KINGDOM OF SAUDI ARABIA
Ministry of Education
Qassim University
College of Science
Department of Chemistry

Extraction of essential oil from *Artemisia campestris* and its application studies

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN CHEMISTRY

By

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بسم الله الرحمن الرحيم

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RECOMMENDATION OF THE COMMITTEE

The committee has approved this **disquisition** as a partial completion of the requirement for the degree of

MASTER OF SCIENCE IN CHEMISTRY

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Dedication

I dedicate the fruit of my efforts to those who supported me through every moment of weakness: my beloved mother, and the one whose hard work paved the way for my life, my dear father. To my siblings and family, the pillars of my journey and my constant companions, whose guidance, encouragement, and belief in me have always been a source of strength, I express my deepest gratitude. This work is dedicated to all of you in appreciation of your unwavering support.

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List of Abbreviations

ABTS	2, 2- azino bis 3-ethylbnzothiazolin-6-sulfonic acid
ADME	Absorption, Distribution, Metabolism, and Excretion
ACEO	Artemisia campestris essential oil
Ache	Acetyl cholinesterase
CPF	Chlorpyrifos
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EFSA	European food safety authority
EO	Essential oil
EMA	European medicines agency
EPA	Environmental protection agency
FDA	Food and drug administration
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometer
GRAS	Generally recognized as safe
Hb	Hemoglbin
Ht	Hematocrit
HPLC	High-performance liquid chromatography
IFRA	International fragrance association
Kx21n(CHU)	21 n automate sysmex kx
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
OP	Organophosphorus
Ops	Organophsphorus pesticides
OPLS4	Optimized potentials for liquid simulations version 4
PCA	Principal component analysis
PLT	Platelet count
RBC	Red blood cell
ROS	Reactive oxygen species
SFE	Supercritical fluid extraction
SP	Standard Precision
SPC	Simple Point Charge
WBC	White blood cell

ABSTRACT

Extraction of essential oil from *Artemisia campestris* and its application studies

By

Ruyuf Moalla Alharbi

This research investigated the protective effects of *Artemisia Campestris* essential oil (ACEO) against chlorpyrifos (CPF) toxicity in rats supported by computational modelling. Rats were divided into four groups: Group 1 (control), Group 2 (CPF treatment for 20 days, 10mg/kg/day), Group 3 (pre-treatment with ACEO for 10 days followed by CPF for 10 days), and Group 4 (ACEO treatment for 20 days). Our findings showed that CPF administration led to hematological disturbances, such as increased white blood cells and decreased red blood cells, hemoglobin, and hematocrit. CPF also disturbed biochemical parameters like acetylcholinesterase (AChE), glycemia, cholesterol, and total bilirubin. Histopathological changes were observed in the brain and spleen. ACEO pre-treatment protected against these disturbances. Histopathological analysis showed that ACEO improved tissue damage in the brain and spleen caused by CPF. In silico ADMET predictions indicated that the major molecules in ACEO exhibited favorable pharmacokinetics. Seven phytoconstituents showed significant binding affinity to human AChE pointing to their potential as neuroprotective agents. The stability of (+)-spathulenol, a lead compound, was studied through dynamic simulations for 200ns, showing promise as an AChE inhibitor similar to Donepezil. This study highlights ACEO's potential as an eco-friendly agent to enhance antioxidant defenses and counteract CPF's harmful effects.

Chapter One

LITERATURE REVIEW

1.1. Introduction

The genus *Artemisia*, a prominent member of the Asteraceae family, encompasses over 500 species distributed widely across the Northern Hemisphere, particularly in temperate and arid regions of Asia, Europe, and North America [1,2]. Plants of this genus are characterized by their aromatic nature and a rich history of ethnomedicinal use, with several species having well-established therapeutic profiles [3]. Among them, *Artemisia annua* is globally recognized for producing artemisinin, an antimalarial compound that has revolutionized malaria treatment [4,5]. Other species like *A. absinthium*, *A. vulgaris*, and *A. dracunculus* are also widely studied for their pharmacological potential [2,6].

One particularly underexplored yet promising species within the genus is *Artemisia campestris* L., commonly known as field wormwood. This perennial herb is native to Europe and extends through North Africa, the Middle East, and Asia [7,8]. It thrives in diverse habitats, including steppes, sandy soils, and coastal zones, often exhibiting significant morphological and phytochemical diversity depending on environmental conditions [9]. Morphologically, *A. campestris* is distinguished by its slender, branched stems, finely divided leaves, and small, yellowish flower heads [10]. The plant is adapted to xerophytic conditions and is resilient against drought and poor soils, making it ecologically significant in its native habitats [11].

Ethnobotanically, *A. campestris* has been used in traditional medicine systems of North Africa, the Mediterranean, and Central Asia for centuries. It has been employed to treat a range of conditions, including gastrointestinal disorders, inflammation, fever, wounds, and parasitic infections [12,13]. Decoctions and infusions prepared from its aerial parts have traditionally been used as vermifuges, antipyretics, and digestive aids [14]. In Algerian and Moroccan folk medicine, the

plant is also used externally to treat skin diseases and internally as a general tonic [15]. These traditional uses are supported by pharmacological investigations, which have reported antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of *A. campestris* extracts and essential oils [16,17].

Central to the therapeutic effects of *A. campestris* is its essential oil (EO), a volatile mixture rich in monoterpenes, sesquiterpenes, and other oxygenated compounds [18,19]. Essential oils, by nature, are complex secondary metabolites that serve plants in ecological roles such as defense and pollinator attraction [20]. In modern science, EOs have garnered significant attention for their potential as natural antimicrobial agents, antioxidants, and anti-inflammatory compounds, especially in the face of growing resistance to synthetic drugs and consumer demand for plant-based remedies [21,22]. The biological activity of EOs is closely linked to their chemical composition, which can vary widely due to genetic, environmental, and seasonal factors [23].

EOs from various *Artemisia* species have been extensively studied for their pharmacological benefits, and *A. campestris* is no exception. Its EO has shown strong antimicrobial activity against a wide range of bacterial and fungal pathogens, in some cases comparable to standard antibiotics [19,24]. Moreover, studies have reported significant antioxidant properties through assays such as DPPH and ABTS, indicating its potential role in oxidative stress management [13]. Recent investigations have also pointed toward cytotoxic effects on cancer cell lines, suggesting the possibility of developing EO-based anticancer formulations [17].

Despite this potential, research on *A. campestris* EO remains scattered and lacks a comprehensive synthesis of its phytochemical diversity, bioactivities, and commercial viability. With the growing global interest in plant-based therapeutics and sustainable bioresources, there is a pressing need to consolidate existing knowledge on this species and identify opportunities for further research and industrial application [25,26]. This review aims to provide a detailed examination of *Artemisia campestris* essential oil, encompassing its botanical and geographical

background, traditional uses, extraction techniques, chemical composition, biological and pharmacological activities, industrial applications, toxicity profile, and future research directions. By integrating findings from ethnopharmacology, phytochemistry, and biomedical sciences, this review highlights *A. campestris* EO as a multifaceted aromatic resource with significant promise for therapeutic and commercial exploitation.

1.2. Botanical Description and Distribution

1.2.1 Morphological Characteristics of *Artemisia campestris*

Artemisia campestris L., commonly known as field wormwood, is a herbaceous or semi-woody perennial plant belonging to the Asteraceae family. It typically grows between 20–100 cm in height and displays a highly branched, erect stem, which can be glabrous or slightly pubescent. The leaves are alternately arranged, finely divided, and covered with silvery hairs, giving the plant a greyish-green appearance. Basal leaves are longer and more deeply lobed than those on the stem. The inflorescences are terminal panicles composed of small, yellowish to reddish capitula (flower heads), each surrounded by involucral bracts. Flowering generally occurs from July to October depending on the geographic region [27].

Figure 1.1. Artemisia campestris L. | Plants

1.2.2 Taxonomic Classification

Artemisia campestris L. belongs to the large and diverse genus *Artemisia*, which is part of the Asteraceae family, one of the largest families of flowering plants. The genus includes more than 500 species, many of which are renowned for their medicinal, aromatic, and ecological importance [28]. Within this genus, *A. campestris* is a polymorphic species, displaying substantial variation across its geographic range. This diversity is reflected in the existence of multiple subspecies and varieties, which differ in morphological features, habitat preferences, and chemical composition. These differences are often driven by local environmental pressures, leading to adaptations that enhance survival in arid or semi-arid ecosystems. The formal taxonomic classification of *A. campestris* is presented in the table below (Table 1).

Table 1. 1. Taxonomic classification of *Artemisia campestris* L.

Taxonomic Rank	Classification
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Order	Asterales
Family	Asteraceae
Genus	*Artemisia*
Species	*Artemisia campestris* L.

This taxonomic placement aligns *A. campestris* with a group of plants that have adapted to thrive in diverse climates, from temperate to arid zones. Understanding the taxonomy of *A. campestris* is not only important for botanical classification but also for guiding research into its phytochemistry and pharmacological potential, as taxonomic differences often correlate with variations in essential oil composition and bioactivity.

1.2.3 Natural Habitats and Global Distribution

A. campestris is widely distributed across the Northern Hemisphere, especially in Europe, North Africa, and temperate regions of Asia. It is also found in parts of North America. This species typically grows in dry, sandy, and rocky soils, including coastal dunes, grasslands, steppes, and degraded lands. It thrives in open, sun-exposed areas and is tolerant of arid to semi-arid climates (Table 2) [29].

Table 1.2. Global distribution of *Artemisia campestris*

Region	Countries	Habitat Type
North Africa	Algeria, Morocco, Tunisia	Arid/semi-arid, steppe, dunes
Europe	France, Spain, Italy, Balkans, Eastern Europe	Coastal areas, grasslands
Asia	Iran, Central Asia, Siberia	Steppes, rocky slopes
North America	USA, Canada	Prairies, sandy wastelands

1.2.4 Chemotypes and Ecological Factors Influencing Composition

The phytochemical composition of *A. campestris* is highly variable and influenced by ecological, climatic, and geographical factors. Several chemotypes have been identified across different regions, distinguished by variations in major constituents of the essential oil, such as camphor, 1,8-cineole, myrcene, borneol, and spathulenol. For example:

- In Algeria, *A. campestris* essential oil is often rich in camphor and 1,8-cineole [30].
- In Tunisia, the plant shows higher levels of α-thujone and borneol [31].
- In Central Asia, chemotypes are dominated by sesquiterpenes such as caryophyllene oxide [32].

1.3. Methods of Extraction and Analysis

1.3.1 Common Extraction Techniques

The essential oils of *Artemisia campestris* are primarily extracted from the aerial parts of the plant using several methods, with hydrodistillation being the most widely employed technique. This method involves immersing plant material in water and boiling it to release volatile compounds, which are then condensed and collected. It is the traditional method recommended by the European Pharmacopoeia and widely used due to its simplicity and effectiveness in preserving thermolabile components (Table 3) [33].

Other techniques include:

- **Steam distillation**: Similar to hydrodistillation but uses steam passed through plant material. It is more energy-efficient and preferred for industrial-scale production.
- **Solvent extraction**: Uses organic solvents (e.g., hexane, ethanol) to extract essential oils, especially when targeting less volatile or heat-sensitive compounds. This method often yields concrete or absolute forms [34].
- **Supercritical fluid extraction (SFE)**: Utilizes supercritical CO_2 for a high-purity extract with minimal degradation. Though more expensive, SFE is gaining popularity for sensitive phytochemical profiles [35].

Table 1.3. Comparison of Essential Oil Extraction Methods for *A. campestris* [33,35]

Extraction Method	Principle	Advantages	Limitations
Hydrodistillation	Boiling plant material in water	Simple, traditional, suitable for volatile oils	Time-consuming, potential thermal degradation
Steam Distillation	Steam passes through plant material	Efficient, scalable	Less suitable for heat-sensitive compounds
Solvent Extraction	Organic solvents extract EOs	Suitable for non-volatile or delicate compounds	Solvent residue, longer process
Supercritical CO_2 (SFE)	Uses supercritical CO_2 to extract	High purity, minimal heat degradation	Expensive equipment, complex setup

1.3.2 Factors Affecting Yield and Composition

The yield and chemical composition of *A. campestris* essential oil are influenced by a combination of **intrinsic** and **extrinsic** factors:

- **Plant part used**: Leaves generally yield more oil and a richer profile than stems or flowers.
- **Developmental stage**: Harvesting during the flowering stage often results in higher oil content and a broader spectrum of bioactive compounds [29].
- **Geographical origin and climate**: Altitude, temperature, and precipitation directly impact secondary metabolite biosynthesis. For instance, plants in arid zones show higher concentrations of oxygenated monoterpenes like camphor and 1,8-cineole [30].
- **Extraction parameters**: Time, temperature, and distillation method significantly affect both yield and quality.

1.4. Chemical Composition of *Artemisia campestris* Essential Oil

1.4.1 Major Chemical Constituents

The essential oil of *Artemisia campestris*, a species in the Asteraceae family, is known for its complex chemical composition. Major constituents identified include monoterpenes, sesquiterpenes, and oxygenated compounds (Table 4). The most prominent chemical components of *A. campestris* essential oil include:

- **Camphor**: A bicyclic ketone with a characteristic odor, camphor is one of the most abundant constituents in *A. campestris* oil. It has antimicrobial, anti-inflammatory, and analgesic properties [36].

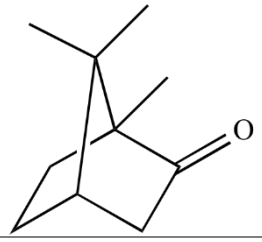

Figure 1.2. Structure of Camphor

- **1,8-Cineole**: Also known as eucalyptol, it is a monoterpene oxide with a cooling, minty aroma. It has antiseptic and bronchodilatory properties [37].

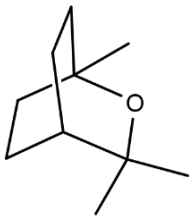

Figure 1.3. Structure of 1,8-Cineole

- **Borneol**: A bicyclic alcohol that contributes to the oil's cooling, minty aroma. It has antifungal and anti-inflammatory properties [36].

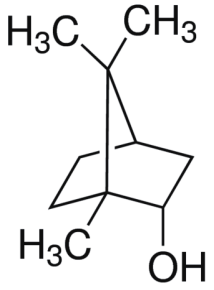

Figure 1.4. Structure of Borneol

- **Thujone**: A ketone found in many Artemisia species, thujone has potential neurotoxic effects but also displays antimicrobial and insecticidal properties [37].

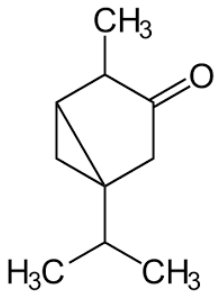

Figure 1.5. Structure of Thujone

- **α-Pinene**: A monoterpene that is found in many essential oils, α-pinene has a fresh pine-like aroma and exhibits anti-inflammatory and antimicrobial activities [36].

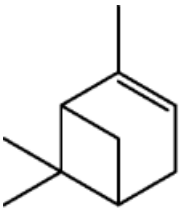

Figure 1.6. Structure of α-Pinene

- **ß-Pinene**: Another monoterpene that contributes to the fragrance profile of the oil and has been shown to possess anti-inflammatory properties [37].

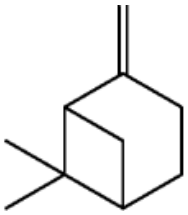

Figure 1.7. Structure of ß-Pinene

- **Linalool**: A terpenoid alcohol with a floral lavender-like scent, linalool has calming, anti-anxiety, and sedative effects [36].

