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Field: Materials Science

Specialization: Organic Chemistry

Presented by:

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Title

The Chemical Composition, *In vitro*, *In silico*, and *In vivo* Studies of the essential oil of the lavender plant found in regions of Algeria

Defended on 06/06/2026 in the presence of the Examination Committee:

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بسم الله الرحمن الرحيم

Dedication:

The Messenger of Allah (peace and blessings be upon him) said: **"Whoever follows a path in pursuit of knowledge, Allah will make easy for him a path to Paradise."**

All praise is due to Allah, by whose grace good deeds are completed, and by whose favor and generosity we attain our goals and aspirations.

After an academic journey spanning years, carrying within it many hardships, struggles, and fatigue, I stand today at the threshold of my graduation. I harvest the fruits of my labor and tip my hat with pride,

To my Mother and Father: Words fail to express the extent of my gratitude. You have always been my foundation and my greatest supporters, providing all the love and encouragement I needed to achieve my dreams. Your patience and sacrifices have been the greatest influence on my success.

To the fragrant flower in the garden of my life, the tender voice that brings me peace: **My Grandmother**. May Allah preserve you and grant you a long life.

To the one through whom Allah strengthened me, my support after my father: **My brother, Cherif Ahmed Cherif**. I ask Allah to grant you success in all your endeavors.

To those who were and remain my backbone and companions, the hearts that understand me without words and the souls that eased my most difficult moments: **Faisal, Ibrahim, Abdel Moneim, and Ahmed.**

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To the "unknown soldiers," my friends **“Abdel Bari , El-Hadj Mohamed”:** I dedicate these words to you as an expression of my deep gratitude. You were always my support throughout my academic journey. The days were not always easy, but your presence by my side made the challenges feel lighter.

And finally, to my ambitious and resilient self, who endured every setback and continued despite all difficulties.

Walid

Dedication:

Allah the Almighty says:

"And the last of their call will be, 'Praise be to Allah, Lord of the worlds.'"

All praise and gratitude be to Allah , in love, thankfulness, and appreciation, for the beginning and the end. Praise be to Allah after the hardship and struggle in pursuit of knowledge and dreams. Today, I stand at the threshold of my graduation, harvesting the fruit of my efforts, and with pride and love, I dedicate this success., To the light that left me too early, whose name I carry with honor and pride; To the one who supported me without limits and gave without expecting anything in return , To the one who overcame difficulties for my sake and always dreamed of seeing the fruit of his efforts reach the highest ranks, To the one whose name accompanies me in every prayer, **My father, may Allah have mercy on him.**

To the one beneath whose feet Allah has placed Paradise; To the one who embraced me with her heart before her hands; To the one who eased my hardships with her prayers, whose tears precede mine and whose joy is my joy, **My mother**, may Allah preserve you and grant me the ability to honor you., To the one through whom Allah strengthened my back; my support after my father, My shield, my protection, my backbone, My brother **Mohamed El-Kebir**, may Allah grant you success., To the shining star in the sky of my life; To the symbol of love and sacrifice; to the shade under which I find comfort; To the one who shared with me both bitter and sweet moments, My dear sister, may Allah grant you lasting happiness., To the granddaughter of the family, the joy and delight of our home, **Amina**, may Allah make you rightly guided and a source of guidance for others., To the loyal friend despite the instability of the world; The companion of the first and last step; The one who was like a rain cloud during the years of hardship, My companion **Walid**., To my friends of many years, my companions in hardship and inspiration for my success, To those who extended their hands to help me when I needed it most; Especially **Massoud, Sadok, Yacoub, Faisal, and Brahim and Manel**, Thank you for your continuous support and for being people I can always rely on.

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Mahmoud

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"**Indeed, those who have believed and done righteous deeds - indeed, we will not allow to be lost the reward of any who did a good deed**." (Al-Kahf: 30). Allah Almighty has spoken the truth.

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Finally, we ask Allah the Almighty to grant us success in doing what He loves and is pleased with, for He is the Guardian of that and the One Capable of it. Praise be to Allah, Lord of the Worlds.

Abstract:

the *Lavandula angustifolia* essential oil was extracted by hydro-distillation with a yield of 2.316% and chemically characterized using (GC–MS), which identified linalool (39.7%), linalyl acetate (9.7%), α-terpineol (8.7%), and camphor (8.3%) as the major constituents.

