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Essential Oil Profiling and Antioxidant Potential of Two Plant Species: GC-MS Analysis, Electrochemical and Spectrophotometric Antioxidant Assays, and In Silico Docking Studies

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﴿ **وَقُلِ ٱعۡمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمۡ وَرَسُولُهُۥ وَٱلۡمُؤۡمِنُونَ** ﴾ سورة التوبة، الآية 105

من قال أنا لها وان أبت رغما عنها أتيت بها لم تكن الرحلة قصيرة ولا ينبغي لها أن تكون

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﴿ **وَقُلِ ٱعۡمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمۡ وَرَسُولُهُۥ وَٱلۡمُؤۡمِنُونَ** ﴾

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Abstract:

This study investigates the chemical composition and antioxidant activity of essential oils extracted from two medicinal plants: Mentha piperita (peppermint) and Ammodaucus leucotrichus (desert cumin), both traditionally used in Algerian folk medicine. Essential oils were obtained by hydro distillation and analysed using Gas Chromatography–Mass Spectrometry (GC-MS), revealing the dominance of menthol and menthone in Mentha piperita, and cumin aldehyde and thymol derivatives in Ammodaucus leucotrichus.

The antioxidant capacity of the oils was evaluated using both the DPPH radical scavenging assay and cyclic voltammetry. Results showed that Mentha piperita had a stronger radical scavenging activity, with an IC_{50} of 49.0 µg/mL, while Ammodaucus leucotrichus exhibited an IC_{50} of 59.1 µg/mL, both outperforming the standard α-tocopherol (IC_{50} = 70.8 µg/mL). Electrochemical analysis confirmed the electron-donating ability of the oils, further supporting their antioxidant potential.

These findings validate the traditional use of both species and highlight their potential as natural sources of bioactive compounds for pharmaceutical and nutraceutical applications. Further studies are recommended to evaluate their broader biological effects and ensure safety for therapeutic use.

Keywords : Mentha piperita, Ammodaucus leucotrichus, Essential Oils, GC-MS, DPPH, Antioxidants Activity, Cyclic Voltammetry.

Résumé

Cette étude porte sur l'analyse chimique et l'évaluation de l'activité antioxydante des huiles essentielles extraites de deux plantes médicinales : Mentha piperita (menthe poivrée) et Ammodaucus leucotrichus (cumin du désert), traditionnellement utilisées dans la médecine populaire algérienne. Les huiles ont été extraites par hydrodistillation et analysées par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Les résultats ont révélé que l'huile de menthe est riche en menthol et menthone, tandis que celle du cumin contient majoritairement du cumin aldéhyde et des dérivés du thymol.

L'activité antioxydante a été évaluée à l'aide du test DPPH et de la volta métrie cyclique. Les résultats ont montré que la menthe poivrée présente une capacité inhibitrice supérieure avec une valeur IC_{50} de 49,0 µg/mL, contre 59,1 µg/mL pour le cumin du désert, surpassant tous deux le standard α-tocophérol (IC_{50} = 70,8 µg/mL). Les analyses électrochimiques ont confirmé la capacité des huiles à donner des électrons, renforçant ainsi leur potentiel antioxydant.

Ces résultats appuient l'usage traditionnel des deux plantes et soulignent leur valeur en tant que sources naturelles prometteuses de composés bioactifs pour les secteurs pharmaceutique et agroalimentaire. L'étude recommande des recherches futures approfondies pour évaluer leurs effets biologiques et leur sécurité d'utilisation thérapeutique.

Mots-clés : Mentha piperita, Ammodaucus leucotrichus, huiles essentielles, GC-MS, DPPH, activité antioxydante, volta métrie cyclique.

الملخص

تتناول هذه الدراسة التحليل الكيميائي وتقييم النشاط المضاد للأكسدة للزيوت الأساسية المستخلصة من نباتين طبيين هما Mentha piperita :(النعناع الفلفلي) و Ammodaucus leucotrichus(الكمون الصحراوي)، المعروفين باستخدامهما التقليدي في الطب الشعبي الجزائري. تم استخلاص الزيوت بالتقطير المائي، وتحليلها باستخدام جهاز GC–MS ، حيث أظهرت النتائج احتواء النعناع على المنثول والمنثون، في حين احتوى الكمون على الكومينالدهيد ومشتقات الثيمول.

تم تقييم الفعالية المضادة للأكسدة باستخدام اختبار DPPH وتحليل الفولطامترية الدورية. أظهرت النتائج أن النعناع الفلفلي يتمتع بفعالية أكبر في تثبيط الجذور الحرة بقيمة IC_{50}ابلغت 49.0 ميكروغرام/مل، مقابل 59.1 ميكروغرام/مل للكمون الصحراوي، وكلاهما تفوّق على α–Tocopherol القياسي .(IC_{50} = 70.8) كما أكدت التحاليل الكهروكيميائية قدرة الزيوت على التبرع بالإلكترونات، مما يعزز من فعاليتها كمضادات أكسدة.

تُعزز هذه النتائج الاستخدامات التقليدية للنباتين، وتبرز قيمتهما كمصادر طبيعية واعدة للمركبات الحيوية في المجالات الدوائية والغذائية. وتوصي الدراسة بإجراء أبحاث مستقبلية أوسع لتقييم تأثيراتهما البيولوجية وسلامة استخدامهما العلاجي.

الكلمات المفتاحية: النعناع الفلفلي، الكمون الصحراوي، الزيوت الأساسية، GC–MS، DPPH، النشاط المضاد للأكسدة، الفولطامترية الدورية.

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List of Abbreviations

α: alfa

β: Beta

ADMET: absorption, distribution, metabolisme, excretion and toxicity

C: concentration

CV: cyclic voltammetry

DMSO: Dimethyl sulfoxide

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

E: energy

GC: Gas chromatograph

GR: glutathione reductase

HPLC: High Performance Liquid Chromatography Chromatogram

IFD: Induced Fit Docking

IC_{50}: concentration causing 50% inhibtion of an activity

IC_{50}: concentration causing 50% inhibtion of an activity

KOH: potassium hydroxide

Kg: Kilo gramme

kb: binding constant

MS: mass spectrometry

M: mole

Mg: milligramme

PH: HydrogenPotential

T°: Temperature

V: Volume

UV: Ultraviolet

Vis: Visible

ΔG: the binding free energy

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GENERAL INTRODUCTION

Since the dawn of civilization, plants have played a vital role in sustaining human life—not only as sources of food and materials but also as powerful agents of healing. Across ancient cultures, medicinal plants were documented and revered as the basis of traditional medicine systems. Despite the rise of synthetic pharmaceuticals, modern science continues to rediscover the value of nature's pharmacy.

Among the most intriguing plant products are essential oils, which are volatile, aromatic compounds known for their diverse biological activities. Rich in terpenes, phenolics, and aldehydes, essential oils have demonstrated antioxidant, antimicrobial, anti-inflammatory, and even anticancer properties, making them highly relevant in the pharmaceutical, cosmetic, and food industries.

In particular, oxidative stress-a condition caused by an imbalance between free radicals and antioxidants-has been linked to numerous chronic diseases including cancer, neurodegenerative disorders, and cardiovascular conditions. This has prompted significant interest in the search for natural antioxidants, especially from medicinal plants.

Algeria, especially its Saharan regions, is home to a rich yet underexplored botanical diversity. Local communities have long used native plants for therapeutic purposes, often without scientific validation. Two such plants are:

- **Mentha piperita (peppermint**), a globally recognized aromatic species known for its soothing and antimicrobial effects.
- **Ammodaucus leucotrichus (desert cumin**), a lesser-known species endemic to North Africa, traditionally used in the treatment of digestive disorders.

This study was designed to explore the phytochemical composition and antioxidant potential of these two species. Essential oils were extracted using hydro distillation, analyzed by Gas Chromatography–Mass Spectrometry (GC-MS), and evaluated through DPPH radical scavenging assay and cyclic voltammetry to provide a dual perspective on their redox behavior.

By bridging traditional knowledge with modern analytical tools, this research aims to confirm the biological significance of these plants and support their sustainable exploitation in pharmaceutical and nutraceutical sectors. It also contributes to the scientific valorization of Algerian medicinal flora

FIRST PART: THERETICAL

CHAPTER ONE:

MEDICINAL PLANTS

I. Generalities about medicinal plants

1. A brief overview of medicinal plants

The use of medicinal plants originated through instinctive behavior, much like that observed in animals. In early times, due to the lack of scientific knowledge regarding the causes of diseases or the methods to treat them using plants, people relied heavily on trial and error. Over time, plants became essential to human life, offering not only shelter, clothing, nourishment, and sensory delights through taste and scent, but also healing substances. These natural resources laid the foundation for the development of complex Traditional Medicine (TM) systems that have evolved over millennia and continue to offer valuable therapeutic solutions today. Ancient civilizations played a crucial role in systematically documenting herbal knowledge and creating extensive herbal pharmacopeia's. While not all the healing claims associated with plants have stood up to modern scrutiny, traditional plant-based remedies are deeply rooted in centuries—if not thousands of years—of empirical observation and practice *(Beyene et al., 2016).*

2. Introduction

Plants play a fundamental role in the ecosystem and are essential to all forms of life on Earth. Medicinal plants, in particular, are found across the globe and have been utilized by human societies since ancient times. Across different cultures and civilizations, people have independently turned to plants for sustenance, protection, clothing, and healing. Through observation and experimentation, early healers—such as priests, shamans, herbalists, and spiritual figures—identified the healing properties of various plants. This tradition continues today in many regions, including parts of Asia, Africa, the Middle East, Australia, and Latin America.

The use of natural herbs and medicinal plants for treating and preventing illnesses—often referred to as “nature’s pharmacy”—has deep historical roots, as recorded in sacred texts such as the Vedas, the Bible, the Qur’an, and the Hadith. These sources reflect humanity’s longstanding connection to plant-based healing. Supporting this, Duke, Duke, and du Cellier (2008) compiled a reference work titled Duke’s Handbook of Medicinal Plants of the Bible, which documents various “faith-based pharmaceuticals” (Singh et al., 2012).

3. History of the Use of Plants as Medicines

The use of medicinal plants dates back over 5,000 years, with some of the earliest written records found on Sumerian clay tablets. These ancient texts include recipes for drug

preparations made from more than 200 plant species. Additional documentation from around 2600 BC in Mesopotamia, inscribed in cuneiform on clay tablets, further supports the early application of plant-based medicine.

In ancient China, around 2500 BC, Emperor Shen Nung compiled descriptions of over 300 medicinal herbs, laying the foundation for the extensive Chinese materia medica tradition. Many of these herbs were also recognized and utilized in other civilizations, including ancient Egypt, Mesopotamia, and various parts of Europe.

Opium stands out as one of the earliest known effective drugs, used thousands of years ago. Civilizations such as the Sumerians, Assyrians, and Babylonians documented herbal remedies in cuneiform on numerous tablets. The famous Code of Hammurabi, dating to the 18th century BC, also includes references to herbal medicines. Egyptian knowledge of medicinal plants was recorded on tomb walls and papyrus scrolls, with the 'Ebers Papyrus' from around 1550 BC being one of the most significant texts. It lists over 600 plant-based prescriptions.

In Europe, the formal study of medicine is often traced back to Hippocrates (460–377 BC), who cataloged over 200 medicinal plants, classifying them by their physiological effects. He is widely regarded as the 'father of medicine'. Later, Celsus (25 BC–50 AD) referenced approximately 250 medicinal plants in his work De re medica. Another pivotal figure, the Greek military physician Dioscorides, wrote De Materia Medica around 77 AD. This text became a cornerstone of European herbal medicine for more than a thousand years, featuring over 900 remedies, mostly derived from plants.

Galen (130–200 AD), a prominent Greek physician and pharmacist, significantly influenced early medical science, including the use of plants in treatment. In the Americas, Indigenous peoples also developed a rich tradition of medicinal plant use. Some modern cancer treatments, like paclitaxel (Taxol®), are derived from North American plants such as the Pacific yew tree (Taxus brevifolia).

African traditional medicine has likewise been practiced for centuries, offering a diverse range of plant-based remedies, though much of this knowledge was historically passed down orally and not well documented.

In the 17th century, the bark of the Cinchona tree was introduced into Europe as an effective treatment for malaria. Its active compound, quinine, was isolated in 1820. During the 18th

century, the foxglove plant was used by William Withering to treat dropsy; its active ingredient, digoxin, is still in use for heart conditions today.

The 19th century marked the beginning of the isolation of 'pure' drugs from plants. Key alkaloids such as quinine (1820), morphine (1806), and ephedrine (1887) were extracted and later synthesized chemically. The second half of the 20th century saw a significant expansion in drug development due to progress in pharmaceutical sciences and the rise of pharmaceutical companies.

While modern medicine uses both synthetic and natural compounds, many of today's widely used drugs are derived directly from plants or inspired by plant-based natural products.

4. Medicinal plants

A medicinal plant refers to any plant that possesses naturally occurring compounds in one or more of its parts, which can be utilized for healing purposes or serve as raw materials in the semi-synthetic production of pharmaceutical drugs. (Yudharaj, P. et al., 2016).

4.1. Classification of medicinal plants:

Out of the roughly 250,000 species of higher plants found on Earth, around 5,000 are recognized for their distinct therapeutic properties, while more than 80,000 species are believed to possess some form of medicinal value. (Joy, P. P. et al., 1998).

Typically, medicinal plants are categorized based on the active compounds found within various storage organs of the plant, such as roots, leaves, flowers, seeds, and other parts. (Lubbe et al., 2011).

There are multiple approaches to classifying medicinal plants, including the following:

4.2 According to the usage

Herb Classification Based on Usage

Herbs can be categorized into four primary types depending on how they are used: medicinal, culinary, aromatic, and ornamental (Lubbe et al., 2011).

A. Medicinal Herbs

These herbs are known for their therapeutic qualities and are commonly used in the production of pharmaceuticals due to their healing properties (Lubbe et al., 2011).

B. Culinary Herbs

Widely utilized in cooking, culinary herbs are favored for their intense flavors. Examples

include mint, basil, and cinnamon (Lubbe et al., 2011).

C .Aromatic Herbs

Aromatic herbs are valued for their pleasant scent, which comes from their leaves or flowers. Their essential oils are often used in the food industry, as well as in cosmetics, perfumes, beverages, and dental products. Notable examples are rosemary, saffron, clove, thyme, and cumin (Lubbe et al., 2011).

D. Ornamental Herbs

These herbs are appreciated for their visual appeal, often chosen for decorative purposes due to their vibrant blossoms and foliage. Lavender and chives are commonly used in this context (Lubbe et al., 2011).

4.3.Based on Their Active Compounds

Herbs can be categorized into five primary groups according to their key active constituents: Aromatic (containing volatile oils), Astringent (rich in tannins), Bitter (composed of phenolic compounds, saponins, and alkaloids), Mucilaginous (high in polysaccharides), and Nutritive (serving as food sources) (Lubbe et al., 2011).

A. Aromatic Herbs (Volatile Oils)

These herbs are known for their pleasant fragrance, which explains the term “aromatic.” They are commonly used in culinary applications, perfumery, and traditional medicine. Aromatic herbs can be further divided into two groups: nervine herbs and stimulants. Examples include garlic, ginger, and fennel (Lubbe et al., 2011).

B. Astringent Herbs

Astringent herbs contain tannins that can bind and precipitate proteins, leading to the contraction and toning of tissues. This action helps control bodily discharges. However, excessive consumption can be hepatotoxic and affect the urinary, circulatory, and digestive systems. These herbs also exhibit antibacterial and pain-relieving properties. Examples include red raspberry and peppermint (Lubbe et al., 2011).

C. Bitter Herbs

These herbs are recognized for their distinct bitter taste, resulting from compounds such as phenols, glycosides, alkaloids, and saponins. Bitter herbs fall into four main subcategories: laxatives (e.g., barberry), diuretics (e.g., parsley), saponin-rich herbs (e.g., alfalfa), and alkaloid-rich herbs (e.g., members of the poppy family) (Lubbe et al., 2011).

D. Mucilaginous Herbs

Characterized by their high polysaccharide content, mucilaginous herbs have a slippery texture and a soothing, mild flavor when diluted. Notable examples include chia seeds,

flaxseeds, aloe vera, and licorice root (Lubbe et al., 2011).

E. Nutritive Herbs

These herbs are named for their nutritional benefits, as they contribute essential nutrients to the diet. Common examples include fruits and vegetables such as apples, bananas, carrots, broccoli, cabbage, onions, and pineapples (Lubbe et al., 2011).

4. 4 Based on their life cycle duration, herbs can be categorized into three types:

A. Annual herbs : complete their entire life cycle—from germination to seed production within a single year. These plants bloom once during the season and then die. Examples of annual herbs that thrive throughout the year include basil, chamomile, and saffron.

B. Biennial herbs require two growing seasons to complete their life cycle. They typically develop foliage in the first year and flower in the second. Common biennials include fennel, lavender, and rosemary.

C. Perennial herbs live for more than two years, enduring through winter and blooming seasonally each year. Notable perennials include thyme, mint, and parsley (Lubbe et al., 2011).

4.5Classification of Medicinal Plants Based on Taxonomy

A. Medicinal plants are categorized into several botanical families

, each contributing significantly to traditional and modern medicine.

Compositae (Asteraceae) Family

Known as the Daisy family, Compositae is recognized as the most extensive group of medicinal plants. It includes species like chamomile, widely used for its therapeutic qualities (Lubbe et al., 2011).

B. Labiatae (Lamiaceae) Family

Also called the mint family, Labiatae includes a variety of aromatic herbs and shrubs. These plants are particularly prevalent in Mediterranean regions, where their essential oil production helps them thrive in hot climates. Notable members include mint, rosemary, thyme, and lavender (Lubbe et al., 2011).

C. Umbelliferae (Apiaceae) Family

Often referred to as the carrot family, Umbelliferae plants are identified by their umbrella-shaped floral arrangements. Many species, such as anise (Pimpinella anisum), fennel (Foeniculum vulgaris), and wild carrot (Daucus carota), produce essential oils that aid in regulating plant temperature during summer (Lubbe et al., 2011).

D. Leguminosae (Fabaceae) Family

The Leguminosae, or pea family, encompasses a wide range of cultivated and wild species used for food, decoration, and medicine. Medicinal representatives include alfalfa (Medicago sativa) and both red and

white clovers (Trifolium pratense and Trifolium repens) (Lubbe et al., 2011).

E. Rosaceae Family

This family, also known as the rose family, includes numerous medicinal species, mostly trees and shrubs that bear edible fruits. Examples include wood strawberry (Fragaria moschata), peach, almond, and apricot (Prunus persica, P. amygdalus , and P. armeniaca) (Lubbe et al., 2011).

F. Rutaceae and Solanaceae Families

Rutaceae (rue family) is a smaller group primarily consisting of fruit-bearing plants and medicinal herbs, such as orange (Citrus aurantium), lemon (Citrus limon), and grapefruit (Citrus paradisi) (Lubbe et al., 2011).

G. Cruciferae (Brassicaceae) Family

This family is marked by its characteristic cross-shaped flowers. It includes numerous therapeutic plants like black mustard (Brassica nigra), white mustard (Sinapis alba), and wild radish (Raphanus raphanistrum) (Lubbe et al., 2011).

H. Liliaceae Family

The lily family is composed mostly of herbaceous plants with medicinal properties. Noteworthy species include aloe (Aloe vera), garlic (Allium sativum), onion (Allium cepa), and meadow saffron (Colchicum autumnale) (Lubbe et al., 2011).

I. Caryophyllaceae and Boraginaceae Families

Caryophyllaceae, known as the pink family, is identified by flowers typically having four or five white or pink petals. Medicinal species include smooth rupture-wort (Herniaria glabra), nailwort (Paronychia argentea), sand spurrey (Spergularia rubra), common chickweed (Stellaria media), and viscid sandwort (Alsine tenuifolia) (Lubbe et al., 2011).

J. Ranunculaceae and Papaveraceae Families

Members of the Ranunculaceae (buttercup family) often bear striking five-petaled flowers. This group includes medicinal plants such as pheasant's eye (Adonis annua), larkspur (Delphinium ajacis), poppy anemone (Anemone coronaria), and lesser celandine (Ranunculus ficaria) (Lubbe et al., 2011).

K. Malvaceae and Other Families

The Malvaceae, or mallow family, is characterized by five-petaled flowers and nut-like fruits. Cotton (Gossypium herbaceum) is an example of a medicinal plant in this group (Lubbe et al., 2011)

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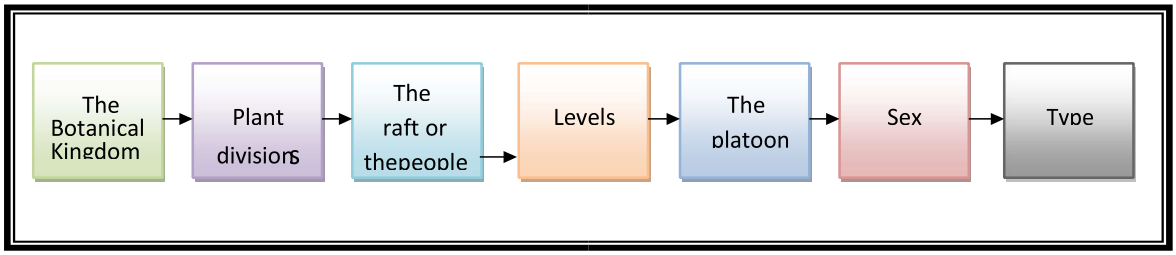


Figure 1:Classification Unit Diagram in Plant Science

5. Sources of Medicinal Plants

Traditional health practitioners (THPs) unanimously identified homestead gardens and vegetation along roadsides as the primary locations for sourcing medicinal plants. Additional sources included local markets, designated forest reserves, and remote woodland areas. In contrast, wetlands were reported as the most challenging environments for locating medicinal herbs. According to all THPs, traditional medical knowledge (TMK) was predominantly inherited from older generations, specifically parents and grandparents. The least cited method of acquiring TMK was through trial and error, which involves the random selection and use of plant species for treatment without prior knowledge of their medicinal value (Ssenku, J. E. et al., 2022).

6. Key Features of Medicinal Plants:

- Medicinal plants possess several notable qualities when utilized for therapeutic purposes, including:

Synergistic Effects:

Plant compounds work together in a coordinated manner, which can enhance the overall effect, counteract adverse reactions, or minimize potential toxicity of individual constituents.

Complementary Role in Conventional Medicine:

In treating serious and complex health conditions such as cancer, plant-derived substances have shown significant effectiveness, often serving as valuable additions to standard medical treatments.

Preventative Properties:

Plant components are also known for their ability to help prevent the onset of certain illnesses. This preventative aspect can reduce the need for chemical-based pharmaceuticals, which are generally prescribed after a disease has developed—thereby also lowering the risk of side effects from synthetic drugs (Rasool et al., 2012)

7 .Elements Influencing the Harvesting and Gathering of Medicinal Plants Cultivation:

The rising market demand for medicinal and aromatic plants is largely due to their exceptional therapeutic value and pharmacological effectiveness. As a result, these plants require enhanced care and more precise management techniques. In situations where scientifically validated cultivation practices are not available, traditional farming methods should be utilized. However, if these are insufficient, new cultivation techniques need to be developed through scientific investigation. Effective agricultural practices should incorporate well-planned crop rotation systems and an environment tailored to meet the specific growth requirements of the plants. Additionally, when feasible, conservation agriculture (CA) methods should be applied, although their use may be limited when faced with unusually high levels of soil moisture and organic matter. (Porwal et al., 2020)

1. Propagation Method:

a) Sexual or Seed-Based Propagation

Sexual propagation refers to the technique of cultivating medicinal plants by planting seeds in appropriately prepared soil. There are two primary approaches for sowing seeds: the dibbling method, which is suited for medium-sized and heavier seeds like coriander and fennel where seeds are placed directly into holes; and the broadcasting method, ideal for smaller seeds such as linseed, isapgol , and sesame. In this method, the seeds are evenly scattered over the soil surface, which has been adequately tilled for growth. (Porwal et al., 2020)

b) Vegetative or Asexual Propagation

Vegetative propagation, an asexual method of plant reproduction, involves growing a new plant from a stem, root, or other part of an existing plant under favorable conditions. One key advantage of this technique is that the offspring are genetically identical to the parent plant. Certain fruit species—like grapes, pomegranates, and lemons—can only be reproduced through vegetative means. This method also ensures that plants mature and bear fruit faster than those grown from seeds. Additionally, grafting and budding can enhance resistance to diseases, while inferior plant varieties can be excluded from propagation.

