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TOPIC

Phytochemical analyses and biological activities of selected medicinal plant extracts: bioactive nutrition and pharmaceutical resources.

Presented and publicly sustained by

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قال الله تعالى: "يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ"

صدق الله العلي العظيم

Dedication

I humbly dedicate this work to my beloved parents, may God bless and protect them, as a token of my deepest love and gratitude. Their unwavering support has been the cornerstone of my journey and the driving force behind the successful completion of this endeavor.

*A very special thanks goes to my dearest mother, « **Omi** », for her endless care, patience, and encouragement, and to my father, « **Habib** », whose wisdom and guidance have always inspired me. Their pride in me today is the greatest reward I could ever receive.*

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Communications

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-Benahmed, F., Rached, W., **MEDJADJI B**., Silarbi, A., & Kharoubi, O. (October 31, 2024 - November 2). Mercury-induced acute nephrotoxicity in rats: Treatment with aqueous extract of

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-Benahmed, F., Rached, W., Silarbi, A., **MEDJADJI B**., Mehrab, E., & Kharoubi, O. (2024). Oxidative stress in *Vicia faba* L. seedlings under different abiotic stresses: Salinity, water deficit, and heavy metal stress [Poster presentation]. II. International Biological & Life Sciences Congress (BIOLIC 2024), Antalya, Turkey.

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-**MEDJADJI Bochra**, (2025, February 23-24). Antibacterial activity of *Tetraclinis articulata* essential oil [Oral presentation]. 2nd National Seminar on Biodiversity, Environmental Risks and Public Health (SNBRESP 25), University Chadli Bendjedid El-Tarf, Algeria.

-**MEDJADJI Bochra**, (2024, December 17-18). Antioxidant potential of *Tetraclinis articulata* essential oil [Oral presentation]. 2nd National Seminar (les Matériaux pour l'Environnement et Développement Durable (MEDD'24)), Université de Relizane, Algeria. Co-authors: Benahmed Fatiha.

LIST OF ABBREVIATIONS

BBB	Blood–brain barrier
BHT	Butylated hydroxytoluene
CAM	Complementary and Alternative Medicine
DHFR	Dihydrofolate reductase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E. faecalis	*Enterococcus faecalis*
EO	Essential oil
E. coli	*Escherichia coli*
FL	Fidelity Level
GC-MS	Gas chromatography coupled with mass spectrometry
HAT	Hydrogen atom transfer
HI	Hemolysis inhibition
IC50	Inhibitory concentration of half-maximal response
ICD-11	Internation classification of diseases
ICF	Informant Consensus Factor
LC-MS	liquid chromatography-mass spectrometry
MEP	Methylerythritol phosphate
MOE	Molecular Operating Environment
MPs	Medicinal plants
MRSA	*Methicillin-Resistant Staphylococcus aureus*
MSSA	*Methicillin-Sensitive Staphylococcus aureus*
MVA	Mevalonate
NSAIDs	Non-steroidal anti-inflammatory drugs
PDB	Protein Data Bank
P-gp	P-glycoprotein
P. verticillata	*Ptychotis verticillata* (Desf.) Duby
RMSD	Root-mean-square deviation
ROS	Reactive oxygen species
SET	Single electron transfer
SFE	Supercritical Fluid Extraction
S. aureus	*Staphylococcus aureus*
SUV	Species Use Value
T. vulgaris	*Thymus vulgaris* L.
UV	Use Value
AACT	Aceto acetyl-CoA thiolase
HMGS	3-hydroxy-3-methyl glutaryl-CoA synthase
HMG-CoA	3-hydroxy-3-methyl glutaryl-CoA
MPs	Medicinal Plants

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INTRODUCTION

Over recent decades, medicinal plants have gained increasing recognition as essential sources for treating a wide range of pathological conditions. The World Health Organization estimates that approximately 80% of populations in various African countries rely on medicinal plants (phytotherapy) as a primary healthcare resource **(WHO, 2019)**. This global interest is driven by the search for novel bioactive compounds, elucidation of their mechanisms, and investigation of their biological properties, aiming to develop new therapeutics for challenging diseases such as cancer, neurodegenerative disorders, and cardiovascular ailments **(Leonoudakis *et al*., 2017; Ci *et al*., 2017)**.

The therapeutic potential of plants is attributed to their production of diverse secondary metabolites, including alkaloids, terpenoids, phenolic acids, flavonoids, tannins, lignins, quinones, and coumarins, which underlie their broad biological activities, such as antimicrobial and anti-inflammatory effects **(Živić *et al*., 2019)**.

In light of the escalating interest in developing safer alternatives to synthetic drugs, which often carry significant side effects, the renewed attention to traditional medicine has been remarkable. Deeply rooted in cultural practices worldwide, including those in Algeria, traditional medicine employs a wide range of natural remedies. Remarkably, the antioxidant potential of plants has been well documented, with mechanisms that involve neutralizing free radicals **(Mansoor *et al*., 2023)**. These pathological conditions are often accompanied by inflammation, a key contributor to aging and disease progression **(Ghosh *et al*., 2015)**.

Given this, global research efforts increasingly emphasize investigating the anti-inflammatory and antioxidant activities of medicinal plants to identify efficacious and safe drug candidates. Such natural agents hold promise for integration into foods and pharmaceuticals, potentially replacing synthetic medications **(Balkrishna *et al*., 2024)**.

Algeria's rich biodiversity spans over 4,000 plant taxa, many of which have traditional medicinal applications **(Belhouala and Benarba, 2021)**. While ethnobotanical studies across various Algerian regions, such as M'Sila, Bechar (Benarba, 2016), and Constantine-Mila (Ouelbani et al., 2016), have documented traditional plant use among local populations, relatively few have explicitly focused on the specialized knowledge possessed by herbalists. This gap is critical given herbalists' expertise and central role in medicinal plant applications **(Weckerle *et al*., 2018)**. This diversity offers vast potential for ethnopharmacological exploration of bioactive compounds. The Relizane region, in particular, is home to several wild and cultivated species traditionally used for therapeutic purposes, including *Thymus vulgaris* L., *Rosmarinus officinalis* L., *Mentha pulegium* L., and *Ptychotis verticillata* Duby. Local herbalists commonly use these species to manage ailments such as digestive and cardiovascular

disorders.

In this context, the present work constitutes the first comprehensive ethnopharmacological investigation in the Relizane region, combining ethnobotanical documentation with phytochemical analysis and multi-faceted biological evaluation. Initially focused on documenting medicinal plant knowledge among traditional healers and herbalists, this study targets explicitly two emblematic species from the region: *Ptychotis verticillata* Duby and *Thymus vulgaris* L.

The research methodology integrates GC-MS for detailed phytochemical characterization of the essential oils, followed by an assessment of their antioxidant, antibacterial, anti-insecticide, and anti-inflammatory properties.

To further elucidate the mechanisms of action, molecular docking studies were conducted. This approach aims to scientifically validate the traditional uses of these plants while exploring their potential for therapeutic development.

This thesis is structured into three main parts:

1. The first part offers a comprehensive literature review of secondary metabolites, their biological activities, molecular Docking, and detailed descriptions of the studied plants.
2. The second part outlines experimental protocols used for qualitative and quantitative biomolecule identification via GC-MS. It evaluates the in vitro antioxidant, anti-inflammatory, antibacterial, insecticidal, and nutritional activity, as well as in silico molecular docking studies.
3. The third part presents Results and discusses the experimental results in the context of existing scientific literature.

LITERATURE REVIEW

1. Ethnobotany, Medicinal Plants: What is the relationship?

A vital symbiotic relationship unites Ethnobotany and medicinal plants. By safeguarding traditional knowledge of plant-based remedies, ethnobotany provides pharmacology with a valuable roadmap for discovering new medicines while preserving an invaluable cultural heritage.

1.1. **. Ethnobotany**

Ethnobotany is an interdisciplinary science that explores the dynamic relationships between people and plants. First coined by Harshberger in 1895, the term has since evolved to encompass not only the traditional uses of plants for food, medicine, and rituals, but also their cultural significance, conservation, and sustainable management **(Alrhmoun *et al.*,2025)**. It lies at the intersection of botany, anthropology, pharmacology, and ecology, providing essential insights into how local communities perceive, classify, and utilize plant resources.

Traditional knowledge associated with plants plays a vital role in primary healthcare, especially in rural and developing regions. According to the World Health Organization **(WHO, 2019)**, approximately 80% of the world's population relies on traditional medicine for basic health needs. In many North African and Mediterranean communities, including Algeria, medicinal plants are deeply embedded in cultural practices, and their use is transmitted orally from generation to generation **(Benarba, 2016)**.

Ethnobotanical surveys are thus crucial for documenting this knowledge before it is lost due to modernization, urbanization, and the erosion of indigenous traditions **(Awoke *et al.*, 2025)**. Moreover, such studies provide a foundation for identifying species with therapeutic potential, which may lead to novel drug discoveries. Indeed, about 25–35% of modern pharmaceutical drugs are derived from plant compounds, and a significant proportion of anticancer agents are of plant origin **(Newman & Cragg, 2020)**.

In Algeria, the flora is estimated at over 3,000 species, many of which are endemic, and offers a valuable yet underexplored reservoir of medicinal biodiversity **(Hemmami *et al.*, 2023)**. Ethnobotanical investigations in different Algerian regions, such as Tlemcen **(Benarba *et al.*, 2014)**, Khenchela **(Bensizerara *et al.*, 2025)**, and the Aurès Mountains **(Sekhri *et al.*, 2017)** have revealed a consistent reliance on local herbal remedies, often administered by traditional healers and herbalists, particularly in remote or underserved areas.

1.2. **Medicinal Plants**

Medicinal Plants (MPs) refer to species or specific plant parts that contain bioactive compounds, commonly called active principles, which can be used for therapeutic purposes

when administered at appropriate dosages and without harmful effects **(Riaz *et al*., 2023)**. These active constituents may serve as precursors in the synthesis of pharmaceutical drugs, exhibit proven therapeutic effects according to biomedical standards, or contain pharmacologically functional molecules **(Chilwant *et al*., 2025)**.

Globally, interest in traditional medicine has significantly increased in recent decades **(Alburyhi *et al*., 2025)**. In Africa, however, traditional medicine still requires greater structural and scientific advancement than more established systems like those in India or China. Due to economic constraints and limited access to conventional healthcare, it is estimated that nearly 80% of the African population depends on medicinal plants for primary healthcare needs **(Seukep *et al*., 2023)**.

The bioactive substances found in medicinal plants include a wide range of naturally occurring compounds such as polyphenols **(El-Saadony *et al*., 2025)**, terpenoids **(Dhar *et al*., 2017)**, steroids, alkaloids **(Seabrooks & Hu, 2017)**, vitamins (A, C, E, and K) **(Turhan & Bör, 2016)**, carotenoids **(Cueto *et al*., 2017)**, tannins **(Huang *et al*., 2017)**, saponins **(Singh & Kaur, 2018)**, pigments **(Kumara *et al*., 2017)**, and various minerals **(Velander *et al*., 2017)**, all of which exhibit a broad spectrum of biological activities. Of the estimated 350,000 plant species identified globally, over 200,000 are found in tropical African ecosystems **(Nguyen *et al*., 2025)**, and approximately 80,000 species are currently used in traditional medicinal practices worldwide **(Monib, 2024)**. These plants are primarily employed in folk medicine systems and pharmacopoeias. MPs have been used since ancient times and remain therapeutically relevant, even in modern healthcare systems **(Sharma *et al*., 2025)**.

Today, MPs are also extensively used in industrial applications, particularly in the production of dietary supplements, food additives, and phytogenic feed additives as natural alternatives to synthetic products in animal husbandry **(Aminullah *et al*., 2025)**. Importantly, medicinal plants and their constituent phytochemicals represent a valuable resource for drug discovery, serving either as lead compounds or structural models for pharmaceutical development **(Chihomvu *et al*., 2025)**.

Their expanding applications in modern society have led to their integration into several health-related domains, including rational phytotherapy **(Zalla, 2024)**, veterinary medicine **(Chapman *et al*., 2017)**, aromatherapy **(Roosta *et al*., 2017)**, nutraceuticals **(Bureau, 2016)**, cosmetics ***(Tareau et al., 2017)***, and animal welfare **(Máthé, 2015)**.

In developed countries, traditional healing practices have been adapted. They are now referred to as "Complementary and Alternative Medicine" (CAM), which is reportedly used by

up to 65% of the population in some nations **(Bhattacharjee *et al.*, 2024)**. Rational phytotherapy based on scientific evidence differs from traditional herbalism by employing standardized doses of dried plant materials (flowers, leaves, stems, roots) or extracts (decoctions, infusions, macerations, distillates, essential oils) for the treatment and prevention of specific symptoms **(Wiesner & Knöss, 2017)**. This approach aligns more closely with conventional medicine **(Xu *et al.*, 2024)**.

In Algeria, numerous native medicinal species have long been utilized to treat a wide range of diseases. Phytotherapy is deeply rooted in Algerian cultural traditions and continues to play a vital role in public health today **(Bensizerara *et al.*, 2025)**. Particularly in arid and mountainous regions, more than 626 plant species have been recorded as medicinal or aromatic plants, many of which are also explored for their pesticidal properties **(Mahato et al., 2025)**.

Among the numerous phytochemical groups derived from medicinal plants, essential oils are among the most valuable and widely studied classes **(da Cruz *et al.*, 2025)**, owing to their complex composition, biological activities, and broad range of therapeutic applications **(Ben Miri, 2025)**.

2. Essential Oils

The term "essential oil" (EO) dates back to the 16th century and originates from the medicinal concept of Quinta essentia, introduced by the Swiss physician Paracelsus von Hohenheim **(Campos, 2024)**.

These oils are also called "essences" because they are easily flammable **(Recsky *et al.*, 2025)**. Indeed, essential oils are concentrated liquids that can ignite quickly due to their chemical composition. Many researchers have attempted to provide a precise definition of exactly what essential oils are, as their complex composition and varied properties make an accurate description difficult **(Cui *et al.*, 2025)**.

According to the French Standardization Agency (AFNOR), an EO is a product obtained from plant raw materials by steam distillation, mechanical processes applied to the citrus peel (epicarp), or "dry" distillation techniques. Essential oils are soluble in alcohol, ether, and fixed oils but are insoluble in water. Typically, these volatile oils are liquid and colorless at room temperature. They have a density below 1, high refractive indices, and notable optical activity. The volatile oils found in aromatic herbs are responsible for the diverse fragrances emitted by plants. EOs can be present in all parts of aromatic plants **(Mohammed *et al.*, 2025)**.

2.1. Essential Oils and Human Health

EOs contain bioactive compounds, such as terpenes, terpenoids, and polyphenols, that provide a broad range of therapeutic effects, including antimicrobial, anti-inflammatory, antioxidant, and psychogenic benefits, thereby significantly contributing to human health **(Kaspute *et al.*, 2024; Arzani *et al.*, 2025)**.

When inhaled, EO compounds enter the respiratory tract, are absorbed into the lungs, and are rapidly transported to the brain, where they influence neurological functions and mood **(Hassid *et al.*, 2025)**.

This inhalation route is a key mechanism in aromatherapy **(Figure 1)**, offering notable health benefits such as stress relief and improved well-being **(Arzani *et al.*, 2025)**. In addition to inhalation, EOs are absorbed through the lungs and the systemic circulation, contributing to their diverse pharmacological effects in the body and to overall health support **(Kaspute *et al.*, 2024)**. Despite these benefits, caution is necessary due to potential adverse effects, including endocrine disruption associated with some EO components **(Tongnuanchan & Benjakul, 2014)**. Understanding EO absorption and transport pathways is thus essential for their safe and effective use in complementary and alternative medicine and for maximizing their health-promoting potential.

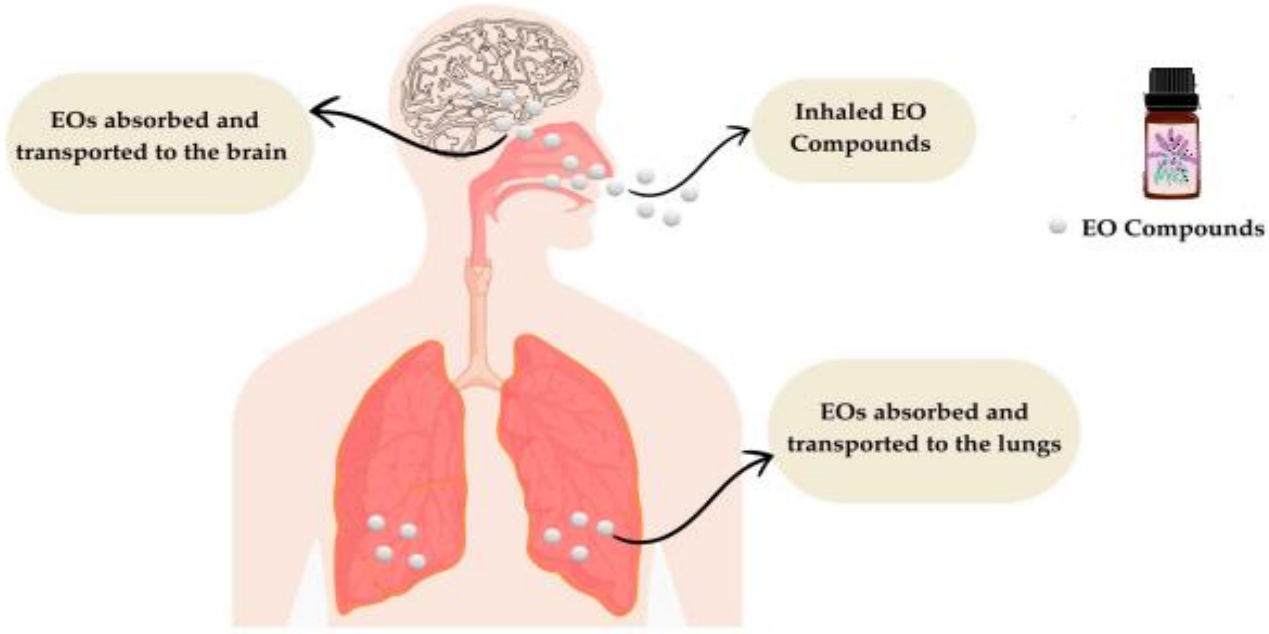


Figure 1. Mechanism of Essential Oil (EO) Absorption and Transport in the Human Body **(Caballero-Gallardo *et al.*, 2025).**

2.2. Terpenes: Key Components of Essential Oils

The pharmacological properties of medicinal plants are attributed mainly to their secondary metabolites, among which essential oils represent particularly bioactive fractions. These volatile extracts, rich in terpenes and phenolic compounds, constitute the primary focus of this investigation. Within this chemical diversity, terpenes emerge as major contributors to the biological activities of essential oils.

(Figure 2) illustrates the classification of terpenes according to their isoprene units and molecular sizes. It highlights those small, volatile terpenes, such as monoterpenes, are primarily detected by gas chromatography-mass spectrometry (GC–MS). In contrast, larger, less volatile compounds, such as diterpenes, require liquid chromatography-mass spectrometry (LC-MS) **(Ren *et al.*, 2025)**. This demonstrates the need for a multimodal analytical approach to comprehensively characterize terpene profiles in edible essential oils, ensuring accurate assessment of their bioactive properties **(Abdelmohsen *et al.*, 2025)**.

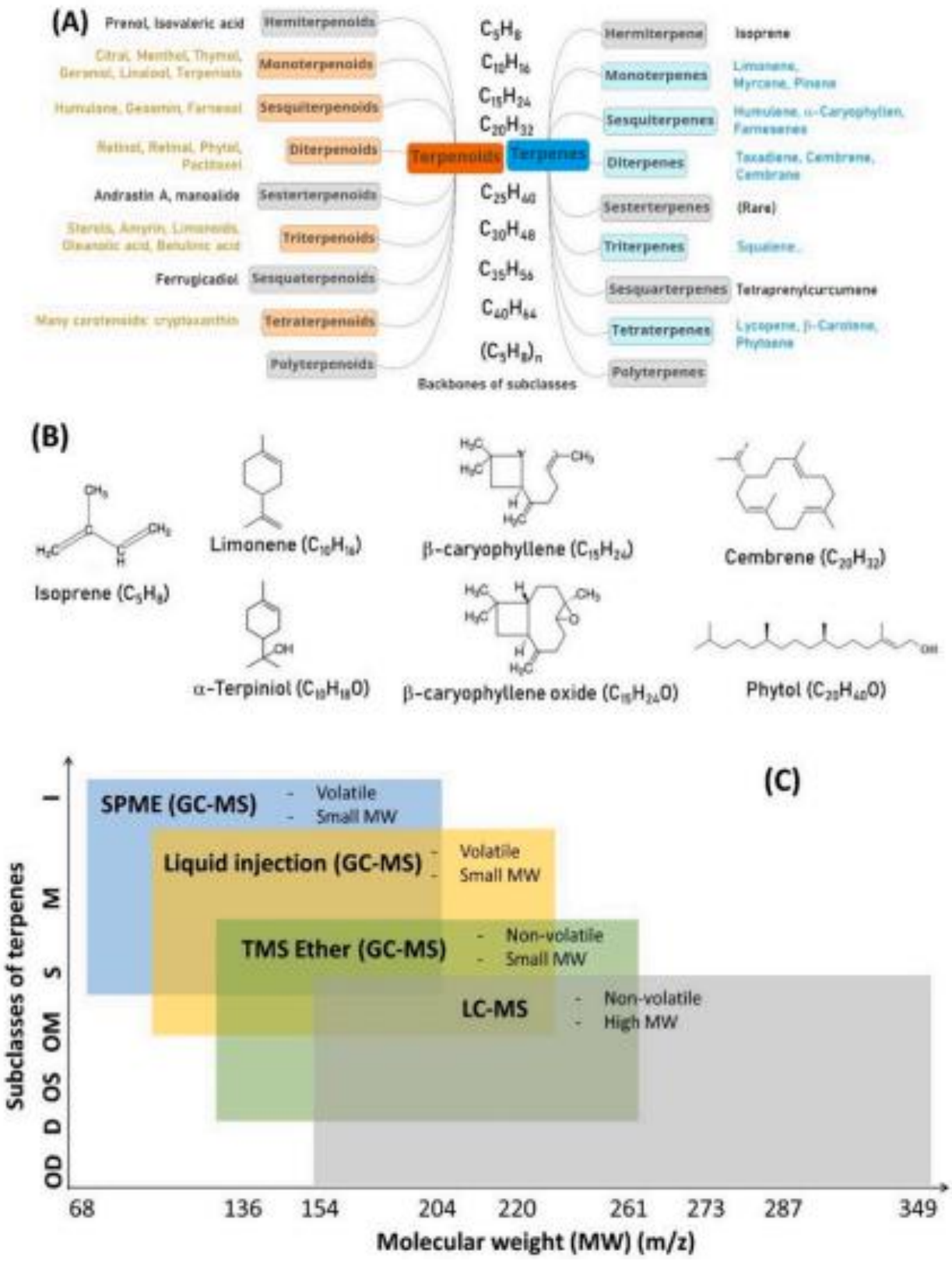


Figure 2. Structures, classification, and overlap in terpenes molecular weight as a function of the analytical methods employed. I = Isoprene, M = Monoterpenes, OM = Oxygenated monoterpenes, S = Sesquiterpenes, OS = Oxygenated sesquiterpenes, D = Diterpenes, OD = Oxygenated diterpenes **(Pham *et al.*, 2024).**

2.2.1. Definition and Significance of Terpenes

Terpenes represent a vast and diverse class of secondary metabolites synthesized by plants through the isoprenoid biosynthetic pathways, specifically **(Semmar, 2024)** the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways **(Figure 3)**. These organic compounds are structurally based on repeating isoprene (C_5H_8) units, the number of which defines the terpene subclasses. Terpenes fulfill critical ecological functions such as attracting pollinators, deterring herbivores, and conferring resistance to abiotic stresses like drought or UV radiation. They contribute significantly to plant aroma, flavor, and medicinal properties **(Wannes *et al.*, 2009)**. Their ecological roles are paralleled by their pharmacological potential, making terpenes essential bioactive metabolites in ethnomedicine and modern phytotherapy.

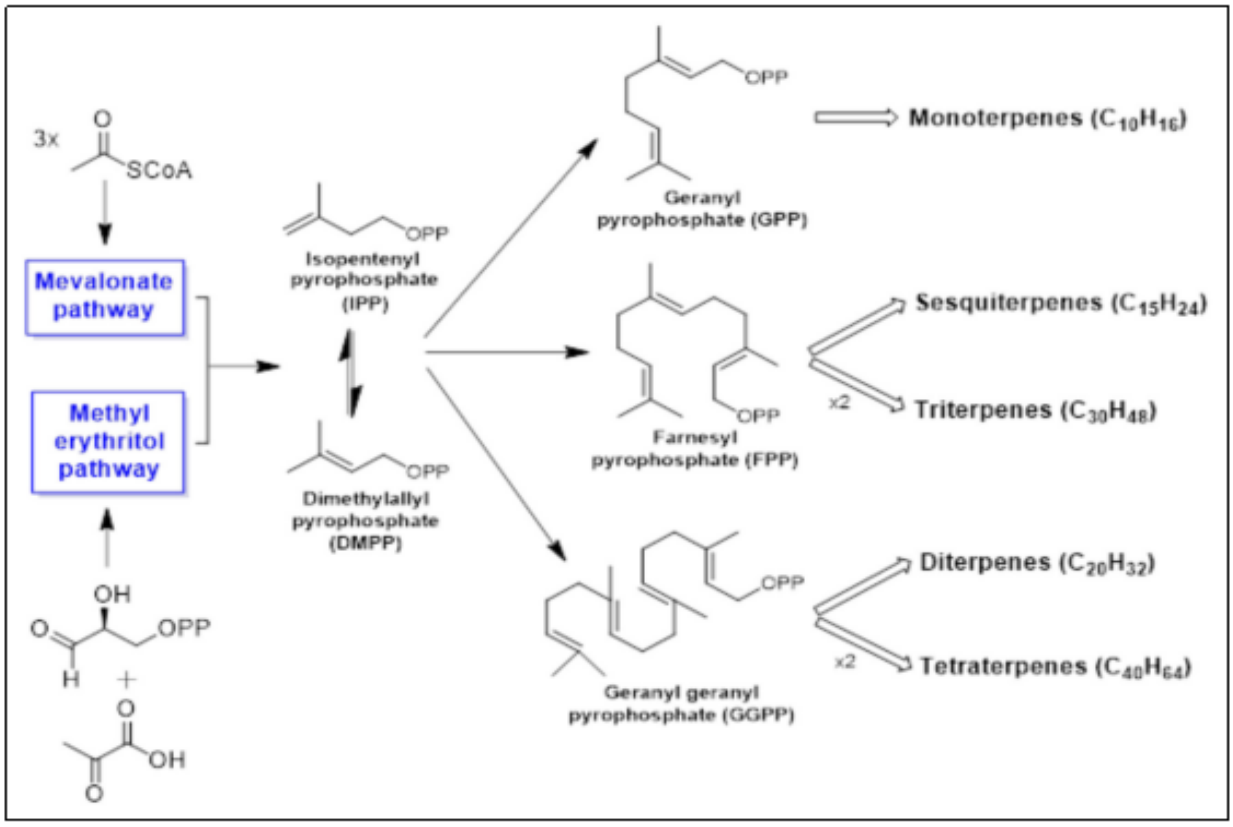


Figure 3. Schematic Representation of Terpenoid Biosynthesis **(Ben Miri, 2025).**

2.2.2. Classification of Terpenes

EOs are rich in terpenes, primarily composed of hemiterpenes, monoterpenes, and sesquiterpenes **(Figure 4)**. These volatile compounds give plants their distinctive aromas and flavors, making them highly valued in culinary, cosmetic, and medicinal fields. EO contains a complex mixture of terpenes, including mono-, sesqui, and diterpene hydrocarbons, as well as their oxygenated derivatives, such as alcohols, aldehydes, and esters **(Baharum *et al.*, 2010)**.

The classification of terpenes is based on the number of carbon atoms and the

arrangement of isoprene units. This classification reflects the metabolic complexity and functional diversity observed in plants, which is key to understanding their bioactive properties **(Shen *et al*., 2023)**.

- Monoterpenes (C_{10}) consist of two isoprene units and represent the simplest and most common form. Examples include thymol, carvacrol, linalool, and limonene, which are frequently found in essential oils and exhibit notable antimicrobial and antioxidant activities.
- Sesquiterpenes (C_{15}) are made up of three isoprene units, such as β-caryophyllene and germacrene D, known for their anti-inflammatory and cytotoxic effects.
- Diterpenes (C_{20}) and higher terpenes, including triterpenes (C_{30}) and tetraterpenes (C_{40}), are larger molecules with diverse biological functions, among them anticancer and neuroprotective activities. Although less common, tetraterpenes and their oxygenated derivatives also play essential roles in plant coloration and photosynthesis **(Ruberto & Baratta, 2000)**.

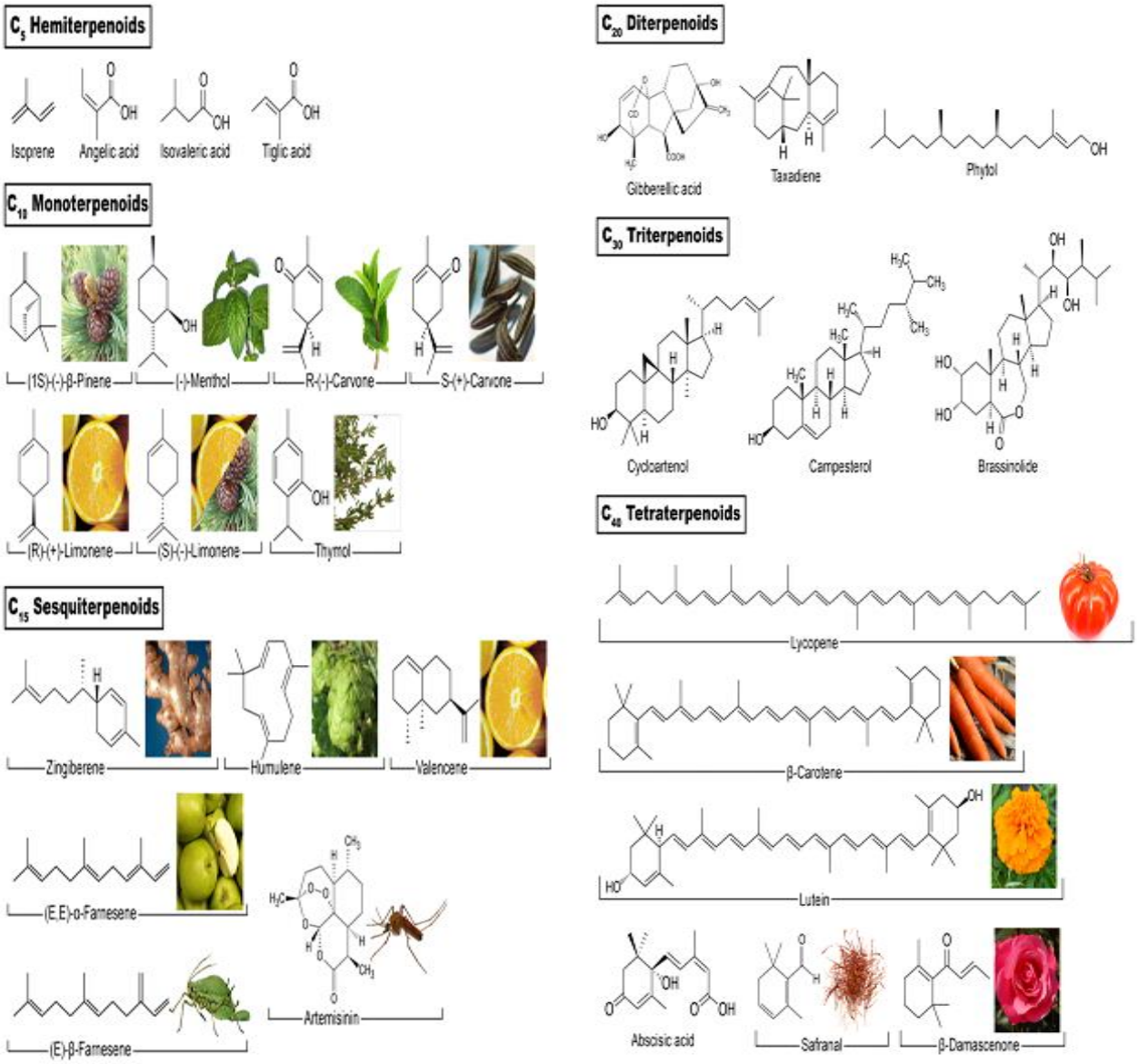


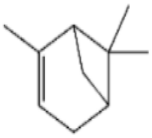
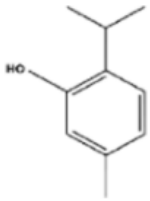
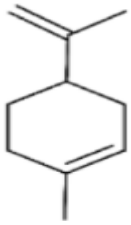
Figure 4. Different classes of terpenoids by chain length and biosynthetic origin, and examples of their odors or other biological applications **(Josh T, 2023).**

2.2.3. Antioxidant and Pharmacological Properties of Terpenes

Terpenes exhibit significant antioxidant activity primarily by neutralizing reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals, and peroxyl radicals **(Mazzone *et al.*, 2025)**. This antioxidative action helps prevent oxidative damage to lipids, proteins, and nucleic acids, which is implicated in the development of chronic diseases, including cancer, neurodegenerative diseases, and cardiovascular disorders **(Table I)**. The molecular mechanisms underlying this activity typically involve Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET), depending on the terpene's molecular structure **(Bhattacharya *et al.*, 2025)**.