The *in vitro* study showed antioxidant activity was evaluated using the DPPH radical scavenging assay, where the essential oil showed moderate antioxidant activity with an IC50 value of 120500 µg/mL compared with BHT (180 µg/mL) and vitamin E (15 µg/mL). Anti-inflammatory activity was assessed using the bovine serum albumin (BSA) anti-denaturation assay, showing a maximum inhibition of 14.2% at 50% concentration, while aspirin exhibited 86.2% inhibition at 40 mg/mL.

The antibacterial activity was determined by the agar well diffusion method against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922. The essential oil produced inhibition zones of 16 mm and 15 mm, respectively, at 50% concentration. Hemolytic activity evaluated using the erythrocyte hemolysis assay demonstrated low hemolysis (<10%) at concentrations ≤ 5 mg/mL, The antidiabetic activity was investigated using the α-amylase inhibition assay and an alloxan-induced diabetic rat model. *In vitro* α-amylase inhibition reached 92.37% at concentration 9 mg/mL. *In vivo* administration of the essential oil (100 mg/kg) reduced fasting blood glucose from 1.06 ± 0.09 g/L in diabetic rats to 0.84 ± 0.38 g/L and decreased HbA1c from 3.44 ± 0.11% to 3.34 ± 0.37%. Liver enzyme analysis showed hepatoprotective activity with ALT reduction from 131.96 ± 51.97 IU/L to 35.12 ± 3.72 IU/L and AST reduction from 218.66 ± 22.06 IU/L to 131.89 ± 9.99 IU/L, Molecular docking analysis revealed favorable interactions between major lavender constituents and α-amylase active-site residues, particularly ASP197, supporting the observed antidiabetic activity. Histopathological examination of liver, kidney, and pancreatic tissues demonstrated partial restoration of tissue in treated diabetic rats.

Keywords:

Lavandula angustifolia Mill.; essential oil; GC–MS; antioxidant activity; anti-inflammatory activity; antibacterial activity; α-amylase inhibition; molecular docking; ADMET; alloxan-induced diabetes.

ملخص:

الزيت الأساسي لنبات *Lavandula angustifolia* تم استخلاصه بالتقطير المائي بمردود بلغت نسبته 2.316% وتم تعرف على تركيبه الكيميائي باستخدام (GC–MS)، والتي حددت linalool (39.7%)، و linalyl acetate (9.7%) ، و α-terpineol (8.7%) ، و camphor (8.3%)كأهم المكونات الرئيسية, تم تقييم النشاط المضاد للأكسدة باستخدام اختبار تثبيط الجذور الحرة DPPH ، حيث أظهر الزيت الأساسي نشاطاً معتدلاً مضاداً للأكسدة بقيمة IC50 بلغت 120500µg/mL مقارنة بمركب BHT (180 µg/mL) وفيتامين E (15 µg/mL) كما تم تقييم النشاط المضاد للالتهابات باستخدام اختبار منع تمسخ ألبومين المصل البقري(BSA) ، حيث أظهر أقصى نسبة تثبيط بلغت 14.2% عند تركيز 50%، بينما أظهر aspirin نسبة تثبيط بلغت 86.2% عند تركيز 40 mg/mL , تم تحديد النشاط المضاد للبكتيريا (agar well diffusion method)ضد Staphylococcus aureus ATCC 25923 وEscherichia coli ATCC 25922. وسجل الزيت الأساسي هالات تثبيط بلغت 16 mm و 15 mm، على التوالي، عند تركيز 50%. وأظهر نشاط التحلل الدموي الذي تم تقييمه باستخدام اختبار (erythrocyte hemolysis assay)تحللاً دموياً منخفضاً (<10%) عند تراكيز ≥ 5 mg/mL تم استقصاء النشاط المضاد لمرض السكري باستخدام اختبار تثبيط إنزيم α-amylase و .(alloxan-induced diabetic rat model) حيث وصل تثبيط إنزيم α-amylase في المختبر (*In vitro*) إلى 92.37% عند تركيز 9 mg/mL. وأدى إعطاء الزيت الأساسي في الجسم الحي (*In vivo*) بجرعة (100 mg/kg) إلى خفض غلوكوز الدم الصائم من 1.06 ± 0.09 g/L في الفئران المصابة بالسكري إلى 0.84 ± 0.38 g/L وخفض HbA1cمن 3.44% ± 0.11 إلى 3.34% ± 0.37. كما أظهر تحليل إنزيمات الكبد نشاطاً واقياً للكبد مع انخفض إنزيم ALT من 131.96 IU/L ± 51.97 إلى 35.12 IU/L ± 3.72 وانخفاض إنزيم AST من 218.66 IU/L ± 22.06 إلى 131.89 IU/L ± 9.99. كشف تحليل Molecular dockingعن تفاعلات ملائمة بين المكونات الرئيسية للخزامى وبقايا الأحماض الأمينية في الموقع النشط لإنزيم α-amylase ، ولا سيما ASP197 . وأظهر الفحص الهيستوباثولوجي لأنسجة الكبد والكلى والبنكرياس استعادة جزئية لنسيج الفئران المعالَجة.