The process involves planting specific plant parts into properly prepared soil to encourage growth. Several types of vegetative structures used in this process include:

- Bulbs: such as garlic and squill

- Corms: including saffron and colchicum
- Tubers: like potatoes and jalap
- Rhizomes: such as turmeric and ginger
- Runners: for example, peppermint
- Suckers: including mint, banana, and pineapple (Porwal et al., 2020)
- **Factors Influencing Cultivation:**

- **Soil**

Soil, the uppermost layer of the Earth's surface, forms through the gradual breakdown of rocks, combined with the activity of plants and microorganisms, which help in creating a weathered layer. For successful cultivation, especially of medicinal plants, the soil must contain adequate levels of organic material, essential nutrients, and other necessary components. Key properties such as soil texture, pH level, moisture retention, fertility, and drainage capacity should be optimized to suit the needs of the specific plant species being grown . Incorporating compost is widely recommended for enhancing soil quality and achieving better yields in medicinal plant cultivation. Soil directly affects several aspects of plant development, including seed germination, plant stability, stem robustness, root depth, and flower production . There are several types of soil, each with different properties: clay, loamy, silt loam, sandy loam, sandy soil, and chalky soil (Porwal et al., 2020).

•Altitude, Temperature and Humidity

Climatic conditions—such as altitude, temperature, humidity, rainfall, and duration of sunlight—play a vital role in shaping the physical, chemical, and biological quality of medicinal plants. Factors like the length of daylight, overall rainfall patterns, average minimum temperatures, and the fluctuations between day and night temperatures significantly affect the plant's biochemical activity and composition (Porwal et al., 2020).

•Rainfall and Irrigation

The successful growth and development of most plants depend on either adequate natural rainfall or a well-organized irrigation system. At each stage of a medicinal plant's life cycle, irrigation and drainage should be carefully tailored to meet the specific requirements of the plant species being cultivated . Water management should align with established local, regional, or national quality standards to ensure optimal plant health. Regular monitoring is essential to guarantee that medicinal plants receive the appropriate amount of water for their growth . However, certain drought-resistant species, such as aloe and acacia, are classified as

xerophytes and can thrive without the need for rainfall or irrigation, making them easier to cultivate in arid conditions (Porwal et al., 2020).

•Fertilizers

Fertilizers are substances composed of both organic and inorganic materials used to enhance soil fertility and promote healthy plant growth. There are several types of fertilizers, each offering different benefits:

Organic Fertilizers of Biotic Origin: These are derived from living organisms and are used to compensate for the natural deficiency of nitrogen-rich compounds and organic matter in the soil. Only materials capable of providing essential nutrients to plants are utilized.

2 Green Leaf Manure:

This type of manure involves mixing fertile organic materials with soil, particularly clay-rich soil. It supplies a wide range of nutrients necessary for the healthy growth of medicinal plants, leading to improved crop yields.

3 Farmyard Manure:

Farmyard manure is produced from a mix of livestock waste, leftover feed, plant stalks, and husks that are not fully consumed. This traditional fertilizer enriches the soil naturally.

4 Compost Manure:

Compost consists of partially decomposed organic matter from both plant and animal sources. Over time, these materials break down and enrich the soil with valuable nutrients.

5 Green Manure:

Green manure is created from fast-growing, herbaceous (non-woody) plants that are plowed back into the soil while still green and tender. These plants enrich the soil with organic content and nitrogen, enhance soil structure, and form a protective layer that reduces erosion and slows down natural degradation processes. The use of green manure can increase the biomass of medicinal plants by 30 to 50 percent.

6 Bio-fertilizers:

These are biologically active substances derived from organisms such as fungi, algae, and bacteria. When introduced into the soil, they facilitate nutrient availability, particularly nitrogen. Common types include symbiotic associations like legume-Rhizobium and Azolla-Anabaena, as well as free-living nitrogen-fixing microorganisms. Many bio-fertilizers include cyanobacteria, a group of primarily photosynthetic, prokaryotic organisms.

7 Chemical Fertilizers:

Chemical fertilizers provide essential nutrients in both macro and micro quantities.

Macronutrients include nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, while micronutrients include elements like iron, manganese, zinc, boron, copper, molybdenum, as well as carbon and oxygen—all vital for plant development (Porwal et al., 2020).

9. Pests and Pest Management

Pesticides, which are substances derived from both chemical and natural sources, are effective in small quantities for controlling pest populations. Pests refer to unwanted organisms—whether plant or animal—that pose a threat to crops and human health. Many insects act as carriers of serious diseases such as typhus and malaria. Additionally, certain pests, including those that affect crops like cotton (Gossypium), maize (Zea mays), wheat (Triticum aestivum), and rice (Oryza sativa), can cause significant agricultural losses. Besides insects, various weeds—such as Ambrosia artemisiiflora and Toxicodendron radicans—along with harmful bacteria, fungi, and rodents like Rattus rattus, are also considered major agricultural pests.

To develop and apply effective pest control strategies, especially those using natural alternatives, it is essential to understand the source and nature of the infestation. Globally, the pesticide industry is extensive, with synthetic chemical pesticides dominating the market, particularly in agricultural sectors (Porwal et al., 2020).

II. General Approaches to Pest Control

1. Natural Control Methods

In nature, predator-prey relationships play a significant role in keeping pest populations in check. Nearly every pest species is naturally regulated to some extent by predatory or parasitic organisms.

The key contributors to natural pest control include predators, parasites, and pathogens that specifically target harmful insects. However, when chemical pesticides are used to eliminate a major pest, they can also unintentionally kill beneficial organisms that help control other pests. This disruption can lead to the emergence of secondary pest problems, as the natural balance between helpful and harmful species is disturbed.

Environmental factors such as seasonal temperature variations, rainfall, soil composition, humidity, and other climatic conditions also influence insect activity and the behavior of their host plants (Porwal et al., 2020).

2. Artificial Pest Control

Artificial pest control methods have been developed and implemented by humans to manage pest populations more effectively. These methods are generally categorized into mechanical, agricultural, chemical, and biological approaches. Each employs specific strategies to reduce or eliminate pests in cultivated areas.

Tableau 1: Techniques and Methods Used in Artificial Pest Control

Controlling techniques	Methods Applied
mechanical control	This approach relies on physical labor and tools to remove and destroy pests. Techniques include hand-picking ,trapping, burning infested material , and eliminating insect life stages such as eggs ,larvae and pupae.
Agricultural control	Involves farming practices such as crop rotation ,deep tillage ,altering environmental conditions, and applying systemic insecticides to deter pest infestations.
Chemical controls	Includes the application of pesticides ,herbicides ,biopesticides ,and antifeedants to directly target and suppress pest populations.

- **Collection of medicinal plant:**

  Regardless of the type of crude drug or the region it is sourced from, the ideal time for harvesting medicinal plants is when they are richest in secondary metabolites—the compounds responsible for their therapeutic effects. Several factors influence the proper collection of these plants, including climatic conditions, the plant's age, daily exposure to sunlight and wind, and the physiological stage of the plant part being harvested .These factors directly impact the potency and yield of the active constituents. For instance, the climate at the time of collection plays a critical role: leaves of Mentha (used in cooking), Eucalyptus (a valuable commercial species), and spearmint (known for memory enhancement) have a higher concentration of essential oils in the spring, whereas their oil content decreases in hot summers or cold winters. Plant age is another key factor. Species of Cinchona from the Rubiaceae family, for example, produce significant quantities of quinine—an alkaloid effective against

malaria—and cinchonine, which has antipyretic properties, when harvested between 6 to 9 years of age

- Additionally, the physiological maturity of plant parts is crucial. Leaves of Digitalis, Tobacco,and Senna are best harvested when they reach full maturity to ensure optimal medicinal value. Likewise, seeds of black cumin (Nigella sativa) are collected when they show both white and black coloration, indicating the appropriate stage for maximum potency (Porwal et al., 2020).

- **Harvesting:**

Harvesting plays a critical role in the cultivation process, significantly influencing the economic value of raw medicinal materials. It is essential to consider the type of plant being harvested and the quality standards it must meet. The timing of collection—ideally during optimal weather conditions or specific times of day—is crucial to maintaining the desired quality of the herb. Harvest intervals depend on the nature of the plant material, and detailed guidelines on timing are typically found in national pharmacopeias, authenticated texts, modern monographs, and other authoritative sources. Notably, the concentration of therapeutic compounds in plants varies with their stage of maturity. Therefore, instead of harvesting the entire plant indiscriminately, it is more effective to base the timing on when the plant's active constituents are most abundant. It is also vital to avoid contaminating the harvest with weeds, toxic species, or other unwanted materials. (Porwal et al., 2020) Medicinal plants should be harvested under dry, favorable conditions—avoiding rainfall, dew, or excessive humidity. Tools, containers, and equipment must be clean and suitable for different types of plant material and soil conditions to prevent contamination. Once collected, the raw medicinal material should be quickly moved to well-ventilated, dry storage areas. Clean, breathable containers such as cloth bags, baskets, or hoppers should be used for transport, ensuring they are free from dirt and foreign substances. (Porwal et al., 2020) When not in use, containers must be stored properly and protected from pests like rodents, birds, and insects. Any mechanical damage or compression of the harvested material—such as over-packing—should be avoided. During harvesting, post-harvest handling, and processing, any decayed or spoiled plant matter should be removed to reduce the risk of microbial contamination and maintain the integrity of the raw material. The sustainable reintroduction of valuable medicinal and aromatic plants can also help in preserving both their therapeutic value and commercial importance. (Porwal et al., 2020)

- **Drying:**

To ensure the long-term preservation and quality of medicinal plant materials intended for dry use, it is essential to minimize their moisture content. Lowering moisture helps prevent the growth of mold and reduces the risk of microbial contamination. The drying process can be carried out through natural means—such as air drying under suitable environmental conditions—or by using artificial methods involving controlled heat and ventilation systems. (Porwal et al., 2020).

1. **Natural drying Methods:**

Natural drying involves exposing the material to sunlight or placing it in the shade. Shade drying is generally favored because it better preserves the natural color of certain medicinal substances—such as digitalis, clove, and senna—as well as their volatile components, like those found in peppermint. However, direct sun drying can be used effectively for substances that are stable under heat and sunlight, including gum acacia, seeds, and fruits (Porwal et al., 2020).

2. **Artificial Drying Methods:**

a) **Tray Drying:**

This method is commonly used for plant materials that do not contain volatile oils, are heat-stable, or require enzyme deactivation. In this process, controlled hot air at a specific temperature is circulated through tray dryers to facilitate the removal of moisture. This technique is particularly effective for drying materials like belladonna roots, cinchona bark, tea leaves, raspberry leaves, and gums (Porwal et al., 2020).

b) **Vacuum Dryin**

Plants such as digitalis leaves and those containing tannic acid, which are sensitive to high temperatures, are dried using vacuum drying to avoid thermal degradation.

c) **Spray Drying:**

pray drying is employed for plant materials that are extremely reactive to temperature fluctuations and exposure to air, helping preserve their integrity during the drying process (Porwal et al., 2020).

Storage:Storage of Medicinal Plant Materials

1) Facility Requirements:

Storage spaces should be well-ventilated, dry, and shielded from light. If needed, they should

also include systems for temperature and humidity regulation, and be safeguarded against pests such as rodents, insects, and larger animals.

2) Structural Conditions:

Floors must be smooth, crack-free, and easy to clean. Medicinal materials should be kept on shelves, away from walls, to ensure proper airflow. Preventive actions must be taken to stop pest infestation, mold development, rotting, or loss of essential oils. Regular inspections are essential.

3) Quality Control:

Ongoing in-process quality checks are necessary to detect and remove any substandard products, contaminants, or foreign substances before and during final packaging. The processed herbs should be packed in clean, dry containers (boxes, sacks, or bags) that comply with standard operating procedures and the regulations of both the producing and importing countries.

4) Packaging Materials:

Containers used for packaging must be clean, intact, non-contaminating, and suitable for the specific plant materials. Fragile items should be packed in sturdy, rigid containers to prevent damage.

5) Storage Conditions for Dried Products:

Dried herbs, including essential oils, should be stored in a dry and well-ventilated building with minimal temperature fluctuations and good airflow.

6) Storage of Fresh and Frozen Materials:

Fresh herbs should be kept at low temperatures, ideally between 2–8°C. Frozen products need to be stored below -20°C to maintain their quality.

7) Storage of Small Quantities:

For small-scale storage, airtight, moisture-proof, and light-resistant containers like metal tins or amber glass jars are ideal.

8) Inappropriate Containers:

Crude drugs should not be stored in paper bags or wooden boxes as they are not suitable for preserving the quality of medicinal plant materials (Small et al., 2007).

1. Plant Mentha Piperita

1.1 Definition of plant *Mentha Piperita*

Peppermint, scientifically named Mentha piperita, is a hybrid herb derived from the crossbreeding of spearmint (Mentha spicata) and water mint (Mentha aquatica). Known for its

intense aroma and sharp, cooling flavor, it also provides a refreshing sensation. The plant was first classified by Carl Linnaeus in 1753. Characterized by its green foliage and purple-violet blossoms, peppermint has square-shaped green roots and stems that are reddish with a hint of greenish-purple underneath. It typically reaches a height of approximately 76 centimeters (around 2.5 feet). This resilient plant can withstand cold and frosty environments. Although it thrives best under direct sunlight, it is also adaptable to partial shade. As a perennial, peppermint can continue to grow for several years, often re-emerging each season when environmental conditions are favorable. (Linnaeus, 1753)

1.2. Botanical taxonomy of *Mentha Piperita*:

- **Scientific name:** *Mentha Piperita*.
- **Synonym:** *Peppermint*.
- **Common name:** *Peppermint*.

Tableau 2: Botanical classification of *Mentha Piperita*

Kingdom	Plant
Division	Mangnoliophyta
Class	Mangnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha*
Species	*piperita*

1.3. Morphological description of *Mentha Piperita:*

Mentha piperita is a herbaceous plant that grows from rhizomes and typically reaches a height of 30 to 90 cm. The stem is smooth and distinctly square-shaped in cross-section. Its rhizomes are exposed, fibrous, and fleshy, spreading densely under ground. The leaves range from 4 to 9 cm in length and 1.5 to 4 cm in width. They are dark green in colour with noticeable reddish veins, a pointed tip, and serrated or jagged edges. Both the leaves and stems are often covered in fine hairs .The plant produces purple flowers that measure about 6–8 mm in length. Each flower has a corolla split into four lobes, with a diameter of

approximately 5 mm. The flowers are arranged in thick, spiked whorls encircling the stem and have a sharply waved appearance. Blooming generally occurs in mid-summer. Chromosome numbers in Mentha piperita vary due to its diploid nature (2N), with recorded chromosome counts including 66, 72, 84, and 120 (Flora et al., 2014). This species exhibits vigorous growth, rapidly spreading once its buds begin to form (Blamey et al., 1989).

Figure 2: Photograph of *Mentha Piperita*.

1.4. Geographical distribution of *Mentha Piperita*:

Peppermint is cultivated across various regions globally, including Europe, Asia, North Africa, and North America. It is also found in parts of Australia, the Galapagos Islands, and New Zealand. In the United States, its presence is notably established around the Great Lakes area, with records dating back to 1843. In Algeria, the plant is widely distributed, with a higher concentration in the northern regions compared to the south.

1.5. Traditional Applications of *Mentha Piperita*:

Mentha piperita, commonly known as peppermint, has long been valued for its medicinal and culinary benefits. Traditionally, it has been consumed as a refreshing, naturally flavored beverage and employed to stimulate appetite. The plant contains approximately 1% volatile essential oils, with menthol making up about 50–60% of its composition, along with other compounds like acetaldehyde and valeric acid. Known also as medicinal mint, peppermint held significance in ancient cultures. The Egyptians used it to prevent vomiting, while the Romans appreciated its aromatic qualities, incorporating it into wines and sauces. Additionally, it was utilized to alleviate ailments such as headaches and digestive issues. Peppermint is recognized for its ability to soothe gastrointestinal spasms, ease nausea, reduce

bloating, and support the nervous system. It also possesses anthelmintic properties. (Duband et al., 1992)

1.6. Previous Research on *Mentha piperita* (Peppermint) in Plant Studies:

Microbial biofilms present a growing challenge in both the medical and food industries, where they contribute significantly to contamination of surfaces and products. These biofilms are also linked to persistent and hard-to-treat infections in humans, often resulting in recurrent health issues. With the increasing resistance of microbes to traditional treatment methods, the effectiveness of standard control strategies is rapidly diminishing.

In response to this issue, a study explored the potential of various commercially available plant extracts as alternative antimicrobial agents. Specifically, eight herbal extracts were tested for their ability to inhibit biofilm formation by both clinical and reference strains of Pseudomonas aeruginosa and Candida albicans. The antimicrobial properties of these extracts against planktonic (free-floating) microbial cells were evaluated using the minimum inhibitory concentration (MIC) assay.

Among the tested extracts, Echinacea angustifolia (coneflower), *Mentha piperita* (peppermint), and Rosmarinus officinalis (rosemary) demonstrated the strongest antimicrobial effects, with MIC values ranging from 0.38 to 2.5 mg/ml. To further investigate their impact on biofilm development, a crystal violet assay was employed. This assay assessed how pre-treatment of surfaces with these extracts affected initial cell adhesion and the subsequent formation of biofilms.

The results indicated that most of the plant extracts reduced microbial surface colonization by at least 50%. However, when applied to already established biofilms, the extracts were generally less effective, suggesting that disrupting mature biofilms is more challenging. Notably, *Mentha piperita* stood out as the only extract that exhibited consistent antibiofilm activity against both microbial species. (Sandasi et al., 2011)

- **In a separate investigation** ,

researchers explored the chemical profile and biological activities—including anti-inflammatory, cytotoxic, and antioxidant properties—of essential oil derived from *Mentha piperita* (MEO) cultivated in China. Gas chromatography–mass spectrometry (GC-MS) analysis revealed that menthol, menthone, and menthyl acetate were the primary constituents of the oil. MEO demonstrated strong anti-inflammatory effects in a croton oil-induced ear edema model in mice. It also significantly suppressed the production of nitric oxide (NO) and prostaglandin E2 (PGE2) in RAW 264.7 macrophages stimulated with lipopolysaccharide (LPS). The oil's cytotoxic potential was evaluated across four human

cancer cell lines. Notably, MEO showed marked activity against human lung carcinoma (SPC-A1), leukemia (K562), and gastric cancer (SGC-7901) cells, with IC50 values of 10.89, 16.16, and 38.76 µg/ml, respectively. Additionally, it exhibited a moderate level of antioxidant capacity. These findings support further in-depth studies and suggest potential applications for peppermint oil in pharmaceutical and clinical contexts. (Sun, Z. et al., 2014).

2. Ammodaucus leucotrichus: Plant Overview

1.2 Definition Ammodaucus leucotrichus:

mmodaucus leucotrichus Coss. & Dur. is a small, glabrous annual plant that typically reaches a height of 10 to 12 cm. It features upright stems marked by fine striations and bears slightly fleshy, finely divided leaves. Its flowers appear in small clusters (umbels) consisting of 2 to 4 branches, each bearing five separate petals. The fruit, a diachene measuring 6 to 10 mm in length, is densely coated with silky white hairs and emits a strong anise-like fragrance. The plant blooms primarily in early spring, from February through April. It is commonly found across the Algerian Sahara and has also been reported in the Canary Islands (El Haci et al., 2021).

In traditional medicine, Ammodaucus leucotrichus is valued for its therapeutic uses in treating various ailments. Prior analyses of the essential oil extracted from its fruit have revealed that perillaldehyde and limonene are the dominant chemical constituents (El Haci et al., 2021).

1.3 Botanical taxonomy of *Ammodaucus leucotrichus:*

- **scientific name:** *Ammodaucus leucotrichus.*
- **Synonym:***Hairy cumin.*
- **common name:***Kûmun-Sûfi.* (MAYOU et al., 2018).

Tableau 3: taxonomic Hierarchy of *Ammodaucus leucotrichus*

Kingdom	Plants
Division	Magnoliophyta
Class	Mangnoliopsida
Order	Araliales
Family	Apiaceae
Genus	*Ammodaucus*
Species	*Leucotrichus*

1.4 Morphological characteristics of *Ammodaucus leucotrichus*:

Ammodaucus leucotrichus is a small annual plant, found both in the wild and under cultivation. It typically grows to a height of 10 to 12 cm. The plant features slender, slightly succulent leaves and displays white flowers arranged in umbels composed of 2 to 4 branches, each bearing five distinct, unfused petals. Its fruit is a diachene, measuring between 6 and 10 mm in length, and is notably covered with a thick layer of soft, white hairs. This hairy texture has earned it the common name "hairy cumin" in certain regions (Abderrezag et al., 2021).

(A)

(B)

Figure 3: Photographs of a plant *Ammodaucus leucotrichus*.(Chatelain et al., 2021).

1.5 Geographical distribution of *Ammodaucus leucotrichus*:

The genus *Ammodaucus*, which is part of this plant family, is considered endemic to the Saharan and Sub-Saharan regions of North Africa. Its distribution spans countries such as

Algeria, Morocco, Tunisia, and Egypt. In Algeria, the only wild-growing species from this genus is *Ammodaucus leucotrichus* Coss & Dur subsp. leucotrichus. Another subspecies, *Ammodaucus leucotrichus* Coss & Dur subsp. nanocarpus, was identified by Bultrán in the Canary Islands (Mohammedi et al., 2018).

1.6 Traditional Applications of Ammodaucus leucotrichus:

In Algeria, Ammodaucus leucotrichus, locally referred to as "Moudrayga" or "El Massoufa," is a medicinal plant commonly available in local herbal markets, especially across the southern regions of the Algerian Sahara. Nomadic populations typically gather the plant's seeds and leaves in the spring season, once the fruits reach maturity. In this region, the leaves are traditionally used to add flavor to tea, while the fruits serve as a spice in various culinary dishes. Both seeds and leaves are also prepared as decoctions or infusions and employed in folk remedies for managing several health conditions, including high blood pressure, chest discomfort, liver and digestive disorders, gastroenteritis, and diabetes (Idm'hand et al., 2020).

1.7 Overview of Previous Research on Ammodaucus leucotrichus:

Research has been conducted on *Ammodaucus leucotrichus*, a plant native to the Algerian Sahara Desert, an area known for its distinct climatic conditions. The plant yielded more than 2.5% essential oil, with Perilla aldehyde emerging as the predominant component, comprising approximately 85% of the oil. This essential oil (EOALF) demonstrated notable antioxidant, antibacterial, and anti-inflammatory properties, mainly attributed to its high content of Perilla aldehyde and the presence of limonene, the second most abundant compound. Given the growing demand in the cosmetics industry for natural bioactive substances, studies were undertaken to assess EOALF's performance when integrated into a standard cream formulation. Results indicated that the essential oil maintained its biological activity over a 28-day period, though a gradual decline was observed. This decrease began soon after the oil's incorporation into the cream, suggesting possible interactions with the formulation matrix. As a result, enhancing the oil's stability and prolonging its efficacy became a priority, with microencapsulation proposed as a promising solution. Despite these challenges, the inherent bioactive potential of EOALF supports its value as a natural ingredient for cosmeceutical applications. (Halla et al., 2018) .

III. General about essential oils

In a separate investigation, an in-depth comparative analysis was conducted on the essential oils (EOs) extracted from various parts—roots, stems/leaves, flowers, and fruits—of A. leucotrichus subsp. leucotrichus, a plant native to the Algerian desert. The findings revealed distinct differences in the profiles of specialized metabolites among the plant organs. Both the content and composition of EOs showed notable variability depending on the plant part analysed. Essential oil yields ranged between 0.52% and 3.04% (w/w, calculated on a dry weight basis) and consisted of complex blends comprising up to 89 unique compounds. The most prominent constituents identified across the different extracts included (Z)-falcarinol, β-bisabolene, myristicin, methyl eugenol, α-pinene, muurola-4,10(14)-dien-1-β-ol, geranyl acetate, perillaldehyde, and limonene. This chemical diversity, which significantly influences both major and minor EO components, underscores the biochemical richness of the species. The variability in EO composition among the plant parts suggests that A. leucotrichus is a promising model for exploring the biosynthetic pathways and regulatory mechanisms governing specialized metabolite production, especially under various environmental or biological stressors. Overall, the diverse and bioactive nature of these EOs positions A. leucotrichus as a valuable resource for potential applications in the pharmaceutical industry. (Neghliz-Benabdelkader et al., 2021)

1. Definition

As defined by the Association Française de Normalisation (AFNOR), essential oils are products derived from plant-based raw materials. They are typically extracted through methods such as steam distillation, mechanical pressing (particularly from the fresh rinds of citrus fruits), or simple distillation. Once obtained, the oils are separated from the aqueous phase using physical means (Bruneton et al., 1999).

