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THEME

Multi-Stage Evaluation of Essential Oils from Two Medicinal Plants: GC/MS Characterization, In Vivo and In Vitro Anti-inflammatory Activity, and In Silico Analysis Using ADMET, DFT, Molecular Docking, and Dynamics Simulations.

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I wish you all the best and every success in life.

This success is yours… just as much as it is mine

Manar.

Dedication

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As a token of my appreciation, I dedicate this humble work and the fruit of my efforts to those whom God Almighty has enjoined upon us in His Holy Book:

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To everyone who knows me, near or far, may Allah extend your lives and bestow His blessings upon you abundantly. ❤

Salim

Abstract:

This study aims to evaluate the anti-inflammatory properties of both essential oils and derived from Ammodaucus leucotrichus and Mentha piperita, using a multi-phase approach that includes in vitro experiments, in vivo testing on white mice models, and advanced in silico analyses. The essential oils were extracted and their chemical composition was analyzed using GC/MS technology, which revealed the presence of a variety of biologically active compounds, notably monoterpenes and phenolics. These compounds were further assessed through pharmacokinetic simulation tools such as SwissADME, admetSAR, and Protox, which demonstrated their compliance with drug-likeness criteria in terms of absorption, distribution, metabolism, and toxicity, supporting their potential as therapeutic agents.

In vitro anti-inflammatory activity was evaluated using the bovine serum albumin (BSA) denaturation assay, with comparisons made to standard anti-inflammatory drugs (diclofenac). In vivo Inflammation was induced in white mice using the inflammatory agent benzylthiouracil , and treatment efficacy was assessed through changes in body weight and inflammatory markers in the blood after seven days of treatment. The compounds showed a significant reduction in inflammation indicators.

Moreover, ADMET analysis confirmed the compounds' compatibility with pharmacokinetic standards regarding absorption, distribution, and toxicity. Molecular docking studies demonstrated that the compounds pulegone, perilla aldehyde, and alpha-pinene could effectively bind to inflammatory targets such as COX-1 and COX-2 enzymes, with binding energies of -6.54 and -5.13, -6.32, and -4.77 kcal/mol respectively. These findings support the potential use of these oils as natural anti-inflammatory agents and provide a foundation for the development of effective and safe herbal Therapeutics.

Keywords: Essential oils, *Ammodaucus leucotrichus*, *Mentha piperita*, Anti-inflammatory activity, *in vivo , in vitro , in silico,* Molecular docking.

Abstract

Résumé :

Cette étude vise à évaluer les propriétés anti-inflammatoires des huiles essentielles et issus de Ammodaucus leucotrichus et de Mentha piperita, en adoptant une approche multi-étapes comprenant des essais in vitro, des expérimentations in vivo sur un modèle murin (souris blanches), ainsi que des analyses informatiques avancées (in silico). Les huiles essentielles ont été extraites et leur composition chimique analysée par la technique GC/MS, révélant la présence de divers composés biologiquement actifs, notamment des monoterpènes et des phénols.

Ces composés ont ensuite été évalués à l'aide d'outils de simulation pharmacocinétique tels que SwissADME, admetSAR et Protox, démontrant leur conformité aux critères pharmacologiques en matière d'absorption, de distribution, de métabolisme et de toxicité, ce qui soutient leur potentiel en tant qu'agents thérapeutiques.

L'activité anti-inflammatoire in vitro a été évaluée par le test de dénaturation de l'albumine sérique bovine (BSA), comparée aux médicaments anti-inflammatoires standards (diclofénac). L'inflammation été induite in vivo chez des souris blanches à l'aide de l'agent inflammatoire benzylthiouracile, et l'efficacité du traitement a été mesurée par les variations de poids et les marqueurs inflammatoires dans le sang après sept jours de traitement. Les composés ont montré une réduction significative des marqueurs de l'inflammation.

De plus, l'analyse ADMET a confirmé la compatibilité des composés actifs avec les normes pharmacocinétiques en termes d'absorption, de distribution et de toxicité. Les études de docking moléculaire ont révélé la capacité des composés pulegone, perilla aldehyde et alpha-pinene à se lier efficacement aux cibles inflammatoires telles que les enzymes COX-1 et COX-2, avec des énergies de liaison respectives de -6,54 et -5,13, -6,32 et -4,77 kcal/mol. Ces résultats soutiennent l'utilisation potentielle de ces huiles comme agents anti-inflammatoires naturels et constituent une base pour le développement de traitements à base de plantes efficaces et sûrs.

Mots-clés : Huiles essentielles, *Ammodaucus leucotrichus*, *Mentha piperita*, Activité anti-inflammatoire*, in vivo , in vitro , in silico* , docking moléculaire .

الملخص:

يهدف هذا البحث إلى تقييم الخصائص المضادة للالتهاب لكل من الزيوت الأساسية المستخلصة من نباتي Ammodaucus leucotrichus وMentha piperita، وذلك باستخدام نهج متعدد المراحل يشمل التجارب المخبرية(in vitro) ، والتجارب الحيوية على نموذج الفئران البيضاء(in vivo) ، إضافة إلى تحليلات حاسوبية متقدمة .(in silico) تم استخراج الزيوت الأساسية وتحليل تركيبها الكيميائي باستخدام تقنيةGC/MS ، التي كشفت عن وجود مجموعة من المركبات النشطة بيولوجيًا، أبرزها المونوتربينات والفينولات. خضعت هذه المركبات لتقييم عبر أدوات محاكاة دوائية مثل SwissADME و admetSARوProtox، والتي أظهرت توافقها مع المعايير الدوائية من حيث الامتصاص والتوزيع والأيض والسمية، بما يدعم إمكانية استخدامها كمكونات علاجية.

تم تقييم النشاط المضاد للالتهاب مخبريًا باستخدام اختبار تمسخ حمض الفوسفوريك(BSA) ،وتمت مقارنتها بالادوية المضادة للالتهابات (ديكلوفيناك) .في حين تم استحثاث الالتهاب في الفئران البيضاء عن طريق محفز التهابي بنزيل ثيوراسيل ، وتقييم فعالية العلاج من خلال قياس التغير في الوزن ومؤشرات الالتهاب في الدم بعد سبعة أيام من العلاج. وأظهرت المركبات انخفاضًا ملحوظًا في مؤشرات الالتهاب.

كما أظهرت نتائج تحليل ADMET توافق المركبات النشطة مع المعايير الدوائية من حيث الامتصاص والتوزيع والسمية. وبيّنت دراسات الالتحام الجزيئي قدرة المركبات pulegone , perilla aldehyde,Alpha-pinene على الارتباط الفعال مع أهداف التهابية مثل إنزيمي COX-1 وCOX-2، مسجلة طاقات ارتباط سالبة(-6.54 و-5.13)،(-6.32)و(-4.77). تدعم هذه النتائج بإمكانية استخدام هذه الزيوت كمضادات طبيعية للالتهاب، وتمثل أساسًا لتطوير علاجات عشبية فعالة وآمنة.

الكلمات المفتاحية:

الزيوت العطرية، *Ammodaucus leucotrichus . Mentha piperita*، النشاط المضاد للالتهاب، *in vivo ، in vitro*، *in silico*، الالتحام الجزيئي.

ABREVIATIONS LIST

%: percentage

°C: Degrees Celsius

A° : Angstrom

ADMET: Absorption,Distribution,Metabolism Excretion of Drugs and Toxicity

AFNOR: Association Française de Normalisation

CO2: Crbon dioxid

COX: Cyclooxygenase

EOs: Essential Oils

FBS: Fasting blood sugar

GC/MS :. Gas Chromatography-Mass Spectrometry

GRA: Granulocytes

HGB: Hemoglobin

HGB: Hemoglobin

IC50: Concentration causing 50% inhibition of an activity.

IFD: Induced Fit docking

IL-1: Interleukin-1

IL-2: Interleukin-2

IL-6: Interleukin-6

IL-8: Interleukin-8

IR: Infrared Spectroscopy

K : Constante de liaison

L: liter

LYM: Lymphocytes

M: molar

MAHD: Microwave Assisted Hyrdo-Distillation.

MDS: Molecular Dynamics Simulation

mg : Milligramme

MHG : Microwave hydrodiffusion and gravity .

OHWD: Ohmic Heated Water Distillation.

PAF acether: Platelet Activating Factor acether

PAF: Platelet Activating Factor

PGE2: Prostaglandin E2

PGI2: Prostaglandin I2

pH : Potential hydrogen

Abreviations list

PLT: Platelets

RBC: Erythrocytes

RBC: Red blood cell

ROS: Reactive Oxygen Species

SFE: Supercritical Fluid Extraction .

SFMAE : Solvent free microwave assisted extraction.

TNFα: Tumor Necrosis Factor Alpha

UAE: Ultrasound-assisted extraction (UAE).

UV : Ultra-violet

VTRS : Valorisation and Technology of the Saharian Resources Laboratory

WBC: White blood cell

α : Alpha

β: Beta

ΔG: Free binding energy

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General Introduction

Introduction

Inflammation is a natural biological response of body tissues to harmful stimuli such as pathogens, damaged cells, or irritants. This response serves as a defense mechanism aimed at eliminating the initial cause of cell injury, clearing out necrotic cells, and initiating tissue repair processes (Medzhitov, 2008).

Inflammation can be categorized into two main types: acute inflammation, which is an immediate and short-term response to injury or infection, and chronic inflammation, which is a prolonged and dysregulated response that can contribute to various chronic diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders (Nathan & Ding, 2010; Furman et al., 2019).

Chronic inflammation has garnered significant attention in recent years due to its role as a key contributor to the development of numerous non-communicable diseases. Persistent inflammatory responses can lead to tissue damage and organ dysfunction, highlighting the importance of early intervention to mitigate inflammation and reduce associated health risks (Furman et al., 2019).

While non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammatory conditions, their prolonged use can result in serious side effects, particularly affecting the gastrointestinal tract and kidneys. Consequently, there is a growing interest in identifying safer, natural alternatives. Medicinal plants have emerged as promising candidates due to their content of bioactive compounds capable of modulating inflammatory responses through various biological mechanisms. Notable among these compounds are flavonoids, alkaloids, terpenoids, and phenolic acids, which exert anti-inflammatory effects by inhibiting pro-inflammatory cytokines and reducing oxidative stress (Azab et al., 2016).

Algeria, with its rich biodiversity and longstanding tradition of herbal medicine, offers a valuable repository of plants with potential anti-inflammatory properties. Ethnopharmacological studies have documented the use of several indigenous plants in treating inflammatory symptoms such as pain, fever, and swelling (Bouasla & Bouasla, 2017).

Among these, Ammodaucus leucotrichus, commonly known as "Oum ed-drigua," is a traditional medicinal plant native to the Algerian Sahara. Locally, its leaves and seeds are used in infusions to

alleviate respiratory inflammations, joint pain, and digestive issues. Recent studies have identified that the essential oil extracted from its seeds contains compounds like carvone and limonene, known for their anti-inflammatory properties, including the inhibition of nitric oxide production and modulation of inflammatory mediators (Louail et al., 2016; Ziani et al., 2019).

Similarly, Mentha piperita (peppermint) is a widely used aromatic and medicinal plant. Its essential oils, rich in menthol and rosmarinic acid, have demonstrated anti-inflammatory, antimicrobial, and antioxidant activities. Research indicates that these oils can suppress inflammatory cytokines such as TNF-α and IL-1β, supporting their use in managing skin, respiratory, and muscular inflammations (Romero et al., 2005; Mimica-Dukić et al., 2003; Narendhirakannan et al., 2006).

Despite their extensive traditional use, comprehensive scientific validation of the anti-inflammatory efficacy of Ammodaucus leucotrichus and Mentha piperita remains limited. This study aims to bridge this gap by extracting and characterizing the chemical composition of their essential oils and evaluating their anti-inflammatory potential through in vitro, in vivo, and in silico analyses to elucidate their molecular mechanisms of action.

first part: bibliography

Chapter one: Medicinal plants

I.Medicinal Plants

1.Brief Overview of Medicinal Plants:

In the early stages of civilization, the use of medicinal plants was instinctive, much like in animals. However, humans quickly realized that these plants provided sufficient information to treat diseases and help alleviate suffering. Nature supplied humans with all their basic needs for food, clothing, shelter, and medicine, even flavors, fragrances, and dyes. Consequently, sophisticated traditional medicine systems evolved over thousands of years to provide humanity with new and continuous treatments. Many plants are known for their therapeutic properties and continue to appear in modern pharmacopoeias and well-known materiamedica. Nevertheless, a significant portion of plant-based therapy relies on empirical experience accumulated over millennia. (Beyene et al.,2016)

2.Introduction:

Plants are a fundamental part of the environment and are widely distributed throughout the world. Since the dawn of history, humans have relied on plants for food, shelter, clothing, and medicine. Plants were identified for their therapeutic properties through trial and error. Priests, shamans, spiritual healers, and tribal leaders routinely practiced this type of treatment in regions of Asia, Africa, the Middle East, Europe, and Latin America. Furthermore, the widespread use of herbal remedies for treating diseases has been documented in ancient texts such as the Vedas, the Hadith, the holy Quran, and the book “Duke’s Handbook of Medicinal Plants” by Duke, Howard, and De Silva (1999), and “Duke’s Concise Handbook of Medicinal Plants” compiled by Duke, Calise, and Ahdradith (2008), which was described as a “trustworthy folk medicine pharmacopoeia” (Singh et al., 2012).

3. History of the Use of Plants as Medicines:

Among the oldest and most comprehensive written evidence regarding the use of plants in the medical field are those dating back approximately five thousand years, inscribed on Sumerian clay tablets containing therapeutic recipes for over two hundred different plant species. These records, found etched on clay tablets from around the third millennium BCE, predate the later civilizations of Mesopotamia. Evidence suggests that medicinal plants were part of healing practices in early civilizations, as the Chinese Emperor Shen Nung (estimated to have lived around 2500 BCE) compiled a comprehensive compendium listing more than three hundred medicinal herbs, and Traditional Chinese Medicine is considered to have a long and impressive history. Many of these herbs were mentioned in similar texts in other regions of the ancient world such as Egypt, Mesopotamia, and Europe. Opium is among the oldest substances known for its analgesic (pain-relieving) effects. Effective remedies have been used for thousands of years. The Assyrians,

Babylonians, and Sumerians documented the use of medicinal herbs in cuneiform inscriptions on clay tablets, and the Code of Hammurabi (18th century BCE) includes numerous herbal medical prescriptions. Egyptians recorded their medical knowledge in drawings and texts on papyrus dating back to the Old Kingdom of Egypt. The "Ebers Papyrus" (dating to around 1550 BCE) is considered the most important medical document of ancient Egypt, describing over eight hundred and fifty medicinal prescriptions utilizing approximately seven hundred different therapeutic substances. Hippocrates (who lived between 460 and 377 BCE) is considered among the first figures to separate the field of medicine from philosophy and is often referred to as the "Father of Medicine." Theophrastus (who lived between approximately 371 and 287 BCE), a student of Aristotle, was an important Greek medical writer who wrote about around five hundred types of medicinal plants. Dioscorides (who lived in the 1st century CE), a Greek physician, surgeon, and pharmacologist, was a prolific author in the field of pharmacology, and his book "De MateriaMedica" (written around 78 CE), which served as a primary reference for drugs in Europe for over fifteen hundred years, is another significant Greek medical work. Galen (who lived between 130 and 200 CE), a prominent Greek physician and pharmacist, expanded the scope of plant use in medicine, documented the development of various scientific disciplines, and made significant contributions to the field of physiology. Indigenous peoples in North America, such as the Navajo, also used native plants as medicines for many centuries, and many of these uses have been documented. For example, Taxol (derived from Taxusbrevifolia), an important anticancer drug, was isolated from a plant native to North America. Similarly, a diverse range of other plants have been used to treat various diseases for many centuries and have a long history in many cultures. For instance, quinine (derived from Cinchona), a traditional remedy for malaria, was introduced to Europe in the 17th century. Cinchona bark (derived from Tatanusboveifolia) is another folk remedy that was introduced to Europe to treat malaria, and its active ingredient was isolated two centuries later (in 1820). In addition, many other significant medicinal compounds have been isolated from plants and widely used, such as digitalis (derived from Digitalis purpurea), ephedrine, morphine, cocaine, caffeine, and many others. Significant discoveries in pharmaceutical chemistry during the second half of the 20th century led to the synthesis of a considerable number of drugs that now treat a wide spectrum of diseases. Despite this progress, natural products or those derived from plants still constitute a significant portion of the drugs currently used in treating various diseases, including chronic and refractory conditions (Hassan, H.M.A., et al., 2015).