الكلمات المفتاحية:

الخزامى(*Lavandula angustifolia Mill*.) ؛ الزيت الأساسي؛GC–MS ؛ النشاط المضاد للأكسدة؛ النشاط المضاد للالتهابات؛ النشاط المضاد للبكتيريا؛ تثبيط ألفا-أميلاز؛ الالتحام الجزيئي؛ ADMET؛ السكري المستحث بالألوكسان .

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List of Abbreviations

Abbreviation	Full Term	Abbreviation	Full Term	Abbreviation	Full Term
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity	GI	Gastrointestinal	FBG	Fasting Blood Glucose
ALAT	Alanine Aminotransferase	H&E	Hematoxylin and Eosin	GC–MS	Gas Chromatography–Mass Spectrometry
ALT	Alanine Aminotransferase	HbA1c	Glycated Hemoglobin	PBS	Phosphate-buffered saline
ANOVA	Analysis of Variance	HCl	Hydrochloric acid	PC	Positive Control
ASAT	Aspartate Aminotransferase	H_2O_2	Hydrogen peroxide	PDB	Protein Data Bank
AST	Aspartate Aminotransferase	HE	Essential oil extract	P-gp	P-glycoprotein
ATCC	American Type Culture Collection	IC50	Half maximal inhibitory concentration	RI	Retention Index
Å	Ångström	Kp	Skin permeability coefficient	RMSD	Root Mean Square Deviation
BBB	Blood–Brain Barrier	LD50	Median lethal dose	SD	Standard Deviation
BOILED-Egg	Brain Or IntestinaL EstimateD permeation	LGA	Lamarckian Genetic Algorithm	SMILES	Simplified Molecular Input Line Entry System
BHT	Butylated Hydroxytoluene	LogP	Partition coefficient	T	Treatment group
BSA	Bovine Serum Albumin	LogS	Solubility coefficient	TBS	Tris-buffered saline
CFU	Colony-Forming Unit	MYC	Myricetin	TPSA	Topological Polar Surface Area
CYP	Cytochrome P450	N	Normal control	UV-Vis	Ultraviolet–Visible
DMSO	Dimethyl sulfoxide	NC	Negative Control	Vit. E	Vitamin E
DPPH	1,1-diphenyl-2-picrylhydrazyl	NSAID	Nonsteroidal Anti-inflammatory Drug	WLOGP	Wildman and Crippen lipophilicity
EI	Electronic Impact	EO	Essential Oil	ZOI	Zone of Inhibition

General Introduction

General Introduction

The success of modern medical sciences relies heavily on drugs originally derived from natural sources [2]. It is well-established that medicinal plants contain a wide array of bioactive compounds possessing antimicrobial, antifungal, anticancer, anti-inflammatory, and antioxidant activities [1].

Lavender (*Lavandula angustifolia*), a perennial aromatic shrub belonging to the *Lamiaceae* family, is widely distributed throughout the Mediterranean basin and is a hallmark of the mountainous landscapes of Northern Algeria[1, 3]. Lavender is renowned for its diverse applications in traditional medicine, aromatherapy, and cosmetics due to its pharmacological properties and secondary metabolites. Key among these compounds are essential oils, flavonoids, and phenolic acids, which have demonstrated significant biological activities, including antioxidant, antibacterial, antifungal, and sedative properties [2].