Some researchers emphasize the need to differentiate between “essence” and “essential oil” (Carette et al., 2000).

Essence: refers to a natural aromatic substance synthesized by the plant itself, stored in various secretory structures, with its composition differing depending on the plant part.

Essential oil: on the other hand, is the concentrated extract of these aromatic substances, typically obtained through steam distillation. In other words, an essential oil is the distilled form of the plant’s original essence.

2 . Distribution and location

Essential oils are found predominantly in higher-order (vascular) plants (Khia et al., 2014). They are characterized by a highly complex chemical makeup, consisting of numerous distinct and intricately structured aromatic compounds. This complexity gives each essential oil a unique composition. These oils can be present in virtually all parts of a plant—including flowers, leaves, fruits, seeds, bark, and stems—with the highest concentrations typically found in the aerial parts such as leaves and flowers (Aboughe Angone et al., 2015). Biosynthesis of essential oils occurs within the cytoplasm of secretory cells. These compounds generally accumulate in specialized glandular cells that are often situated on the plant's surface and protected by a cuticle. The storage of these secondary metabolites takes place in dedicated anatomical structures, such as glandular trichomes, secretory cavities, and canals. In members of the Asteraceae family, for example, these structures play a central role in the accumulation and storage of essential oils (Teucher et al., 2003).

3. Physio-chemical properties of essential oils

Essential oils consist of aromatic compounds with low molecular weights, which contribute to their unique physical behavior (Degryse et al., 2008). At ambient temperature, they exist in liquid form and are notably volatile—an attribute that distinguishes them from non-volatile, fixed oils. These oils are lipophilic, readily dissolving in alcohol and most organic solvents, and although they can be carried by water vapor, their solubility in water is minimal(Couic-Marinier et al., 2013).

4. Chemical composition and biosynthesis of essential oils

Essential oils are chemically complex mixtures composed of numerous compounds, primarily classified into two major groups with distinct biosynthetic origins. The first group includes terpene-based compounds such as monoterpenes and sesquiterpenes, while the second group comprises aromatic compounds derived from phenylpropanoids—though these are less common and include molecules like cinnamyl alcohol (Bakkali et al., 2008; Couic-Marinier et al., 2013).

Additionally, essential oils may contain a variety of degradation products from non-volatile substances, including acids, alcohols, aldehydes, and esters.

The biosynthesis of these constituents follows two main metabolic pathways: the mevalonic acid pathway, responsible for the formation of terpenoids, and the shikimic acid pathway, which leads to the production of phenylpropanoids (Singh et al., 1990).

These distinct routes highlight the biochemical diversity and intricacy underlying essential oil composition

5. Toxicity of essential oils

Similar to pharmaceutical drugs, each essential oil carries a balance between therapeutic benefits and potential risks, which must be evaluated in relation to the individual using them. For example, essential oils containing furocoumarins or pyro coumarins—commonly found in citrus oils—can cause erythematous skin reactions when applied topically or ingested and subsequently exposed to sunlight. Prolonged exposure may even increase the risk of skin cancer (Bakkali et al., 2008).

Ingestion of essential oils rich in monoterpenes over extended periods can lead to inflammation and eventual damage to the nephrons, the key filtering units of the kidneys—an effect known as nephrotoxicity. Additionally, topical use of essential oils in cosmetics or perfumes may result in skin irritation, allergic reactions, or photosensitivity. This is particularly evident with oils from thyme, oregano, and savory, which are high in thymol or carvacrol and known for their strong irritant properties (Bakkali et al., 2008).

Due to their chemical structure typically lipophilic and low in molecular weight—essential oil molecules are readily absorbed and distributed throughout the body. However, despite limited scientific research on their toxicity, it's crucial to recognize their potential harmful effects, including liver (hepatotoxicity), nerve (neurotoxicity), and skin toxicity. Given the wide range of uses for essential oils, there is also a considerable risk of poisoning, whether due to accidental exposure or improper use (Lucette, 2001).

6. Biological activities of essential oils

The beneficial properties of essential oils have been recognized and utilized for centuries, although their traditional use was largely empirical and lacked scientific validation. Today, however, their applications are increasingly grounded in scientific research, particularly studies that explore their antimicrobial and antioxidant capabilities. Essential oils are widely acknowledged for their antiseptic and antimicrobial effects, and many have also demonstrated anti-toxic, antiviral, antiparasitic, and anti-venomous actions. More recently, some have even shown promising anti-cancer potential.

In herbal medicine, essential oils are employed primarily for their ability to combat bacterial infections, such as those caused by endocanal pathogens (Billerbeck, 2008) or imbalances in the vaginal microbiota (Duarte, 2007). They are also effective against fungal infections, particularly those caused by dermatophytes. However, it's important to note that certain essential oils exhibit cytotoxic activity as well (Bakkali, 2008), which aligns them with broad-spectrum antimicrobial agents like disinfectants and antiseptics.

In agriculture and food safety, essential oils and their bioactive constituents are being investigated as natural alternatives to synthetic chemicals. They show potential as protective agents against plant-pathogenic fungi and as antimicrobial preservatives to prevent contamination in food products (Smith, 2002).

7.Methods for Extracting Essential Oils:

1. Traditional and Conventional Techniques:

A variety of approaches exist for obtaining essential oils from plants. Despite their clear importance, many regions around the world still heavily depend on outdated technologies for processing essential oils. The most commonly employed and conventional methods include hydro distillation (HD), steam distillation (SD), solvent extraction, evaporation, cohobation , and maceration (Rassem et al., 2016).

1.1 **Hydro Distillation (HD):**

Hydro distillation is one of the oldest and most straightforward traditional techniques used to extract essential oils. This method involves immersing plant materials in water or another solvent, followed by heating the mixture to release the oils through evaporation. As the mixture heats up, essential oil components vaporize alongside water or solvent vapors . These vapors are then condensed and collected, allowing the oils to be separated from the water in a decanter. This technique operates under the principle of isotropic distillation, where the boiling points of oil and water align during the process.

HD is considered a form of steam distillation, adapted and popularized in France (Rassem et al., 2016).

- There are three main variations of hydro distillation:
  Immersion in water
  Direct injection of steam
  A combination of water immersion and steam injection

The duration of distillation depends on the type of plant material used. Extended processing times tend to yield smaller amounts of oil and can result in the extraction of undesirable high-boiling compounds and oxidation byproducts (Rassem et al., 2016).

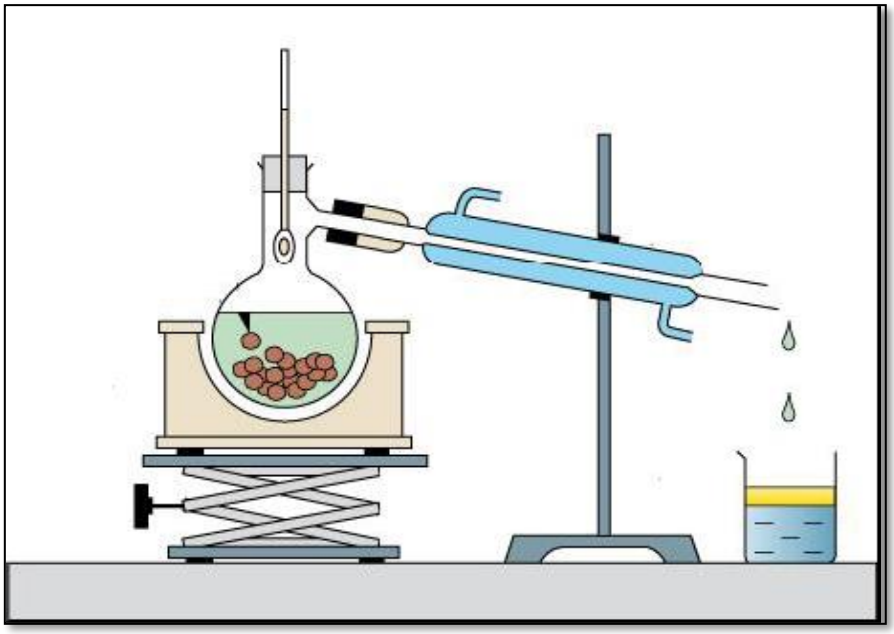


Figure 4:The schematic subsidize apparatus for hydro distillation. (Rassem et al., 2016).

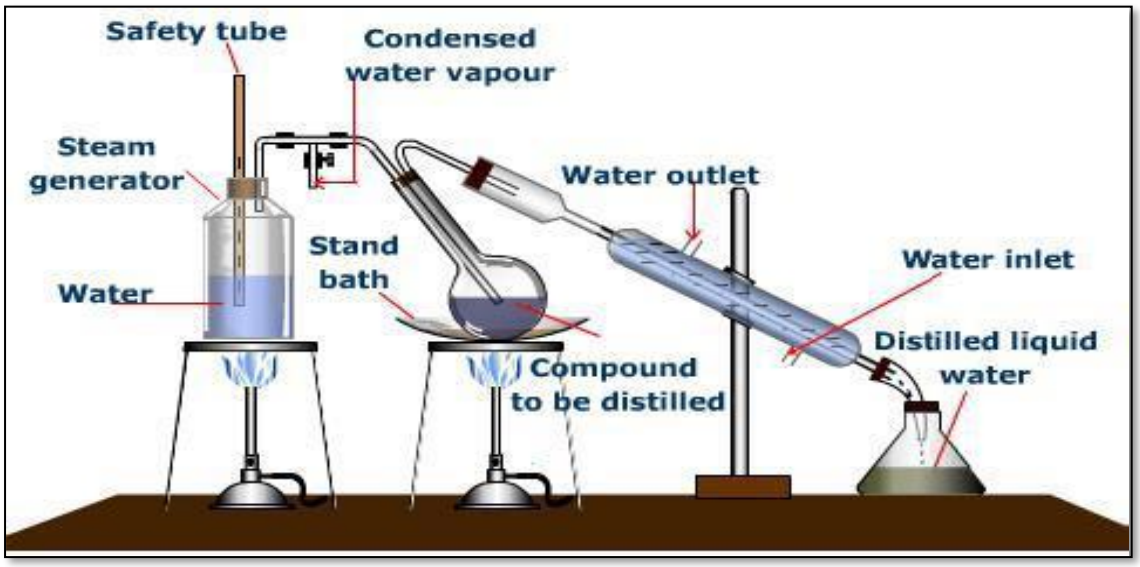


Figure 5: The schematic subsidize apparatus for Steam distillation(Rassem et al., 2016).

1.2 Solvent extraction:

Solvent extraction—also referred to as liquid–liquid extraction or partitioning—is a separation technique that relies on the differing solubilities of a compound's components in two immiscible liquids, such as water and an organic solvent (Figure 6) (Rassem et al., 2016)

In essential oil recovery through this method, the plant material containing the oils is placed in an extraction unit equipped with perforated trays. A solvent is then repeatedly passed over the material to dissolve and extract the desired compounds. This technique is commonly used

in the production of perfumes, vegetable oils, and biodiesel .Solvent extraction is particularly useful for processing delicate plant materials, as it enables the recovery of larger quantities of essential oils at a reduced cost. It remains one of the most widely employed techniques for preparing plant samples for analysis. However, the efficiency and yield of the extraction largely depend on the solubility of the compounds in the chosen solvent and the level of heat applied during the process .Despite being a straightforward and generally effective approach, solvent extraction comes with notable drawbacks, including lengthy extraction times, high solvent usage, and issues with consistency and reproducibility (Rassem et al., 2016).

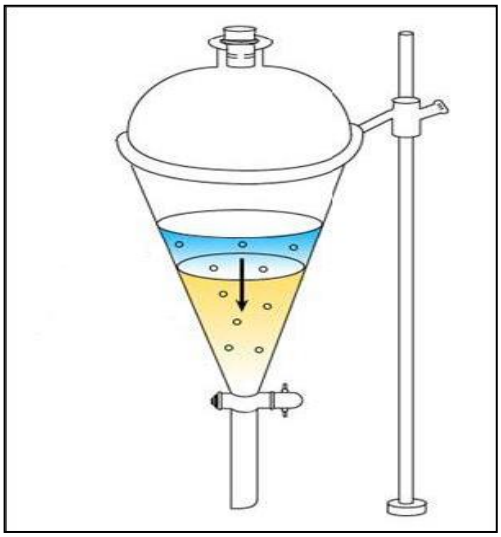


Figure 6: Solvent Extraction (Rassem et al., 2016).

1.3 Soxhlet Extraction:

The Soxhlet extractor, invented by Franz von Soxhlet in 1879, is a laboratory device primarily designed to extract lipids from solid materials (Figure 7). This technique is typically employed when the target compound has low solubility in a solvent, and the impurities remain insoluble in that same solvent (Rassem et al., 2016).

The Soxhlet extractor operates without the need for constant monitoring, efficiently recycling a small amount of solvent to extract larger quantities of material. It works by repeatedly contacting the solid with a liquid solvent that is heated to create vapor. This vapor condenses and fills the extraction chamber, allowing the solvent to dissolve the target compound. Once the liquid reaches a specific level, a siphon draws the solution back into the distillation flask, carrying the extracted substances with it (Rassem et al., 2016).

This cycle is repeated until the extraction is nearly complete. One of the main advantages of Soxhlet extraction is the continuous replenishment of the solvent, which helps prevent

saturation and ensures more effective extraction by maintaining a constant flow of fresh solvent through the sample (Rassem et al., 2016).

Additionally, because the temperature within the system is typically close to the boiling point of the solvent, the heat accelerates the extraction process, improving the efficiency of the procedure. However, Soxhlet extraction also has some drawbacks. The process can take several hours or even days to complete, involves using large volumes of solvent, and the heat applied during distillation can lead to losses through thermal degradation and volatilization of the extracted compounds (Rassem et al., 2016).

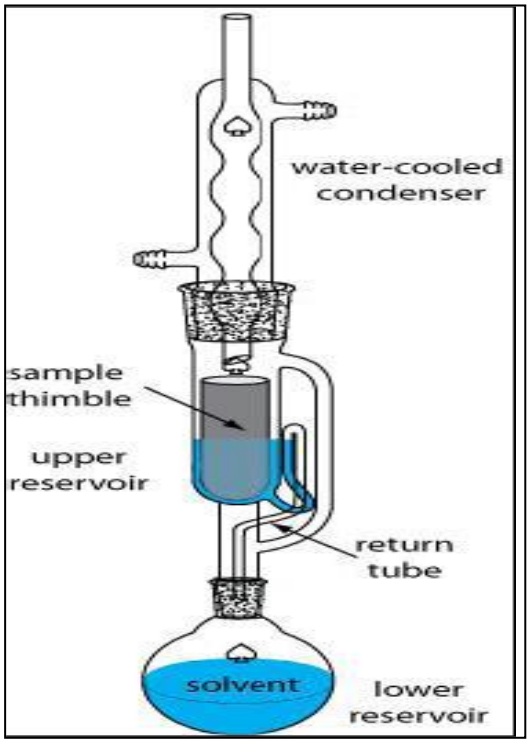


Figure 7:Soxhlet Extraction (Rassem et al., 2016).

1.4 Cold Pressing method:

The term "cold pressing" refers to the process of extracting oil by applying low heat and pressure through an expeller. This method is considered one of the most effective ways to extract essential oils and is widely used for many carrier and essential oils. It ensures that the oil remains 100% pure and preserves all the beneficial properties of the plant (Figure 8). (Rassem et al., 2016).

In this process, the raw material is subjected to a mechanical extraction method that minimizes the application of heat. Another name for this technique is scarification. Cold pressing is commonly employed to extract essential oils from plants, flowers, seeds, as well as

citrus oils like lemon and tangerine (Rassem et al., 2016).

The process begins by removing the outer layers of the plants that contain the oil through a scrubbing technique. The entire plant is then pressed to extract the oil from the pulp, allowing the essential oil to be released from its natural storage areas. The oil rises to the surface and is separated from the rest of the material through centrifugation (Rassem et al., 2016).

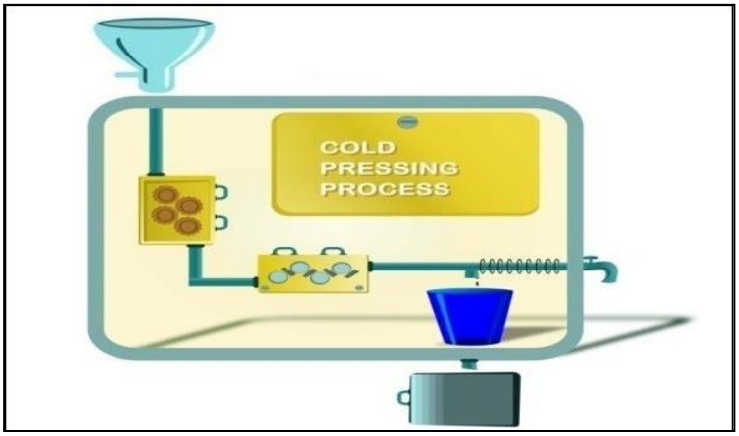


Figure 8: Cold Pressing Method (Rassem et al., 2016).

2. Advanced Methods for Extracting Essential Oils (Non-Traditional Approaches):

Modern, non-conventional extraction techniques are considered environmentally sustainable or "green." These innovative methods have been developed to address the shortcomings of traditional extraction processes. They offer several advantages, including reduced extraction time, lower energy and solvent requirements, minimal environmental impact, and enhanced yield and quality of essential oils. Importantly, they also eliminate the use of hazardous chemicals. (Murti et al., 2023)

2.1 Extraction Using Supercritical Fluids:

Supercritical Fluid Extraction (SFE) represents an advanced technique for separating specific compounds from a mixture by utilizing a supercritical fluid—typically a gas or liquid—under conditions where it exhibits both gas-like and liquid-like properties. By adjusting temperature and pressure, the solvent power of the supercritical fluid can be fine-tuned. This method is particularly useful for extracting volatile oil components that are unsuitable for recovery via traditional steam distillation. A diagram illustrating the SFE setup is provided in Figure 9. (Murti et al., 2023)

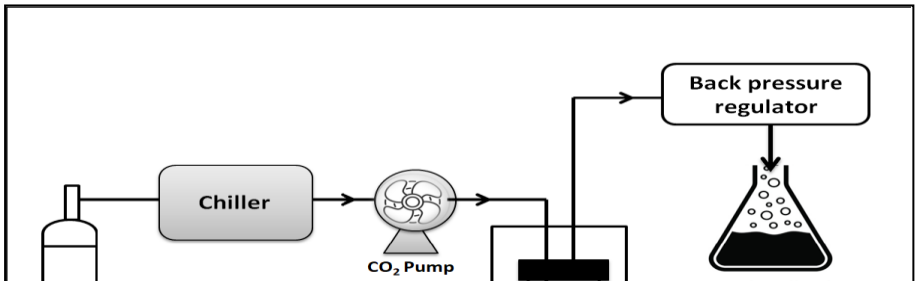


Figure 9: Schematic representation of the supercritical fluid extraction (SFE) system.**(Murti et al., 2023).**

Carbon dioxide (CO_2) is one of the most commonly utilized solvents, thanks to several advantageous properties. These include its relatively low critical temperature (31.1 °C) and pressure (73.8 bar), along with its non-flammable, non-corrosive, non-toxic nature, cost-effectiveness, and high purity. Its use in extraction processes, particularly from plant materials, is facilitated through pressure-based methods. (Murti et al., 2023).

However, a limitation of CO_2 is its non-polarity, which makes it ineffective for extracting polar compounds on its own. To overcome this, it is often employed as a supercritical fluid in combination with co-solvents such as dimethyl ether, ethanol, methanol, nitrous oxide, propane, ethane, freons, ethylene, and others. (Murti et al., 2023).

This extraction technique offers numerous advantages, including the production of high quality extracts with superior biological and functional characteristics when compared to those obtained via hydro distillation. Additionally, it is environmentally sustainable, as the non-toxic solvents used do not leave behind harmful residues. The volatile nature of the solvent allows for its rapid recovery after the extraction process. (Murti et al., 2023).

Furthermore, substances with high boiling points can be removed at lower temperatures, making this method suitable for extracting heat-sensitive (thermolabile) compounds. Nonetheless, one significant disadvantage is the technical complexity of the system, which contributes to increased equipment costs. (Murti et al., 2023).

2.2 Subcritical Liquid Extraction:

Subcritical Liquid Extraction (SLE) occurs when a liquid is subjected to pressures exceeding its critical pressure (Pc) while its temperature remains below the critical temperature (Tc). A diagram illustrating the SLE setup is provided in Figure 10 (Murti et al., 2023).

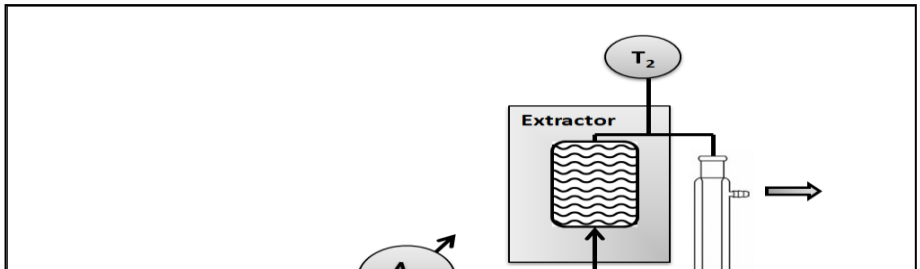


Figure 10: Schematic representation of the subcritical liquid extraction (SLE) system. (Murti et al., 2023).

In this extraction method, water (H_2O) and carbon dioxide (CO_2) are employed as solvents to isolate essential oils. Operating under subcritical conditions offers distinct benefits, such as reduced fluid density, lower viscosity, and enhanced diffusivity between gas and liquid phases. Due to its low operating temperature, this technique is particularly well-suited for preserving heat-sensitive (thermolabile) compounds. Compared to traditional extraction methods, it is significantly faster, requiring only 15 minutes to complete the process (Murti et al., 2023).

2.3 Microwave-Assisted Hydro distillation:

Microwave-Assisted Hydro distillation (MWHD) is an innovative technique that replaces conventional electric heating with microwave energy to warm the solvent. This method functions by altering the polarity of water molecules, which are then agitated by microwaves, generating heat. The microwaves interact directly with aromatic plant materials and the electromagnetic field, converting electromagnetic energy into thermal energy (Murti et al., 2023).

This energy transfer can lead to changes in the cellular structure of plant tissues. A visual diagram of the MWHD system can be seen in Figure 11. In this process, both heat and mass move outward from the inner plant cells toward the exterior. Several factors influence the process's efficiency, including duration, temperature, the chemical and physical properties of the target compounds, the dielectric characteristics of the mixture, and the type of solvent used.

MWHD has found application in industrial settings due to its versatility, especially when optimized conditions are met. It is suitable for processing a wide range of plant-based materials and is known for its rapid extraction rates while consuming minimal solvent. The method is also environmentally friendly, as it produces lower carbon dioxide emissions. Additionally, it is gentle on heat-sensitive (thermolabile) compounds, with its efficiency largely determined by the dielectric properties of both the water and the plant material involved (Murti et al., 2023).

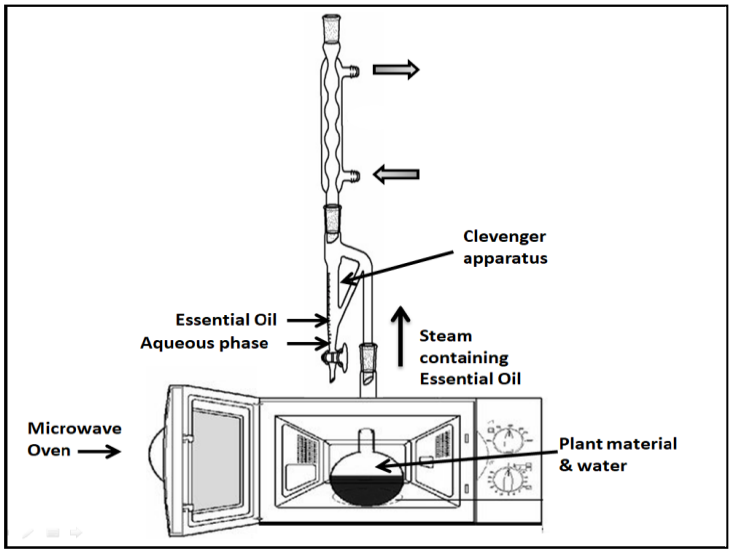


Figure 11: Schematic representation of the microwave-assisted hydro distillation (MWHD) system(Murti et al., 2023).