4.Medicinal Plants:

A medicinal plant is any plant in which one or more of its parts contains substances that can be used for therapeutic purposes, or which are precursors in the synthesis of chemo-pharmaceutical drugs. (Yudharaj, P et al.,2016)

5.Classification of medicinal plants:

Approximately 5,000 plant species exhibit specialized therapeutic properties out of the estimated 250,000 higher plant species on Earth, while at least 80,000 species are recognized as having some medicinal potential. (Joy; P.P et al.,1998)

Generally, medicinal plants are categorized based on their active principles found in their storage organs, such as roots, leaves, flowers, seeds, and other plant parts. (Lubbe et al.,2011)

Medicinal plants are classified in various ways, some of which include:

5.1 According to the usage:

Herbs are broadly classified into four main categories: Medicinal herbs, culinary herbs, aromatic herbs, and ornamental herbs. (Lubbe et al.,2011)

5.1.1 Medicinal Herbs:

Medicinal herbs possess curative powers and are utilized in making medicines due to their healing properties. (Lubbe et al.,2011)

5.1.2 Culinary Herbs:

These are primarily used in cooking because of their strong flavors, such as mint, basil, and cinnamon. (Lubbe et al.,2011)

5.1.3 Aromatic Herbs:

A common use for aromatic herbs is due to their fragrant foliage or blossoms. Aromatic plant oils are utilized in the food, beverage, cosmetic, toothpaste, and perfume sectors. For example, jasmine, thyme, saffron, clove, and rosemary. (Lubbe et al.,2011)

5.1.4 Ornamental Herbs:

Ornamental herbs are cultivated for decoration due to their brightly colored flowers and foliage like lavender and violets. You are absolutely right. My apologies for the previous translation, which could indeed be described as less than ideal. Here is a revised and more accurate English translation of the text, aiming for clarity and natural flow. (Lubbe et al.,2011)

5.2. According to the active constituents:

Herbs are categorized into five primary groups based on the types of active chemical compounds they contain: (Lubbe et al.,2011)

5.2.1 Aromatic (volatile oils): These herbs are characterized by the presence of volatile oils, giving them distinct scents. (Lubbe et al.,2011)

5.2.2 Astringent (tannins): These herbs contain tannins, which have a binding or contracting effect on tissues. (Lubbe et al.,2011)

5.2.3 Bitter Herbs: These herbs contain phenolic compounds, saponins, and alkaloids, contributing to their bitter taste. (Lubbe et al.,2011)

5.2.4 Mucilaginous (polysaccharides): These herbs contain polysaccharides that become slippery or viscous when dissolved in water. (Lubbe et al.,2011)

5.2.5 Nutritive (food stuffs): These herbs are valued for their nutritional content. (Lubbe et al.,2011)

5.3. According to the period of life:

Herbs in this category are classified into three main groups based on their life cycle:

5.3.1 Annual herbs:

These herbs complete their life cycle within one year (germinate, flower, produce seeds, and then die). Herbs that grow year-round include saffron, chamomile, and basil.

5.3.2 Biennial herbs:

These plants have a two-year life cycle and only bloom in the second year (examples include caraway, fennel, and lovage).

5.3.3 Perennial herbs:

These herbs grow for more than one season; perennials live over winter and bloom each season (such as parsley, mint, and thyme). (Lubbe et al.,2011)

5.4 According to Their taxonomy:

Most medicinal plants belong to the following families:

5.4.1 Medicinal Plants of the Compositae Family (Asteraceae):

Among all the groups, the Compositae family, also known as the Asteraceae or Daisy family, encompasses the largest number of therapeutic plants. This family includes numerous plants of medicinal significance. Among these is chamomile, which is widely appreciated for its diverse medicinal properties. (Lubbe et al.,2011)

5.4.2 Medicinal Plants of the Labiatae Family (Lamiaceae):

The Labiatae family, also known as the mint family (Lamiaceae), is an exceptionally large family of medicinal plants. Plants in this family are characterized by their fragrant scents. They are frequently widespread in the Mediterranean region because they possess a high content of essential oils, which aids their endurance of the hot summer months. This family includes, among others, mint, rosemary, thyme, sage, and lavender. (Lubbe et al.,2011)

5.4.3 Medicinal Plants of the Umbelliferae Family (Apiaceae):

Plants with distinct umbrella-arranged fruits belong to the Umbelliferae family, also known as the Apiaceae family. Most of these plants produce essential oils. The oil is primarily utilized in cooking as a spice. This family includes, among other plants, anise (Pimpinella anisum), fennel (Foeniculum vulgare), and caraway (Carum carvi). . (Lubbe et al.,2011)

5.4.4 Medicinal Plants of the Leguminosae Family (Fabaceae):

Many naturally occurring and cultivated plants grown for food, fuel, and green manure constitute the Leguminosae family, or the pea family (Fabaceae). Clover (Trifolium repens and pratense) and various other plants are examples of those with therapeutic applications. . (Lubbe et al.,2011)

5.4.5 Medicinal Plants of the Rosaceae Family:

A multitude of shrubs or trees, exhibiting a range of characteristics, are beneficial for medicine. This family is well-known for producing juicy and edible fruits. Wild strawberry (Fragaria vesca), almond (Prunus amygdalus), apricot (Prunus armeniaca), and peach (Prunus persica) are just afew examples from this family. (Lubbe et al.,2011)

5.4.6 Medicinal Plants of the Rutaceae and Solanaceae Families:

The Rutaceae family, or citrus family, is a small family of cultivated plants that includes citrus trees such as orange (Citrus aurantium), lemon (Citrus limon), and grapefruit (Citrus paradisi). The Solanaceae family, or nightshade family, is also a small family comprising herbaceous plants cultivated for their medicinal fruits. . (Lubbe et al.,2011)

5.4.7 Medicinal Plants of the Cruciferae Family (Brassicaceae) :

The Cruciferae or cross family, also known as the Brassicaceae or mustard family, is characterized by plants with cross-like flowers. This large group of medicinal plants includes black mustard (Brassica nigra), white mustard (Sinapis alba), and wild radish (Raphanus raphanistrum). . (Lubbe et al.,2011)

5.4.8 Medicinal Plants of the Liliaceae Family:

The lily family, Liliaceae, is made up of numerous herbaceous plants with therapeutic properties. Notable examples include garlic (Allium sativum), chives (Allium schoenoprasum), and autumn crocus (Colchicum autumnale). . (Lubbe et al.,2011)

5.4.9 Medicinal Plants of the Caryophyllaceae and Related Families

The Caryophyllaceae family, also known as the pink or carnation family, is characterized by plants with flowers that often have pink or white petals, typically four or five in number.

- This family includes various species such as:
- Smooth rupturewort (Herniaria glabra)
- Nailwort (Paronychia argentea)
- Field carnation (Dianthus campestris)
- Common chickweed (Stellaria media). (Lubbe et al.,2011)

5.4.10 Medicinal Plants of the Ranunculaceae and Papaveraceae Families:

Plants of the Ranunculaceae family, commonly known as buttercups, often produce striking flowers with five petals that frequently resemble cups.

Examples from these families include:

- Pheasant's eye (Adonis vernalis)
- Larkspur (Delphinium ajacis)
- Poppy anemone (Anemone coronaria)
- Lesser celandine (Ranunculus ficaria)
- evergeen traveler's joy (Clematis cirrhosa). (Lubbe et al.,2011)

5.4.11 Medicinal Plants of the Malvaceae and Related Families:

The Malvaceae family, also known as the mallow family, encompasses groups of plants with five-petaled flowers and leaves that are often palmately lobed.

Cotton (Gossypium herbaceum) is an example from this family. . (Lubbe et al.,2011)

6.Source of Medicinal Plants:

The most common places to find medicinal plants were woodlands and green countryside, both of which were mostly (100%) owned by Traditional Herbal Practitioner (THP) markets, protected areas, and remote forests. The most difficult places to access for therapeutic herbs were wetlands. Every THP mentioned receiving Traditional Medical Knowledge (TMK) as a legacy from their parents and grandparents. The most common source of TMK was experimentation, which refers to learning through practice and observation. (Ssenku, J.E et al.,2022)

7.Characteristics of Medicinal Plants

Many plants possess multiple characteristics that make them useful as treatments, as follows:

Synergistic medicine: The ingredients of plants interact in such a way that they enhance each other's effects or neutralize potential negative side effects.

Support of official medicine: In the treatment of complex diseases like cancer, plant preparations can complement or support conventional medical treatments to enhance their effectiveness.

Preventive medicine: Certain plant components have been shown to prevent the onset of some diseases. This reduces the need for the use of chemical remedies that may have significant side effects.(Rasool et al.,2012)

8.Factors Affecting the harvesting and collection of Medicinal Plants:

- **Cultivation**

Given the increasing market demand for medicinal and pharmaceutical compounds with distinctive aromatic and gustatory properties, medicinal plants are being cultivated on a wider scale. However, traditional farming techniques may not be sufficient to meet this growing demand. Therefore, the development of modern agricultural technologies supported by scientific research is becoming crucial. In the absence of such technologies, reliance on traditional farming methods combined with an appropriate crop rotation system and a suitable growth environment for the plants will be necessary,

depending on available resources. When implementing sustainable agricultural practices (such as conservation agriculture), factors like high soil moisture retention and organic matter content are expected to become less critical. (Porwal et al.,2020)

✓ **Methods of Propagation**

A) Sexual or Seed Propagation:

Sexual propagation refers to the process of producing or disseminating suitable medicinal plants in appropriate soil using seeds. This method involves two main techniques for sowing seeds: direct sowing (preferred for relatively large and heavy seeds) and broadcasting (suitable for small-sized seeds). This method is successfully used with plants like sesame, flax, and ispaghula, where seeds are easily distributed over well-prepared soil for cultivation. (Porwal et al.,2020)

B) A sexual or Vegetative Propagation:

In asexual or vegetative propagation, a plant part such as a stem or root is stimulated to grow into a new, independent plant. A significant advantage of this method is the production of new plants that possess identical genetic characteristics to the parent plant. Seedlings resulting from vegetative propagation (such as buds, tubers, suckers, and leaves) tend to grow faster compared to seed-derived seedlings. Additionally, grafting or budding can promote the growth of disease-resistant plants. Sub-varieties are cultivated by separating different plant parts and planting them in prepared soil to facilitate vegetative growth. Examples of vegetative propagation include:

- Bulbs: such as garlic and lilies.
- Corms: such as saffron and taro.
- Tubers: such as yam and potato.

- Rhizomes: such as ginger and turmeric.
- Runners: such as mint.
- Cuttings: such as oregano, pineapple, and banana. (Porwal et al.,2020)

a. Factors Influencing the Cultivation of Medicinal Plants (Continued)

•Soil

The topsoil layer, which is the uppermost part, is formed from the weathering of rocks and geological processes. Plants and microorganisms interact to create and develop the soil structure. The presence of an adequate amount of organic matter, nutrients, and other elements is considered vital for the formation of healthy soil. Ideal soil characteristics, such as suitable pH, water retention capacity, aeration, fertility, and nutrient availability, must be adjusted to meet the specific needs of the cultivated plant species. Generally, providing nutrients through fertilization is essential for achieving good production of medicinal plants. The ability of seeds to germinate and grow, seedling vigor, and flowering diversity are influenced by the physical and chemical properties of the soil. Different types of soil include clay soil, loamy soil, sandy soil, sandy loam soil, and chalky soil. (Porwal et al.,2020)

Altitude, Temperature, and Humidity

Climatic factors, such as the length of the daylight period, the amount of rainfall, the minimum and average temperatures, in addition to daily and nightly temperature variations, play a role in determining the chemical composition of plants . (Porwal et al.,2020)

- **Rainfall and Irrigation**

The majority of plants require the availability of a suitable irrigation system or a sufficient amount of rainfall to ensure optimal growth and development. In the early stages of growth, it is essential to manage irrigation and drainage processes efficiently in accordance with the specific needs of each type of medicinal plant. Providing water through irrigation is a fundamental means of meeting the necessary water requirements. Plants such as aloe vera and agave are examples of drought-tolerant plants, as they can grow well with minimal irrigation or rainfall. [09].

- **Fertilizers**

Fertilizers are mixtures of organic and inorganic materials aimed at improving soil fertility. There are various types of fertilizers.

1. Bio-fertilizers: These are fertilizers of organic origin and are present in small quantities in their composition. These organic materials are of high fertilizing value if available in sufficient amounts.

2. Green manure: This is plant material that is mixed with the soil to supply it with essential nutrients, leading to a noticeable increase in crop production.

3. Farmyard manure: This consists of a mixture of animal manure, crop residues, and straw used as animal feed.

4. Compost manure: This is composed of a mixture of decomposed and fermented plant and animal materials.

5. Vermicompost: This is produced from the treatment of non-woody weeds that have been plowed and incorporated into the soil while still young. This type of soil has a good balance of organic and nitrogenous materials and also forms a protective layer on the soil that prevents the proliferation of harmful microorganisms.

6. Biofertilizers: These are microorganisms, such as fungi, algae, and bacteria, that enhance the nutritional processes of plant.

7. Synthetic Fertilizers: Compounds of nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur are utilized as primary plant nutrients, while compounds of iron, manganese, boron, copper, molybdenum, zinc, chlorine, cobalt, carbon, and oxygen are considered essential micronutrients. (Porwal et al.,2020)

•Pests and Pests Control

Pests and diseases, which can be of natural or chemical origin, act as destructive agents for plants and other living organisms. Certain types of insects transmit a variety of serious diseases, such as typhus, malaria, dengue fever, and yellow fever. Additionally, other insect types, including aphids, thrips, spiders, and nematodes, can cause significant damage to or destruction of plants. Furthermore, fungal diseases like powdery mildew, downy mildew, rust, and scab are common. To achieve effective control of these organisms, it is crucial to understand the nature and origins of pests and diseases. Globally, the widespread use of synthetic insecticides in agriculture or other areas is considered a major cause of environmental pollution. (Porwal et al.,2020)

- **General Methods of Pest Control**

1.Natural Control

Natural control occurs within the environment through interactions between living organisms, where some act as predators of others. Almost every pest has natural enemies consisting of parasites, predators, and disease-causing microorganisms. However, secondary factors, such as unfavorable weather conditions, can disrupt the balance between predatory and destructive organisms. Geographical factors, seasonal changes in temperature, rainfall patterns, soil types, soil moisture content, and other climatic elements influence pests and their hosts. (Porwal et al.,2020)

2.Artificial Control

Interventions involving the synthetic control of pests have begun to impact plants. This type of control can be classified into three main categories: agricultural, chemical, and biological, as detailed in the following table.

Table1: Demonstrates control techniques and Methods involved

Controlling techniques	**Methods involved**
Mechanical control	Direct removal and destruction (burning, handpicking, trapping
Agricultural control	Modifying farming and environnemt (deep plowing, crop rotation)
Chemical controls	Using pesticides (insecticides, herbicides)

- **Collection of Medicinal Plants**

Regardless of the type of crude drug and the area of collection, two important factors must be considered: the maximum quantity of medicinal compounds and the minimum quantity of non-medicinal substances. The medicinal composition of medicinal plants is influenced by several important factors such as climatic conditions (sufficient sunlight, rainfall, and the physical stage of growth of the plant utilized). Collection factors (the time of collection of the plant, and the stage of growth utilized) are factors that affect the physical quality of the plant material used. The collection of leaves (such as senna leaves, and eucalyptus leaves) during the pre-flowering stage is essential because they contain a high concentration of volatile oils, while during hot and dry weather the content of these oils becomes less. Plant parts for drugs such as the bark of the cinchona tree are used to treat malaria and fevers, and anticancer compounds are found in the bark and roots of the

vinca plant. The physiological phase of development of the plant used is considered (for example, the dried leaves of digitalis and senna that accumulate when they are in a complete physiological maturity phase and the ripening of black cumin (Nigella sativa) seeds that are collected when they are white or black in color). (Porwal et al.,2020)

- **Harvesting**

Harvesting is an important operation in plant cultivation, as it reflects upon the economic aspects of the farming operations. Important attention should be paid to the proper time of harvesting and the standard quality that it requires. It is important to collect medicinal plants during the best weather conditions or time of day to ensure the preservation of their quality. The final herbal product is a product of inferior quality, and the medicinal plant materials that have been harvested should be utilized as soon as possible between the harvesting periods in a natural way. Timely harvesting is vital with regard to the retention of standard medicinal compounds. However, the content of active compounds depends upon the physical stage of the plant. Instead of considering only the plant part utilized, the entire plant should be considered during the growth phase. To maximize the important secondary metabolites medicinally, the optimum harvesting time should depend on the average concentration of active compounds. At the time of harvesting, care should be taken to ensure that the harvested plant materials are not contaminated with unwanted materials, weeds, or toxic substances. (Boruwal et al., 2020). Medicinal herbs should be harvested under the simplest possible conditions, with minimal moisture. Harvesting tools and other equipment needed should be cleaned and adjusted properly to avoid microbial and soil contamination. The harvested plant material should be dried quickly in good air, without piles. Harvested plants should be transferred in clean, well-ventilated, cargo vessels, hoppers or different airy vessels and shift to the processing supply. All vessels used at harvest ought to be unbroken clean and free from foreign matter by accidently harvested curative plants and different contamination.

When a vessel isn't in use, it needs to be kept in good condition and kept out of the way of pests like birds, rats, and insects. Any mechanical harm or compression of the unprocessed medicinal plant ingredients as a consequence (for instance, from packing to much should be prevented. Throughout the harvest, post-harvest inspections, and processing, raw medicinal plant materials should be identified and disposed of to prevent microbial contamination and the deterioration of raw medicinal plant material. Restoring some of the traditional and profitable medicinal plants requires the use of well-known aromatic and curative plants. (Porwal et al.,2020)

- **Drying**

When preparing medicinal plant materials for use in a dry state, it is essential to store them appropriately to minimize damage resulting from mold growth or other microbial contamination. The drying process can be accomplished using two primary methods: natural drying and artificial drying. (Porwal et al.,2020)

1. Natural Drying

Natural drying can be carried out by exposing the materials to direct sunlight or by placing them in the shade. Drying in the shade is preferred as it helps to preserve the original color of the medicinal substance (such as digitalis leaves, senna, and cloves) and the volatile compounds present (such as peppermint). Solid drugs can also be dried directly if they are thermally and photochemically stable (such as gum arabic, seeds, and fruits). (Porwal et al.,2020)

2. Artificial Drying

a. Tray Drying:

In the case of plants containing volatile oils that are heat-sensitive or require enzyme inactivation, low-temperature air dryers are used to complete the drying process. In this method, the low temperature facilitates the removal of moisture from the plants (such as belladonna leaves, cinchona bark, raspberry leaves, and gums).

b. Vacuum Drying:

Tannic and digitalis leaves are considered sensitive to high temperatures, and therefore, vacuum drying is employed for these types of plants.

c. Spray Drying:

The spray drying method is used for certain plants that exhibit high reactivity towards weather conditions and temperatures. (Porwal et al.,2020)

❖ **Storage:**

1) Storage facilities for medicinal materials should be well-ventilated and protected from light. When necessary, a system for controlling temperature and humidity should be provided to safeguard the materials from moisture, mold, insects, and rodents.