Several terpenes, such as thymol, carvacrol, and α-pinene, have demonstrated vigorous radical-scavenging activity in vitro, often comparable or superior to that of synthetic antioxidants like BHT **(Ferreira *et al.*, 2025)**.

Table I. Monoterpenes : Botanical Sources, Therapeutic Uses, and Chemical Profiles.

Name of Terpene	Source	Medical Applications	Chemical Structure	References
α-Pinene	Oils of aromatic plants like *Eucalyptus* and *Salvia ros*	Antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, antinociceptive, antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, anti-inflammatory, anti-Leishmania, and analgesic effects		**(Khanam *et al.*, 2025)**
Thymol	Essential oil of *Thymus vulgaris* L.	Anti-inflammatory, antiviral, antibacterial, and anti-septic, treatment of burns and ulcerations		**(Peng *et al.*, 2024)**
Limonene	Oils of *Citrus sinensis*, *Citrus paradisi*, *Citrus limon*, and *Anethum graveolens* L. (dill)	Anti-inflammatory, anti-oxidant, antinociceptive, anticancer, antidiabetic, anti-hyper analgesic, anti-viral, and gastro-protective effects		**(Eddin *et al.*, 2023)**

2.3. Phenolic Compounds

Polyphenols, with over 8,000 known phenolic structures, constitute a complex group of secondary metabolites synthesized exclusively in the plant kingdom. They are characterized by the presence of at least one benzene ring attached directly to one or more free hydroxyl groups or conjugated with other functional groups such as ethers, esters, glycosides, etc. **(Wang *et al.*, 2025)**.

They are broadly classified into flavonoids and non-flavonoids based on their chemical structures **(Figure 5)**, particularly the presence or absence of the C6–C3–C6 backbone typical of flavonoids **(Arzani *et al.*, 2025)**. Flavonoids include diverse subclasses such as flavones, flavanones, and anthocyanidins, known for their potent antioxidant and health-promoting properties, with quercetin being a notable dietary flavonol **(Abd El-Hack *et al.*, 2025)**. Nonflavonoids comprise phenolic acids, stilbenes, lignans, and tannins, many of which

demonstrate significant bioactivity and polymeric complexity **(De Rossi et al., 2025)**.

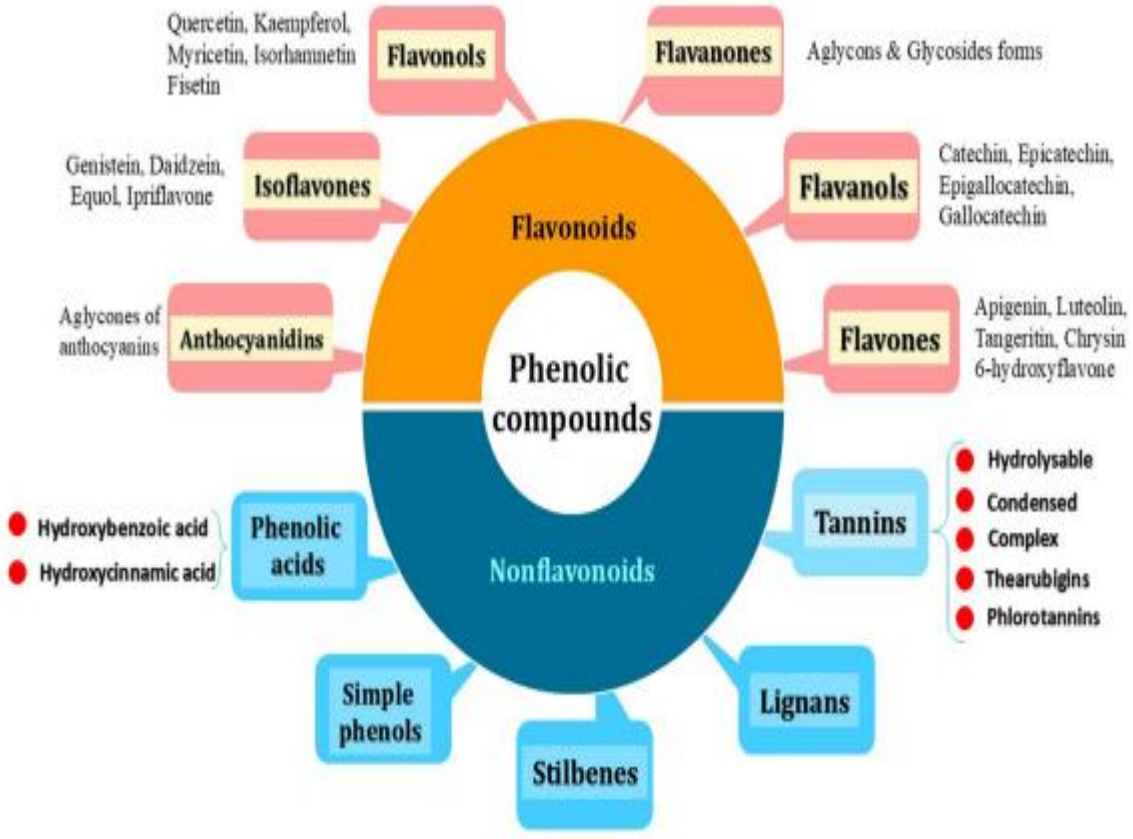


Figure 5. Classes of polyphenols **(Arzani *et al.*, 2025).**

The chemical diversity of polyphenols accounts for their wide range of biological activities and their importance in plant physiology and human health **(Benjeddi *et al.*, 2025)**.

3. In vitro biological activities

3.1. Antioxidants

The antioxidant activity of plant-derived compounds, especially those found in EOs, is primarily attributed to secondary metabolites such as terpenoids and phenolic compounds **(Câmara *et al.*, 2024)**. Terpenes are a diverse class of organic compounds produced by plants and are recognized for their strong antioxidant potential. Their mode of action as antioxidants includes **(Ahmadinejad *et al.*, 2017)**:

- Scavenging free radicals: Terpenes can donate hydrogen atoms or electrons to neutralize free radicals, interrupting radical chain reactions and preventing oxidative damage to biomolecules.
- Chelating metal ions: By binding transition metal ions (such as Fe^{2+} and Cu^{2+}), terpenes inhibit metal-catalyzed formation of highly reactive hydroxyl radicals via Fenton

reactions.

- Modulating antioxidant defense pathways: Certain terpenoids may enhance endogenous antioxidant defenses by influencing cellular signaling pathways related to oxidative stress response.

As illustrated **(Figure 6)**, essential oils exhibit antioxidant activities that block the initiation and progression of lipid peroxidation chain reactions. This prevents the oxidation of fatty acids and lysophospholipids, thereby protecting lipid integrity and cellular health. Analytical techniques such as shotgun lipidomics enable the detection of changes in lipid peroxidation products, facilitating the interpretation of EO antioxidant efficacy. This highlights the critical role of essential oils as natural antioxidants against oxidative stress and lipid damage in biological systems.

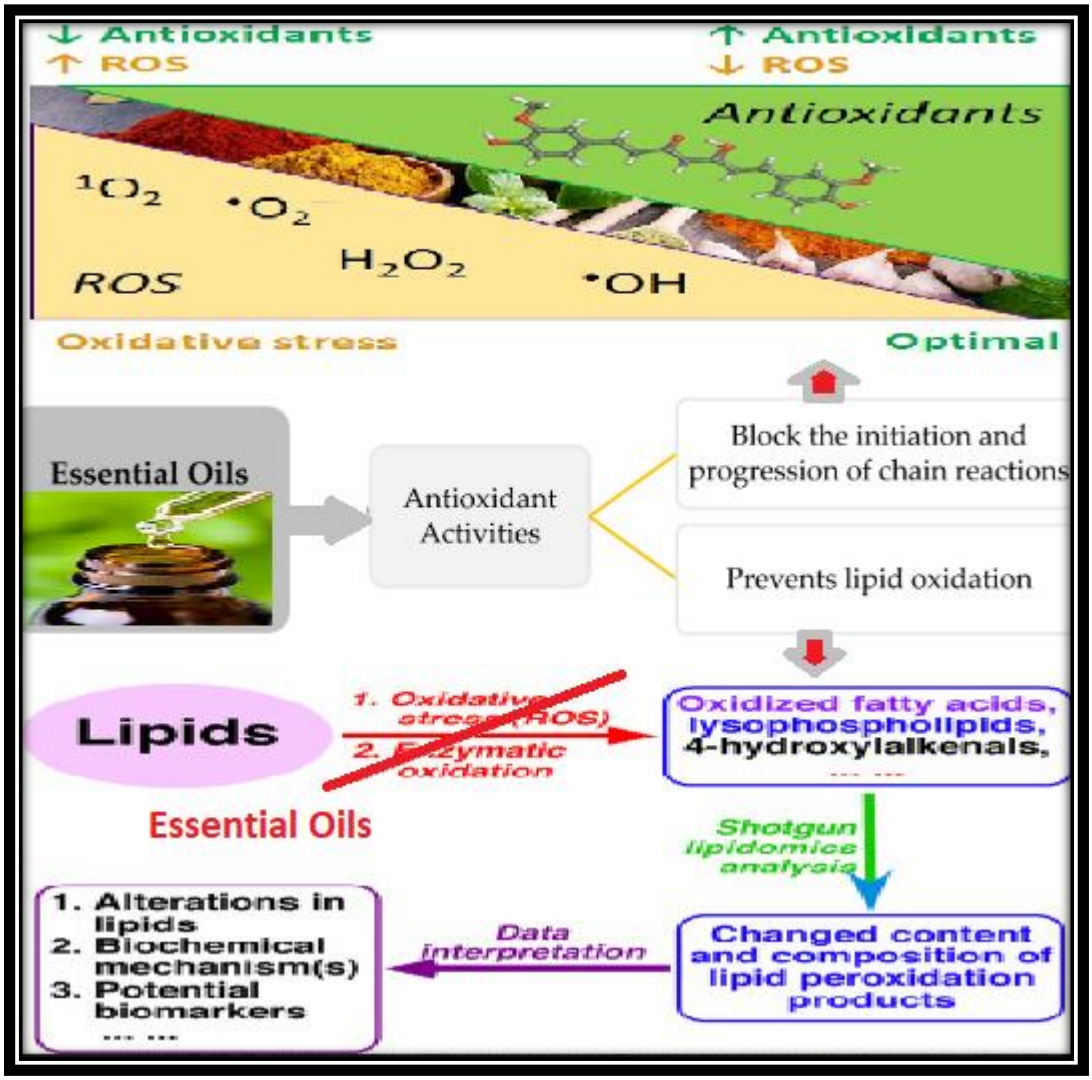


Figure 6. Antioxidant Mechanisms of Essential Oils.

3.2. Anti-Inflammatory Activity

EOs contain bioactive terpenes and terpenoids that exhibit potent anti-inflammatory effects through multiple mechanisms **(Câmara *et al.*, 2024)**. These compounds modulate inflammatory responses by inhibiting key enzymes such as cyclooxygenases (COX-1, COX-2) and lipoxygenases (LOX), thereby reducing the production of pro-inflammatory mediators, including prostaglandins, leukotrienes, and nitric oxide **(Iqbal *et al.*, 2024)**. Moreover, EOs interfere with signaling pathways such as NF-κB and TNF-α, leading to downregulation of cytokine expression (IL-1β, IL-6, IL-8) and decreased immune cell recruitment at inflammatory sites **(Caballero-Gallardo *et al.*, 2025)**. **(Figure 7)** illustrates the multi-targeted action of different EOs on neuroendocrine-immune interactions. *Thymus vulgaris* L. (*T. vulgaris*) and *Ptychotis verticillata* Duby (*P. verticillata*) EOs influence the hypothalamic-pituitary-adrenal (HPA) axis by modulating neurotransmitters such as serotonin (5-HT), dopamine (DA), and gamma-aminobutyric acid (GABA), which indirectly regulate stress and inflammatory responses. EOs exert their effects by inhibiting NF-κB signaling in cytokine-producing cells, thereby reducing pro-inflammatory cytokine release. These actions are further linked to the adrenal gland's activation of glucocorticoids, which suppress excessive inflammation.

Additionally, the small molecular size of terpene compounds enhances their cellular penetration and bioavailability, making them effective natural alternatives for managing inflammation with minimal side effects **(Kaspute *et al.*, 2024)**. In this context, aromatherapy with EOs provides a holistic complementary medicine strategy, particularly beneficial for chronic inflammatory conditions, offering therapeutic benefits beyond symptomatic relief **(Diogo Gonçalves, 2025)**.

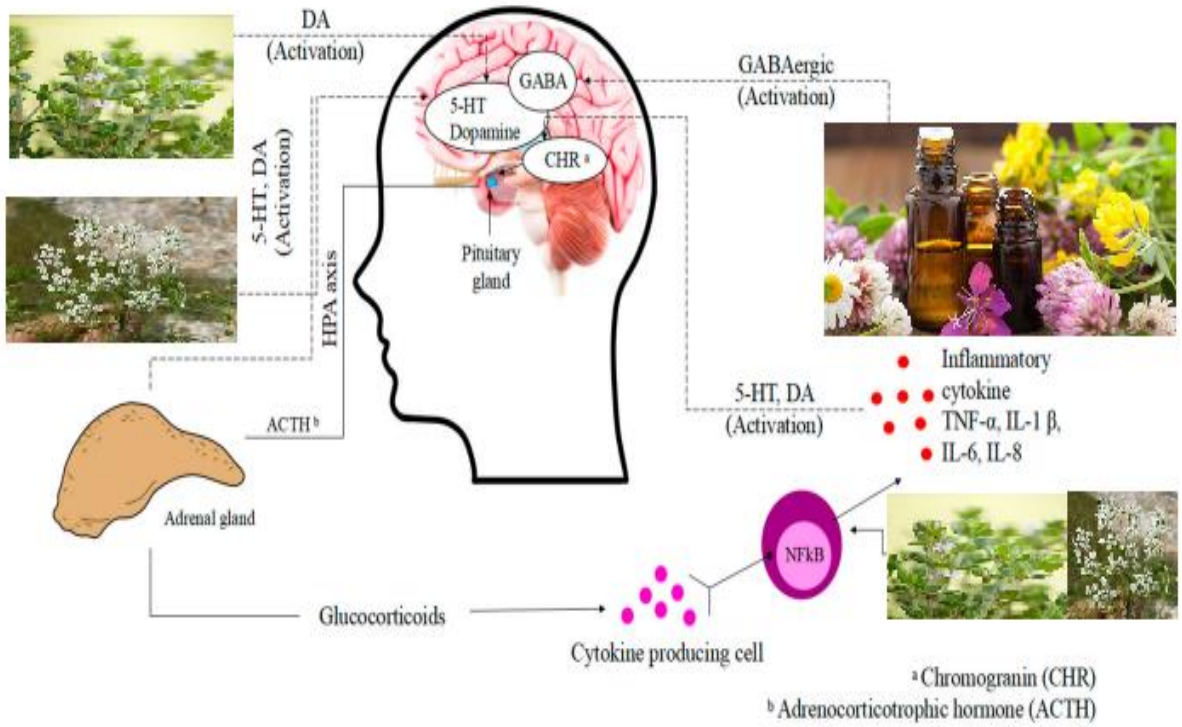


Figure 7. Multi-target anti-inflammatory mechanisms of essential oils (EOs) through neuroendocrine-immune interactions.Adopted from Bochra Medjadji. (**Caballero-Gallardo *et al.*, 2025).**

3.3. Antibacterial Activity

Plant-derived natural substances form the core of traditional medicine practices today. The rising prevalence of antibiotic resistance is the primary driver of the need to discover and develop new antimicrobial agents **(Cushnie *et al.*, 2011)**. Plants produce a wide variety of bioactive compounds, such as terpenoids, flavonoids, alkaloids, and antimicrobial peptides, that are traditionally used in medicine worldwide. These natural substances exhibit antimicrobial activity by targeting bacterial membranes, inhibiting essential enzymes, and interfering with microbial metabolism **(Figure 8)**. The growing problem of antibiotic resistance motivates the search for and development of novel plant-derived antimicrobial agents. Plant-derived antimicrobials can act alone or synergistically with conventional antibiotics to improve effectiveness and reduce the development of resistance **(Rakholiya *et al.*, 2025)**.

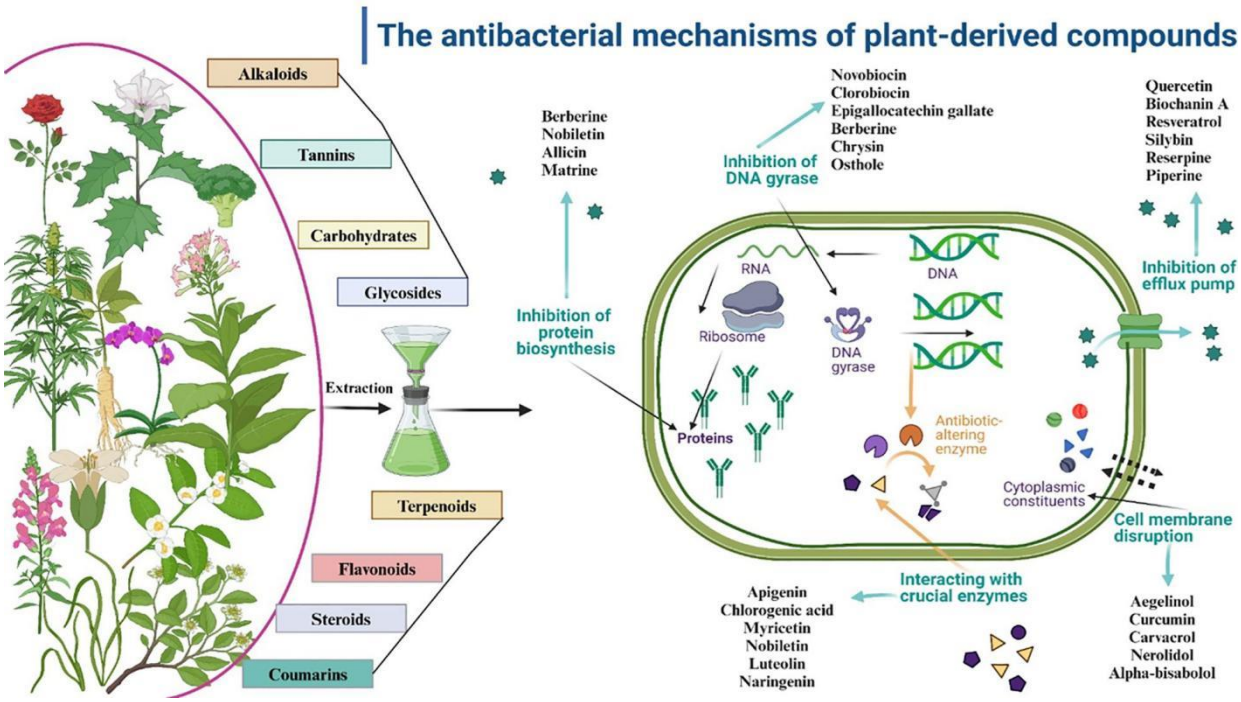


Figure 8. Antibacterial Mechanisms of Plant-Derived Compounds and Their Bioactive Classes (El-Saadony et al., 2025).

The studies have focused on plant-derived antimicrobial agents such as polyphenols and essential oils, which have a long-standing history of use in traditional medicine to overcome infections **(Bouyahya *et al.*, 2017)**. Earlier research on polyphenols, primarily flavonoids, has demonstrated their bactericidal activity through three main mechanisms: damaging the bacterial plasma membrane via decreasing its fluidity, inhibiting bacterial energy metabolism, and suppressing nucleic acid synthesis **(Djahra *et al.*, 2012; Zacchino *et al.*, 2017)**.

Among these, terpenes are among the most essential classes of plant-derived compounds with remarkable antibacterial potential. As shown in **(Figure 9)**, terpenes exert multi-targeted antibacterial actions: they disrupt the bacterial cell membrane, leading to leakage of vital intracellular components; inhibit oxidative phosphorylation and oxygen uptake, thus reducing bacterial respiration; interfere with quorum sensing pathways, limiting biofilm formation and cell-to-cell communication; suppress ATP synthesis and H^+-ATPase activity, causing energy depletion; inhibit protein synthesis by targeting essential bacterial proteins; and act as efflux pump inhibitors, thereby restoring antibiotic efficacy against multidrug-resistant strains **(Maestre-Muñiz *et al.*, 2021)**.

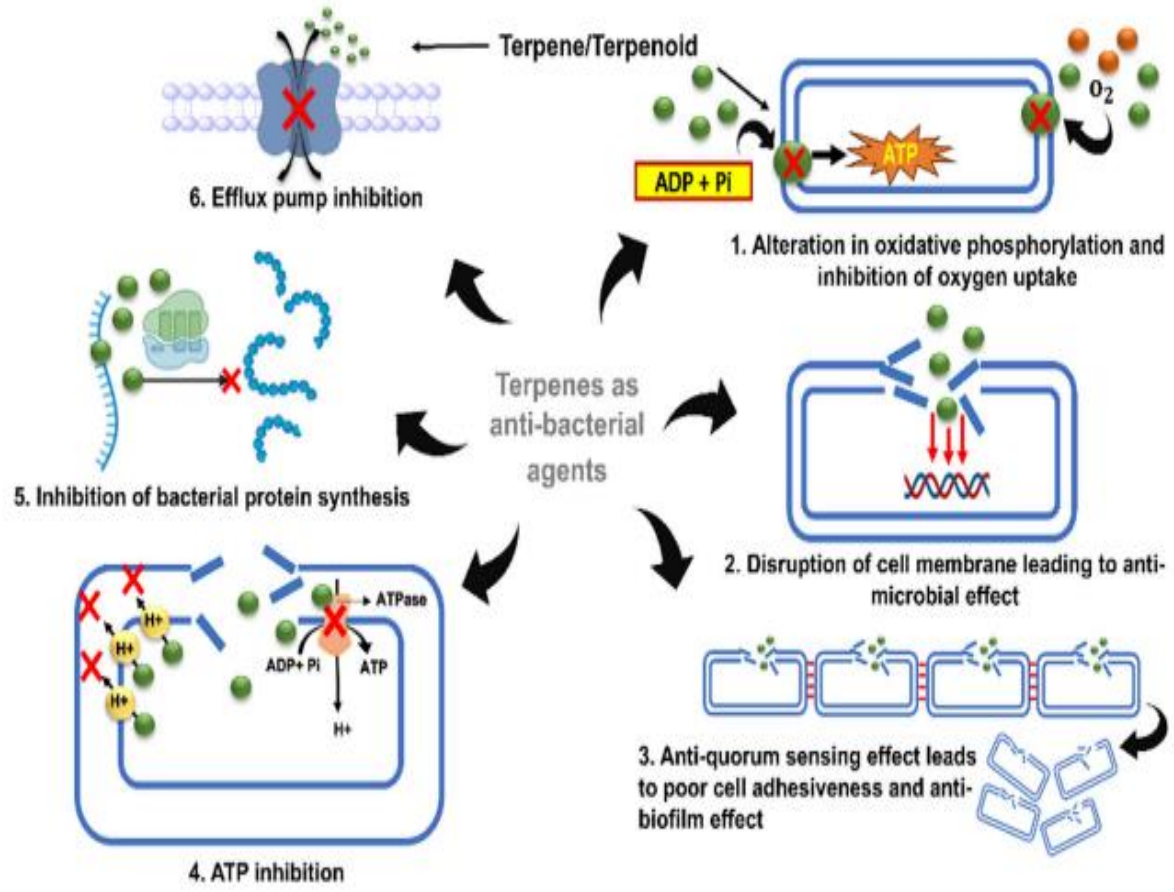


Figure 9. Multi-target antibacterial of terpenes and terpenoids against resistant bacterial **(Khanam *et al.*, 2025).**

3.4. Insecticidal Activity

3.4.1. Insecticidal Agents and Target Pests

Insecticidal agents are substances that induce mortality or behavioral disruption in insects, their larvae, or eggs, which are often agricultural pests or vectors of diseases **(Araújo *et al.*, 2023)**

Many essential oils extracted from aromatic plants possess natural insecticidal properties **(Chebbac *et al.*, 2023)**. These oils, obtained mainly from leaves, flowers, seeds, or stems, contain bioactive compounds such as monoterpenes, sesquiterpenes, phenolics, and aldehydes. Key components like thymol, carvacrol, citronellal, eugenol, and linalool have been shown to interfere with insect nervous systems, similar to synthetic insecticides **(Jankowska *et al.*, 2017)**. Thus, essential oils and their constituents act as effective natural insecticides with diverse modes of action **(Figure 10)**, offering an eco-friendly alternative to conventional pesticides.

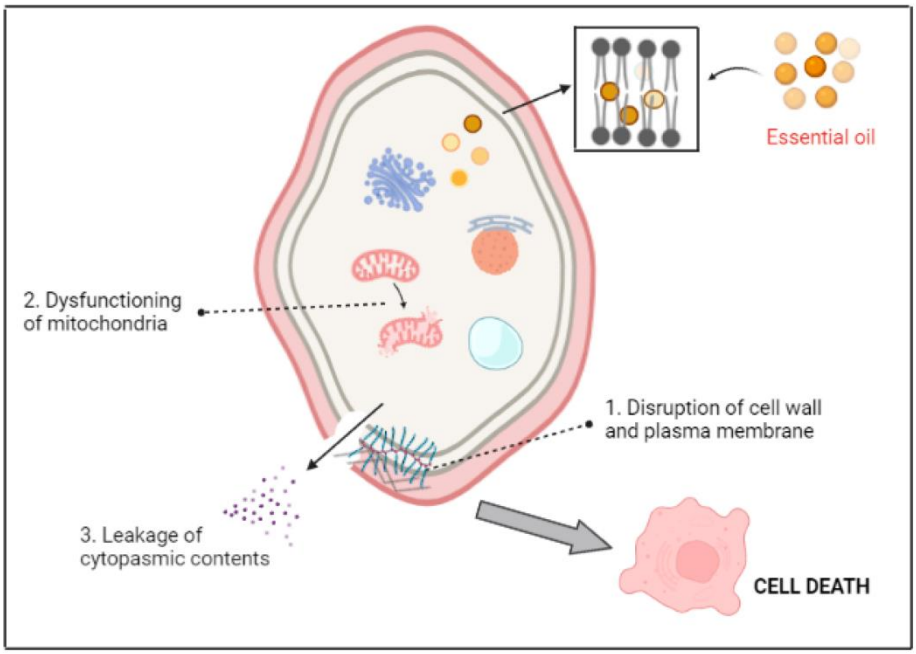


Figure 10. The Insecticidal Mechanisms of Essential Oils **(Gupta *et al.*, 2020).**

3.4.2. Plant-Derived Insecticidal Compounds

Plants produce a wide range of secondary metabolites with insecticidal properties, including EOs, alkaloids, terpenes, and phenolics. These bioactive compounds can act as repellents, antifeedants, growth regulators, or direct toxins to insect pests, often exhibiting lower environmental impact and reduced risk of resistance development compared to synthetic insecticides **(Ediagbonya *et al.*, 2025)**.

Insects develop resistance to conventional chemical insecticides through various mechanisms **(Figure 11).**

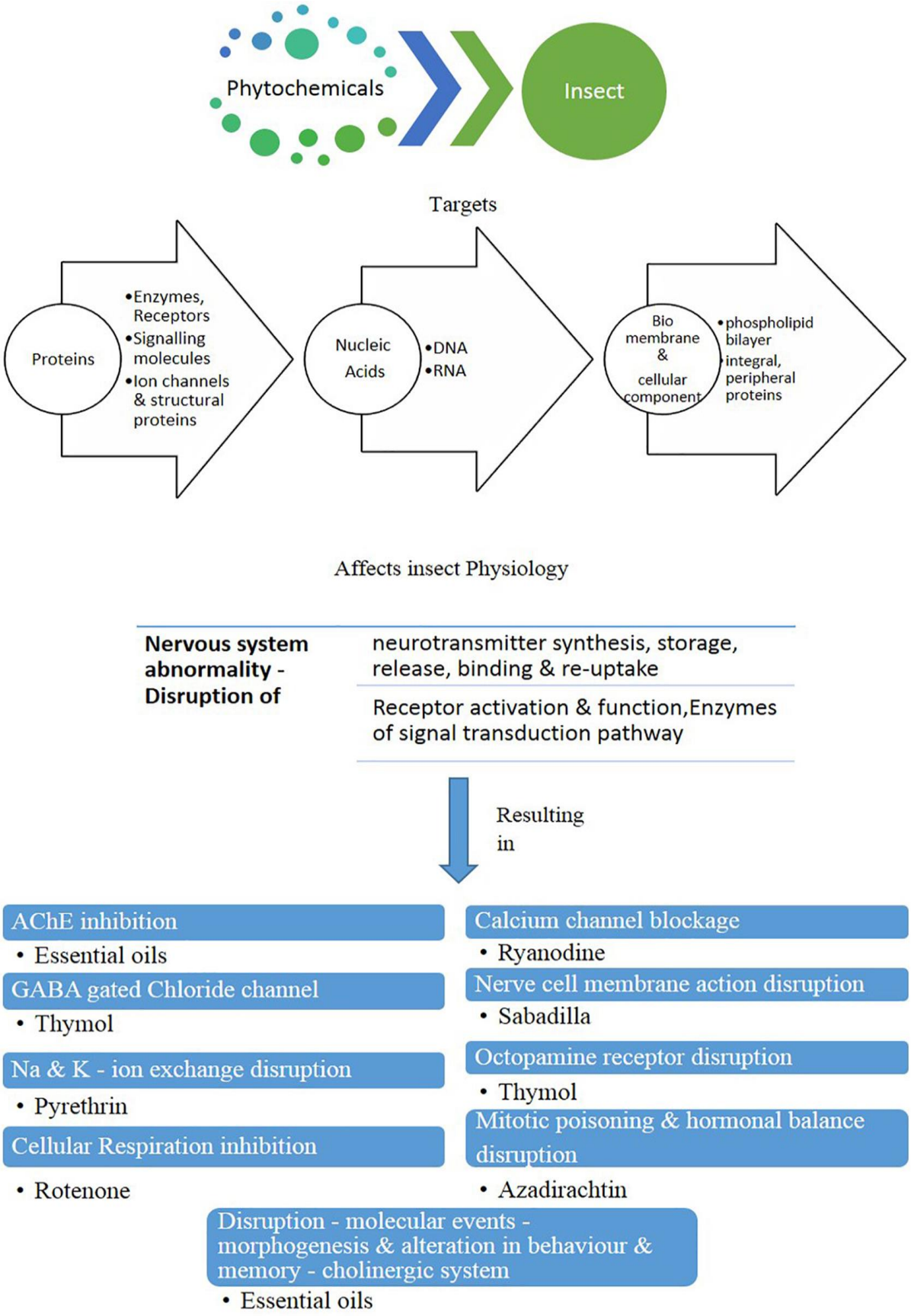


Figure 11. Mode of action of phytochemicals in the insect body **(Senthil-Nathan, 2020).**

Research continues to characterize plant extracts and essential oils with effective insecticidal activity against diverse pest species. Such natural products are promising components of integrated pest management strategies aiming for sustainability and ecological balance.

4. In silico biological activity

4.1. Molecular Docking

Molecular docking is a computational technique used to predict how a molecule **(Raval and Ganatra, 2022)**, called a ligand, interacts with a biological target, usually a protein. This method aims to model the binding of a ligand to the active site of an enzyme or receptor, evaluating both the spatial position and the interaction affinity **(Campbell *et al.*, 2003)**.

The principle of docking is based on simulating physicochemical interactions such as hydrogen bonds, hydrophobic interactions, Van der Waals forces, and electrostatic forces to identify the most stable conformation of the ligand-receptor complex **(Naqvi *et al.*, 2018)**. For each possible binding pose, a scoring function is calculated to estimate binding strength and rank complexes by their likelihood of forming.

Docking is widely used in drug discovery for virtual screening of large chemical libraries, enabling the rapid identification of potential candidates that can then be experimentally validated. This method is also used to study molecular mechanisms of action, optimize pharmaceutical compounds, and predict the effects of mutations at active sites **(Friedman, 2022)**.

Commonly used tools and software include AutoDock, MOE, Glide, and GOLD, which offer various conformational search algorithms and scoring functions **(Reddy *et al.*, 2020)**. The validity of docking results is often confirmed by comparison with experimental structures from X-ray crystallography or NMR. A significant limitation of docking is the relative rigidity of the target and ligand during modeling, which can limit accuracy, especially for highly mobile or flexible targets.

4.2. Studied Plants and Botanical Classification

This study focuses on two medicinal plant species most frequently cited by herbalists in the Wilaya of Relizane: *Ptychotis verticillata* (Desf.). Duby (syn. *Ammoides pusilla* (Brot.) Breistr.) and *Thymus vulgaris* L.

These species belong to two distinct but pharmacologically important botanical families: Apiaceae and Lamiaceae, respectively. Both are traditionally used to treat various ailments, particularly respiratory, digestive, and inflammatory disorders **(Firdaus *et al.*, 2025)**.