2.4 Ultrasound-Assisted Extraction :

Ultrasound-Assisted Extraction is a highly efficient technique used to selectively isolate volatile oils from plant materials. This method operates on the principle of cavitation—where microscopic bubbles are generated in the solvent medium as a result of ultrasound wave propagation. These bubbles collapse energetically, increasing the permeability of the plant matrix and allowing the solvent to penetrate more effectively, which in turn enlarges the contact surface area for extraction (Murti et al., 2023).

In this process, plant materials such as leaves, seeds, or flowers are submerged in a solvent—commonly water, methanol, or ethanol—and exposed to ultrasonic waves (as illustrated in Figure 12). The method is particularly effective in extracting essential oil compounds. Several parameters significantly influence both the quality and the extraction yield, including ultrasonic frequency, duration of ultrasonic exposure, immersion period, temperature during extraction, ultrasonic pulse cycle, as well as the nature and size of the plant material used (Murti et al., 2023).

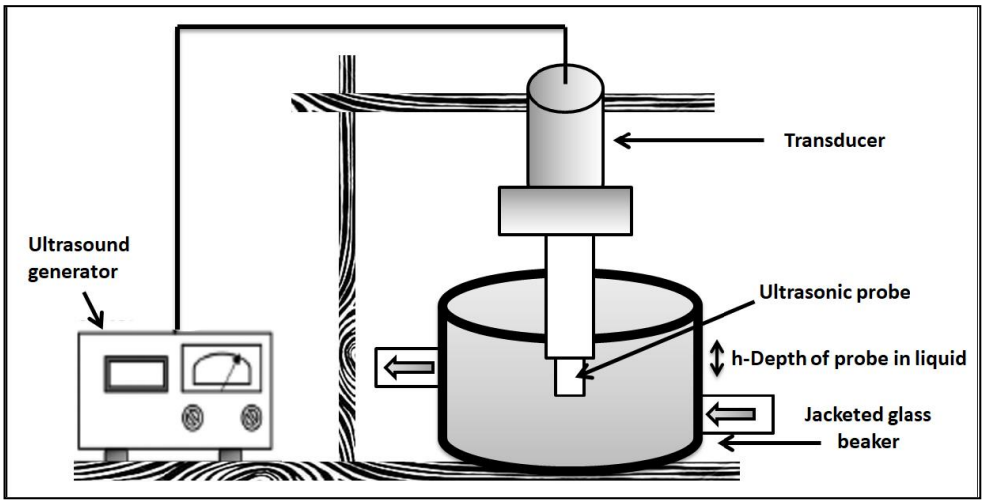


Figure 12: Schematic representation of the ultrasound-assisted extraction (UAE) system(Murti et al., 2023).

2.5 Microwave Extraction Without Solvents:

This method integrates microwave energy with dry distillation principles, relying on the plant's inherent moisture to act as a natural solvent (Murti et al., 2023).

Before extraction, the botanical material is hydrated by soaking it in water for approximately two hours. Once moistened, it is exposed to microwave radiation, which facilitates the release of essential oils. These oils are then captured through a condensation system. Compared to conventional techniques, microwave treatment induces higher internal pressure and thermal stress in the plant tissues, accelerating their breakdown. The equipment used in this process includes a control panel that regulates key parameters such as radiation intensity, pressure, and temperature (see Figure 13) (Murti et al., 2023).

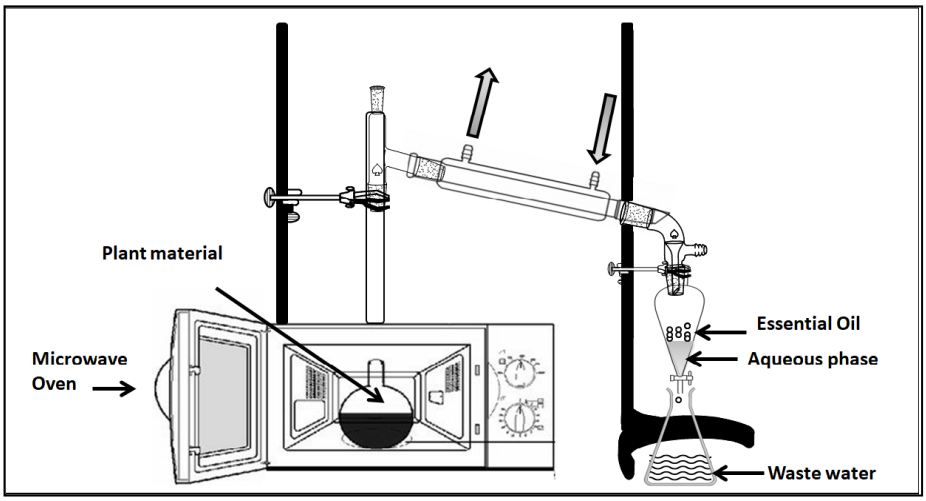


Figure 13: Schematic representation of the solvent-free microwave extraction (SFME) system(Murti et al., 2023).

2.6 Microwave Hydro-Diffusion and Gravity (MHG):

Microwave hydro-diffusion and gravity (MHG) represents an innovative and eco-friendly approach for extracting volatile oils. This method employs microwave energy in combination with natural gravitational force to facilitate the movement of volatile compounds from the inner cells of plant material to its surface. These compounds are then collected without the use of any external solvents. The MHG system, typically operated under atmospheric pressure, is illustrated in Figure 14. It was initially developed for small-scale experimental applications and limited production (Murti et al., 2023).

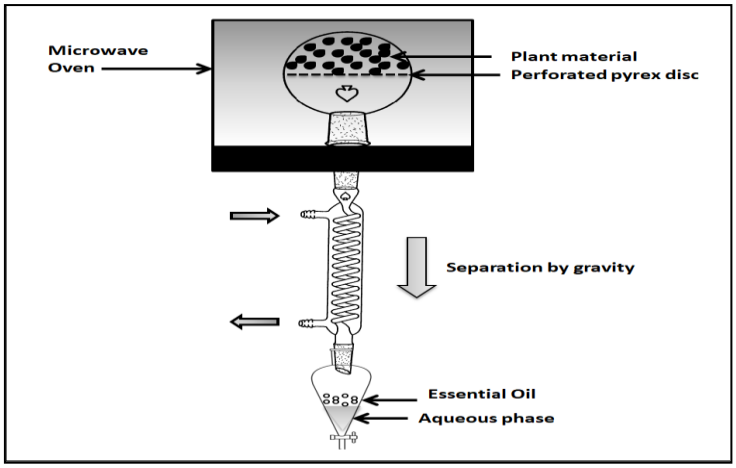


Figure 14: Schematic representation of the microwave hydro-diffusion and gravity (MHG) extraction system (Murti et al., 2023).

2.7 Ohmic Heating-Based Water Distillation (OHWD):

Ohmic heated water distillation (OHWD) is an advanced technique for extracting essential oils that employs Joule (ohmic) heating, offering greater energy efficiency by consuming less power per milliliter. Achieving uniform treatment throughout the process depends on precise input modeling for optimal control. The design of the OHWD extraction setup is illustrated in Figure 15. In this method, the rate of heating is directly related to both the square of the applied electric field and the electrical conductivity of the substance being processed (Murti et al., 2023).

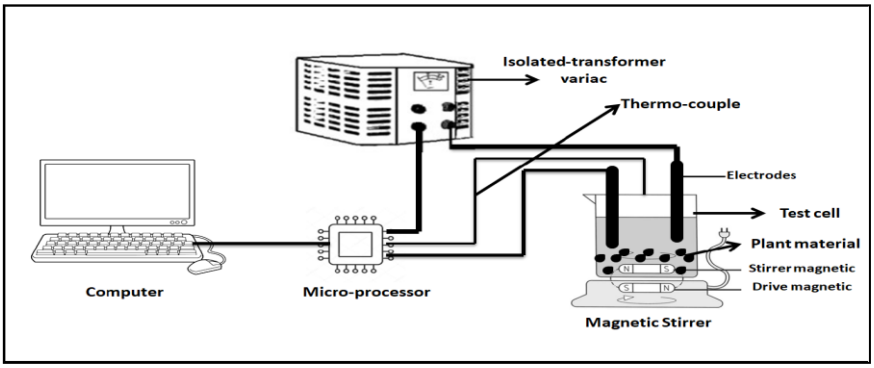


Figure 15: Schematic representation of the Ohmic heated water distillation (OHWD) extraction system (Murti et al., 2023).

2.8 Solar Distillation:

Solar energy plays a valuable role in agriculture by reducing both costs and environmental impact. Advancements in technology have made it possible to harness sunlight—an abundant renewable resource—to improve the efficiency of the distillation process. The thermal energy required for processing each unit of plant material remains largely consistent with traditional methods. Solar distillation systems incorporate several key components, including an oil separator, distillation still, condenser, focus concentrator, and a Scheffler-type fixed steam receiver (Figure 16) (Murti et al., 2023). The performance of the system depends on solar intensity as well as the thermal and optical efficiency of the distillation apparatus. This technique offers a cost-effective and eco-friendly approach to extracting essential oils from medicinal plants (Murti et al., 2023).

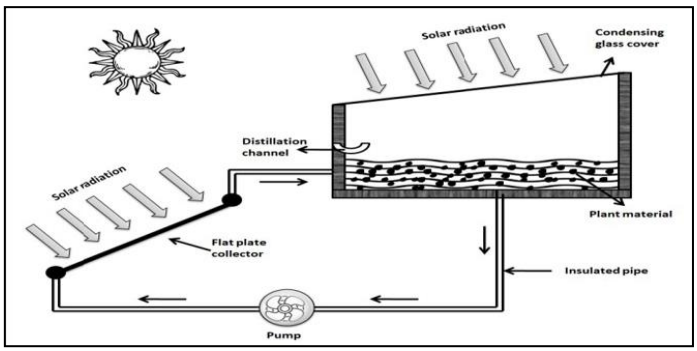


Figure 16: Schematic representation of the solar distillation (SD) extraction system **(Murti et al.,)**

CHAPTER TOW:

ANTIOXIDANTS

I. ANTIOXIDANTS

1. General Perspective on Antioxidants

Antioxidants play a critical role in maintaining cellular homeostasis by neutralizing reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated as natural by-products of cellular metabolism. These reactive species can inflict oxidative damage on vital biomolecules including nucleic acids, proteins, lipids, and carbohydrates (Phaniendra et al., 2015).

While small quantities of ROS/RNS are essential for physiological functions such as pathogen defense and cellular signaling, their excessive accumulation leads to oxidative stress, contributing to various chronic diseases such as cancer, neurodegeneration, cardiovascular disorders, and diabetes (Liguori et al., 2018).

Human bodies are equipped with endogenous antioxidant systems including enzymes (superoxide dismutase, catalase, glutathione peroxidase) and small molecules (glutathione, uric acid), but these systems are often insufficient to completely counteract oxidative stress. Hence, the consumption of exogenous antioxidants from plant-based foods becomes indispensable for reinforcing the body's defense mechanism (Carvalho et al., 2021).

2. Definition of Antioxidants

Antioxidants are molecules capable of donating electrons to free radicals without becoming destabilized themselves, thus terminating the radical chain reactions. Biologically, an antioxidant is defined as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell & Gutteridge, 2015). They act primarily as free radical scavengers, hydrogen donors, single oxygen quenchers, or metal chelators.

Antioxidants are broadly distributed in fruits, vegetables, spices, and medicinal plants, where they are found in forms such as polyphenols, flavonoids, carotenoids, and vitamins (Lobo et al., 2010).

3. Types of Antioxidants

Antioxidants can be categorized based on their mechanisms of action into three primary types:

3.1. Primary (Preventive) Antioxidants

These antioxidants inhibit the initiation of oxidation by neutralizing free radicals through hydrogen or electron donation, thus preventing the formation of new radicals. Enzymatic

antioxidants like catalase and superoxide dismutase fall under this category, as they catalyze the decomposition of ROS (Yadav et al., 2016).

3.2. Secondary (Chain-breaking) Antioxidants

Secondary antioxidants interrupt the propagation of free radical chains by reacting with peroxyl radicals and converting them into stable products. These include phenolic compounds and tocopherols, which are abundant in plant oils and leafy greens (Yadav et al., 2016).

3.3. Repair and De Novo Antioxidants

This group includes complex enzymatic systems that repair biomolecular damage after oxidation has occurred. Examples include DNA repair enzymes (e.g., PARP) and lipid-repairing enzymes (e.g., phospholipase A2) (Pham-Huy et al., 2008).

4. Classification of Antioxidants by Source

4.1. Natural Antioxidants

Natural antioxidants are derived from biological sources either endogenously synthesized or acquired from the diet. Endogenous antioxidants include enzymatic agents like:

- **Superoxide dismutase (SOD):** Converts superoxide radicals (O2•−) into hydrogen peroxide.
- **Catalase:** Decomposes hydrogen peroxide into water and oxygen.
- **Glutathione peroxidase:** Reduces hydrogen peroxide and lipid peroxides.

Exogenous antioxidants are commonly obtained from food and include:

- **Vitamin C (Ascorbic Acid):** A water-soluble antioxidant that scavenges ROS.
- **Vitamin E (α-Tocopherol):** A lipid-soluble antioxidant that protects cell membranes.
- **Polyphenols and flavonoids:** Found in berries, tea, and grapes (Flieger et al., 20

A- Scorbick acid C6H8O6.

B- VitaminEC29H50O2 – D-alpha-tocopherol E

4.2. Synthetic Antioxidants

These are industrially produced antioxidants widely used in food preservation, pharmaceuticals, and cosmetics. Examples include:

- **Butylated hydroxyanisole (BHA)**
- **Butylated hydroxytoluene (BHT)**
- **Tertiary butylhydroquinone (TBHQ)**

- **Propyl gallate (PG)** Figure 17

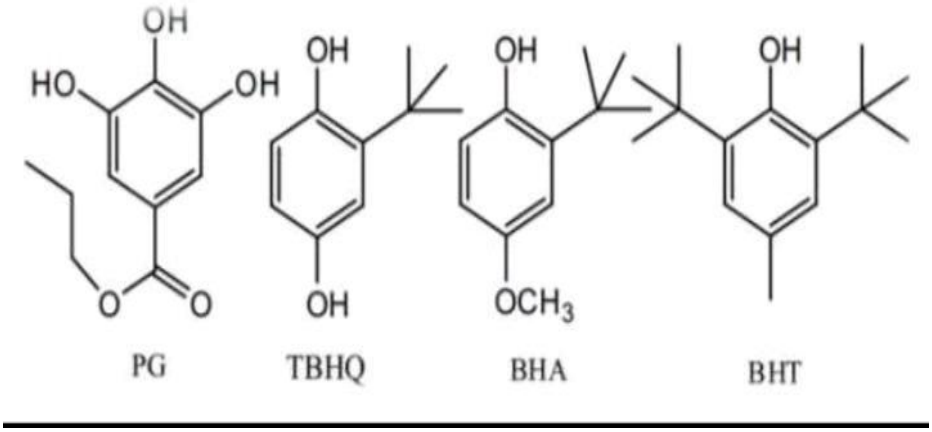


Figure 17 : Antioxidants used in the food industry

Although effective, some synthetic antioxidants raise concerns regarding their potential toxicity and carcinogenicity at high concentrations, prompting ongoing evaluations of their safety (Flieger et al., 2021; Rasheed & Azeez, 2019).

II. FREE RADICALS

1. Definition and Origin of Free Radicals

Free radicals are atoms, molecules, or ions that contain one or more unpaired electrons in their outermost orbitals, making them highly reactive and short-lived. These species strive to attain electron stability by capturing electrons from nearby stable molecules, which in turn become radicals themselves, perpetuating a destructive chain reaction (Pham-Huy et al., 2008).

In biological systems, free radicals are formed endogenously as a result of normal metabolic processes (e.g., mitochondrial respiration, phagocytosis, prostaglandin synthesis), and exogenously through exposure to pollutants, radiation, cigarette smoke, pesticides, and certain medications (Valko et al., 2007). The most common radicals include:

- **Reactive Oxygen Species (ROS):** Superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2).
- **Reactive Nitrogen Species (RNS):** Nitric oxide (NO•), peroxynitrite ($ONOO^-$), and nitrogen dioxide (NO_2•).

2. Biological Roles of Free Radicals

Despite their notoriety, free radicals are not inherently harmful. At low to moderate levels, they serve several physiological roles, including:

- **Immune Defense:** Neutrophils and macrophages produce ROS to destroy invading pathogens through the respiratory burst mechanism.
- **Cell Signaling:** Radicals act as secondary messengers in redox signaling pathways, modulating transcription factors such as NF-κB and AP-1.
- **Apoptosis Regulation:** Controlled ROS production can trigger programmed cell death, a mechanism essential in preventing cancer development.

However, when their production overwhelms the antioxidant defense system, oxidative stress ensues, leading to cellular damage and pathological conditions (Liguori et al., 2018).

3. Types of Free Radicals

Free radicals can be classified based on their reactivity, stability, and origin:

3.1. Highly Reactive (Short-Lived) Radicals

These radicals exhibit extremely short lifespans (nanoseconds to picoseconds) due to their high reactivity:

- **Hydroxyl Radical (•OH):** Formed via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\bullet}OH + OH^-$); one of the most damaging ROS, capable of non-selective attacks on DNA, lipids, and proteins.
- **Superoxide Anion ($O_2{\bullet}^-$):** Produced by mitochondria during aerobic respiration; it is moderately reactive and can be dismutated by SOD to form H_2O_2.

These radicals are typically detected and studied through electron paramagnetic resonance (EPR) and spin trapping techniques (Fitter, 2002).

3.2. Relatively Stable (Longer-Lived) Radicals

These radicals are more chemically stable due to delocalization of the unpaired electron, often through resonance:

- **DPPH• (2,2-diphenyl-1-picrylhydrazyl):** A nitrogen-centered radical commonly used in antioxidant activity assays due to its stability and violet color.
- **TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl):** A nitroxide radical known for its longevity and use in organic synthesis and EPR studies.
- **Persistent Organic Radicals:** Such as triphenylmethyl radicals, stabilized by steric hindrance and electron delocalization.

These radicals are used as models in experimental studies to investigate antioxidant mechanisms and radical interactions (Kochar et al., 2024).

3.3. Lipid and Environmental Radicals

- **Lipid Radicals (L•):** Generated during lipid peroxidation processes initiated by ROS, leading to membrane damage and cytotoxic by-products like malondialdehyde (MDA).
- **Environmental Radicals:** Such as carbon-centered radicals from combustion or smoking, and chlorine/nitrogen radicals from disinfectants and fertilizers.

4. Mechanisms of Radical-Induced Damage

Free radicals cause damage through several pathways:

- **Lipid Peroxidation:** Initiated by the abstraction of a hydrogen atom from polyunsaturated fatty acids, leading to membrane rigidity and loss of function.
- **Protein Oxidation:** Results in structural changes, fragmentation, or aggregation, impairing enzymatic and signaling functions.
- **DNA Damage:** Causes strand breaks, base modifications, and cross-linking, potentially leading to mutations and carcinogenesis.

The cumulative oxidative damage has been implicated in the pathogenesis of numerous diseases including:

- **Neurodegenerative disorders:** Alzheimer's, Parkinson's, and Huntington's diseases.
- **Cardiovascular diseases:** Atherosclerosis, hypertension, and stroke.
- **Cancer:** Through DNA mutations and aberrant signaling pathways.
- **Aging:** As per the free radical theory of aging, oxidative stress accelerates cellular senescence and tissue degeneration (Harman, 1956; Barnham et al., 2004).:

III. Oxidative Stress and Disease Pathogenesis

1. Definition of Oxidative Stress

Oxidative stress is defined as a physiological condition where the balance between the generation of reactive oxygen/nitrogen species (ROS/RNS) and the antioxidant defense mechanisms is disrupted in favor of the pro-oxidants, leading to potential damage of cellular components such as lipids, proteins, and DNA (Sies, 1997). It is not merely an overproduction of radicals, but rather the failure of the organism to counteract their harmful effects effectively.

This imbalance can be acute or chronic, and its severity plays a decisive role in determining the outcome—ranging from reversible cellular adaptation to irreversible cell injury and death (Valko et al., 2007).

2. Molecular Targets of Oxidative Stress

2.1. Lipids

Polyunsaturated fatty acids (PUFAs) in the phospholipid bilayer are prime targets of ROS. Lipid peroxidation, initiated by hydroxyl radicals (•OH), leads to the formation of reactive aldehydes like **malondialdehyde (MDA)** and **4-hydroxynonenal (4-HNE)**, which disrupt membrane integrity, signaling pathways, and cellular metabolism (Ayala et al., 2014).

2.2. Proteins

ROS modify proteins via oxidation of amino acid residues (particularly cysteine, methionine, and tyrosine), cross-linking, fragmentation, or nitration (via RNS like peroxynitrite). These changes impair enzymatic activity, disrupt signaling pathways, and lead to protein aggregation—a hallmark in many degenerative diseases (Stadtman & Levine, 2003).

2.3. DNA and RNA

Free radicals, especially •OH, can cause oxidative DNA lesions, including **8-hydroxy-2'-deoxyguanosine (8-OHdG)**, single-strand breaks, base modifications, and cross-linking. Such damage can result in mutations, altered gene expression, and oncogenesis (Cooke et al., 2003).

3. Oxidative Stress in Human Diseases

Oxidative stress plays a central role in the pathogenesis of a wide spectrum of chronic diseases. Below are some prominent examples:

3.1. Neurodegenerative Disorders

The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption, abundant lipid content, and relatively low antioxidant defenses.

- **Alzheimer's Disease (AD):** Characterized by excessive ROS, β-amyloid aggregation, and lipid peroxidation, which impair synaptic function and neuronal viability (Butterfield et al., 2006).

- **Parkinson's Disease (PD):** Involves dopaminergic neuron loss, where dopamine metabolism and mitochondrial dysfunction generate ROS, exacerbating neurotoxicity (Dias et al., 2013).

3.2. Cardiovascular Diseases

Oxidative modification of **low-density lipoprotein (LDL)** is a key step in atherogenesis. Oxidized LDL promotes endothelial dysfunction, foam cell formation, and plaque instability—contributing to atherosclerosis, myocardial infarction, and stroke (Madamanchi et al., 2005).

3.3. Cancer

DNA mutations from oxidative damage can activate oncogenes or deactivate tumor suppressor genes. ROS also modulate signaling cascades (e.g., MAPK, PI3K/Akt) that control cell proliferation, apoptosis, and metastasis (Reuter et al., 2010).

3.4. Diabetes Mellitus

Hyperglycemia enhances mitochondrial ROS production, leading to glycation of proteins and oxidative stress, which impairs insulin signaling and contributes to complications such as neuropathy, nephropathy, and retinopathy (Brownlee, 2001).

3.5. Inflammatory and Autoimmune Diseases

ROS are involved in the activation of **NF-κB**, a transcription factor regulating pro-inflammatory cytokines. Sustained oxidative stress contributes to chronic inflammation seen in diseases like rheumatoid arthritis, lupus, and inflammatory bowel disease (Mittal et al., 2014).

3.6. Aging

The free radical theory of aging, proposed by Harman (1956), postulates that cumulative oxidative damage to DNA, proteins, and lipids accelerates aging processes and functional decline. Recent evidence links telomere shortening and mitochondrial dysfunction to chronic oxidative stress (López-Otín et al., 2013).

4. Cellular Defense Mechanisms Against Oxidative Stress

To counteract oxidative stress, cells have evolved multi-layered antioxidant systems:

4.1. Enzymatic Antioxidants

- **Superoxide Dismutase (SOD):** Converts superoxide ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2).
- **Catalase (CAT):** Breaks down H_2O_2 into water and oxygen.
- **Glutathione Peroxidase (GPx):** Reduces lipid hydroperoxides using glutathione.

4.2. Non-Enzymatic Antioxidants

- **Endogenous:** Glutathione (GSH), uric acid, bilirubin, coenzyme Q10.
- **Exogenous:** Vitamins C, E, and A; polyphenols; carotenoids; flavonoids.

IV. Evaluation of Oxidative Stress and Antioxidant Capacity

1. Importance of Oxidative Stress Assessment

Measuring oxidative stress and the antioxidant capacity of biological systems or plant extracts is crucial in biomedical and phytochemical research. These assessments help:

- Understand disease mechanisms involving ROS/RNS.

- Evaluate the antioxidant potential of natural compounds.
- Monitor the impact of dietary or pharmaceutical interventions.