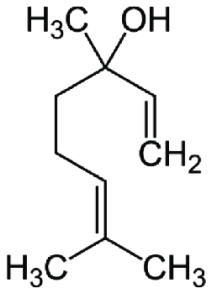

Figure 1.8. Structure of Linalool

Table 1.4: Major Chemical Constituents of *Artemisia campestris* Essential Oil

Compound	Chemical Formula	Percentage in Essential Oil	Properties
Camphor	$C_{10}H_{16}O$	10-40%	Antimicrobial, anti-inflammatory
1,8-Cineole	$C_{10}H_{18}O$	5-20%	Antiseptic, bronchodilator
Borneol	$C_{10}H_{18}O$	5-15%	Antifungal, anti-inflammatory
Thujone	$C_{10}H_{16}O$	1-10%	Antimicrobial, insecticidal
α-Pinene	$C_{10}H_{16}$	2-10%	Anti-inflammatory, antimicrobial
ß-Pinene	$C_{10}H_{16}$	2-10%	Anti-inflammatory
Linalool	$C_{10}H_{18}O$	1-5%	Calming, anti-anxiety

1.4.2 Variation in Composition Based on Geography, Climate, and Harvest Time

The chemical composition of *A. campestris* essential oil can vary significantly depending on several environmental and seasonal factors:

- **Geographical Variation**: Studies have shown that *A. campestris* from different regions exhibit variations in the levels of major constituents. For example, plants grown in Mediterranean climates tend to have higher concentrations of camphor and 1,8-cineole, while those from temperate regions may show increased levels of borneol and thujone [37].
- **Climate Conditions**: Temperature, humidity, and rainfall during the growing season can influence the biosynthesis of essential oil constituents. Dry climates may lead to higher concentrations of monoterpenes, whereas more humid conditions can result in higher levels of oxygenated compounds like camphor and borneol.
- **Harvest Time**: The timing of the harvest can also affect the oil's composition. Essential oils extracted from plants harvested during peak flowering or early fruiting periods may contain higher levels of certain volatile compounds. For instance, the concentration of 1,8-cineole can vary significantly depending on whether the plant is harvested in the early morning or late afternoon [36].

1.4.3 Chemometric Analysis and Cluster Grouping

Chemometric methods, such as principal component analysis (PCA), hierarchical clustering, and multivariate analysis, have been widely used to study the variation in the chemical composition of essential oils. These techniques help identify patterns in the data that may be associated with geographic location, harvest time, and climatic factors.

- **Cluster Analysis**: Through chemometric analysis, researchers can group essential oils into clusters based on their chemical similarity. This allows for the identification of patterns that correlate with specific environmental conditions [36].
- **Principal Component Analysis (PCA)**: PCA can be used to reduce the dimensionality of large datasets, helping to visualize the differences and similarities in the chemical composition of essential oils from different regions and harvest times [36].

Such analyses have revealed that *A. campestris* essential oils from plants grown in similar climates often exhibit closely related chemical profiles, while oils from geographically distant populations show distinct compositional differences [37].

1.5. Biological and Pharmacological Activities

The essential oil of *Artemisia campestris* has been studied for a wide range of biological and pharmacological activities [38]. These activities include antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, and other protective effects. Below is a detailed overview of the different biological activities associated with *A. campestris* essential oil.

1.5.1. Anticancer and Cytotoxic Effects

Artemisia campestris essential oil has shown promising anticancer and cytotoxic properties in various in vitro and in vivo studies. The oil has been reported to inhibit the growth of several cancer cell lines and demonstrates selective cytotoxicity.

Evidence from *in vitro* and *in vivo* models:

Several studies have demonstrated that *A. campestris* essential oil possesses anticancer potential. In vitro assays have revealed that the oil significantly inhibits the proliferation of cancer cells, including breast, lung, and colon cancer cell lines.

The oil induces cell cycle arrest, apoptosis, and inhibits tumor cell migration and invasion. For example, a study by Rashed et al., [36] showed that the essential oil of *A. campestris* exhibited a dose-dependent inhibition of cell growth in human colorectal cancer cells (HCT116). The oil also induced apoptosis, as evidenced by increased caspase-3 and caspase-9 activity. *In vivo* studies have further supported these findings, with essential oil demonstrating antitumor effects in animal models, including a reduction in tumor size and weight when administered to mice with induced breast cancer [37].

Synergistic Effects with Chemotherapeutic Agents:

Artemisia campestris essential oil has been found to enhance the effects of standard chemotherapeutic agents. Studies show that the combination of *A. campestris* essential oil with agents like doxorubicin or cisplatin leads to increased cytotoxicity and improved anticancer effects, possibly through the modulation of drug resistance mechanisms in cancer cells (Table 5).

Table 1.5: Anticancer and Cytotoxic Effects of *Artemisia campestris* Essential Oil [36,37]

Study Model	Effect	Mechanism of Action
In vitro (various cancer cell lines)	Inhibition of cell proliferation	Induction of apoptosis, cell cycle arrest
In vitro (HCT116 cells)	Cytotoxicity and growth inhibition	Caspase activation, apoptosis
In vivo (mice with breast cancer)	Reduced tumor size and weight	Tumor growth inhibition, apoptotic pathways

1.5.2. Other Biological Activities

Apart from its anticancer and cytotoxic properties, *Artemisia campestris* essential oil has demonstrated several other beneficial biological activities:

Insecticidal and Larvicidal Effects:

A. campestris essential oil has been shown to possess strong insecticidal and larvicidal properties, particularly against mosquitoes and agricultural pests. Studies indicate that the essential oil can effectively kill insect larvae and act as a repellent. The oil's compounds, such as 1,8-cineole and camphor, contribute to these effects (Table 6).

Antidiabetic Potential:

The oil has been reported to exhibit antidiabetic effects, as it can help regulate blood sugar levels. In animal models, *A. campestris* essential oil has been shown to improve insulin sensitivity and reduce blood glucose levels.

Hepatoprotective Effects:

Artemisia campestris essential oil has demonstrated hepatoprotective properties, protecting the liver from oxidative damage and toxic agents. In experimental models, it significantly reduced liver enzyme levels, indicating its potential in preventing liver damage [37].

Neuroprotective Potential:

The essential oil's neuroprotective effects have been highlighted in studies related to neurodegenerative diseases. It has been shown to alleviate symptoms associated with oxidative stress-induced neurodegeneration, which is promising for conditions like Alzheimer's disease [36].

Table 1.6: Other Biological Activities of *Artemisia campestris* Essential Oil

Activity	Model/Condition	Effect	Mechanism of Action
Insecticidal /Larvicidal	Mosquitoes, agricultural pests	Effective larvicidal and repellent	Neurotoxic effects through inhibition of acetylcholinstrase and disruption of the insect nervous system, leading to paralysis and death
Antidiabetic	Animal models (rats)	Reduced blood glucose levels	Improvement of insulin sensitivity
Hepatoprotective	Animal models (liver damage)	Reduced liver enzyme levels	Antioxidant effects, detoxification
Neuroprotective	Animal models (neurodegeneration)	Alleviation of neurodegenerative symptoms	Antioxidant, anti-inflammatory

1.6. Industrial and Commercial Applications

The essential oil of *Artemisia campestris* has garnered significant attention for its diverse industrial and commercial applications due to its potent biological properties. These applications span multiple sectors, including food preservation, cosmetics, personal care products, and bio-pesticides.

1.6.1. Use in Food Preservation and Flavoring

Artemisia campestris essential oil is recognized for its antimicrobial and antioxidant properties, making it a valuable ingredient in food preservation and flavoring. The oil's ability to inhibit microbial growth and prevent spoilage has led to its exploration as a natural preservative in food products. It is especially useful in extending the shelf life of perishable goods such as meat, dairy, and baked products.

- **Preservative Properties**: The essential oil's antimicrobial properties, particularly against bacteria and fungi, make it suitable for use in food products to prevent contamination and extend freshness [36]. The oil's active compounds, such as camphor and 1,8-cineole, are effective against a wide range of spoilage microorganisms.
- **Flavoring Agent**: The distinctive aroma of *A. campestris* essential oil, which is a blend of herbal, camphorous, and minty notes, has been utilized as a natural flavoring agent in food products. It is particularly popular in beverages, confectionery, and sauces, where its unique flavor profile can enhance the taste.

1.6.2 Applications in Cosmetics and Personal Care Products

The essential oil of *Artemisia campestris* has found multiple uses in the cosmetics and personal care industries due to its beneficial properties for skin and hair. Its antimicrobial, anti-inflammatory, and antioxidant effects make it an excellent addition to formulations aimed at promoting skin health and addressing common cosmetic concerns.

- **Skin Care**: The oil's antimicrobial properties help in treating acne, fungal infections, and skin irritations. Its anti-inflammatory effects make it a suitable ingredient for soothing and calming the skin [37]. Additionally, its antioxidant activity protects the skin from oxidative stress, which is a major contributor to premature aging.
- **Hair Care**: *A. campestris* essential oil has been used in hair care products to promote scalp health, reduce dandruff, and improve hair growth. Its antifungal properties help combat scalp conditions caused by fungal infections, while its antimicrobial effects prevent bacterial growth [36].
- **Perfume and Fragrance**: Due to its unique aroma, *A. campestris* essential oil is also utilized in the perfume industry to create distinctive fragrances. The

oil's refreshing scent, which combines herbal and minty notes, is incorporated into both men's and women's fragrances.

1.6.3 Potential as a Bio-pesticide or Eco-friendly Agent

With the increasing demand for environmentally friendly agricultural practices, *Artemisia campestris* essential oil has emerged as a potential bio-pesticide. Its insecticidal, larvicidal, and repellent properties make it an effective and eco-friendly alternative to chemical pesticides (Table 7).

- **Insecticidal and Larvicidal Effects**: As mentioned previously, *A. campestris* essential oil has been shown to effectively kill insect larvae and act as a repellent. These properties have been utilized in the development of bio-pesticide formulations to control pests in crops.
- **Eco-friendly Pesticide**: The oil's low toxicity to humans and animals, along with its biodegradable nature, makes it an attractive alternative to synthetic chemical pesticides. Unlike conventional pesticides, *A. campestris* essential oil breaks down naturally in the environment, posing minimal risk of pollution and ecosystem disruption [37].
- **Plant Protection**: Additionally, its application as a plant protectant helps control fungal diseases in crops, reducing the need for chemical fungicides. This is especially beneficial in organic farming practices, where the use of synthetic chemicals is restricted.

Table 1.7: Industrial and Commercial Applications of *Artemisia campestris* Essential Oil

Application Area	Use	Active Compounds	Benefits/Properties
Food Preservation and Flavoring	Preservative and flavoring	Camphor, 1,8-Cineole	Antimicrobial, antioxidant, enhances flavor
Cosmetics and Personal Care	Skin care, hair care, fragrance	Camphor, Linalool, Borneol	Antimicrobial, anti-inflammatory, antioxidant, soothing
Bio-pesticide and Eco-friendly Agent	Insecticidal, larvicidal, plant protection	1,8-Cineole, Camphor	Biodegradable, effective against pests and fungi, low toxicity

1.7. Toxicity and Safety Aspects

Despite the promising pharmacological properties of *Artemisia campestris* essential oil, its safety and toxicity profile must be carefully evaluated, especially for long-term use in humans and animals. Various studies have been conducted to assess the acute and chronic toxicity, cytotoxicity, genotoxicity, safe dosage ranges, and regulatory considerations for its use in different applications.

1.7.1 Acute and Chronic Toxicity Data

Acute toxicity studies assess the immediate harmful effects of a substance after a single dose or exposure. Research on *Artemisia campestris* essential oil suggests that, while the oil exhibits beneficial effects, it can be toxic at high concentrations. In animal studies, oral administration of the essential oil has resulted in mild to moderate toxicity symptoms, including gastrointestinal irritation, lethargy, and in severe cases, death at extremely high doses [37]. A study by Rashed et al., [36] in rats showed that a single high dose (greater than 500 mg/kg body weight) of *A. campestris* essential oil led to toxic signs like excessive salivation and respiratory

distress. However, lower doses (below 200 mg/kg body weight) did not produce any significant adverse effects.

Chronic toxicity refers to the long-term effects of repeated exposure to a substance. Long-term exposure to *A. campestris* essential oil, particularly at doses exceeding the recommended therapeutic levels, has been associated with liver damage and kidney dysfunction in some animal models. However, the oil appears to be relatively safe when used in low concentrations and in short-term applications, such as in cosmetic formulations and food products.

1.7.2 Cytotoxicity and Genotoxicity Assessments

Cytotoxicity refers to the potential of a substance to damage or kill cells. Several in vitro studies have assessed the cytotoxic effects of *A. campestris* essential oil on human cell lines. While the essential oil exhibits anticancer activity, it has also shown cytotoxic effects on non-cancerous cells at higher concentrations. Studies using human fibroblast and liver cells (HepG2) indicated that *A. campestris* oil can induce cell death, but these effects were generally dose-dependent [40]. At therapeutic doses, the oil demonstrated selective toxicity towards cancer cells, making it a promising anticancer agent.

Genotoxicity refers to the ability of a substance to cause genetic damage, leading to mutations or cancer. Few studies have directly assessed the genotoxicity of *A. campestris* essential oil. However, in some preliminary studies, the oil's major constituents, such as camphor and 1,8-cineole, have been shown to possess genotoxic potential at high concentrations, particularly in bacterial mutagenesis assays [40]. While the oil does not exhibit significant genotoxicity at typical exposure levels, caution should be exercised when using it in high doses or over prolonged periods.

1.7.3 Safe Dosage Ranges and Regulatory Considerations

Safe Dosage Ranges:

- Cosmetic Use

According to guidelines from regulatory bodies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), essential oils are generally recognized as safe (GRAS) for cosmetic use in concentrations ranging from 0.1% to 5% depending on the product. For skin-care products, *A. campestris* essential oil is typically included at concentrations of 0.5% to 2% in formulations.

- Oral Consumption

For oral consumption, it is recommended to keep the dose of *A. campestris* essential oil below 0.5 mL per day, based on its potential for toxicity at higher doses [37]. Doses above this level may lead to gastrointestinal distress, liver, and kidney dysfunction, particularly with prolonged use.

Regulatory Considerations:

In the European Union, essential oils are subject to the regulations set by the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA). These bodies require the submission of safety data on essential oils, including toxicity assessments, before they can be used in food, medicinal, or cosmetic products.

In the United States, essential oils are typically classified as GRAS (Generally Recognized as Safe) by the FDA for use in food products and cosmetics, provided they are used in low concentrations and do not exceed established safety limits.

Toxicological Studies and Certifications

Before using *A. campestris* essential oil in consumer products, particularly food and medicinal products, it is essential to conduct comprehensive toxicological studies.

These studies should include dermal irritation tests, patch tests, and long-term animal studies to ensure consumer safety. Additionally, certification from recognized bodies such as the International Fragrance Association (IFRA) or the Environmental Protection Agency (EPA) is necessary for ensuring the safe application of *A. campestris* essential oil in various industries (Table 8).