The El Oued region in southeastern Algeria is characterized by an arid Saharan climate, including high temperatures, intense solar radiation, low humidity, and limited rainfall. These environmental conditions may influence the biosynthesis of secondary metabolites in aromatic plants. Lavandula angustifolia collected from this region was therefore selected for phytochemical and biological investigation. The genus Lavandula is known to be rich in bioactive compounds, particularly oxygenated monoterpenes such as linalool and linalyl acetate, which contribute to its reported antioxidant, anti-inflammatory, and antimicrobial activities [1]. Medicinal and aromatic plants are widely recognized as important sources of biologically active secondary metabolites with therapeutic potential [4].

This study aims to characterize the phytochemical composition and evaluate the antioxidant, antibacterial, and antifungal activities of Lavender (*Lavandula angustifolia*) leaf extracts collected from the Medea region. By assessing the biological efficacy of these extracts, we seek to understand their pharmacological potential and explore new natural sources for therapeutic and industrial applications.

Our work is structured as follows:

Part I: Theoretical Part

Chapter I: General overview of *Lavandula angustifolia*.

Chapter II: Biological Activity.

Chapter III: Molecular docking and Pharmacokinetic Evaluation.

Part II: Practical Part

Chapter IV: Materials and Methods.

Chapter V: Results and Discussion.

Finally, a General Conclusion summarizes the findings and suggests future research perspectives.

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Part I:

Theoretical Part

I. Chapter I:

General overview of *Lavandula angustifolia*.

1-Definition of Medicinal Plants:

Medicinal plants are plants where some or all parts contain active substances with therapeutic value. They are used in a direct form such as roots, stems, leaves, flowers, fruits, seeds, sap, or the entire herb as practiced in folk medicine. Alternatively, they can be used in a concentrated form by isolating their active ingredients, which are then incorporated into many pharmaceutical preparations and are responsible for their therapeutic effectiveness.[5]

2-Classification of Medicinal Plants:

Medicinal plants are classified into several different groups, each sharing common characteristics, such as commercial or therapeutic classifications. However, morphological classification remains the most prominent in the study of botany, as it is based on the specific part of the plant used and the concentration of the active ingredient within it.[6]

3-Introduction to Lavender (*L. angustifolia*):

Lavender, known in Latin as *Lavandula*, is a plant belonging to the *Lamiaceae* (Mint) family [7]

It has several common names depending on the geographical region; in some Mediterranean regions, it is known by various local dialects, while in different parts of the world, it is referred to by names such as "Al-Khuzama" in Arabic-speaking countries, "Lofant" in parts of Eurasia, and simply "Lavender" in Britain, Australia, and the United States [8].

However, the meaning of the general term for the plant is quite interesting. According to historical etymology [9], the name *Lavandula* is believed to derive from the Latin verb "lavare," which means "to wash," referring to its ancient use in purifying and scenting baths. Its original home is the Mediterranean basin, specifically in mountainous and coastal areas, where its medicinal and aromatic properties have been known for thousands of years. At the beginning of the twentieth century, the cultivation of this plant was expanded to many developing and industrialized countries, particularly in temperate and subtropical climates [10].

Today, Lavender trees and shrubs are found in approximately 80 countries worldwide. It is estimated that there are millions of lavender plants globally, with the Mediterranean region, Southern Europe, and North Africa constituting its primary distribution areas [11].