Numerous in vitro and in vivo methods have been developed for this purpose, each with its strengths and limitations.

2. Assays for Lipid Peroxidation

2.1. Thiobarbituric Acid Reactive Substances (TBARS)

- **Principle:** Quantifies malondialdehyde (MDA), a byproduct of lipid peroxidation, by reacting with thiobarbituric acid (TBA) to form a pink MDA-TBA adduct (λmax = 532 nm).
- **Application:** A biomarker of oxidative damage in tissues or cells

4. Antioxidant Capacity Indexes

- **ORAC (Oxygen Radical Absorbance Capacity):** Evaluates the ability to scavenge peroxyl radicals using fluorescein as a probe.
- **TEAC (Trolox Equivalent Antioxidant Capacity):** Expresses antioxidant capacity in Trolox (vitamin E analogue) equivalents.

V. The Role of Diet and Phytochemicals in Antioxidant Defense

1. Nutritional Antioxidants: A First Line of Defense

Diet plays a fundamental role in modulating the oxidative status of the human body. A balanced intake of fruits, vegetables, whole grains, and medicinal herbs is essential to supply the body with exogenous antioxidants, which work in synergy with endogenous defense mechanisms.

Key Dietary Antioxidants:

- **Vitamin C (Ascorbic acid):** Water-soluble; scavenges ROS and regenerates vitamin E (Carr & Frei, 1999).
- **Vitamin E (Tocopherols and Tocotrienols):** Lipid-soluble; protects cell membranes from lipid peroxidation.
- **Polyphenols:** A broad group including flavonoids, phenolic acids, tannins, and lignans.
- **Carotenoids:** Such as β-carotene, lycopene, and lutein, protect against singlet oxygen and lipid peroxyl radicals.

2. Mechanisms of Action of Phytochemicals

Phytochemicals act through various mechanisms:

- **Direct free radical scavenging** (donating electrons or hydrogen atoms).

- **Metal chelation** (e.g., flavonoids bind Fe^{2+} and Cu^{+}).
- **Enhancing endogenous enzymes** via gene expression (e.g., activation of Nrf2 pathway).
- **Inhibition of pro-oxidant enzymes** like xanthine oxidase, NADPH oxidase, and iNOS.

Example: Curcumin (from turmeric) and resveratrol (from grapes) have been shown to activate the Nrf2-ARE pathway, promoting the expression of detoxifying enzymes such as glutathione S-transferases (GSTs) and heme oxygenase-1 (HO-1) (Lee & Johnson, 2004).

3. Functional Foods and Antioxidant-Rich Herbs

Numerous medicinal plants and functional foods are recognized for their antioxidant potential:

Tableau 4: Antioxidant Mechanisms and Active Compounds of Various Plants and Herbs

Plant/Herb	Active Compounds	Antioxidant Mechanism
***Mentha piperita* (Peppermint)**	Menthol, flavonoids	Scavenging, metal chelation
Ammodaucus leucotrichus	Terpenoids, phenolics	Radical scavenging
***Camellia sinensis* (Green tea)**	Catechins (EGCG)	Inhibition of ROS-generating enzymes
***Curcuma longa* (Turmeric)**	Curcumin	Nrf2 activation, anti-inflammatory
***Vitis vinifera* (Grapes)**	Resveratrol, anthocyanins	Chain-breaking antioxidants

4. Synergistic Effects and Bioavailability

- **Synergism:** Antioxidants can regenerate one another. For instance, vitamin C regenerates oxidized vitamin E.
- **Bioavailability Issues:** Not all antioxidants are absorbed efficiently; factors such as metabolism, solubility, and gut microbiota impact their efficacy.
- **Solution:** Nanoformulations and liposomal delivery systems are being explored to enhance absorption (Sahu et al., 2018).

Several studies show an inverse relationship between antioxidant-rich diets and the risk of chronic diseases:

- **Cardiovascular diseases:** High intake of flavonoids reduces oxidative LDL and improves endothelial function (Hollman et al., 2010).

- **Neurodegenerative disorders:** Polyphenols delay cognitive decline by modulating oxidative and inflammatory pathways.
- **Cancer prevention:** Carotenoids and polyphenols inhibit mutagenesis and cell proliferation..

7. The Role of Antioxidants in Disease Prevention and Therapy

1. Antioxidants and Oxidative Stress-Related Diseases

Oxidative stress has been implicated in the pathogenesis of numerous chronic diseases. Free radicals attack biological molecules, leading to cell damage, inflammation, and genetic mutations. Antioxidants, by neutralizing these radicals, play a key role in preventing or alleviating the progression of these diseases.

2. Antioxidants in Cardiovascular Diseases

a) **Mechanism:** ROS oxidize low-density lipoprotein (LDL), triggering atherosclerotic plaque formation.

b) **Protective Role of Antioxidants:**
   - Vitamin E prevents lipid peroxidation in vascular membranes.
   - Polyphenols (e.g., quercetin) improve endothelial function and reduce inflammation.

c) **Clinical Evidence:** Diets rich in fruits and vegetables (e.g., Mediterranean diet) are associated with reduced cardiovascular risk (Kris-Etherton et al., 2002).

3. Antioxidants and Neurodegenerative Diseases

Diseases like Alzheimer's and Parkinson's are characterized by neuronal damage due to ROS/RNS accumulation.

- **Mechanism:** Oxidative stress damages mitochondria and DNA in neurons, leading to protein aggregation (e.g., amyloid-β).
- **Neuroprotective Antioxidants:**
  - Curcumin: inhibits amyloid aggregation and oxidative damage.
  - Resveratrol: activates SIRT1 and modulates mitochondrial function.
- **Research Insight:** Flavonoids cross the blood-brain barrier and exert neuroprotective effects (Spencer, 2009).

4. Antioxidants and Cancer Prevention

ROS can induce mutations in DNA, promoting tumorigenesis.

- **Antioxidant Action:**
  - Scavenging of mutagenic free radicals.

- Regulation of cell cycle and apoptosis (via p53, Bcl-2).

- **Phytochemicals like:**
  - Lycopene (from tomatoes): inhibits prostate cancer cell proliferation.
  - EGCG (from green tea): suppresses tumor growth and angiogenesis.
- **Caution:** Some antioxidants may interfere with chemotherapy or act as pro-oxidants in high doses.

5. Antioxidants in Metabolic Disorders (e.g., Diabetes)

Chronic hyperglycemia leads to ROS overproduction, contributing to diabetic complications.

a. **Mechanisms:**
   - Oxidative stress damages pancreatic β-cells.
   - Promotes vascular complications via protein glycation and lipid oxidation.

b. **Therapeutic Role:**
   - α-lipoic acid and vitamin C improve insulin sensitivity and reduce neuropathy.
   - Polyphenols regulate glucose metabolism and reduce inflammation.

6. Respiratory and Inflammatory Diseases

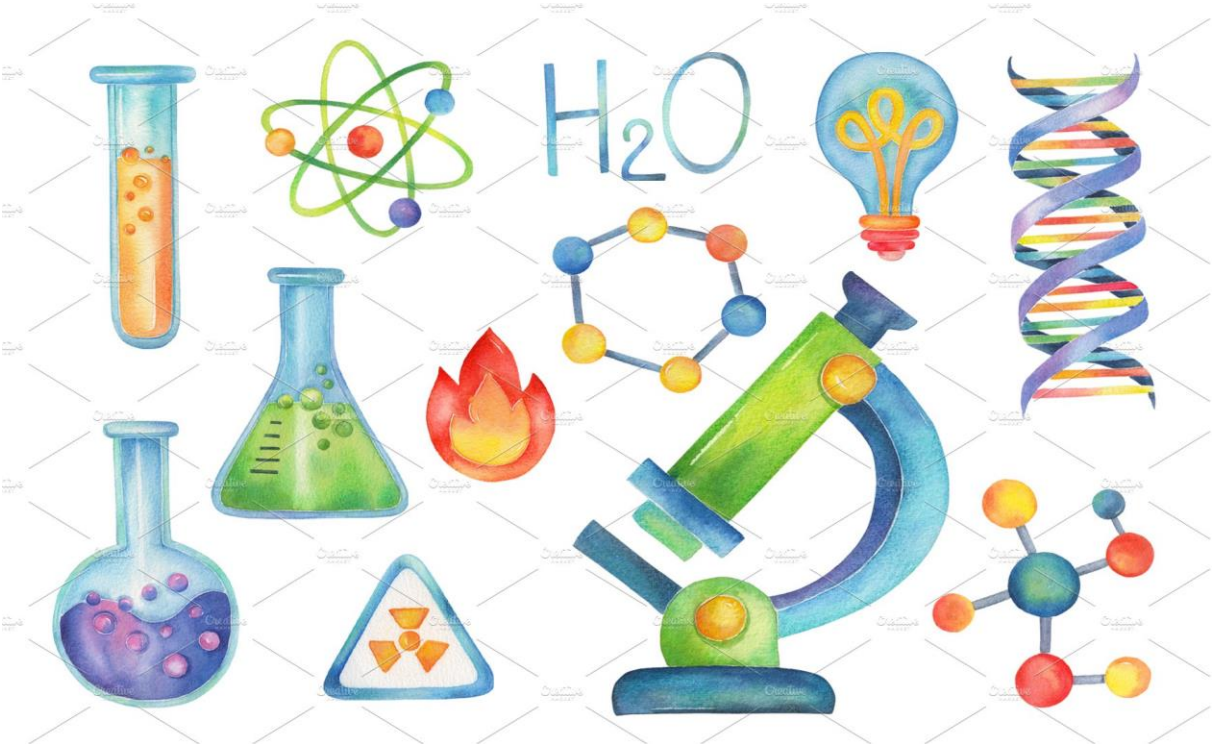
- **Asthma and COPD:** Oxidative stress contributes to airway remodeling and inflammation.
- **Antioxidants like NAC (N-acetylcysteine):** Restore glutathione levels and reduce mucus viscosity.
- **Vitamin C and flavonoids:** Modulate inflammatory cytokine production (IL-6, TNF-α).

SECOND PART:

EXPERIMENTAL

CHAPTER ONE:

MATERIALS AND METHODS



1. Introduction:

This study was conducted collaboratively between two specialized laboratories: the Valorisation and Technology of the Saharian Resources Laboratory (VTRS) at the Faculty of Exact Sciences, Department of Chemistry, University of El Oued, and the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimique (CRAPC) in Ouargla, Algeria. The VTRS lab facilitated essential oil extraction, conducted *in vitro* and *in-silico* assays. Meanwhile, CRAPC handled the precise GC/MS analysis of the extracted oils. This collaborative effort ensured thorough and accurate experimentation, combining expertise and resources from both institutions.

2. Plant Material:

2.1. Ammudaucus leucotrichus:

The plant *Ammudoucus leucotrichus* was harvested at different time intervals during the month of June 2023 in a desertic forest region located in southeastern Algeria, specifically in the province of El Oued. The area exhibits the following specifications:

- Geographic coordinates: 33 degrees north, 6 degrees east.
- Astronomical location: Latitude 32 degrees north.
- Altitude above sea level: 82 meters.
- Distance from sea level: 230 kilometers.
- Bioclimatic character: Desertic.

2.2. Mentha piperita:

During various time intervals in May 2023, the peppermint plant (*Mentha piperita*) was harvested in a desertic woodland area located in the southeastern region of Algeria. Specifically, in Elbayadah city, which falls under the jurisdiction of the El Oued province. The region possesses the following specifications:

- Geographical coordinates: 33°N, 6°E.
- Astronomical location: Latitude 33°N.
- Elevation above sea level: 84 meters.
- Distance from sea level: 310 kilometers.
- Bioclimatic type: Desertic.

3. Chemicals and reagents:

Dimethyl sulfoxide (DMSO) (HPLC-grade from Sigma-Aldrich) was utilized as a solvent in the experimental procedures. Additionally, 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical commonly employed in antioxidant assays, served as the reagent for assessing antioxidant activity. Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (electrochemical grade, 99% purity, Sigma-Aldrich) was employed as the supporting electrolyte, maintaining a concentration of 0.1M. All other reagents utilized were of analytical grade.

4. Materials and Methods:

4.1. Essential Oils Extraction:

4.1.1. Apparatus:

The essential oil extraction process required the use of specialized equipment to ensure accuracy and efficiency. In this study, the following apparatus was utilized:

- ✓ Adventurer – Pro AV53 sensitive balance
- ✓ Heating flask
- ✓ Refrigerant
- ✓ Clevenger apparatus
- ✓ Separating funnel
- ✓ Rotary evaporator

The Clevenger apparatus, a key component in the extraction process, is depicted in Figure 18

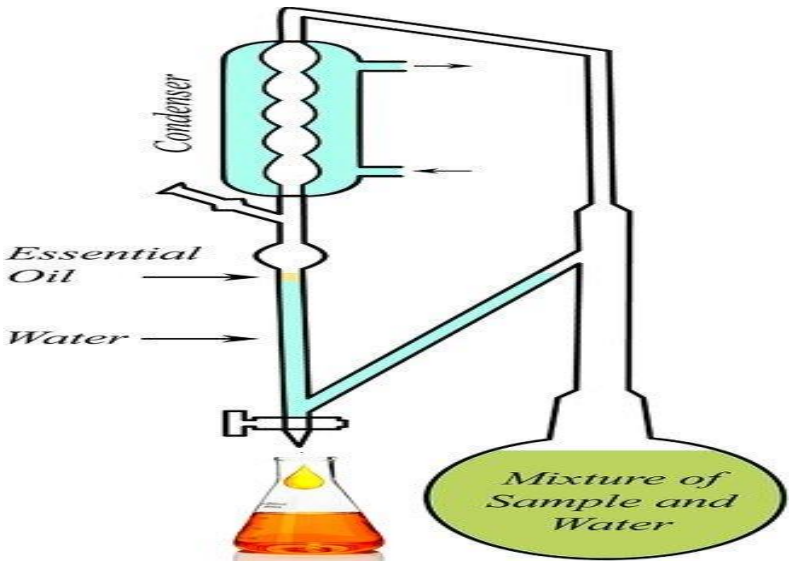


Figure 18: Schematic representation of the Clevenger apparatus used in the essential oil extraction process (Biswa *et al.*, 2023)

4.1.2. Procedure:

The procedure for essential oil extraction involved a series of meticulous steps to ensure optimal results. The extraction process was conducted as follows:

- ✓ Cleaning of the plant sample to remove any impurities.
- ✓ Weighing the plant sample to a quantity of 100 g using a sensitive balance.
- ✓ Washing the plant sample with water to prevent burning, followed by placement in a heating flask.
- ✓ Addition of small pieces of boiling regulator to the flask.
- ✓ Direct heating of the mixture at a temperature of 100 degrees Celsius for three and a half hours.
- ✓ Conducting the steam distillation process and subsequent separation of oil and water phases using liquid-liquid separation with diethyl ether.
- ✓ Drying of the organic oily phase with anhydrous sodium sulphate.
- ✓ Filtration of the dried oil phase through filter paper to remove diethyl ether particles.
- ✓ Evaporation of the filtered oil phase using a rotary evaporator to remove any remaining organic solvent.
- ✓ Storage of the obtained essential oil in small brown bottles and refrigeration at a temperature of 5°C.

4.2. Yield of Essential Oil Extraction:

Two distinct methodologies are commonly employed to ascertain the yield of essential oil extraction, each offering unique perspectives on the efficiency of the process.

4.2.1. Volumetric Yield - Mass-based: (Naima *et al.*, 2019)

This method entails an assessment of the mass of the utilized plant material intended for essential oil extraction, juxtaposed against the volume of the resultant oil. The yield is then computed utilizing the following Equation 1:

(1)
$$R_{EO} = \frac{V_{EO}(ml)}{m_0(g)} \times 100$$

Where: R_{EO}: Essential oil yield, m_0: Mass of the utilized plant sample, V_{EO}: Volume of the extracted essential oil

4.2.2. Mass-based Yield - Mass-based: (Larbi et al., 2018)

Alternatively, the essential oil extraction yield is defined as the quotient of the mass of the extracted essential oil and the mass of the plant material utilized. This yield is calculated using Equation 2:

$$R_{EO} = \frac{m_{EO}(g)}{m_0(g)} \times 100 \quad (2)$$

Where: R_{EO}: Essential oil yield, m_0: Mass of the utilized plant sample, m_{EO}: Mass of the extracted essential oil

These methodologies have been systematically applied to derive essential oil yields for all examined plant specimens.

4.3. Characterization of Essential Oils:

The characterization of essential oils comprises two fundamental components: physicochemical properties and Gas Chromatography-Mass Spectrometry (GC/MS) analysis.

4.3.1. Physicochemical Properties:

Here, we delve into the fundamental traits of essential oils, including their relative density, acidity, ester content, and refractive index. These properties offer valuable insights into the composition and behavior of essential oils, helping us understand their chemical makeup and how they interact with their environment (Atti-Santos *et al.*, 2005).

- *Relative Density (AFNOR NF T75-111 Standard):*

At 20°C, 1 mL of the essential oil is measured using a pipette, and its mass is then determined. The procedure is repeated for distilled water, and density is calculated using the following Equation 3 (Valarezo *et al.*, 2015):

$$d = \frac{m_{EO}}{m_{H_2O}} \quad (3)$$

Where: m_{EO}: Mass of the extracted essential oil, m_{H_2O}: Mass of the distilled water

- *Acidity Index (AFNOR NF T75-111 Standard):*

To determine the acid value, representing the concentration of free acids in 1 g of the essential oil, a titration method with potassium hydroxide (KOH) solution is employed (Sahoo *et al.*, 2007).

Initially, a small aliquot (0.5 mL) of the essential oil is mixed with 2 to 3 drops of phenolphthalein indicator in a small vessel. Subsequently, titration is performed with 0.5 N

KOH solution until the appearance of a faint pink colour, indicating complete neutralization of the acids. The acid value is then calculated using the following Equation 4.

$$I_a = \frac{56.11 \times V \times C}{m} \quad (4)$$

Where: V: the volume of the KOH solution, C: Concentration of KOH, m: the mass of the essential oil

➢ *Ester Index (AFNOR NF T75-111 Standard):*

The determination of free acids resulting from ester hydrolysis within the essential oil involves a titration process utilizing 0.5 N potassium hydroxide (KOH) solution (Alajtal *et al.*, 2018).

- Begin by placing 0.5 mL of the essential oil into a small vessel.
- Add 1 mL of 0.5 N potassium hydroxide (KOH) solution to the vessel to initiate the titration process.
- Place the mixture in a gas-evacuated water bath for a specified duration to facilitate reaction.
- After cooling, introduce 0.5 mL of distilled water and add 3 drops of phenolphthalein indicator to the mixture.
- Titrate the excess potassium hydroxide (KOH) using 0.5 N hydrochloric acid (HCl) until a colour change is observed, indicating neutralization.
- Quantify the volume of hydrochloric acid (HCl) required to neutralize the excess potassium hydroxide (KOH).
- Calculate the ester content using the following Equation 5:

$$I_e = \frac{2805}{m}(V_0 - V_1) - I_a \quad (5)$$

Where: V_0 (ml): Volume of hydrochloric acid (HCl) without essential oil, V_1 (ml): Volume of hydrochloric acid (HCl) in the presence of essential oil, I_a: Acid value, I_e: Ester value and m: Mass of the essential oil sample.

➢ *Refractive Index (AFNOR NF T75-111 Standard):*

The refractive index of an essential oil is defined as the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light ray, with a specific wavelength, transitioning from air into the essential oil, while the latter is maintained at a constant temperature (Singh, 2002)

The refractive index of the essential oil is directly measured using a refractometer at a reference temperature 20°C.

4.3.2. Gas Chromatography-Mass Spectrometry (GC/MS) analysis:

The coupling (GC/MS) technique stands as the most frequently employed method within the field of essential oils, facilitating the concurrent separation, identification, and quantitative measurement of the various constituents present in extracted oils.

➢ *Principle:*

The principle is founded on the varying affinities of compounds within the mixture towards two phases: a stationary phase and a mobile phase. This technique relies on the distribution of constituents between a stationary phase and a gas phase. The stationary phase comprises a silicone-based liquid that permeates an inert and granular solid material, housed within a typically coiled steel or glass column measuring 1 to 3 meters in length and 2 to 4 millimetres in diameter. The mobile phase consists of an inert carrier gas such as nitrogen, helium, or argon.

The column is maintained at a high temperature via a furnace. Under the influence of temperature, constituents vaporize and become separable. The basis of separation lies in the discrepancy of partition coefficients of volatile compounds between the stationery and gas phases. A detection system generates a signal at the exit of each molecule from the column, manifesting as the recording of peaks corresponding to each constituent.

Gas chromatography is coupled with a mass spectrometer (MS); this coupling relies on computerized comparison of the spectrum of an unknown peak with one or more reference libraries, enabling its identification.

➢ *Apparatus:*

The identification of the chemical constituents of our essential oils was performed using a gas chromatographic system (HP 5890-SERIE II) equipped with an HP5 MS capillary column (30 meters in length, 0.25 mm internal diameter, and 0.25 µm film thickness) coupled with a mass spectrometer (HP-MSD 5972).

N_2 was employed as the carrier gas for the analysis of the two essential oils. Spectra were recorded at an emission energy of 70 eV, and spectral analysis of the compounds was conducted by comparison with their counterparts using the WILEY275 (Shamma, 1972; Kiryakov, 1968; Chiu *et al.*, 1982; Guinaudeau *et al.*, 1975) spectral libraries.

➢ *Procedure:*

The carrier gas (N_2) is introduced at a flow rate of 1 mL/min, with the injected volume of the essential oil being 1 µL in split injection. The injector and detector temperatures are set at 250°C and 320°C, respectively. The oven temperature is programmed to initially reach 60°C and held for 8 minutes, then gradually increased to 250°C at a rate of 2°C/min, maintained isothermally at 250°C for 15 minutes, and finally elevated to 300°C at a rate of 10°C/min.

4.4. *In Vitro* DPPH inhibition assays:

4.4.1. Cyclic Voltammetric Measurements:

✓ *Apparatus:*

The apparatus used for voltammetric measurements is a Potentiostat/Galvanostat Model PGZ301 (Radiometer Analytical SAS) connected to a three-electrode electrochemical cell,

- A glassy carbon electrode with a diameter of 5.2 mm².
- A mercury/mercurous chloride/saturated KCl reference electrode.
- A platinum auxiliary electrode with a diameter of 3 mm².

All of this is controlled by a Pentium IV microcomputer (CPU 4.0 GHz and RAM 2 GB) equipped with VoltaMaster4 software, version 7.08.

✓ *Reagents:*

- Acetonitrile solution
- Electrolyte support (Tetra-n-butylammonium tetrafluoroborate, 99%)
- Nitrogen (Gas)
- 2,2-diphenyl-1-picrylhydrazyl

✓ *Procedure:*

The reaction between the essential oils and the DPPH takes place in the electrochemical cell. The reaction medium is an acetonitrile solution.

Before each measurement, the working electrode is polished using p4000 abrasive paper. After polishing, the electrode is washed with ultra-pure water and dried with air. The surface of the electrode thus appears mirror-like. Then, the electrochemical cell is equipped with the reference electrode and the auxiliary electrode, as well as the working electrode. The cell is filled with 10 ml of a solution consisting of DMSO solution containing the essential oils under study. Nitrogen gas is bubbled through the solution for 10 to 15 minutes to remove oxygen from the cell. A variable potential is applied at a fixed scan rate and at a temperature

of 28 ± 2°C. The obtained voltammogram is analysed to access the interaction parameters between the studied essential oil and the DPPH.

The ability of the test sample to scavenge the DPPH radicals (% Inhibition of DPPH) was calculated using Equation 6 (Lanez *et al.*, 2023; Lanez *et al.*, 2019),

$$\% \ radical \ scavenging \ activity = \frac{i_0 - i}{i_0} \times 100 \quad (6)$$

where i_0 and i are the anodic peak current densities of the radical in the absence and presence of *Ammudoucus leucotrichus* and *Mentha pipertia* essential oils, respectively

4.4.2. Spectroscopic Measurements:

✓ *Apparatus:*

UV-Vis measurements were performed using a UV-Vis spectrometer (Shimadzu 1800) and a quartz cell with a volumetric capacity of 5 ml. Data acquisition was carried out with a Pentium IV microcomputer (CPU 4.0 GHz and RAM 2 GB) equipped with UV probe software version 2.34 (Shimadzu). The data is processed using OriginLab 9.0 software.

✓ *Reagents:*

- DMSO solution
- 2,2-diphenyl-1-picrylhydrazyl

✓ *Procedure:*

The electronic spectra of DPPH in the acetonitrile solution were obtained in the absence and presence of increasing concentrations of *Ammudoucus leucotrichus* and *Mentha pipertia* essential oils.