The frequent citation of these species in ethnobotanical surveys underscores their strong cultural and therapeutic significance in local phytotherapy. However, despite their extensive traditional use, a review of the scientific literature reveals that *P. verticillata* has been comparatively less explored pharmacologically than *T. vulgaris*. **(Li *et al*., 2024)**, which is more extensively documented for its antimicrobial, antioxidant, and anti-inflammatory properties. This justifies a deeper scientific exploration of these species to validate and, if warranted, valorize their bioactive potential. The botanical classification of the studied species is presented in **(Table II)**.

Table II. Botanical Classification of the Studied Plant Species.

Taxonomic	***Ptychotis verticillata* (Desf.) Duby**	***Thymus vulgaris* L.**
Kingdom	Plantae	Plantae
Phylum	Spermatophyta (Seed plants)	Spermatophyta (Seed plants)
Subphylum	Angiosperms	Angiosperms
Class	Eudicotyledons	Eudicotyledons
Subclass	Rosids	Asterids
Superorder	Apiales	Lamiales
Order	Apiales	Lamiales
Family	Apiaceae	Lamiaceae
Genus	*Ptychotis*	*Thymus*
Species	*Ptychotis verticillata* (Desf.) Duby	*Thymus vulgaris* L.
Synonym	*Ammoides pusilla* (Brot.) Breistr.	–
Common Name –Arabic	النوخة(Noukha)	الزعتر(Zaatar)
Common Name –French	Ptychotis / Ammoïdes	Thym commun
Common Name –English	Ptychotis / Wild caraway	Common thyme

Figure 12. *P. verticillata* **(Tela, 2025).**

Figure 13. *T.* *vulgaris* **(Lepage, 2025).**

5.1. *Ptychotis verticillata* (Desf.) Duby

5.1.1. Botanical Description and Habitats

Ptychotis verticillata (Desf.) Duby (*P. verticillata)***,** belonging to the Apiaceae family, is an annual herbaceous plant typically 15–35 cm tall, with a glaucous (bluish-green) appearance. It has a slender, pivoting root system. The plant bears finely divided leaves and umbels of small flowers characteristic of the Apiaceae family. It grows naturally in Mediterranean regions, particularly in dry, rocky, or calcareous soils typical of arid and semi-arid environments **(Rhizlan *et al*., 2024).** Flowering usually occurs in spring to early summer. It adapts well to well-drained soils and open sunny habitats.

5.1.2. Geographic Distribution

P. verticillata is native to the Mediterranean basin, including parts of North Africa and southern Europe. It has been documented in countries such as Morocco, Algeria, and parts of southern France. It thrives in Mediterranean climates with dry summers and mild, wet winters, occupying calcareous rocky landscapes and open fields **(Taibi *et al*., 2023).**

5.1.3. Traditional uses and pharmacological properties

Traditionally, *P. verticillata* has been used in Moroccan and wider Mediterranean ethnomedicine for its antibacterial and antioxidant properties. Studies on related *Ammoides/Ptychotis* species report their use of the plants for infections and inflammation. Extracts from roots and aerial parts show bioactivity, including antimicrobial and antioxidant effects **(Taibi *et al*., 2024).** While detailed clinical studies remain limited, its traditional use in treating microbial infections and oxidative stress is well noted.

5.1.4. Phytochemistry

Phytochemical investigations reveal that *P. verticillata* contains essential oils, flavonoids, and phenolic compounds, which contribute to its bioactivity. Monoterpenes and sesquiterpenes, typical of Apiaceae species, characterize essential oils. The plant also contains bioactive antioxidants responsible for its medicinal effects **(Taibi *et al*., 2024).** However, detailed metabolomic studies and modern chemical profiling specific to *P. verticillata* are limited, underscoring the need for further research.

5.2. *Thymus vulgaris* L.

5.2.1. Botanical Description and Habitats

Thymus vulgaris L. (*T. vulgaris*) is a perennial, woody subshrub, usually reaching heights of 20-40 cm. It has small, opposite, oval to lanceolate evergreen leaves measuring approximately 5-15 mm in length. The plant produces dense clusters of small, tubular flowers, ranging in color from pale pink to purple, that bloom primarily from late spring to mid-summer.

It grows in dry, rocky, and sunny habitats typical of the Mediterranean basin but is widely cultivated in temperate regions worldwide **(Forster *et al*., 2023**). *T. vulgaris* is well adapted to calcareous soils and tolerates drought conditions, often found in maquis and garrigue biomes **(Cianfaglione *et al*., 2022)**.

5.2.2. Geographic Distribution

Native to the Mediterranean region, *T. vulgaris* is widely distributed across southern Europe, North Africa, and Western Asia. It has been naturalized in many temperate regions globally, including North America and parts of Asia. The species thrives in Mediterranean climates with hot, dry summers and mild, wet winters **(Lopez *et al*., 2021).**

5.2.3. Traditional Uses and Pharmacological Properties

Thymus vulgaris L. has a long history of use in traditional medicine due to its antiseptic, antibacterial, and respiratory benefits. It is commonly used in infusions and essential oil preparations for the treatment of coughs, bronchitis, and digestive disorders **(Oren *et al*., 2023)**. Recent pharmacological studies have confirmed its antibacterial efficacy against multidrug-resistant strains such as *Staphylococcus aureus* and *Escherichia coli* **(Hussien *et al*., 2025)**. Additionally, thyme extracts exhibit significant antioxidant and anti-inflammatory effects, which are linked to their phenolic compounds and terpenoids **(Kim & Park, 2023)**. Clinical studies also suggest potential neuroprotective and anticancer activities, particularly via modulation of oxidative stress pathways and apoptosis **(Chen *et al*., 2022)**.

5.2.4. Phytochemistry

The chemical composition of *T. vulgaris* is characterized predominantly by its essential oils (0.5-2.5% oil content), rich in thymol, carvacrol, p-cymene, and γ-terpinene **(Alvarez *et al*., 2023)**. These monoterpenoids are responsible for the plant's characteristic aroma and many of its bioactivities. Beyond volatile oils, the plant contains flavonoid derivatives such as luteolin and apigenin, phenolic acids including rosmarinic acid, and tannins **(Hassan *et al*., 2021)**. Advanced metabolomic profiling has identified novel polyphenols and minor sesquiterpenes contributing to thyme’s pharmacological properties **(Grigore-Gurgu *et al*., 2025)**. The variation in phytochemical profiles depends on environmental factors and chemotype, and recent studies have improved the understanding of their biosynthetic pathways **(Lopez-Garcia *et al*., 2023)**.

MATERIAL & METHODS

1. Ethnobotanical Study, Biological Activities, and Pharmaceutical Formulation of Selected Medicinal Plants from Relizane, Algeria

An ethnobotanical survey was conducted among traditional herbalists in the Wilaya of Relizane to document medicinal plant usage and evaluate their ethnopharmacological significance. Data were collected via structured interviews and questionnaires focusing on plant species used, methods of preparation, and therapeutic applications. Quantitative ethnobotanical indices, including Spearman's rank correlation, Use Value (UV), Fidelity Level (FL), and Informant Consensus Factor (ICF), were calculated to assess the cultural importance and consensus regarding particular plants.

Based on the highest Use Values (UV), reflecting frequent citation and importance in the local pharmacopeia, two medicinal plants, *P.verticillata* and *T.vulgaris*, were selected for biological investigation. These species were not only highly cited in the field survey but also reported extensively in recent literature as possessing significant bioactive properties.

Extractions of the selected plants were performed using solvent extraction techniques, deliberately avoiding essential oil distillation, to isolate the bioactive compounds for biological testing. Extracts were subsequently subjected to a series of in vitro biological activity assays designed to evaluate their pharmacological potentials, including antioxidant and antimicrobial activities.

Following the positive outcomes of the biological evaluations, a pharmaceutical ointment was formulated incorporating the active extracts from *P. verticillata* and *T. vulgaris*.

This topical preparation aims to utilize the established bioactivities, creating a vehicle for potential therapeutic use. The ointment was carefully characterized for physicochemical properties and tested for efficacy correlated with the evaluated biological activities.

To this end, the following points were addressed **(Figure 14):**

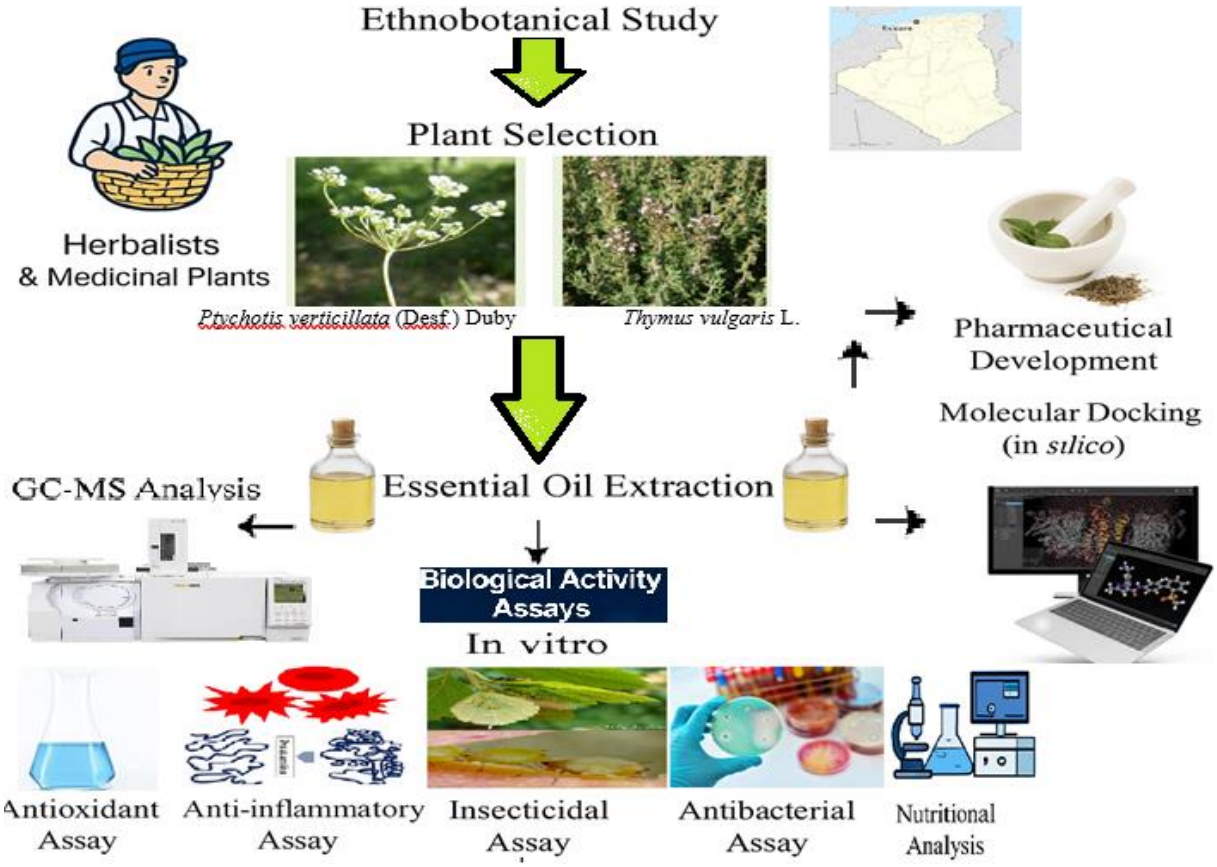


Figure 14. Laboratory Objectives and Work Structure.

2. Ethnobotanical study

2.1. Study Area

Algeria spans approximately 2,381,741 square kilometers, with the Sahara Desert covering about 84% of its territory, making it one of the largest deserts in the world (Benarba et al., 2015).

The Relizane province is situated in the western part of northern Algeria, at 35°44′N latitude and 0°33′E longitude, with elevations ranging from 40 to 200 meters. Covering an area of 4,851 km², it is home to an estimated population of around 998,518 inhabitants distributed across 38 municipalities and 13 districts (Daïras), according to the 2021 census **(Figure 15)**. The average population density is approximately 206 inhabitants per square kilometer **(Bouguerra *et al.*, 2024)**.

The province exhibits ecological diversity characterized by two primary zones: the plains situated centrally, comprising the Low-Chellif and Mina plains, and the mountainous regions, represented in the north by the Dahra Mountains, in the south by the Ouarsenis Mountains, and extending east to west to the Beni Chougrane Mountains.

Relizane's topography is divided into three physical zones: mountainous, plain, and piedmont areas. The mountainous regions occupy the southern and extreme northern portions, covering 38% of the province. These consist of three massifs with elevations between 600 and 1,200 meters: the Dahra Mountains in the north, Beni Chougrane Mountains to the southwest, and Ouarsenis Mountains to the southeast. Plains, accounting for 32% of the area, are located in the Low-Chellif and Mina regions, forming a corridor between mountainous units at elevations from 40 to 200 meters. The Piedmonts cover 30% of the territory and serve as transitional areas between the plains and mountain ranges.

Major rivers drain the province: Oued Chlef flows east to west, while Oued Mina and Oued Rhiou flow from south to north, eventually discharging into Oued Chlef.

Relizane experiences a Mediterranean climate characterized by hot, dry summers and mild, wet winters. It is divided into arid and semi-arid climatic zones, further subdivided into semi-arid "cool" zones in the north, arid "cool" zones in the plains and piedmonts, and semi-arid "warm" zones east of the Ouarsenis Mountains. The average annual rainfall is approximately 600 mm ; however, drought conditions over the past decade have reduced this to about 240 mm (www.andi.dz).

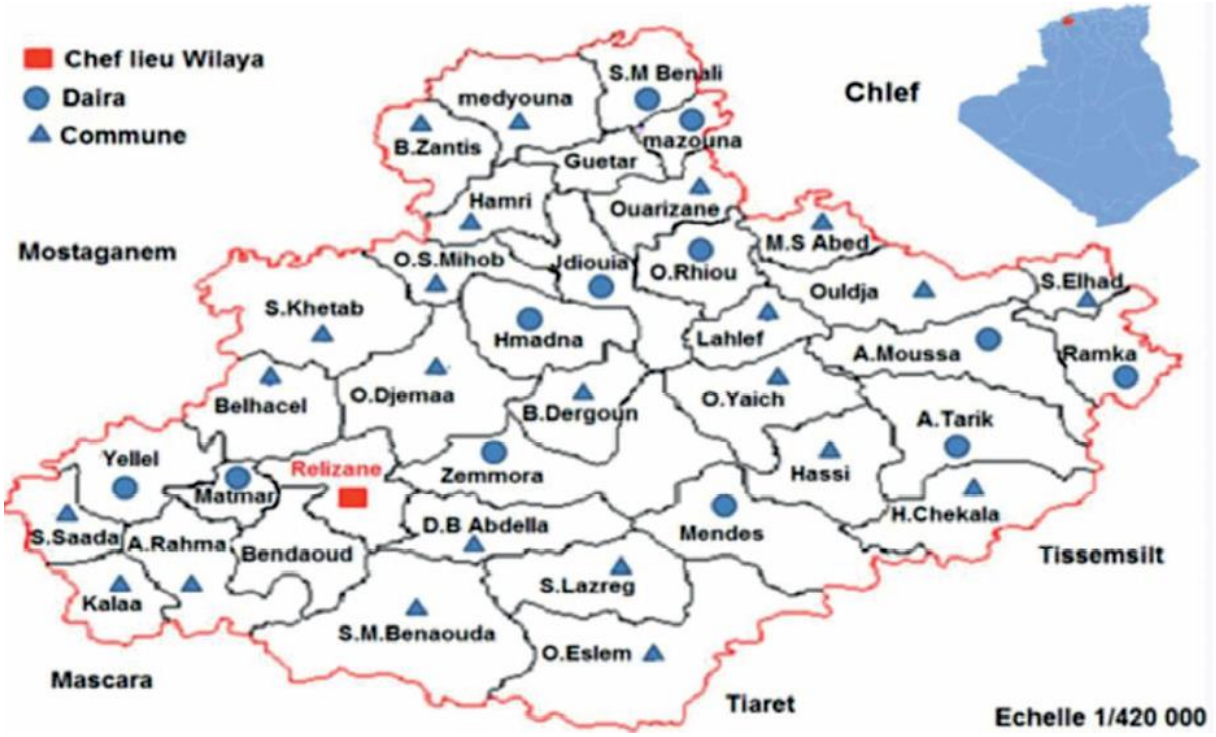


Figure 15. Location of the study area **(www.andi.dz).**

2.2. Data Collection

The ethnobotanical study was conducted in the province of Relizane through face-to-face interviews with practicing herbalists from various regions. Before the interviews, participants were informed about the objectives and scope of the study. A total of 60 herbalists were surveyed using a semi-structured questionnaire adapted from Maache *et al*. (2024), comprising two main sections.

The first section gathered sociodemographic data from each informant, including age, gender, educational background, and residence. The second section focused on medicinal plant use. It included questions on the botanical and local names of plants, their ecological status (local or imported, cultivated or spontaneous), the parts used, modes of preparation and administration, the ailments treated, dosage details, and any additives used. Local plant names were recorded in the native Dardja language to preserve cultural authenticity.

Interviews were conducted as guided field walks, a qualitative data collection method involving direct observation and note-taking during interactions with informants **(Maache *et al*., 2024)**. Participants were encouraged to respond freely and without time constraints, fostering a relaxed atmosphere that adhered to the ethical standards outlined by the International Society of Ethnobiology (ISE). The interviews were carried out in the local dialect, typically in public or easily accessible community settings.

Informed consent was obtained from all herbalists before participation, ensuring their voluntary agreement for the collection, analysis, and publication of the ethnobotanical data **(Albuquerque *et al*., 2014)**.

2.3. Ailment categories

According to the interviewees' responses, the ailments cited for treatment with medicinal plants were grouped into 10 categories, defined by major organ systems. Each citation to a specific part of a medicinal plant species was treated as an individual use-report **(Benarba *et al*., 2015)**. The categories were: circulatory system, digestive system, respiratory system, urinary system, reproductive system, endocrine system, musculoskeletal system, integumentary system (skin and its appendages), nervous system, and sensorial system (eye/ear).

2.4. Data Analysis

The collected ethnobotanical data were analyzed using IBM SPSS Statistics Version 22. The statistical approaches included the following:

✓ **Spearman's Rank Correlation Analysis**

This nonparametric test was used to examine associations among study variables, such as the relationship between participants' age and the number of plants they reported knowing. This method helps identify the presence and strength of monotonic relationships between ethnobotanical indicators **(Sampaio *et al*., 2024)**.

✓ **Use Value (UV) of Species**

The Use Value (UV) index is widely used to assess the relative importance of plant species based on local knowledge and usage as herbal remedies **(Zenderland *et al*., 2019)**. UV reflects both the frequency with which a species is cited and the diversity of its reported uses, highlighting species of ethnobotanical significance. The UV is calculated by the formula **(Hoffman and Gallaher, 2007)**:

$$UV = \frac{\sum U}{N}$$

Where:

- U = Number of use citations for a given species
- N = Total number of informants interviewed

✓ **Fidelity Level (FL)**

Fidelity Level (FL) measures the degree of consensus among informants regarding the use of a particular plant species to treat a specific ailment. It is expressed as a percentage indicating the proportion of informants who agree on a plant's use for a particular condition relative to those who mention the plant for any purpose **(Hoffman and Gallaher, 2007)**. The FL is calculated as follows:

$$FL(\%) = \frac{N_P}{N} \times 100$$

Where:

- Np = Number of informants who cited the plant for a particular use
- N = Total number of informants who cited the plant for any use

✓ **Informant Consensus Factor (ICF)**

ICF quantifies the agreement or uniformity of knowledge among informants about plants used within specific illness categories. Low ICF values (close to 0) indicate random selection or low agreement, while high values (close to 1) signify clear consensus and well-defined ethnobotanical practices **(Boudjelal *et al*., 2013)**. The ICF is calculated using the

formula proposed **(Heinrich *et al.*, 1998)**:

$$ICF = \frac{Nur - Nt}{Nur - 1}$$

Where:

- Nur = Total number of use reports for all species in a particular ailment category
- Nt = Number of plant species used for that ailment category

3. Plant Materials

The aerial parts (leaves and flowers) of *P. verticillata* (Apiaceae) and *T. vulgaris*. (Lamiaceae) were collected in May 2024 from the Zemmoura region of Relizane, Algeria (35°39′55″ N, 0°46′07″ E;561 m). Mr. Yacine Boulenouar conducted botanical identification for the Forest Department of Relizane. Voucher specimens (PV-ZM2024-01 and TV-ZM2024-02) have been deposited at the Herbarium of the University of Ahmed Zabana.

Fresh plant materials were air-dried in a shaded, well-ventilated room at 20–25°C and 45–50% relative humidity for 15 days to preserve volatile compounds **(Miara *et al.*, 2019)**. The dried aerial parts were then ground into a fine powder (<1 mm) using an electric grinder (IKA® A11 Basic). The powdered samples were stored in amber glass containers at 4°C to prevent photodegradation until hydrodistillation **(Rebbas *et al.*, 2012)**.

4. Essential Oil Extraction

The dried aerial parts (200 g) of *P.verticillata* and *T.vulgaris* were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus. This traditional extraction technique involves boiling the plant material in water, where the steam vaporizes volatile oil components, which subsequently condense to form a mixture of essential oil and hydrolate (water) **(El-Assri *et al.*, 2021; Benabderrahmane *et al.*, 2024)**.

Water served as the extraction solvent during this process. After distillation, the essential oils were separated from the hydrolate, dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, and stored in sealed vials protected from light at 4 °C until further analysis. The yield of essential oil was determined as a percentage relative to the dry plant material weight, following the method described by **Khebri (2011) and Benabderrahmane *et al.* (2024)**. The calculation formula for yield (%) is:

$$EO\ yield(\%) = \frac{weight\ of\ the\ extracted\ essential\ oil\ (g)}{weight\ of\ the\ dried\ plant\ material\ (g)} \times 100$$

5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

5.1. Instrumentation and Conditions

GC-MS analyses were performed at the Physico-Chemical Analysis Technical Platform (PTAPC-CRAPC) in Laghouat using a SHIMADZU GCMS-QP2020 system. The chromatograph was equipped with a fused silica capillary column Rxi®-5ms (30 m length × 0.25 mm internal diameter, with 0.25 µm film thickness). The stationary phase consisted of 5% diphenyl and 95% dimethyl polysiloxane, a standard phase chemically equivalent to those used in commercial columns such as HP-5ms and DB-5ms.

A volume of 0.5 µL of essential oil was injected in split mode (split ratio 1:80). Injector and detector temperatures were set at 250 °C and 310 °C, respectively. The oven was programmed to start at 50 °C with an initial hold of 2 minutes, then increase at 3 °C/min to 310 °C, where it was held for 2 minutes. Helium gas (purity 99.995%) was used as carrier gas at a constant flow rate of 1 mL/min.

The mass spectrometer operated under electron ionization (EI) mode at 70 eV, with the ion source temperature maintained at 200 °C. Mass spectra were recorded over a mass-to-charge ratio (m/z) range of 45–600.

5.2. Identification of Essential Oil Components

Individual compounds in the essential oils were identified by comparing their linear retention indices (LRI) and mass spectra with reference data. LRIs were calculated relative to a homologous series of n-alkanes (C8–C33) run under identical chromatographic conditions. These indices and mass spectral data were compared with published literature values **(Adams, 2007; Babushok *et al.*, 2011)** and spectral libraries from NIST (NIST17.lib), Wiley, and FFNSC1.2.lib to confirm compound identities.

This methodology adheres to standard, widely accepted protocols for hydrodistilled essential oil extraction, followed by chemical profiling using GC-MS. It ensures reproducibility and facilitates meaningful comparison with existing scientific literature.

6. In Vitro biological activity

6.1. Evaluation of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant potential of the essential oils was assessed using the free radical scavenging method based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, first described by **Blois (1958)**. This technique relies on the reduction of the deep-violet DPPH radical (DPPH) by hydrogen donation from antioxidant compounds (Figure 16), leading to the formation of a

pale-yellow non-radical form (DPPH-H) and a decrease in absorbance **(Athamena *et al.*, 2010)**.

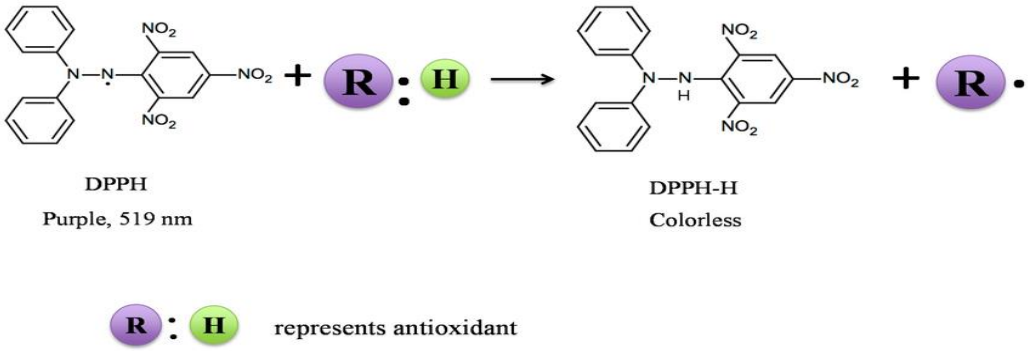


Figure 16. Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant **(Babushok *et al.*, 2011).**

✓ **Principle**

DPPH is a stable free radical characterized by an absorption peak between 512 and 517 nm. Antioxidants present in the sample reduce the DPPH radical by donating hydrogen atoms, resulting in discoloration proportional to their free radical-scavenging capacity **(Sanchez-Moreno, 2002)**.

✓ **Methodology**

A freshly prepared methanolic solution of DPPH (6×10^{-6} mol/L) was used. For each assay, 100 µL of essential oil extract was mixed with 3 mL of the DPPH solution. The mixture was incubated in the dark at room temperature for 30 minutes to prevent photooxidation. After incubation, absorbance was measured at 517 nm against a methanol blank.

A control sample consisting of 100 µL methanol and 3 mL DPPH solution was included to determine the baseline absorbance.

✓ **Calculation of Radical Scavenging Activity**

The percentage inhibition of the DPPH radical by the sample was calculated using the formula:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where A control is the absorbance of the DPPH solution without extract, and A sample is the absorbance with the sample.

✓ **Determination of IC_{50}**

The half-maximal effective concentration (IC_{50}) was determined graphically by plotting

the percentage of inhibition against increasing concentrations of the extract. IC_{50} represents the concentration required to reduce 50% of the initial DPPH radical concentration. Lower IC_{50} values indicate more potent antioxidant activity. Ascorbic acid (vitamin C) was used as a positive control antioxidant for comparison under identical assay conditions **(Molyneux, 2004)**.

6.2. Anti-Inflammatory Effect

6.2.1. Hemolysis Inhibition Assay

The anti-inflammatory potential of the plant extracts was evaluated by their ability to prevent heat-induced hemolysis of human erythrocytes. This well-established model reflects the stabilization of the red blood cell (RBC) membrane under thermal stress. This assay is widely used as an in vitro indicator of anti-inflammatory properties, as stabilization of the erythrocyte membrane is analogous to that of lysosomal membranes, thereby preventing the release of pro-inflammatory mediators **(Li *et al.*, 2024)**.

Fresh human blood was obtained from a healthy volunteer who had refrained from taking any anti-inflammatory or related medications for at least two weeks prior to blood collection to avoid interference. The blood was immediately centrifuged at 3000 rpm for 3 minutes to separate erythrocytes, which were then washed three times in isotonic 0.9% saline to remove plasma and buffy coat components. A 10% (v/v) suspension of erythrocytes was prepared in 10 mM sodium phosphate buffer at physiological pH 7.4.

For the assay, varying concentrations of the plant extract were tested by mixing 50 µL of erythrocyte suspension with 2950 µL of phosphate buffer containing the extract. The samples were incubated at 54°C for 20 minutes to induce hemolysis. Following incubation, the samples were centrifuged at 2500 rpm for 3 minutes, and the extent of hemolysis was quantified by measuring the absorbance of the supernatant at 540 nm, corresponding to released hemoglobin. A negative control (erythrocyte suspension incubated at 54°C without extract) was included to account for maximum hemolysis, while diclofenac sodium served as a positive anti-inflammatory control.

The percentage of HI% was calculated according to the formula:

$$HI\% = \frac{A_0 - A_S}{A_0} \times 100$$

where:

- A0 is the absorbance of the control (complete hemolysis)

- As is the absorbance of the sample treated with the plant extract

This method provides a practical and rapid screening tool for evaluating membrane-stabilizing and therefore potential anti-inflammatory effects of natural products **(Li *et al.*, 2024)**.

6.2.2. Protein Denaturation Inhibition Assay

Protein denaturation is a fundamental cause of inflammation and tissue damage. The inhibition of protein denaturation is frequently employed as an in vitro measure of anti-inflammatory activity, as many anti-inflammatory agents exert effects by stabilizing proteins against denaturation **(Chandra *et al.*, 2012)**.

Following the reported protocol, the protein denaturation inhibition assay was conducted by preparing a reaction mixture comprising 200 µL of fresh egg albumin, 2800 µL of phosphate-buffered saline (PBS, pH 6.4), and 2000 µL of the plant extract or standard drug solution at various concentrations. The mixture was incubated at 37°C for 20 minutes to mimic physiological conditions, then heat-induced denatured by increasing the temperature to 70°C for 5 minutes.

After cooling to room temperature, the turbidity resulting from protein denaturation was quantified by measuring absorbance at 660 nm. Diclofenac sodium was used as a reference anti-inflammatory drug to validate the assay.

The percentage inhibition of PDI% was calculated using the equation:

$$PDI\% = \frac{A_1 - A_2}{A_1} \times 100$$

where:

- A1 is the absorbance of the control (denatured protein without treatment)
- A2 is the absorbance of the sample (protein treated with extract)

This assay provides insight into the protective capacity of the extract on protein conformation, suggesting potential therapeutic relevance in inflammatory conditions **(Chandra *et al.*, 2012)**.

6.3. Insecticidal Activity

The insecticidal efficacy of essential oils derived from *T. vulgaris* and *P. verticillata* was evaluated against adult individuals of *Aphis spiraecola*. Groups consisting of 10 adult insects were carefully selected and transferred onto host plant leaves, following protocols established for the bioassay of essential oils against insect pests **(Khater, 2000)**.

The leaves with insects were placed inside ventilated Petri dishes to ensure adequate air circulation and minimize stress on the insects. To maintain optimal humidity throughout the experimental period, a cotton disc moistened with distilled water was positioned at the bottom of each dish. Petri dishes were sealed at the top, balancing containment with sufficient ventilation to avoid hypoxic conditions.

For each treatment concentration (1%, 0.5%, and 0.25% v/v), three replicate Petri dishes were prepared to ensure experimental reproducibility. Three untreated control boxes were included for comparison. The essential oils were applied by fine-spraying directly onto the leaves harboring the insects, ensuring even coverage and direct contact between the oil and the pests. Subsequently, the Petri dishes were maintained at ambient room temperature under controlled laboratory conditions during the experiment **(Ayvaz *et al*., 2010)**.

Insect mortality was monitored at set intervals: 24, 48, and 72 hours post-application. Visual counting of dead insects was performed manually using a stereomicroscope, a standard and reliable approach in entomological toxicity assessments. This time-course observation enabled detection of both immediate and delayed lethal effects of the essential oils on *Aphis spiraecola*.

The observed mortality rates were corrected for natural mortality occurring in untreated control groups using Abbott's formula to accurately reflect the treatment's effectiveness **(Ainane *et al*., 2019)** :

$$M = \frac{M_I - M_C}{100 - M_C} \times 100$$

Where:

M = corrected mortality (%)

MI = observed mortality (%) in treated groups

MC = mortality (%) observed in control groups

This formula accounts for background mortality, ensuring results accurately reflect the bioactivity of the essential oils.

Through this methodical approach, the insecticidal properties of *Thymus vulgaris* L. and *Ptychotis verticillata* Duby essential oils were robustly assessed, demonstrating their potential as natural, eco-friendly agents for pest management. This study supports the inclusion of these essential oils in integrated pest control strategies to reduce reliance on synthetic insecticides and promote sustainable agriculture.

6.4. Antibacterial Activity

6.4.1. Bacterial Strains

The antibacterial potential of essential oils (EOs) and their combinations was evaluated against a panel of pathogenic bacterial strains frequently associated with human infections. The test panel included both Gram-positive and Gram-negative bacteria, sourced from the American Type Culture Collection (ATCC). The chosen strains were *Methicillin-Resistant Staphylococcus aureus* (MRSA, ATCC 43300), *Methicillin-Sensitive Staphylococcus aureus* (MSSA, ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Escherichia coli* (ATCC 25922). All strains were maintained on selective agar slants at appropriate conditions, ensuring viability and purity.

6.4.2. Agar diffusion technique

To assess the antimicrobial activity of essential oils from *P. verticillata* and *T. vulgaris* sourced from the same region, we employed the aromatogram method **(Figure 17)**, which utilizes the diffusion of discs on a gelled medium.