Table 1.8: Toxicity and Safety Aspects of *Artemisia campestris* Essential Oil

Toxicity Aspect	Findings	Safety Recommendations
Acute Toxicity	Mild to moderate toxicity at high doses (500 mg/kg in rats)	Avoid high doses, limit to <200 mg/kg for oral use
Chronic Toxicity	Liver and kidney dysfunction with prolonged use at high doses	Limit long-term use and adhere to recommended concentrations
Cytotoxicity	Selective toxicity to cancer cells, but non-cancer cells affected at higher concentrations	Use in therapeutic doses for anticancer purposes, avoid high concentrations in non-cancer treatments
Genotoxicity	Potential genotoxic effects at high doses (camphor, 1,8-cineole)	Monitor for prolonged or high-dose use
Safe Dosage	0.1% to 5% for cosmetics, <0.5 mL/day for oral use	Follow regulatory guidelines for safe concentrations
Regulatory Considerations	GRAS for food and cosmetic use within safety limits	Comply with EFSA, EMA, and FDA guidelines for product formulation

1.8. Challenges and Future Perspectives

Despite the growing interest in *Artemisia campestris* essential oil for its wide range of biological and industrial applications, several challenges remain that hinder its full potential. These challenges include gaps in current research, issues related to standardization and quality control, the need for clinical validation and formulation development, and concerns about the sustainability of harvesting and

cultivation practices. Addressing these challenges will be crucial for ensuring the safe, effective, and sustainable use of *A. campestris* essential oil in various fields.

1.8.1. Gaps in Current Research

While *Artemisia campestris* essential oil has demonstrated promising biological and pharmacological activities, there are significant gaps in our understanding of its full therapeutic potential and mechanisms of action. Some of the key research gaps include:

Comprehensive Toxicological Data: Although some studies have evaluated the toxicity of *A. campestris* essential oil, more detailed and extensive research is needed to understand its long-term effects on human health, particularly with chronic exposure. Research on its interaction with other pharmaceutical compounds or potential adverse effects in vulnerable populations (e.g., pregnant women, children) is also lacking.

Pharmacokinetics and Bioavailability: The absorption, distribution, metabolism, and excretion (ADME) properties of *A. campestris* essential oil and its components have not been well studied. Future research should focus on the pharmacokinetics of the oil's bioactive compounds to determine how they are absorbed and metabolized in the human body.

Mechanisms of Action: While many studies have demonstrated the biological effects of *A. campestris* essential oil, the underlying molecular mechanisms, such as receptor interactions, signal transduction pathways, and enzyme modulation are not yet fully understood. Further research is needed to elucidate these mechanisms and identify potential targets for therapeutic interventions.

1.8.2. Standardization and Quality Control Issues

One of the major challenges in the use of *Artemisia campestris* essential oil is the lack of standardization and quality control in its production and application. Essential oils are highly susceptible to variations in composition based on factors

such as geographic origin, climatic conditions, and harvesting methods. These variations can result in inconsistent therapeutic effects and efficacy.

Geographical Variability

A. campestris essential oil's chemical composition varies significantly depending on the region where it is grown, the time of harvest, and even the specific cultivar. This geographical variability makes it difficult to standardize the oil for commercial and therapeutic use, potentially affecting product efficacy and safety.

Batch-to-Batch Consistency

To ensure consistent quality and therapeutic efficacy, stringent quality control measures must be implemented during the harvesting, extraction, and packaging processes. Analytical techniques like gas chromatography (GC) and high-performance liquid chromatography (HPLC) are crucial for ensuring the consistency of key active compounds in each batch.

Standardization of Active Compounds

There is a need to establish standardized concentrations of key active compounds, such as camphor and 1,8-cineole, to ensure the oil's efficacy in various applications. This would enable manufacturers to produce consistent, high-quality products.

1.8.3.Need for Clinical Validation and Formulation Development

While the antimicrobial, anticancer, and antioxidant properties of *A. campestris* essential oil have been supported by *in vitro* and animal studies [41], clinical trials are essential to validate its efficacy and safety in human populations. Some key areas requiring further research include:

Clinical Trials

Rigorous clinical studies are needed to confirm the oil's therapeutic potential in humans, especially for conditions such as cancer, inflammation, and

diabetes. These trials should focus on determining optimal dosages, treatment durations, and potential side effects in humans.

Formulation Development

The development of effective and stable formulations that deliver the therapeutic benefits of *A. campestris* essential oil is another critical area of research. Given the volatility and potency of essential oils, advanced formulation technologies such as nanoencapsulation, liposomal delivery systems, and controlled-release formulations should be explored to enhance bioavailability and reduce potential toxicity.

Synergistic Effects

Investigating the potential synergistic effects of *A. campestris* essential oil in combination with other therapeutic agents (e.g., chemotherapeutic drugs) could open new avenues for its use in integrated therapies. Clinical studies exploring these combinations are necessary to determine their effectiveness and safety in treating complex conditions like cancer and infectious diseases.

1.8.4. Sustainable Harvesting and Cultivation Practices

As the demand for *Artemisia campestris* essential oil grows, ensuring the sustainability of its production is crucial to prevent overharvesting and protect biodiversity [42]. Several factors need to be addressed to ensure that the cultivation and harvesting of *A. campestris* are environmentally sustainable:

Overharvesting and Conservation

In some regions, the collection of wild *A. campestris* plants for essential oil extraction has led to overharvesting and potential threats to natural populations. Sustainable harvesting practices, such as limiting the amount of wild plants harvested and promoting the cultivation of *A. campestris*, are necessary to prevent depletion of natural resources.

Cultivation Practices

Encouraging the cultivation of *A. campestris* under controlled conditions can help reduce the pressure on wild populations. However, the cultivation of *A. campestris* needs to be done with careful consideration of local ecosystems and soil health. Organic farming practices should be promoted to avoid the use of harmful pesticides and fertilizers, which could reduce the quality of the essential oil.

Environmental Impact

The extraction of essential oils can have environmental implications, especially if solvents or chemical extraction methods are used [43]. Sustainable extraction methods, such as steam distillation, should be prioritized to minimize the environmental footprint of essential oil production.

Table 1.9: Key Challenges and Future Perspectives for *Artemisia campestris* Essential Oil

Challenge	Current Status	Future Perspective
Research Gaps	Limited pharmacokinetics, mechanistic studies, and clinical data	Focus on in vivo studies, clinical trials, and molecular mechanisms
Standardization and Quality Control	High variability due to geography, harvesting, and extraction methods	Development of standardized quality control protocols and analytical methods
Clinical Validation and Formulation Development	Lack of clinical trials for human efficacy and safety	Conduct rigorous human trials and develop stable, bioavailable formulations
Sustainable Harvesting and Cultivation	Overharvesting and unsustainable cultivation practices in some regions	Promote sustainable farming, conservation efforts, and eco-friendly extraction methods

References

[1] M.J. Abad, L.M. Bedoya, L. Apaza, P. Bermejo. The *Artemisia* L. genus: a review of bioactive essential oils. Molecules 2012, 17(3), 2542–66. https://doi.org/10.3390/molecules17032542.

[2] K.S. Bora, A. Sharma. The genus *Artemisia*: a comprehensive review. Pharm. Biol. 2011, 49(1), 101–9. https://doi.org/10.3109/13880209.2010.497815.

[3] R.X. Tan, W.F. Zheng, H.Q. Tang. Biologically active substances from the genus *Artemisia*. Planta Med. 1998, 64(4), 295–302. https://doi.org/10.1055/s-2006-957438.

[4] D.L. Klayman. Qinghaosu (artemisinin): an antimalarial drug from China. Science 1985, 228(4703), 1049–55. https://doi.org/10.1126/science.3887571.

[5] Y. Tu. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat Med. 2011, 17(10), 1217–20. https://doi.org/10.1038/nm.2471.

[6] A. Judžentienė, J. Būdienė. Chemical composition of essential oils of *Artemisia vulgaris* L. (mugwort) from North Lithuania. Chemija 2006, 17(1), 12–6.

[7] W. Dhifi, S. Bellili, S. Jazi, N. Bahloul, W. Mnif. Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines (Basel) 2016, 3(4), 25. https://doi.org/10.3390/medicines3040025.

[8] V. Roussis, M. Tsoukatou, P.V. Petrakis, J.B. Harborne. Volatile constituents of four *Helichrysum* species growing in Greece. Biochem. Syst. Ecol. 2000, 28(2), 163-175. https://doi.org/10.1016/S0305-1978(99)00046-0.

[9] A. Saravanakumar, P. Periyasamy, H.T. Jang. In vitro assessment of three different *Artemisia* species for their antioxidant and anti-fibrotic activity. Biochem. Biophys. Adv. 2019, 5, 101040. https://doi.org/10.1016/j.bcab.2019.101040.

[10] J. Palá-Paúl, M.J. Pérez-Alonso, A. Velasco-Negueruela, J. Varadé, A.M. Villa, J. Sanz, J.J. Brophy. Analysis of the essential oil composition from the different parts of *Eryngium glaciale* Boiss. from Spain. J. Chromatogr. A 2005, 1072(1), 129-134. https://doi.org/10.1016/j.chroma.2005.09.029.

[11] A. Tantaoui-Elaraki, L. Beraoud. Inhibition of growth and aflatoxin production in *Aspergillus parasiticus* by essential oils of selected plant materials. J. Environ. Pathol Toxicol. Oncol. 1994, 13(1), 67-72.

[12] W. Peng, Y.-J. Liu, N. Wu, T. Sun, X.-Y. He, Y.-X. Gao, C.-J. Wu. *Areca catechu* L. (Arecaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. J. Ethnopharmacol. 2015, 173, 46-60. https://doi.org/10.1016/j.jep.2015.02.010.

[13] A. Ben Hsouna, N. Ben Halima, S. Smaoui, N. Hamdi. Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Lipids in Health Dis. 2017, 16(1), 146. https://doi.org/10.1186/s12944-017-0487-5.

[14] K. Belhouala, B. Benarba. Medicinal plants used by traditional healers in Algeria: A multiregional ethnobotanical study. Front. Pharmacol. 2021, 12, 760492. https://doi.org/10.3389/fphar.2021.760492.

[15] O. Said, K. Khalil, S. Fulder, H. Azaizeh. Ethnopharmacological survey of medicinal herbs in Israel, the Palestinian area, and the Golan Heights. J. Ethnopharmacol. 2002, 83(3), 251–65. doi:10.1016/S0378-8741(02)00253-2.

[16] L. Bendifallah, O. Merah. Phytochemical and biocidal properties of *Artemisia campestris* subsp. *campestris* L. (Asteraceae) essential oil at the southern region of Algeria. Nat. Prod. Environ. Res. 2023, 3, 100035. https://doi.org/10.1016/j.napere.2023.100035.

[17] K. Chebbac, A. El Moussaoui, M. Bourhia, A.M. Salamatullah, A. Alzahrani, R. Guemmouh. Chemical analysis and antioxidant and antimicrobial activity of essential oils from *Artemisia negrei* L. against drug-resistant microbes. Evid Based Complementary Alternat. Med. 2021, 2021, 5902851. https://doi.org/10.1155/2021/5902851.

[18] M. Ben Farhat, A. Landoulsi, R. Chaouch-Hamada, J.A. Sotomayor, M.J. Jordán. Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. Ind. Crops Prod. 2013, 51, 12-18. https://doi.org/10.1016/j.indcrop.2013.06.047.

[19] B. Cicioğlu Aridoğan, H. Baydar, S. Kaya, M. Demirci, D. Ozbaşar, E. Mumcu. Antimicrobial activity and chemical composition of some essential oils. Arch. Pharmacal Res. 2002, 25(6), 860-864. https://doi.org/10.1007/BF02977005.

[20] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar. Biological effects of essential oils—a review. Food Chem. Toxicol. 2008, 46(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106.

[21] S. Burt. Essential oils: Their antibacterial properties and potential applications in foods—A review. Inter. J. Food Microbiol. 2004, 94(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

[22] M. Soković, J. Glamočlija, P.D. Marin, D. Brkić, L.J.L.D. van Griensven. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules 2010, 15(11), 7532–7546. https://doi.org/10.3390/molecules15117532.

[23] C. Juliano, A. Mattana, M. Usai. Composition and variability of essential oils of *Artemisia arborescens* from different locations in Sardinia. Fitoterapia 2000, 71(3), 269–75. doi:10.1016/S0367-326X(00)00138-1.

[24] S. Das, B. Vörös-Horváth, T. Bencsik, G. Micalizzi, L. Mondello, G. Horváth, T. Kőszegi, A. Széchenyi. Antimicrobial Activity of Different *Artemisia* Essential Oil Formulations. Molecules 2020, 25(10), 2390. doi: 10.3390/molecules25102390.

[25] M.B. Goudjil, S. Zighmi, D. Hamada, Z. Mahcene, S.E. Bencheikh, S. Ladjel. Biological activities of essential oils extracted from *Thymus capitatus* (Lamiaceae). S. Afr. J. Bot. 2020, 130, 375–382. https://doi.org/10.1016/j.sajb.2019.11.020.

[26] I. Limam, R. Ghali, M. Abdelkarim, A. Ouni, M. Araoud, M. Abdelkarim, ... F. Ben-Aissa Fennira. Tunisian *Artemisia campestris* L.: a potential therapeutic agent against myeloma-phytochemical and pharmacological insights. Plant Methods 2024, 20(1), 59. https://doi.org/10.1186/s13007-024-01185-4

[27] L.G. Lucca, P.R.T. Romão, M. Vignoli-Silva, V.F. da Veiga-Junior, L.S. Koester. In vivo acute anti-inflammatory activity of essential oils: A review. Mini-Reviews Med. Chem. 2022, 22(11), 1495–1515. https://doi.org/10.2174/1389557521666211123091541.

[28] I. Dib, F.E. El Alaoui-Faris. *Artemisia campestris* L.: Review on taxonomical aspects, cytogeography, biological activities and bioactive compounds. Biomed. Pharmacother. 2019, 109, 1885–1893. https://doi.org/10.1016/j.biopha.2018.10.149.

[29] N. Bencheikh, F.Z. Radi, J. Fakchich, M. Elachouri, et al. Ethnobotanical, phytochemical, toxicological, and pharmacological properties of *Ziziphus lotus* (L.) Lam.: A comprehensive review. Pharmaceuticals 2023, 16(4), 575. https://doi.org/10.3390/ph16040575.

[30] X. Chen, C. Qin, X. Li, F. Zhang, R.J. Linhardt, P. Sun, A. Zhang. Chemical composition and biological activities of essential oil isolated by HS-SPME and UAHD from fruits of bergamot. LWT - Food Sci. Technol. 2019, 103, 293–300. https://doi.org/10.1016/j.lwt.2019.01.020.

[31] S. Zrira, J.M. Bessiere, C. Menut, A. Elamrani, B. Benjilali. Chemical composition of the essential oil of nine *Eucalyptus* species growing in Morocco. Flavour Frag. J. 2004, 19(2), 172–175. https://doi.org/10.1002/ffj.1289.

[32] M.I. Rocha, M.J. Gonçalves, C. Cavaleiro, M.T. Cruz, C. Pereira, P. Moreira, L. Salgueiro, A. Figueirinha. Chemical characterization and bioactive potential of *Artemisia campestris* L. subsp. *maritima* (DC) Arcang. essential oil and hydrodistillation residual water. J. Ethnopharmacol. 2021, 278, 114146. https://doi.org/10.1016/j.jep.2021.114146.

[33] N. Bousbia, M.A. Vian, M.A. Ferhat, E. Petitcolas, B.Y. Meklati, F. Chemat. Comparison of two isolation methods for essential oil from rosemary: Hydrodistillation and microwave hydrodiffusion and gravity. Food Chem. 2009, 114, 355–362. https://doi.org/10.1016/j.foodchem.2008.09.106.

[34] E. Reverchon, I. De Marco. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluids 2006, 38(2), 146–166. https://doi.org/10.1016/j.supflu.2006.03.020.

[35] J. Azmir, I.S.M. Zaidul, M.M. Rahman, K.M. Sharif, A. Mohamed, F. Sahena, ... A.K.M. Omar. Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng. 2013, 117(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014.