The ability of the test sample to scavenge the radicals (% Inhibition of the radical) was calculated using Equation 7 (Filote *et al.*, 2022),

$$\% \ radical \ scavenging \ activity = \frac{A_0 - A}{A_0} \times 100 \quad (7)$$

where A_0 and A are the absorbances of the radical in the absence and presence of the essential oils, respectively.

4.5. *In-Silico* analysis:

4.5.1. Software

Computational simulations, including Induced Fit Docking, molecular dynamics studies, and MM-GBSA calculations, were conducted employing the Glide module, Induced Fit Docking module, Prime module, and the Desmond module within the Maestro version 11.7 user

interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015). The simulations were executed on a DELL Intel(R) Core(TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32.0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

4.52. ADMET and drug-likeness evaluation:

Drug candidates should possess favourable ADMET properties and ideally non-toxic. Therefore, the major identified compounds from the essential oils extract were evaluated of their ADME profile, including physicochemical, lipophilicity, water solubility, pharmacokinetics, drug-like nature, medicinal chemistry, and several other parameters using SwissADME (Riyadi *et al.*, 2021; Daina *et al.*, 2017) module provided in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss). Furthermore, the toxicity aspect of designed compound was also predicted using ProTox (Banerjee *et al.*, 2018) webserver (https://comptox.charite.de/protox3/).

4.5.3. Docking setup:

- *Ligands preparation:*

The three-dimensional configurations of the major compounds isolated from *Ammudoucus leucotrichus* and *Mentha pipertia* essential oils were obtained from the National Library of Medicine (NCBI) database (Kim *et al.*, 2016), accessible through the NCBI website (https://pubchem.ncbi.nlm.nih.gov/).

Ligand preparation involved an energy optimization process to derive the most energetically favourable conformations for each compound. Utilizing LigPrep module within the Schrödinger suite (Schrödinger, 2024), this optimization procedure ensured the attainment of the lowest energy state for the studied drugs, including Montbretin A (a co-crystallized ligand). The ionization states were established at a pH of 7.0 ± 2.00, as computed by the Epik classic module, while maintaining specified chirality and generating relevant tautomeric forms. Furthermore, partial atomic charges were computed using Optimized Potentials for Liquid Simulations OPLS4 force field (Lu *et al.*, 2021).

- *Receptor Preparation:*

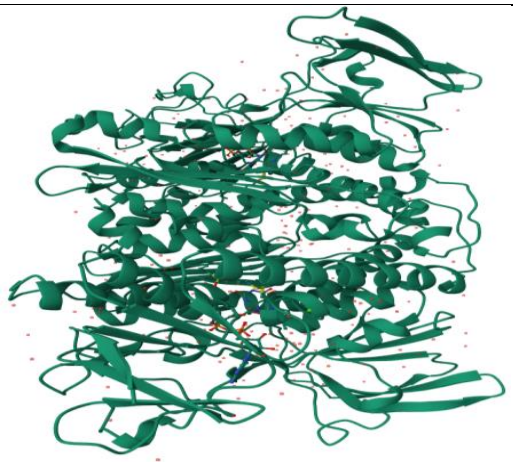
The crystallographic data for Human pancreatic glutathione reductase (Table 1) (PDB ID: 1BWC) (Gallwitz *et al.*, 1999) was retrieved from the Protein Data Bank (http://www.rcsb.org) (Rose *et al.*, 2017), adhering to specific parameters such as a resolution of 1.35Å and R value-free of 0.211. Processing of the protein structure was executed through

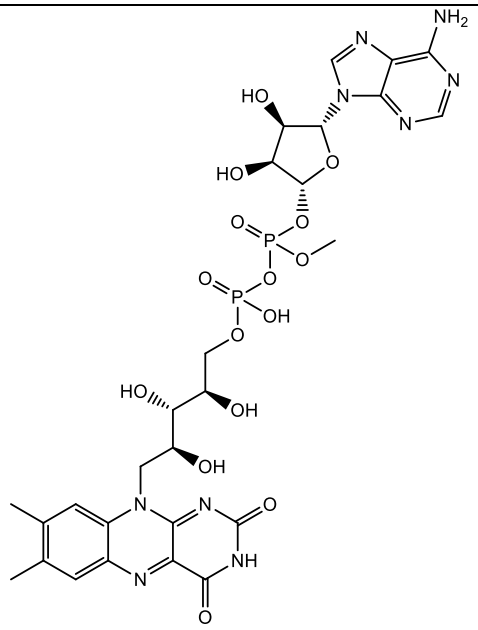
the “protein preparation Workflow” module within the Schrödinger suite (Madhavi Sastry *et al.*, 2013), involving consecutive stages of import and processing, review and modification, and refinement.

In the initial stage, the Prime tool was employed to address missing residues and side chains, maintaining the pH of PROPKA at 7.0 ± 2.00. Subsequent steps included the optimization and assignment of hydrogen bonds, along with the removal of water molecules beyond 8 Å. Restrain minimization utilizing the Optimized Potentials for Liquid Simulations (OPLS4) force field was performed to achieve a low-energy state for the protein (Lu *et al.*, 2021). This phase of protein preparation signifies an energy optimization methodology, presenting the protein in its energetically favourable state for subsequent *in-silico* studies.

The "Receptor grid generation" panel facilitated the creation of a grid encompassing the active site of the protein, delimited by the co-crystallized ligand Montbretin A. Default parameters were maintained, and the grid centre was generated at the coordinates X = -6.65; Y = 6.99; Z = -20.65.

Tableau 5:. Target receptor information chosen for docking studies

Human glutathione reductase	Detaille’s	
	PDB ID	1BWC
	Mutation	No
	Resolution (Å)	2.10
	R-Value Free	0.230
	R-Value Work	0.180
	R-Value Observed	0.180
	Organism	Homo sapiens
	Expression System	Escherichia coli
	Space Groupe	C 2 2 2_1
	Sequence Length	478
Co-Crystallized Ligand	**Detaille’s**	

	Name	FLAVIN-ADENINE DINUCLEOTIDE
Formula	**$C_{27}H_{33}N_9O_{15}P_2$**	

➢ *Molecular Docking:*

Computational simulations, including Induced Fit Docking, molecular dynamics studies, and MM-GBSA calculations, were conducted employing the Glide module, Induced Fit Docking module, Prime module, and the Desmond module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015). The simulations were executed on a DELL Intel(R) Core(TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32,0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

The molecular docking tool employed for all docking studies was Glide (Grid-based Ligand docking with Energetics), a module within the Schrödinger suite (Yang *et al.*, 2021). The prepared ligands underwent docking onto the specified protein site utilizing the Glide module, in Standard Precision (SP) modes (Friesner *et al.*, 2006).

➢ *Induced Fit Docking (IFD):*

The Induced Fit docking (IFD) module of the Maestro molecular modelling suite has been noted as a reliable and effective docking approach to consider flexibility in both ligands and the binding pocket residues in the binding pocket of target receptors (Khelil *et al.*, 2020). During the IFD process, Glide/SP (Standard Precision) was performed for each ligand, the Prime refinement step specifically addressed the side chains of residues within a 5 Å radius of the ligand. Noteworthy is the retention of a maximum of 20 poses for each ligand.

➢ *Molecular Dynamics Simulation (MDS):*

The best compound in each plant exhibiting the highest IFD docking score was chosen for Molecular Dynamics Simulation (MDS). Recognizing the limitations of ligand docking in representing the biological system under aqueous conditions (Korb *et al.*, 2012), a 100 ns simulation time for MDS was executed using the Desmond module within the Schrödinger suite (Bowers *et al.*, 2006).

The MDS protocol comprised three essential steps: system builder, minimization, and MDS. In the system builder phase, the protein and ligand complex were selected and immersed in a biological environment. The transferable intermolecular potential with 3 points (TIP3P) solvent model, with the boundary condition maintained in an orthorhombic box form.

throughout the process with dimensions of 10 x 10 x 10 Å (Akbar *et al.*, 2022). The OPLS-3e force field was consistently applied (Roos *et al.*, 2019). The neutralization of model was conducted by addition of counter ions when needed and 0.15 M of NaCl salt was included to mimic the physiological state.

Subsequently, the NPT ensemble was utilized for energy minimization, maintaining pressure and temperature at 1.0132 bar and 300 K, respectively. Finally, MDS was conducted for the minimized protein-ligand complex (Halder *et al.*, 2023).

➢ *Free Energy (MM-GBSA) Calculation:*

Upon completion of the dynamic simulation, an assessment of the free binding energy between the protein and the ligand was systematically undertaken utilizing the Prime MM-GBSA module within the Maestro molecular modelling suite (Wang *et al.*, 2021). The calculation of ligand binding affinities was accomplished through the Molecular Mechanics/Generalized Born Surface Area ΔG (MM-GBSA ΔG) metric applied to the optimized Receptor-Ligand Complex. This computation, facilitated by the VSGB solvation model and the OPLS4 force field, stands as a methodologically rigorous approach for the comprehensive evaluation of molecular interactions and binding strengths (Gorla *et al.*, 2021).

CHAPTER TWO: RESULTS AND DISCUSSION

1. Introduction:

This chapter delves into the findings regarding the extraction yield, characterization, and comprehensive exploration of the antioxidant properties inherent in two essential oils derived from the aerial constituents of *Ammudoucus leucotrichus* and *Mentha piperita*. The overarching aim of this investigation was to assess the potential antioxidant efficacy exhibited by essential oils as significant agents in the domain of novel pharmaceutical development.

The elucidations provided herein furnish intricate insights into the chemical constitution of the essential oils, thereby enabling their utilization in the *in-silico* study. This involved the application of advanced computational techniques such as Induced Fit Docking (IFD) and Molecular Dynamics Simulation (MDS) for each compound, capabilities not feasible in traditional *in vitro* and *in vivo* studies.

2. Extraction Yield:

illustrates the yields of essential oils obtained through hydro distillation of the aerial parts of *Ammudoucus leucotrichus* and *Mentha piperita*. variety of Eloued. Figure 19

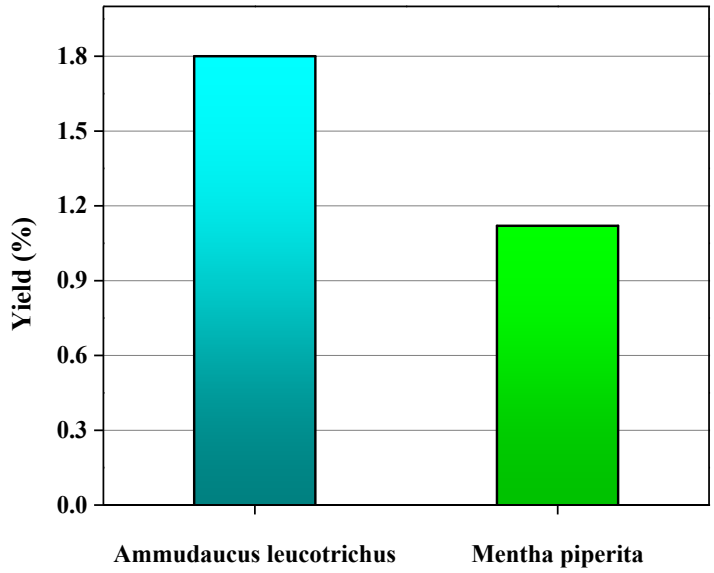


Figure 19: The yields of extracted essential oils

We observe that *Ammudoucus leucotrichus* exhibits a higher essential oil yield than *Mentha piperita* (1.80% and 1.12% respectively). However, the difference between the two yields is non-significant.

Nevertheless, findings reported by (Manssouri *et al.*, 2020) indicate lower yields of essential oils extracted by hydro distillation from *Ammudoucus leucotrichus* at ambient temperature.

Similar results to ours were, however, obtained by (Naima *et al.*, 2019) for the essential oil extracted from the same variety using the same method.

Furthermore, significantly higher yields were achieved by (Samber *et al.*, 2015) during the hydro distillation of *Mentha piperita*.

These variations in results can be explained by the fact that essential oil yields are influenced by several factors during extraction: either factors related to the plant (species, variety, chemical composition, etc.) or factors associated with experimental conditions (extraction process, extraction duration, etc).

3. Chemical composition of essential oils:

3.1. Organoleptic characteristics:

Through the conducted work, it has been revealed that the essential oil extracted from the studied plants exhibits the following (Table 6) organoleptic properties:

Tableau 6: The organoleptic characteristics of essential oils

Plant	Smell	Aspect	Colour
Ammudoucus leucotrichus	A strong smell	liquid at room temperature	Blue
Mentha piperita	A pleasant fragrance	liquid at room temperature	Light-yellow

3.2. Physicochemical Properties:

The physicochemical properties were meticulously determined according to the standards of the French Association for Standardization (AFNOR) using established methodologies to measure relative density, refractive index, acidity index, and ester index, as depicted in the following Table 7.

Tableau 7: The physicochemical properties of essential oils

Plant	Relative Density	Refractive Index	Acidity Index	Ester Index
Ammudoucus leucotrichus	0.887	1.4268	4.30	40.91
Mentha piperita	0.933	1.4601	4.46	41.88

By comparing these results to those obtained by (Naima *et al.*, 2019) (density 0.953, refractive index 1.474, acidity index 4.93 and ester index 45.96) and (Mimica-Dukić *et al.*, 2003) (density: 0.834 ± 0.02, 0.835 ± 0.02, refractive index, at 25°C 1.4596 ± 0.03, 1.4622 ± 0.04), and considering that the refractive index was measured at a temperature of 23°C, we can conclude that these values are in accordance with the standards described by AFNOR (Afnor, 1982).

3.3. Gas Chromatography-Mass Spectrometry (GC/MS) analysis:

The analysis focused on the essential oil constituents extracted from two specific plant species using gas chromatography-mass spectrometry (GC/MS). Mass spectra corresponding to each chromatographic peak were juxtaposed with spectra from relevant scientific literature and the Weily electronic database for mass spectra (Horai *et al.*, 2010). Retention indices were employed to ascertain compound identities. Moreover, utilizing the identical non-polar HP5 stationary phase in the gas chromatography column facilitated the preservation of consistent peak numbers and elution sequences for the compounds under investigation.

Figure 20 and Figure 21 depict the chromatograms illustrating the essential oil compositions of *Ammudoucus leucotrichus* and *Mentha piperita*, respectively, as obtained through gas chromatography-mass spectrometry (GC/MS) analysis:

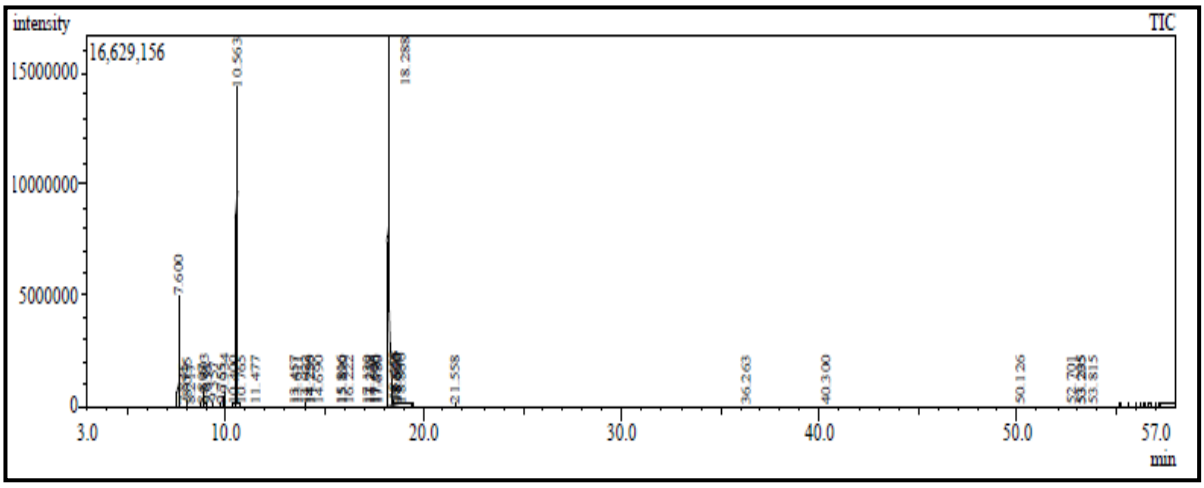


Figure 20: Chromatogram of the essential oil of *Ammudoucus leucotrichus* plant obtained by GC/MS

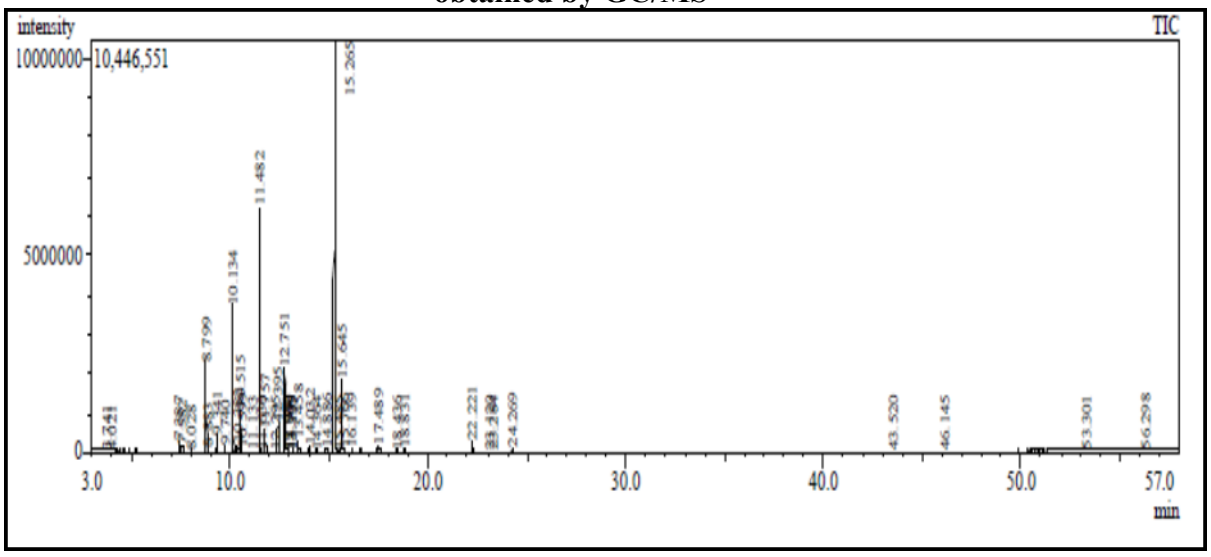


Figure 21: Chromatogram of the essential oil of *Mentha piperita* plant obtained by GC/MS

Upon scrutinizing the two chromatograms, notable resemblances are evident, with discernible bands interspersed with overlapping ones. Generally, distinct regions can be identified, delineating three segments:

1. The initial segment, delimited within the time frame of (4 min to 12.4 min), hosts several bands denoting the presence of hydrocarbon monoterpenes.
2. The succeeding segment, spanning from (12.4 min to 24 min), encompasses the predominant bands, indicative of oxygenated monoterpenes prevalent in the plant's essential oil.
3. The final segment, spanning (24 min to 32.5 min), exhibits minimal banding, suggesting a negligible presence of hydrocarbon sesquiterpenes in the plant's essential oil.

The pertinent compounds in each essential oil, extracted from *Ammudoucus leucotrichus* and *Mentha piperita*, have been identified and collated as follows

3.3.1. *Ammudoucus leucotrichus:*

The hydrodistillation extraction of *Ammudoucus leucotrichus* yielded a Bleu oil with a yield of 1.80%. Gas chromatography-mass spectrometry (GC/MS) analysis identified 24 compounds, collectively constituting 99.91% of the oil's composition (as presented in Table 8). The aromatic oil derived from *Ammudoucus leucotrichus* comprises 64.8% oxygenated monoterpenes and 34.86% hydrocarbon monoterpenes. Notably, perillaldehyde predominates at 64.66%, succeeded by D-Limonene at 26.99%, with a minor presence of α-pinene at 5.8%. At concentrations below 1%, additional compounds were detected, such as β-pinene at 0.66%,

β-Ocimene at 0.65%, camphene at 0.4%, and minimal quantities (0.08%) of both methyl perillate and bornyl acetate.

Tableau 8. Essential oil constituents of *Ammudoucus leucotrichus* identified by GC/MS

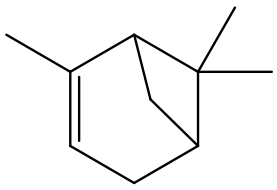
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	**Alpha-Pinene**	**932**	**925**	**5.8**
02	Alpha-Fenchene	945	934	0.02
03	Camphene	946	941	0.4
04	Thuja-2,4(10)-diene	953	948	0.04
05	Sabinene	969	969	0.03
06	Beta-Pinene	974	972	0.66
07	Myrcene	988	989	0.15
08	Alpha-Phellandrene	1002	1003	0.04
09	Beta-Ocimene	1009	1022	0.65
10	para-Cymene	1020	1024	0.06
11	**D-Limonene**	**1024**	**1029**	**26.99**
12	1,3,8-p-Menthatriene	1108	1122	0.02
13	Alpha-Campholenal	1122	1127	0.02
14	trans-Pinocarveol	1135	1141	0.05
15	Pentyl-Benzene	1152	1147	0.03
16	Myrtenal	1195	1198	0.01
17	Verbenone	1204	1212	0.02
18	Cumin aldehyde	1238	1244	0.02
19	Carvone	1239	1249	0.02
20	Linalool acetate	1257	1257	0.03
21	**Perilla aldehyde**	**1269**	**1285**	**64.66**
22	Bornyl acetate	1287	1291	0.08
23	Benzyl isobutanoate	1297	1305	0.03
24	Methyl perillate	1392	1399	0.08
Total		**99.91**		

Our findings are consistent with those reported by (Louail *et al.*, 2016; Sonboli *et al.*, 2012; Baser *et al.*, 1993). However, when comparing our results with other studies, some differences in the quantitative measurement of the main components of the essential oil and the nature of the identified components were observed. For instance, studies by (Moulay *et*

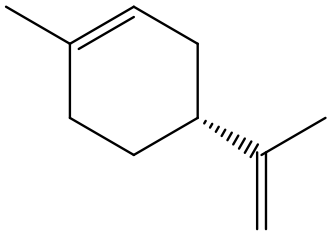
al., 2014; Louail *et al.*, 2016) indicated the presence of a small quantity of α-pinene, which does not align with our results.

Additionally, (Moulay *et al.*, 2014) mentioned that the essential oil of *Ammudoucus leucotrichus* primarily contained perillaldehyde (88.7%) and limonene (8.26%), contrasting with our findings where peril aldehyde predominates and limonene is less prominent. According to (Louail *et al.*, 2016), the tested *Ammudoucus leucotrichus* oil contained 59.12% perillaldehyde and 23.89% limonene. Hence, the origin of the oil from Algeria (specifically Eloued region) serves as a source of the active component (perillaldehyde), commonly utilized in the fragrance and cosmetics industries due to its scent (Wang *et al.*, 2010).

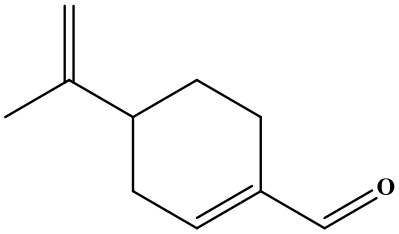
For a visual representation of the top major compounds (more than 1%) and their structures, refer to Figure 22 , which depicts the chemical structures and IUPAC names of the predominant constituents identified in the essential oil of *Ammudoucus leucotrichus*.



2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
(Alpha-Pinene)



(R)-1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene
(D-Limonene)



4-(prop-1-en-2-yl)cyclohex-1-enecarbaldehyde
(Perilla aldehyde)

Figure 22: Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Ammudoucus leucotrichus*

3.3.2. Mentha piperita:

The application of hydrodistillation for extracting the essential oil from *Mentha piperita*, commonly referred to as peppermint, yielded a light-yellow oil with a 1.2% yield. Analysis revealed the presence of 34 chemical compounds (as presented in Table 9), constituting 98.24% of the total oil composition. These compounds were categorized into 70.3%

oxygenated monoterpenes, 3.6% hydrocarbon monoterpenes, 0.18% oxygenated sesquiterpenes, and 22.96% other compounds. Notably, three primary compounds were

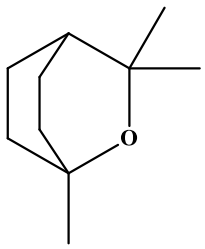
identified, each exceeding 20%: trans-Sabinene hydrate (27.51%), Linalool acetate (21.90%), and Pulegone (20.77%).