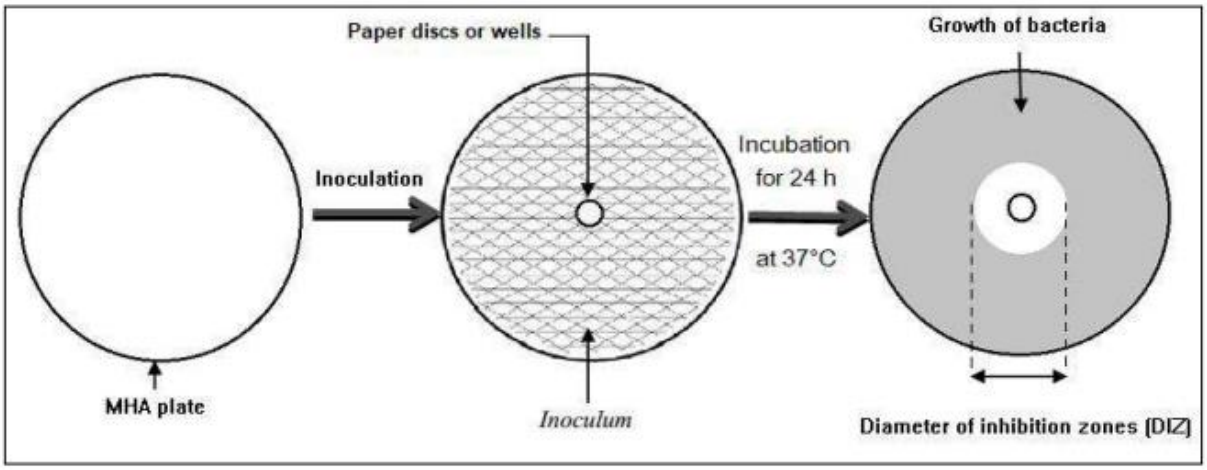


Figure 17. Diagrammatic representation of the agar diffusion method principle **(Andrews, 2001).**

This method is recognized for its effectiveness in evaluating the antibacterial properties of essential oils against various microorganisms, enabling the determination of the sensitivity of different bacterial species to specific essential oils. Studies have shown that the aromatogram serves as a preliminary step in identifying essential oils with significant antibacterial effects **(Andrews, 2001)**. The antibacterial activity of essential oils was evaluated against five reference pathogenic strains using the disc diffusion method. This technique involves placing two 6 mm diameter discs on Petri dishes containing Mueller-Hinton agar, which has been previously inoculated with target strains cultured for 18 to 24 hours. Subsequently, 30 µL of each oil was aseptically applied to each disc. After incubation at 37°C for 24 to 48 hours,

antibacterial antagonism was assessed by measuring the diameter of the inhibition zone around each disc.

6.4.3. Antibacterial Synergy Assay by Disk Diffusion Method

The antibacterial activity and synergistic interactions between the essential oils of *P. verticillata* and *T. vulgaris* were evaluated using the disk diffusion method. This technique involved depositing sterile paper disks, each of 6 mm diameter, onto Petri dishes containing Mueller-Hinton agar previously inoculated with 18- to 24-hour-old bacterial cultures of the target strains (*Escherichia coli, Staphylococcus aureus,* and *Enterococcus faecalis*).

A mixture of the two essential oils was prepared in equal parts (15 µL of each oil, for a total of 30 µL) and aseptically applied to the disks on the agar surface. The plates were incubated at 37°C for 24 to 48 hours. Following incubation, the antibacterial antagonism or synergy was assessed by measuring the diameter of the inhibition zones formed around each disk using a calibrated ruler.

This method allowed the determination of the enhanced inhibitory effect of the oil combination compared to individual oils, reflecting potential synergistic mechanisms such as membrane disruption and enzyme inactivation. The assay was performed in triplicate to ensure the reliability of the results.

6.5. Nutritional Analysis

To thoroughly characterize the nutritional composition of the plant samples, a comprehensive set of standardized physicochemical analyses was performed. The parameters determined included pH, moisture content, protein, fat, total carbohydrates, reducing sugars, mineral ash, and energy values.

The pH was measured on a 10% aqueous suspension at 20°C using a calibrated potentiometric pH meter **(Barbosa, 1994)**.

Moisture content was assessed by oven drying at a controlled temperature until constant weight, as described in the standard methods (ISO 712:2009) **(Arquier, 2023)**.

Protein quantification employed the Kjeldahl method, which measures total nitrogen to estimate crude protein content **(Kirk, 1950)**.

Lipid extraction and quantification were performed by the Soxhlet method, a widely accepted technique for total fat determination **(Pérez-Palacios *et al.*, 2008)**.

Total carbohydrates were estimated by the Bertrand titration method, involving oxidation-reduction reactions to quantify carbohydrate levels (Bertrand, late 19th century)

(Pant *et al.*, 2015).

Reducing sugars were quantified using refractometry, which measures the refractive index to estimate fermentable sugar content **(Elangovan *et al.*, 2014)**.

Mineral content was determined according to the NA 733-1991 standard by incinerating the samples at high temperature and weighing the residual inorganic matter **(Kuan *et al.*, 2011)**. Finally, energy values were calculated from measured macronutrient contents using Atwater conversion factors, expressed in kcal and kJ per 100 g dry weight **(Atwater and Benedict, 1902)**.

All analyses were conducted under controlled laboratory conditions to ensure validity and reproducibility **(Figure 18)**.

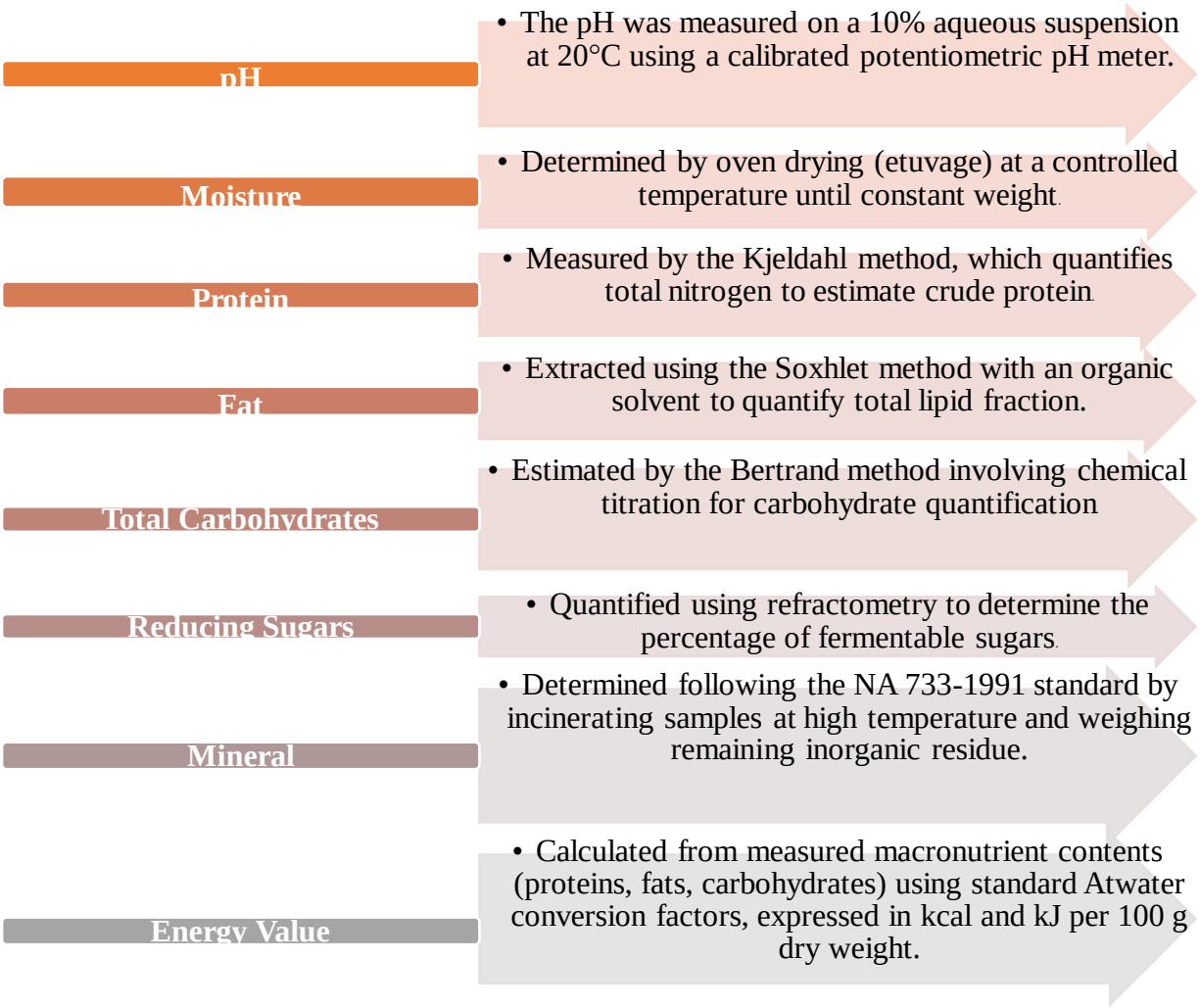


Figure 18. Nutritional analysis methods conducted on *P. verticillata* and *T. vulgaris* samples.

7. In silico activity

7.1. Molecular Docking

7.1.1. Ligand Preparation

The major phytoconstituents identified from *P. verticillata* and *T. vulgaris* (**Figure 19**), were selected for the molecular docking study. The 2D structures of the ligands were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then built and optimized using HyperChem 8.0. The optimization was first carried out with the MM+ force field, followed by the AM1 semi-empirical method, until the minimum-energy stable conformation was reached. The optimized structures were then converted into .mdb format and imported into MOE **(Khalil *et al.*, 2019)** for docking analysis.

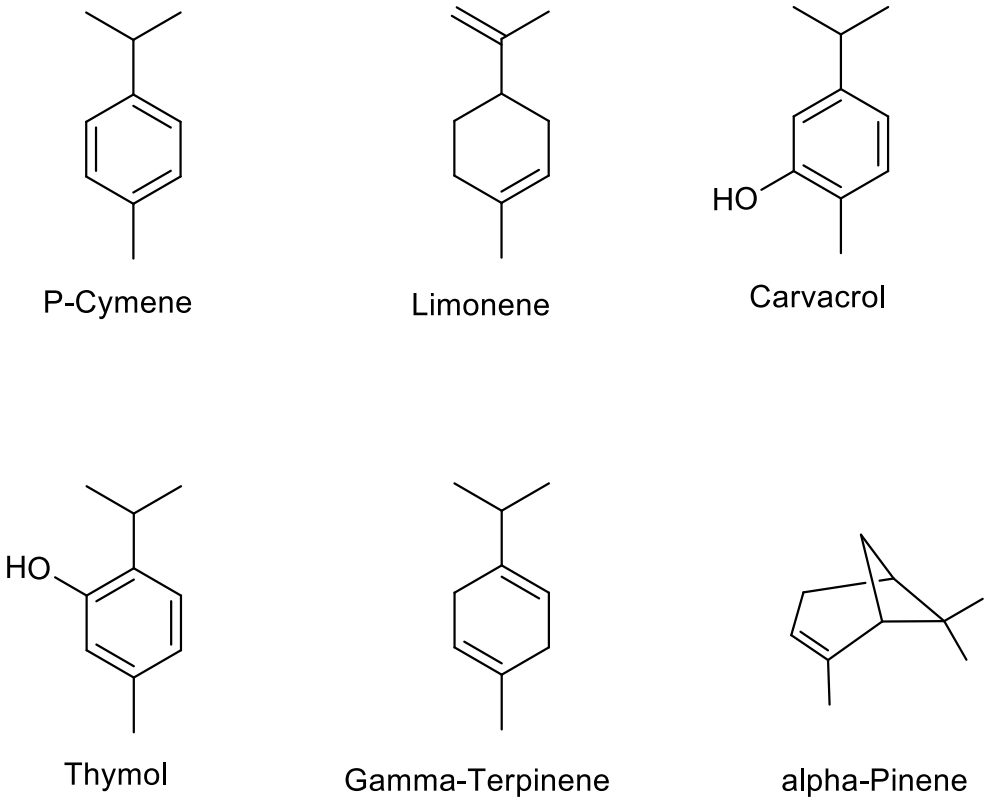


Figure 19. Chemical structures of selected compounds **(https://pubchem.ncbi.nlm.nih.gov/).**

7.1.2. Target Preparation

Three enzymes were retrieved from the Protein Data Bank (PDB): the DNA gyrase subunit B from *Escherichia coli* (PDB ID:4PRX) **(Stanger *et al.*, 2019)**, dihydrofolate reductase (DHFR) from *Staphylococcus aureus* (PDB ID: 6E4E), and the FIC proteins from *Enterococcus faecalis* (PDB ID: 6EP5) **(Veyron *et al.*, 2019)**. The selected targets have resolutions of 1.80 Å, 1.90 Å, and 1.93 Å, respectively (Table 2), indicating good structural quality according to Clément and colleagues **(Clément and Slenzka, 2006)**.

The protein structures were imported into MOE (Molecular Operating Environment, 2015.10) **(Khalil *et al.*, 2019)** and first examined for completeness and structural integrity. Duplicate chains were removed, and water molecules located farther than 4.5 Å from the binding pocket were excluded. Hydrogen atoms were added, and partial charges were assigned. Binding sites were identified using the Site Finder module in MOE, with the co-crystallized ligands serving as references for validation.

The docking protocol was validated by redocking the native ligands into their respective binding pockets, and the method's accuracy was assessed by calculating the root-mean-square deviation (RMSD) between the crystallographic and docked poses. An RMSD value of less than 2.0 Å was considered acceptable for reliable docking. As shown in **Table III**, all PDB structures yielded acceptable RMSD values, confirming the reliability of the docking protocol. This protocol was then applied to the selected compounds from *P. verticillata* and *T. vulgaris*.

Table III. Receptor properties and RMSD value.

Receptors (PDB)	Sequence Length	Chains	Resolution (Å)	Co-crystallized ligand	RMSD (Å)
4PRX	398	A	1.80	ADP	1.28
6E4E	182	A	1.90	MMV	1.75
6EP5	207	A, B, C, D and F	1.93	ADP	0.82

7.2. ADME-T and Drug-Likeness prediction

In silico methods are commonly used to predict various pharmacokinetic and toxicological parameters. This serves as a crucial preliminary step for analyzing new compounds and identifying potential drug candidates **(Coen and Schaffer, 2003)**. To evaluate the compounds, a comprehensive set of parameters was used to predict them. The SwissADME web tool **(Daina *et al.*, 2017)** was used to assess oral bioavailability and other key properties, including human intestinal absorption, P-glycoprotein (P-gp) substrate, cytochrome P450 (CYP) inhibition, and blood–brain barrier (BBB) permeability. Additionally, drug-likeness was evaluated using well-known rules from Lipinski **(Lipinski *et al.*, 1997)**, Veber **(Veber *et al.*, 2002)**, and Egan **(Egan *et al.*, 2000)**. Furthermore, ADMETlab 3.0 **(Xiong *et al.*, 2021)** was employed to evaluate various toxicity and safety parameters. These included: Oral Rat Acute Toxicity (LD50), Ames toxicity, Mutagenicity, Hepatotoxicity, and Skin irritation.

8. Statistical Analysis

Data are presented as mean ± standard deviation (SD) with three independent replicates (n = 3). Statistical analyses were performed using IBM SPSS Statistics (version 22). The student's t-test was applied to determine significant differences. For antioxidant and antibacterial assays, a significance threshold of $p < 0.05$ was adopted, whereas for anti-inflammatory assays, a more stringent threshold of $p < 0.001$ was used. Data organization and preliminary processing were conducted with Microsoft Excel. All further statistical analyses and graphical representations were carried out using GraphPad Prism (version 10).

RESULTS & DISCUSSION

1. Ethnobotanical analysis

1.1. Informants' socio-demographic profile

1.1.1. Demographic Profile of Herbalists

The demographic characteristics of the 60 herbalists interviewed in this study are summarized in **Table IV**. The sample size aligns closely with previous ethnobotanical investigations conducted in Algeria. For instance, **Miara *et al*. (2019)** interviewed 51 herbalists in the Bordj Bou Arreridj province, **Boudjelal *et al*. (2013)** engaged 83 herbalists in M'sila, and **Ouelbani *et al*. (2016)** surveyed 45 herbalists in the Constantine and Mila regions. This consistency highlights the representativeness of our sample within the context of Algerian ethnobotanical research.

Table IV. Medicinal plant utilization patterns in relation to socio-demographic characteristics.

Characteristic	Number	Percentage (%)
Sex		
Male	52	86.67
Female	8	13.33
Age Groupe		
20–29	5	8.33
30–39	10	16.67
40–49	16	26.67
> 50	29	48.33
Educational level		
Illiterate	16	26.67
Primary	22	36.66
Secondary	14	23.33
University	8	13.33
Residence		
Urban	29	48.33
Rural	31	51.67

1.1.2. Gender Distribution

The practice of traditional medicine in the study area is predominantly male-dominated,

with men accounting for 86.67% of herbalists, while women account for only 13.33%. This finding contrasts with the broader literature suggesting that women generally possess greater knowledge of medicinal plants than men **(Gaoue *et al*., 2017)**. The observed gender disparity is attributed mainly to prevailing cultural norms and societal roles that restrict women's involvement in economic activities outside their primary responsibilities as caregivers. Indeed, women are often the leading health providers and caretakers within households in many traditional communities **(Gaoue *et al*., 2017)**. Similar trends have been reported across various Algerian regions, where men overwhelmingly dominate traditional medicine practice (84–93.75%), compared to a minority female participation (6.25–14%) **(Miara *et al*., 2019; Boudjelal *et al*., 2013)**.

1.1.3. Age Distribution

Herbalism is practiced across all age groups, except those under 20 years old, with the majority of practitioners (48.33%) being over 50 years old. This elder cohort has generally acquired its knowledge of medicinal plants through intergenerational transmission. The other age groups were distributed as follows: 40–49 years (26.67%), 30–39 years (16.67%), and 20–29 years (8.33%). This age-related pattern supports the hypothesis that medicinal plant knowledge tends to increase with age due to lifelong accumulation and experience **(Gaoue *et al*., 2017)**. However, the preservation of this invaluable traditional knowledge is currently under threat due to inconsistent intergenerational transmission and growing skepticism, particularly among youth, regarding the efficacy and relevance of traditional medicine **(Bendif *et al*., 2018)**. Our findings reaffirm those of **Miara *et al*. (2019)** and underscore the critical role that older herbalists play as custodians of ethnobotanical heritage.

1.1.4. Educational Level

Regarding formal education, 36.66% of the herbalists attained only primary schooling, while a significant 26.67% were illiterate. On the other hand, 23.33% completed secondary education and 13.33% possessed university degrees. The relationship between educational attainment and ethnobotanical knowledge is complex, but an inverse correlation is often observed, with higher formal education associated with diminished traditional plant knowledge **(Gaoue *et al*., 2017)**. Our data tend to support this observation, as more formally educated individuals may favor biomedicine and place less reliance on or have less faith in traditional remedies **(Bouasla & Bouasla, 2017)**. These results concur with previous studies conducted in Algeria **(Miara *et al*., 2019)**.

1.1.5. Place of Residence

Herbalists in the study area were nearly evenly distributed between rural (51.67%) and urban (48.33%) settings. This suggests that ethnobotanical knowledge and practice are preserved and actively maintained irrespective of urban or rural residency. Similar findings have been reported by **Djahafi *et al*. (2021)**. This challenges the common assumption that urbanization invariably leads to the loss of traditional medicinal plant knowledge **(Gaoue *et al*., 2017)**. **Furusawa and Kaneko (2009)** also emphasized that the relationship between urbanization and ethnobotanical knowledge is nuanced, in which modern and indigenous knowledge systems may coexist and integrate without a direct loss of traditional expertise.

1.2. Botanical Diversity

Our survey identified 50 medicinal plant species distributed among 29 botanical families, all recognized for their therapeutic properties. Among these, the family Lamiaceae was the most prominent, comprising 11 species (22%), followed by Asteraceae and Apiaceae, each represented by 3 species (6%). Additionally, the families Malvaceae, Cupressaceae, Poaceae, Rhamnaceae, Rubiaceae, Rutaceae, and Myrtaceae were each represented by 2 species (4%). Each of the remaining families was represented by a single species **(Figure 20)**.

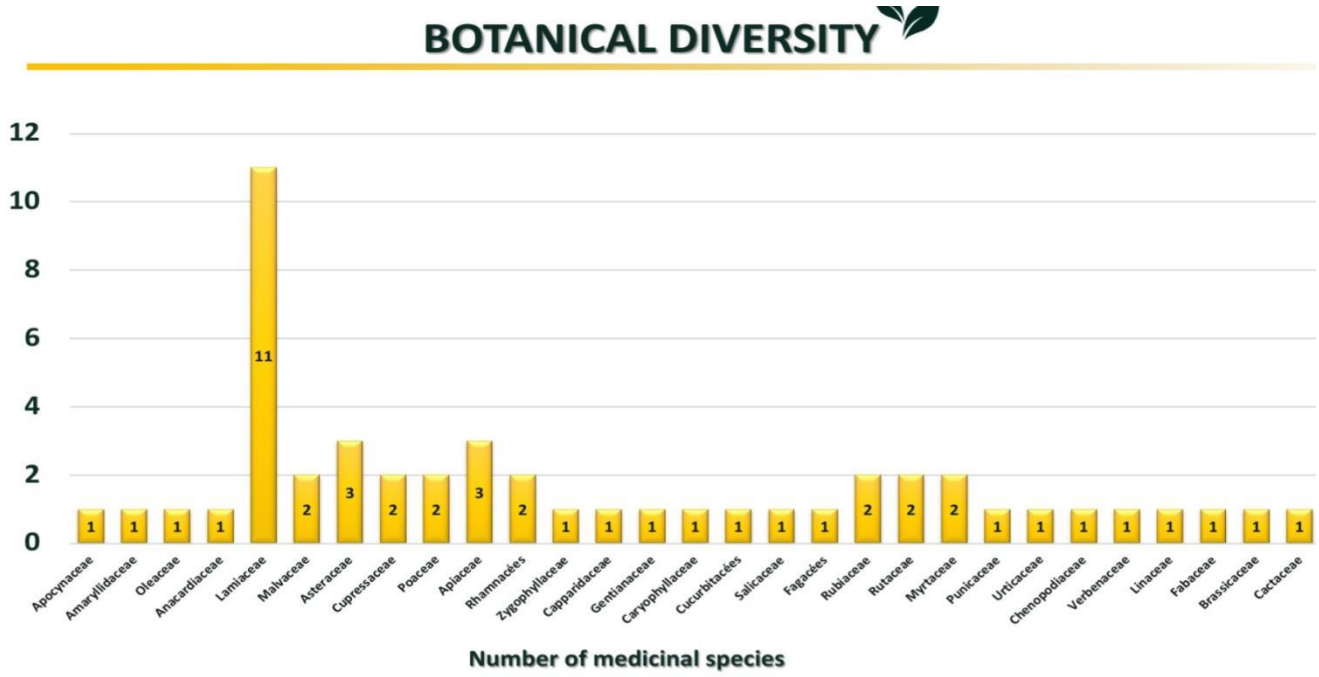


Figure 20. Distribution of medicinal plant species used by herbalists across botanical families.

Our findings align with several other ethnobotanical surveys conducted in Algeria. For

example, in the regions of Tiaret **(Djahafi *et al.*, 2021)**, Mascara **(Benarba *et al.*, 2015)**, Bordj Bou Arreridj **(Miara *et al.*, 2019)**, Constantine and Mila **(Ouelbani *et al.*, 2016)**, and Adrar and Bechar **(Benarba, 2016)**, the majority of medicinal plants used by herbalists and local healers also belonged primarily to these three dominant families: *Lamiaceae*, *Asteraceae*, and *Apiaceae*. These families are similarly well represented in the medicinal floras of neighboring countries, such as Morocco **(El-Ghazouani *et al.*, 2021)**, Tunisia **(Karous *et al.*, 2021)**, and Turkey **(Palabaş Uzun & Şimşir Bozdağ, 2022)**.

These three families are well established in our regions both in terms of the number of taxa and their ethnobotanical importance. They constitute over 20% of the estimated 4,120 taxa recorded in the Algerian flora, including 648 species of Asteraceae, 184 of *Lamiaceae*, and 163 of *Apiaceae*. The prominence of *Lamiaceae* and *Apiaceae* in local ethnobotany is attributed not only to their species richness but also to their significant economic importance, particularly the abundance of aromatic plants in these families. This importance is further supported by the diverse range of bioactive compounds they contain **(Karous *et al.*, 2021)**.

These families synthesize a wide variety of phytochemicals including terpenoids, phenolic acids, phenylpropanoids, polyacetylenes, and alkaloids **(Pollastro & Gaeta, 2020; Bendif *et al.*, 2018)**. Similarly, the prevalence of terpene compounds, such as sesquiterpene lactones, in Asteraceae species accounts for their abundance and efficacy, providing an explanation for their widespread application in treating various ailments. This biochemical diversity again reflects the considerable size and diversity of the *Asteraceae* family **(Karous *et al.*, 2021)**.

1.2.1. Plant parts used

Aerial parts accounted for the majority (42%) of plant materials used, likely due to their ease of access and richness in bioactive compounds, followed by leaves and fruits (14% each). Other parts, such as flowers (8%), roots and stems (4%), and pericarp/cortex (2%), are used less frequently. This aligns with previous research highlighting the prevalence of aerial components in traditional medicine **(Figure 21)**.

1.2.2. Methods of preparation

Our survey found infusion (50%) and decoction (26%) to be the most common preparation methods, while other techniques (raw, powder, oil, maceration, etc.) were less frequent **(Figure 21)**. This aligns with ethnobotanical studies across Algeria **(Belhouala and Benarba, 2021)** and neighboring regions (Morocco, Spain) **(González *et al.*, 2010)**. The preference for infusions and decoctions likely stems from their disinfectant properties (via

heating) and enhanced extraction efficiency **(Benarba *et al.*, 2015)**.

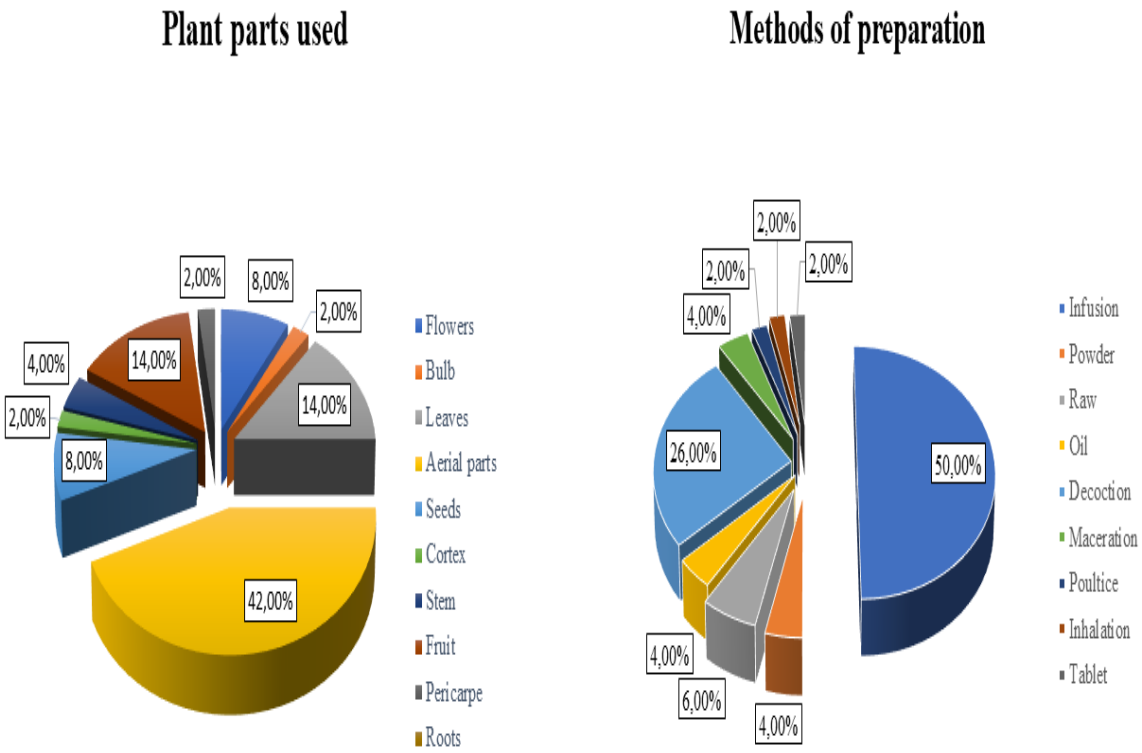


Figure 21. Proportional distribution of medicinal plant preparation methods and plant parts used, as reported by traditional herbalists in Relizane.

1.3. Disease categories and therapeutic applications

The medicinal plants reported by herbalists were classified according to the International Classification of Diseases (ICD-11), 2024 edition, available on the World Health Organization website. This study also identified 11 different types of diseases treated by the aforementioned medicinal plants **(Table V)**. These included the following: 01 (certain infectious or parasitic diseases): predominantly sexually transmited infections;03 (diseases of the blood or blood forming organs): anaemia;04 (diseases of the immune system): allergic;05 (endocrine nutritional or metabolic diseases): diabetes;06 (mental behavioural or neurodevelopmental disorders): stress;08 (diseases of the nervous system): migraine;11 (diseases of the circulatory system): hypertension, cholesterol hypertension;12 (diseases of the respiratory system): cough, cold problems;13 (diseases of the digestive system): gastric infection, haemorrhoids, gingivitis, diarrhoea;14 (diseases of the skin): eczema, dandruff;16 (diseases of the genitourinary system): amenorrhoea, urinary calculus.

Table V. Assessing the Therapeutic of Medicinal Plants in Relizane, Algeria: A Focus on ICD-11 Disease Classifications.

ICD-11	Disease types	Plant Species	Specified disease categories
05	Endocrine, nutritional, or metabolic diseases	*Olea europaea*	Diabetes
		Marrubium vulgare	Diabetes
		Ajuga-iva	Diabetes
		Atemisia herba-alba Asso.	Diabetes
		Triticum aestivum L.	Diabetes
		Coriandrum sativum L.	Diabetes
		Capparis spinosa L.	Diabetes
		Centaurium erythraea Rafn.	Diabetes
		Rubia montana L.	Diabetes
		Linum usitatissium L.	Diabetes
11	Diseases of the circulatory system	*Allium sativum* L.	Hypertension
		Pistacia lentiscus	Hypertension
		Mentha spicata L.	Hypertension
		Ocimum basilicum L.	Hypertension
		Hibiscus sabdariffa	Hypertension
		Apium graveolens L.	Hypertension
		Peganum harmala	Hypertension
		Urtica dioica L.	Hypertension
		Mentha suaveolens Her	Hypertension
		Atriplex halimus L.	Hypertension
		Myrtus communis L.	Hypertension
		Punica granatum L.	Cholesterol hypertension
		Lepidium sativum L.	Cholesterol hypertension
12	Diseases of the respiratory system	*Thymus vulgaris*	Cough
		Mentha pulegium L.	Cough
		Citrus sinensis L.	Cough
		Eucalyptus globulus Labill.	Cough
		Ptychotis verticillata Duby	Cold problems
		Citrus limum Riss.	Cold problems
14	Diseases of the skin	*Neurium oleander* L.	Eczema
		Lavandula angustifolia	Eczema
		Verbena officinalis L.	Eczema
		Opuntia ficus -indica	Dandruff
13	Diseases of the digestive system	*Rosmarinus officinalis* L.	Gastric infection
		Juniperus oxycedrus L.	Haemorrhoids
		Malva sylvestris L.	Gingivitis
		Castanea sativa	Diarrhoea
16	Diseases of the genitourinary system	*Salvia officinalis* L.	Amenorrhoea
		Paronychia argentea	Urinary calculus
		Santolina rosmarinifolia L.	Urinary calculus
01	Certain infectious or parasitic diseases	*Juniperus communis* L.	Predominantly sexually transmitted infections
08	Diseases of the nervous system	*Ecballium*	Migraine
		Elaterium	
		Matricaria chamomilla L.	Migraine
		Populus nigra L	Migraine
06	Mental behavioural or neurodevelopmental disorders	*Rhamnus alaternus* L.	Stress
03	Diseases of the blood or blood-forming organs	*Ceratonia siliqua* L.	Anaemia
		ziziphus jujuba	Anaemia
		Rubia tinctorum L.	Anaemia
04	Diseases of the immune system	*Glycyrrhiza glabra* L.	Allergic
		Hordeum vulgare L.	Allergic

In the research domain, ICD-11 codes help evaluate the effectiveness and safety of traditional medicine interventions and support clinical studies integrating traditional medicine with Western medicine **(Reddy and Fan, 2021)**. Classifying medicinal plants according to ICD-11 helps identify knowledge gaps and highlights plants that warrant further research for the development of new drugs. Future studies can focus on identifying the active compounds in these plants that have therapeutic effects on specific diseases. Additionally, this classification suggests the potential to expand the use of traditional medicinal plants by testing their efficacy and safety for treating various conditions, thereby contributing to the development of new treatments based on traditional medicine.

Medicinal plants span a wide range of biological systems, addressing both simple infections, such as colds, and chronic conditions, such as diabetes and hypertension. The emphasis on common diseases, including sexually transmitted infections, diabetes, and hypertension, underscores the significant role medicinal plants can play in managing prevalent health issues.