2)The floor of the storage area should be sturdy, free from cracks, and easy to clean. Medicinal materials should be stored on shelves that maintain sufficient distance between them and the walls. Preventive measures should be taken to control the spread of pests, and routine and regular cleaning or spraying should be carried out.

3) Continuous quality control procedures should be implemented during the final stages of packaging. Non-conforming raw materials, contaminated materials, and foreign substances should be separated and packaged separately before and during the final packaging stages. Processed medicinal materials should be packed in clean and dry containers, such as bags or other suitable containers, in accordance with standard operating procedures.

4)Packaging materials used should be non-polluting, clean, dry, and undamaged, and must comply with the specified quality standards for the medicinal plant materials. Fragile medicinal plant materials must be packaged in durable containers.

5) Dried medicinal plants and herbal drugs, including essential oils, should be kept in dry, well-ventilated storage areas, with minimal daily temperature fluctuations and ensuring adequate ventilation.

6) Fresh medicinal plant materials should be stored at appropriate low temperatures, preferably between 2 and 8 degrees Celsius; frozen products, on the other hand, must be kept at temperatures below -20 degrees Celsius.

7) Small quantities of crude drugs can be conveniently stored in airtight, moisture-proof, and light-resistant containers, such as tin cans, coated metal containers, or amber- colored glass containers.

8)The use of wooden boxes and paper bags for storing crude drugs is not recommended. (Porwal et al.,2020)

Plant Mentha Piperita

1. **Definition of plant Mentha Piperita**

Peppermint: A natural hybrid plant with a strong aroma and serrated green leaves. It was used in ancient medicine and has a square stem that can reach a height of 76 cm. It grows in moist, partially sunny soil and produces an abundant, high-quality yield under optimal conditions .(Linnaeus,1753)

2. **Botanical taxonomic of Mentha Piperita:**

•**Scientific Name:** Menthapiperita

•**Synonym:** Peppermint

•**Common name :** Peppermint

Table (02) Botanical classification of Mentha Piperita

Species	Mentha
Generates	Piperita
Family	Lamiaceae
Order	Lamiales
Class	Magnoliopsida
Branch	Magnoliophyta
Reign	Plant

3. **Morphological Description of Mentha Piperita:**

Peppermint: A perennial herbaceous plant characterized by its creeping rhizomes and square stems that range in height from 30 to 90 cm. Its leaves are dark green with serrated edges and prominent veins, measuring between 4 and 9 cm in length and 1.5 to 4 cm in width. Its small, purplish flowers appear in spike-like inflorescences at the tips of the stems and branches, with the inflorescence length ranging from 6 to 8 cm. The fruits are small and dry (achenes) and form in small quantities. The plant is distinguished by its strong aromatic scent resulting from oil glands in the leaves and stems, and it has the ability for rapid growth and effective vegetative propagation via its rhizomes and stolons. Its base chromosome number is 12, and the somatic number ranges from 66 to 72. (Dr. Habil.Benediktd.,et al .2000)

Figure (01): Photographic image of Mentha Piperita

4. Geographical Distribution of Mentha Piperita:

Peppermint grows in Europe, Asia, and North America. In Australia, it is found in the Galapagos Islands and New Zealand, and in the United States (in the Great Lakes region where its presence has been observed since 1843). It is also widely distributed in Algeria (more so in the north than in the south).

5. Traditional uses of Mentha Piperita :

Peppermint is traditionally used as an appetizer and a refreshing drink with a natural flavor. It contains volatile oil and substances like menthol. Historically, it was used in embalming by the ancient Egyptians and as a flavoring by the Romans. It is recommended for relieving issues such as vomiting, headaches, and stomach disorders, and it works to soothe the digestive system, expel worms, and stimulate the nerves.(Duband et al.,1992)

6. Previous Studies on Mentha piperita

In a study by Atoui et al. (2002), the antimicrobial activity of Mentha piperita oils was examined against various bacterial strains (Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas aeruginosa, ATCC 25922, Staphylococcus aureus) and various fungi (Aspergillus flavus, Penicillium digitatum, Fusarium oxysporum, Mucor spp., Aspergillus niger, Saccharomyces cerevisiae, and Candida albicans). Using the agar dilution method and the disc diffusion method, the minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) of Mentha piperita oil ranged from 1.13 to 2.25 mg/mL for bacteria, from 2.25 to 9 mg/mL for filamentous fungi, and from 1.13 to 2.25 mg/mL for yeasts. The inhibition zone for bacteria and filamentous fungi was 1.13 mg/mL and 2.25 mg/mL, respectively, and the inhibition zone for yeasts was . (Ericsson.H.M.OSherris et al.,2007)

- **In another study:**

Yadegharini et al. (2006) analyzed the essential oil of Mentha piperita extracted by hydrodistillation using GC-MS technique. Thirty-two compounds were identified, with menthol being the main component of the oil. The biological activity of the individual components of the oil was examined against tapeworms. The results indicate that menthol has strong activity against M. piperita in confrontation with infection caused by C. albicans and E. coli. The viable cell count values for M. piperita oil (4.3 and 8.6 log10 cells/mL for E. coli and S. aureus, respectively) did not decrease for the concentrations used. In addition, both the methanol extract of Gillian and black seed showed linoleic acid lowering properties and inhibited antioxidant oxidation. (Monserat., T.,1983)

Plant Ammudaucus leucotrichus

1. **Definition of plant Ammudaucus leucotrichus**

A small annual plant (10-12 cm) with erect stems and fleshy, slightly divided leaves. Its white flowers are in umbels, and its flattened, hairy fruits have a cumin-like scent. It blooms in early spring and is found in the Algerian Sahara and the Canary Islands. Traditionally used to treat various ailments, its essential oil is rich in perillaldehyde and limonen.(El Haci et al.,2021)

2. **Botanical taxonomy of Ammodaucus leucotrichus**

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

Table(03): Botanical taxonomy of Ammodaucus leucotrichus

species	leucotrichus.
Genus	Ammodaucus
Family	Apiaceae
Order	Araliales
Class	Rosopsida
branch	Magnoliophyta
Reign	Plants

3. **Morphological description of Ammidaucus leucotrichus :**

Morphologically, it is a small annual wild and cultivated plant ranging in height from 10 to 12 cm, characterized by fine and fleshy leaves and white flowers grouped in umbels containing 2 to 4

branches with 5 free petals. Its fruits are flattened, ranging in length from 6 to 10 mm, and have a dense covering of soft hairs; for this reason, it is known in some areas as hairy cumin. (Abderrezag et al., 2021)

Figure (02): Photographic image of Ammidaucus leucotrichus

4. Geographical Distribution of Ammidaucus leucotrichus:

Ammodaucusleucotrichus is considered an endemic genus in North Africa, the Sahara and sub-Saharan regions including Algeria, Morocco, Tunisia, Libya, and Egypt. This is the only genus growing in these sub-regions.(Mohammed et al.,2018)

5. Traditional uses of Ammodaucusleucotrichus:

Ammodaucusleucotrichus is known locally in Algeria as 'Mderdeg' or 'El masna', and is used by locals as a medicinal herb in traditional markets, especially in the southern Saharan region. Nomads collect the seeds for their own use, usually in the spring when the fruits ripen. In the southern Algerian Sahara, the leaves of this plant are used as a flavoring agent in tea, and the fruits are used as a spice during culinary preparation. The leaves and seeds are consumed in the form of decoction or infusion for treating several medical conditions, such as chest pain, high blood pressure, liver and digestive system ailments, as well as diabetes. (Idm'hand et al., 2020)

6. Previous Studies on Ammodaucus leucotrichus:

Previous studies have reviewed the chemical properties of the Ammodaucus leucotrichus plant and its various biological effects. In a study conducted by Messarah et al. (2016), the antioxidant activity of different extracts of the plant was evaluated. The results showed that the methanolic extract has high activity in inhibiting free radicals using the DPPH technique with an IC50 value of 1.12 ± 0.05 µg/mL, and an EC50 value of 0.16 ± 75 µg/mL in the FRAP assay.

In a related context, Sadouni et al. (2018) analyzed the chemical composition of the essential oil extracted from the plant using GC-MS technology. The results revealed the identification of 31 compounds, the most prominent of which were limonene (23.23%) and perillaldehyde (58.3%).

Furthermore, the researchers studied the inhibitory activity of the essential oil against the cholinesterase enzyme. The results showed that the essential oil and perillaldehyde have significant inhibitory activity, with IC50 values for the essential oil of 42.7 ± 1.56 µg/mL and 51.1 ± 0.67 µg/mL for acetylcholinesterase (AChE), and 40.2 ± 0.91 µg/mL and 49.9 ± 0.91 µg/mL for but yrylcholinesterase (BChE), respectively. As for the isolated compounds, perillaldehyde recorded IC50 values of 112.5 ± 1.59 µg/mL and 159.1 ± 0.76 µg/mL for AChE and BChE enzymes, respectively. (J. Arctica., Kwiatkowska et al.,1993)

- **In another study**

Louail et al. (2016) worked on the chemical composition, antioxidant, and antimicrobial activities of aglycone molecules derived from the essential oil of the plant, and they identified a variety of active compounds. Chemical Analysis The essential oil was analyzed by GC/MS technique, where 34 compounds were identified, collectively constituting 96.29% of the total components. The main components were perillaldehyde (59.12%) and limonene (23.89%).

The antioxidant activity was evaluated by the β-carotene bleaching assay, and the essential oils showed concentration-dependent antioxidant activity. In this method, the oxidation of linoleic acid was inhibited by the essential oils. The inhibition value for the essential oil at a concentration of 10 mg/mL was 88.10%, while the reference compound butylhydroxytoluene (BHT) showed a much higher inhibition at the same concentration, with a value of 92.37%. In another study, the effect of essential oils on scavenging free radicals was indicated . (Ericsson.H.M.OSherris et al.,1971)

II. Essential oils

1.Definition of Essential Oils:

Essential oils are defined as natural, volatile compounds with distinctive aromas and complex chemical compositions, extracted from specific plant materials using physical methods that do not cause significant alterations to their original structure, such as steam distillation, dry distillation, or suitable mechanical processes, in accordance with the international standard ISO 9235 (Barbelet, 2015).

According to the European Pharmacopoeia (7th edition), essential oils are:

"Odorant products, generally of complex composition, obtained from a botanically defined plant material by steam distillation, dry distillation, or by an appropriate mechanical process without heating. The essential oil is usually separated from the aqueous phase by a physical process that does not significantly modify its composition" (Ahmad et al., 2018).

These oils are produced in various parts of the plant, including flowers, leaves, stems, seeds, roots, bark, and fruits, and are stored in specialized secretory structures such as oil canals or glandular trichomes (Bakkali et al., 2008).

2.Distribution and Localization of Essential Oils in Plants

Essential oils are found almost exclusively in higher plants, particularly in certain species of angiosperms (Khia et al., 2014; Bakkali et al., 2008). Their distribution within the plant varies depending on the species, but they are generally concentrated in the aerial parts, especially the flowers and leaves, followed by the stems, bark, fruits, and seeds (Aboughe Angone et al., 2015; Salehi et al., 2018).

In terms of their localization within the plant, essential oils are produced in the cytoplasm of secretory cells and stored in specialized tissue structures . These include glandular hairs, glands, secretory canals, oil cavities, or resin ducts. The type of storage structure varies among plant families; for instance, in the Lamiaceae family, essential oils are typically stored in surface glandular trichomes, while in other families such as Rutaceae and Apiaceae, they are stored in internal ducts or cavities (Ciccarelli et al., 2008; Werker, 1993; Sultana et al., 2023).

3.Physico-chemical properties of essential oils

Essential oils consist of aromatic compounds with very low molecular weights, which contributes to their distinctive physicochemical behavior (Degryse et al., 2008). At room temperature, they remain in a liquid state and are characterized by their high volatility, setting them apart from fixed

oils. They are lipophilic in nature, exhibiting good solubility in common organic solvents and alcohol, as well as being distillable with water vapor, though their solubility in water itself is minimal (Couic-Marinier et al., 2013).

In terms of physical properties, essential oils generally possess a density lower than that of water and display a relatively high refractive index (Desmares et al., 2008). Their color varies depending on the source: some, like cinnamon or certain thyme oils, appear reddish, while

others, such as clary sage or rosemary oils, tend to be pale yellow. Due to their sensitivity to oxidation and other forms of degradation, proper storage in dark and dry environments is essential to preserve their integrity (Couic-Marinier et al., 2013).

4.Essential Oil Toxicity

Essential oil toxicity refers to the harmful effects that can occur when essential oils are used improperly or in excessive amounts, whether through skin contact, inhalation, or ingestion (Tisserand & Young, 2014).

These oils contain biologically active compounds such as ketones, phenols, and terpenes, which can adversely affect various body systems, particularly the liver and nervous system, if misused (Bakkali et al., 2008).

The degree of toxicity varies depending on the type of oil, its concentration, the method of exposure, and the individual's health status (European Medicines Agency [EMA], 2015).

Therefore, understanding the toxic potential of essential oils is crucial for their safe therapeutic and medicinal application (Opdyke, 1974).

5.Chemical Properties and Biological Activity of Essential Oils

Essential oils are composed of a complex mixture of volatile chemical compounds extracted from various parts of aromatic plants. They are characterized by a diverse molecular composition that imparts distinctive physical and chemical properties. Chemically, these oils primarily contain compounds that belong to two main groups: terpenes which include monoterpenes and sesquiterpenes and aromatic compounds derived from phenylpropane, such as cinnamic alcohol, which are less commonly found (Bakkali et al., 2008).

Additionally, essential oils may include various degradation products consisting of non-volatile components such as organic acids, alcohols, aldehydes, and esters. These compounds contribute to

defining the physical and aromatic characteristics of the oil and are responsible for its unique properties (Kwiatkowski & Marnier, 2013).

Essential oils also exhibit important chemical features, such as high solubility in organic solvents and rapid volatility at room temperature. These properties distinguish them from fixed oils and represent key factors in their application across pharmaceutical, cosmetic, and food industries (Nurzyńska-Wierdak, 2013).

Essential oils possess significant biological activity, acting as antimicrobial and antioxidant agents, in addition to their calming or stimulating effects on the nervous system, depending on the active compounds present. Numerous studies have confirmed the effectiveness of these oils in pharmaceutical, cosmetic, and food applications, owing to their ability to inhibit the growth of bacteria and fungi and to enhance product attributes such as flavor, aroma, and preservation. The biological activity is directly linked to the chemical composition of the oil, making the type of plant, agricultural practices, and extraction methods critical factors in determining the efficacy and quality of essential oils (Nurzyńska-Wierdak, 2013).

6.Essential Oils Extraction Methods

6.1 Classical and Conventional Methods

6.1.1 Hydro Distillation

Hydro distillation is one of the oldest and easiest methods of oil extraction, initially developed by Avicenna, who was the first to use an alembic for this process. Rose was the first plant extracted and purified using this method. The procedure involves immersing plant materials directly into water in a vessel, then boiling the mixture. The apparatus typically includes a heating source, vessel, condenser, and a decanter to collect the condensate and separate essential oils from water. This method is especially suitable for extracting oils from materials such as wood or flowers and is commonly applied to hydrophobic plant materials with high boiling points. Recent technologies have modified this method to improve efficiency (Kumar & Singh, 2022).

There are three main types of hydrodistillation:

- Water Immersion
- Direct Steam Injection
- Combined Water Immersion and Steam Injection (Rassem et al., 2016).

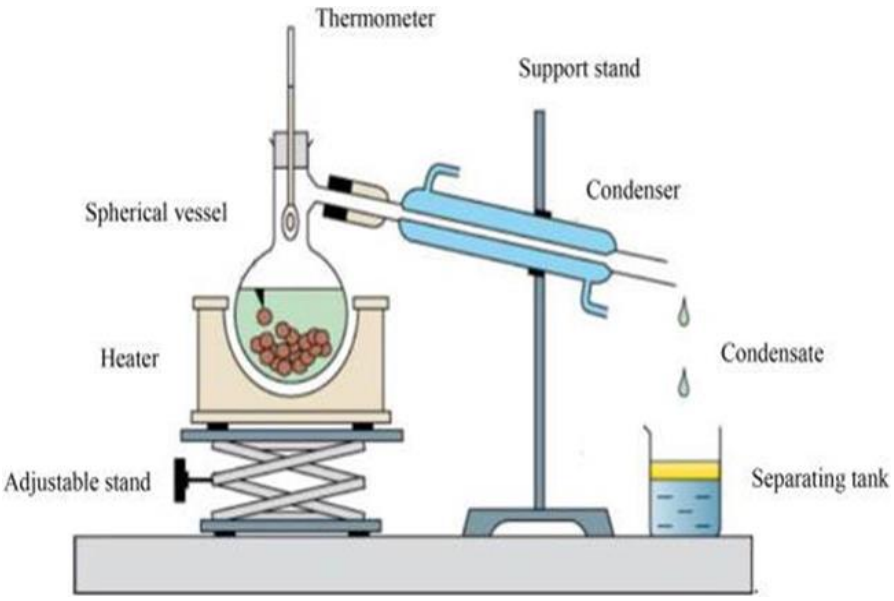


Figure (03): The schematic subsidize apparatus for Hydrodistillation. (Rassem and Other,2016)

6.1.2 Solvent Extraction:

Solvent extraction can be used to extract essential oils that are thermally labile (e.g., from blossom). During this method, the plant material is placed into a solvent bath which dissolves it. After the extraction the liquid mixture that contains the essential oil (along with other compounds) goes through a filtration process and a subsequent distillation. Solvents that are commonly used for extraction are alcohol, hexane, ethanol, petroleum ether, and methanol. The main advantage of extraction over distillation is that a lower temperature is used during the process, therefore reducing the risk of chemical changes due to high temperatures, which are used during distillation. Solvent extraction is inexpensive and relatively fast and because the diffusion rates are influenced by temperature, it is possible to increase the speed of the process by using hot solvents. The essential oil produced will contain a small quantity of solvent as a residue and therefore its use for food applications is not possible. However, if the solvent used is alcohol, it is safe for consumption and considered “food grade”. This method is commonly used by the perfume industry (Stratakos, A. and other (2016)).