Tableau 9: Essential oil constituents of *Mentha piperita* identified by GC/MS

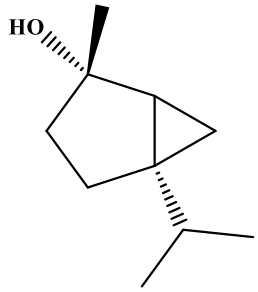
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	Alpha Pinene	924	932	0.46
02	Camphene	940	946	0.22
03	Sabinene	968	969	0.22
04	β Pinene	971	974	0.91
05	Myrcene	988	988	0.58
06	Alpha Terpinene	1015	1014	0.05
07	para-Cymene	1023	1020	0.04
08	Limonene	1027	1024	0.46
09	**1,8-Cineole**	**1030**	**1026**	**16.08**
10	trans-beta-Ocimene	1037	1044	0.41
11	δ Terpinene	1058	1054	0.15
12	Terpinolene	1088	1086	0.10
13	**trans-Sabinene hydrate**	**1101**	**1098**	**27.51**
14	3-Octanol, acetate	1123	1120	0.03
15	trans-Sabinol	1141	1137	0.03
16	**Menthone**	**1154**	**1148**	**1**
17	iso-Menthone	1165	1158	0.07
18	**Borneol**	**1167**	**1165**	**1.66**
19	l neoiso' Isopulegol	1173	1167	0.11
20	Terpinen-4-ol	1178	1174	0.22
21	**Alpha-Terpineol**	**1192**	**1186**	**2.54**
22	neoiso-Dihydrocarveol	1230	1126	0.22
23	**Pulegone**	**1243**	**1233**	**20.77**
24	**Linalool acetate**	**1257**	**1254**	**21.90**
25	Lavandulyl acetate	1292	1288	0.33
26	Piperitenone	1347	1340	0.06
27	Linalool isotretinoin	1383	:1373	0.48
28	Caryophyllene (E)	1424	1417	0.79
29	Alpha Humulene	1459	1452	0.12
30	Germacrene D	1488	1484	0.25
31	gamma Cadinene	1521	1513	0.04
32	Elmole	1555	1548	0.18
33	Cinnamaldehyde	1591	1599	0.19
34	Khusimone	1600	1604	0.06
Total		**98.24**		

Furthermore, α-Terpineol, Menthone, and Borneol were among the compounds present in concentrations exceeding 1%. Our findings are largely consistent with certain other studies, albeit with variations observed in the proportions of major compounds. For instance, (Gavahian *et al.*, 2015) reported significant levels of 1,8-Cineole (7.2%), Menthone (24.7%), and Pulegone (4.5%). Similarly, (Smaoui *et al.*, 2016) obtained results in alignment with ours,

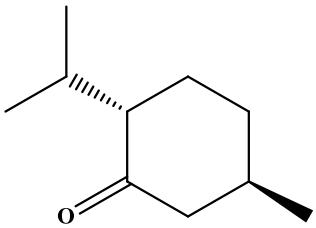
revealing predominant compound percentages of 1,8-Cineole (2.8%), Menthone (33%), and Pulegone (1.6%).



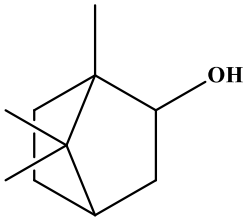
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (1,8-Cineole)



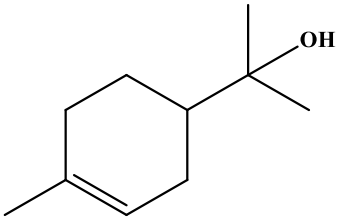
(2*S*,5*R*)-5-isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol (trans-Sabinene hydrate)



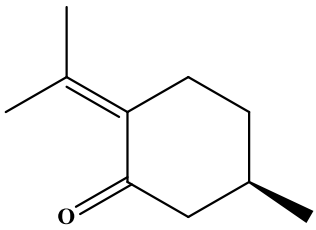
(2*S*,5*R*)-2-isopropyl-5-methylcyclohexanone (Menthone)



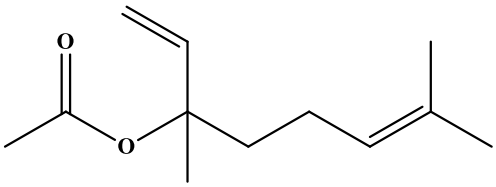
1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (Borneol)



2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (alpha -Terpineol)



(*R*)-5-methyl-2-(propan-2-ylidene)cyclohexanone (Pulegone)



3,7-dimethylocta-1,6-dien-3-yl acetate (Linalool acetate)

Figure 23: Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Mentha piperita*

However, discrepancies were noted in the analysis conducted by (Ahmad *et al.*, 2014), where the presence of Menthol at 34.82% differed from our findings. In a separate study by (Hossain *et al.*, 2014) on peppermint plants cultivated in Oman, the detection of Eucalyptol,

absent in our study, was noteworthy. For an illustrative overview of the major compounds identified, Figure 23, displaying the structures and IUPAC nomenclature of these constituents.

4. In Vitro Antioxidant activity :

4.1. Using superoxide anion radical:

In the present study, the antioxidant activity of *Ammudoucus leucotrichus oil* and *Mentha pipertia* by the irability to scavenge $O_2^{\bullet-}$ radicals. Kinetic profiles were established, and half-maximal inhibitory concentration (IC_{50}) values were determined to quantify the efficacy of these compounds in scavenging $O_2^{\bullet-}$ radicals. The anti oxidant capacity *Ammudoucus leucotrichus oil* and *Mentha pipertia* of is expressed as (IC_{50}), denoting the concentration (mg/ml) of the samples required to inhibit the formation of $O_2^{\bullet-}$ radicals by 50%. *(CAMPOS CHISTE, Renan, et al.2015)*

- % $O_2^{\bullet-}$radical scav enging activity= $\frac{ip_{a0}-ip_a}{ip_{a0}} = aC = aC(\frac{mg}{ml}) + b$

Where **ipa0** and **ipa** are the anodic peak current densities of the superoxide anion radical in the absence and presence of *Ammudoucus leucotrichus* oil and *Mentha pipertia* respectively. The equations derived from the linear calibration plots within the investigated concentration range for *Ammudoucus leucotrichus*, *Mentha pipertia*, and α-tocopherol are consolidated in **Table 10**. In this table, 'y' denotes the anodic peak current density of ($O_2^{\bullet-}$), while 'x' represents the sample concentrations expressed as mg/ml. *(STOIN, Uri, et al.2013)*

Tableau 10: IC_{50} values (µg/ml) obtaine dosing $O_2^{\bullet-}$radicals scavenging activity

Couponed	Equtation	R^2	IC_{50} (µg/ml)
Ammudoucus leucotrichus	y=5.509x -17.353	0.946	15.718
Mentha Piperita	y=2.859x+5.063	0.987	12.225
α-Tocophero	y =141.113x + 0.149	0,950	18.83

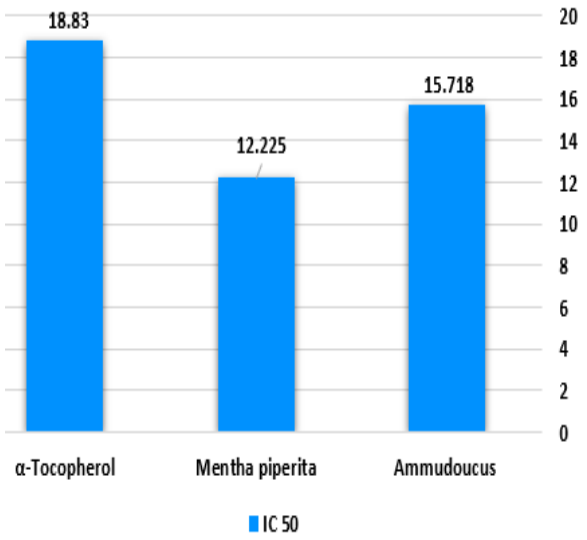


Figure 24: Classification of O2 •− inhibition capacity according to (IC_{50}) values

Based on the results presented in Table10,oil *Ammudoucus leucotrichus* and *Mentha pipertia* demonstrated significant radical scavenging abilities. The classification of these compounds according to their antioxidant activity (IC_{50}), is represented in the following curve: figure 24

Mentha piperita exhibited the strongest anti oxidant activity, with an (**IC_{50}**) of 12.225 µg/ml, indicating a highly effective radical-scavenging capability. *Ammodaucus leucotrichus* also demonstrated anti oxidant potential but was less potent than *Mentha piperita,* with an (IC_{50}) of 15.718 µg/ml. α-Tocopherol was significantly less effective, with an (IC_{50}) of 18.83 µg/ml, suggesting that the tested essential oils are superior antioxidants

4.2. Cyclic Voltammetry study:

Typical CV behavior of O2 •− in DMSO/0.1M TBATFB in the potential window of 0.0 to -1.6 V at a glassy carbon electrode in the absence and presence of a solution of different concentrations *Ammudoucus leucotrichus* different concentrations (0.1, 0.2, 0.3, 0.4, 0.5mg/ml),*Mentha pipertia* (0.1, 0.2 ,0.3 ,0.4, 0.5 mg/ml) in the same solvent.

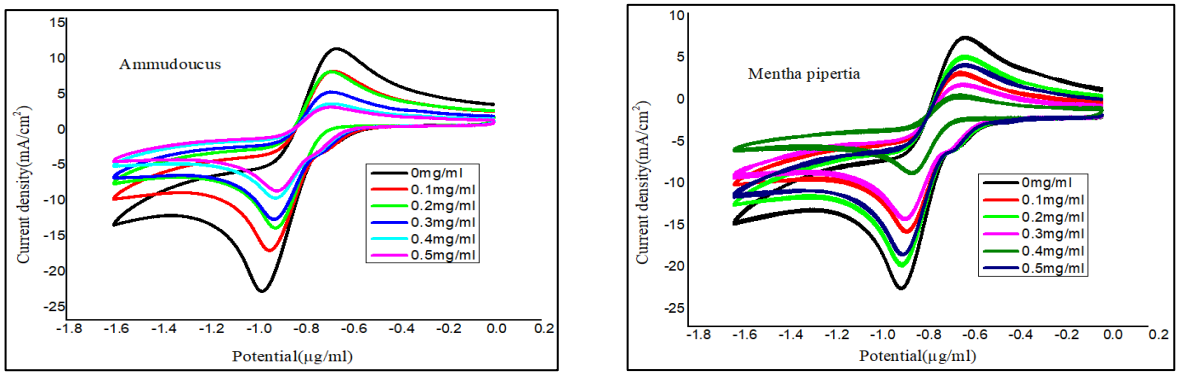


Figure 25: **Cyclic voltammograms of oxygen saturated DMSO/0.1 M TBATFB on a GC electrode in the presence of a different concentration of *Ammudoucus leucotrichus* and *Mentha pipertia*, scan rate 0.1 V.s-1, T= 28**

The third image present cyclic voltammetry curves, which allow for the electro chemical valuation of the interaction between the compounds and superoxide radicals ($O_2^{\bullet -}$) at different concentrations:

Ammodaucus leucotrichus significantly reduces the current intensity associated with free radical presence, indicating strong radical scavenging activity and confirming its potent anti oxidant effect. *Mentha piperita* shows a moderate response, confirming its antioxidant potential but at a low erefficiency than Ammodaucus leucotrichus.*(AMORATI, Riccardo; FOTI, Mario C.;2013)*

The addition of varying concentrations of *Ammudoucus leucotrichus* and *Mentha pipertia*, into a Dimethyl sulfoxide (DMSO) solution saturated with commercial oxygen leads to a significant decrease in peak current density, as illustrated in Figure 26. This notable reduction in peak current density can be attributed to the decrease in free O2•− radical concentration resulting from the formation of O2•− −*Ammudoucus leucotrichus* and O2•− −*Mentha pipertia* oil. The kb values are determined from the curves of the variation of log (1/C) versus log (i/i0-i) plotted using equation III.2: *(LOUAIL, Zineb, et al.2020)*

$$\log\frac{1}{C} = logKb + log\frac{i}{i_0 - i}$$

C: concentration of the compound tested (mol.l-1).

kb: binding constant (l/mol).

i and i_0 :intensity of the anodic current in the presence and absence of the compound tested respectively.

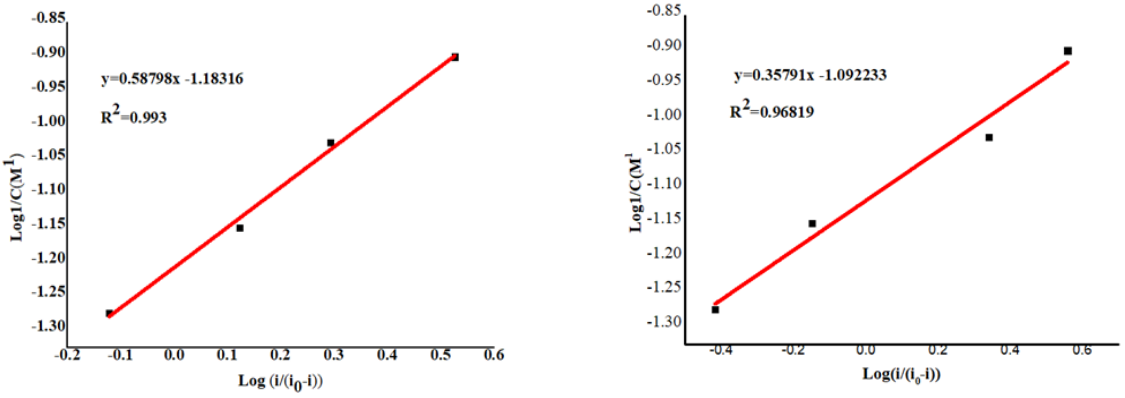


Figure 26: **.Plots of log (1/ C) versus log (i/i0-i) for O2•− with a variable concentration of *Ammudoucus leucotrichus* and *Mentha pipertia* in DMSO/0.1 TBATFB, used to calculate the binding constants of productsO2•− −*Ammudoucus leucotrichus* and O2•− −*Mentha pipertia.***

- **Binding free energy: The binding free energy change was calculated using the following Equation III.3**

$$\Delta G = -RT \ln Kb$$

Where: ΔG is the binding free energy in KJ.mol-1, R is the gas constant, 8.32 J.mol-1K -1, and T is the absolute temperature, 298K. The binding constants were determined, and subsequently, the free binding energy was calculated by analysing the intercept obtained through linear curve fitting of the logarithmic transformation of the reciprocal of the concentration (log(1/C)) versus log(i/i0 − i). Table 11 *(BHAVANIRAMYA, Sundaresan, et al. 2020)*

Tableau 11: Binding constants and values of binding free energies of O2•− −*Ammudoucus leucotrichus*, O2•− −*Mentha piperita* T= 298 K

Adduct	Equation	R^2	K^{M-1}	-ΔG(KJ.mol-1)
Ammudoucus	y=0.35791x-1,092	0.968	12.37	-6.23
Mentha Piperita	y=0.58798x-1.183	0.993	15.25	-6.75

The negative values of ΔG indicate that the binding processes are thermo dynamic ally favourable, as expected for spontaneous reactions. However, the magnitudes of ΔG differ slightly among the adducts, suggesting variations in the strength of interactions between O2•− and the different derivatives. This analysis suggests that the nature of the substituent in the derivatives affects the binding affinity with O2•−. *(SITZMANN, Judith, et al.2014)(Louail, Z., et al. 2016)*

4.2.1. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH):

The DPPH Radical(2,2-Diphenyl-1-picrylhydrazyl) is one of the most used substrates, has high sensitivity, for a direct, rapid, and reliable assessment of the antioxidant capacity, and gives reproducible results due to its stability and simplicity of analysis.

The DPPH• is characterized by its adaptation to various sample in a short time and sensitive enough to detect active ingredients at low concentrations *(Molyneux, 2004)*., for this purpose it has been used for the screening of antioxidant capacities

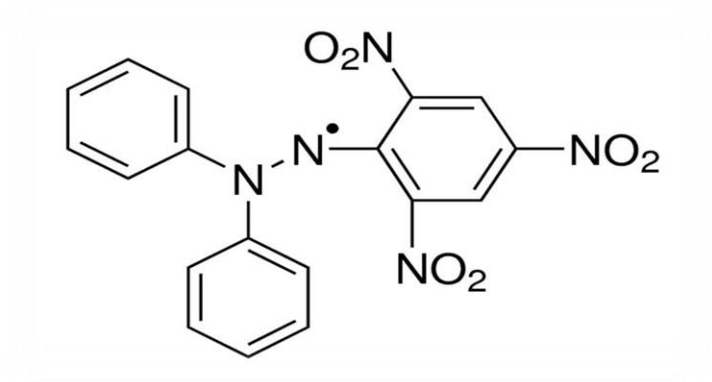


Figure 27: Chemical structure of the free radical DPPH (2,2-Diphenyl-1-picrylhydrazyl)

4.2.2. Electro chemical DPPH radical interaction study:

4.2.2.1.Inhibitory concentration (IC50):

In this study, the antioxidant activity of compounds (*Ammudoucus leucotrichus; Mentha piperita)* was evaluated through their ability to capture DPPH radicals. Kinetic profiles were determined, and the (IC_{50}) value was calculated for each compound to measure its free radical resistance efficiency according to the equation .

established, and half-maximal inhibitory concentration (**IC_{50}**)values were determined to quantify the efficacy of these compounds in scavenging DPPH. Radical, Where **ipa0** and **ipa** are the anodic peak current densities of the DPPH radical in the absence and presence of *Ammudoucus leucotrichus* and *Menhta piperita.(Chevion et al., 2000; Goyal & Bishnoi, 2011).*

Tableau 12: **IC_{50} values (µg/ml) obtained using DPPH radical scavenging activity from**

Compound	Equation	R^2	IC_{50}(µg/ml)
Ammudoucus	**y=3.556x -17.485**	**0.984**	**18.976**
Mentha Piperita	**y=6.264x -28.951**	**0.944**	**12.605**
α-Tocopherol	**y = 1325,7x + 5.236**	**0.967**	**33.77**

4.2.2.2.Electrochemical method

Based on the results presented in **Table 12** compounds (*Ammudoucus leucotrichus ;Mentha piperita)* demonstrated significant radical scavenging abilities.

The classification of these compounds according to their antioxidant activity (IC_{50}), is represented in the following curve : **figure28**

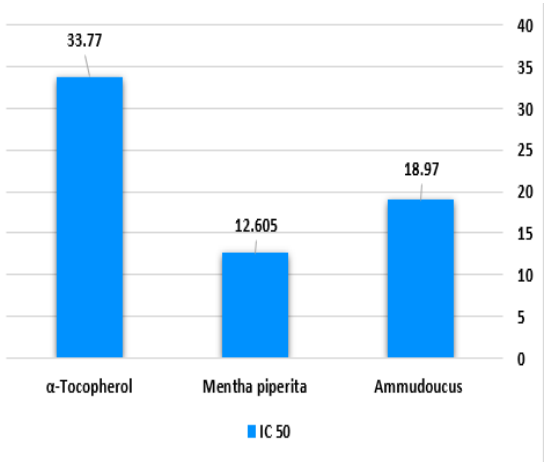


Figure 28: **Classification of DPPH radical inhibition capacity according to IC50 values by electro chemical method**

The results demonstrate that *Mentha piperita* has the highest antioxidant potential, followed by *Ammodaucus leucotrichus*, while α-Tocopherol, though a standard antioxidant, showed the least activity in comparison. This suggests that both plant extracts may serve as effective natural antioxidants.*(Goyal & Bishnoi, 2011; Chevion et al., 2000).*

- **Binding constant:**

The typical cyclic voltammetry (CV) behavior of DPPH in DMSO/0.1M TBATFB is observed within the potential window of 0.0 to -1.6 V at a glassy carbon electrode, both in the absence and presence of solutions at varying concentrations

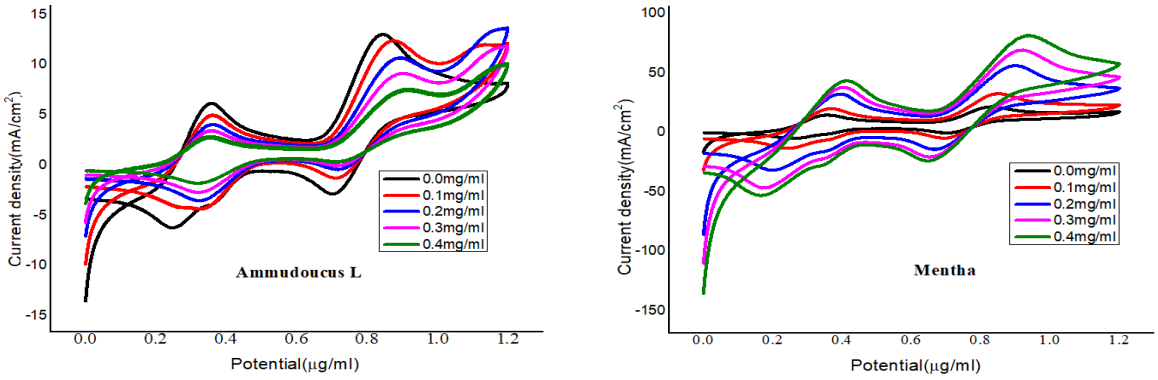


Figure 29: Cyclic voltammograms of DPPH• in the presence of increasing concentrations

The figure shows the effect of adding the two compounds (*Ammudoucus leucotrichus* and *Mentha piperita)* on the peak oxidation current density of the redox couple in a DPPH solution in DMSO . The decrease in the anodic current density is attributed to the reaction of DPPH with these compounds, leading to the formation of products such as DPPH•−

Ammudoucus leucotrichus, DPPH•−Mentha piperita. The calculation of the association constant (kb) is based on the changes in log(1/C) versus log(i/i0 - i), according to the equation as shown in the figure29. In addition, the changes in the peak potential values can be used to determine the mechanism of the reaction between the compounds and DPPH .*(Chevion et al., 2000; Kaurinovic & Vastag, 2019).*

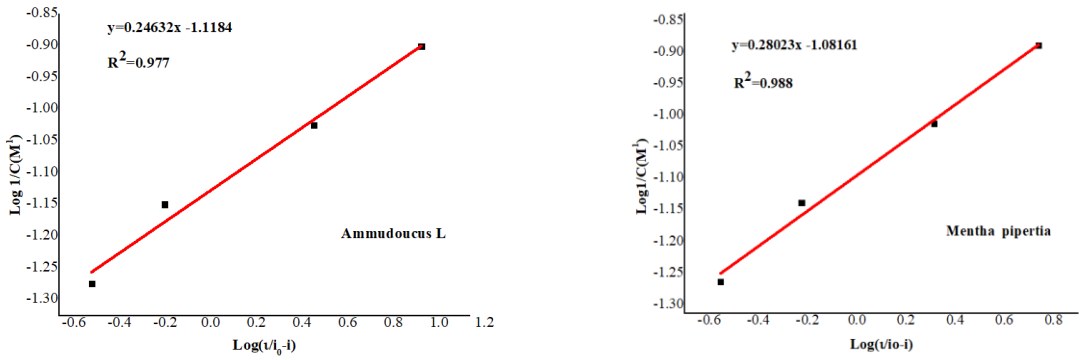


Figure 30: .Plots of Log (1/ C) versus Log (i /i_0-i) for DPPH with a variable concentration of Ammudoucus leucotrichus and Mentha piperita(D) in DMSO/0.1M Bu4NBF4, used to calculate the binding constants of products DPPH•− *Ammudoucus leucotrichus,* DPPH•− *Mentha piperita,*

- **Binding free energy:**

The binding free energy change was calculated using equation

The intercept of the linear curve fit of log(1/C) versus log(i/i0 − i) (equation III.2) gave the binding constants from which the free binding energy was calculated.