1.4. Association between age and number of plants known

Spearman's rank correlation analysis revealed a strong, statistically significant positive relationship between herbalists' age and the number of medicinal plants. They reported knowing ($\rho = 0.646$, p-value < 0.001, $n = 60$). This suggests that older individuals tend to possess more extensive ethnobotanical knowledge than younger individuals. **(Figure 22)** The finding can be explained by the cumulative nature of traditional knowledge over time, as well as the role of elders in preserving and orally transmitting cultural practices and plant-based remedies across generations.

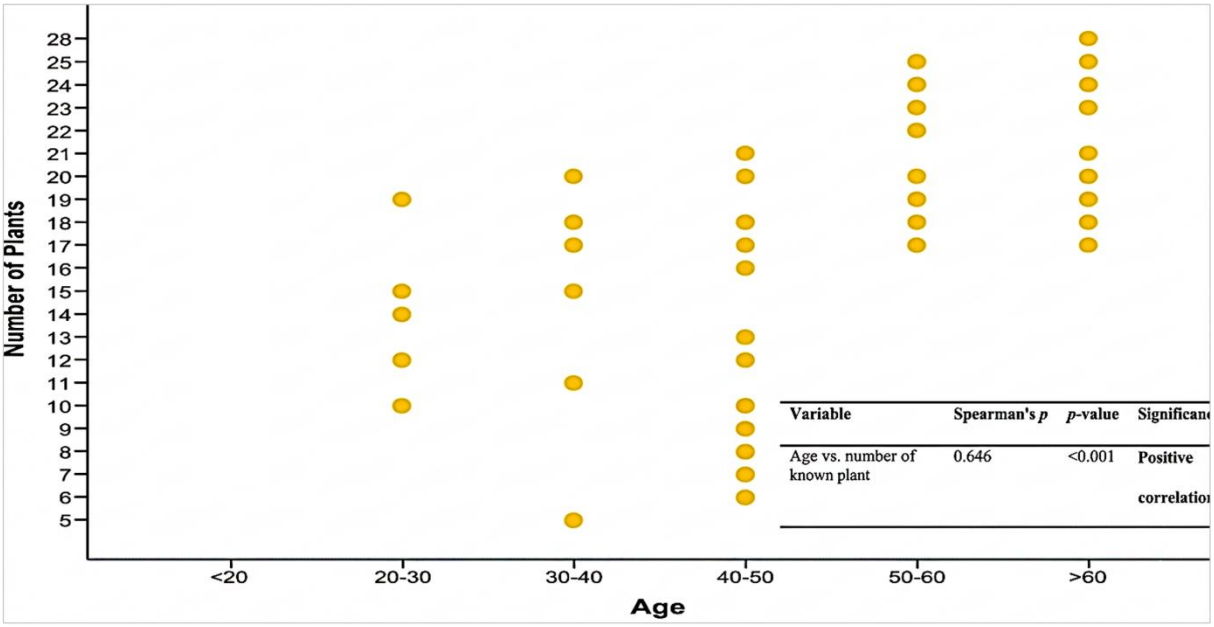


Figure 22. Relationship between Age and number of plants known: Significant Positive Correlation.

Similar trends have been reported in previous ethnobotanical studies conducted in various regions. For instance, a study in northwestern Algeria found that knowledge of medicinal plants was more prevalent among older individuals, highlighting the role of age in the retention of traditional knowledge **(Ali *et al*., 2023)**.

1.5. Use-value (UV)

The results of UV **(Table VI).** demonstrated *Ptychotis verticillata* Duby remarkable high Species Use Value (SUV) of 0.96, which indicates that it has become a plant of great significance in the research area's traditional medicine. This high SUV highlights the plant's

widespread use to treat a variety of illnesses, indicative of its crucial importance in regional ethnomedicine. noted *Ptychotis verticillate* Duby significant ethnobotanical value, emphasizing its many therapeutic uses and potential. strengthened the plant's standing in local herbal medicine by offering a thorough analysis of its pharmacological characteristics and traditional use.

Table VI. Documentation of Traditional Medicinal Plants: Uses Reported by Herbalists and Supported by Literature, with Use Values (UV) and Fidelity Levels (FL).

Plant species	Plant families	Plant parts used	Method of use	Recommended uses	UV	FL%
Ajuga iva L.	Lamiaceae	Leaves	Decoction	Diabetes **(Nazim *et al*., 2020)**	0.11	28.57
Allium sativum L.	Amaryllidaceae	Bulb	Raw	Diabetes **(Barkaoui *et al*., 2017)**	0.18	81.81
Apium graveolens L.	Apiaceae	Aerial parts, leaves	Infusion, decoction	Pathologies of the urinary system, Cardiovascular diseases, Pathologies of the digestive system (**Jamila and Mostafa, 2014)**	0.6	69.44
Artemisia herba-alba Asso.	Asteraceae	Leaves, Aerial parts, flowers	Infusion, decoction, powder	Diabetes, Pathologies of the digestive system, ermocosmotology **(Jamila and Mostafa, 2014)**	0.8	79.16
Atriplex halimus L.	Chenopodiaceae	Aerial parts	Infusion, lotion	Eczema **(Boudjelal *et al*., 2013)**	0.83	87.5
Capparis spinosa L.	Capparidaceae	Fruits	Infusion, powder	Diabetes **(Jouad *et al*., 2001)**	0.16	30
Castanea sativa Mill.	Fagaceae	Fruits	Decoction	Diarrhea **(Benarba *et al*., 2015)**	0.16	20
Centaurium erythraea Rafn.	Gentianaceae	Aerial parts	Decoction	Diabetes, power problems **(Jamila and Mostafa, 2014)**	0.26	62.5
Ceratonia siliqua L.	Lamiaceae	Fruit, cortex	Powder	Pathologies of the digestive system, Pathologies of the respiratory system, Dermocosmotology **(Jamila and Mostafa, 2014)**	0.25	51.21
Citrus limon L.	Rutaceae	Fruits, flowers, leaves	Juice, infusion, decoction	Problems of hearing and the ear, Cold problems, Head problems **(Jamila and Mostafa, 2014)**	0.75	71.11
Citrus sinensis L.	Rutaceae	Leaves, fruits	Juice, decoction	Pathologies of the digestive system, Skeleton–muscular system problems **(Jamila and Mostafa, 2014)**	0.66	87.5

Coriandrum sativum L.	Apiaceae	Seeds, Aerial parts	Infusion, powder, decoction	Pathologies of the digestive system, Pathologies of the urinary system, Allergy **(Jamila and Mostafa, 2014)**	0.5	36.76
Ecballium Elaterium L.	Cucurbitaceae	Fruit	Insufflation	Hepatic diseases **(Boudjelal *et al*., 2013)**	0.16	40
Eucalyptus globulus Labill.	Myrtaceae	Leaves	Infusion, decoction	Colds and flu, antitussive **(Boudjelal *et al*., 2013)**	0.76	84.78
Glycyrrhiza glabra L.	Fabaceae	Stem, seeds	Infusion, decoction, powder	Pathologies of the digestive system, Pathologies of the respiratory system, Allergy **(Jamila and Mostafa, 2014)**	0.15	66.67
Hibiscus sabdariffa L.	Malvaceae	Seeds, flowers	Infusion, decoction, maceration	cholesterol, hypertension, stress, depilation **(Benarba *et al*., 2015)**	0.16	10
Hordeum vulgare L.	Poaceae	Cortex, seeds	Powder, decoction	Diabetes, Allergy, Fever **(Jamila and Mostafa, 2014)**	0.41	20
Juniperus communis L.	Cupressaceae	Aerial parts	Infusion, decoction, Maceration	Acne, diabetes, eczema, infections genital and urinary, rheumatism, difficult digestion **(Fadil *et al*., 2015)**	0.91	18.18
Juniperus oxycedrus L.	Cupressaceae	Aerial parts	Tablet	Anti-inflammatory, eye infections **(Boudjelal *et al*., 2013)**	0.66	15
Lavandula angustifolia Mill.	Lamiaceae	Leaves, flowers	Infusion, decoction, maceration, inhalation	Carminative, diuretic, antiepileptic, antirheumatic, analgesic, anticonvulsant, sedative, spasmolytic, antioxidant, antibacterial **(Jouad *et al*., 2001)**	0.51	32.25
Lepidium sativum L.	Brassicaceae	Seeds	Infusion, decoction, powder	Cardiovascular diseases, Pathologies of the digestive system, Pathologies of the respiratory system **(Jamila and Mostafa, 2014)**	0.06	25
Linum usitatissium L.	Linaceae	Stem	Powder, maceration	Pathologies of the digestive system, Pathologies of the respiratory system, Allergy **(Jamila and Mostafa, 2014)**	0.31	26.31
Malva sylvestris L.	Malvaceae	Aerial parts	Decoction	Anti-inflammatory, weight loss **(Boudjelal *et al*., 2013)**	0.43	19.23
Marrubium vulgare L.	Lamiaceae	Aerial parts	Infusion, powder	Antidiabetic, leishmanicidal, digestive disorders ***(Boudjelal et al*., 2013)**	0.82	25

Matricaria chamomilla L.	Asteraceae	Flowers	Infusion, ecoction, poultice,maceration, inhalation	Menstrual pain, migraine, rheumatism, skin irritations, anemia **(Fadil *et al*., 2015)**	0.31	52.63
Mentha pulegium L.	Lamiaceae	Stem, leaves, Flowers	Infusion, decoction Inhalation	Colds, nausea, toothache, stomach ulcer, menstruation painful **(Fadil *et al*., 2015)**	0.68	45.71
Mentha spicata L.	Lamiaceae	Stem, leaves,Flowers	Infusion, decoction	Culinary use, stomach lazy, vomiting, gas intestinal, neuralgia **(Fadil *et al*., 2015)**	0.75	77.78
Mentha suaveolens Ehrh.	Lamiaceae	Leaves, roots, aerial parts	Infusion, powder, decoction	Cardiovascular diseases, Pathologies of the respiratory system, Pathologies of the digestive system **(Jamila and Mostafa, 2014)**	0.58	22.22
Myrtus communis L.	Myrtaceae	Aerial parts	Infusion, decoction	Antihypertensive **(Boudjelal *et al*., 2013)**	0.38	26.08
Neurium oleander L.	Apocynaceae	Flowers	Infusion, powder	Antitumor, leishmanicidal, eczema **(Boudjelal *et al*., 2013)**	0.15	33.33
Ocimum basilicum L.	Lamiaceae	Aerial parts	Infusion	Hypertension **(Jouad *et al*., 2001)**	0.1	16.67
Olea europaea L.	Oleaceae	Leaves	Infusion, oil, decoction	Dermocosmotology, Pathologies of the respiratory system, diabetes **(Jamila and Mostafa, 2014)**	0.6	30.55
Opuntia ficus -indica L.	Cactaceae	Flowers, leaves, roots	Infusion, decoction, maceration	Pathologies of the respiratory system, Pathologies of the digestive system, Pathologies of the urinary system **(Jamila and Mostafa, 2014)**	0.28	16.66
Paronychia argentea Lam.	Caryophyllace	Aerial parts	Infusion, decoction	Antilithiasis **(Boudjelal *et al*., 2013)**	0.41	72
Peganum harmala L.	Zygophyllaceae	Seeds	Infusion	Diabetes, hypertention **(Jouad *et al*., 2001)**	0.41	24
Pistacia lentiscus L.	Anacardiaceae	Leaves	Oil, decoction, dried leaves	Hypertension and diabetes **(Nazim *et al*., 2020)**	0.71	46.51
Populus nigra L	Salicaceae	Aerial parts, Leaves	Infusion, decoction, powder	Pathologies of the digestive system, Pathologies of the respiratory system, Head problems **(Jamila and Mostafa, 2014)**	0.1	66.67

Ptychotis verticillata Duby	Apiaceae	Ieaves, roots, Aerial parts	Infusion, decoction, powder	Diabetes, Pathologies of the digestive system, Cold problems, Pathologies of the respiratory system, cancer, Pathologies of the circulatory system, Problems of hearing and the ear **(Jamila and Mostafa, 2014)**	0.96	43.10
Punica granatum L.	Punicaceae	pericarpe	Infusion	Renal disease **(Jouad *et al.*, 2001)**	0.23	42.85
Rhamnus alaternus L.	Rhamnaceae	Aerial parts	Decoction	Hepatic diseases **(Boudjelal *et al.*, 2013)**	0.35	31.66
Rosmarinus officinalis L.	Lamiaceae	Aerial parts	Infusion, decoction	Antihypertensive, hepatic diseases, antitumoral, Eczema **(Boudjelal *et al.*, 2013)**	0.25	8.33
Rubia montana L.	Rubiaceae	Aerial parts	Infusion	Diabetes, renal disease **(Jouad *et al.*, 2001)**	0.78	74.46
Rubia tinctorum L.	Rubiaceae	Roots	Decoction, raw	Anemia, female sterility **(Benarba *et al.*, 2015)**	0.13	37.5
Salvia officinalis L.	Lamiaceae	Aerial parts	Infusion, powder	Antidiabetic, antihypertensive, weight loss, eczema **(Boudjelal *et al.*, 2013)**	0.06	25
Santolina rosmarinifolia L.	Asteraceae	Aerial parts	Infusion, decoction, powder	Wound healing, digestive disorders **(Boudjelal *et al.*, 2013)**	0.05	33.333
Thymus vulgaris L.	Lamiaceae	Stem, Leaves	Infusion, decoction	Anemia, parasites, intestinal gas, rheumatic disorders **(Fadil *et al.*, 2015)**	0.88	56.60
Triticum aestivum L.	Poaceae	Cortex, seeds, leaves	Infusion, powder, decoction	Diabetes, Allergy, Pathologies of the digestive system **(Jamila and Mostafa, 2014)**	0.51	32.25
Urtica dioica L.	Urticaceae	Leaves, seeds	Infusion	Diabetes, hypertention **(Jouad *et al.*, 2001)**	0.05	33.33
Verbena officinalis L.	Verbenaceae	Stem, leaves	Infusion, decoction, poultice, aceration, inhalation	Antioxidant, antifungal, antimicrobial, diuretic, expectorant, anti-rheumatic, anti-inflammatory **(Fadil *et al.*, 2015)**	0.05	66.67
Ziziphus jujuba Mill.	Rhamnaceae	Fruits, leaves	Raw, infusion	Anemia **(Moulessehoul *et al.*, 2023)**	0.48	68.69

highlighted the plant's considerable value in ethnomedicinal traditions and its usefulness in treating a variety of ailments, further supporting these findings. **(Taibi *et al.*, 2024)** further affirmed the therapeutic potential and traditional importance of *Ptychotis verticillate* Duby, while talking about the latest developments in our knowledge of the plant's pharmacological advantages and its ongoing significance in contempo

rary herbal medicine. This also explains the species' high SUV in our study. *Juniperus communis* L. and *Thymus vulgaris* L. also showed high SUVs of 0.91 and 0.88, respectively, indicating their frequent use among herbalists. Notably, *Thymus vulgaris* was ranked just after *Ptychotis verticillata* Duby in our study, highlighting its significance in traditional medicine. *Thymus vulgaris* L. is considered one of the most highly valued plants, with a significant use value (UV) reported in Mediterranean countries such as Spain **(Calvo and Cavero, 2014)**, Italy **(Idolo *et al*., 2010)**, and Portugal **(Neves *et al*., 2009)**. This herb is particularly renowned in Mediterranean regions for its efficacy in treating respiratory disorders, such as bronchitis, colds, and influenza, thanks to its active compounds, thymol and carvacrol, which exhibit strong antiviral and antibacterial properties. Our findings align with previous studies that also reported a high use value for *Thymus vulgaris* L. For instance, a study found that *Thymus vulgaris* L. had the highest UV of 0.883 among local informants **(Benarba *et al*., 2015)**, being frequently used (55%) to treat various respiratory ailments, including bronchitis, allergies, colds, flu, and cough **(Choudhary *et al*., 2024)**. The presence of bioactive compounds such as thymol, carvacrol, p-cymene, eugenol, phenols, luteolin, and tetramethoxylated further explains its therapeutic potential, as these compounds are

known for their antiviral, anti-inflammatory, antioxidant, and antibacterial properties.

In addition, other species such as *Mentha spicata* L. (0.75) and *Citrus limum Riss.* (0.75) were also reported with high SUVs, reflecting their widespread use in traditional medicine for treating digestive issues and respiratory infections. These findings are consistent with existing literature, which underscores the therapeutic uses of these plants, especially in regions with strong herbal traditions.

Conversely, certain plants recorded low SUVs, such as *Salvia officinalis* L. (0.06) and *Lepidium sativum* L. (0.06), possibly indicating that these plants are either less well known or are used for more specific medicinal purposes. This pattern may reflect limited availability in the region or a gap in traditional knowledge about their medicinal benefits, as observed in other lesser-used plants that may be at risk of knowledge erosion.

It is noteworthy that plants with high SUVs might face increased anthropogenic pressure, potentially leading to their rarity in the future if conservation measures are not implemented. For instance, plants like *Olea europaea* are commonly used in traditional medicine for their antioxidant and anti-inflammatory benefits, highlighting the importance of preserving such species to ensure their continued use **(Brahmi *et al.*, 2016)**.

1.6. Fidelity level (FL)

The fidelity level indicates which medicinal plant species respondents most favor for treating specific categories of ailments **(Caunca & Balinado, 2021)**. Our findings revealed that the relative usage frequency of 50 plant species ranged from 8.33% to 87.5% **(Table VI).** *Citrus sinensis* L. and *Atriplex halimus* L. exhibited the highest Fidelity Level for treating cough and hypertension at 87.5%, followed by *Eucalyptus globulus Labill, with* 84.78% for treating cough, and *Allium sativum* L. with 81.81% for treating hypertension. According to previous studies, plants with high FL values are frequently utilized as natural pharmaceutical resources and should be prioritized in future conservation programs, bioassays, and phytopharmaceutical research.

The Fidelity Level (FL) analysis revealed diverse medicinal plant use depending on the type of disease. For metabolic and endocrine disorders, *Artemisia herba-alba Asso.* had the highest FL (79.16%) for diabetes treatment, highlighting its significant role in traditional medicine within the studied community. Following this, *Rubia montana* L. and *Centaurium erythraea Rafn.* demonstrated FLs of 74.46% and 62.5%, respectively, indicating their frequent and practical use for the same condition. These findings are consistent with recent studies conducted in Mediterranean countries like Italy and Morocco, which also reported the

effectiveness of these plants in managing diabetes **(Devangan *et al*., 2021)**.

Regarding circulatory system diseases, *Atriplex halimus* L. was the plant most frequently used to manage hypertension, achieving the highest FL of 87.5%. *Allium sativum* L. and *Mentha spicata* L. also showed significant FLs of 81.81% and 77.78%, respectively, further supporting their widespread use in the management of hypertension. These results align with studies from Tunisia and Greece, which also underscore the antihypertensive properties of these plants **(Benamara *et al*., 2022)**.

In respiratory diseases, Citrus sinensis L. was highly effective for treating cough, as reflected in its FL of 87.5%. Similarly, *Eucalyptus globulus Labill.* had an FL of 84.78%, underscoring its everyday practicality for respiratory issues. These outcomes are supported by research from Spain and Turkey, which also highlight the antitussive and anti-inflammatory properties of these plants **(González *et al*., 2010)**.

1.7. Informant Consensus Factor (ICF)

The ICF values reflect herbalists' agreement on the use of plants for specific diseases **(Table VII)**. Higher ICF values (close to 1) indicate strong consensus and frequent use of certain plants, suggesting a solid traditional knowledge base **(Weckerle *et al*., 2018)**. For example, infectious diseases and mental disorders showed perfect agreement (ICF = 1), highlighting the reliability of plant remedies.

Table VII. Herbalists' consensus index (ICF) values for plant selection across various disease categories.

Disease Category	Nur	Nt	ICF
Certain infectious or parasitic diseases	23	1	1
Diseases of the blood or blood-forming organs	19	3	0.89
Diseases of the circulatory system	53	12	0.78
Diseases of the digestive system	11	4	0.70
Diseases of the genitourinary system	21	3	0.9
Diseases of the immune system	9	2	0.875
Diseases of the nervous system	6	3	0.60
Diseases of the respiratory system	49	6	0.89
Diseases of the skin	32	4	0.90
Endocrine, nutritional, or metabolic diseases	31	10	0.70
Mental, behavioral, or neurodevelopmental disorders	17	1	1

High ICF values were also found for skin diseases (0.90) and respiratory diseases (0.89), suggesting focused plant use. In contrast, nervous system disorders had a lower ICF value (0.60), indicating more variability in plant selection. These results suggest that high ICF values may represent areas of reliable traditional knowledge, suitable for further research **(Zenderland *et al.*, 2019)**.

2. Essential Oils

2.1. Yields and Organoleptic Characteristics

The essential oils were extracted by hydrodistillation from the aerial parts of *Ptychotis verticillata* Duby and *Thymus vulgaris* L. The extraction yields, along with the organoleptic and physicochemical characteristics of the obtained oils, are presented in **(Table VIII)**.

Table VIII. Yields and Organoleptic Characteristics of Essential Oils Extracted from the Aerial Parts of Plants.

Plant Species	Raw Extract	Yield (%)	Appearance	Color	Odor
P. verticillata	Crude Essential Oil	2.5	Viscous liquid	White	Strong, characteristic aromatic scent
T. vulgaris	Crude Essential Oil	2.01	Viscous liquid	Yellow	Intense, pungent, typical thyme aroma

The essential oil yield of *P. verticillata* was measured at 2.5%, which is consistent with previously reported values ranging between 2.2% and 2.7% depending on geographic origin and extraction conditions **(Benabderrahmane *et al.*, 2024; Bendif *et al.*, 2018)**. The oil exhibited a viscous texture and a white color, typical of this species' essential oil profile, which is characterized by a complex mixture of monoterpenes and sesquiterpenes, contributing to its strong aromatic odor. *T. vulgaris* essential oil showed a yield of 2.01%, aligning with literature values generally reported between 1.8% and 2.3% **(Hosseinzadeh *et al.*, 2015)**. The oil appeared as a viscous, yellow liquid and emitted a potent, pungent odor characteristic of thyme EOs, primarily attributable to bioactive compounds such as thymol and carvacrol **(Karous *et al.*, 2021)**.

These organoleptic characteristics and physicochemical properties confirm the quality and compositional consistency of the extracted essential oils, which are relevant for subsequent biological activity evaluations.

2.2. Identification of the essential oils components

The GC-MS analysis yielded experimental retention indices, which were subsequently compared with literature values. Each peak in the electron ionization (EI) mass spectrum was

also matched against established mass spectral libraries **(Benabderrahmane *et al.*, 2024)**. **(Figures 23** and **24)** show the GC-MS chromatograms of the essential oils of *Ptychotis verticillata* Duby and Thymus vulgaris L., respectively. Each peak in the chromatograms corresponds to a specific compound, identified by mass spectral library matching and linear retention index (LRI) comparison.

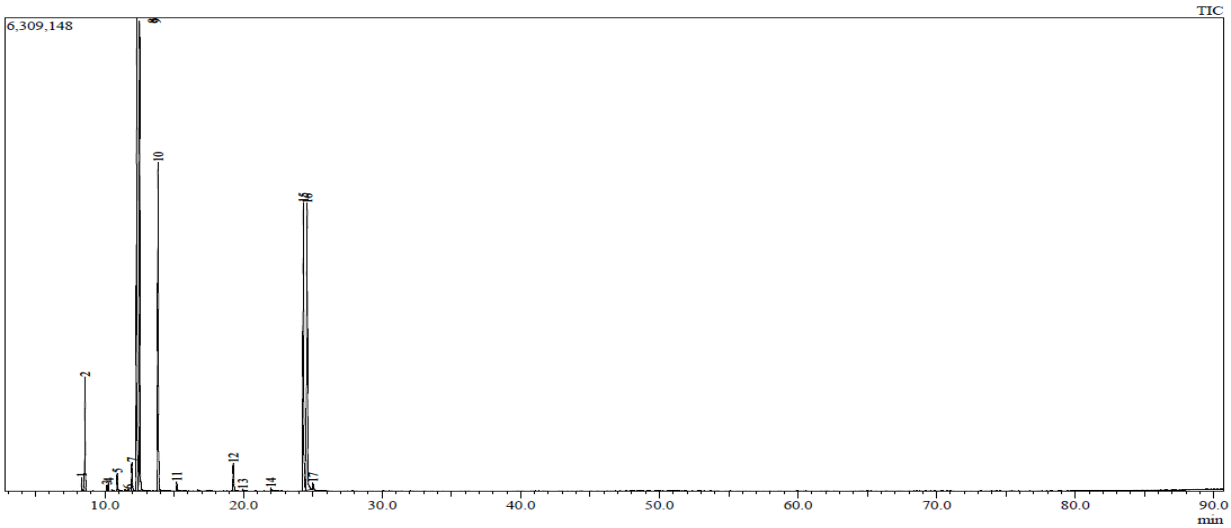


Figure 23. GC-MS of Essential Oil from *P. verticillata* : Analysis of Chemical Composition.

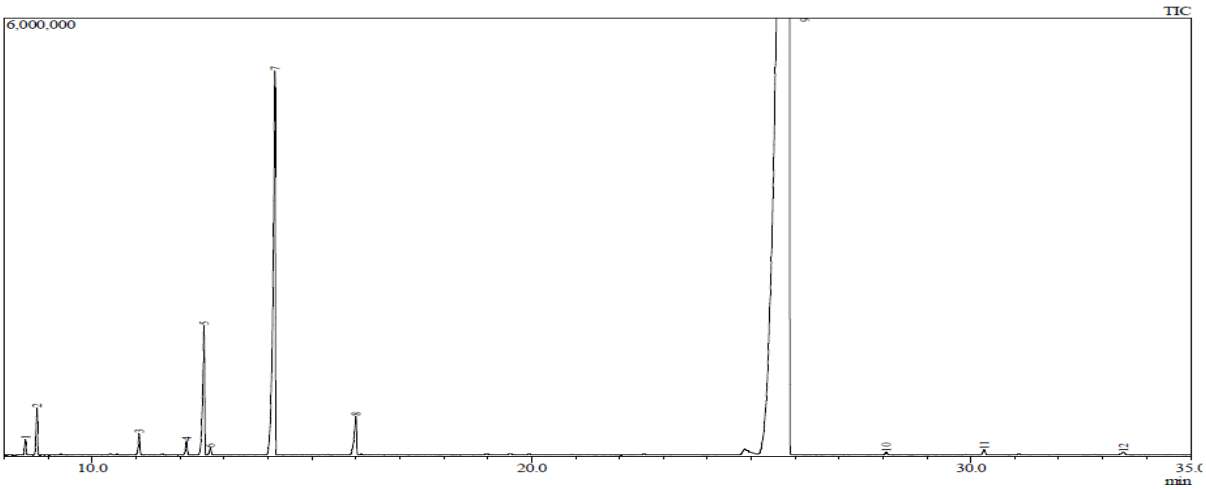


Figure 24. GC-MS of Essential Oil from *T. vulgaris*: Analysis of Chemical Composition.

This study delineates the chemical compositions of *the essential oils of P. verticillata and T. vulgaris*, as summarized in (**Table VIX)**.

Table IX. The essential oil constituents of *Ptychotis verticillata* Duby and *Thymus vulgaris L.*

N⁰	*P. verticillata*				*T.vulgaris*			
	Compounds	*Retention-time*	*Percentage(%)*	LRI	Compounds	Retention-time	Percentage(%)	LRI
1	Alpha-Thujene	8.475	0.43	919	Alpha-Pinene	8.477	0.17	961
2	Alpha-Pinene	10.318	4.04	925	Trans-beta-Ocimene	8.739	0.52	968
3	Sabinene	10.427	0.22	965	beta-Myrcene	11.065	0.25	1027
4	beta.-Pinene	11.080	0.33	968	2-Carene	12.144	0.17	1052
5	Myrcene	12.166	0.66	984	p-Cymene	12.543	1.89	1061
6	Delta-3-Carene	12.640	0.05	1003	Limonene	12.686	0.09	1064
7	Alpha-Terpinene	12.808	1.09	1009	gamma-Terpinene	14.155	6.46	1098
8	p-Cymene	12.866	23.20	1017	Linalool	16.003	0.69	1139
9	Limonene	14.372	22.01	1021	Thymol	25.858	89.58	1361
10	gamma-Terpinene	15.413	14.89	1051	3-Decyne	28.077	0.05	1413
11	Terpinolene	19.508	0.35	1081	Caryophyllene	30.305	0.08	1466

12	Terpinen-4-ol	20.491	1.34	1169	Camphene	33.472	0.03	1546
13	Alpha-Terpineol	22.180	0.08	1184				
14	Carvacrol methyl ether	22.589	0.13	1228				
15	Thymol	24.891	15.41	1280				
16	Carvacrol	25.289	15.45	1286				
17	Phenol, 2-methyl-5-(1-methylethyl)-	25.461	0.35	1295				
	Total identified		100.00		Total identified		100.00	
	Total monoterpenes		99.68		Total Monoterpenes		99.82	
	Monoterpene hydrocarbons		67.27		Monoterpene hydrocarbons		9.55	
	Oxygented monoterpenes		32.41		Oxygented monoterpenes		90.27	
	Total sesquiterpenes		-		Total sesquiterpenes		0.08	
	Sesquiterpene hydrocarbons		-		Sesquiterpene hydrocarbons		0.08	
	Oxygented sesquiterpenes		-		Oxygented sesquiterpenes		-	
	Others		0.35		Others		0.05	

Lists the identified compounds along with their respective peak numbers, retention times, percentages, and LRIs. The peak numbers in the chromatograms **(Figures 23** and **24**) directly correspond to the compound order in the table. For example, in *P. verticillata*, peak No. 8 at a retention time of 12.866 min corresponds to p-Cymene (23.20%, LRI 1017), while in *T. vulgaris*, peak No. 9 at 25.858 min corresponds to Thymol (89.58%, LRI 1361), the primary compound.

This comprehensive alignment between chromatographic data and compound identification provides a robust interpretation of the essential oil composition. The GC-MS analysis successfully identified 17 compounds in *P.verticillata* and 12 in *T. vulgaris*, accounting for 100% of the detected components. These essential oils exhibit distinct chemical profiles indicative of their potential applications across various fields, including pharmacology and food preservation.

The analysis revealed a comprehensive composition of these oils, highlighting the presence of key bioactive compounds. Notable constituents identified in *P. verticillata* include cymene (23.20%) Global studies have also observed similar chemical compositions, such as in Saudi Arabia, where *P. verticillata* essential oils showed high concentrations of p-cymene and limonene **(Laouer *et al.*, 2003)**, limonene (22.01%), thymol (15.41%), and carvacrol (15.45%).

In contrast, *T. vulgaris* exhibited a significantly higher concentration of thymol (89.58%), along with other components such as γ-terpinene (6.46%) and p-cymene (1.89%). Both essential oils were characterized by a high percentage of monoterpenes, with *P. verticillata* containing 99.68% and T. vulgaris containing 99.82%. The predominance of oxygenated monoterpenes in *T. vulgaris* (90.27%) compared to *P. verticillata* (32.41%) suggests a greater potential for antimicrobial activity, as oxygenated compounds are often associated with enhanced bioactivity. In Algeria, studies have demonstrated the antifungal potential of *P. verticillata*, particularly against *Fusarium avenaceum*, in which its essential oil was shown to inhibit mycelial growth effectively **(Chakroun *et al.*, 2021)**.

This aligns with findings highlighting thymol's role as a potent antimicrobial agent, reinforcing its significance in both local and global contexts **(Belaiba *et al.*, 2024)**.

A study in Tunisia identified high concentrations of p-cymene, limonene, and γ-terpinene in *P. verticillata* essential oil, confirming the dominance of hydrocarbon monoterpenes **(Dudek *et al.*, 2016)**, while extensive research on the chemical composition of *P. verticillata* revealed significant levels of thymol and γ-terpinene, which contribute to its biological activities **(Chakroun *et al.*, 2021)** .

These findings are consistent with global studies that emphasize the diverse applications of essential oils in food preservation and natural medicine due to their bioactive compounds' efficacy against pathogens and spoilage organisms **(Tefiani *et al.*, 2016)**.

The high thymol content in *T. vulgaris* is particularly noteworthy, as thymol is well-documented for its potent antimicrobial properties against a range of pathogens, including bacteria and fungi. The essential oil from *P. verticillata*, which also contains thymol, exhibits a more diverse chemical profile that may contribute to its antioxidant and antimicrobial efficacy.

Studies have shown that compounds such as limonene and cymene can enhance the overall antimicrobial activity of essential oils.

The synergistic effects of these components could provide a broader spectrum of activity against various microorganisms, making these essential oils suitable candidates for further research into their applications in food preservation and natural antiseptics.

The GC-MS results underscore the significance of both *P. verticillata* and *T. vulgaris* as sources of potent essential oils with diverse chemical compositions and potential health benefits.

2.3. Evaluation of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of the plant extracts and EOs was evaluated using the DPPH radical scavenging method. Results are expressed as percentage inhibition (%) of the DPPH radical and half-maximal inhibitory concentration (IC_{50}) values. The IC_{50} value indicates the concentration required to inhibit 50% of the radical activity; lower IC_{50} values indicate more substantial antioxidant potential.