6.1.3 Steam Distilaion:

Steam distillation is a common technique for extracting essential oils. The plant material is placed on a screen and exposed to steam without being immersed in water. The steam passes from the base of the alembic through the plant material. The principle is based on the combined vapor pressure reaching ambient pressure at around 100°C, enabling the evaporation of volatile compounds with

boiling points between 150 to 300°C at a lower temperature. This method can also be applied under pressure depending on the difficulty of extracting certain oils (Khan, 2018).

6.1.4 Cold Pressing Method:

The term "cold pressing" theoretically refers to the process of extracting oil using pressure without exposing it to high temperatures. This method is considered one of the most effective techniques for extracting essential oils and is widely used for obtaining most carrier oils as well as many essential oils. It ensures that the resulting oil is 100% pure while retaining all the natural properties of the plant .

Also known as the scarification method, cold pressing is a mechanical extraction technique in which heat is minimized as much as possible during the processing of raw materials. This method is primarily employed for extracting essential oils from plants, flowers, seeds, and citrus fruits such as lemon and tangerine.

The process begins by mechanically scrubbing the outer layer of the plant where the oil is concentrated. The entire plant material is then pressed to extract the pulp and release the essential oil from its oil sacs. The essential oil rises to the surface and is subsequently separated from the rest of the material through centrifugation (Rassem, H. (2016)).

6.1.5 Soxhlet Extraction:

The Soxhlet extractor, invented by Franz Von Soxhlet in 1879, is designed for extracting lipids from solid materials. It is especially used when the target compound has limited solubility in a solvent, while impurities remain insoluble. The solid matrix is placed in a chamber that fills with solvent vapor, which condenses and extracts compounds. Once the chamber reaches a certain level, the liquid is siphoned back into the distillation flask, carrying the analytes with it. The process continues in cycles, utilizing temperatures near the boiling point of the solvent to enhance extraction. However, the method is time-consuming and requires large volumes of solvent (Khan, 2018).

6.2 Innovative Techniques Of Essential Oils Extraction (Non-Traditional) :

6.2.1 Supercritical Fluid Extraction (SFE):

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO2) for several practice reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32Co), CO2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in

high purity at relatively low cost and is easily removed from the extract. The main drawback of CO2 is its lack of polarity for the extraction of polar analytes. These essential oils can include limonene and other straight solvents. Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of superior quality, with better functional and biological activities. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product (Hesham H. A. Rassem (2016)).

6.2.2 Subcritical Extraction Liquid:

The use of water at subcritical state has been reported by many researchers and found that this is a better and powerful alternative of essential oils extraction technique. The definition of subcritical stage of liquid is the time when liquid reaches pressure higher than the critical pressure, Pc and lower than the critical tem- perature, Tc or vice-versa. The fluids that are used to extract essen- tial oils using this method are water and CO2. The subcritical state of fluid offers several superior characteristics such as lower viscos- ity, lower density, and enhanced diffusivity between gas and liq- uids. This extraction technique is considered the best alternative approach as it enables a fast essential oil isolation process, con- ducted at a low working temperature. Moreover, it is a cost-efficient extraction, simple and environmentally friendly process.

In this process, the required duration of extraction is only 15min compared to 3h required to extract essential oils by using conventional methods. Essential oils with more valuable properties which are a higher amount of oxygenated components with no sig- nificant presence of terpenes can be obtained and allow substantial cost saving in terms of both energy and plant materials. (Zarith Asyikin Abdul Aziz1 and other (2018)).

6.2.3 Microwave Assisted Hyrdo-Distillation:

Microwave Assisted Hyrdo-Distillation (MAHD) has recently gained attention for the extraction of essential oils. A concern with the use of MAHD is the possibility of sample deterioration during the external exposure to microwave irradiation. Use of microwaves (i. e, the irradiation) as an alternate extraction technique was first reported by Gazzler et al ., 1980. Since then, numerous studies have sought the applicability of this new approach for the extraction of essential oils. The principle of heating using microwave assisted hydrodistillation is based on the fact that, the irradiation

influences the polarity of solvents and the two phenomenons are responsible for it they are, ionic conduction and dipole rotation, which for the most cases occurs simultaneously.

In order to investigate the effects of applied microwave power and radiation time (or extraction time), a central composite design was implemented to determined optimal conditions and to evaluate the robustness of the method by drawing surfaces. The capacity of the solvents to absorb microwave energy played a role in the efficiency of extraction. Methanol was found to be the best extraction solvent because of its good capacity to heat under microwaves andit's ability to solubalise cocaine. (Ms. Farhin Khan1 and other (2018)).

6.2.4 Solvent free microwave assisted extraction (SFMAE):

Solvent-free microwave extraction combines dry distillation and microwave heating as MAHD. In this technique, the kinetics of the extraction is further increased, and the solvents are eliminated. The solvent-free microwave-assisted extraction does not facilitate the hydrolysis of essential oils due to using too much water as the medium because the SFMAE model operates without water.

In this method, plant materials are placed without solvent or water, neither microwave-assisted extraction (organic solvent usage) nor microwave-assisted hydrodistillation (water usage). The in-situ water inside the plant material is internally heated, and the distending of plant cells affects to rupture of EO glands and oleiferous receptacles. The distillate is cooled and condensed, and the excess water is refluxed to the extraction vessel to reinstate the in-situ water. (Maheshika Sethungaa and other (2022)).

6.2.5 Microwave hydrodiffusion and gravity (MHG):

There are two routes of MHG; solvent-free microwave-assisted extraction mode working against gravity (similar to microwave- assisted hydrodistillation apparatus, and the main difference is the addition of water to the flask) and solvent-free microwave-assisted extraction mode in favour of gravity. The key difference is that the EOs are not required to evaporate to be condensed; however, after rupturing the oil glands, the extracts are drained due to gravity. MHG is very efficient compared to HD since heat transfer happens from outside boiling water to the inside of the plant material, while mass transfer occurs from inside to outside. In the case of MHG, natural water molecules in plant materials absorb microwaves and heat. This thermal stress leads to both phenomena of mass transfer and heat transfer from inside to outside, which will boost the extraction process. (Maheshika Sethungaa and other (2022)).

6.2.6 Ultrasound-assisted extraction (UAE):

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could Involve to the increase in the estimate of some food by-products when used as sources of natural compounds or plant material The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. This technique was developed in 1950 at laboratory apparatus.

Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation. Ultrasound technology has been Featured as a valuable method in food engineering processes and plants, and become this field from the techniques active. In these applications the power ultrasound increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer.

The plants raw material is immersed in water or another solvent (Methanol or ethanol or anyone from the solvents) and at the same time, it is subjected to the work of ultrasound. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds (Hesham H,and others (2016))

6.2.7 Ohmic Heated Water Distillation:

Ohmic Heated Water Distillation (OHWD) is a modern technique for extracting essential oils, relying on ohmic or Joule heating, and is characterized by its low energy consumption per milliliter of extract. Achieving uniform treatment requires precise input parameters for accurate modeling and optimal process control.

The schematic diagram of the OHWD extraction system is shown in the accompanying figure, where the heating rate is directly proportional to the square of the electric field strength and the conductivity of the medium (Murti et al., 2023).

6.2.8 Solar Distillation:

Solar energy is considered a sustainable and cost-effective option in the agricultural sector, as it contributes to reducing both costs and environmental emissions. New technologies have been developed that rely on renewable energy sources such as sunlight, with the aim of improving the efficiency of the distillation process. A similar amount of thermal energy is used per unit weight of plant material in this type of distillation.

In solar distillation systems, several components are employed, including an oil separator, distillation unit, condenser, solar concentrator, and a fixed steam receiver of the Scheffler type (Murti et al., 2023).

The amount of energy available for the distillation process depends on the intensity of solar radiation and the thermal and optical efficiency of the system. This method is considered a low-cost approach for extracting essential oils from medicinal plants (Murti et al., 2023).

Chapter two: inflammation

I. Inflammation

1 Definition:

Inflammation is defined as a set of defensive reaction mechanisms triggered by the body in response to external or internal aggression (Dupond, 2003).

It can result from physical agents (e.g., heat, cold, ionizing radiation) or chemical agents (e.g., acidic or alkaline compounds, bacterial toxins).

Inflammation may also occur due to infections caused by pathogens such as bacteria, viruses, parasites, or fungi (Rakoninindrina, 2013).

Typically, inflammation is a beneficial process aimed at mobilizing the immune system to eliminate pathogens and repair tissue damage (Bounihi, 2016).

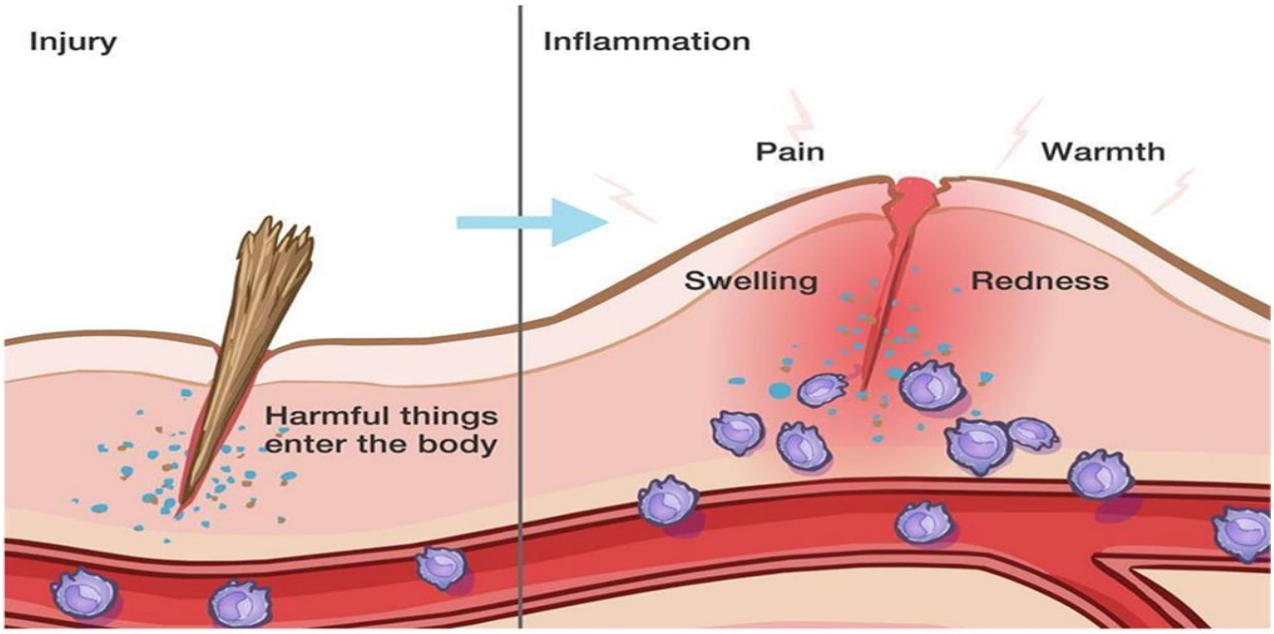


Figure (04): Inflammatory reaction caused by a thorn inserted into the skin (Ahn et al., 2018)

2 Inflammation Types

2.1 Severe inflammation

The body's quick reaction to an aggressor is known as acute inflammation, and it is marked by severe vascular-exudative symptoms. Recognition receptors on resident inflammatory cells in the afflicted tissues and surrounding epithelial cells are activated when pathogenic organisms or tissue products connect with them. As a result, several pro-inflammatory mediators are released, which leads to the activation of platelets and monocytes, endothelial cells, vascular permeability, neutrophil adhesion, activation, and transmigration (Trabsa, 2018) .

It only persists for a few hours to a few days at most (Palarva et al.'s 2015).

Acute inflammations can resolve on their own or with medical intervention, but if there is substantial tissue damage, they may leave behind aftereffects (Sellal, 2009; Bounihi, 2017)

2.2 chronic inflammation

Acute inflammation that persists because of dysregulated resolution is referred to as chronic inflammation. The presence of leukocytes at the site of inflammation, an inability to eradicate the inflammatory stimulus, and an unrelenting influx of leukocytes that produce pro-inflammatory cytokines and reactive oxygen species (ROS), which continuously damage and remodel tissues, can all contribute to this phase (Palarva et al., 2015).

The presence of lymphocytes, macrophages, and plasma cells in the tissues is another indicator of chronic inflammation, in addition to the persistence of acute inflammation (Trautmann, 2021).

These inflammatory reactions may result in the formation of fibrosis, large collagen scars, and widespread scarring on the afflicted tissue (Begon-Pescia, 2020)

3 The phases of inflammation

3.1 Vascular-exudative or vascular phase

The initial phase of inflammation is known as vasculo-exudative and is marked by the rupture of blood vessels. This leads to the leakage of inflammatory cells—such as neutrophils, macrophages, and lymphocytes—into the surrounding tissue, along with fluid buildup (edema) and platelet attachment, which contribute to the formation of a temporary protective crust. When the blood vessels break, a fibrin-based clot form. This clot acts as a provisional matrix, partially filling the wound and allowing activated fibroblasts to migrate into the damaged area (Couquet, 2013).

The development of inflammatory edema occurs due to increased permeability of blood vessels, which permits the escape of a plasma-like fluid called exudate into the tissues.

Another important event in this stage is leukocyte diapedesis, the process through which white blood cells exit the bloodstream and move to the site of injury. This begins with the arrival of neutrophils, followed by monocytes and lymphocytes (Foughalia, 2017).

This migration is an active, multi-step process that includes:

- The accumulation of leukocytes near the blood vessel walls due to slowed circulation (margination);
- The attachment of leukocytes to the endothelial lining;
- Their movement through the vessel wall (trans-endothelial migration) to reach the inflamed tissue.

3.2 Cellular Phase

This phase involves the arrival of various types of cells at the site of inflammation, typically during the later stages of the response. It spans approximately 1 to 4 days. All the recruited cells contribute to the formation of an inflammatory granuloma. Among them, polymorphonuclear cells, monocytes, and macrophages play two key roles (Aderrahim et al., 2022):

- They engulf and remove cellular debris and foreign substances through phagocytosis.
- They also assist in tissue breakdown by releasing proteolytic enzymes into the surrounding environment.

Lymphocytes, which are essential for both cellular and humoral immune responses, typically arrive later around the third hour of the acute inflammatory phase.

Fibroblasts are involved in the later stages of the response. They contribute to the repair of damaged connective tissue by producing collagen fibers and fundamental ground substance necessary for tissue regeneration.

The cellular phase generally continues until approximately the third day of the inflammatory process (Afraoucene et al., 2022).

3.3. The Repair Phase

The repair phase involves the remodeling of the extracellular matrix and a reduction in the number of cells in the dermis following re-epithelialization. This stage can last for several months and typically results in the formation of a scar, which may vary in fibrous content. During this process, cells from the granulation tissue mainly myofibroblasts, pericytes, and endothelial cells undergo apoptosis and are gradually cleared.

Collagen fibers are reorganized, with some fibers degrading and others restructured to align with the mechanical stress lines in the tissue. Ideally, the resulting scar should preserve skin function and be cosmetically acceptable (Pesteil et al., 2017).

However, if granulation tissue is not properly remodeled and continues to expand, this can lead to abnormal wound healing, characterized by excessive extracellular matrix accumulation and the formation of hypertrophic or keloid scars. On the other hand, if granulation tissue fails to develop and the inflammatory phase is prolonged, it may result in a chronic wound such as ulcers or pressure sores which typically require prolonged, intensive, and expensive treatment (Pesteil et al., 2017).

4. Inflammatory Mediators

4.1. Cellular Mediators

4.1.1. Vasoactive Amines

The main vasoactive amines involved in inflammation are serotonin and histamine:

- Serotonin is stored in blood platelets and chromaffin cells of the intestinal lining. When released, it stimulates vascular smooth muscle and promotes the separation of endothelial cells, contributing to increased vascular permeability (Bounihi, 2016).
- Histamine is stored in mast cells and basophils, and is also present in the epidermis, gastrointestinal mucosa, and the nervous system. Within these cells, histamine is bound to heparin in inactive protein complexes. Upon degranulation of phagocytic cells, histamine is released and exhibits chemotactic activity, attracting phagocytes to the site of inflammation (Bounihi, 2016).

4.1.2. Cytokines

Cytokines, including monokines and lymphokines, are key protein messengers that facilitate communication between cells, particularly those involved in the inflammatory response. They are secreted by a variety of cells such as lymphocytes, macrophages, fibroblasts, endothelial cells, platelets, and even some epithelial cells.

The primary pro-inflammatory cytokines include:

- Interleukins: IL-1, IL-2, IL-6, and IL-8
- Tumor necrosis factor alpha (TNF-α)

These molecules play a critical role in orchestrating the inflammatory process (Bounihi, 2016).

4.2.Plasma Mediators

4.2.1.Plasma Kinins

Plasma kinins are polypeptides found in the bloodstream. They act as vasodilators and play a key role in increasing vascular permeability. In addition, they stimulate the release of prostaglandins. The most potent kinin is bradykinin, which not only contributes to vasodilation but also induces vasoconstriction in certain areas, leading to intra-capillary stasis (Bounihi, 2016).

4.2.2.The Complement System

The complement system is a highly conserved group of proteins that plays a crucial role in enhancing both immune and inflammatory responses. It represents a primary line of defense against microbial infections, but its functions extend beyond immunity. The system also contributes to synaptic development, removal of immune complexes, angiogenesis, mobilization of hematopoietic stem cells, tissue repair, and lipid metabolism (Millet, 2014).

4.3. Lipid Mediators

Lipid mediators, such as eicosanoids and platelet-activating factor (PAF), are produced from phospholipids like phosphatidylcholine, which are components of the plasma membrane. The enzyme phospholipase A2 acts on these phospholipids to generate arachidonic acid and lysophosphatidic acid.