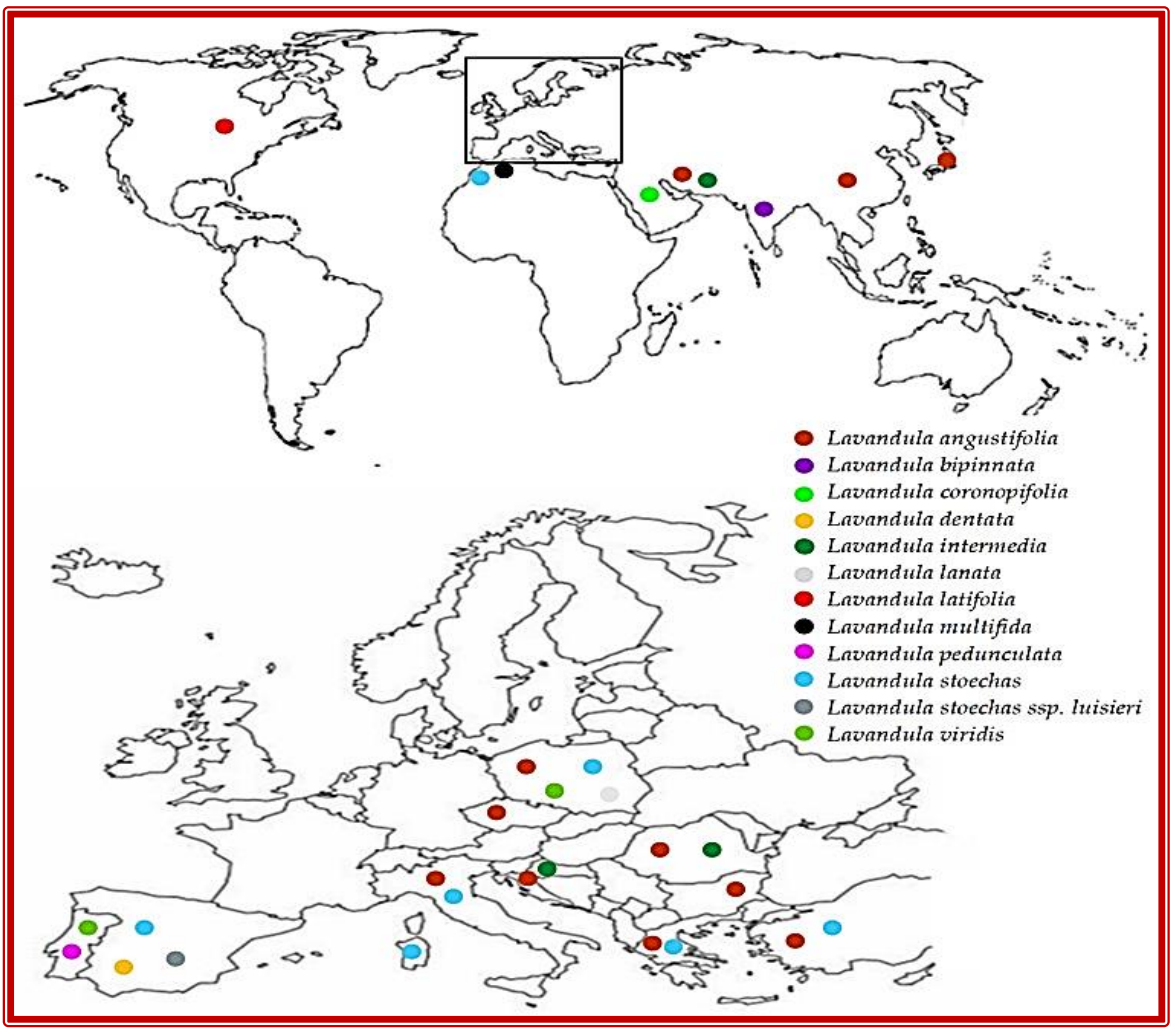

Figure I-1: World distribution regions of Lavandula angustifolia [1]

4-Scientific Classification of Lavender:

The lavender plant was classified as early as 1753 by Linnaeus (and later refined by various taxonomists), as follows [12]:

Kingdom	Plantae
Order:	*Lamiales*
Family	*Lamiaceae* (Mint family)
Subfamily	*Nepetoideae*
Tribe	*Lavanduleae*
Genus	*Lavandula*
Species	*angustifolia*

Table I-1:Classification of the *Lavandula* plant

5. Importance of the Lavender Plant:

The Lavender plant has been known for over two thousand years in the Mediterranean and European regions for its numerous therapeutic virtues. Over the past years, lavender has been the subject of extensive research aimed at exploring all its beneficial properties, specifically those that directly benefit human health [10]. Various parts of the plant have been used, including the leaves, seeds (buds), flowers, and essential oils [10], to treat fevers, headaches, ulcers, respiratory disorders, and digestive issues [13].

Lavender also possesses antifungal, anti-parasitic, anti-inflammatory, and immune-modulating properties, and is widely known for its sedative and calming effects [13]. It is used to treat a wide range of allergic conditions (as an antihistamine), microbial and viral infections, as well as cardiovascular conditions (such as mild hypertension) and as a neuroprotective agent [14]. Lavender extracts are also believed to have inhibitory effects on several cancer cell lines, and compounds extracted from the leaves and flowers have been described as effective in treating appetite loss and skin problems. The floral waters and oils are widely used as expectorants and hold great importance in treating intestinal and urinary issues [15].