As illustrated, the inhibition percentage of DPPH radicals increased in a dose-dependent manner for all samples. EOs exhibited significantly higher DPPH radical scavenging effects compared to aqueous extracts.

At a concentration of 75 mg/mL, both essential oils and extracts recorded inhibition percentages approaching those of the standard antioxidant, ascorbic acid. Specifically, the essential oils showed inhibition percentages comparable to those of ascorbic acid, indicating their substantial antioxidant potential.

The IC_{50} values presented in Table X demonstrate that essential oils generally exhibited higher IC_{50} values compared to ascorbic acid, reflecting a somewhat lower scavenging efficiency.

For instance, *P. verticillata* and *T. vulgaris* essential oils showed IC_{50} values of 80.869 ± 13.31 and 59.906 ± 17.76, respectively, compared to 37.542 ± 0.62 for ascorbic acid. This difference in activity may be attributed to variation in phenolic and flavonoid contents within the essential oils, which are critical contributors to antioxidant capacity **(El Jemli *et al.*, 2016)**.

Secondary metabolites such as flavonoids and phenolic acids including caffeic acid, gallic acid, and chlorogenic acid play crucial roles in preventing oxidative damage by scavenging reactive oxygen species (hydroxyl, superoxide, alkoxyl, and peroxyl radicals), chelating pro-oxidant metal ions (e.g., iron and copper), and inhibiting enzymes responsible for free radical generation **(Van Acker *et al.*, 1996; Krishnaveni *et al.*, 2013)**.

The essential oils under study reduced the stable DPPH radical to a yellow-colored diphenyl-picrylhydrazine, achieving 50% scavenging with IC_{50} values within the aforementioned range. The presence of compounds such as 1,8-cineole and caryophyllene, known for their potent free radical scavenging properties, partly explains this activity **(Ruberto and Baratta, 2000)**.

Table X. IC_{50} Values of Different Essential Oils, and Ascorbic Acid for DPPH Radical Scavenging.

	IC_{50} values (mg/ml)
Ascorbic acid	37.54 ± 0.62
P. verticillata	80.87 ± 13.31
T. vulgaris	59.91 ± 17.76

Values are expressed as mean ± SD (n = 3); $p < 0.05$ is considered significant.

The EO exhibited higher IC_{50} values compared to ascorbic acid, indicating comparatively lower radical scavenging efficiency. This discrepancy is attributed to differences in the phenolic and flavonoid profiles of the oils, which significantly influence antioxidant activity **(El Jemli *et al.*, 2016)**. Key secondary metabolites such as caffeic acid, gallic acid, and chlorogenic acid are known to mitigate oxidative damage by neutralizing reactive oxygen species and chelating pro-oxidant metals **(Van Acker *et al.*, 1996; Krishnaveni *et al.*, 2013)**.

Furthermore, compounds such as 1,8-cineole and caryophyllene, present in the oils, contribute to their free radical-scavenging properties **(Ruberto and Baratta, 2000)**. significant difference in antioxidant activities among the tested samples ($p = 0.0187$).

Here, the p-value of < 0.05 indicates that the observed differences in IC_{50} values are statistically significant and unlikely to be due to random variation. Based on conventional thresholds, this level of significance corresponds to a single asterisk, indicating moderate but

meaningful confidence that the sample activities differ.

These findings align with previous reports of moderate antioxidant effects of EOs, in which higher IC_{50} values than those of commercial antioxidants were observed, yet bioactivity remained significant **(Mimica-Dukić *et al.*, 2010)**. Variations between *P. verticillata* and *T. vulgaris* likely reflect distinct phytochemical compositions and concentrations of bioactive terpenoids and phenolics. Moreover, no significant difference was noted between the antioxidant activities of essential oils and their corresponding methanolic extracts, corroborating the robustness of these results **(Wannes *et al.*, 2010)**.

2.4. Anti-inflammatory properties demonstrated in vitro

The anti-inflammatory potential of the essential oils *P. verticillata* and *T. vulgaris* was investigated using two widely accepted in vitro models; the erythrocyte membrane stabilization test revealed that both *P.verticillata* and *T. vulgaris* EOs exhibit concentration-dependent inhibitory effects on red blood cell lysis **(Table XI)**.

At a concentration of 100 µg/ml, *P. verticillata* inhibited hemolysis by 18.3 ± 0.95%, increasing to 31.1 ± 0.76% at 200 µg/ml, 43.4 ± 1.21% at 300 µg/ml, and 56.4 ± 0.60% at 400 µg/ml, reaching 65.5 ± 1.35% at 500 µg/ml. Similarly, *T. vulgaris* demonstrated higher inhibition values at all concentrations, starting from 22.2 ± 0.72% at 100 µg/ml, increasing to 35.4 ± 0.71% at 200 µg/ml, 48.8 ± 1.16% at 300 µg/ml, and 61.3 ± 0.70% at 400 µg/ml, and achieving 70.2 ± 0.80% at 500 µg/ml. These effects were compared with the standard anti-inflammatory drug diclofenac, which exhibited 63.0 ± 1.0% inhibition at 100 µg/ml and 75.0 ± 1.0% at 200 µg/ml. The results indicate that both EOs, particularly *T. vulgaris*, effectively protect erythrocyte membranes against hypotonic stress, with activities comparable to those of the reference drug. The essential oils were also evaluated for their ability to prevent heat-induced denaturation of egg albumin. Both *P. verticillata* and *T. vulgaris* displayed dose-dependent inhibitory activity. At 100 µg/ml, *P. verticillata* showed 14.0 ± 1.0% inhibition, increasing to 25.0 ± 1.0% at 200 µg/ml, 34.0 ± 0.8% at 300 µg/ml, and 44.0 ± 0.9% at 400 µg/ml, reaching 51.0 ± 1.0% at 500 µg/ml. In contrast, *T. vulgaris* exhibited higher inhibition values, starting at 35.0 ± 1.5% at 100 µg/ml, increasing to 46.0 ± 1.2% at 200 µg/ml, 55.0 ± 1.1% at 300 µg/ml, and 65.0 ± 1.2% at 400 µg/ml, and reaching 73.0 ± 1.3% at 500 µg/ml. Diclofenac, used as the standard, showed 210.0 ± 2.0% and 275.3 ± 1.5% inhibition at 100 and 200 µg/ml, respectively.

Table XI. Anti-inflammatory activity of essential oils from *P. verticillata* and *T. vulgaris*

assessed via protein denaturation inhibition (%) at various extract concentrations (ug/ml).

Concentrations (µg/ml)	% Inhibition			
	Of haemolysis		Protein denaturation	
	P . verticillata	*T. vulgaris*	*P.verticillata*	*T. vulgaris*
100	18.3 ± 0.95	22.2 ± 0.72	14.0 ± 1.0	35 ± 1.5
200	31.1 ± 0.76	35.4 ± 0.71	25.0 ± 1.0	46.0 ± 1.2
300	43.4 ± 1.21	48.8 ± 1.16	34.0 ± 0.8	55.0 ± 1.1
400	56.4 ± 0.60	61.3 ± 0.70	44.0 ± 0.9	65.0 ± 1.2
500	65.5 ± 1.35	70.2 ± 0.80	51.0 ± 1.0	73.0 ± 1.3
			Diclofenac	
100	63.0 ± 1.0		210.0 ± 2.0	
200	75.0 ± 1.0		275.3 ± 1.5	

Values are expressed as mean ± SD (n = 3). Statistical significance was determined using Student's t-test; p < 0.001 considered significant.

The erythrocyte membrane stabilization assay is widely used as an in vitro model to evaluate anti-inflammatory potential by measuring the ability of compounds to protect red blood cells (RBCs) from hemolysis induced by hypotonic solutions. This assay reflects the stabilization of lysosomal membranes, preventing the release of inflammatory mediators during inflammation **(Yesmin *et al*., 2020)**,

Protein denaturation inhibition assays assess the ability of compounds to prevent heat-induced protein unfolding, a process implicated in inflammatory diseases. Essential oils that inhibit protein denaturation are considered to have therapeutic potential for the management of inflammation **(Sam *et al*., 2015)**. The anti-inflammatory mechanisms involving both membrane stabilization and inhibition of protein denaturation observed in your *P. verticillata* and T. vulgaris oils align with established scientific findings that plant-derived bioactives can modulate inflammation through multiple cellular targets.

2.5. Evaluation of the insecticidal properties of the oils under investigation

The insecticidal assays conducted on *P. verticillata* and *T. vulgaris* essential oils revealed significant bioactivity, both concentration- and time-dependent. At the highest tested concentration (10 µl/cm³), *T. vulgaris* essential oil induced a mortality rate of 72.37% after 24 hours, which sharply declined to 7.41% at 48 hours and was negligible by 72 hours. Similarly, *P. verticillata* at (10 µl/cm³) concentration caused 48.32% mortality at 24 hours, decreasing to 3.70% at 48 hours and 0% at 72 hours. Lower concentrations (5 µl/cm³, 2.5 µl/cm³)

corresponded with reduced mortality rates for both oils, consistent with a dose-response relationship commonly observed in essential oil bioassays **(Figure 25)**.

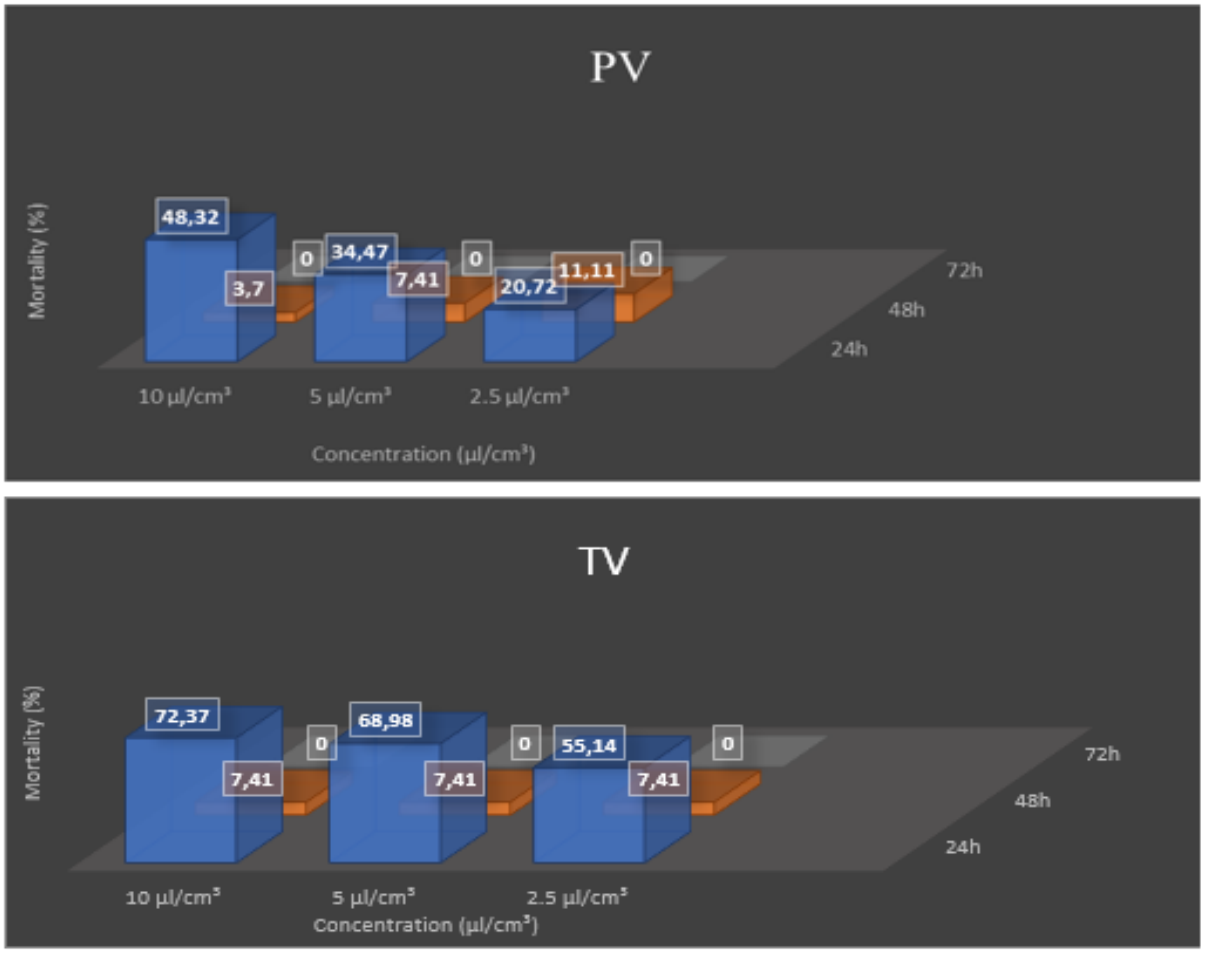


Figure 25. Insecticidal Activity of *P. verticillata* (PV) and *T. vulgaris* (TV) Essential Oils Against Aphis spiraecola at Different Doses and Time Intervals.

These findings align with previous studies demonstrating that essential oils from Thymus species exert potent insecticidal effects primarily through their major phenolic constituents such as thymol and carvacrol. For instance, carvacrol-rich thyme oils have shown substantial fumigant toxicity against stored-grain pests such as Sitophilus oryzae, achieving mortality rates up to 100% at higher concentrations over extended exposure periods, largely due to acetylcholinesterase inhibition, leading to neurotoxicity in insects **(Darrag *et al.*, 2021)**.

Furthermore, the synergistic interaction between thymol and other constituents such as p-cymene enhances the overall insecticidal efficacy, as demonstrated against Musca domestica adults, where p-cymene boosts thymol's toxicity **(Yoon and Tak, 2023)**.

The observed decline in mortality over time in our study may reflect the volatility and rapid degradation of essential oil components under test conditions, a phenomenon reported in other investigations of botanical insecticides **(Gayess *et al.*, 2025)**.

Additionally, the variation in efficacy between *T. vulgaris* and *P. verticillata* could be attributed to differences in their chemical profiles; *P. verticillata* essential oil contains a unique blend of bioactive molecules that may act synergistically or antagonistically, influencing insecticidal potency ***(Mojarab-Mahboubkar et al.,* 2015)**.

The control group exhibited no mortality, confirming that the observed insecticidal effects were attributable to the essential oils. These results support the potential application of *T. vulgaris* and *P. verticillata* essential oils as eco-friendly bio-insecticides.

Further chemical characterization and mechanistic studies are warranted to isolate the active constituents responsible and to optimize formulations for practical pest management.

2.6. Antibacterial Effects and Synergistic Interaction

The present study revealed that *the essential oils of T. vulgaris L. and P. verticillata* exhibit potent antibacterial activity against a variety of pathogenic bacteria.

Notably, the oils were effective against all tested strains, including *E. coli*, *methicillin-sensitive* and *resistant S. aureus* (*MSSA* and *MRSA*), and *E. faecalis*. Among the two oils, *P. verticillata* generally demonstrated greater antibacterial potency, particularly against Gram-positive strains. Furthermore, the combination of *T. vulgaris* and *P. verticillata* oils resulted in significantly larger inhibition zones than either oil alone and even outperformed several standard antibiotics. These results highlight the promising potential of both oils individually and in synergy as effective antimicrobial agents, especially against multidrug-resistant bacteria. To assess this antibacterial activity, the agar diffusion method was employed, and detailed inhibition zone measurements were obtained for each treatment.

The antibacterial activity of *Thymus vulgaris* L. (TV) and *Ptychotis verticillata* Duby (PV) essential oils was evaluated against four pathogenic bacterial strains using the agar diffusion method. The inhibition zones observed for each treatment, including the individual oils, their combination (Cmb), and standard antibiotics, are illustrated in **(Figure 26)**, providing a visual comparison of their efficacy. To support these observations, **(Table XII)** presents the corresponding mean diameters (± standard deviation) of the inhibition zones, offering detailed quantitative data for each bacterial strain and treatment. The size of the inhibition zones indicated antimicrobial potency, with larger zones reflecting greater activity. Both essential oils demonstrated inhibitory effects against all tested strains, with inhibition zones ranging from 27.43 ± 1.56 mm to 46.93 ± 8.71 mm.

Table XII. Mean ± Standard Deviation (mm) of Inhibition Zone Diameters for Various Bacterial Strains Treated with *Thymus vulgaris* L. and *Ptychotis verticillata* Duby Essential

Oils and Standard Antibiotics.

Bacteria	*T. vulgaris* (TV)	*P. verticillata* (PV)	Combination (Cmb)	Antibiotic 1	Antibiotic 2
E.coli	29.83 ±1.44	27.43 ±1.56	42.27 ±1.82	Ciprofloxacin 25.67 ±1.02	Gentamicin 20 ±0.93
SASM	36.97 ±2.02	46.93 ±8.71	46.23 ±2.51	Oxacillin 26.83 ±0.84	Vancomycin 20.83 ±1.56
SARM	41.47 ±2.36	43.3 ±4.47	48.97 ±4.02	Cefoxitin 22.63 ±1.71	Vancomycin 20.63 ±2.42
E.faecalis	32 ±2	44.3 ±3.8	36.13 ±1.18	Ampicillin 22.9 ±1.4	Vancomycin 20.5 ±2

Values are expressed as mean ± SD (n = 3). Statistical significance was determined using Student's t-test; $p < 0.05$ was considered significant.

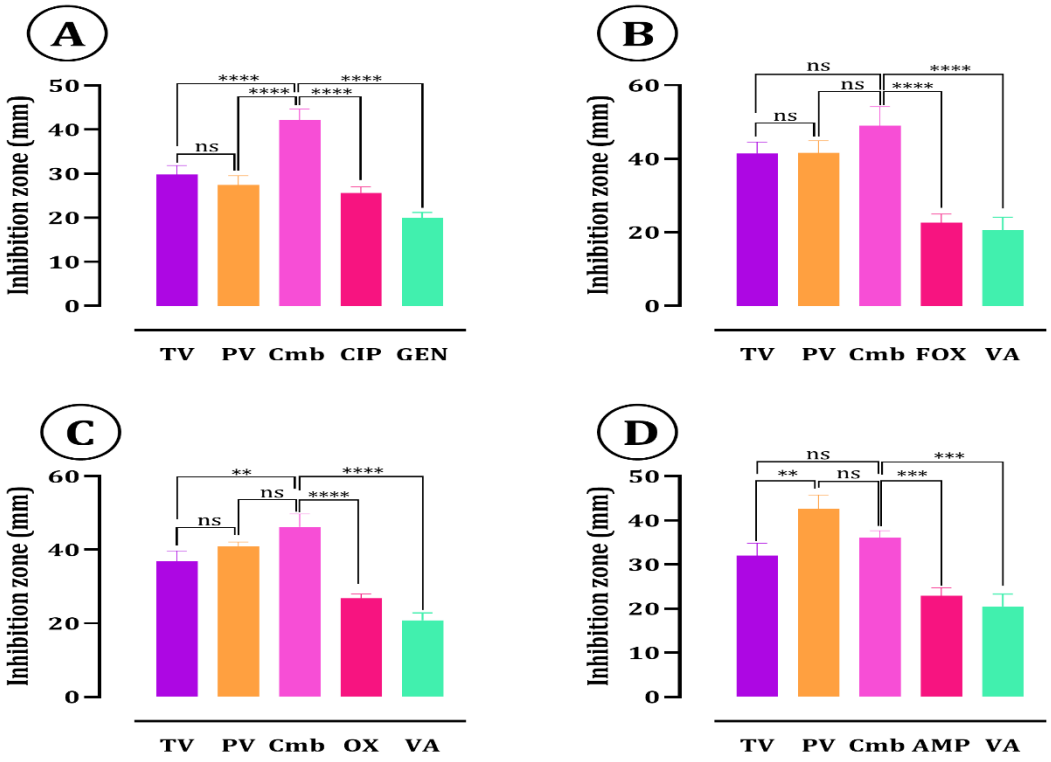


Figure 26. Antibacterial Activity of *Thymus vulgaris L. (TV), Ptychotis verticillata Duby (PV),* and Their Synergy *(Cmb)* with different Antibiotics, Against Different Bacteria: A) *E. coli*, B) *SASM*, C) *SARM*, D) *E.faecalis.*

These results align with previous findings where *T. vulgaris* L. essential oil exhibited inhibition zones up to 42 mm against clinical isolates, confirming its potent antimicrobial activity **(Fani and Kohanteb, 2017)**, The broad-spectrum antibacterial potential observed is likely attributed to their rich content of phenolic compounds, particularly thymol and carvacrol, which are well-documented for their ability to disrupt bacterial membranes and induce cell lysis.

These compounds integrate into the lipid bilayer of bacterial membranes, causing increased permeability, loss of proton motive force, and ultimately cell death **(Raei *et al.*, 2017)**. Their efficacy against various bacterial pathogens, including multidrug-resistant strains, has been extensively reported **(Sateriale *et al.*, 2023)**.

A comparative analysis between Gram-positive and Gram-negative strains revealed that Gram-negative bacteria, such as Escherichia coli, were generally more susceptible to the essential oils. This increased sensitivity may be linked to structural differences in bacterial cell walls; Gram-negative bacteria possess a thinner peptidoglycan layer and outer membrane porins that may facilitate the penetration of active components like thymol and carvacrol **(Taibi *et al.*, 2024)**

However, some studies also report variable susceptibility depending on bacterial species and oil composition **(Sateriale *et al.*, 2023)**. When comparing the individual oils, *P. verticillata* displayed slightly higher antimicrobial activity than *Thymus vulgaris* L., likely due to a higher concentration of active biomolecules and possible synergistic effects among its constituents, which enhance the oil's overall potency **(Taibi *et al.*, 2024)**.

Gas chromatography-mass spectrometry analyses have revealed that *P. verticillata* essential oil contains significant levels of thymol, carvacrol, and other bioactives, which contribute to its antibacterial efficacy **(Bnouham *et al.*, 2012)**.

The combination of both essential oils resulted in significantly enhanced antibacterial activity, with inhibition zones exceeding those of the individual oils. Notably, the combined oils showed greater efficacy than conventional antibiotics, including ciprofloxacin, gentamicin, oxacillin, and vancomycin, against strains including *E. coli, MRSA, MSAA*, and *Enterococcus faecalis*.

This enhanced effect is likely due to synergistic interactions between thymol, carvacrol, and other bioactive compounds, which together target multiple bacterial pathways, disrupt membrane integrity, and inhibit biofilm formation. The antibiofilm activity of thymol and carvacrol, particularly against carbapenemase-producing Gram-negative bacilli, further supports their therapeutic potential in controlling resistant infections **(Rúa *et al.*, 2019)**.

These findings underscore the potential of *T. vulgaris* L. and *P. verticillata* essential oils as natural alternatives or adjuncts to conventional antibiotics, especially in managing infections caused by multidrug-resistant bacteria.

However, interactions between thymol and carvacrol can vary depending on concentration and bacterial strain, with some studies reporting antagonistic effects in certain combinations, highlighting the need for careful optimization **(Rúa *et al.*, 2019)**. Further investigations, including isolation of active compounds and in vivo assessments, are warranted to confirm these results and support their application in pharmaceutical and food safety contexts.

2.7. Nutritional Profile and Potential Phytotherapeutic Applications

The parameters analyzed in the samples provided a comprehensive overview of the nutritional composition of *P. verticillata* and *T. vulgaris.* **(Table XIII)**. These data encompass essential physicochemical characteristics, including pH, moisture, macronutrient content (proteins, fats, carbohydrates), reducing sugars, mineral content (ash), and derived energy values. This detailed profiling provides an in-depth understanding of the plants' nutritional potential and serves as a basis for evaluating their applicability as functional foods or phytotherapeutic agents.

Table XIII. Nutritional Composition of *Ptychotis verticillata* Duby and *Thymus vulgaris* L.

Parameter	**Unit**	***P.verticillata* Duby**	***Thymus vulgaris* L.**
pH (10%, 20°C)	pH units	5.02	5.34
Moisture	%	6.41	7.63
Proteins	%	10.23	13.46
Fat content	%	2.37	1.57
Total carbohydrates	%	71.85	67.20
of which reducing sugars	%	4.20	2.80
Mineral content (ash)	%	8.34	8.87
Energy value	Kcal/100 g	322.65	312.77
Energy value	kJ/100 g	1348.67	1307.37

The measured pH values were slightly acidic, with 5.02 for *P. verticillata* and 5.34 for *T. vulgaris*, consistent with conditions that favor the preservation of bioactive compounds and support the antimicrobial properties commonly attributed to these species **(Taibi *et al.*, 2024)**.

Moisture content was relatively low, at 6.41% and 7.63%, respectively, which is advantageous for the preservation of plant material and inhibits microbial proliferation. These values align with those reported in studies on dried medicinal plants, where low moisture content is critical for maintaining constituent stability **(Banik *et al.*, 2020)**.

Proteins account for a significant portion of the macronutrient composition, measuring 10.23% in *P. verticillata* and 13.46% in *T. vulgaris*, highlighting their considerable nutritional value. Such findings are in accordance with published nutritional profiles of aromatic herbs rich in health-promoting bioactive compounds and essential nutrients **(Series, 2024)**.

Lipid content was minimal (2.37% and 1.57%), which is typical for these types of plants, while carbohydrates were the predominant macronutrient, forming 71.85% and 67.2% of the composition. The reducing sugar fraction was higher in *P. verticillata* (4.2% versus 2.8%), contributing to the plants' energy content and potentially influencing biological activities **(Shahrajabian and Sun, 2023)**.

The mineral fraction ranged between 8.34% and 8.87%, falling within favorable levels for physiological functions. Key minerals such as calcium, iron, potassium, and magnesium reported widely in *T. vulgaris* underpin the nutritional and therapeutic attributes of these plants **(Mofunanya, 2016)**.

Energy values calculated from macronutrient data were comparable, with 322.65 kcal/100 g and 312.77 kcal/100 g, reflecting a moderate caloric contribution characteristic of dried aromatic plants. These results confirm that these species serve not only as traditional remedies but also possess meaningful nutritional potential **(Naeem *et al.*, 2025)**.

Both plants are rich sources of bioactive terpenoids, namely carvacrol in *Ptychotis verticillata* Duby and thymol in *T. vulgaris*, compounds that impart strong antimicrobial, antioxidant, and anti-inflammatory effects, as validated by numerous investigations **(Boutalaka *et al.*, 2025)**. This underscores the significance of combining nutritional and pharmacological characteristics, potentially enhancing therapeutic efficacy through synergistic interactions.

The observed combination of nutritional richness and biomedical properties suggests that a dry mixture of *P. verticillata* and *T. vulgaris* leaves could be effectively used as a phytotherapeutic infusion. Such a formulation may provide complementary nutritional benefits alongside synergistic anti-inflammatory and antimicrobial effects, reinforcing ethnopharmacological traditions and promoting health **(El-Saadony *et al.*, 2025)**.

2.8. Molecular Docking Simulation

2.8.1. Binding site identification

The binding sites of each target were identified with the site finder tool in MOE, and

the results are presented in **Table III** below.

Table XIV. The binding site residues of the selected targets.

Target ID	Site Number	Residues
4PRX	**Site 1**	Chain A: MET25, TYR26, ILE27, GLY28, ASP29 THR34, GLY35, HIS37, HIS38, GLU42, VAL43, ASP45, ASN46, ALA47, ASP49, VAL71, ASP73, ARG76, GLY77, ILE78 PRO79, HIS83, GLU86, ALA90, VAL93, ILE94, VAL97, HIS99, ALA100, GLY101, GLY102, LYS103 SER108, TYR109, LEU115, HIS116, GLY117, VAL11, GLY119, VAL120, THR165, ILE273, PRO274, GLN275, ARG276, GLN335, THR336, LYS337, ASP338.
6E4E	**Site 1**	Chain A: LEU5, VAL6, ALA7, ILE14, GLY15, PHE16, GLU17, ASN18, GLN19, LEU20, PRO21, TRP22, HIS23, LEU24, PRO25, ASP27, LEU28, VAL31, GLY43, ARG44, LYS45, THR46, SER49, ILE50, LEU54, LEU62, THR63, SER64, HIS77, PHE92, GLY93, GLY94, GLN95, THR96, LEU97, PHE98, GLU100, ASP120, THR121.
6EP5	**Site 3**	Chain A: LYS78, GLY79, PHE81, PHE83, TYR111, MET114, ASN115, HIS118, ASN124, GLY125, THR128, ARG129, TYR154, LEU155, MET158, GLU159, PRO162, GLU197.

2.8.2. Evaluation of in silico antibacterial activity

Molecular docking of the six compounds identified from *P. verticillata* (p-cymene, limonene, carvacrol, thymol, γ-terpinene, and α-pinene) and three compounds from *T. vulgaris* (including thymol, γ-terpinene, and p-cymene, which were common to both plants) was carried out against three X-ray crystal structures of the selected targets. The detailed outcomes of docking simulations are presented in (**Tables XIV, XV**, and **XVI**). The molecular interactions of the top-ranked compounds with the active site of selected targets were illustrated using the parameters of the BIOVIA DS Visualizer (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, 2021).

2.8.2.1 Docking of compounds with DNA gyrase from *E. coli* (4PRX)

As described in **(Table XV)**, most of the compounds displayed various forms of non-covalent interactions toward the active sites of enzymes, including hydrogen bonds and

hydrophobic interactions (pi-cation and pi-H). Notably, all the selected compounds exhibit an appreciable binding affinity toward 4PRX, with docking scores ranging between –4.69 and –4.94 kcal/mol. closely resembling that of the native ligand ADP (score energy = -6.25 Kcal/mol).

As can be seen from **(Table XV)**, among the tested ligands, p-cymene achieved the most favorable docking score (–4.94 kcal/mol), forming multiple hydrophobic interactions with residues ILE78, ILE94, and PRO79, including both alkyl and π-alkyl contacts. Similarly, carvacrol (–4.89 kcal/mol) and thymol (–4.79 kcal/mol) engaged in a combination of hydrogen bonding, electrostatic π-cation interactions, and hydrophobic contacts. Notably, carvacrol formed a conventional hydrogen bond with HOH535 (2.75 Å) and a π-cation interaction with LYS103 **(Figure 27)**. In contrast, thymol established a strong hydrogen bond with ASN46 (2.76 Å) and a π-cation contact with LYS103. α-pinene (–4.88 kcal/mol) exhibited several hydrophobic interactions involving ILE94, VAL120, and LYS103, in addition to π–alkyl contact with TYR109, suggesting a well-defined accommodation in the binding pocket. γ-terpinene (–4.78 kcal/mol) showed weaker binding but maintained consistent hydrophobic interactions with ILE78 and ILE94 **(Figure 28)**. Limonene (–4.69 kcal/mol), while displaying the least favorable score, still interacted with key residues such as ILE94, ILE78, VAL120, and LYS103 through alkyl contacts **(Figure 29)**. In contrast, the native ligand ADP demonstrated the strongest binding affinity (–6.25 kcal/mol), forming an extensive hydrogen-bonding network with ASN46, GLY102, LYS103, VAL120, THR165, ASP73, and TYR109, alongside π–alkyl interactions with ILE78. Notably, several tested compounds shared common interactions with the native ligand (ADP), thereby reinforcing their potential inhibitory effects. Specifically, p-cymene, limonene, and γ-terpinene all displayed hydrophobic interactions with ILE78, which was also observed in the ADP complex. Similarly, limonene and α-pinene shared interactions with VAL120, while carvacrol, thymol, and α-pinene exhibited overlapping contacts with LYS103 and TYR109, both of which are key residues engaged by the native ligand. Interestingly, our findings show that the studied compounds bind to the same active site residues as ADP, specifically ASN46, PRO79, ILE94, LYS103, VAL120, and TYR109 **(Fang *et al.*, 2016)**. This overlapping binding highlights a mechanism of competitive inhibition, where the compounds mimic ADP to occupy the active site, thereby preventing the enzyme from completing its normal catalytic cycle.

Table XV. Docking score and interactions between the compounds and native ligands with active site residues of antibacterial target *E. coli*.