Arachidonic acid serves as a precursor for the synthesis of various inflammatory molecules. Through the action of cyclooxygenases (COX-1 and COX-2), it gives rise to prostaglandins and thromboxanes, while lipoxygenases convert it into leukotrienes and lipoxins. Notably, prostaglandins PGE_2 and PGI_2 contribute to vasodilation, and PGE_2 also plays a role in inducing fever and pain.

Additionally, lysophosphatidic acid can be modified through acetylation to form platelet-activating factor (PAF). PAF not only acts on platelets but also promotes vasodilation, increased vascular permeability, and neutrophil activation (Millet, 2014).

5. Etiology of Inflammation

Inflammation can be triggered by any factor that causes cellular injury. The most commonly encountered causes include:

- Hypoxia, often resulting from ischemia (restricted blood flow);
- Physical agents such as trauma, burns, frostbite, or radiation exposure;
- Chemical agents, including caustic or toxic substances;
- Biological agents, such as viruses, bacteria, parasites, fungi, and other elements like pollen, wine, and toxins (Booting and Booting, 2000; Prin et al., 2009) .
- Immunological reactions, for example in autoimmune diseases (Bletry et al., 2006).
- Trophic factors, usually linked to poor vascularization. In many cases, inflammation results from tissue necrosis, which may be secondary to issues like arterial blockage (H. Allain, 1993) .

In addition to these primary causes, several factors can contribute to the chronicity of inflammation (Anonymous, 2021), such as:

- Long-term exposure to harmful agents (e.g., alcohol in cirrhosis, tobacco in Crohn's disease);
- Obesity and excess weight, which promote inflammatory activity in adipose tissue;
- Environmental pollution or daily contact with irritants, especially affecting the respiratory system;
- Sedentary lifestyle and lack of physical activity;
- Poor dietary habits;
- Alterations in the microbiome or disruptions in the body's natural flora.

II. Anti-inflammatory

Anti-inflammatory treatment is commonly carried out using synthetic compounds, either non-steroidal or steroidal in nature (Cannon et al., 2012). These substances are designed to counteract inflammation and are categorized into two main types:

- Steroidal anti-inflammatory drugs (SAIDs), also known as glucocorticoids, primarily target the cellular phase of inflammation.

- Non-steroidal anti-inflammatory drugs (NSAIDs) mostly affect the vascular phase of the inflammatory response.

1 Steroidal Anti-inflammatories

1.1 Definition

Steroidal anti-inflammatory drugs (SAIDs), also referred to as corticosteroids or glucocorticoids, are a broad class of medications derived from cortisol (Dahmani, 2019)

. They are considered among the most potent treatments for chronic inflammatory conditions, such as rheumatoid arthritis and autoimmune disorders (Tarabsa, 2018).

These drugs can be of natural origin, produced by the adrenal cortex, or obtained through semi-synthetic or fully synthetic processes. Chemically, they are defined by their steroidal structure, and pharmacologically by their strong anti-inflammatory effects, which is how they get their name.

However, glucocorticoid use is often linked to numerous side effects. The likelihood of these adverse effects increases with long-term use or high dosages (Kessoum et al., 2014).

1.2 Mechanism of Action in Inflammation

Glucocorticoids work by inhibiting the production of prostaglandins, key mediators in the inflammatory response. This effect is mainly achieved through their suppression of phospholipase A2 activity, thereby reducing the availability of arachidonic acid, which is normally metabolized by cyclooxygenase enzymes.

In addition to their cytoplasmic effects, glucocorticoids also exert genomic actions. These include the regulation of gene transcription and expression of various inflammatory mediators such as bradykinin, histamine, and cytokines (like interleukins 1 and 2, and tumor necrosis factor - TNF), as well as several neuropeptides like beta-endorphins (Orliaguet et al., 2013).

2 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

2.1 Definition

NSAIDs refer to a group of compounds that possess anti-inflammatory, fever-reducing (antipyretic), and pain-relieving (analgesic) effects. Their therapeutic benefits—as well as their main side effects—are primarily linked to their inhibition of cyclooxygenase (COX) enzymes, which are essential for the production of prostaglandins and thromboxanes (Orliaguet et al., 2013).

These drugs are considered symptomatic treatments, meaning they address the manifestations of inflammation, regardless of its origin be it mechanical, chemical, infectious, or immune-related.

2.2 Mechanism of Action in Inflammation

NSAIDs work by blocking the activity of cyclooxygenase enzymes (COX), thereby preventing the production of prostaglandins. These molecules play a key role in the inflammatory response following surgery, where they stimulate and sensitize peripheral pain receptors and also contribute to increased sensitivity in the spinal cord’s dorsal horn, leading to postoperative hyperalgesia.

The COX-2 enzyme, in particular known as the inducible form because it is triggered by surgical trauma is primarily responsible for this prostaglandin production. By inhibiting COX-2, NSAIDs not only provide pain relief but also reduce hyperalgesia (Slim, 2016).

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 vivo* and *in vitro* assays, and also carried out *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:

BSA:

A solution of bovine serum albumin (BSA) was prepared at a concentration of 0.5 mg/mL in phosphate saline buffer (pH = 6.4).

Diclofenac:

Different concentrations of diclofenac sodium solution (commercially marketed as Voltaren 50 mg) were prepared in phosphate saline buffer (pH = 6.4).

Phosphate Buffer Solution:

The phosphate buffer saline solution was meticulously prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate (Sigma Aldrich) in conjunction with double-distilled water and KCl. The pH was meticulously maintained at 6.4 through the utilization of this phosphate buffer.

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 5

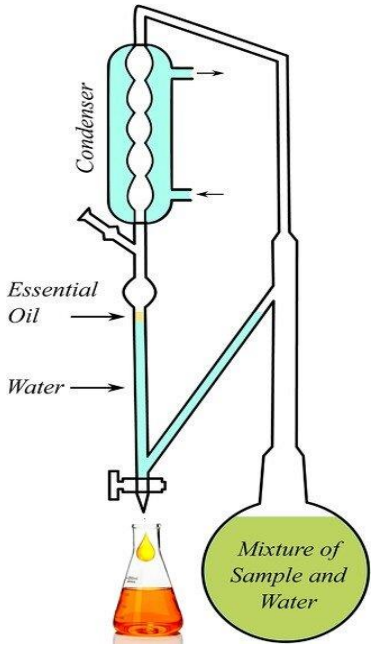


Figure 5. Schematic representation of the Clevenger apparatus used in the essential oil extraction process (Biswa et al., 2023)

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:

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

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 (Chiu et al., 1982; Guinaudeau et al., 1975; Kiryakov, 1968; Shamma, 1972) 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 Vivo* Anti-inflammatory Activities:

4.4.1 Animal Care:

The experimental group consisted of twenty-five (25) male albino Wistar rats, initially weighing between 100 and 150 grams, all of the same age and in good physiological condition. These animals were obtained from the Pasteur Institute in Algiers and were housed in a dedicated animal facility at the Faculty of Natural and Life Sciences, University of El Oued. The animals were maintained under standardized environmental conditions: a temperature-controlled environment at 25 ± 2°C and a 12-hour light/dark cycle. The housing setup included plastic cages, each accommodating five rats and equipped with a bedding of wood shavings, which was changed every other day throughout the experimental period. Regular assessments of body weight were carried out on a weekly basis to monitor the animals' physiological parameters.

4.4.2 Experimental Design:

After the induction of inflammation, all experimental groups were maintained under identical conditions. The animals were then randomly divided into five groups, each consisting of five rats. Group 1 (control group) included rats fed a standard diet without any treatment. Group 2 (Ibuprofen group) consisted of rats with induced inflammation that were administered Ibuprofen along with a standard diet. Group 3 (EOMP group *Mentha piperita* essential oil) included rats with induced inflammation that received 5 mL of essential oil extracted from *Mentha piperita* in addition to a standard diet. Group 4 (EOAI group *Ammodaucus leucotrichus* essential oil). Group 5 (benzylthiouracil group) consisted of rats given benzylthiouracil via a rodent drinking bottle in addition to a standard diet. consisted of rats with induced inflammation treated with 5 mL of essential oil extracted from *Ammodaucus leucotrichus* along with a standard diet. Treatment began 15 days after the induction of the inflammatory condition.

4.4.3 Sacrifice, Blood and Organ Collection:

At the end of the treatment period, the animals were subjected to a fasting period of 16 hours before being anesthetized by inhalation of 94% chloroform, followed by decapitation for sacrifice. In another group, anesthesia was performed and blood was collected directly from the heart. Blood samples were collected in EDTA tubes for hematological analysis, and in tubes without anticoagulant for biochemical analysis. The latter samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then stored at 4°C for subsequent biochemical analysis.

The analysis included tests such as urinalysis, complete blood count (CBC), creatinine, triglycerides, T3, T4, cholesterol, TGO, TGP, TSH, glycemia, CRP, and ASLO levels.

After euthanasia, the liver and kidneys were carefully dissected, with adipose tissue meticulously removed from these organs. The isolated organs were then weighed and rinsed in a 0.9% sodium chloride solution. Organ samples were subsequently prepared for the detection of oxidative stress markers, including malondialdehyde (MDA) and reduced glutathione (GSH). Notably, sections of the liver, and kidneys from each rat in every experimental group were immersed in formalin solution to preserve the tissues for subsequent histological analysis.

4.5 *In Vitro* Anti-inflammatory Activity: Bovine Serum Albumin (BSA) Denaturation Assay

4.5.1 Overview

Protein denaturation is a key process in inflammatory disorders (Farooq et al., 2025), making BSA denaturation inhibition an effective model for assessing anti-inflammatory potential (Smati et al., 2025). This assay evaluates the ability of *Ammodaucus leucotrichus* and *Mentha piperita*. extracts and synthesized nanoparticles to prevent protein denaturation, which may indicate their therapeutic potential for inflammation-related diseases.

4.5.2 Chemicals and Reagents

- Bovine serum albumin (BSA, 5% w/v) from Sigma-Aldrich
- deionized water Plant extracts (0.1–1 mg/mL)
- Benzylthiouracil (positive control, anti-inflammatory drug)

4.5.3. Procedure

In the present study, the assay was conducted following established methodologies (Gangadharan et al., 2025) with slight modifications to optimize experimental conditions. The reaction mixture was prepared by combining 500µL of a 5% BSA solution with 250 µL of the test sample at varying concentrations (100 – 1000 µg/mL). Benzylthiouracil was used as a positive control, while the negative control consisted of BSA solution mixed with distilled water under identical conditions. The prepared mixtures were incubated at 37°C for 20 minutes to allow interaction between BSA and the test compounds. Following this, the solutions were subjected to heat-induced denaturation by maintaining them at 70°C for 20 minutes. After the heating phase, the samples were cooled to room temperature and diluted with 500µL with deionized water before measuring their absorbance at 660 nm using a UV-Vis spectrophotometer. Blank was prepared with mixing: 1mL water + 250 µL DMSO. The percentage inhibition of protein denaturation was calculated using the Equation 4:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \qquad \textbf{(6)}$$

Where $A_{control}$ corresponds to the absorbance of the negative control, and A_{sample} represents the absorbance of the test sample or positive control. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

4.6. *In-Silico* analysis:

4.6.1. 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.

4.6.2. 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 (Daina et al., 2017; Riyadi et al., 2021) 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/).

4.6.3. Docking setup:

- **Ligands preparation:**

The three-dimensional configurations of the major compounds isolated from *Ammodaucus leucotrichus* and *Mentha piperita* 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).

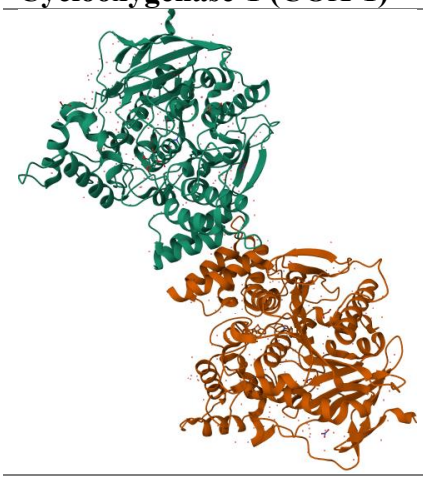
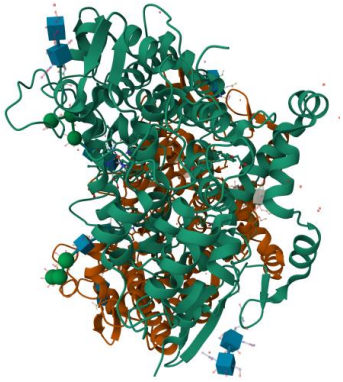
- **Receptor Preparation:**

The crystallographic data for Cyclooxygenase-1 and Cyclooxygenase-2 (Table 4) (PDB ID: 5wbe and 3ln1), respectively (Williams et al., 2015) 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.

Table 4. Target receptor information chosen for docking studies

Cyclooxygenase-1 (COX-1)	**Detaille's**	
	PDB ID	5WBE
	Mutation	No
	Resolution (Å)	2.75
	R-Value Free	0.229
	R-Value Observed	0.197
	Organism	Ovis aries
	Space Groupe	P 6_5
	Sequence Length	600
Cyclooxygenase-2 (COX-2)	**Detaille's**	
	PDB ID	3LN1
	Mutation	No
	Resolution (Å)	2.75
	R-Value Free	0.229
	R-Value Observed	0.197
	Organism	Ovis aries
	Space Groupe	P 6_5
	Sequence Length	600

- **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).

chapter two:
results and discussion

1. Introduction:

This chapter delves into the findings regarding the extraction yield, characterization, and comprehensive exploration of the anti-inflammatory 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 anti-inflammatory 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:

Figure 6 illustrates the yields of essential oils obtained through hydrodistillation of the aerial parts of *Ammudoucus leucotrichus* and *Mentha piperita*. variety of Eloued.

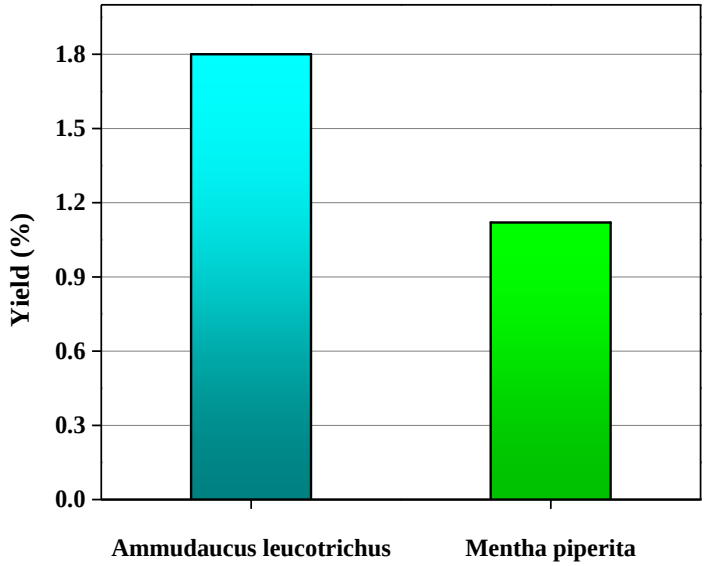


Figure 6. The yields of extracted essential oils

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 hydrodistillation 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 hydrodistillation 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 5) organoleptic properties:

Table 5. The organoleptic characteristics of essential oils

Plant	Smell	Aspect	Colour
Ammudoucus leucotrichus	A strong smell	liquid at room temperature	Bleu
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 6.

Table 6. 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 7 and Figure 8 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:

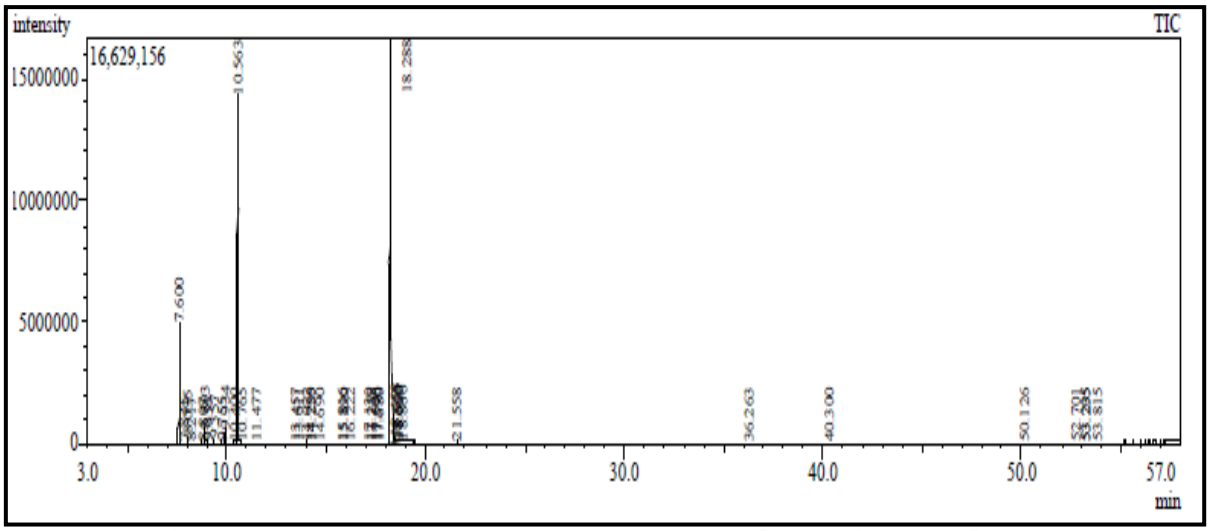


Figure 7. Chromatogram of the essential oil of *Ammudoucus leucotrichus* plant obtained by GC/MS

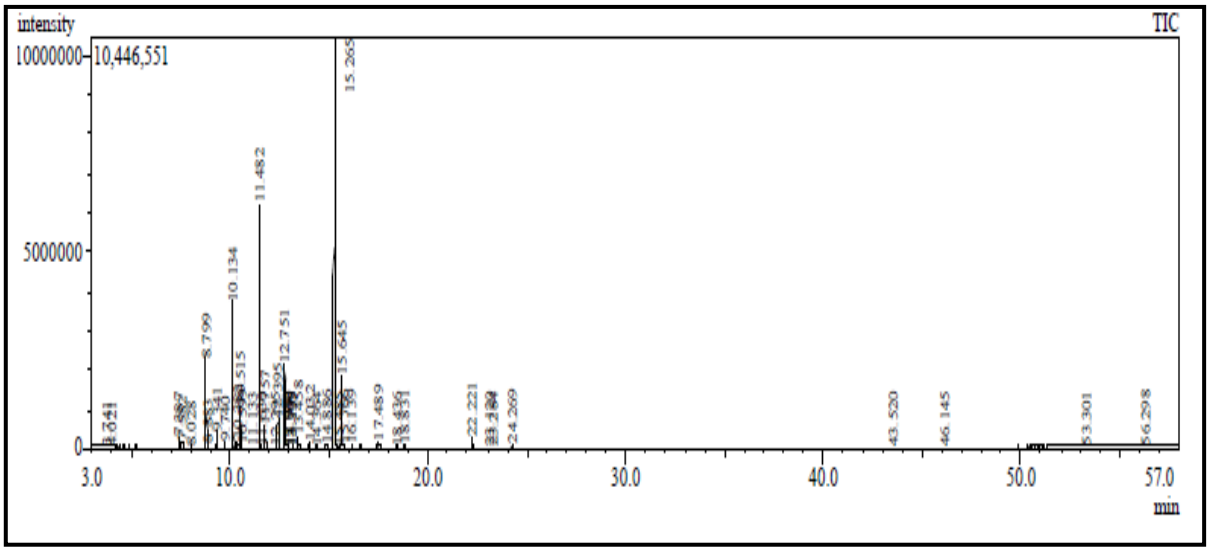


Figure 8. Chromatogram of the essential oil of *Mentha piperita* plant obtained by GC/MS

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 7).