In addition, The flowers produce the essential oil used in soaps, detergents, and perfumes, while other by-products are used in organic fertilizers and soil conditioning. Its extracts have also been used as natural insect repellents [16].

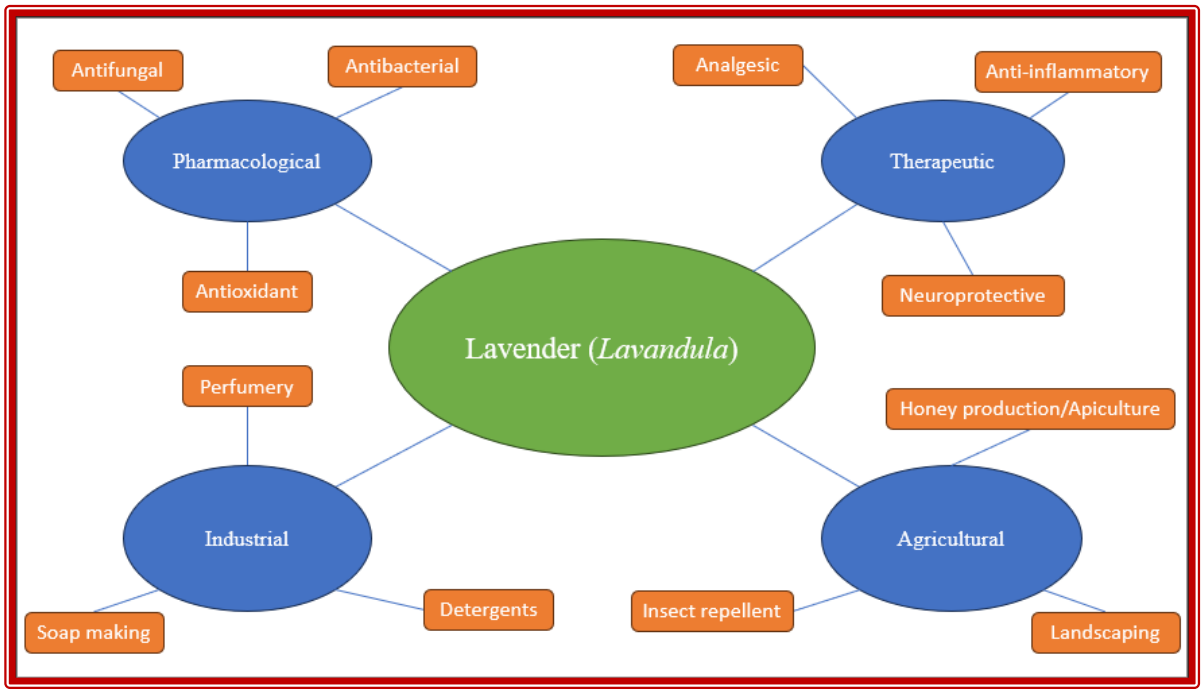

Figure I-2:The importance of Lavender (Lavandula)

6- Morphological Description of *Lavandula angustifolia*:

Lavender is a rapid-growing, evergreen suffruticose perennial characterized by a highly branched, lignified (woody) base [8]. It is a medium-sized shrub, typically attaining a vertical height of 30 to 90 cm, with a dense, globose canopy reaching a lateral spread of 60 to 120 cm [17].

The specimen initiates its reproductive phase (flowering) within 1 to 2 years of cultivation. By approximately the third year, the plant reaches physiological maturity, becoming fully productive and capable of yielding a significant annual biomass of floral spikes and essential oils, often reaching several kilograms of fresh weight per individual plant [10]. Although it lacks the multi-centurial longevity of arborescent species, *L. angustifolia* remains commercially and biologically viable for a lifespan exceeding 20 to 30 years under optimal edaphic and climatic conditions [18].

6-1- Root System of *L. angustifolia*:

The root system of Lavender consists of a primary, lignified taproot from which an extensive network of highly developed lateral roots emerges [17]. In mature specimens, the lateral root surface area can extend significantly to optimize nutrient and moisture acquisition, often reaching a horizontal spread of 80 to 150 cm [8].