E. coli (PDB ID: 4PRX)							
Compounds	**S-score (kcal/mol)**	**Bonds between atoms of compounds and the active site residues of 4prx**					
		Atom of a compound	**Involved receptor atoms**	**Involved receptor residues**	**Categories**	**Type of interaction bond**	**bond Distance (Å)**
p-cymene	-4.94	C1	/	PRO79	Hydrophobic	Alkyl	5.09
		C1	/	ILE94	Hydrophobic	Alkyl	4.52
		C10	/	ILE78	Hydrophobic	Alkyl	5.22
		/	/	ILE78	Hydrophobic	Pi-Alkyl	4.59
Limonene	-4.69	/	/	ILE94	Hydrophobic	Alkyl	4.84
		C8	/	ALA100	Hydrophobic	Alkyl	4.30
		/	/	LYS103	Hydrophobic	Alkyl	5.35
		C8	/	ILE94	Hydrophobic	Alkyl	4.01
		C9	/	ILE78	Hydrophobic	Alkyl	4.67
		C10	/,	ILE94	Hydrophobic	Alkyl	4.89
		C10	/	VAL120	Hydrophobic	Alkyl	3.99
Carvacrol	-4.89	H 1	O	HOH535	Hydrogen Bond	Conventional H-Bond	2.75
		/	NZ	LYS103:	Electrostatic	Pi-Cation	4.59
		/	C	TYR109	Hydrophobic	Pi-Alkyl	5.33
		/	/	ILE94	Hydrophobic	Pi-Alkyl	5.06
		/	/	LYS103	Hydrophobic	Pi-Alkyl	5.29
Thymol	-4.79	O1	HD22	ASN46	Hydrogen Bond	Conventional H-Bond	2.76
		/	NZ	LYS103	Electrostatic	Pi-Cation	4.48
		C10		PRO79	Hydrophobic	Alkyl	4.89
		/	/	ILE94	Hydrophobic	Pi-Alkyl	4.90
		/	/	LYS103	Hydrophobic	Pi-Alkyl	5.48
γ-terpinene	-4.78	/	/	ILE78	Hydrophobic	Alkyl	4.63
		C1	/	ILE94	Hydrophobic	Alkyl	4.29
		C10	/	ILE78	Hydrophobic	Alkyl	4.96
α-pinene	-4.88	/	/	LYS103	Hydrophobic	Alkyl	5.34
		/	/	ILE94	Hydrophobic	Alkyl	4.38
		C7	/	ILE94	Hydrophobic	Alkyl	4.91
		C7	/	VAL120	Hydrophobic	Alkyl	4.18
		C8	/	VAL120	Hydrophobic	Alkyl	4.88
		C10	/	LYS103	Hydrophobic	Alkyl	4.31
		C10	/	TYR109	Hydrophobic	Pi-Alkyl	5.21

Native ligand (ADP)	-6.25	O2A	HD22	ASN46	Hydrogen Bond	Conventional H-Bond	2.11
		O3'	H	GLY102	Hydrogen Bond	Conventional H-Bond	2.84
		O3B	HZ1	LYS103	Hydrogen Bond	Conventional H-Bond	2.34
		O2A	H	VAL120	Hydrogen Bond	Conventional H-Bond	2.01
		N1	HG1	THR165	Hydrogen Bond	Conventional H-Bond	3.06
		H61	OD2	ASP73	Hydrogen Bond	Conventional H-Bond	2.17
		H1'	OH	TYR109	Hydrogen Bond	Carbon H-Bond	2.57
		/	/	ILE78	Hydrophobic	Pi-Alkyl	4.72
		/	/	ILE78	Hydrophobic	Pi-Alkyl	4.72
		H1B	O	HOH535	Hydrogen Bond	Conventional H-Bond	2.56

2.8.2.2. Docking of compounds with dihydrofolate reductase from *S.aureus* (6E4E)

The results obtained after the docking calculations for the selected compounds with the binding pocket of the *S. aureus* target are shown in **(Table XVI).** The docking interactions of the selected compounds with dihydrofolate reductase (*S. aureus*, PDB: 6E4E) revealed binding affinities ranging from –4.87 to –5.95 kcal/mol, compared with the native ligand MMV (–7.35 kcal/mol). Several compounds shared key amino acid interactions with the native ligand, supporting their potential inhibitory effect.

Carvacrol exhibited the strongest binding (–5.95 kcal/mol), forming hydrogen bonds with ASP27 and water-mediated interactions, in addition to hydrophobic and π-stacked contacts with PHE92, LEU20, LEU5, ALA7, and VAL31 **(Figure 29)**. Importantly, ASP27 and PHE92, which directly interact with MMV, are critical residues involved in the enzyme's catalytic function. As seen in Figure 4 and Table 4, thymol (–5.00 kcal/mol) also interacted with PHE92 through both hydrogen bonding and π- alkyl interactions, while also binding to ILE50 and LEU20, residues also contacted by the native ligand MMV. Limonene and α-pinene displayed moderate binding (–5.16 and –5.19 kcal/mol, respectively), primarily via hydrophobic

interactions with ALA7, LEU20, ILE14, and PHE98, of which LEU20 and ILE14 overlap with MMV contacts. On the other hand, p-Cymene (–4.87 kcal/mol) engaged hydrophobic residues ALA7, LEU20, LEU5, and VAL31 **(Figure 30)**, several of which are also involved in native ligand stabilization.

According to literature reports **(Dale *et al.*, 1997)**, key residues such as ASP27, PHE92, LEU20, LEU5, ILE50, and ALA7 play critical roles in ligand recognition and the catalytic function of DHFR. The overlap between the compound–residue interactions observed in this study and those reported for MMV suggests that these natural compounds may act as competitive inhibitors by binding within the same catalytic pocket.

Table XVI. Docking score and interactions between selected compounds and native ligands with active site residues of antibacterial target *S. aureus.*

		***S. aureus* (PDB ID:6E4E)**					
Compounds	**S-score (kcal/mol)**	**Bonds between atoms of compounds and the active site residues of**					
		Atom of a compound	**Involved receptor atoms**	**Involved receptor residues**	**Categories**	**Type of interaction bond**	**bond Distance (Å)**
p-cymen	-4.87	C10	/	ALA7	Hydrophobic	Alkyl	3.83
		C1	/	LEU20	Hydrophobic	Alkyl	5.21
		C10	/	LEU5	Hydrophobic	Alkyl	4.54
		C10	/	VAL31	Hydrophobic	Alkyl	4.52
		/	/	ALA7	Hydrophobic	Pi-Alkyl	4.57
		/	/	VAL31	Hydrophobic	Pi-Alkyl	5.25
Limonene	-5.16	C9		ALA7	Hydrophobic	Alkyl	4.22
		/	/	ILE14	Hydrophobic	Alkyl	5.40
		/	/	LEU20	Hydrophobic	Alkyl	5.14
		C9	/	ILE14	Hydrophobic	Alkyl	4.57
		C9	/	LEU20	Hydrophobic	Alkyl	5.20
		C10	/	PHE98	Hydrophobic	Pi-Alkyl	4.8
Carvacrol	-5.95	O	H1	HOH307	Hydrogen Bond	Conventional H-Bond	2.46
		H14	OD2	ASP27	Hydrogen Bond	Conventional H-Bond	2.15
		/	/	PHE92	Hydrophobic	Pi-Pi Stacked	4.97
		C10	/	ALA7	Hydrophobic	Alkyl	3.73

		C1	/	LEU20	Hydrophobic	Alkyl	4.90
		C10	/	LEU5	Hydrophobic	Alkyl	4.58
		C10	/	VAL31	Hydrophobic	Alkyl	4.40
		/	/	ALA7	Hydrophobic	Pi-Alkyl	4.17
Thymol	-5.00	H14	O	PHE92	Hydrogen Bond	Conventional H-Bond	2.52
		C10	/	ILE50	Hydrophobic	Alkyl	4.83
		C10	/	PHE92	Hydrophobic	Pi-Alkyl	4.51
		/	/	LEU20	Hydrophobic	Pi-Alkyl	4.70
γ-terpinene	-5.00	/	/	LEU20	Hydrophobic	Alkyl	4.89
		C1	/	ILE14	Hydrophobic	Alkyl	4.27
		C1	/	PHE98	Hydrophobic	Pi-Alkyl	5.42
α-pinene	-5.199	C7	/	ILE14	Hydrophobic	Alkyl	3.87
Native ligand (MMV)	-7.35	O26	H1	HOH385	Hydrogen Bond	Conventional H-Bond	2.59
		H71	O	LEU5	Hydrogen Bond	Conventional H-Bond	1.77
		H72	O	PHE92	Hydrogen Bond	Conventional H-Bond	2.26
		H82	OD1	ASP27	Hydrogen Bond	Conventional H-Bond	1.98
		/	/	PHE92	Hydrophobic	Pi-Pi Stacked	5.58
		C9	/	LEU20	Hydrophobic	Alkyl	4.29
		/	/	ILE50	Hydrophobic	Alkyl	5.34
		/	/	PHE92	Hydrophobic	Pi-Alkyl	4.12
		/	/	LEU5	Hydrophobic	Pi-Alkyl	5.23
		/	/	ALA7	Hydrophobic	Pi-Alkyl	4.12
		/	/	ILE50	Hydrophobic	Pi-Alkyl	5.29

2.8.2.3. Docking of compounds with *E. faecalis* FIC protein (6EP5)

As shown in **(Table XVII)**, the molecular docking analysis of the selected compounds with the active site of E. faecalis DHFR (PDB ID: 6EP5) demonstrated favorable binding affinities, with S-scores ranging from –4.29 to –5.86 kcal/mol, compared to the native ligand ADP (–7.20 kcal/mol). Among the tested compounds, p-cymene (–5.86 kcal/mol) and limonene (–5.35 kcal/mol) exhibited the strongest binding scores, supported by multiple hydrophobic and π-alkyl interactions with residues LYS78, PHE83, MET158, and TYR154 **(Figure 29)**. Notably, carvacrol and thymol established strong hydrogen bonds with critical binding site

residues (GLU197 and ASN115, respectively) **(Figures 30 and 31)**, which are important for ligand interaction and enzyme regulation. γ-terpinene and α-pinene also demonstrated consistent hydrophobic interactions with LYS78, MET158, PHE81, and PHE83 **(Figure 32)**, residues that are also engaged by ADP. These residues are also implicated in ADP binding, suggesting overlapping or adjacent binding sites that contribute to ligand interactions with the enzyme.

Table XVII. Docking score and interactions between selected compounds and native ligands with active site residues of antibacterial target *Enterococcus faecalis*.

		***E. faecalis* (PDB ID:6EP5)**					
Compounds	**S-score (kcal/mol)**	**Bonds between atoms of compounds and the active site residues of**					
		Atom of a compound	**Involved receptor atoms**	**Involved receptor residues**	**Categories**	**Type of interaction bond**	**bond Distance (Å)**
p-cymen	-5.86	/	ND1	HIS118	Electrostatic	Pi-Cation	4.91
		/	/	HIS118	Hydrophobic	Pi-Pi T-shaped	5.01
		C1	/	LYS78	Hydrophobic	Alkyl	4.41
		C1	/	PHE83	Hydrophobic	Pi-Alkyl	4.83
		/	/	LYS78	Hydrophobic	Pi-Alkyl	5.25
		/	/	MET158	Hydrophobic	Pi-Alkyl	5.17
Limonene	-5.35	/	/	MET158	Hydrophobic	Alkyl	4.73
		C8	/	LEU155	Hydrophobic	Alkyl	5.21
		C8	/	MET158	Hydrophobic	Alkyl	4.9
		C9	/	PRO162	Hydrophobic	Alkyl	4.86
		C10	/	LYS78	Hydrophobic	Alkyl	4.21
		C9	/	PHE81	Hydrophobic	Pi-Alkyl	4.59
		C9	/	PHE83	Hydrophobic	Pi-Alkyl	5.01
		C10	/	PHE83	Hydrophobic	Pi-Alkyl	5.47
		C8	/	TYR154	Hydrophobic	Pi-Alkyl	5.09
Carvacrol	-5.02	H14	OE2	GLU197	Hydrogen Bond	Conventional H-Bond	2.52
		C1	/	MET158	Hydrophobic	Alkyl	5.11
		/	/	LYS78	Hydrophobic	Pi-Alkyl	5.11
		/	/	MET158	Hydrophobic	Pi-Alkyl	5.43
Thymol	-4.94	H14	OD1	ASN115	Hydrogen Bond	Conventional	1.99

						H-Bond	
		C10	/	LEU155	Hydrophobic	Alkyl	5.27
		C10	/	TYR154	Hydrophobic	Pi-Alkyl	5.21
		/	/	MET158	Hydrophobic	Pi-Alkyl	4.67
γ-terpinene	-5.35	/	/	LYS78	Hydrophobic	Alkyl	4.93
		/	/	MET158	Hydrophobic	Alkyl	5.09
		C1	/	LYS78	Hydrophobic	Alkyl	4.52
		C10	/	LEU155	Hydrophobic	Alkyl	4.93
		C10	/	MET158	Hydrophobic	Alkyl	4.27
		C1	/	PHE83	Hydrophobic	Pi-Alkyl	4.78
		C10	/	TYR154	Hydrophobic	Pi-Alkyl	4.70
α-pinene	-4.29	/	/	LYS78	Hydrophobic	Alkyl	4.25
		/	/	MET158	Hydrophobic	Alkyl	4.90
		C8	/	LYS78	Hydrophobic	Alkyl	4.02
		C10	/	LYS78	Hydrophobic	Alkyl	3.88
		C7	/	PHE81	Hydrophobic	Pi-Alkyl	5.16
		C7	/	PHE83	Hydrophobic	Pi-Alkyl	5.16
		C8	/	PHE83	Hydrophobic	Pi-Alkyl	5.31
		C10	/	HIS118	Hydrophobic	Pi-Alkyl	4.72
Native ligand (ADP)	-7.202	N3	HD21	ASN115	Hydrogen Bond	Conventional H-Bond	2.04
		O5'	HE2	HIS118	Hydrogen Bond	Conventional H-Bond	2.42
		H	O1A	ASN124	Hydrogen Bond	Conventional H-Bond	2.49
		H	O3A	ASN124	Hydrogen Bond	Conventional H-Bond	2.83
		H	O3A	GLY125	Hydrogen Bond	Conventional	1.92

					H-Bond	
	H2'	OH	TYR154	Hydrogen Bond	Conventional H-Bond	2.15
	H61	O	LYS78	Hydrogen Bond	Conventional H-Bond	2.65
	O4'	HE1	HIS118	Hydrogen Bond	Carbon Hydrogen Bond	2.23
	H1'	OD1	ASN115	Hydrogen Bond	Carbon Hydrogen Bond	2.36
	/	HG3	LYS78	Hydrophobic	Pi-Sigma	2.91
	/	/	LYS78	Hydrophobic	Pi-Alkyl	4.72

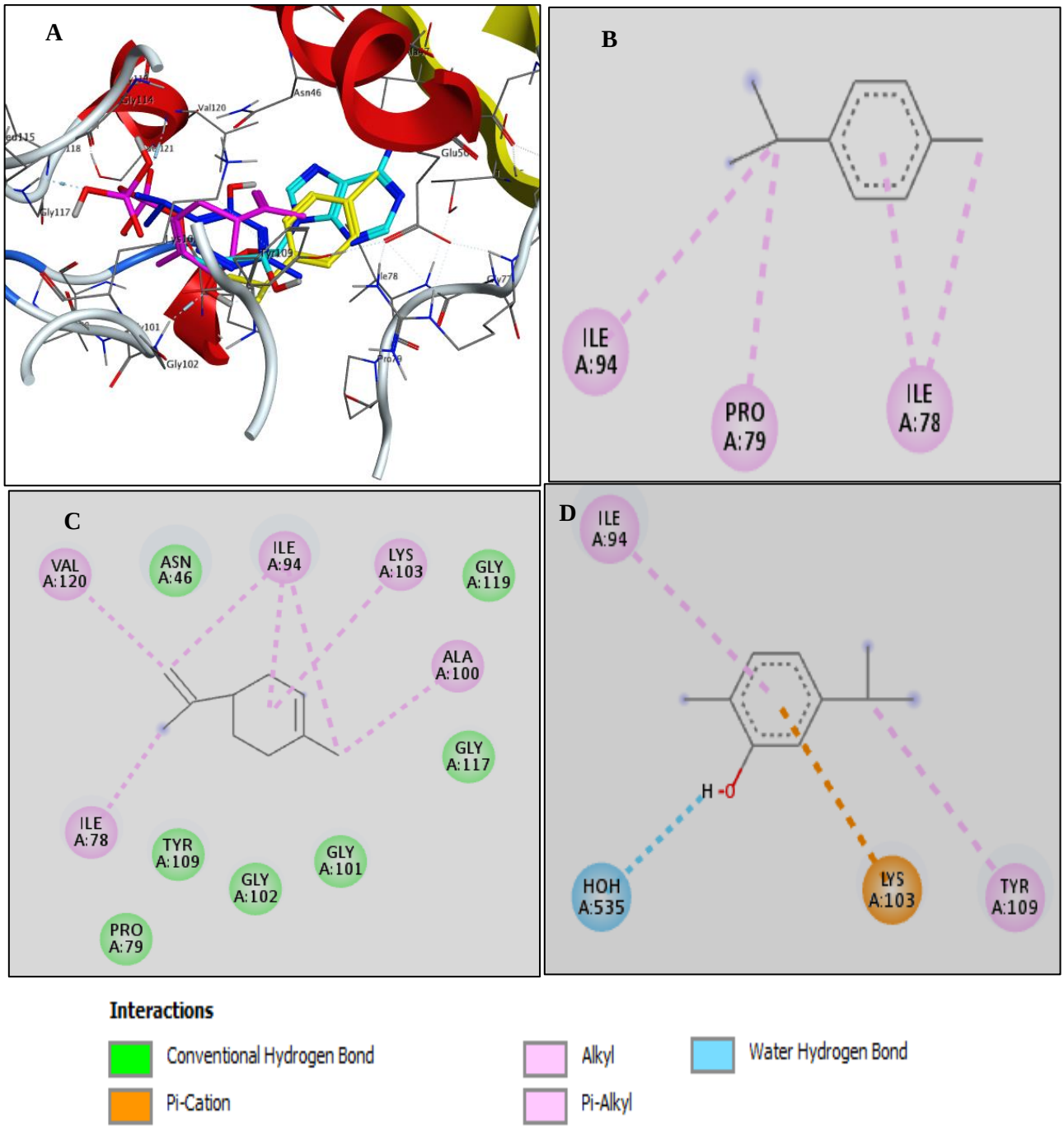


Figure 27. Docking of compounds against *E. coli* (4PRX) : ADP, p-cymene, limonene, carvacrol (3D & 2D interactions).

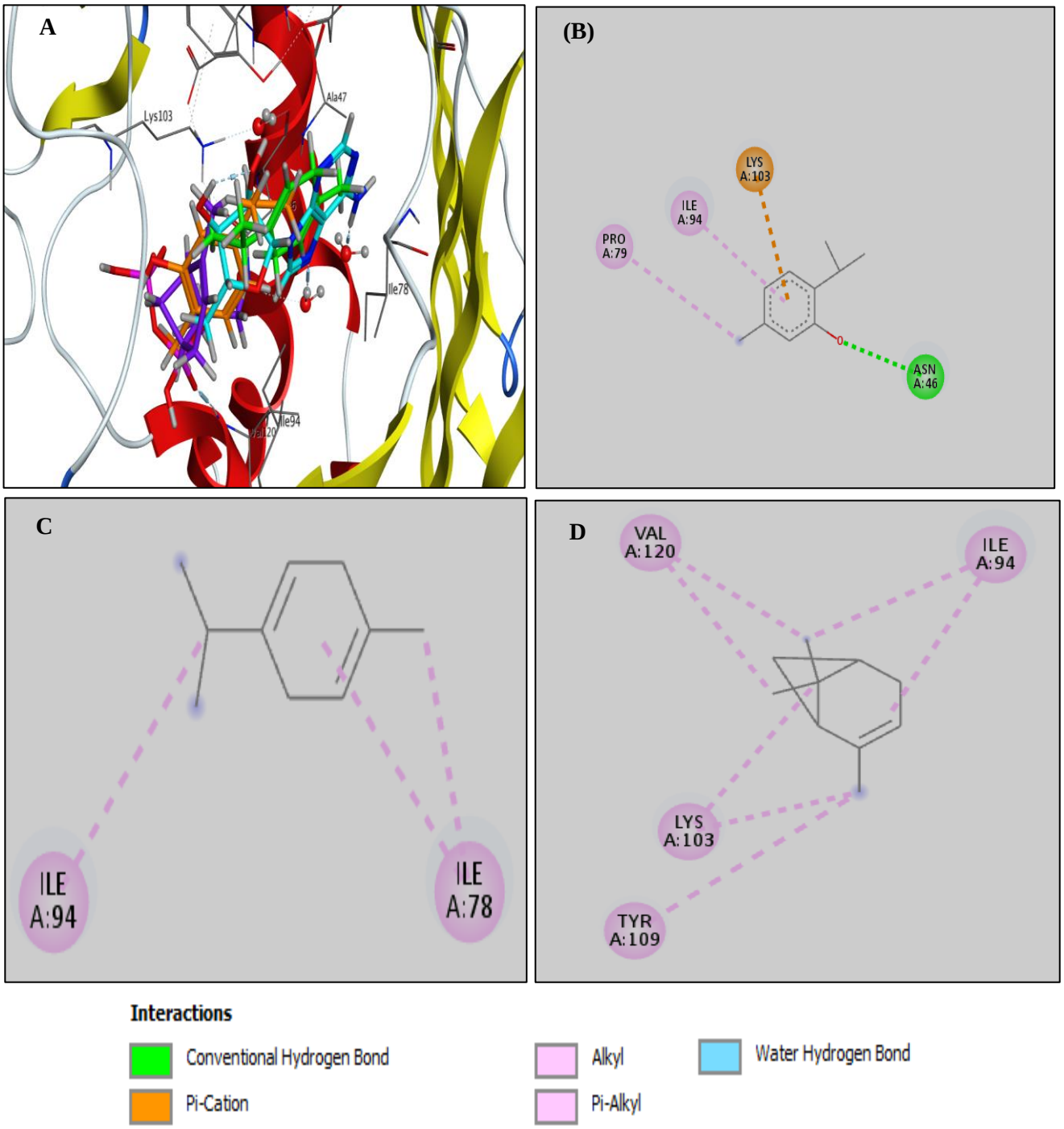


Figure 28. Docking of compounds against *E. coli* (4PRX): ADP, thymol, γ-terpinene, α-pinene (3D & 2D interactions).

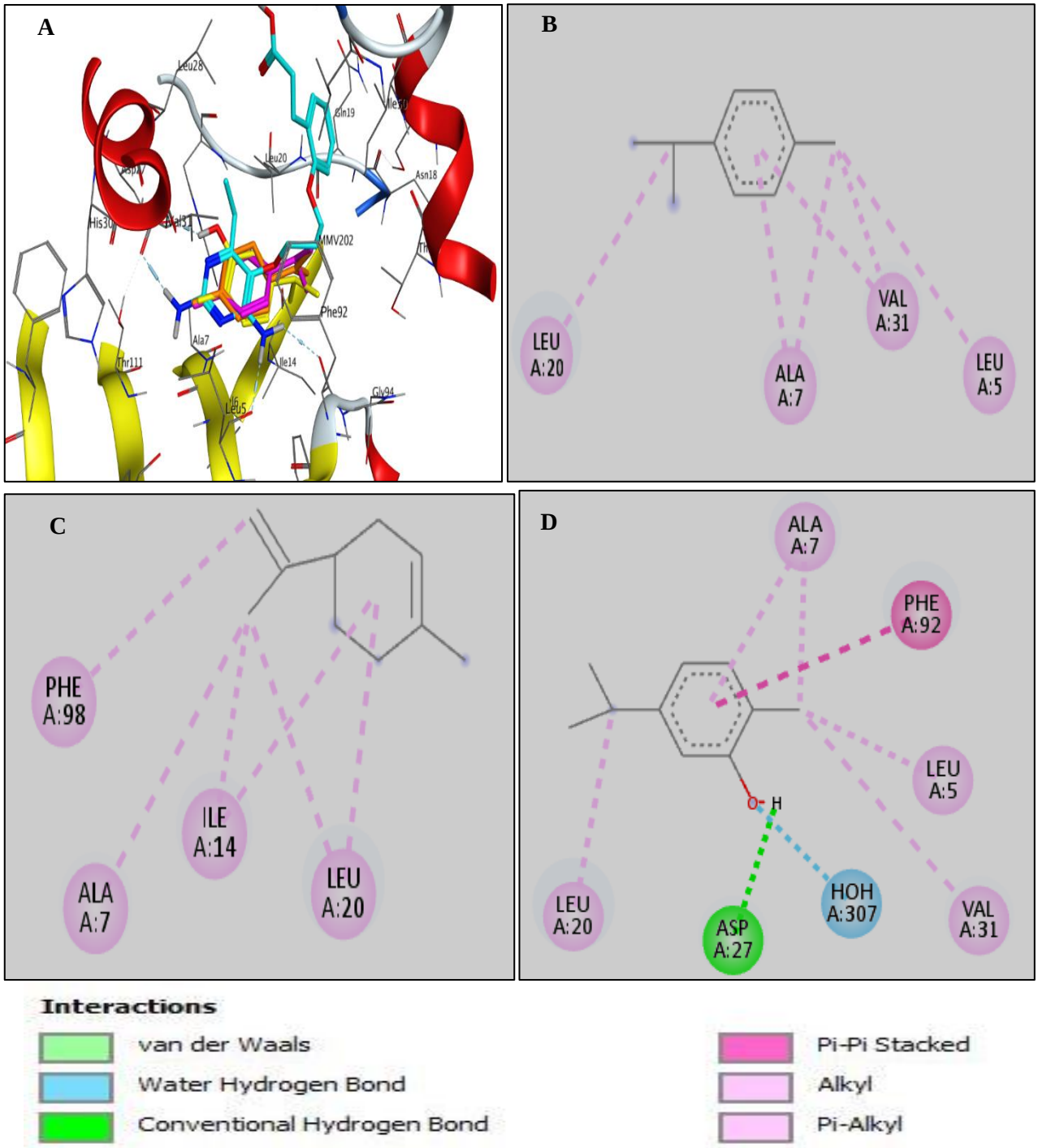


Figure 29. Docking of compounds against *S. aureus* (6E4E): MMV, p-cymene, limonene, carvacrol (3D & 2D interactions).

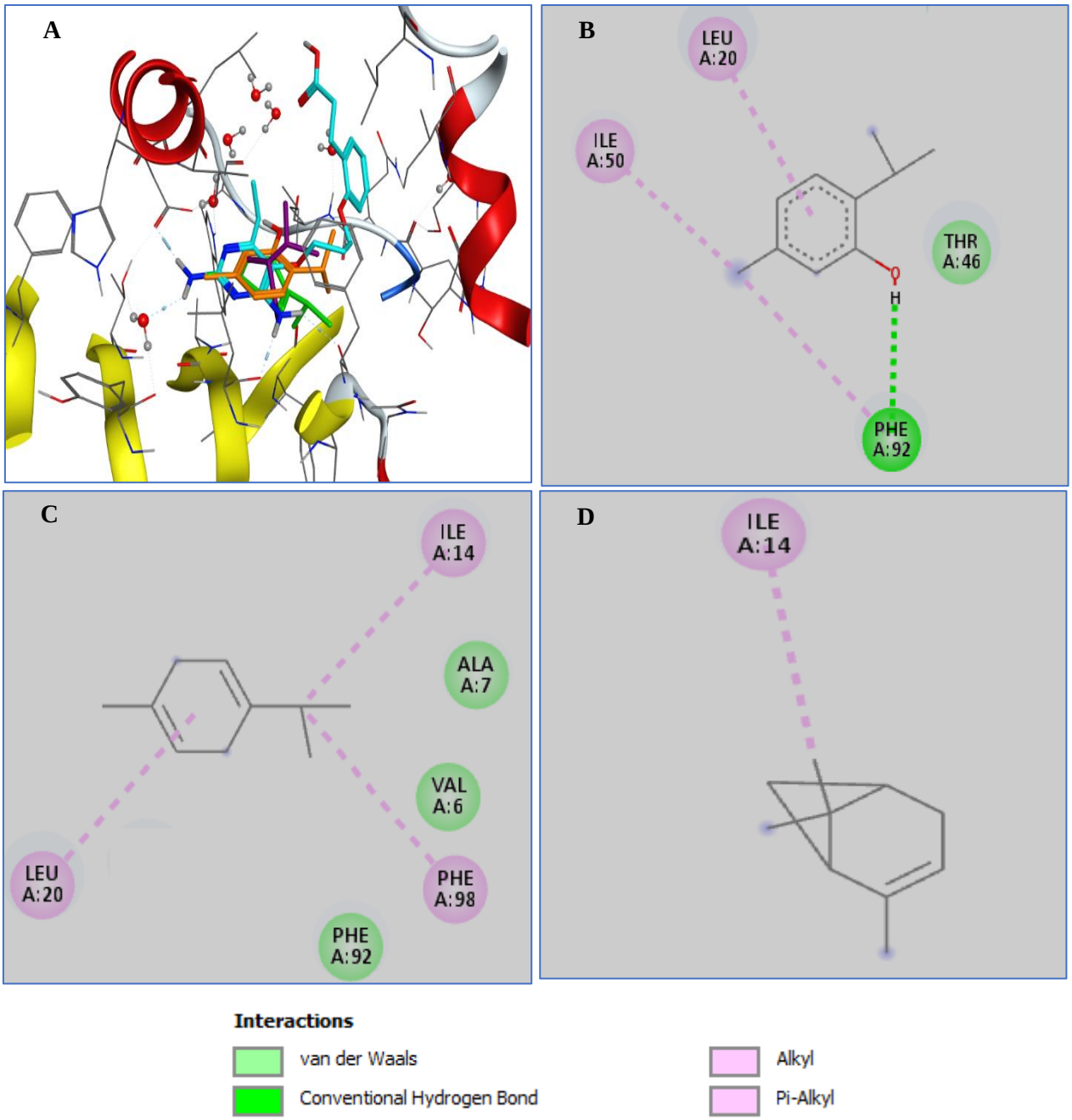


Figure 30. Docking of compounds against *S. aureus* (6E4E): MMV, thymol, γ-terpinene, α-pinene (3D & 2D interactions).

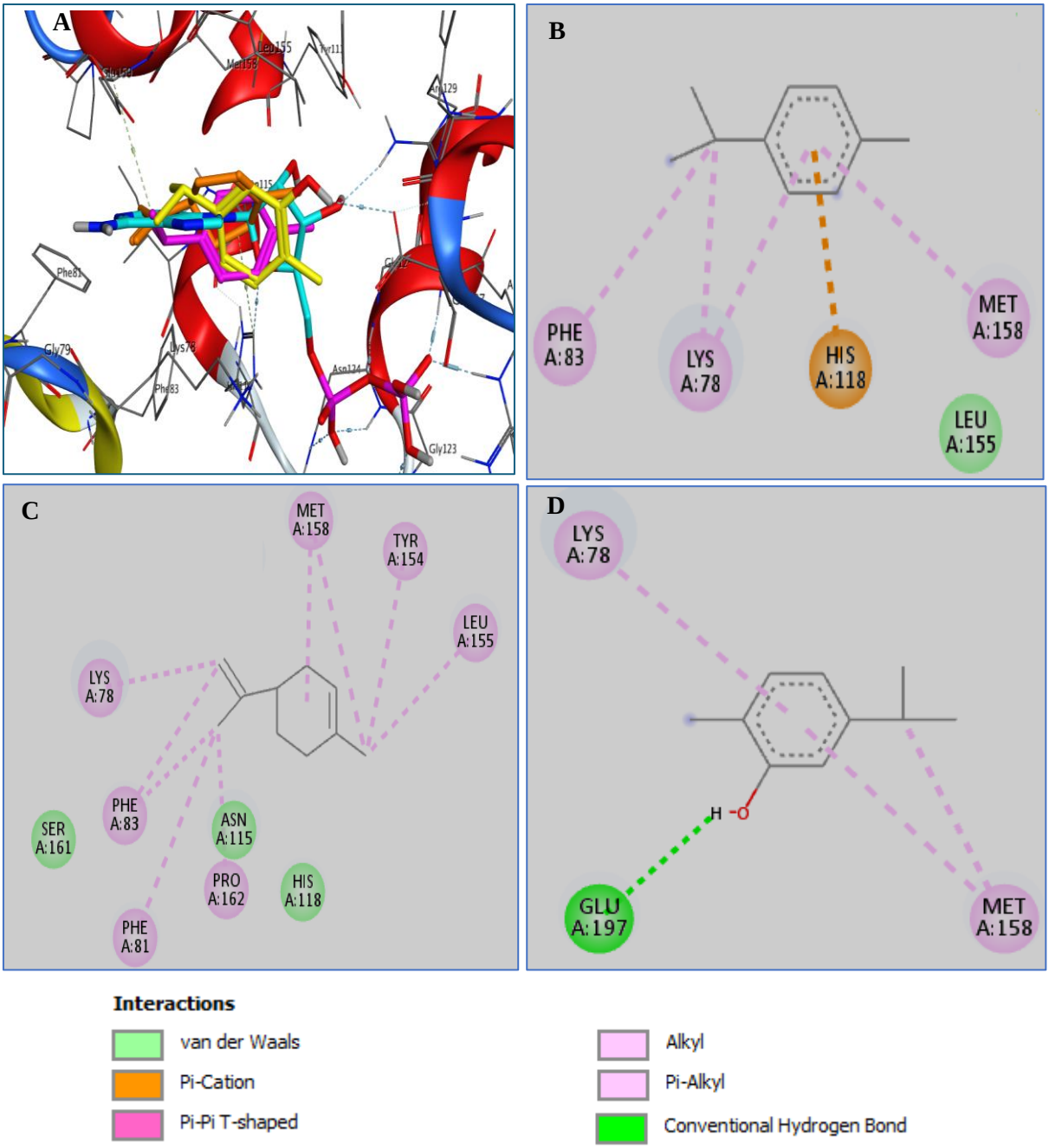


Figure 31. Docking of compounds against *E. faecalis* (6EP5): ADP, p-cymene, limonene, carvacrol (3D & 2D interactions).