Tableau 13: Binding constants and values of binding free energies of products DPPH•− Ammudoucus leucotrichus and DPPH•− Mentha piperita, at T= 28

Adduct	Equation	R^2	K(M^{-1})	-ΔG(KJ.mol^1)
Ammudoucus	y=0.24632x-1,118	0.977	13.13	6.38
Mentha Piperita	y=0.28023x-1.183	0.988	15.25	6.75

Regarding the value of -ΔG, the values range from- 6.38 to -6.75 kJ/mol. Lower values of-ΔG indicate stronger interactions between DPPH and the corresponding addition product When comparing the -ΔG values among different addition products, it is evident that DPPH exhibits the strongest interaction within the range of -6.38 to -6.75 kJ/mol. The greater the interaction between DPPH and the compounds, the more negative the -ΔG value, indicating greater stability of the resulting addition product. There fore, based on these -ΔG values, it

can be concluded that *Ammodaucus leucotrichus* forms the most stable addition products with DPPH, while *Mentha piperita* forms the least stable addition products.*(Kaurinovic & Vastag, 2019; Kumar et al., 2020).*

4.3. Electronic spectroscopy DPPH radical interaction study:

4.3.1. Inhibitory concentration (IC_{50}):

In the present study, the antioxidant activity of *Ammudoucus leucotrichus*, *Mentha piperita,* and αtocopherol was assessed by their ability to scavenge DPPH radicals. The kinetic profiles were established by measuring the absorbance decrease of the DPPH solution (monitored at a specific wavelength, often 517 nm) after the addition of various concentrations of the samples. This method has been widely used in toxicological screening due to its reliability in evaluating radical-scavenging activity. *(Al-Saadi et al., 2018; Kedare & Singh, 2011)*

The half-maximal inhibitory concentration (**IC_{50}**) was determined from linear calibration plots of % inhibition versus sample concentration, according to Equation :This approach is commonly applied in phytochemical and toxicological research to quantify antioxidant capacity based on DPPH scavenging. *(Zamreek & Barkil, 2017; Sebaa et al., 2018).*

$$\%\ \text{DPPH radical scavenging activity} = \frac{A_0 - A}{A_0} \times 100 = aC\left(\frac{mg}{ml}\right) + b$$

Where A0 and A are the absorbances of the DPPH solution in the absence and presence of the tested samples, respectively; is the concentration (mg/mL); and , are parameters obtained from the linear fit. Table 14 summarizes the linear equations, determination coefficients

(R^2), and the resulting IC_{50} values for *Ammudoucus leucotrichus, Mentha* x *piperita,* and α-tocopherol (taken as a reference antioxidant).

Tableau 14: shows all four compounds show radical-scavenging activities.

Compound	Equation	R2	IC_{50} (µg/ml)
Ammudoucus	Y=3.556x -17.485	0.976	59.108
Mentha piperita	Y=6.264x -28.951	0.914	49.045
α-Tocopherol	Y=4.264x -20.951	0.988	70.77

The classification of these compounds *Ammudoucus leucotrichus* and *Mentha piperita* according to their antioxidant activity (IC_{50}), is represented in the following curve: Figure 31

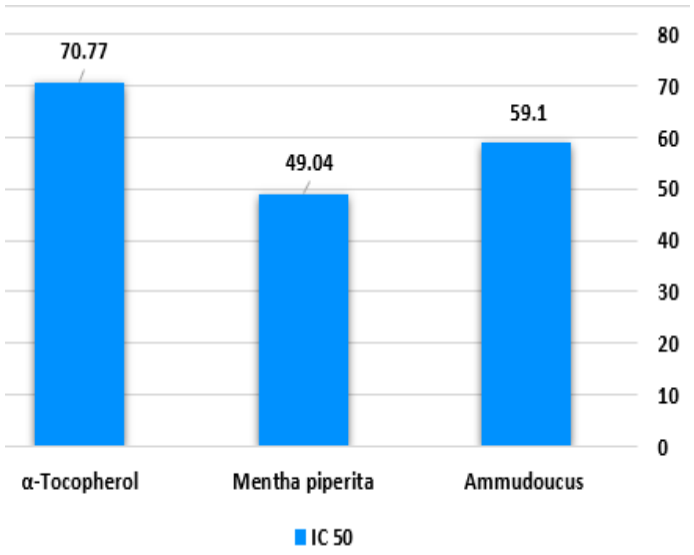


Figure 31: Classification of DPPH• inhibition capacity according to IC_{50} values from Electronic spectroscopy method

- ***A-Tocopherol*** recorded an IC_{50} = 70.77 µg/ml, indicating the weakest antioxidant activity among the tested compounds since it requires the highest concentration to achieve half-maximal inhibition.
- ***Mentha piperita*** showed an IC_{50} = 49.045 µg/ml, demonstrating stronger antioxidant activity compared to α-Tocopherol.
- ***Ammodaucus leucotrichus*** exhibited an IC_{50} = 59.108 µg/ml, making it less effective than Mentha piperita but still more potent than α-Tocopherol.

These differences in (IC_{50}) values could be attributed to the variation in the phenolic content, flavonoids, and other active compounds in each extract, as well as differences in the mechanism of interaction with the DPPH radical. Furthermore, recent studies that advanced methods, such as electrochemical and spectroscopic approaches, provide enhanced accuracy in antioxidant screening . *(Saba et al., 2024).*

5. In-Silico analysis:

5.1. ADMET and drug-likeness prediction:

Modern drug discovery involves assessment of competence of the dynamic molecules and their strength to reach target site in bioactive form, which involves cellular, animal and human clinical trials which are highly priced and encumbered with risks (Ranjith & Ravikumar, 2019; Ranjith D & Viswanath S, 2019). Presently computer aided drug development encouraged the estimate of absorption, distribution, metabolism and excretion of drugs (ADME), they postulate anticipatory and dependable data very quickly and compliment for

experimental approaches (Ranjith & Ravikumar, 2019; Sliwoski et al., 2014). It has been determined that the initial appraisal of ADME properties in the discovery period diminishes remarkably the fraction of pharmacokinetics related failures in the clinical phase (Hay et al., 2014; Ranjith & Ravikumar, 2019).

In the present study we evaluated the ADME properties of the major compounds present in both essential oils using SwissADME web tool. A total of 4 potent phytoconstituents were analysed to study general characteristics (Table 15), Physicochemical properties , lipophilicity and water solubility characteristics, pharmacokinetic parameters , drug likeness rule and bioavailability score and medicinal chemistry properties (Table 19). General characteristics of the studied compounds revealed all the compounds having molecular weight less than 500 Da, which is a good prime property to be called as drug likeness of the small molecules and they still have a low molecular weight.

Tableau 15: General characteristics of the phytoconstituents of essential oils

SI. No	Compounds	**Molecular formula**	**Canonical SMILES**	**Molecular weight (g/mol)**
1	trans-Sabinene hydrate	$C_{10}H_{18}O$	CC(C12CCC(C2C1)(C)O)C	154.25
2	Linalool acetate	$C_{12}H_{20}O_2$	C=CC(OC(=O)C)(CCC=C(C)C)C	196.29
3	Perilla aldehyde	$C_{10}H_{14}O$	O=CC1=CCC(CC1)C(=C)C	150.22
4	D-Limonene	$C_{10}H_{16}$	CC1=CCC(CC1)C(=C)C	136.23

Tableau 16: Physicochemical properties of the phytoconstituents of essential oils

Properties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene
Num. heavy atoms	11	14	11	10
Num. arom. heavy atoms	0	0	0	0
Fraction Csp3	1.00	0.58	0.50	0.60
Num. rotatable bonds	1	6	2	1
Num. H-bond acceptors	1	2	1	0
Num. H-bond donors	1	0	0	0
Molar refractivity	46.90	60.17	47.32	47.12
TPSA (Å²)	20.23	26.30	17.07	0.00

Tableau 17: Lipophilicity characteristics of the phytoconstituents of essential oils

Properties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene
iLOGP	2.47	3.08	2.16	2.72
XLOGP3	2.12	3.93	3.13	4.57
WLOGP	2.19	3.24	2.49	3.31
MLOGP	2.45	2.95	2.10	3.27
SILICOS-IT	2.44	2.98	2.64	2.97
Consensus Log Po/w	2.33	3.24	2.50	3.37

Tableau 18: Water Solubility characteristics of the phytoconstituents of essential oils

Small molecules	ESOL				Ali				SILICOS-IT			
	Log S (ESOL)	Solubility		Class	Log S (Ali)	Solubility		Class	Log S (SILICOS-IT)	Solubility		Class
		mg/ml	mol/L			mg/ml	mol/L			mg/ml	mol/L	
trans-Sabinene hydrate	-2.07	1.33e-0	8.59e-3	S	-2.18	1.03e-0	6.67e-3	S	-1.91	1.92e-0	1.24e-2	S
Linalool acetate	-3.14	1.43e-1	7.30e-4	S	-4.18	1.29e-2	6.58e-5	MS	-2.52	5.97e-1	3.04e-3	S
Perilla aldehyde	-2.61	3.68e-1	2.45e-3	S	-3.16	1.04e-1	6.96e-4	S	-1.83	2.22e-0	1.48e-2	S
D-Limonene	-3.50	4.33e-2	3.18e-4	S	-4.29	6.93e-3	5.09e-5	MS	-2.26	7.54e-1	5.53e-3	S

Tableau 19: Pharmacokinetics parameters of the phytoconstituents of essential oils

GI absorption	**High**	**High**	**High**	**Low**	**Low**
BBB permeant	Yes	Yes	Yes	Yes	No
P-gp substrate	No	No	No	No	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log Kp (Skin Permeation) (cm/s)	-5.74	-4.71	-4.99	-3.89	-16.29

Tableau 20: Druglikeness rule and bioavailability score of the phytoconstituents of essential oils

Proprieties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene
Lipinski	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations
Ghose	No; 1 violation: MW<160	Yes	No; 1 violation: MW<160	No; 1 violation: MW<160
Veber	Yes	Yes	Yes	Yes
Egan	Yes	Yes	Yes	Yes
Muegge	No; 2 violations: MW<200, XLOGP3>3.5	No; 1 violation: MW<200	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2
Bioavailability score	0.55	0.55	0.55	0.55

Table 21. Medicinal Chemistry properties of the Phytoconstituents of essential oils

Proprieties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene
PAINS	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	1 alert: isolated_alkene	1 alert: isolated_alkene	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5
Synthetic accessibility	2.82	2.75	3.19	3.46

Lipophilicity property of the compounds portrays an important role for molecular discovery activities in multifarious domains. The quantitative descriptor of the lipophilicity is the partition coefficient P of a given molecule between *n*-octanol and water system (Daina et al., 2014). Because of its amphiphilic nature, *n*-octanol is considered a good mimic of phospholipid membrane characteristics (Liu et al., 2011). Multifarious algorithms are accessible to compute log Po/w, which rely on factual methodologies. The classic log P predictors branched in to two division, first ones split molecular structures into molecular fragments includes fragmental approach e.g. KLOGP (Klopman et al., 1993), KOWWNIN (Meylan & Howard, 2000) or atomic approach e.g. ALOGP (Ghose et al., 1998; R. Wang et al., 1997), XLOGP (Cheng et al., 2007; Moriguchi et al., 1994). The second division gathers the topological methods in which, the molecules description is related to its topology being as count or flags for specific atoms, groups or structural properties e.g. MLOGP (Brenk et al., 2008; Moriguchi et al., 1992), the prediction attained by manifold linear regression trained on large molecular data sets. The SILICOS-IT is a hybrid technique which combines both molecular fragments and topological parameters, which confide on 27 fragments and 7 topological descriptors, it was disciplined on 23,455 molecules with experimental *n*-octanol/water partition values (Daina et al., 2014). The version three of the XLOGP atomic model is established on a system of 87 fragments and two corrective factors. If the input structures are similar to a reference compound, the fragments differentiating them are treated and the corresponding log P contributions added to the reference structure log P value (Cheng et al., 2007). Lipophilicity estimated as consensus Log P, which is the average value of all Log P evaluated with various lipophilicity criteria, determined as most lipophilic whereas trans-Sabinene hydrate as least lipophilic and water solubility of the small molecules ranged from highly water soluble (trans-Sabinene hydrate).

The SwissADME model returns “Yes” or “No” if the compound under examination has greater probability to be a substrate or non-substrate of P-gp or inhibitor or non-inhibitor of Cytochrome P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4).

The pharmacokinetics and drug likeness performed using SwissADME showed a low level of GI absorption and BBB permeant with D-Limonene while a high absorption detected with trans-Sabinene hydrate and Linalool acetate. All the compounds present in the essential oils are not the substrates for P-gp (Table 19), so they are not suscepte efflux mechanism performed by this transporter which is used by many tumours cell lines as a drug-resistance mechanism (Ranjith & Ravikumar, 2019)

All of the small molecules returned as non-inhibitors for inactivation for CYP isoenzymes. The skin permeability coefficient (Log Kp), a multiple linear regression, the more negative the log Kp (with Kp in cm/s), the less skin permeant is the molecule. Among the phytoconstituents, trans-Sabinene hydrate (-4.96) is the least permeant and D-Limonene (-5.4) is highly permeant respectively. This SwissADME section gives access to five different rule-based filters, with diverse ranges of properties inside of which the molecule is defined as drug-like. The Lipinski (Pfizer) filter is the pioneer rule-of-five implemented and with the Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer) methods. Multiple estimations allow consensus views or selection of methods best fitting the end-user's specific needs in terms of chemical space or project-related demands. Any violation of any rule described here appears explicitly in the output panel. All the four compounds followed the filtered rule invoked in the SwissADME; the violation shown by the molecules are minimal.

SwissADME interpretation posts 0 PAINS alert of the 4 studied compounds. Brenk considered compounds that are smaller and less hydrophobic and not those defined by "Lipinski's rule of 5" to widen opportunities for lead optimization. This was after exclusion of compounds with potentially mutagenic, reactive and unfavourable groups such as nitro groups, sulphates, phosphates, 2-halopyridines and thiols (Brenk et al., 2008). All the compounds examined flouted Brenk's rule with only one alert, all the compounds failed Lead-likeness criteria due to their molar weight.

In silico toxicity study aims to help in optimizing compounds regarding their toxicity proprieties. The study could offer an important improvement to the awareness of the full perspective of virtual screening for the identification of target compounds with negligible or no toxicity, which may open a path for the selection of novel nontoxic phytoconstituents present in *Ammudoucus leucotrichus* and *Mentha piperita* essential oils with high antidiabetic activity. In silico toxicity study of the chosen compounds was performed using the ProTox-II web server (Drwal et al., 2014). It aims to predict hepatotoxicity (Dili), carcinogenicity (Carcino), immunotoxicity (Immuno), mutagenicity (Mutagen), cytotoxicity (Cyto), median lethal dose (LD_{50}), and toxicity class (TC).

According to in silico toxicity profiles presented in Table 21, the toxicity class of all the phytoconstituents was detected to be equal to 5 except the Linalool acetate which predicted to be 6. Also, all the compounds were nontoxic in all the type of toxicities. the standard of type 2 diabetes was predicted to be immunotoxin with a toxicity class of 6.

Tableau 21: In silico toxicity profiles of the studied compounds

Molecule	Dili	Carcino	Immuno	Mutagen	Cyto	LD_{50} ($mg.Kg^{-1}$)	TC
trans-Sabinene hydrate	Inactive	Inactive	Inactive	Inactive	Inactive	2000	4
Linalool acetate	Inactive	Inactive	Inactive	Inactive	Inactive	12000	6
Perilla aldehyde	Inactive	Inactive	Inactive	Inactive	Inactive	1720	4
D-Limonene	Inactive	Inactive	Inactive	Inactive	Inactive	4400	5

5.2. Molecular Docking Study:

In the current study , the binding interaction of all the major compounds presented in *Ammudoucus leucotrichus* and *Mentha piperita* essential oils compounds with glutathione reductase was further investigated by carrying out a molecular docking analysis of the binding interaction between human pancreatic glutathione reductase with compounds: Perilla aldehyde, D-Limonene, alpha-Pinene, Pulegone, Menthone, trans-Sabinene hydrate, alpha-Terpineol, Borneol, Linalool acetate, 1,8-Cineole (the major phytochemicals present in the two plants with yield > 1) using Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015).

Each compound was docked with human pancreatic glutathione reductase (GR) to understand their interactions at the enzyme's active site. Of all the major compounds present in *Ammudoucus leucotrichus* and *Mentha piperita* essential oils. (Table 22). This result indicates that the plant is a rich source of compounds which, either individually or synergistically, resulted in the decrease of glutathione reductase activity as observed in the *in vitro* and *in vivo* experimental glutathione reductase inhibition study. Also, the interactions were spontaneous as observed from the negative Gibb's free energy values (Avwioroko et al., 2020).

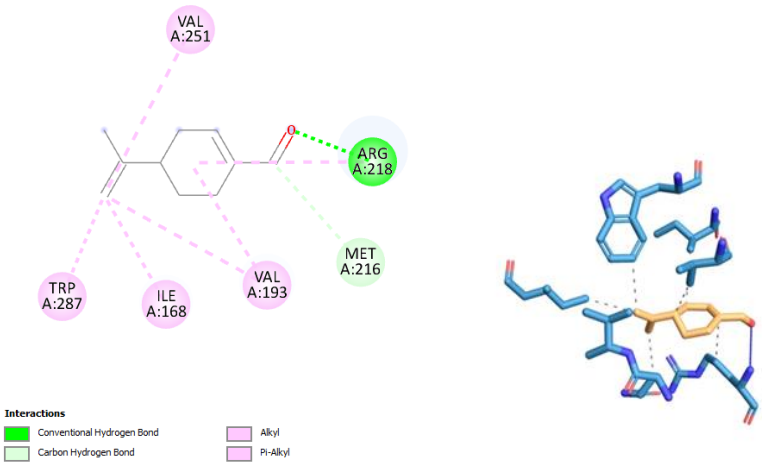
From studies on the X-ray crystal structure and enzyme kinetics of glutathione reductase, it was found that GR active site is characterized by the existence of three special amino acid residues namely ASP-197, GLU-233 and ASP-300 which contribute to the overall catalytic action of the enzyme towards starch hydrolysis (Anigboro et al., 2021). ASP-197 acts as a catalytic nucleophile during starch hydrolysis,GLU-233 residue acts as acid–base catalyst, while ASP-300 plays the role of optimizing the orientation of the substrate (Anigboro et al., 2021).

Tableau 22: Docking score of the studied compounds

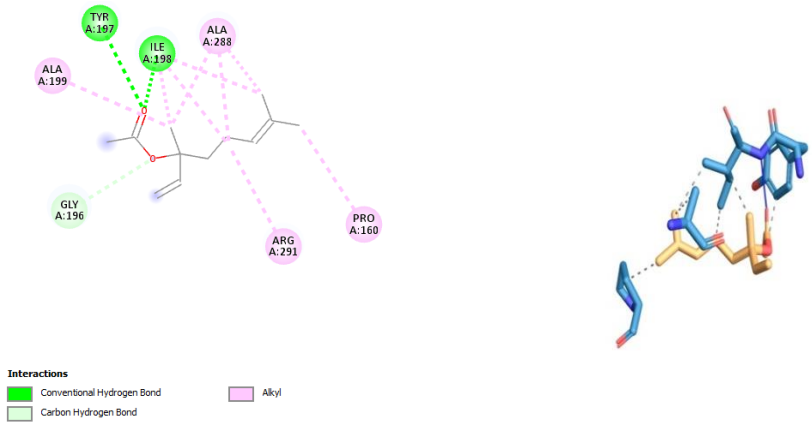
Compound	Rigid Docking Score (Kcal/mol)	IFD scores (Kcal/mol)
Perilla aldehyde	-8.40919	-7.16844
D-Limonene	-7.62852	-7.12662
alpha-Pinene	-7.59983	-6.14262
Pulegone	-5.59606	-6.26562
Menthone	-6.80255	-8.69487
trans-Sabinene hydrate	-6.59115	-8.20287
alpha-Terpineol	-6.31935	-7.57926
Borneol	-5.86484	-6.93228
Linalool acetate	-7.46544	-9.41073
1,8-Cineole	-4.50886	-5.92368

The results in Figure 32 revealed that the best two compounds in all the plants interacted with a similar set of amino acid residues at the active site of the protein just as the standard GR inhibitor did. It is noticed in their interaction with the enzyme's key catalytic residues (TYR-62, ILE-168, ALA-342, PRO-415, ARG-218, for Linalool acetate and ARG-218, MET-216, VAL-193, VAL-251, ILE-168, and TRP-287for Perilla aldehyde). Compound Perilla aldehyde, showed interaction with multiple amino acids within or near the catalytic site of glutathione reductase, indicating a strong binding pattern. In contrast, Linalool acetate showed interaction with fewer residues, specifically TYR-62, ILE-168, ALA-342, PRO-415, ARG-218, which may suggest binding at a different domain or orientation. This perhaps denotes that the inhibitory effect contributed by Linalool acetate to the overall inhibitory effect of the other compounds may be that of non-competitive inhibition, whereas that of Perilla aldehyde (the compound with the best binding affinity) may be that of competitive mode of inhibition. Overall, the in-silico analysis of the ligand protein interaction of these compounds with amino acid residues present in the glutathione reductase active site domain (such as TRP-58, TRP-59, TYR-62, GLN-63, ASP-197, GLU-233, ASP-300 etc.) gave credence to the inhibitory effect of the compounds reported in this study. Furthermore, it also confirmed the possibility of the binding interaction between glutathione reductase and the phytoconstituents of Ammudoucus leucotrichus and Mentha piperita essential oils investigated and observed using spectroscopic methods reported. The best ligand-binding poses in the catalytic domain of

glutathione reductase obtained after docking as well as the amino acid residues involved in the interaction are as illustrated in Figure 32.



Docking complex and interaction plot for compound Perilla aldehyde with GR



Docking complex and interaction plot for compound Linalool acetate with GR

Figure 32: Molecular interactions of studied compounds with humane pancreatic glutathione reductase

CONCLUSION

Conclusion:

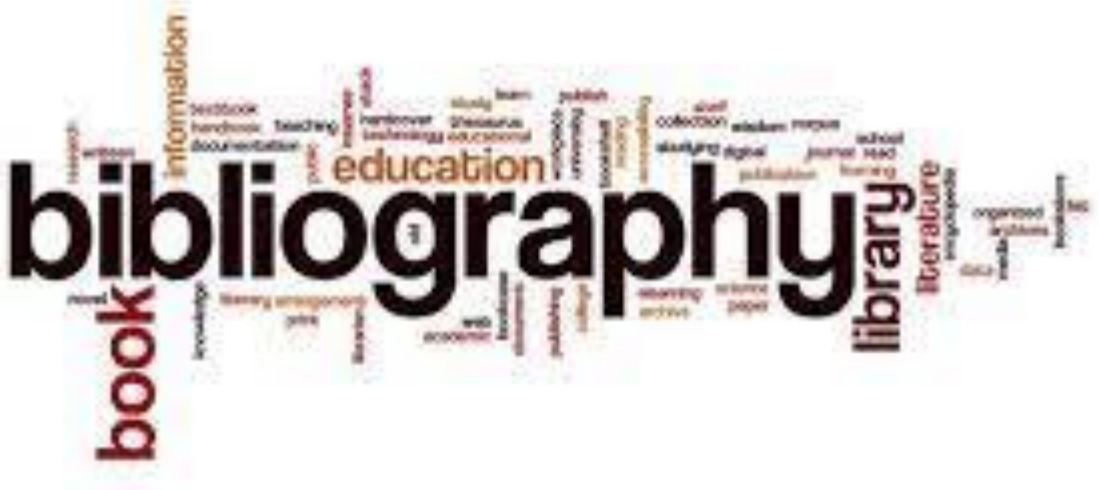
This research provided a comprehensive investigation into the chemical composition and antioxidant capacity of essential oils extracted from Mentha piperita and Ammodaucus leucotrichus, two medicinal plants with valuable ethnobotanical backgrounds in Algerian traditional medicine.

Through hydro distillation and GC-MS analysis, we identified major phytochemicals such as menthol and menthone in Mentha piperita, and cumin aldehyde and thymol derivatives in Ammodaucus leucotrichus. These compounds are known to possess strong antioxidant properties, which were further confirmed by the results of both the DPPH assay and cyclic voltammetry analysis.

The DPPH assay revealed that Mentha piperita had a lower IC_{50} value (49.0 µg/mL) compared to Ammodaucus leucotrichus (59.1 µg/mL), indicating a stronger radical scavenging capacity. Moreover, the voltametric profiles showed that both oils possess effective electron-donating ability, which further supports their potential therapeutic applications.

These findings support the traditional uses of both plants and suggest their potential as natural sources of antioxidants for pharmaceutical, nutraceutical, and cosmetic applications. In addition, this work contributes to the scientific validation of Algerian medicinal plants and highlights the need to preserve and sustainably exploit native flora.

For future research, we recommend extending the investigation to include antimicrobial, anti-inflammatory, cytotoxic, and in vivo studies. Furthermore, assessing the safety, dosage, and possible side effects of these essential oils will be crucial before any commercial or clinical application.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