Figure I-3 : A photograph of lavender roots

6-2- Stem and Cauline Structure:

The primary stem of Lavender is typically erect and relatively short, transitioning into a heavily lignified base as the plant matures. It attains a basal diameter ranging from 3 to 8 cm . The cortical tissue (bark) exhibits a coloration spanning from light grey to dark brown, varying significantly according to the specimen's age and environmental exposure. Characteristically, the stem is highly branched at the base, forming a dense, woody framework that is notably resistant to most phytophagous insect infestations due to the high concentration of secondary metabolites (essential oils) [10].

Figure I-4: A photograph of a lavender stem

6-3- Leaves:

The leaves are arranged oppositely (or in whorls) on short, woody stems. Both surfaces are typically a silvery-green or grayish-green due to the presence of fine star-shaped hairs, which give the plant its characteristic soft texture. The length of the leaf usually ranges between 2 to 6 cm.

The leaves are narrow and linear (lanceolate), and unlike many other plants, they are often entire (smooth) rather than serrated. Young leaves emerge with a vibrant green or silvery hue, and as they mature, they maintain a symmetrical, slender shape that is highly aromatic when bruised [8].

Figure I-5: A photograph of lavender leaves

6-4- Flowers:

The flowers are violet, lavender, or blue-purple (rarely white or pink) and are bisexual. They are arranged in compact spikes (verticillasters) held atop long, leafless stalks (peduncles) [8]. The length of an individual tubular flower typically ranges from 10 to 12 mm [19]. They possess a distinctive aroma due to linalool and linalyl acetate, which attract a high diversity of pollinators, including honeybees and several species of bumblebees [20].

Figure I-6: A photograph of lavender flowers

6-6- Seeds:

The seed (nutlet) consists of a hard, smooth outer coat (testa) and an internal embryo containing high concentrations of essential lipids [8]. While the flowers are the primary source of oil, the seeds are essential for cultivar propagation and contain trace amounts of linalyl acetate, contributing to the plant's overall aromatic profile and defense mechanisms against certain soil-borne pathogens.

7- Cultivation Requirements for *Lavandula* Species:

The plant adapts morphologically to its climatic factors and topography [8]. It grows exceptionally well in dry, well-drained, and stony soils, and can even thrive in poor or chalky soils, provided they are not compacted. Lavender requires minimal supplemental water once established and demands full solar exposure (at least 6–8 hours of direct sunlight) [21].

It grows naturally in regions where the average annual rainfall ranges between 300 mm and 800 mm; however, it has been successfully cultivated in areas with rainfall as low as 200 mm if the soil retains some moisture. Lavender can thrive at altitudes ranging from sea level up to 1,500 meters [8], and performs best in temperatures ranging from 15°C to 30°C, though some species are frost-hardy. It cannot tolerate waterlogged areas or poorly drained soils, as these lead to root rot. The ideal pH range for its growth is between 6.5 and 8.0 (slightly alkaline). When planting, a distance of at least 30 to 90 cm must be left between each plant, depending on the specific variety [22].

8- Chemical Composition of *Lavandula* Species:

Lavender is a distinctive plant that has between 100 and 150 bioactive chemicals, which are found in different areas of the plant in variable amounts. It is especially well known for its volatile essential oils, which give it its distinctive herbal and flowery scent [13].

The constituents of Lavender can be classified primarily into terpenoids and non-terpenoid compounds. The terpenoids include monoterpenes and sesquiterpenes, while the non-terpenoid components include phenolic acids, flavonoids, and coumarins [16]. Among the most biologically active and significant compounds in Lavender are Linalool, Linalyl acetate, and 1,8-Cineole. These compounds are lipophilic (fat-loving) and highly soluble in organic solvents such as alcohols and esters; they contribute effectively to the plant's antimicrobial, antifungal, and sedative properties [23].

The phytochemical matrix of lavender essential oil (*Lavandula angustifolia*) is composed of a diverse array of bioactive metabolites that determine its therapeutic and aromatic profile [13]. These constituents can be classified into several primary functional groups:

Esters (Linalyl acetate, 25%–45%): Defined as organic compounds derived from the condensation of an alcohol and a carboxylic acid, esters are the primary contributors to the oil's sweet, fruity fragrance and provide significant sedative and antispasmodic effects [24, 25].

Monoterpenes (Linalool, 20%–45%): These are terpene-based alcohols consisting of a ten-carbon backbone; they are the fundamental building blocks responsible for the oil's potent antimicrobial properties and its characteristic floral heart note [13, 24].