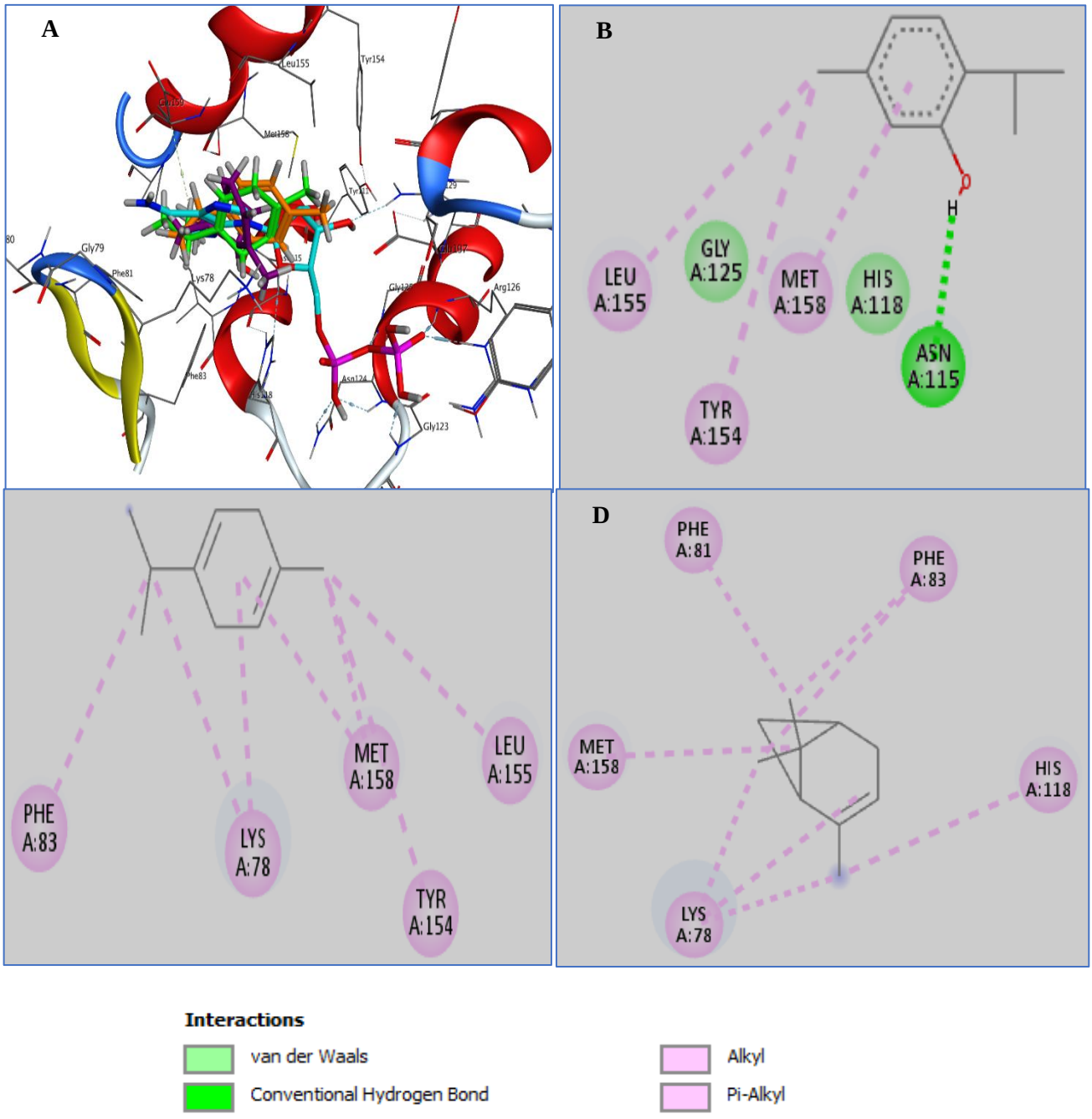


Figure 32. Docking of compounds against *E. faecalis* (6EP5): ADP, thymol, γ-terpinene, α-pinene (3D & 2D interactions).

2.8.3. ADME-T and Drug-Likeness prediction

The pharmacokinetic and drug likeness properties of the selected compounds were assessed using ADME-Tlab predictions **(Table XVIII)**. Among the compounds, carvacrol and thymol showed high predicted gastrointestinal absorption (GI-ABS), suggesting good oral bioavailability. In contrast, p-cymene, limonene, γ-terpinene, and α-pinene demonstrated low predicted GI absorption. In addition, all compounds possess the features to cross the blood-

brain barrier (BBB). None of the compounds is predicted to be P-glycoprotein substrates (P-gp). This is a favorable result, as it means the compounds are unlikely to be actively pumped out of cells, which could enhance their bioavailability.

Regarding Cytochrome P450 (CYP) Inhibition, all compounds act as non-inhibitors of CYP1A2, except for carvacrol and thymol were predicted to inhibit it. Limonene was predicted to inhibit CYP2C19 and CYP2C9, whereas α-pinene inhibited CYP2C9, and p-cymene inhibited CYP2D6. These results suggest potential metabolic interactions, particularly for limonene, which shows multi-isoenzyme inhibition. In contrast, γ-terpinene did not inhibit any CYP isoform, suggesting a lower possibility of metabolic interactions.

The drug-likeness properties were calculated based on the following parameters: hydrogen bond donors <7 (n-HD: (0~7)), hydrogen bond acceptors <12 (n-HA: (0~12)). Molecular weight belongs to the interval (MW):100~500 g/mol, MLogP and WLogP <5 and nROTB <11.

As shown in 6, all compound Regarding Cytochrome P450 (CYP) Inhibition, all compounds act as non-inhibitors of CYP1A2, except carvacrol and thymol were predicted to inhibit it. Limonene was predicted to inhibit CYP2C19 and CYP2C9, whereas α-pinene inhibited CYP2C9, and p-cymene inhibited CYP2D6. These results suggest potential metabolic interactions, particularly for limonene, which shows multi-isoenzyme inhibition. In contrast, γ-terpinene did not inhibit any CYP isoform, suggesting a lower likelihood of metabolic interactions.

The drug-likeness properties were calculated based on the following parameters: hydrogen bond donors <7 (n-HD: (0~7)), hydrogen bond acceptors <12 (n-HA: (0~12)). Molecular weight belongs to the interval (MW):100~500 g/mol, MLogP and WLogP <5 and nROTB <11.

It can be observed from **(Table XVII)**, all the compounds respect the physicochemical properties for drug likeness according to the Lipinski, Veber, and Egan rules. All compounds exhibited very low Topological Polar Surface Area (TPSA) values (0–20.23 $Å^2$), well below the threshold of 140 $Å^2$, indicating good membrane permeability and oral absorption potential. On the other hand, their molecular weights (MW) were also in the ideal drug-like range (100–500 g/mol). MLogP and WLogP values were between 2.76–4.47 and 2.82–3.31, respectively, falling within the acceptable range (0–5), suggesting balanced hydrophilicity and lipophilicity. Moreover, all compounds had minimal hydrogen bonding (0–1), further supporting membrane permeability. Furthermore, the very low number of rotatable bonds n-ROT (0–1), indicates limited molecular flexibility, which generally enhances oral bioavailability.

Table XVIII. Pharmacokinetics and Druglikeness predictions.

Compound	Pharmacokinetics								
	GI-ABS	**BBB**	**P-gp substrate**	**CYP1A2 inhibitor**	**CYP2C19 Inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	
p-cymene	Low	Yes	No	No	No	No	Yes	No	
Limonene	Low	Yes	No	No	Yes	Yes	No	No	
carvacrol	High	Yes	No	Yes	No	No	No	No	
thymol	High	Yes	No	Yes	No	No	No	No	
γ-terpinene	Low	Yes	No	No	No	No	No	No	
α-pinene	Low	Yes	No	No	No	Yes	No	No	
Compound	**Physicochemical properties**						**Drug Likeliness**		
	TPSA (Å2)	**n-ROT**	**MW (g/mol)**	**MLog P** / WLog P	**n-HA**	**n-HD**	**Lipinski**	**Veber**	**Egan**
	(0~140)	(0~10)	(100~500)	(0~5)	(0~12)	(0~7)			
p-cymene	0.00	1	134.22	4.47 / 3.12	0	0	Yes	Yes	Yes
Limonene	0.00	1	136.23	3.27 / 3.31	0	0	Yes	Yes	Yes
carvacrol	20.23	1	150.22	2.76 / 2.82	1	1	Yes	Yes	Yes
thymol	20.23	1	150.22	2.76 / 2.82	1	1	Yes	Yes	Yes
γ-terpinene	0.00	1	136.23	3.27 / 3.31	0	0	Yes	Yes	Yes
α-pinene	0.00	0	136.23	4.29 / 3.00	0	0	Yes	Yes	Yes

TPSA: Topological Polar Surface Area, n-ROT: Number of Rotatable, MW: Molecular Weight, Log P: Logarithm of partition coefficient of compound between n-octanol and water, n-HA: Number of hydrogen bond acceptors, n-HD: Number of hydrogen bond donors

The toxicity of the selected compounds was evaluated by ADMETLab, and the results obtained are listed in **(Table XIX)**.

As presented in **(Table XIX)**, various toxicity parameters were evaluated for the tested compounds. The AMES mutagenicity test was used to assess mutagenicity potential, as it correlates with carcinogenicity. Rat Oral Acute Toxicity was used for the preliminary in vivo safety evaluation of the drug candidates. Meanwhile, human hepatotoxicity was used for the prediction of liver toxicity. Skin sensitisation was used to predict the potential adverse effect for dermally applied compounds. The scoring system for these parameters is as follows: 0 to 0.3 is excellent, 0.3 to 0.7 is medium, and 0.7 to 1.0 is poor.

The Ames mutagenicity test indicated that all compounds fell within the excellent to medium range (0.19–0.39), suggesting they have a low risk of causing genetic mutations.

Regarding rat oral acute toxicity (LD50), p-cymene, limonene, and thymol fell within the excellent range and are predicted to be the least toxic. Carvacrol, α-pinene, and γ-terpinene all fell into the medium-risk category. Human hepatotoxicity predictions were moderate for all compounds (0.35–0.49), suggesting a low probability of hepatotoxicity.

In contrast, skin sensitization scores revealed the most notable concern. Limonene (0.89) and α-pinene (0.79) were predicted to have high probability of causing skin sensitization. Carvacrol and thymol also showed relatively high values (0.71 and 0.64, respectively), while p-cymene and γ-terpinene demonstrated moderate sensitization potential.

Table XIX. Toxicity prediction.

Properties	**Reference [13]**	**Compounds**					
		p-cymene	**limonene**	**carvacrol**	**thymol**	**γ-terpinene**	**α-pinene**
Toxicity							
AMES mutagenicity	0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor	0.23	0.35	0.39	0.30	0.19	0.26
Rat Oral Acute Toxicity (LD50) (mol/kg)		0.25	0.16	0.417	0.30	0.36	0.32
Human Hepatotoxicity		0.49	0.48	0.48	0.45	0.35	0.48
Skin Sensitisation		0.55	0.89	0.71	0.64	0.56	0.79

CONCLUSION & PERSPECTIVES

This thesis has scientifically confirmed the therapeutic potential of the essential oils of *Ptychotis verticillata* Duby and *Thymus vulgaris* L., traditionally used in the Relizane region. The ethnobotanical study conducted among herbalists highlighted the vital place of these plants in the local pharmacopeia, emphasizing the richness of traditional knowledge that guided this research. Phytochemical analysis revealed a wealth of major bioactive compounds, including thymol and carvacrol, known for their pharmacological properties.

Biological evaluations demonstrated that these EOs possess significant antioxidant activity, making them promising candidates for combating oxidative stress in various diseases. Their anti-inflammatory effect was demonstrated through mechanisms that inhibit cellular inflammatory processes, thereby validating their use in the treatment of inflammatory conditions.

The observed insecticidal efficacy opens the door to more ecologically friendly agricultural applications. At the same time, the antimicrobial activity, enhanced by synergy between the two oils, underscores their potential to combat bacterial infections, including those resistant to conventional antibiotics.

Molecular docking studies reveal the molecular interactions underlying these biological effects, confirming the relevance of the identified compounds as competitive enzyme inhibitors. Additionally, pharmacokinetic and toxicological predictions indicate a favorable safety profile, while highlighting the need for caution due to a moderate risk of skin sensitization.

This work illustrates an integrated approach that combines local knowledge and modern scientific methods to value Algerian plant resources. To extend and deepen these promising results, several research directions can be considered:

- The isolation, identification, and characterization of the bioactive compounds responsible for the observed pharmacological effects, to better understand their molecular mechanisms of action.
- The study of potential synergistic interactions among the different compounds presents in essential oils to optimize their therapeutic activity.
- The expansion of ethnobotanical surveys to other regions and medicinal plant species to document and preserve a broader traditional heritage.
- The integration of these plants into public health strategies aimed at promoting accessible and natural medicines, especially in rural areas.

These perspectives encourage a harmonious integration of traditional knowledge and modern scientific approaches, thus guiding the sustainable development of phytomedicines in Algeria.

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APPENDIX

يهدف هذا الاستبيان الى المساهمة في معرفة النباتات المستخدمة في الطب التقليدي، كما أنوه الى ان نتائج الاستبيان تؤخذ بجدية من طرفنا لذا نرجو منكم الاجابة بدقة وبكل موضوعية، وذلك بوضع العلامة (x) في الخانة التي تتفق مع رأيكم وأكم جزيل الشكر مسبقا.

1- معلومات شخصية

السن (سنة): اقل من 20 ☐ 20- 30 ☐ 30- 40 ☐ 40- 50 ☐ 50- 60 ☐ اكبر من 60 ☐

النشاط: بطال ☐ عامل ☐

المستوى المعيشي: منخفض ☐ متوسط ☐ مرتفع ☐

الجنس: انثى ☐ ذكر ☐

المستوى التعليمي: امي ☐ ابتدائي ☐ متوسط ☐ ثانوي ☐ جامعي ☐ الزاوية ☐

الحالة العائلية: أعزب ☐ متزوج ☐ مطلق ☐ أرمل ☐

الإقامة: مدينة ☐ قرية ☐ الريف ☐

2- طريقة العلاج

حينما تصاب بمرض هل تستعين؟

بالطب التقليدي ☐ التعليل: فعال ☐ اقل تكلفة ☐ سهل المنال ☐ الادوية غير فعالة ☐

بالطب الحديث ☐ التعليل: فعال ☐ اكثر دقة ☐ سهلة الإجراءات ☐

اذا كنت تستعين بهما معا، بماذا تبدأ للتداوي؟ الطب التقليدي ☐ الطب الحديث ☐

مصدر معلوماتك حول الطب التقليدي: المطالعة ☐ العائلة ☐ خبرة الآخرين ☐ بائع الاعشاب ☐

3- معلومات حول النبات

الاسم الشائع للنبات: ..... ، الاسم العلمي (اختياري): .....

نوع النبات: بري ☐ مزروع ☐ نبات دخيل ☐

مصدر النبات: بائع الاعشاب ☐ الجني شخصيا ☐

موسم الجني: .....

يستعمل لغرض: دواء ☐ مستحضر زينة ☐ غذائي ☐ غير ذلك .....

يستعمل في حالة: طازج ☐ جاف ☐

اذا كان جاف، اذكر طريقة التجفيف: في الظلام ☐ معرض للشمس ☐

الجزء المستعمل من النبات: الساق ☐ الأزهار ☐ الأوراق ☐ الفواكه ☐ الجذور ☐ القشور ☐ الحبوب ☐ الجزء الهوائي ☐ النبات كامل ☐

النبات يستخدم: وحده ☐ هناك اضافات اخرى .....

طريقة التحضير: مطبوخ ☐ نقيع ☐ مسحوق ☐ ازالة ☐ زيت ☐ عصير ☐ مرهم ☐ غير ذلك .....

طريقة الاستعمال: البلع ☐ الدلك ☐ الغسل ☐ الدهن ☐ الحقن ☐ الاستنشاق ☐ الكمد ☐ المك ☐ غير ذلك .....

الجرعة المستعملة: ملعقة ☐ حفنة ☐ كأس ☐ غير ذلك .....

الجرعة اليومية: الطفل: مرة/اليوم ☐ مرتين/اليوم ☐ 3مرات/اليوم ☐ غير ذلك .....

البالغ: مرة/اليوم ☐ مرتين/اليوم ☐ 3مرات/اليوم ☐ غير ذلك .....

المسن: مرة/اليوم ☐ مرتين/اليوم ☐ 3مرات/اليوم ☐ غير ذلك .....

مدة العلاج: يوم ☐ اسبوع ☐ شهر ☐ حتى الشفاء ☐ غير ذلك .....

طريقة الحفظ: معرض للشمس ☐ في الظلام ☐ غير ذلك .....

4- دواعي الاستعمال

نوع المرض: الأمراض الجلدية ☐ بدقة: .....

امراض الجهاز التنفسي ☐ بدقة: .....

اضطرابات القلب والاوعية الدموية ☐ بدقة: .....

اضطرابات الجهاز البولي والتناسلي ☐ بدقة: .....

الام العظام والمفاصل ☐ بدقة: .....

امراض الجهاز الهضمي ☐ بدقة: .....

امراض الغدد الملحقة بالجهاز الهضمي ☐ بدقة: .....

اضطرابات عصبية ☐ بدقة: .....

غير ذلك ☐ بدقة: .....

تشخيص المرض يكون من طرف: الطبيب ☐ بائع الاعشاب ☐ انت شخصيا ☐ غير ذلك .....

نتيجة العلاج: شفاء ☐ تحسن ☐ غير فعال ☐ غير ذلك .....

الاثار الجانبية ان وجدت: .....

السمية ان وجدت: .....

احتياطات الاستعمال: .....

Figure 33. Questionnaire for Ethnobotanical Data Collection.

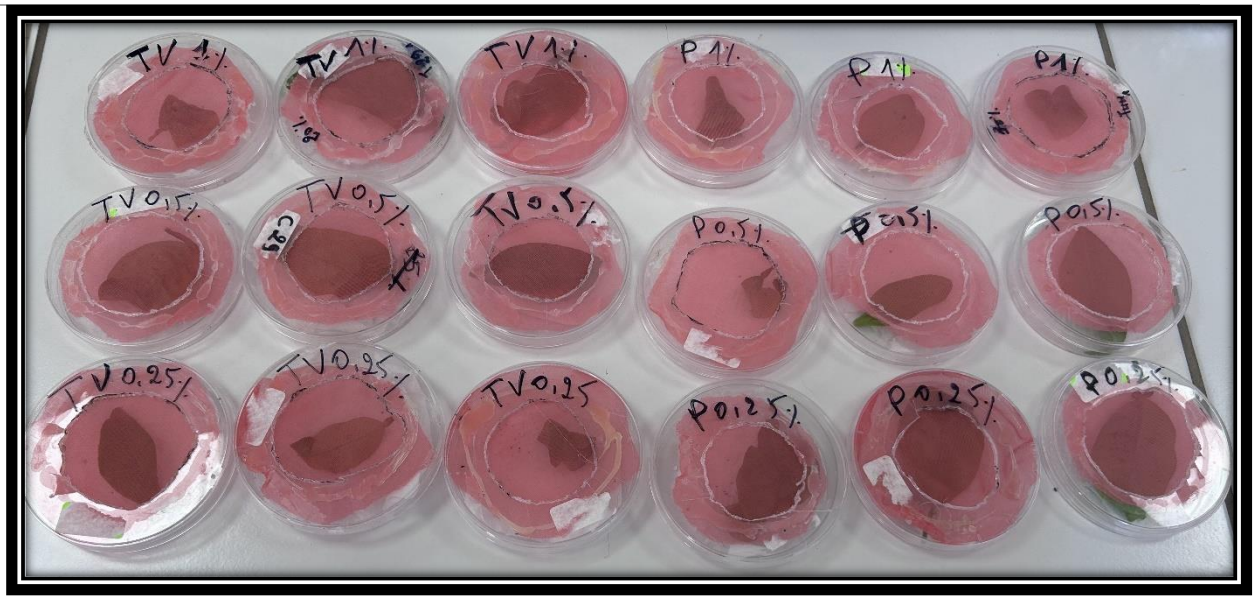

Figure 34. Insecticidal Activity Assay Using Filter Paper Impregnation and Larval Mortality Observation.

الملخص

انطلقت هذه الدراسة من دراسة اثنوبوتانية، التي أجريت بين العشابين في ولاية غليزان الجزائر، وتهدف الى تسليط الضوء على الأهمية الثقافية والعلاجية لنوعين رئيسين من النباتات الطبية: النوخة والزعتر. وقد خضع هذان النوعان، اللذان يستعملان على نطاق واسع في الطب العشبي المحلي، لتحليل مفصل لزيوتهما الأساسية المستخلصة بواسطة التقطير المائي من اجل تقييم أنشطتهما البيولوجية في المخبر، مدعومة بدراسات الارساء الجزئي بحاسوب وتنبؤات الخصائص الحركية الدوائية والسمية.

أنتجت عملية التقطير المائي عوائد من الزيوت العطرية بلغت على التوالي 1.2% (وزن/وزن) لـنبتة النوخة و1.5% (وزن/وزن) لنبتة الزعتر وأكدت تحاليل الكروماتوغرافيا بالغاز وطيف الكتلة (GC-MS)على التعقيد الكيميائي. حيث تم التعرف على الثيمول كمركب رئيسي بنسبة 38.4% في الزعتر والكارفورول بنسبة 29.7% في النوخة، إلى جانب مركبات مونوثربنية أخرى.

أظهرت الاختبارات البيولوجية نشاطًا مضادًا للأكسدة ملحوظًا عبر استنزاف جذور الـ DPPH، إذ كانت قيم IC50 تصل إلى 45.3 ± 2.1 ميكروغرام/مل لـ النوخة و38.7 ± 1.8 ميكروغرام/مل لـ الزعتر، مقارنةً بالحمض الأسكوربيكي 0.9 ± 12.4ميكروغرام/مل. كما لوحظت تأثيرات مضادة للالتهابات مهمة، خاصة عن طريق استقرار أغشية كريات الدم الحمراء وتثبيط دمار البروتينات، مع معدلات تصل إلى 65.2 ± 4.3% و69.8 ± 3.7% على التوالي عند 100 ميكروغرام/مل، وكذلك 61.5 ± 2.9% و67.3 ± 3.1% بالنسبة لنفس التركيز. كما أظهرت الزيوت العطرية فعالية كعامل مبيد للحشرات ضد *Aphis spiraecola*، حيث تسببت في وفاة 78% بعد 24 ساعة على تركيز 0.5%. فيما يخص النشاط المضاد للبكتريا، أظهرت هذه الزيوت تثبيطًا ملحوظًا ضد سلالات مُمرضة تشمل *Escherichia coli* و *Staphylococcus aureus*الحساسة والمقاومة للميثيسيلين، و*faecalis* Enterococcus، منتجة مناطق تثبيط بمقاييس 18.5 ± 0.7 ملم، 20.3 ± 0.9 ملم، 15.9 ± 1.0 ملم و19.1 ± 0.8 ملم على التوالي. كما عزز تفاعل تآزري بين زيوت النوخة والزعتر من قدرتهما كمضادات حيوية طبيعية، متفوقين أحيانًا على بعض المضادات الحيوية التقليدية.

كشفت تحاليل التوصيل الجزيئي على ثلاث إنزيمات بكتيرية هدف عن ارتباطات واعدة بين -7.6 و -9.1 كيلو كالوري/مول للمركبات الرئيسية المكتشفة، مما يشير إلى آليات تثبيط تنافسية ذات صلة. توقعت الدراسات الحاسوبية فيزيولوجية التسمم وخصائص الدواء أن يكون هناك امتصاص معوي عالي(85% <) ، نفاذية معتدلة للحاجز الدموي الدماغي، وخطورة منخفضة للتسمم الحاد (DL50 > 2000 ملغم/كغم)، بالإضافة إلى خطر معتدل للتحسس الجلدي لبعض الجزيئات.

كذلك أظهر التحليل الغذائي لنباتات تركيبة متوازنة تتميز بمحتوى مرتفع من الكربوهيدرات الكلية، ومستويات متوسطة من البروتينات، ومحتوى منخفض من الدهون، ودرجة الحموضة منخفضة مما يدعم استعمالها التقليدي في الطب العشبي، ويشير الى امكان توظيفها كمكملات غذائية.

بناءا على هذه المعطيات، تم تطوير صيغة دوائية موضعية تتضمن الزيوت الأساسية لكم من النوخة والزعتر. يهدف هذا المنتج الطبيعي الى اسغلال الخصائص المضادة للميكروبات والمضادة للالتهاب لهذه الزيوت، مما يجعله بديلا واعدا لعلاج الحالات الجلدية الالتهابية، ونظرا لانخفاض سميتها سمكن أيضا استعمال هذه النباتات على شكل مستخلص منقوع كمكمل غذائي متاح للفئات الحساسة .

تمثل هذه الدراسة مثالا على التكامل الصارم بين المعارف التقليدية والمنهجيات العلمية الحديثة، وتشجع على الاستغلال المستدام للموارد النباتية الطبية الجزائرية، مؤكدة أهمية مواصلة البحوث الطبية الحيوية من اجل تثمين كامل لامكاناتها العلاجية.

الكلمات المفتاحية: اثنوبوتانيك، زيوت أساسية، النوخة، الزعتر، أنشطة بيولوجية، الارساء الجزئي، صيدلانيات .

ABSTRACT

This study was initiated to conduct an ethnobotanical survey among herbalists in the Wilaya of Relizane, Algeria, highlighting the cultural and therapeutic significance of two major medicinal plants: *Ptychotis verticillata* Duby and *Thymus vulgaris* L. These species, widely used in local phytotherapy, were subjected to a detailed analysis of their essential oils extracted by hydrodistillation to evaluate their in vitro biological activities, supported by molecular docking studies and pharmacokinetic and toxicological (ADMET) predictions.

The hydrodistillation process yielded 1.2% (w/w) and 1.5% (w/w) essential oils from *Ptychotis verticillata* Duby and *Thymus vulgaris* L., respectively. Gas chromatography-mass spectrometry (GC-MS) analyses confirmed the chemical complexity and richness of these mixtures, identifying thymol as the predominant compound at 38.4% in *T. vulgaris* and carvacrol at 29.7% in *P. verticillata*, along with other monoterpenes.

Biological assays demonstrated significant antioxidant activity via DPPH radical scavenging, with IC50 values of 45.3 ± 2.1 µg/mL for *P. verticillata* and 38.7 ± 1.8 µg/mL for *T. vulgaris*, compared to ascorbic acid (IC50 = 12.4 ± 0.9 µg/mL). Notable anti-inflammatory effects were evidenced by erythrocyte membrane stabilization and inhibition of protein denaturation, with inhibition rates of 65.2 ± 4.3% and 69.8 ± 3.7%, and 61.5 ± 2.9% and 67.3 ± 3.1%, respectively, at 100 µg/mL. The essential oils also exhibited effective insecticidal activity against *Aphis spiraecola*, inducing 78% mortality at 0.5% after 24 hours. Regarding antimicrobial efficacy, these oils exhibited marked inhibition against pathogenic strains, including Escherichia coli, methicillin-sensitive and resistant Staphylococcus aureus, and Enterococcus faecalis, producing inhibition zones of 18.5 ± 0.7 mm, 20.3 ± 0.9 mm, 15.9 ± 1.0 mm, and 19.1 ± 0.8 mm, respectively. Synergistic interaction between the essential oils of *Ptychotis verticillata* Duby and *Thymus vulgaris* L. enhanced their potential as natural antimicrobial agents, in some cases outperforming certain conventional antibiotics.

Molecular docking analyses of three bacterial target enzymes revealed promising binding affinities of -7.6 to -9.1 kcal/mol for the major identified compounds, suggesting relevant competitive inhibition mechanisms. In silico studies of pharmacokinetic and toxicological properties predicted high intestinal absorption (>85%), moderate blood-brain barrier permeability, low acute toxicity (LD50 > 2000 mg/kg), and a moderate risk of skin sensitization for some molecules.

Nutritional analysis of the plants revealed a balanced composition, characterized by high total carbohydrate content, moderate protein levels, low lipid content, and a slightly acidic pH, supporting their traditional use in phytotherapy and as potential nutritional supplements.

Based on these data, a topical pharmaceutical formulation named "Derma Phyto Calm" was developed, incorporating essential oils of *Ptychotis verticillata* Duby and Thymus vulgaris L. enriched with natural vitamin E to enhance antioxidant stability. This natural product aims to exploit the oils' antimicrobial and anti-inflammatory properties, offering a promising phytotherapeutic alternative for treating inflammatory and infectious skin conditions, including intimate areas. Furthermore, given their low toxicity, these plants could also be used in infusion form as an accessible nutritional supplement for sensitive populations.

This research exemplifies a rigorous integration of traditional knowledge with contemporary scientific methodology, encouraging the sustainable exploitation of Algerian medicinal plant resources and emphasizing ongoing biomedical research to valorize their therapeutic potential fully.

Keywords : Ethnobotany, Essential Oils**,** *Ptychotis verticillata* Duby, *Thymus vulgaris* L., Biological Activities, Molecular Docking, Pharmaceutical.

RESUME

Cette étude initiée par une étude ethnobotanique conduite auprès des herboristes de la Wilaya de Relizane en Algérie met en évidence l'importance culturelle et thérapeutique de deux plantes médicinales majeures : *Ptychotis verticillata* Duby et *Thymus vulgaris* L. Ces espèces, largement utilisées dans la phytothérapie locale, ont fait l'objet d'une analyse approfondie de leurs huiles essentielles extraites par hydrodistillation afin d'évaluer leurs activités biologiques in vitro, validées par des études de modélisation moléculaire (docking) et des prédictions pharmacocinétiques et toxicologiques (ADMET).

Le procédé d'hydro-distillation a permis d'obtenir des rendements en huiles essentielles respectifs de 1,2% (p/p) pour *Ptychotis verticillata* Duby et 1,5% (p/p) pour *Thymus vulgaris* L. Les analyses par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS) ont confirmé la complexité chimique et la richesse de ces mélanges, identifiant le thymol comme composé prédominant à 38,4% dans *T. vulgaris* et le carvacrol à 29,7% dans *P. verticillata,* accompagnés d'autres monoterpènes.

Les tests biologiques ont démontré une activité antioxydante significative via la capture des radicaux DPPH, avec des valeurs d'IC50 de 45,3 ± 2,1 µg/mL pour *P. verticillata* et 38,7 ± 1,8 µg/mL pour *T. vulgaris*, comparées à l'acide ascorbique (IC50 = 12,4 ± 0,9 µg/mL). Des effets anti-inflammatoires notables ont été observés, notamment par la stabilisation des membranes érythrocytaires et l'inhibition de la dénaturation des protéines, avec des taux d'inhibition respectifs de 65,2 ± 4,3 % et 69,8 ± 3,7 %, ainsi que 61,5 ± 2,9 % et 67,3 ± 3,1 % à 100 µg/mL. Les huiles essentielles ont également montré une activité insecticide efficace contre *Aphis spiraecola*, provoquant 78% de mortalité après 24 heures à une concentration de 0,5%. En ce qui concerne l'efficacité antimicrobienne, ces huiles ont affiché une inhibition marquée contre des souches pathogènes incluant *Escherichia coli, Staphylococcus aureus sensible* et *résistant* à la *méthicilline*, ainsi qu'Enterococcus *faecalis*, produisant des zones d'inhibition de 18,5 ± 0,7 mm, 20,3 ± 0,9 mm, 15,9 ± 1,0 mm et 19,1 ± 0,8 mm respectivement. Une interaction synergique entre les huiles essentielles de *Ptychotis verticillata* Duby et de *Thymus vulgaris* L. a renforcé leur potentiel en tant qu'agents antimicrobiens naturels, surpassant dans certains cas certains antibiotiques conventionnels.

Les analyses de docking moléculaire sur trois enzymes bactériennes cibles ont révélé des affinités de liaison prometteuses comprises entre -7,6 et -9,1 kcal/mol pour les principaux composés identifiés, Les études in silico des propriétés pharmacocinétiques et toxicologiques ont prédit une forte absorption intestinale (> 85%), une perméabilité modérée à la barrière hémato-encéphalique, une faible toxicité aiguë (DL50 > 2000 mg/kg) ainsi qu'un risque modéré de sensibilisation cutanée pour certaines molécules.

L'analyse nutritionnelle des plantes a révélé une composition équilibrée caractérisée par une teneur élevée en glucides totaux, des protéines modérées, de faibles lipides et un pH légèrement acide.

À partir de ces données, une formulation pharmaceutique topique, l'onguent « Derma Phyto Calm », a été développée, intégrant les huiles essentielles de *Ptychotis verticillata* Duby et *Thymus vulgaris* L., enrichie en vitamine E naturelle afin d'améliorer sa stabilité antioxydante. Ce produit naturel vise à exploiter les propriétés antimicrobiennes et anti-inflammatoires des huiles pour offrir une alternative phytothérapeutique prometteuse dans le traitement des affections cutanées inflammatoires et infectieuses, y compris sur les zones intimes. Par ailleurs, compte tenu de leur faible toxicité, ces plantes peuvent également être utilisées en infusion comme complément nutritionnel accessible aux personnes sensibles.

Cette recherche illustre une intégration rigoureuse des savoirs traditionnels avec une méthodologie scientifique contemporaine, encourageant l'exploitation durable des ressources végétales médicinales algériennes et soulignant l'importance de poursuivre les investigations biomédicales pour valoriser pleinement leur potentiel thérapeutique.

Mots-clés : Ethnobotanique, Huiles essentielles, *Ptychotis verticillata* Duby, *Thymus vulgais* L., activités biologiques, Docking moléculaire, Pharmaceutique.