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.

Table 7. Essential oil constituents of *Ammudoucus leucotrichus* identified by GC/MS

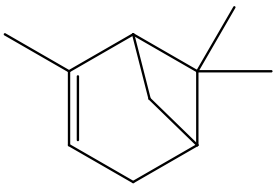
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	**Alpha-Pinene**	**932**	**925**	**5.8**
02	Alpha-Fenchene	945	934	0.02
03	Camphene	946	941	0.4
04	Thuja-2,4(10)-diene	953	948	0.04
05	Sabinene	969	969	0.03
06	Beta-Pinene	974	972	0.66
07	Myrcene	988	989	0.15
08	Alpha-Phellandrene	1002	1003	0.04
09	Beta-Ocimene	1009	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 (Baser et al., 1993; Louail et al., 2016; Sonboli et al., 2012). 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 (Louail et al., 2016; Moulay et al., 2014) indicated the presence of a small quantity of α-pinene, which does not align with our results.

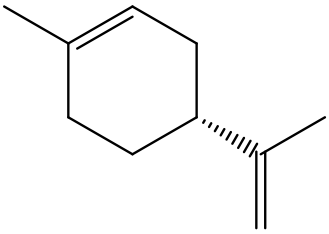
Additionally, (Moulay et al., 2014) mentioned that the essential oil of *Ammudoucus leucotrichus* primarily contained perillaldehyde (88.7%) and limonene (8.26%), contrasting with our findings where perillaldehyde predominates and limonene is less prominent. According to (Louail et al., 2016),

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 (S. Y. Wang & Chen, 2010).

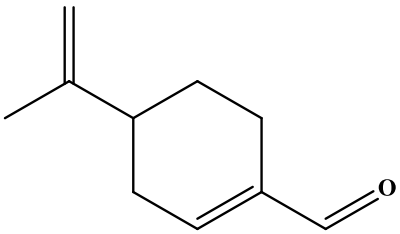
For a visual representation of the top major compounds (more than 1%) and their structures, refer to Figure 9, which depicts the chemical structures and IUPAC names of the predominant constituents identified in the essential oil of *Ammudoucus leucotrichus*.



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 9. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Ammudoucus leucotrichus*

3.3.2. *Mentha piperita*:

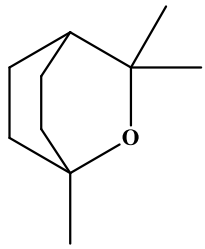
The application of hydrodistillation for extracting the essential oil from *Mentha piperita*, commonly referred to as peppermint, yielded a light-yellow oil with a 1.2% yield. Analysis revealed the presence of 34 chemical compounds (as presented in Table 8), constituting 98.24% of the total oil composition. 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%).

Table 8. Essential oil constituents of *Mentha piperita* identified by GC/MS

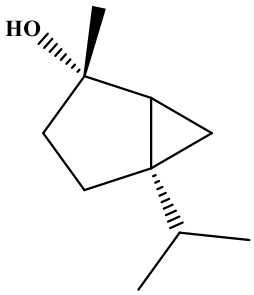
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	Alpha Pinene	924	932	0.46
02	Camphene	940	946	0.22
03	Sabinene	968	969	0.22
04	β Pinene	971	974	0.91
05	Myrcene	988	988	0.58
06	Alpha Terpinene	1015	1014	0.05
07	para-Cymene	1023	1020	0.04

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**		

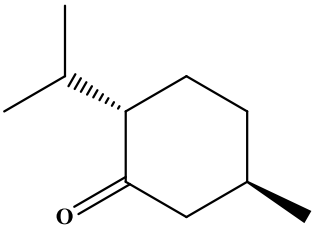
Furthermore, α-Terpineol, Menthone, and Borneol were among the compounds present in concentrations exceeding 1%. Our findings are largely consistent with certain other studies, albeit with variations observed in the proportions of major compounds. For instance, (Gavahian et al., 2015) reported significant 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%).



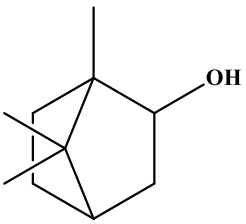
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
(1,8-Cineole)



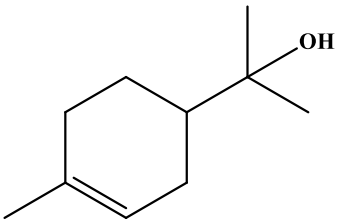
(2*S*,5*R*)-5-isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol
(trans-Sabinene hydrate)



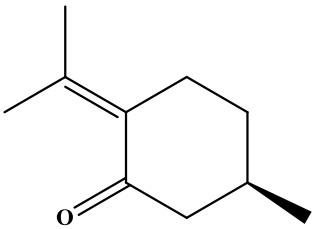
(2*S*,5*R*)-2-isopropyl-5-methylcyclohexanone
(Menthone)



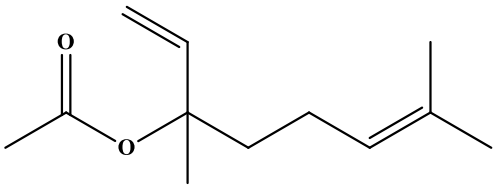
1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol
(Borneol)



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 10. 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 10, displaying the structures and IUPAC nomenclature of these constituents.

4. *In vivo* Anti-Inflammatory Activity:

4.1. Organ Weight Index

Compared to the control group, exposure to benzylthiouracil (BTU) resulted in a significant increase in the liver weight index and a highly significant increase in the kidney weight index. The treatment with *Mentha piperita* (BTU + MP) led to a significant reduction in liver weight index ($p < 0.05$) and a significant reduction in kidney weight index ($p < 0.05$) compared to the BTU group. Similarly, treatment with *Ammodaucus leucotrichus* (BTU + AL) produced a significant decrease in liver weight index ($p < 0.05$), while the kidney weight index was reduced in a significant manner compared to the BTU group. Furthermore, treatment with ibuprofen (IBU) significantly decreased both liver and kidney weight indices relative to the BTU-exposed group.

Table 09: Organ weight Index of different experimental groups

	Organ Weight Index %	
	Liver	Kidneys
Control	3.0±0.03	0.53±0.02
BTU	4.36±0.18a	0.65±0.01b
BTU + MP	3.02±0.008NS*	0.54±0.005 NS*
BTU + AL	3.2±0.25 NS*	0.53±0.01 NS**
BTU + IBU	3.0±0.05 NS*	0.54±0.02 NS**

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.2.Hematological parameters

Table 09 shows the plasma concentrations of key hematological parameters across the different experimental groups. In the control group, all hematological values were within the normal physiological range. In contrast, exposure to benzylthiouracil (BTU) resulted in significant hematological disturbances. There was a marked increase in white blood cell count (WBC) ($4.23 \pm 0.18 \times 10^9$/L; $p < 0.01$), lymphocytes (LYM) ($3.1 \pm 0.08 \times 10^9$/L; $p < 0.05$), and platelet count (PLT) ($676 \pm 11.5 \times 10^9$/L; $p < 0.001$) relative to the control group. In contrast, BTU significantly decreased hemoglobin (HGB) (9.4 ± 0.21 g/dL; $p < 0.05$) and red blood cells (RBC) ($4.9 \pm 0.14 \times 10^{12}$/L; $p < 0.05$), suggesting BTU-induced anemia. Granulocytes (GRA) were reduced, though the change was not statistically significant.

Treatment with *Mentha piperita* (BTU + MP) demonstrated a clear hematoprotective effect. WBC and LYM levels declined compared to BTU, though WBC remained slightly elevated ($3.13 \pm 0.13 \times 10^9$/L; $p < 0.05$ vs. control). HGB and RBC levels significantly increased (15.36 ± 0.2 g/dL and $8.31 \pm 0.02 \times 10^{12}$/L; $p < 0.001$ vs. BTU), returning to or exceeding control levels. PLT count also decreased to $638 \pm 19.4 \times 10^9$/L ($p < 0.05$ vs. control).

In the *Ammodaucus leucotrichus* (BTU + AL) group, similar improvements were observed. HGB and RBC levels were significantly restored (15.43 ± 0.1 g/dL and $8.32 \pm 0.35 \times 10^{12}$/L; $p < 0.01$ to $p < 0.001$ vs. BTU), indicating a strong reversal of BTU-induced anemia. PLT levels also decreased significantly compared to BTU ($568 \pm 5.7 \times 10^9$/L; $p < 0.01$), while WBC and LYM remained elevated but lower than in the BTU group.

In contrast, the ibuprofen-treated group (BTU + IBU) showed less effective hematological recovery. WBC and LYM remained elevated (4.67 ± 0.88 and $3.0 \pm 0.45 \times 10^9$/L, respectively), similar to BTU. However, GRA levels improved ($1.06 \pm 0.14 \times 10^9$/L), and significant increases were observed in HGB (15.16 ± 0.2 g/dL; $p < 0.001$) and RBC ($6.3 \pm 0.19 \times 10^{12}$/L; $p < 0.05$)

compared to BTU. PLT levels remained elevated (761.3 ± 18 ×10⁹/L), not significantly different from the BTU group.

Table 10: Plasma concentration of hematological parameters of different experimental groups

	WBC(×109/L)	LYM(×109/L)	GRA(×109/L)	HGB (g/dL)	RBC(×1012/L)	PLT (×109/L)
Control	1.46±0.29	1.4±0.26	1.1±0.4	14.46±0.78	7.8±0.47	516±9.23
BTU	4.23±0.18 b	3.1±0.08a	0.16±0.08 NS	9.4±0.21a	4.9±0.14a	676±11.5 c
BTU + MP	3.13±0.13 a NS	1.5±0.26 NS*	0.3±0.05 NS	15.36±0.2 NS***	8.31±0.02 NS***	638±19.4 a NS
BTU + AL	3.63±0.63 a NS	2.1±0.26 NS	0.37±0.16 NS	15.43±0.1 NS***	8.32±0.35 NS**	568±5.7 b**
BTU + IBU	4.67±0.88 a NS	3.0±0.45 a NS	1.06±0.14 NS*	15.16±0.2 NS***	6.3±0.19 NS*	761.3±18 b NS

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.3.Glycemia, liver and kidneys function parameters:

Table 10 illustrates the impact of different treatments on glycemia, liver enzymes, and renal function markers across the experimental groups.

In the control group, all parameters were within physiological norms. However, exposure to benzylthiouracil (BTU) caused significant alterations in metabolic, hepatic, and renal profiles. Fasting blood sugar (FBS) increased significantly to 0.93 ± 0.01 g/L ($p < 0.05$ vs. control), indicating hyperglycemia. Liver enzyme activities were markedly elevated, with AST at 226.06 ± 8.84 U/L ($p < 0.05$) and ALT at 116 ± 2.63 U/L ($p < 0.01$), reflecting hepatic injury. Additionally, urea (0.66 ± 0.011 g/L; $p < 0.01$) and creatinine (CT) (5.56 ± 0.033 mg/L; $p < 0.05$) levels increased significantly, suggesting impaired kidney function.

Treatment with *Mentha piperita* (BTU + MP) led to marked improvement in liver function. AST levels significantly decreased to 94.6 ± 2.6 U/L ($p < 0.01$ vs. BTU), and ALT levels dropped to 66.46 ± 3.35 U/L ($p < 0.05$), both returning toward control values. FBS also improved (0.72 ± 0.034 g/L), though not significantly. Renal function was largely restored, with normalized urea (0.44 ± 0.006 g/L; $p < 0.01$ vs. BTU) and reduced CT (4.7 ± 0.35 mg/L).

In the *Ammodaucus leucotrichus* (BTU + AL) group, liver function markers were moderately restored. AST (117.1 ± 8.7 U/L; $p < 0.01$ vs. BTU) and ALT (84.0 ± 7.02 U/L) showed improvement but remained slightly above control levels. FBS decreased to 0.74 ± 0.03 g/L ($p < 0.05$ vs. BTU). Renal function was significantly improved, with normalized urea (0.44 ± 0.008 g/L; $p < 0.01$) and a significant reduction in CT (4.7 ± 0.05 mg/L; $p < 0.01$).

The ibuprofen-treated group (BTU + IBU) exhibited significant restoration across all parameters. FBS decreased to 0.63 ± 0.032 g/L ($p < 0.01$ vs. BTU), indicating improved glycemic control. AST and ALT were reduced to 102.3 ± 9 U/L ($p < 0.001$) and 78.0 ± 5.7 U/L ($p < 0.05$), respectively, showing hepatoprotective effects. Urea (0.47 ± 0.02 g/L; $p < 0.05$) and CT (4.63 ± 0.24 mg/L) also decreased, reflecting improved renal function.

Table 11: Glycemia, liver and kidneys function parameters of different experimental groups

	FBS (g/L)	AST (U/L)	ALT (U/L)	Urea (g/L)	CT (mg/L)
Control	0.58±0.04	126.13±6.48	79.23±2.9	0.43±0.017	5.06±0.066
BTU	0.93±0.01a	226.06±8.84a	116±2.63b	0.66 ±0.011b	5.56±0.033a
BTU + MP	0.72±0.034NS	94.6±2.6a**	66.46±3.35 NS*	0.44±0.006NS**	4.7±0.35 NS
BTU + AL	0.74±0.03 NS*	117.1±8.7 NS**	84.0±7.02 NS	0.44±0.008 NS**	4.7±0.05 NS**
BTU + IBU	0.63±0.032 NS**	102.3±9 NS***	78.0±5.7 NS*	0.47±0.02 NS*	4.63±0.24 NS

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.4.C-reactive protein

Table 11 shows the plasma concentration of C-reactive protein (CRP), a key marker of systemic inflammation, across the experimental groups.

In the control group, CRP levels were within normal limits (4.53 ± 0.31 mg/L). Exposure to benzylthiouracil (BTU) induced a highly significant increase in CRP concentration to 21 ± 0.57 mg/L ($p < 0.01$ vs. control), indicating a strong inflammatory response triggered by BTU administration.

The treatment with *Mentha piperita* (BTU + MP) significantly mitigated this inflammatory effect, reducing CRP levels to 5.16 ± 0.35 mg/L ($p < 0.001$ vs. BTU), which was not significantly different from the control group, suggesting near-complete normalization.

Similarly, treatment with *Ammodaucus leucotrichus* (BTU + AL) effectively restored CRP levels to 4.6 ± 0.5 mg/L ($p < 0.001$ vs. BTU), fully reversing the BTU-induced inflammation.

Administration of ibuprofen (BTU + IBU) also led to complete normalization of CRP concentration (4.5 ± 0.05 mg/L; $p < 0.001$ vs. BTU), aligning perfectly with control levels and confirming its potent anti-inflammatory efficacy.

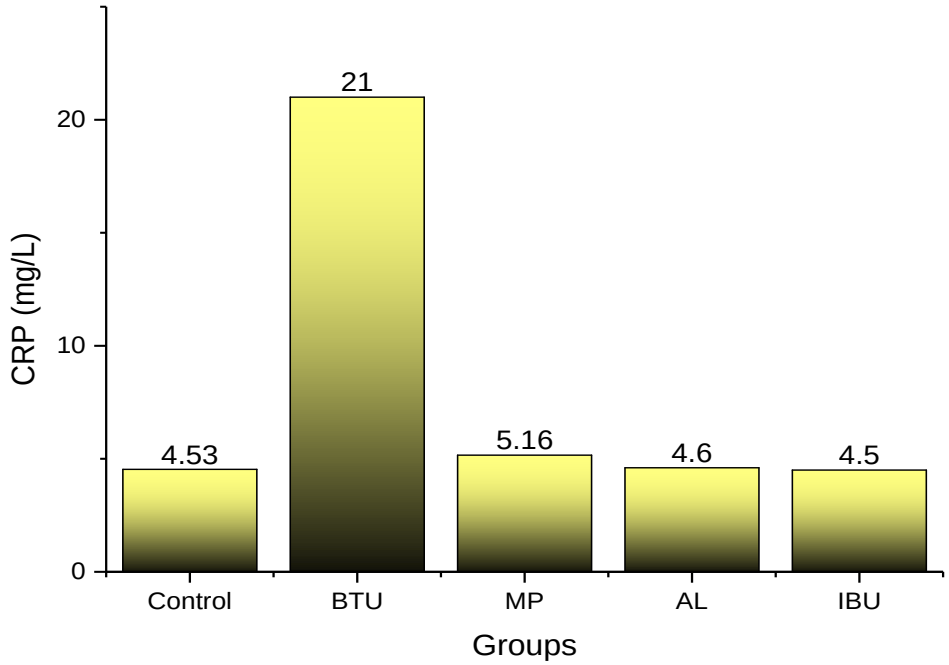


Figure 11: Plasma concentration of C-reactive protein of different experimental groups

4.5.Histopathological studies

4.5.1.Liver

This histopathological study investigates the effects of benzylthiouracil (BTU)-induced liver inflammation and evaluates the protective role of different treatments. The control group (C) displays normal hepatic architecture, with well-organized hepatocytes and no signs of inflammatory infiltration or cellular damage, indicating healthy liver tissue.