[36] A.A. Rashed, A.Z. Abd Rahman, D.N. Gunasegavan Rathi. Essential oils as a potential neuroprotective remedy for age-related neurodegenerative diseases: A review. Molecules 2021, 26(4), 1107. https://doi.org/10.3390/molecules26041107.

[37] C.C. Hoch, J. Petry, L. Griesbaum, T. Weiser, K. Werner, M. Ploch, A. Verschoor, G. Multhoff, A.B. Dezfouli, B. Wollenberg. 1,8-cineole (eucalyptol): A versatile phytochemical with therapeutic applications across multiple diseases. Biopharm. Drug Dispos. 2023, 44(4), 115467. https://doi.org/10.1016/j.biopha.2023.115467.

[38] I. Limam, R. Ghali, M. Abdelkarim, *et al.* Tunisian *Artemisia campestris* L.: a potential therapeutic agent against myeloma-phytochemical and pharmacological insights. Plant Methods 2024, 20, 59. https://doi.org/10.1186/s13007-024-01185-4

[39] F.M. Abdoul-Latif, A. Ainane, I.H. Aboubaker, J. Mohamed, T. Ainane. Exploring the potent anticancer activity of essential oils and their bioactive compounds: Mechanisms and prospects for future cancer therapy. Pharmaceuticals 2023, 16(8), 1086. https://doi.org/10.3390/ph16081086.

[40] N. Ren, M. Atyah, W.-Y. Chen, C.-H. Zhou. The various aspects of genetic and epigenetic toxicology: Testing methods and clinical applications. J. Transl. Med. 2017, 15, 110. https://doi.org/10.1186/s12967-017-1218-4.

[41] A. Akrout, L. A. Gonzalez, H. El Jani, P. C. Madrid. Antioxidant and antitumor activities of Artemisia campestris and Thymelaea hirsuta from southern Tunisia. Food Chem. ToxicoL 2011, 49, 342-347. https://doi.org/10.1016/j.fct.2010.11.003

[42] A. Alami, M. Fadil, F. zahrae Radi, M. El Hallabi, M.O. Alami, A. Farah. (2025). Exploring Artemisia Essential Oils as Natural Larvicides for Culex Mosquito Control. J. Nat. Pest. Res. 2025, 13, 100141. https://doi.org/10.1016/j.napere.2025.100141

[43] M.H.B. Nabi, M.M. Ahmed, M.S. Mia, S. Islam, & W. Zzaman. Essential Oils: Advances in Extraction Techniques, Chemical Composition, Bioactivities, and Emerging Applications. Food Chem. Adv. 2025, 8, 101048. https://doi.org/10.1016/j.focha.2025.101048

Chapter Two

Neuroprotective effects of *Artemisia campestris* essential oil against chlorpyrifos-induced toxicity in rats supported by computational modelling

2.1. Introduction

Chlorpyrifos or CPF ((*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinol) phosphorothioate) is a lipophilic chlorinated OP ester, commonly used in agriculture as a protecting agent for crops and in food production [1,2]. CPF acts by inhibiting AChE, crucial enzyme for nerve function of insects, but it remains an agent that seriously threatens and destroys human health (respiratory, cardiovascular and nervous organs), as well as environmental safety [1,3]. The metabolism of CPF produces ROS *via* its bioactivation, contributing to its prominent genotoxicity and neurotoxicity [4-6]. Every year, approximately 2 million tons of pesticides, primarily organophosphorus, are used, resulting in an estimated 2.5 million cases of acute poisoning and 200,000 deaths [1,7]. Moreover, their broad use can affect the applicators' health, and induces harmful on the environment [8]. Recent studies indicate that pesticide exposure cause oxidative stress and enhance the production of reactive oxygen species (ROS), leading to free radical formation and lipid peroxidation in the tissues of mammals and other organisms [9-11]. Organophosphorus pesticides (OPs) are the most commonly used in agriculture for insect control [12-14]. Underestimating their use is insidious and can lead to health risks, particularly OP-related poisoning [15]. OP compounds are nerve agents that act by damaging the AChE enzyme irreversibly [16]. Its inhibition in the central and peripheral nervous systems, resulting in acetylcholine accumulation and excessive activation of muscarinic and nicotinic receptors, which can ultimately result in death [17]. Moreover, many authors show that these

compounds also modify the activities of the antioxidant enzymes [17]. Essential oils (EOs) and their beneficial chemical phytocompounds from medicinal herbs have an important function in traditional pharmacopeia practices [18-21]. EOs showed broad-range of bioactivities and have been classified as effective antioxidants [22-26]. Since pesticide exposure impairs endogenous defence mechanisms and increases ROS production, leading to the pathogenesis of neurological disorders and depletion of antioxidant systems, EOs may exert a counteracting effect by scavenging and neutralizing ROS [27-29].*Artemisia* species are among the promising herbal medicines that has been extensively used in the local folk medicine, due to their important medicinally valuable secondary metabolites and essential oils served for the treatment of a plethora ailments in humans [30,31]. The EO from *Artemisia Campestris* has been proven for its powerful antioxidant properties [32,33]. Its phytochemical composition was previously investigated by our team and GC–MS analysis revealed the presence of a total of 61 components such as *β*-pinene and *β*-eudesmol followed by α-pinene, *γ*-terpinene, terpinene-4-ol, *β*-myrcene, trans-*β*-ocimene and *α*-terpineol [34]. The originality of our study is to demonstrate simulation and molecular docking, a novel approach not developed for the effects of *ACEO* against CPF induced nervous toxicity and disrupts AChE activity, and there are no reports in the field. Therefore, the present study was conducted to investigate the effect of ACEO on CPF-induced nervous and splenic toxicities in rats supported with an in-silico modelling including pharmacokinetic, molecular docking and dynamic simulation analysis on the top major phytoconstituents of ACEO.

2.2. Material and Methods

Preparation of ACEO

ACEO was prepared by hydrodistillation (2h) of 250 g of ground samples using a Clevenger-type apparatus. After drying over anhydrous sodium sulfate, the essential oil was stored at 4 °C. The chemical composition of ACEO was initially identified through GC-MS analysis in previous study [34]. With these compounds

analyzed by GC-MS, we will conduct a comprehensive in silico pharmacokinetics modelling to evaluate their potential therapeutic applications.

Animal experiments

Adult female albino *Wistar* rats were handled under the used standard laboratory conditions. Food and water were available *ad libitum*. All animal experiments were conducted according to the protocols and guidelines for animal treatment and experimentation. All animal procedures were performed in accordance with the Ethical Committee Guidelines for Laboratory Animal. After two weeks of acclimatization, the rats were allocated randomly to four experimental groups:

Groupe I: Control rats receiving water and a standard diet *ad libitum*

Groupe II: The rats received CPF by gavage (10 mg /Kg/day) for 20 days

Group III: The rats received a pre-treatment by ACEO with 200 mg/kg of bw for 10 days and then a post treatment by the CPF (10 mg /Kg/day) during the last 10 days by gavage

Groupe IV: The rats received ACEO at the dose of 200mg/Kg/day by gavage for 20 days.

At the end of experimentation, the rats were sacrificed after anesthesia with chloral hydrate. Some blood samples were collected in heparinized tubes used for the determination of the hematological parameters. Other blood samples are taken without anticoagulant. Serum samples were drawn from blood after centrifugation at 4000 rpm for 15 min. They were kept at -20 °C until biochemical analyses. The brain and spleen were quickly excised, minced with ice cold saline, blotted on filter paper which were used for histopathological analysis.

Hematological parameters

Hematology White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular

hemoglobin concentration (MCHC) and blood platelet count (PLT) were measured with an automate sysmex Kx-21N (CHU Habib Bourguiba Sfax).

Biochemical parameters

AchE, glycemia, cholesterol and total bilirubine parameters were measured using commercial reagents kits in the CHU Hedi Chaker Sfax.

Histopathological study

Brain and spleen samples were fixed with 10% formalin, dehydrated with alcohols of increasing concentrations, then included in paraffin. Fine sections were made with a microtome and stained with hematoxylin-eosin (H&E) for microscopic observation.

Molecular Docking and dynamic study

The inhibitory effects of the essential oils on AChE were evaluated through a molecular docking study using human AChE (PDB ID: 4EY7). All phytocompounds were prepared with the Glide LigPrep panel v6.7 (Schrodinger Suite 2024-1), where their 3D structures were energy-minimized, chiralities were corrected, protonation states were generated at physiological pH, and multiple conformers were produced. The AChE crystal structure was refined using the Protein Preparation Wizard, which corrected missing atoms, loops, and side chains, adjusted bond orders, and removed unwanted water molecules or heteroatoms. The docking grid was created with the Glide Receptor Grid Generation tool, and docking was carried out using the Glide SP (v10.2) protocol to predict optimal binding modes [35,36]. Molecular dynamics simulations were performed using Desmond (G 6.9.137) to examine the stability of the protein–ligand complexes. Each system was solvated in an orthorhombic box using the SPC water model [37], with a 10 Å buffer around the complex. The OPLS4 force field was applied for both the protein and ligand. Counterions were added to neutralize the system, along with 0.15 M NaCl to mimic physiological ionic strength. Energy minimization was performed to remove steric clashes, followed by equilibration under NVT and NPT ensembles. The

temperature was maintained at 300 K using the Nosé–Hoover thermostat, and pressure was kept at 1 atm with the Martyna–Tobias–Klein barostat. Long-range electrostatics were treated using the Particle Mesh Ewald method, and all covalent bonds involving hydrogen were constrained with the SHAKE algorithm. Production runs were carried out for 100 ns with a 2 fs time step, and the trajectories were analyzed for RMSD, RMSF, hydrogen bonding patterns, and the overall stability of the complex throughout the simulation. The Desmond simulations were performed on a Linux workstation running Ubuntu 22.04.2 LTS, equipped with an Intel® Xeon® W-2245 CPU (8 cores / 16 threads, 3.9 GHz base, 4.7 GHz turbo) and an NVIDIA RTX A4000 GPU (Driver Version 535.183.01, CUDA Version 12.2), providing efficient GPU-accelerated performance for molecular dynamics computations.

ADMET analysis

In silico ADMET studies of the major selected phytocompounds were assessed to predict their druglikeness and pharmacokinetic properties using https://biosig.lab.uq.edu.au/pkcsm/ and http://www.swissadme.ch/ server tools.

Statistical analysis

The results obtained have been expressed as mean values ± standard error of mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed Tukey post hoc test. Values of $p<0.05$ were considered significant.

2.3. Results

Effects on the variation of some body growth parameters of control and treated rats

The exposure to CPF showed a remarkable decrease in body growth during the treatment (**Fig. 1**). The pretreatment with ACEO restores the growth of treated rats compared to the group of rats receiving only the pesticide. There is also a significant decrease in absolute weight at the end of treatment for treated rats compared to control. However, the pretreatment with ACEO significantly reduced these disturbances of growth.

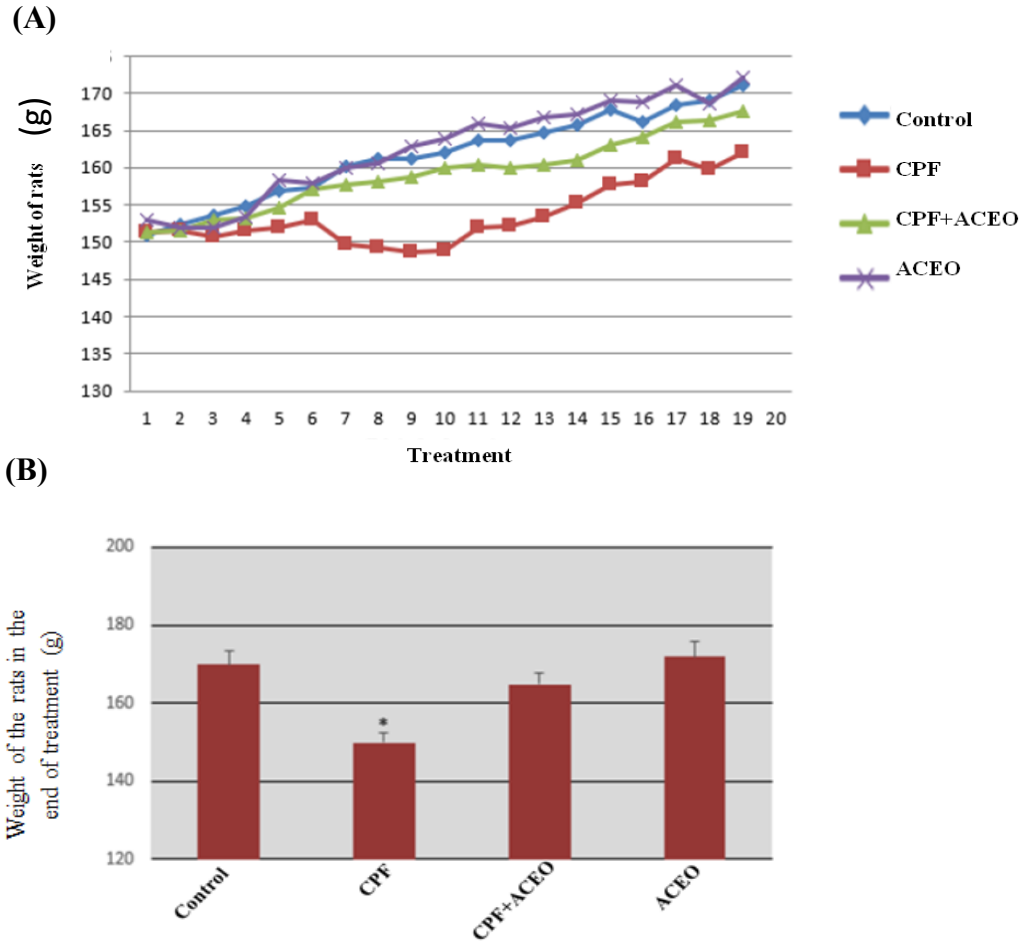

Fig.2.1. Growth curve (A) and body weight (B) after treatment of control and treated rats

Values were expressed as means± SEM (n=5).

* $p \leq 0.05$

Effects on some hematological parameters

Our results demonstrated a significant decrease in RBC, Hb and Ht levels, as well as a significant increase in WBC in group treated with CPF compared to control (Table 1). ACEO remarkably reduced this disturbance of hematologic factors induced by CPF

Table 2.1. Effects of CPF, ACEO and their combination (CPF+ACEO) on some hematological parameters

Parameters & treatment	Control	CPF	CPF+ACEO	ACEO
RBC (10^6/µL)	7.95±0.13	7.18±0.08**	7.39±0.05 *#	7.79±0.19
WBC (10^3/µL)	12.17±1.1	14.06±0.72*	11.52±0.55#	11.83±0.82
Hb (g/dl)	14.47±0.21	13.4±0.22**	13.75±0.18 *#	14.05±0.33
Ht (%)	44.25±0.71	41.7±0.67*	42.27±0.12 #	43.97±1.19
VGM (fl)	55.65±0.45	57±0.21	57.15±0.44	56.42±0.43
MCH (pg)	18.2±0.26	18.27±0.085	18.6±0.24	18.02±0.28
MCHC (g/dl)	32.72±0.28	32.05±0.21	32.52±0.44	31.95±0.39

Values were expressed as means± SEM (n=5).

* $p \leq 0.05$; ** $p \leq 0.01$ vs control; ACEO+CPF vs CPF : # $p \leq 0.05$

Effects on some biochemical parameters

The administration of CPF induced an increase in blood glucose, total bilirubin and total cholesterol levels as well as a decrease of cholinesterase activity and these in comparison with the control rats (Table 2). The pretreatment with ACEO significantly restored these biochemical parameters already mentioned in comparison with rats treated with CPF only.

Table 2.2. Effects of CPF, ACEO and their combination (CPF+ACEO) on serum parameters of control and treated rats.

Values were expressed as means± SEM (n=5).

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ vs control; *ACEO*+CPF vs CPF : # $p \leq 0.05$; ## $p \leq 0.01$

Parameters & treatment	Control	CPF	CPF+ACEO	ACEO
Glycemia (mmol/l)	5.91±0.14	6.12±0.38*	5.62±0.06 #	5.81±0.17
Cholesterol (mmol/l)	0.75±0.03	0.86±0.04*	0.83±0.01 #	0.79±0.1
Total bilirubin (mmol/l)	6.06±0.91	6.83±0.83*	6.2±0.17	5.9±1.35
AchE (mmol/l)	319.75±8.37	33.5±0.35***	61.4±11.61*** ##	346.2±58.32

Histopathology of the brain and the spleen

The microscopic observation of the brain histopathology (**Fig. 2**) demonstrated a conservation of cellular architecture and normal vessels in control group. In addition, there were no changes in the appearance of the brain in rats treated with ACEO compared to control. However, CPF treated rats revealed the presence of edema and an alteration of the architecture with the presence of clear periplasmic halos marker of cerebral edema which is an accumulation or excess of fluid. These alterations are subsequently corrected by the administration of ACEO. The microscopic observation of the spleen histopathology (**Fig. 3**) demonstrated that control rats showed normal spleen morphology. CPF induced evidence of spleen alterations and tissue damage compared to control. On the other hand, the combination of CPF and ACEO revealed improvement in spleen as compared to treated rats.

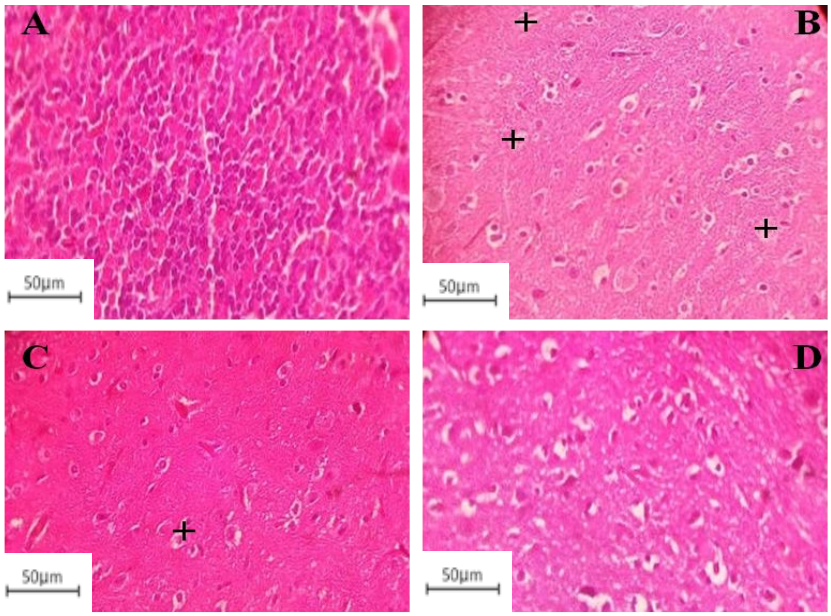

Fig.2.2. Representative photographs of brain showing the protective effect of ACEO on CPF induced neurotoxicity in rats. Control (A), rats treated with CPF (B), rats treated with the combination of CPF+ACEO (C) and rats treated with ACEO (D) (400x)

+: Edema

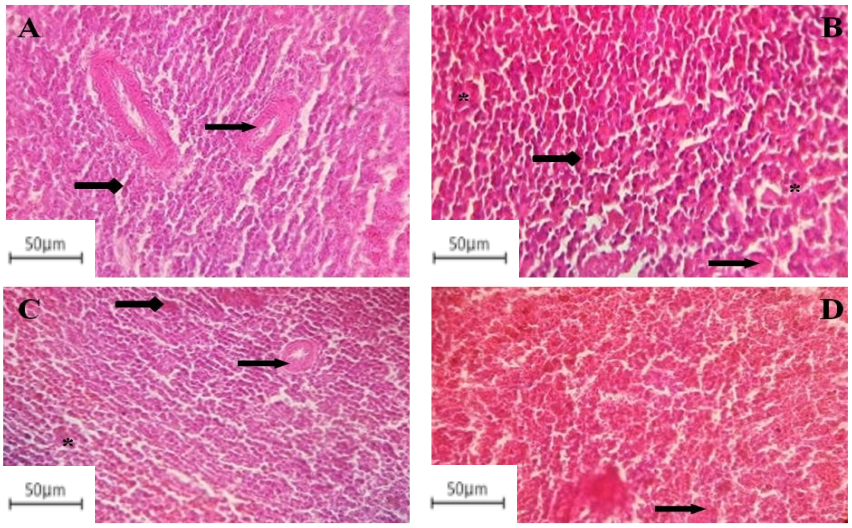

Fig.2.3. Representative photographs of spleen showing the protective effect of ACEO on CPF induced splenocytes toxicity in rats. Control (A), rats treated with CPF (B), rats treated with the combination of CPF+ ACEO (C), rats treated with ACEO (D) (400x)

→ : Red pulp; *: Inflammatory aspects; ➡ : White pulp

ADMET prediction studies

Conducting computational drug-likeness and pharmacokinetics predictions is a crucial and very important stage in accelerating the drug discovery process through effective filtering and prioritization of potential candidates [38-44]. In our case, eleven majors volatile phytoconstituents (Fig. 4) from ACEO were selected for their pharmacokinetics prediction using both pkCSM and Swiss ADME online tools [45-47]. The forecast of drug-likeness properties provides that all selected compounds obeyed to Lipinski's five rules. Our predictive results (Table 3) showed that the selected molecules displayed good absorption properties with negative water solubility values, Caco2 permeability and intestinal absorption (> 93%) are in acceptable range, moderate to potent skin permeability (log Kp) and were no P-glycoprotein inhibitor. Their distribution parameters indicated all compounds exhibited high BBB permeability with CNS values lesser than -3. Regarding their metabolism behavior towards the CYP-coenzymes as listed in Table 3, all phytocompounds were no inhibitor of any sub-enzymes. Total Clearance and Renal OCT2 substrate were also predicted as descriptive of their excretion characteristics. The toxicity results indicated that almost all compounds are defined as inactive with regard to hepatotoxicity and mutagenicity, but only compounds 4, 7, 9, 10 and 11 are represent a positive skin sensitization. The bioavailability radar graph (Fig. 4) indicate that all compounds were completely located in the in the pink area of oral bioavailability, as evidenced by their good oral bioavailability.The gastrointestinal absorption and brain penetration properties of the selected compounds (Fig. 4) revealed that they belong to the yellow region (red point) meaning their ability to passively traverse the blood–brain barrier (BBB) and that they are P-gp (PGP-).

Table 2.3. ADMET parameters of ACEO major compounds

Entry	1	2	3	4	5	6	7	8	9	10	11	Reference
Absorption												
Water solubility	-	-	-	-	-	-	-	-2.039	-	-	-4.712	-
Caco2 permeability	1.398	1.385	1.394	1.397	1.417	1.403	1.202	1.489	1.566	1.313	1.329	*>0.9*
Intestinal absorption	96.041	95.525	94.475	95.898	95.185	95.301	93.857	94.183	96.057	94.86	96.364	*<30% is poorly*
Skin Permeability (log	-	-	-	-	-	-	-	-2.418	-	-	-1.982	*>-2.5 is low*
P-glycoprotein inhibitor	-	-	-	-	-	-	-	-	-	-	-	-
Distribution												
VDss (human)	0.667	0.685	0.366	0.396	0.337	0.412	0.103	0.207	0.056	0.495	0.386	*Low is <-0.15, High is*
Fraction unbound	0.425	0.35	0.389	0.484	0.386	0.424	0.489	0.565	0.382	0.306	0.138	-
BBB permeability	0.8	0.818	0.777	0.723	0.772	0.741	0.55	0.305	0.552	0.61	0.635	*Poorly is <-1, High is >0.3*
CNS permeability	-	-	-	-2.37	-	-	-	-2.807	-	-	-1.783	*Penetrate is >-2, Unable is*
Metabolism												
CYPs inhibitors	-	-	-	-	-	-	-	-	-	-	-	-
Excretion												
Total Clearance	0.043	0.03	0.438	0.213	0.441	0.217	1.269	1.219	0.583	0.895	1.032	-
Renal OCT2 substrate	-	-	-	-	-	-	-	-	-	-	-	-
Toxicity												
AMES toxicity, Hepatotoxicity	-	-	-	-	-	-	-	-	-	-	-	-
Skin Sensitization	-	-	-	+	-	-	+	-	+	+	+	-

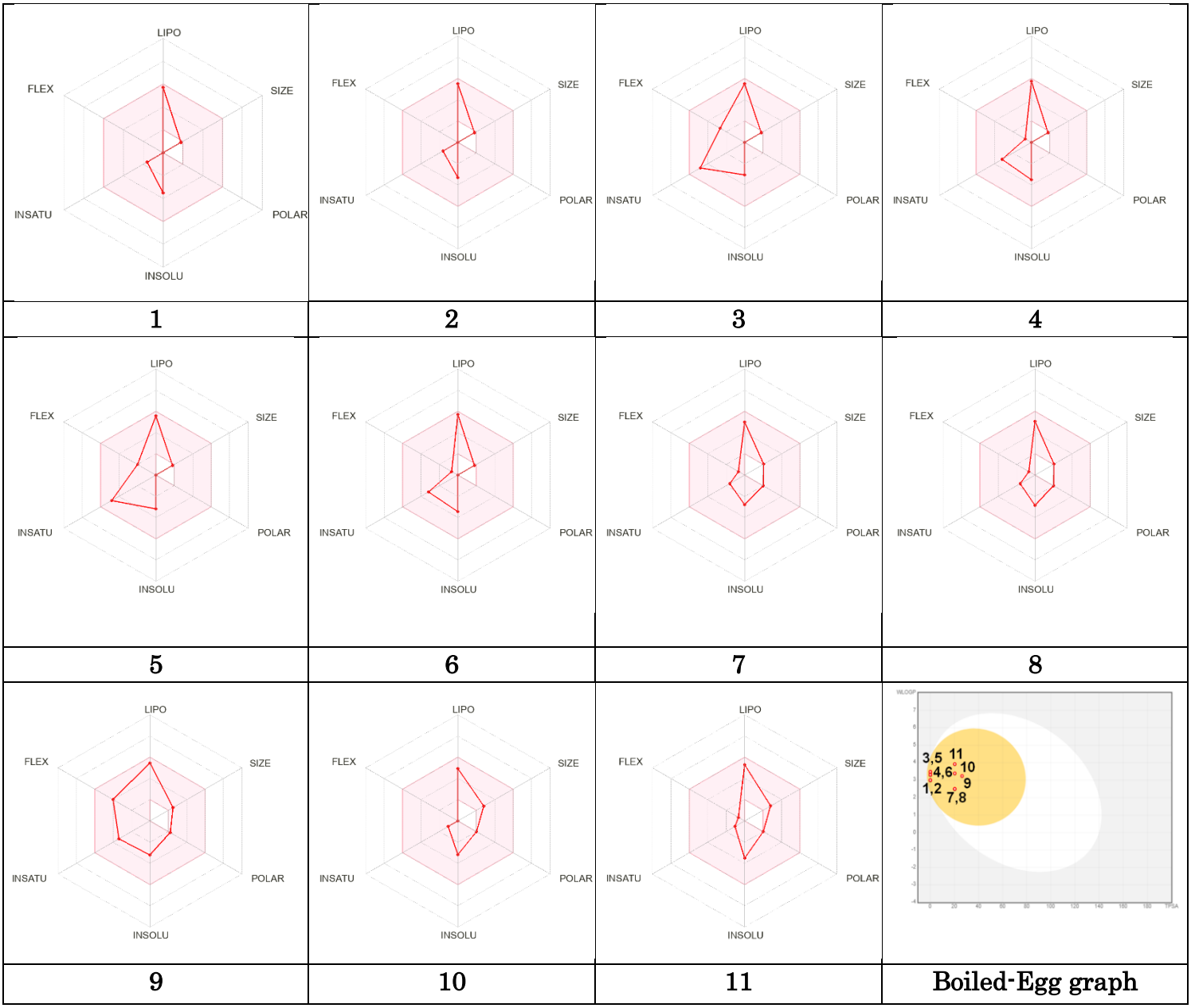

Fig.2.4. Radar plot and Boiled-Egg graph of the ACEO major compounds. Major volatile compounds present in the ACEO, *α*-Pinene (1), *β*-Pinene (2), *β*-Myrcene (3), R(+)- Limonene (4), trans-*β*-Ocimene (5), *γ*-Terpinene (6), Terpinene-4-ol (7), *α*-Terpineol (8), Geranyl acetate (+) (9), (+)-Spathulenol (10), *β*-Eudesmol (11).

Molecular Docking Analysis

Phytochemical screening of plant extracts often reveals significant biological activity, but the specific phytocompound responsible for this activity remains elusive. To bridge this knowledge gap, molecular docking studies are conducted on the major identified compounds to establish a correlation between the phytocompound and the observed AChE inhibitors activity (Neuroprotective effects). Alzheimer's disease, a devastating neurodegenerative disorder, is characterized by the progressive decline of cognitive functions, memory loss, and neuronal death [48]. One of the key enzymes implicated in the pathogenesis of Alzheimer's disease is AChE. This enzyme plays a crucial role in the breakdown of acetylcholine, a neurotransmitter essential for learning, memory, and cognitive functions. The decrease in acetylcholine levels is thought to contribute to the cognitive decline and memory impairment characteristic of Alzheimer's disease. Inhibiting AChE activity has been a promising therapeutic strategy for Alzheimer's disease. AChE inhibitors, such as donepezil, rivastigmine, and galantamine, have been developed to increase acetylcholine levels in the brain, thereby improving cognitive functions and slowing disease progression [49].

Table 2.4. Docking score in Kcal/mol of the studied identified phytocompounds and Donepezil against AChE enzyme.

Phytocompounds	Docking score (kcal/mol)
α-pinene	-6.081
β-pinene	-5.157
β-myrcene	-2.584
R-(+)-Limonene	-4.605
Trans-*β*-ocimene	-3.704
γ-Terpinene	-5.682
Terpinene-4-ol	-6.445
α-Terpineol	-5.618
Geranyl acetate	-5.845
(+)-Spathulenol	**-6.74**
β-Eudesmol	-6.68
Donepezil	-12.167

The molecular docking was carried out to inspect the binding affinity and prospective interactions between the identified phytocompounds and the active site of AChE (PDB ID: 4EY7). To ensure the reliability of the docking workflow, the protocol was validated using the co-crystallized ligand donepezil from the human AChE crystal structure (PDB ID: 4EY7). The ligand was extracted from the binding site, prepared using the same LigPrep settings applied to the test compounds, and redocked into the receptor with the Glide SP protocol. The predicted binding pose was then compared with the original crystallographic orientation. The superimposition analysis showed a very close alignment between the two poses, with an RMSD value of 0.111 Å (**Fig. 5**). This low deviation confirms that the docking protocol accurately reproduces the experimentally observed binding mode and is suitable for evaluating the phytocompounds in the study. The molecular docking results, as shown in **Table 4**,

reveal the binding affinity of the identified phytocompounds against the AChE enzyme.