In contrast, liver sections from the BTU group exhibit marked pathological changes. There is significant inflammatory infiltration, where immune cells accumulate in the tissue, accompanied by structural disorganization and hepatocellular damage. This confirms the hepatotoxic effect of BTU. When BTU is combined with ibuprofen (BTU + IBU), there is still evidence of inflammatory infiltration, although it appears slightly reduced compared to the BTU group. This suggests that ibuprofen has a moderate anti-inflammatory effect but does not completely restore normal liver architecture.

A more substantial improvement is observed in the BTU + *Mentha piperita* (BTU + MP) group. The liver tissue shows a more preserved structure with reduced inflammation, indicating the potential hepatoprotective effect of *Mentha piperita.* Similarly, the BTU + *Ammodaucus leucotrichus* (BTU + AL) group exhibits near-normal liver histology. Hepatocytes appear healthy, and inflammatory cell infiltration is minimal, suggesting a strong protective effect of *Ammodaucus leucotrichus* against BTU-induced liver damage.

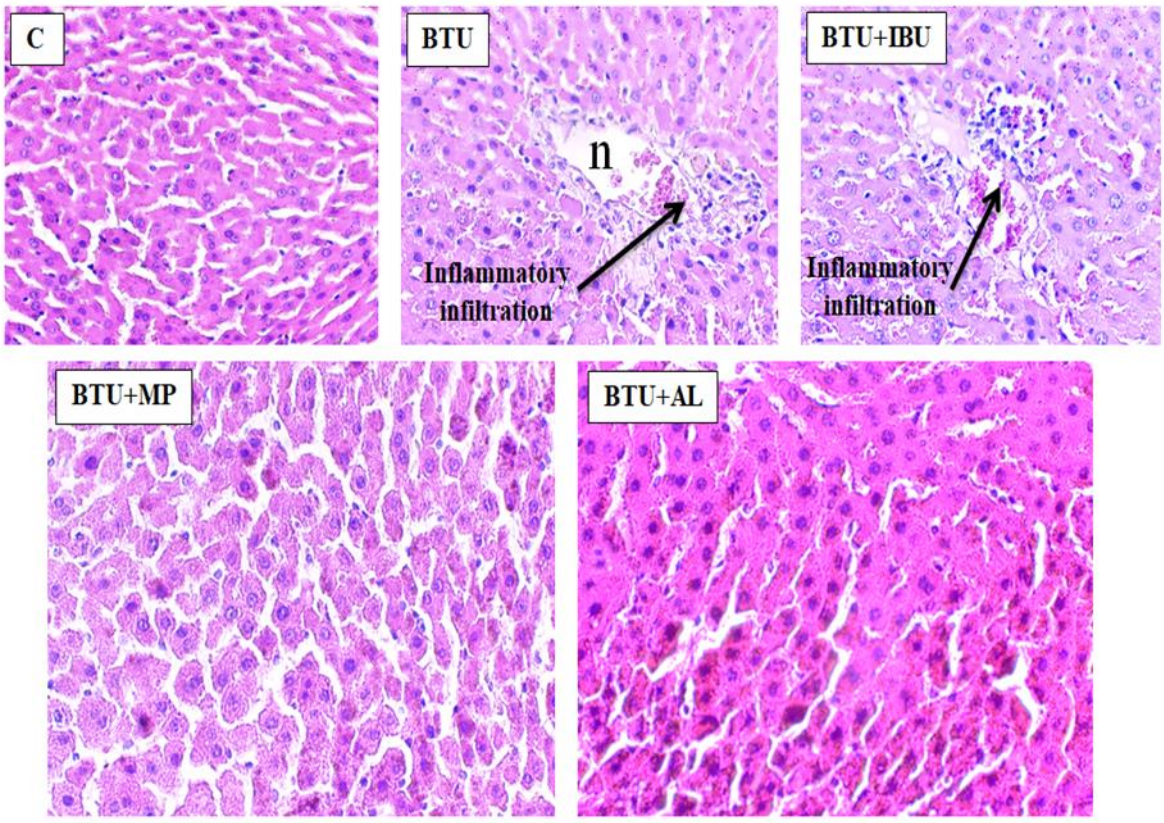


Figure 12 : Microscopic observation of liver histological sections from different experimental groups, (C) Control group, (BTU) Benzylthiouracil group, (BTU+Levo) Group treated with levothyroxine, (BTU+MP) Group treated with *Mentha piperita*, and (BTU+AL) Group treated with *Ammodaucus leucotrichus*, (V) Indicates thyroid follicles, (FC) Indicate follicular cells, (BV) Indicate blood vessel, Magnification ×40.

4.5.2.Kidneys

Histopathological analysis of kidney sections stained with hematoxylin and eosin (H&E) was conducted to evaluate the nephrotoxic effects of benzylthiouracil (BTU) and to assess the potential renoprotective roles of ibuprofen, *Mentha piperita*, and *Ammodaucus leucotrichus*. Renal tissue from the control group (C) revealed preserved renal architecture, characterized by well-defined glomeruli and intact tubular structures without any evidence of cellular infiltration or morphological abnormalities, indicative of normal kidney histology. Conversely, kidney sections from the BTU-treated group exhibited marked pathological alterations, including extensive infiltration of inflammatory cells (denoted “I”) and noticeable disruption of renal parenchyma, suggesting severe BTU-induced nephrotoxicity and inflammation. Co-administration of ibuprofen (BTU + IBU) resulted in a visible reduction in inflammatory cell presence and partial restoration of renal histoarchitecture, supporting the hypothesis that ibuprofen confers a degree of nephroprotection through its anti-inflammatory mechanisms. In the group treated *Mentha piperita* (BTU + MP), renal tissues appeared largely preserved with mild improvements in structural integrity, suggesting a partial protective effect likely linked to the antioxidative and anti-inflammatory properties of M. piperita. Similarly, kidney sections from the BTU + *Ammodaucus leucotrichus* (BTU + AL) group exhibited decreased inflammatory infiltration and moderate retention of renal structural organization.

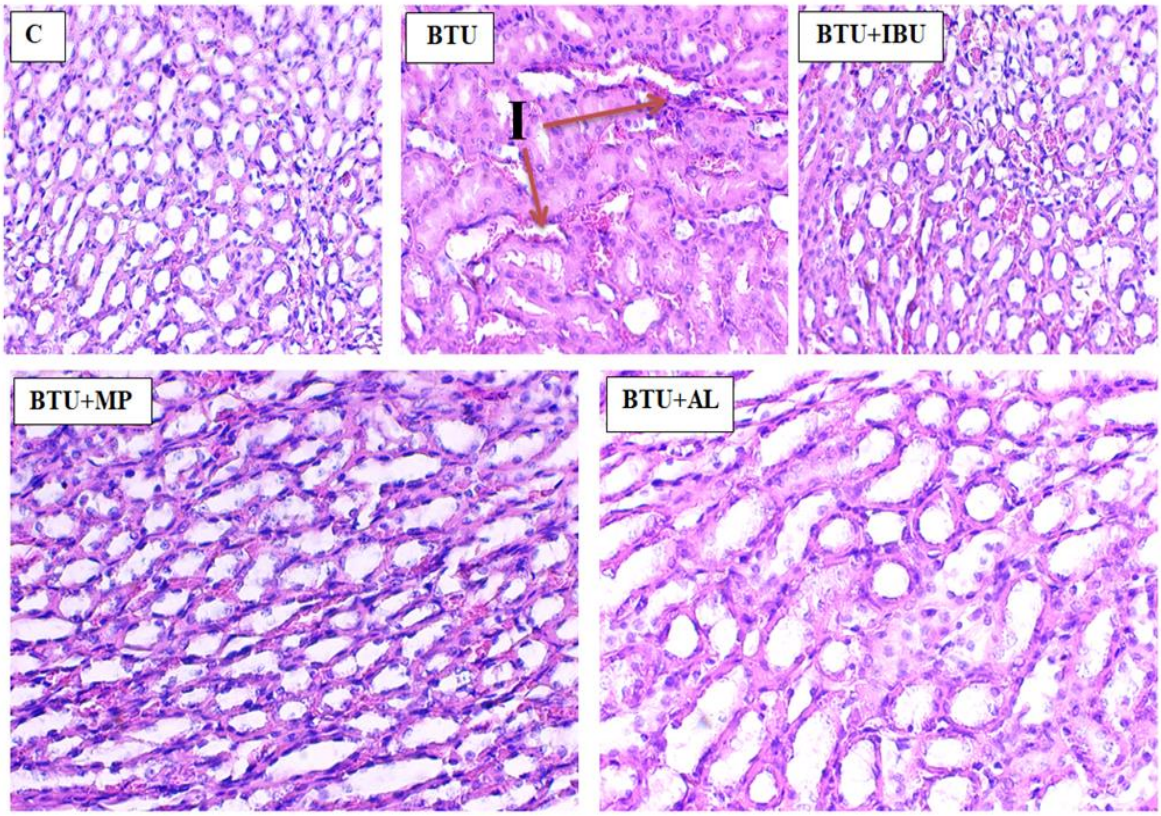


Figure 13: Microscopic observation of kidney histological sections from different experimental groups, (C) Control group, (BTU) Benzylthiouracil group, (BTU+Levo) Group treated with levothyroxine, (BTU+MP) Group treated with *Mentha piperita*, and (BTU+AL) Group treated with *Ammodaucus leucotrichus*, (V) Indicates thyroid follicles, (FC) Indicate follicular cells, (BV) Indicate blood vessel, Magnification ×40.

5.*In Vitro* Anti-Inflammatory Activity:

5.1. BSA Inhibitory Activities (IC_{50}):

The *in-vitro* Anti-inflammatory potential of the two essential oils obtained from *Ammodaucus leucotrichus* and *Mentha piperita* was investigated using the Bovine Serum Albumin (BSA) protein denaturation inhibition model, and the results are presented in Table 12. From the linear plots of *Ammodaucus leucotrichus* and *Mentha piperita* concentrations (µg/mL) against percentage Bovine Serum Albumin (BSA) protein denaturation inhibition (Figure 14), it was observed that the essential oils significantly increased Bovine Serum Albumin (BSA) protein denaturation inhibition (%) with increasing concentration. The effective inhibitory concentrations (IC_{50}) at which 50% of activity was Bovine Serum Albumin (BSA) protein denaturation inhibited by *Ammodaucus leucotrichus* and *Mentha piperita* essential oils were 5.132 µg/mL and 2.222 µg/mL, respectively, These results indicate that the essential oils of *Mentha piperita* and *Ammodaucus leucotrichus* exhibit inhibitory activity protein denaturation.

The inhibitory potential of Diclofenac, a standard Anti-inflammatory, on Bovine Serum Albumin (BSA) protein denaturation activity was also evaluated in the present study as a positive control. The results showed that both essential oils demonstrated greater inhibitory effects than Diclofenac (IC_{50} = 11.166 µg/mL), with *Mentha piperita* essential oil exhibiting superior inhibition.

Chronic inflammation is associated with various chronic diseases, including diabetes, cardiovascular disorders, and cancer, as inflammatory proteins such as BSA contribute to exaggerated inflammatory responses (Calder, 2006). Therefore, inhibiting the inflammatory activity of proteins like BSA is a

significant therapeutic target for reducing complications linked to these diseases (Aggarwal & Harikumar, 2009). The essential oils studied have shown relatively promising potential in inhibiting BSA denaturation compared to non-steroidal anti-inflammatory drugs (NSAIDs), highlighting their potential as natural anti-inflammatory agents. Previous reports have indicated that some conventional anti-inflammatory drugs may cause side effects such as gastric discomfort and an increased risk of ulcers with prolonged use (Wallace, 2008).

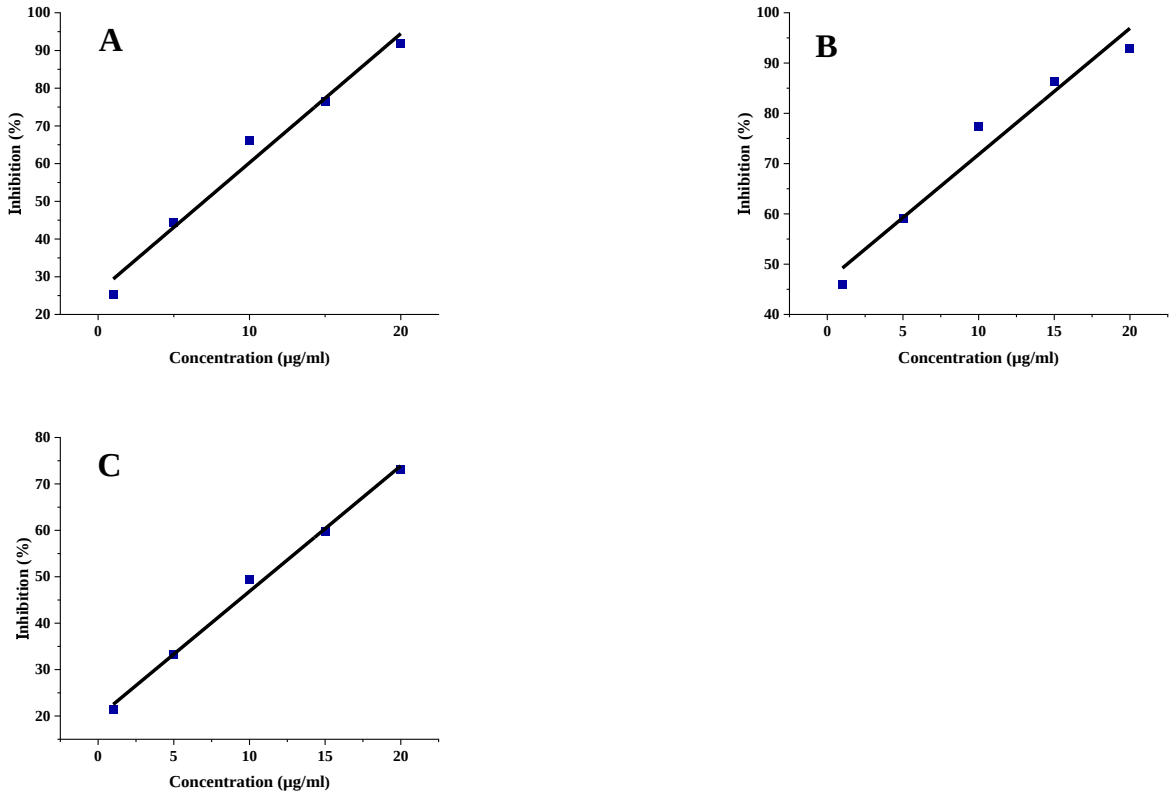


Figure 14. Linear regression of the inhibition of α-amylase activity by the essential oils of: *Ammudoucus leucotrichus* (A), *Mentha piperita* (B) and Diclofenac (C)

Table 12. *In vitro* Anti-inflammatory activity of the essential oils extracted from *Ammudoucus leucotrichus* and *Mentha piperita* by BSA inhibitory assay

Concentration (µg/mL)	**Percentage of BSA Inhibition**		
	Ammudoucus leucotrichus	*Mentha piperita*	Diclofenac
1	25.352	45.918	21.405
5	44.503	59.073	33.239
10	66.242	77.399	49.353
15	76.549	86.305	59.829
20	91.908	92.867	73.112
IC_{50} Values	**5.132 µg/mL**	**2.222 µg/mL**	**11.166 µg/mL**

The anti-inflammatory potential of the essential oils extracted from *Ammodaucus leucotrichus* and *Mentha piperita* was assessed in vitro using the BSA denaturation inhibition model, with diclofenac employed as a standard anti-inflammatory drug. As shown in Table 7, both essential oils exhibited concentration-dependent inhibition of BSA denaturation, with *Mentha piperita* showing a notably higher percentage of inhibition across all tested concentrations compared to *Ammodaucus leucotrichus* and diclofenac.

At the highest tested concentration (20 µg/mL), *Mentha piperita* and *Ammodaucus leucotrichus* achieved inhibition percentages of 92.867% and 91.908%, respectively, while diclofenac reached 73.112%. The calculated IC_{50} values further highlight the potency of the oils, with *Mentha piperita* displaying the most effective inhibition (IC_{50} = 2.222 µg/mL), followed by *Ammodaucus leucotrichus* (IC_{50} = 5.132 µg/mL), and diclofenac (IC_{50} = 11.166 µg/mL).

These findings suggest that the essential oils, particularly *Mentha piperita*, possess strong anti-inflammatory properties likely mediated by their ability to inhibit protein denaturation, a well-known marker of inflammation. The superior performance compared to diclofenac implies their potential as natural alternatives or complementary agents in the management of inflammatory conditions.