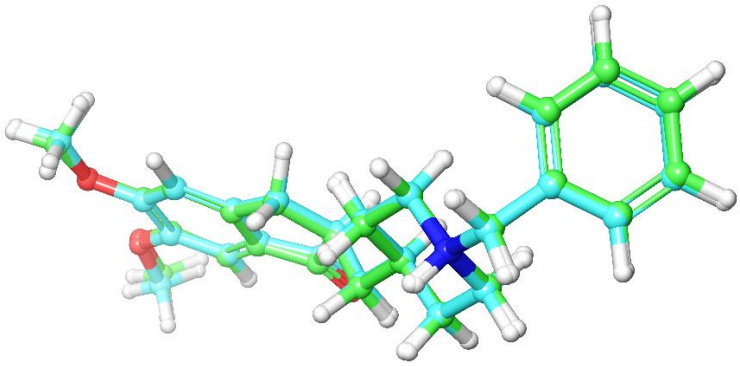

Fig.2.5. Validation of the docking protocol using the co-crystallized ligand donepezil in AChE (PDB ID: 4EY7).

The experimental pose (shown in green color) is overlaid with the Glide-redocked pose (shown in blue color). The two conformations align closely, yielding an RMSD of 0.111 Å, confirming that the docking procedure reliably reproduces the crystallographic binding orientation.

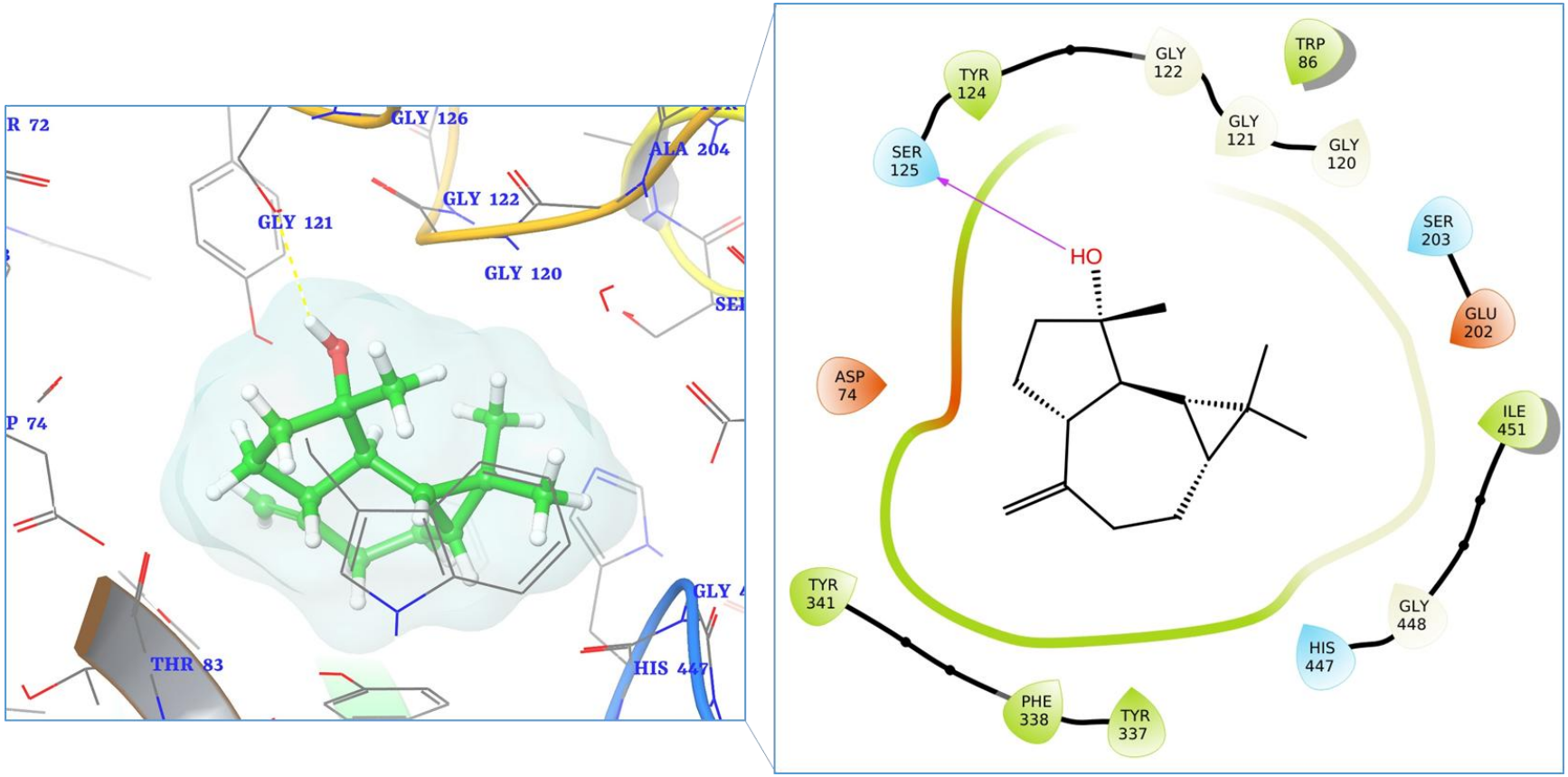

Fig.2.6. 2D and 3D Binding poses of (+)-spathulenol in the active of site human AChE enzyme (PDB code: 4EY7) (*Hydrogen bond interaction is shown by pink arrow line*).

The docking scores, expressed in kcal/mol, indicate the strength of interaction between each phytocompound and the AChE active site. The results suggest that several phytocompounds exhibit promising binding affinity, with 2.5 Å while the (+)- spathulenol-AChE Complex (green) generally stays below 2.0 Å. score of -6.74 kcal/mol. *β*-eudesmol and Terpinene-4-ol also shows a strong interaction, with a docking score of -6.68 and -6.445 kcal/mol respectively. In comparison, the docking score of donepezil, a known AChE inhibitor, is -12.167 kcal/mol, indicating a strong binding affinity. The binding interaction (**Fig. 6**) of (+)-spathulenol shows that it is situated in the same binding cavity as Donepezil with the active site of AChE, revealing that it forms a hydrogen bond with Ser125 through its terminal hydroxyl group. Additionally, van der Waals forces of interaction are observed with several residues, including Tyr124, Gly122, Gly121, Gly120, Ser203, Glu202, Ile451, Gly448, His447, Tyr337, Phe338, and Tyr341. According to the X-ray crystal structure, it was observed that the Donepezil hydrophobic scaffold also shows van der Waals forces of interaction with Gly120, Gly121, Tyr124, and Gly126. Among the phytocompounds identified in the *Artemisia campestris* essential oil, (+)-spathulenol stands out as a likely major contributor to the observed AChE inhibitory activity. Its binding affinity and interaction pattern suggest a stronger and more favorable engagement with the AChE active site compared with the Donepezil. Since acetylcholinesterase inhibition is a well-recognized mechanism for enhancing cholinergic neurotransmission, this activity is often linked to neuroprotective potential, particularly in conditions involving cognitive decline. Therefore, the prominent inhibitory behavior of (+)-spathulenol indicates that it may play a central role in the neuropharmacological properties associated with *A. campestris* essential oil.

Molecular Dynamic Simulation

The binding affinity and interactions observed in the docking studies provide a static view of the complex, but do not account for the dynamic fluctuations that occur in the system. To gain a deeper understanding of the molecular interactions and stability of the complexes, molecular dynamics (MD)

simulations were performed. A 200 ns molecular dynamics simulation of the (+)-spathulenol-AChE and Donepezil-AChE complexes was carried out to investigate the dynamic behavior of these compounds in the binding site. This simulation provides further insights into the molecular interactions of these compounds in motion, allowing for the evaluation of the stability and dynamics of the complexes. The MD simulation results can provide valuable information on the conformational changes, hydrogen bonding, and Hydrophobic interactions that occur between the compounds and the enzyme over time. This can help to identify the key residues and interactions that are responsible for the stability and inhibitory activity of the compounds. Furthermore, the simulation can also provide insights into the flexibility and adaptability of the compounds and the enzyme, which can influence the binding affinity and inhibitory activity.

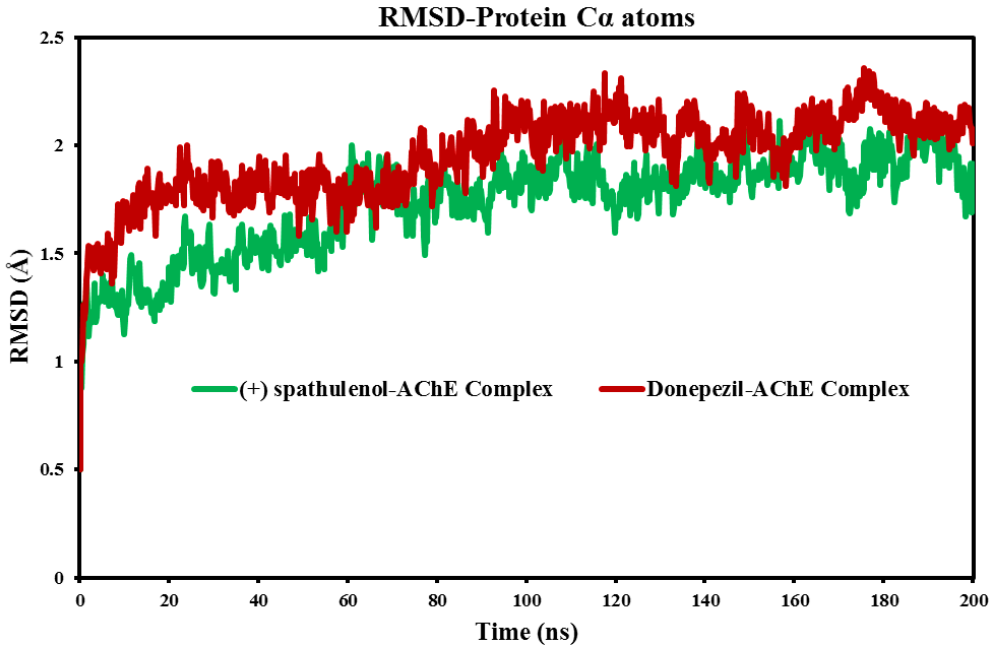

Fig.2.7. Time dependent RMSD of AChE protein Cα atoms in complex with (+)-spathulenol and Donepezil

One of the key metrics used to evaluate the structural integrity of protein-ligand complexes over a given period is the root-mean-square deviation (RMSD). This parameter provides valuable insights into the stability of these complexes by

tracking the fluctuations in the atomic positions of the protein-ligand system as it evolves over time. During MD simulations, the positions of the atoms in the complex are repeatedly sampled and recorded at regular intervals, generating a trajectory of the system's behavior. By analyzing these atomic coordinates, the RMSD can calculate, which serves as a quantitative measure of the complex's stability and conformational changes over time. A small RMSD value is indicative of a structurally stable complex that maintains its native conformation, whereas a higher RMSD value signifies that the complex undergoes significant fluctuations or conformational changes over the course of the simulation. Throughout the simulation, the RMSD values for the Donepezil-AChE Complex (red) predominantly range from around 1.0 to the lower range of RMSD in the (+)-spathulenol-AChE Complex suggests that its atomic positions remained more consistent with the reference structure compared to those in the Donepezil-AChE Complex (**Fig. 7**). After the initial spike, the RMSD of both complexes decreases and stabilizes between 20-40 ns, indicating a period of relative structural consistency. The RMSD of the (+)-spathulenol-AChE Complex remains relatively consistent and low between 140-150 ns, indicating a stable conformation. The Donepezil-AChE Complex appears to have a higher RMSD on average than the (+)-spathulenol -AChE Complex. This suggests that the Donepezil-AChE Complex experiences moderate deviations from the initial structure, potentially indicating flexibility or less stability compared to the (+)-spathulenol-AChE Complex.

The Root Mean Square Fluctuation (RMSF) analysis is a valuable tool used to assess the conformational flexibility of a protein structure during molecular dynamics simulations. RMSF analysis involves calculating the mean deviation of the atomic positions of individual amino acids from their average positions over a specified time period. This yields a profile of atomic fluctuations, where regions with high RMSF values indicate greater flexibility and those with low RMSF values suggest more rigid structures. The RMSF data for the (+)-spathulenol-AChE complex and the Donepezil-AChE complex reveals comparable overall flexibility, as evidenced by similar average RMSF values.

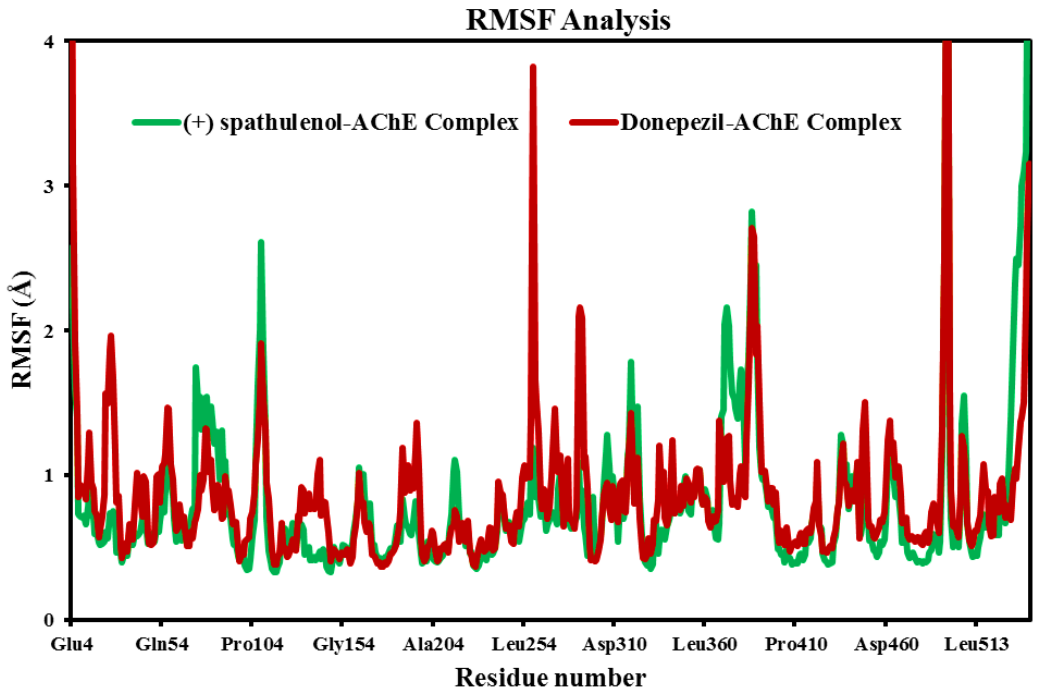

Fig.2.8. RMSF of individual amino acids of EGFR protein Cα atoms in complex with (+)-spathulenol and Donepezil

of 0.81 Å and 0.85 Å respectively. However, there are moderate differences observed in their dynamics. The (+)-spathulenol-AChE complex exhibits a slightly higher maximum RMSF of 5.71 Å compared to 5.15 Å for the Donepezil-AChE complex, indicating potential differences in their effects on binding modes. Higher RMSF values are observed in loop regions as well as in terminal amino acids, including Glu4, Asp5, Asn265, Arg493, Asp494, and Ala542 (**Fig. 8**). Despite these variances, both complexes demonstrate similar minimum RMSF values, suggesting a comparable ability to stabilize specific regions of the AChE protein. During the simulation, both the (+)-spathulenol-AChE complex and the Donepezil-AChE complex were found to interact with key amino acids, including Tyr72, Asp74, Leu76, Trp86, Gly121, Tyr124, Ser125, Trp286, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, and Tyr341 within the AChE protein. Notably, the RMSF values of these interacting residues remained below 2.5 Å throughout the simulation, indicative of their stable interactions with the ligands. This stability suggests that (+)-spathulenol effectively bind to these

amino acids within the AChE protein, influencing its dynamics and potentially modulating its activity.