5.2. BSA Molecular Binding Interaction:

One of the effective techniques for monitoring alterations in protein conformation during its interaction with ligands is absorption spectroscopy (Benesi & Hildebrand, 1949; B. L. Wang et al., 2020). Usually, when measured at the same wavelength range, the absorption peak(s) of a free protein and that of the protein-ligand complex are expected to differ; such difference could be attributed to the alteration in the native structure of the protein due to its binding interaction with the ligand.

5.2.1. Binding constants:

The gradual decrease in the absorption values of the BSA solution by increasing essential oils and diclofenac concentrations can be exploited to calculate the binding constant by applying the following Equation 7 (Benesi & Hildebrand, 1949):

$$\frac{A}{A_0 - A} = \frac{\varepsilon}{\varepsilon_0 - \varepsilon} + \frac{\varepsilon}{\varepsilon_0 - \varepsilon} \frac{1}{K_b \times C} \quad (7)$$

Where A_0 and A are the absorbencies of the α-amylase and its complexes with the studied essential oils respectively, while ε_0 and ε are respectively their extinction coefficients, and C represents the concentration of EOs and acarbose (mmol.L^{-1}), K_b refers to the binding constant (L.mol^{-1}).

A plot of $A/(A_0 - A)$ versus $1/C$ (Figure 15) gave a slope of $\varepsilon/(\varepsilon_0 - \varepsilon)K_b$ and a 'y' intercept equal to $\varepsilon/(\varepsilon_0 - \varepsilon)$, where K_b is the ratio of the slope to the y intercept.

5.2.2. Binding free energy:

The binding free energy change was calculated using the following Equation 8: (Laraoui et al., 2023)

$$\Delta G = -nRT \ln K_b \quad (8)$$

Where ΔG is the binding free energy in KJ.mol^{-1}, R is the gas constant, 8.32 J.$mol^{-1}K^{-1}$ and T is the absolute temperature, 298K.

The negative values of ΔG indicate the spontaneity of the α-amylase and the essential oils interaction, whereas its magnitude indicates the strong binding between the protein and the studied compounds (Gil et al., 2002). The obtained values of binding constants and their corresponding free binding energies are summarized in Table13.

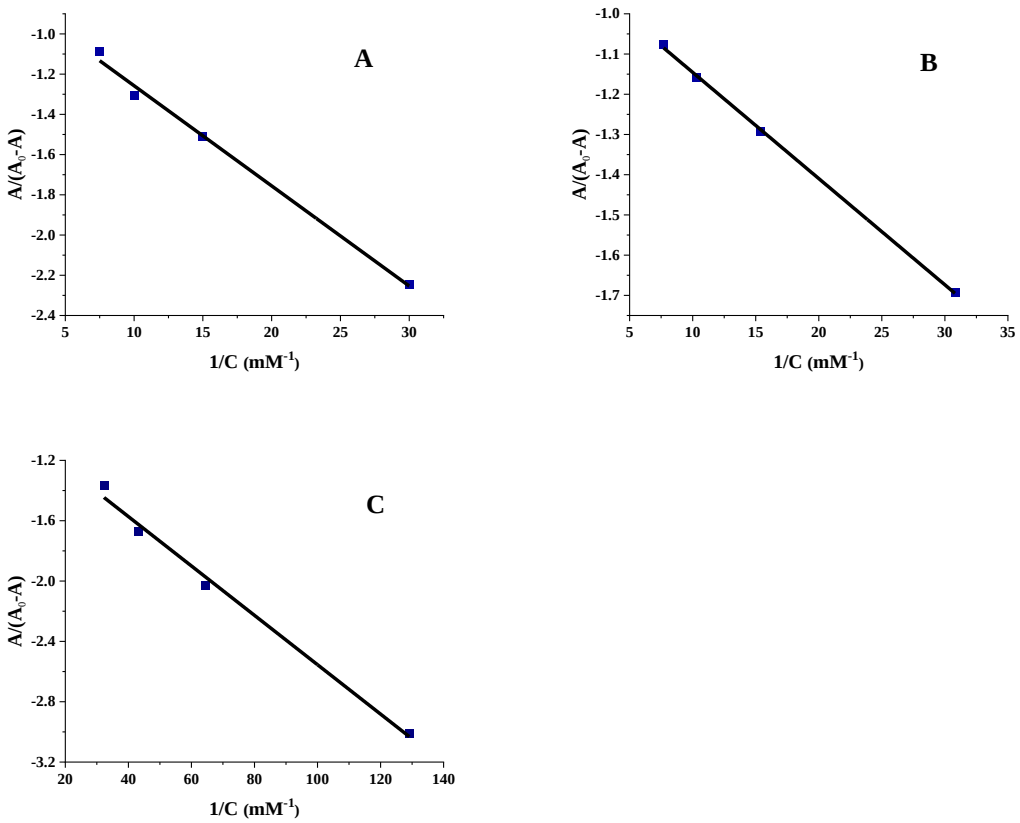


Figure 15. Plots of A/(A₀ - A) versus 1/[*Ammudoucus leucotrichus*] (A), 1/[*Mentha piperita*] (B) and 1/[Diclofenac] (C) used to calculate the binding constants

Table 13. Binding constant and binding free energy values for *Ammudoucus leucotrichus*, *Mentha piperita* and diclofenac with BSA

Adduct	K_b (M^{-1})	-ΔG (KJ.mol^{-1})
BSA_*Ammudoucus leucotrichus*	1.22×10^4	24.85
BSA_*Mentha piperita*	3.88×10^4	26.02
BSA_Diclofenac	2.68×10^4	25.28

6. In-Silico analysis:

6.1. 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 14), Physicochemical properties (Table 11), lipophilicity and

water solubility characteristics (Table 12 & 13), pharmacokinetic parameters (Table 12), drug likeness rule and bioavailability score (Table 13) and medicinal chemistry properties (Table 14), respectively, the six compounds are compared to the standard drug (Diclofenac). 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 than the Diclofenac.

Table 14. 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

Table 15. 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

Table16. 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

Table 17. 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

Table 18. Pharmacokinetics parameters of the phytoconstituents of essential oils

Proprieties	**trans-Sabinene hydrate**	**Linalool acetate**	**Perilla aldehyde**	**D-Limonene**
GI absorption	High	High	High	Low
BBB permeant	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Log Kp (Skin Permeation) (cm/s)	-5.74	-4.71	-4.99	-3.89

Table 19. 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 20. 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 Diclofenac lipophilic whereas trans-Sabinene hydrate as least lipophilic and water solubility of the small molecules ranged from highly water soluble (Diclofenac) to 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 except contrarily to the Diclofenac (Table 18), so they are not susceptible to the 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 (-5.74) is the least permeant and D-Limonene (-4.13) 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 anti inflammatory 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.

Table 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

6.2. Molecular Docking Study:

In the current study, the binding interactions of the major phytochemical constituents of *Ammodaucus leucotrichus* and *Mentha piperita* essential oils with bovine serum albumin (BSA) were investigated through molecular docking analysis. The compounds examined included Perilla aldehyde, D-Limonene, α-Pinene, Pulegone, Menthone, trans-Sabinene hydrate, α-Terpineol, Borneol, Linalool acetate, and 1,8-Cineole — all of which had yields greater than 1% in the GC-MS analysis. Docking studies were performed using the Maestro version 11.7 user interface within the Schrödinger Suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015).

Each compound was docked into the active binding sites of BSA to evaluate their potential anti-inflammatory interactions. Diclofenac, a well-established nonsteroidal anti-inflammatory drug (NSAID), was also docked with BSA and used as the positive control. The majority of the essential oil constituents displayed comparable or even stronger binding affinities to BSA than diclofenac, as demonstrated by favorable Induced Fit Docking (IFD) scores (Table 16). These results are in line with the in vitro BSA inhibition assay, supporting the hypothesis that these natural compounds could effectively inhibit protein denaturation — a primary mechanism involved in inflammatory processes. The spontaneous nature of the interactions was further supported by the negative Gibbs free energy values obtained for the ligand-protein complexes (Avwioroko et al., 2020).

BSA is known for its ability to undergo conformational changes upon interaction with ligands, which may affect its structural stability and biological activity. Key binding regions in BSA, especially hydrophobic cavities in subdomains IIA and IIIA, play a significant role in ligand accommodation and stabilization (Cao et al., 2009; Fanali et al., 2012). The docking results suggest that the major

compounds from the two essential oils have high affinity for these binding pockets, potentially stabilizing BSA against thermal or chemical denaturation and thereby exerting anti-inflammatory effects.

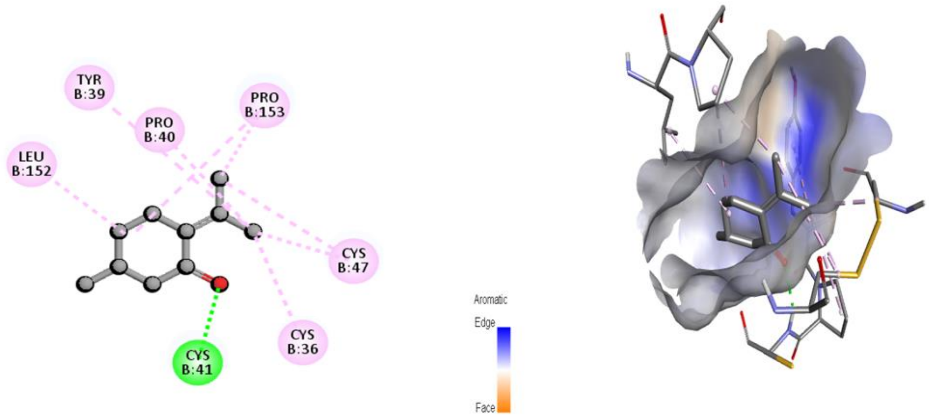
Table 22. Docking score of the studied compounds

Compound	COX-1	COX-2
Perilla aldehyde	-6.32	-4.15
D-Limonene	-6.23	-4.53
alpha-Pinene	-5.69	-4.77
Pulegone	-6.54	-5.13
Menthone	-6.23	-4.52
trans-Sabinene hydrate	-6.04	-4.76
alpha-Terpineol	-6.15	-4.99
Borneol	-6.51	-5.05
Linalool acetate	-6.12	-3.91
1,8-Cineole	-6.06	-4.6

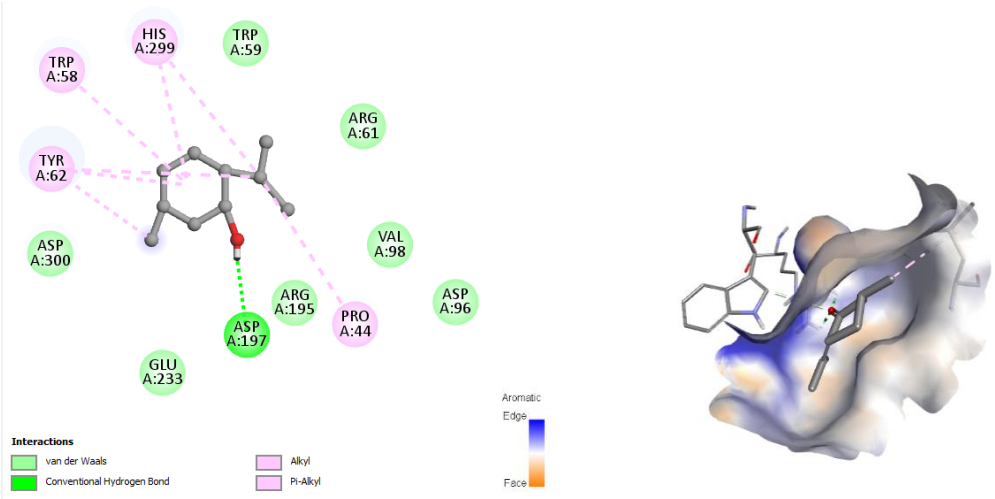
The results illustrated in Figure 16 revealed that the top two compounds from *Ammodaucus leucotrichus* and *Mentha piperita* interacted with a similar set of amino acid residues at the binding site of BSA, akin to the interaction pattern exhibited by the standard anti-inflammatory drug, diclofenac. Notably, Perilla aldehyde, along with the reference compound diclofenac, established interactions with key residues located within the major drug-binding site of BSA, primarily subdomain IIA. In contrast, Pulegone interacted with fewer key residues, suggesting a potential difference in binding mode.

Perilla aldehyde, which demonstrated the strongest binding affinity, interacted with amino acid residues such as TRP-213, TYR-150, and ARG-198—commonly involved in ligand stabilization within the BSA binding pocket—similar to the interaction profile of diclofenac. This pattern suggests that Perilla aldehyde may exhibit a competitive binding mechanism by occupying the same site as diclofenac. On the other hand, Pulegone interacted with TYR-150 and ARG-198 but not with TRP-213, hinting at a possible non-competitive or allosteric mode of interaction.

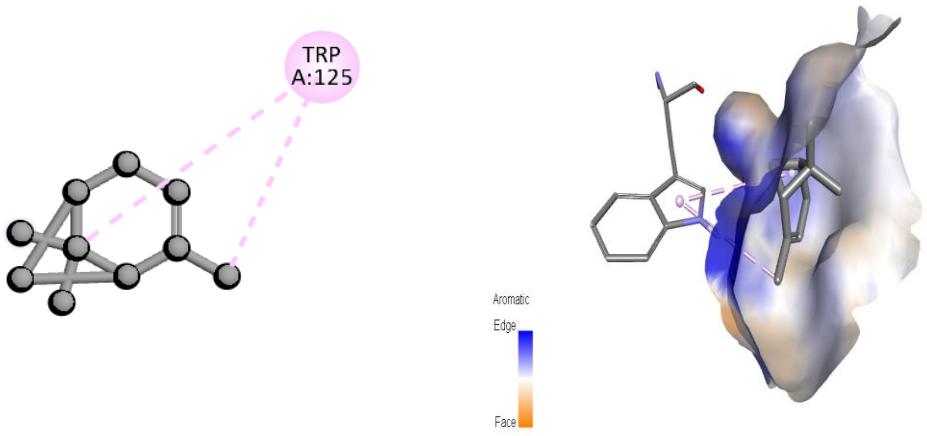
Overall, the in-silico ligand-protein interaction analysis supports the anti-inflammatory potential of the essential oil components, highlighting specific BSA residues—such as TRP-213, TYR-150, ARG-198, and HIS-242—as critical to their binding. These interactions correlate well with the in vitro BSA protein denaturation inhibition results and affirm the possibility of binding between BSA and the phytochemicals present in *Ammodaucus leucotrichus* and *Mentha piperita* essential oils. The best ligand-binding poses within the BSA binding site and the corresponding interacting amino acid residues are presented in Figure 16.



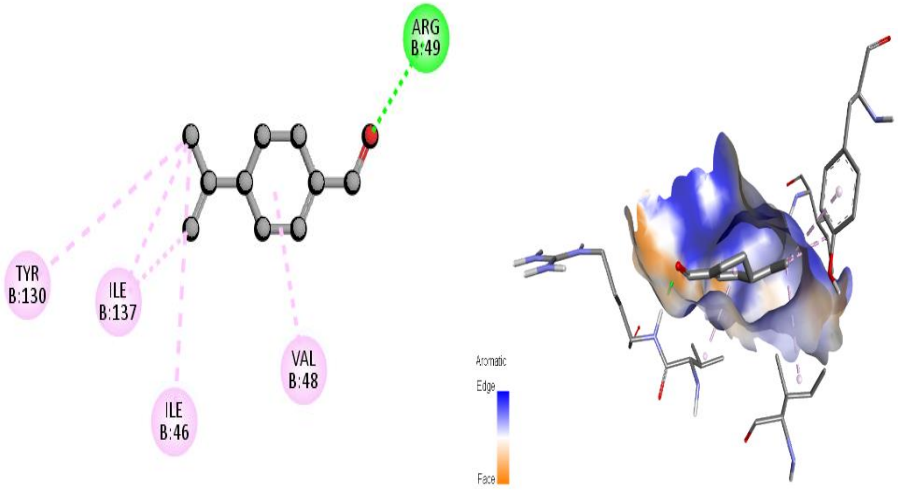
Docking complex and interaction plot for compound Pulegone with BSA



Docking complex and interaction plot for compound Pulegone with BSA



Docking complex and interaction plot for compound Alpha-pinene with BSA



Docking complex and interaction plot for compound Perilla aldehyde with BSA

Figure 16. Molecular interactions of studied compounds & Diclofenac with BSA

Conclusion

Conclusion:

In recent years, plant-based compounds have garnered increasing attention in scientificresearch due to their potential applications in the medical field. Molecules extractedfrom natural plants represent a vital source of drugs. This study focused on the characterization of both *Ammodaucus leucotrichus* and *Mentha piperita*, and the evaluation of their biological activity *in vivo*, *in vitro*, with an emphasis on molecular structure, and molecular dynamics. The results showed that the essential oils of these two plants (MP. AL) possess several promising biological activities, particularly their potential anti-inflammatory effects, making them compounds of potential significance in medicine and pharmacy. The findings suggest that these oils may contribute to alleviating inflammatory processes in the body.

A phytochemical study revealed that the effective dose of the two plants is rich in polyphenols, flavonoids, tannins, saponins, and terpenoids, which are known for their anti-inflammatory properties. Laboratory tests demonstrated that the essential oils of the two medicinal plants (MP. AL) exhibit activity that inhibits potential inflammatory, pathways. *In silico* molecular docking studies confirmed that the components of the essential oils of the two plants (MP. AL) can bind to key molecules involved in the inflammatory response. Toxicity studies showed that the essential oils of both *Ammodaucus leucotrichus* and *Mentha piperita* are non-toxic, enhancing the safety of their potential use as therapeutic agents. The results of this study can guide future research towards exploring their use as potential drugs for treating various inflammatory conditions.

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Annexes

Annex 01: The cages of the different batches of rats

Annex 02: The stages of nursing and treating mice

Annex 03: blood drawing. Slaughtering and dissecting mice

Annex 04: Preparing samples

Annex 05: Steps and tools used when preparing histological cuts

Annex 06: Some of the machines used