During molecular dynamics MD simulation, significant binding interactions were observed in both the (+)-spathulenol-AChE complex and the Donepezil-AChE complex, elucidating their respective modes of interaction with AChE. In the (+)-spathulenol-AChE complex, notable hydrogen bond interactions were observed primarily at residues Tyr124 and Ser125, indicating specific points of contact between the ligand and the protein. Strong hydrophobic interactions were prominent at Trp86, Trp286, Phe338, and Tyr341, underscoring their role in stabilizing the complex through nonpolar contacts. Moderately strong ionic interactions predominantly occurred at Ser125, with a smaller fraction at Asp74, suggesting electrostatic attractions within the complex. Additionally, water bridges were notably formed with Phe295 and Arg296, potentially aiding in the stabilization of the complex through solvation effects (**Fig. 9**). In contrast, in the Donepezil-AChE complex, hydrogen bond interactions were predominantly observed at Ser125, while strong hydrophobic interactions were noted at Trp86, Trp286, and Tyr341, similar to the observations in the (+)-spathulenol-AChE complex. Ionic interactions were evident at Asp74, indicating electrostatic attractions, and water bridges were observed at Phe295, Arg296, Tyr341, and Gly342, hinting at additional water-mediated interactions contributing to the stability of the complex. These comprehensive binding interaction profiles offer valuable insights into the specific molecular mechanisms underlying the interactions between (+)-spathulenol and Donepezil with AChE, which are crucial for understanding their pharmacological effects and potential therapeutic applications. The interaction analysis reveals that (+)-spathulenol demonstrates binding interactions akin to those observed with Donepezil within the AChE protein in physiological conditions. Such parallels in molecular interactions imply that (+)-spathulenol.

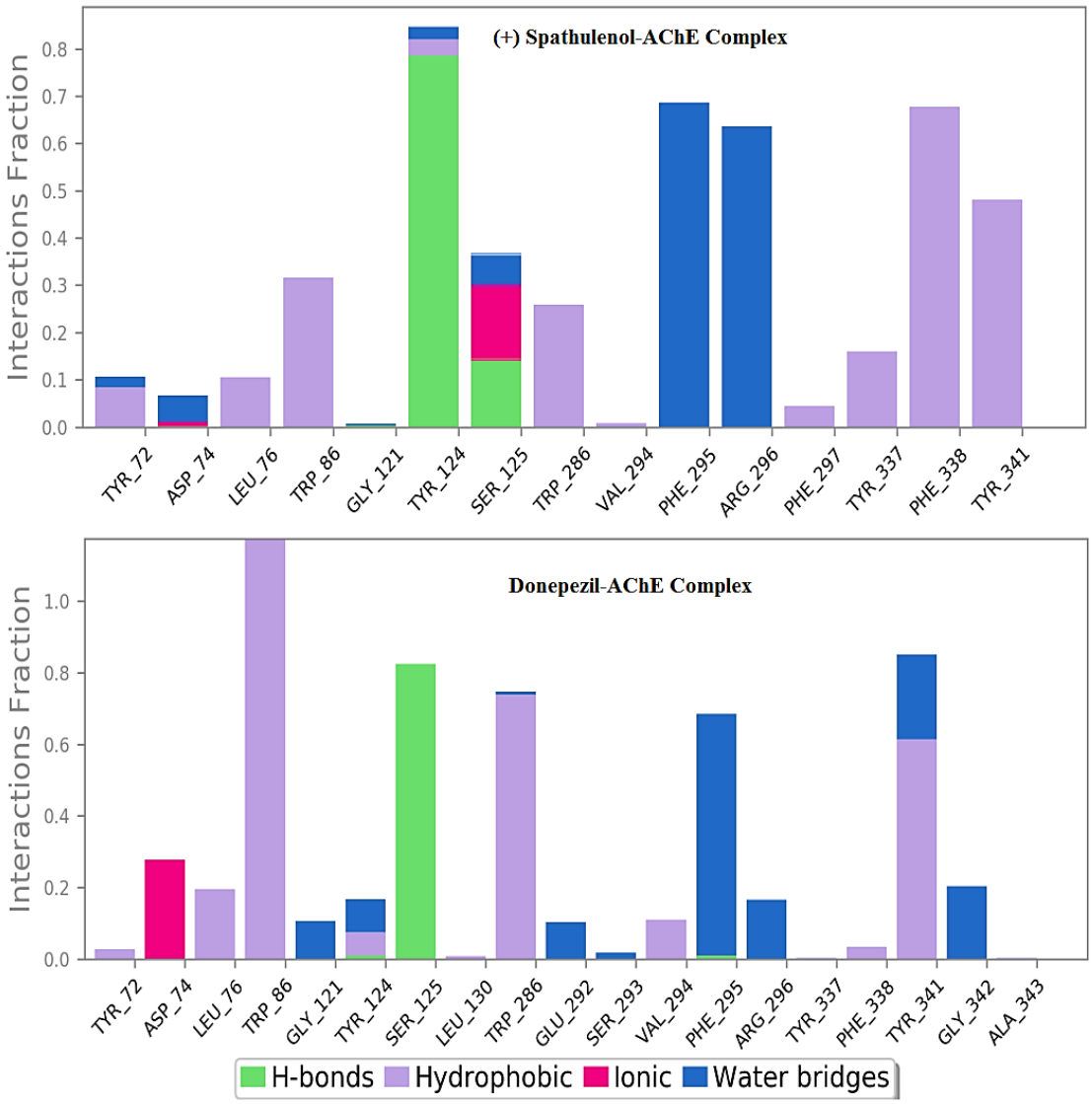

Fig.2.9. Binding interaction analysis of (+)-spathulenol-AChE complex and the Donepezil-AChE complex during 200ns simulation.

holds promise as an inhibitor of AChE activity, akin to Donepezil, a well-established therapeutic agent for Alzheimer's disease. This similarity in binding profiles suggests that (+)-spathulenol could potentially exhibit comparable efficacy in modulating AChE function, thereby presenting itself as a promising candidate for further investigation and development as a therapeutic agent for neurodegenerative disorders.

2.4. Discussion

The benefits of medicinal plants for well-being and health have become an essential tributary of great importance and hold immense potential in pharmacotherapy to protect humans against several disease [50-52]. In the present experiment, we evaluated the antioxidant capacity of ACEO against toxicity induced by CPF. For this purpose, WBC, RBC, Gly, Ch, TB, AChE and histopathological study were performed in different group of rats.

CPF administration induced a significant decrease in RBC, Hb and Ht levels, as well as a significant increase in WBC as compared to control. Belhattab, R, et al., [53] and Zouari, N, et al., [54] suggested that the decrease in RBC indicated either excessive damage or inhibition of erythrocyte formation. The pretreatment of rats treated with *A. campestris* EO reduced this disruption of hematological parameters induced by CPF. Our results are in agreement with the work of Saoudi et al., [55] who reported that *A. campestris* reduced the damage induced by deltamethrin. Our results indicated that deltamethrin induced a decrease in Hb Hajlaoui, H, et al., [56] and Feriani et al., [57]. Our study revealed that treatment of rats with CPF induced a remarkable increase in Glycemia, Total Bilirubin and total cholesterol levels as well as a decrease in cholinesterase activity compared to control group. Our results were in agreement with Boutabia, L, et al., [58]. The disruption of AChE activity induced by exposure to CPF could alter cholinergic synaptic function and thus affect the transmission of nerve information [59]. Degradation of acetylcholine is performed by AchE, which breaks it down into choline and acetate [60]. The combination of CPF and ACEO caused a significant increase in those parameters as compared treated rats with CPF alone. However, there were no differences detected for the cited parameters of treated animals with the ACEO compared to control group. Our results were in agreement with Saoudi et al., [55] who revealed that ACEO in combination with deltamethrin decreased the AChE level as compared to deltamethrin alone.

The antioxidant capacity of ACEO is verified also according to subsequent studies Hazzit, M, et al., [59] by the presence of a richness in monoterpenes (50, 24%) whose hydrocarbon monoterpenes reveal 38,85% (α-pinene, *β*-pinene, *β*-myrcene, R-(+)-limonene, trans-*β*-ocimene, *γ*-terpinene, terpinene-4-ol, α-terpineol, geranyl acetate, (+)-spathulenol and *β*-eudesmol).

Neurotoxicity is defined as a functional alteration of the nervous system originating from exposure to physical, biological or chemical agents Ghanmi, M, et al., [60]. Our histological study showed a normal architecture in both brain and spleen of the control group. CPF selectively caused the presence of edema and an alteration of the architecture with the presence of clear periplasmic in brain moreover alterations, inflammatory aspects and tissue damage in spleen of treated animals as compared to control. Our results are consistent with those of Sbayou, H, et al., [61] indicated that rats treated with deltamethrin showed in cerebral cortex abnormal cellular morphology accompanied by lipid deposits. Our results indicated that *ACEO* protected histological alterations in both brain and kidney against the administration of deltamethrin [55].

Reference

[1] Wołejko, E, et al., *Chlorpyrifos occurrence and toxicological risk assessment: a review*, International Journal of Environmental Research and Public Health 2022. 19: p. 12209. https://doi.org/10.3390/ijerph191912209

[2] Shi, T, et al., *Rapid biodegradation of the organophosphorus insecticide chlorpyrifos by Cupriavidus nantongensis X1T*, International Journal of Environmental Research and Public Health 2019. 16: p. 4593. https://doi.org/10.3390/ijerph16234593

[3] Bootharaju, M.S, et al., *Understanding the degradation pathway of the pesticide chlorpyrifos by noble metal nanoparticles*, Langmuir 2012. 28: p. 2671-2679. https://doi.org/10.1021/la2050515

[4] Ubaid Ur, R, et al., *Comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: evidence of mechanisms, exposures and mitigation strategies*, Science of The Total Environment 2021. 755: p. 142649. https://doi.org/10.1016/j.scitotenv.2020.142649

[5] Lin, J.W, et al., *Chlorpyrifos induces neuronal cell death via both oxidative stress and Akt activation downstream-regulated CHOP-triggered apoptotic pathways*, Toxicology in Vitro 2022. 86: p. 105483. https://doi.org/10.1016/j.tiv.2022.105483

[6] Jeon, H.J, et al., *Developmental toxicity of chlorpyrifos-methyl and its primary metabolite, 3,5,6-trichloro-2-pyridinol, to early life stages of zebrafish (Danio rerio)*, Ecotoxicology and Environmental Safety 2023. 249: p. 114352. https://doi.org/10.1016/j.ecoenv.2022.114352

[7] Sharma, A, et al., *Worldwide pesticide usage and its impacts on ecosystem*, SN Applied Sciences 2019.1: p. 1-16.

[8] Jabłońska-Trypuć, A, et al., *The impact of pesticides on oxidative stress level in human organism and their activity as an endocrine disruptor*, Journal of Environmental Science and Health Part B 2017. 52: p. 483-494. https://doi.org/10.1080/03601234.2017.1303322

[9] Mishra, B.P, et al., *Antioxidant status and oxidative stress in organophosphate pesticide poisoning*, Journal of Dental and Medical Sciences 2013. 7: p. 20-24. https://doi.org/10.9790/0853-0762024

[10] Shukla, S, et al., *Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate and herbicide in zebrafish*, Toxicology Reports 2017. 4: p. 240-244. https://doi.org/10.1016/j.toxrep.2017.05.002

[11] Sule, R.O, et al., *A common feature of pesticides: oxidative stress – the role of oxidative stress in pesticide-induced toxicity*, Oxidative Medicine and Cellular Longevity 2022. 2022: p. 1-31. https://doi.org/10.1155/2022/5563759

[12] Mahajan, R, et al., *Organophosphate pesticide: usage, environmental exposure, health effects, and microbial bioremediation, In: Microbial Biodegradation and Bioremediation*. Elsevier 2022: p. 473-490. https://doi.org/10.1016/B978-0-323-85455-9.00013-8

[13] Wang, Y, et al., *Organophosphorus pesticides management strategies: prohibition and restriction multi-category multi-class models, environmental*

transformation risks, and special attention list, Toxics 2025. 13: p. 16. https://doi.org/10.3390/toxics13010016
[14] Fatimah, N, et al., *Evaluation of suitability and biodegradability of the organophosphate insecticides to mitigate insecticide pollution in onion farming*, Heliyon 2024. 10: p. e32580. https://doi.org/10.1016/j.heliyon.2024.e32580
[15] da Silva, F.D.A, et al., *Molecular docking analysis of acetylcholinesterase corroborates the protective effect of pralidoxime against chlorpyrifos-induced behavioral and neurochemical impairments in Nauphoeta cinerea*, Computational Toxicology 2018. 8: p. 25-33. https://doi.org/10.1016/j.comtox.2018.07.003
[16] Abou-Donia, M.B, et al., *O-isopropyl methylphosphonofluoridate neurotoxicity: critical review*, Critical Reviews in Toxicology 2016. 46: p. 845-875. https://doi.org/10.1080/10408444.2016.1220916
[17] Rodríguez-Fuentes, G, et al., *Impacts of oxidative stress on acetylcholinesterase transcription and activity in embryos of zebrafish (Danio rerio) following chlorpyrifos exposure*, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2015. 172: p. 19-25. https://doi.org/10.1016/j.cbpc.2015.04.003
[18] Panda, S, et al., *Essential oils and their pharmacotherapeutic applications in human diseases*, Advances in Traditional Medicine 2022. 22: p. 1-15. https://doi.org/10.1007/s13596-020-00477-z
[19] Vambe, M, et al., *Biological properties, chemical profiles and safety of essential oils from South African aromatic plants: a literature review*, Journal of Essential Oil Research 2024. 37: p. 1-19. https://doi.org/10.1080/10412905.2024.2428698
[20] Ben Miri, Y, et al., *Essential oils: chemical composition and diverse biological activities: a comprehensive review*, Natural Product Communications 2025. 20: p. 1-29. https://doi.org/10.1177/1934578X241311790
[21] Subudhi, L, et al., *Anti-inflammatory activity of essential oil from medicinal plants: an insight into molecular mechanism, in-silico studies and signaling pathways*, Phytomedicine 2025. 138: p. 156364. https://doi.org/10.1016/j.phymed.2025.156364
[22] Kadri, A, et al., *Chemical constituents and antioxidant activity of the essential oil from aerial parts of Artemisia herba-alba grown in Tunisian semi-arid region*, African Journal of Biotechnology 2011. 10: p. 2923-2929. https://doi.org/10.5897/AJB10.2491
[23] Kadri, A, et al., *Chemical constituents and antioxidant properties of Rosmarinus officinalis L. essential oil cultivated from South-Western Tunisia*, Journal of Medicinal Plants Research 2011. 5: p. 5999-6004. https://doi.org/10.5897/JMPR11.423
[24] Kadri, A, et al., *Chemical composition and antioxidant activity of Marrubium vulgare L. essential oil from Tunisia*, African Journal of Biotechnology 2011. 10: p. 3908-3914. https://doi.org/10.5897/AJB11.301
[25] Zarai, Z, et al., *The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of Marrubium vulgare L. essential oil grown in Tunisia*,

Lipids in Health and Disease 2011. 10: p. 161. https://doi.org/10.1186/1476-511X-10-161
[26] Tit, D.M, et al., Antioxidant activity of essential oils, Antioxidants 2023. 12: p. 383. https://doi.org/10.3390/antiox12020383
[27] Kong, A.S.Y, et al., *Anti- and pro-oxidant properties of essential oils against antimicrobial resistance*, Antioxidants 2022. 11: p. 1819. https://doi.org/10.3390/antiox11091819
[28] Spisni, E, et al., *Essential oil molecules can break the loop of oxidative stress in neurodegenerative diseases*, Biology 2023. 12: p. 1504. https://doi.org/10.3390/biology12121504
[29] Sajad, M, et al., *Role of nutraceutical against exposure to pesticide residues: power of bioactive compounds*, Frontiers in Nutrition 2024. 11: p. 1342881. https://doi.org/10.3389/fnut.2024.1342881
[30] Bisht, D, et al., *Phytochemistry and pharmacological activity of the genus Artemisia*, Archives of Pharmacal Research 2015. 44: p. 439-474. https://doi.org/10.1007/s12272-021-01328-4
[31] Taljaard, L, et al., *Artemisia species and their active constituents for treating schistosomiasis*, Revista Brasileira de Farmacognosia 2023. 33: p. 875-885. https://doi.org/10.1007/s43450-023-00407-5
[32] Saoudi, M, et al., *Antioxidant and protective effects of Artemisia campestris essential oil against chlorpyrifos-induced kidney and liver injuries in rats*, Frontiers in Physiology 2021. 12: p. 618582. https://doi.org/10.3389/fphys.2021.618582
[33] Bendifallah, L, et al., *Phytochemical and biocidal properties of Artemisia campestris subsp. campestris L. (Asteraceae) essential oil at the southern region of Algeria*, Journal of Natural Pesticide Research 2023. 4: p. 100035. https://doi.org/10.1016/j.napere.2023.100035
[34] Saoudi, M, et al., *Chemical components, antioxidant potential and hepatoprotective effects of Artemisia campestris essential oil against deltamethrin-induced genotoxicity and oxidative damage in rats*, General Physiology and Biophysics 2017. 36: p. 331-342. https://doi.org/10.4149/gpb_2016057
[35] Bharadwaj, K.K, et al., *Nutraceutical flavonoid arrests cell cycle progression of human ovarian cancer (SKOV3) cells by inhibiting the histone deacetylase 2 protein*, Journal of Biomolecular Structure and Dynamics 2023. 42: p. 13421-13436. https://doi.org/10.1080/07391102.2023.2275187
[36] Desai, N.C, Aret al., *Design, synthesis, biological profile and molecular modeling and MD simulation studies of heterocyclic benzimidazole and thiazolidine-4-one based 5-arylidene analogues as prospective antimicrobial agents*, Journal of Molecular Structure 2024. 1299: p. 137166. https://doi.org/10.1016/j.molstruc.2023.137166
[37] Shaw, D.E, et al., *Desmond molecular dynamics system. Maestro-Desmond interoperability tools*, Schrödinger Release 2021-1.
[38] Othman, I.M, et al., *New substituted pyrazolones and dipyrazolotriazines as promising tyrosyl-tRNA synthetase and peroxiredoxin-5 inhibitors: design, synthesis, molecular docking and structure-activity relationship*

(SAR) analysis, Bioorganic Chemistry 2021. 109: p. 104704. https://doi.org/10.1016/j.bioorg.2021.104704
[39] Othman, I.M, et al., *Synthesis, structure-activity relationship and in silico studies of novel pyrazolothiazole and thiazolopyridine derivatives as prospective antimicrobial and anticancer agents*, ChemistrySelect 2021. 6: p. 7860-7872. https://doi.org/10.1002/slct.202101622
[40] Hammouda, M.B, et al., *Design, synthesis, biological evaluation and in silico studies of novel 1,2,3-triazole linked benzoxazine-2,4-dione conjugates as potent antimicrobial, antioxidant and anti-inflammatory agents*, Arabian Journal of Chemistry 2022. 15: p. 104226. https://doi.org/10.1016/j.arabjc.2022.104226
[41] Noumi, E, et al., *Illicium verum L. (Star Anise) essential oil: GC/MS profile, molecular docking study, in silico ADME profiling, quorum sensing, and biofilm-inhibiting effect on foodborne bacteria*, Molecules 2023. 28: p. 7691. https://doi.org/10.3390/molecules28237691
[42] Noumi, E, et al., *Chemical composition, antibacterial and antioxidant activities, in silico ADMET, molecular docking and dynamics simulations of Artemisia campestris essential oil*, Arabian Journal of Chemistry 2022. 15: p. 103925. https://doi.org/10.1016/j.arabjc.2022.103925
[43] Saoudi, M, et al., *Artemisia campestris essential oil attenuates oxidative stress and liver damage induced by chlorpyrifos in rats*, Environmental Science and Pollution Research 2021. 28: p. 42374-42384. https://doi.org/10.1007/s11356-021-14920-0
[44] Hammouda, M.B, et al., *Artemisia campestris essential oil: chemical composition, antioxidant activity and computational studies*, Natural Product Research 2021. 35: p. 5231-5236. https://doi.org/10.1080/14786419.2020.1809772
[45] Ben Hsouna, A, et al., *Chemical composition and antimicrobial activities of essential oils from Artemisia campestris L. and Artemisia herba-alba Asso*, Journal of Food Safety 2011. 31: p. 236-243. https://doi.org/10.1111/j.1745-4565.2010.00287.x
[46] Boubaker, H, et al., *Essential oil of Artemisia campestris L. inhibits mycelial growth of Botrytis cinerea and triggers defense mechanisms in tomato plants*, Plant Physiology and Biochemistry 2016. 103: p. 156-164. https://doi.org/10.1016/j.plaphy.2016.02.041
[47] Cheraif, I, et al., *Inhibition of cell communication and the induction of apoptosis by essential oils from three medicinal plants in human dermal fibroblasts*, Planta Medica 2012. 78: p. 958-964. https://doi.org/10.1055/s-0031-1298537
[48] Bouyahya, A, et al., *Synergistic effects of essential oils on human pathogens and their mechanisms of action: a review*, Phytomedicine 2020. 59: p. 152905. https://doi.org/10.1016/j.phymed.2019.152905
[49] Noumi, E, et al., *Chemical composition, antioxidant, anti-inflammatory and anticancer activities of essential oils of Artemisia campestris L. and Artemisia herba-alba Asso*, Journal of Food Biochemistry 2021. 45: p. e13505. https://doi.org/10.1111/jfbc.13505

[50] El Ouadi, Y, et al., *Essential oil composition of Artemisia campestris L. and its allelopathic effect on seed germination and seedling growth of weeds*, Allelopathy Journal 2017. 42: p. 181-194. https://doi.org/10.26651/allelo.j/2017-42-2-1102
[51] Boughendjioua, H, et al., *Chemical composition and antimicrobial activity of essential oils from Artemisia campestris L. growing in Algeria*, Journal of Essential Oil Bearing Plants 2017. 20: p. 1577-1588. https://doi.org/10.1080/0972060X.2017.1379290
[52] Taoufik, O, et al., *Chemical composition and antimicrobial activity of essential oil of Artemisia campestris L. from Morocco*, International Journal of Pharmacognosy and Phytochemical Research 2015. 7: p. 243-249.
[53] Belhattab, R, et al., *Composition and antimicrobial activity of essential oil from Artemisia campestris L. growing in Algeria*, Journal of Materials and Environmental Science 2014. 5: p. 693-698.
[54] Zouari, N, et al., *Chemical composition and antimicrobial activity of essential oil from Artemisia campestris L. growing wild in Tunisia*, Journal of Medicinal Plants Research 2010. 4: p. 1232-1239. https://doi.org/10.5897/JMPR10.149
[55] Saoudi, M, et al., Deltamethrin induced oxidative stress in kidney and brain of rats: Protective effect of *Artemisia campestris* essential oil, Biomedicine & Pharmacotherapy 2017. 11: p. 955-963. https://doi.org/10.1016/j.biopha.2017.08.030
[56] Hajlaoui, H, et al., *Chemical composition and biological activities of essential oils from three Tunisian Artemisia species*, International Journal of Essential Oil Therapeutics 2010. 4: p. 99-106.
[57] Feriani, A, et al., *Zygophyllum album saponins prevent atherogenic effect induced by deltamethrin via attenuating arterial accumulation of native and oxidized LDL in rats*, Ecotoxicology and Environmental Safety 2020. *193*: 110318. https://doi.org/10.1016/j.ecoenv.2020.110318
[58] Boutabia, L, et al., *Chemical composition and antimicrobial activity of essential oil of Artemisia campestris L. from Algeria*, Asian Journal of Chemistry 2011. 23: p. 2327-2330.
[59] Hazzit, M, et al., *Chemical composition and antibacterial activity of essential oils of Artemisia campestris L. and Artemisia herba-alba Asso*, Journal of Essential Oil Research 2009. 21: p. 337-340. https://doi.org/10.1080/10412905.2009.9700180
[60] Ghanmi, M, et al., *Chemical composition and antimicrobial activity of essential oil of Artemisia campestris L. from Morocco,* Natural Product Research 2012. 26: p. 1748-1755. https://doi.org/10.1080/14786419.2011.565962
[61] Sbayou, H, et al., *Chemical composition and biological activities of essential oil from Artemisia campestris L. growing wild in Morocco*, Journal of Essential Oil Bearing Plants 2018. 21: p. 1000-1011. https://doi.org/10.1080/0972060X.2018.1496677

Chapter Three

3.1. Conclusions

ACEO represents a natural substance in the treatment of different diseases and it has many activities such as antioxidant and anti-inflammatory. These activities are very effective as its chemical compounds have good affinities and interact with some key receptors, which are involved in many pathologies. The phenolic compounds of ACEO enhance endogenous antioxidant enzymes, such as SOD, CAT, and GPx. The extract inhibits NF-κB signalling, leading to decreased production of inflammatory cytokines particularly TNF-α and IL-6. Some components can chelate pro-oxidant metal ions, limiting ROS formation. The properties of ACEO that have been previously evaluated and our results encourage the use of this plant. The major selected phytoconstituents were assessed for their ADMET analysis revealing satisfactory bioavailability and pharmacokinetic properties. Significant interactions formed were formed with (+)-spathulenol within the AChE active site. (+)-Spathulenol-AChE complex was stable under the conditions during MD simulation. Overall, our results identify this plant, and particularly its ACEO, as a promising candidate for further investigation in the context of Alzheimer disease management. Nevertheless, additional in vivo and clinical research must be subjected to examine the safety of ACEO, to be a candidate drug and to validate the predicted observations.

3.2. Recommendations

- It is recommended to expand the pharmacological investigation of *Artemisia campestris* essential oil (ACEO) by assessing additional neuro-inflammatory markers such as TNF-α, IL-1ß, IL-6, NF-κB, and GFAP to better elucidate the mechanisms underlying the protective effects observed against chlorpyrifos (CPF).

- The multi-biomarker approach should be enhanced by incorporating oxidative stress parameters not measured in the present work, particularly MDA, GSH, CAT, GPx, and SOD.

- Given the promising activity on acetylcholinesterase (AChE) and the predicted capacity to cross the blood–brain barrier, future research should explore other targets associated with neurodegeneration, including ß-secretase (BACE-1) and hyperphosphorylated Tau.

- Bioguided fractionation of ACEO is recommended to correlate specific active phytoconstituents particularly (+)-spathulenol, ß-eudesmol, and terpinene-4-ol with the in vivo biological responses.

3.3. Future Works

- Measure additional oxidative stress and inflammation markers to better understand how ACEO protects the brain and spleen.

- Assess how ACEO is absorbed, distributed, metabolized, and eliminated in the body.

- Validate molecular docking results by conducting laboratory-based enzyme inhibition assays.

Chapter Four

Summary in English

This research investigated the protective effects of *Artemisia Campestris* essential oil (ACEO) against chlorpyrifos (CPF) toxicity in rats supported by computational modelling. Rats were divided into four groups: Group 1 (control), Group 2 (CPF treatment for 20 days, 10mg/kg/day), Group 3 (pre-treatment with ACEO for 10 days followed by CPF for 10 days), and Group 4 (ACEO treatment for 20 days). Our findings showed that CPF administration led to hematological disturbances, such as increased white blood cells and decreased red blood cells, hemoglobin, and hematocrit. CPF also disturbed biochemical parameters like acetylcholinesterase (AChE), glycemia, cholesterol, and total bilirubin. Histopathological changes were observed in the brain and spleen. ACEO pre-treatment protected against these disturbances. Histopathological analysis showed that ACEO improved tissue damage in the brain and spleen caused by CPF. In silico ADMET predictions indicated that the major molecules in ACEO exhibited favorable pharmacokinetics. Seven phytoconstituents showed significant binding affinity to human AChE pointing to their potential as neuroprotective agents. The stability of (+)-spathulenol, a lead compound, was studied through dynamic simulations for 200ns, showing promise as an AChE inhibitor similar to Donepezil. This study highlights ACEO's potential as an eco-friendly agent to enhance antioxidant defenses and counteract CPF's harmful effects.

SUMMURY

الملخص بالعربية

يهدف هذا البحث إلى دراسة التأثيرات الوقائية للزيت العطري لنبات *Artemisia campestris* (ACEO) ضد السمية الناتجة عن مبيد الكلوربيريفوس (CPF) في الجرذان، وذلك بدعم من النمذجة الحاسوبية. تم تقسيم الجرذان إلى أربع مجموعات: المجموعة الأولى (مجموعة الشاهد)، المجموعة الثانية (معالجة بـ CPF لمدة 20 يومًا بجرعة 10 ملغ/كغ/يوم)، المجموعة الثالثة (معالجة مسبقة بـ ACEO لمدة 10 أيام تليها معالجة بـ CPF لمدة 10 أيام)، والمجموعة الرابعة (معالجة بـ ACEO لمدة 20 يومًا.

أظهرت النتائج أن إعطاء CPF أدى إلى اضطرابات دموية تمثلت في زيادة عدد كريات الدم البيضاء وانخفاض عدد كريات الدم الحمراء والهيموغلوبين والهيماتوكريت. كما تسبب CPF في اضطراب بعض المؤشرات البيوكيميائية مثل نشاط إنزيم الأستيل كولين إستيراز(AChE) ، ومستوى الغلوكوز في الدم، والكوليسترول، والبيليروبين الكلي. كما لوحظت تغيرات نسيجية مرضية في الدماغ والطحال.

وقد أظهرت المعالجة المسبقة بـ ACEO تأثيرًا وقائيًا ضد هذه الاضطرابات. كما بيّن التحليل النسيجي أن ACEO ساهم في تحسين التلف النسيجي في الدماغ والطحال الناتج عن التعرض لـCPF.

وأشارت تنبؤات ADMET الحاسوبية إلى أن الجزيئات الرئيسية في ACEO تمتلك خصائص حركية دوائية ملائمة. كما أظهرت سبعة مركبات نباتية قدرة ارتباط ملحوظة بإنزيم الأستيل كولين إستيراز البشري(AChE) ، مما يشير إلى إمكانية استخدامها كعوامل واقية للأعصاب.

كما تمت دراسة استقرار المركب الرئيسي (+)-spathulenol باستخدام محاكاة الديناميكيات الجزيئية لمدة 200 نانوثانية، حيث أظهر نتائج واعدة كمثبط لإنزيم AChE بطريقة مشابهة لدواء Donepezil.

وتبرز هذه الدراسة الإمكانات الواعدة للزيت العطري لنبات *Artemisia campestris* كعامل صديق للبيئة قادر على تعزيز الدفاعات المضادة للأكسدة والحد من التأثيرات الضارة لمبيد الكلوربيريفوس.

استخلاص الزيت العطري من نبات الشيح ودراسة تطبيقاته

رسالة مقدمة لاستكمال متطلبات الحصول على درجة ماجستير العلوم في الكيمياء

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