Terpene Alcohols (Terpinen-4-ol, 2%–5%): These act as secondary aromatic alcohols that, while present in smaller volumes, are essential for the synergistic biological activity of the oil and contribute to its woody, herbal undertones [25].

Ketones (Camphor, < 1%): Characterized by a carbonyl group (C=O) bonded to two carbon atoms, ketones in true lavender are kept at trace levels to ensure the oil's safety, as they are known for their strong mucolytic and neuroactive potential [25, 26].

Oxides (1,8-cineole, < 2%): These are cyclic ethers formed through the oxidation of terpenes; they provide a refreshing, camphoraceous medicinal note and are often studied for their ability to enhance respiratory function [26].

Collectively, these compounds are highly lipophilic and exhibit excellent solubility in organic solvents such as alcohols and esters [24]. This chemical diversity allows lavender oil to interact effectively with various biological membranes, facilitating its widespread use in pharmacological and plant-protection research [13].

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II. Chapter II:

Biological Activity.

1. Antioxidant Activity:

1-1. Definition of the Active Antioxidant Compounds:

In many plants, approximately 80% of antioxidant compounds are phenolic [27], concentrated in the outer tissues [1] and responsible for plant pigmentation [27]. However, in the case of Lavender Essential Oil, the antioxidant activity is primarily driven by oxygenated monoterpenes [28] rather than heavy phenolic acids [1]. These volatile compounds are characterized by their ability to interact with free radicals and prevent oxidative damage to cells [29].

1-2. Monoterpenes and oxygenated monoterpenes:

1-2-1. Definition and Functional Groups:

Monoterpenes are a class of terpenes consisting of 10 carbon atoms, which result from the biochemical union of two five-carbon isoprene units [30, 31]. While basic monoterpenes are pure hydrocarbons with the molecular formula $C_{10}H_{16}$, oxygenated monoterpenes (often referred to as terpenoids) are derivatives that contain at least one oxygen atom within their chemical structure [31, 32].

The addition of oxygen allows for the formation of various functional groups that define the oil's therapeutic and aromatic profile:

Alcohols: Such as Linalool ($C_{10}H_{18}O$), which is a major constituent in lavender oil known for its sedative and antioxidant properties [31].

Esters: Such as Linalyl acetate, which is formed through the esterification of linalool and is highly characteristic of high-quality lavender oil [33].

Ketones: Such as Camphor ($C_{10}H_{16}O$), which contributes to the oil's analgesic and antiseptic effects [31].

Ethers/Oxides: Such as 1,8-Cineole ($C_{10}H_{18}O$), also known as eucalyptol, which is recognized for its anti-inflammatory and decongestant activities [31].

1-2-2. Chemical Characteristics:

Molecular Formula: Oxygenated monoterpenes generally follow the molecular pattern $C_{10}H_xO_y$, reflecting the modification of the C_{10} hydrocarbon base by oxygen-containing groups [31].

Biosynthesis: They are synthesized from two isoprene building blocks (C_5H_8) via the mevalonate or MEP pathways, typically forming the precursor geranyl pyrophosphate (GPP) [30, 32].

Volatility: These molecules are highly volatile at room temperature due to their low molecular weight and high vapor pressure, making them the primary constituents of steam-distilled essential oils [32, 33].

Solubility and Polarity: The presence of oxygen atoms makes these compounds more polar than their pure hydrocarbon counterparts (such as limonene or pinene). Consequently, oxygenated monoterpenes exhibit significantly higher water solubility and are often more biologically active due to their improved interaction with cellular targets [30].

1-3. Free Radicals:

1-3-1. Definition:

Free radicals are atoms or molecules containing unpaired electrons in their outer shell [34]. Because they lack a stable electron configuration, they are highly reactive, constantly seeking other molecules to bond with to achieve stability [35]. The most significant free radicals in living cells are derived from oxygen, known as Reactive Oxygen Species (ROS) [36].

1-3-2. Types and Sources:

Free radicals arise from normal metabolic processes and are derived from oxygen and nitrogen molecules [37]:

- **Reactive Oxygen Species (ROS):** Includes Singlet Oxygen (O_2),Superoxide ($O_2^{\bullet-}$), Hydroxyl radical ($OH^{\bullet}$), and Hydrogen Peroxide (H_2O_2) [38].
- **Reactive Nitrogen Species (RNS):** Includes Nitric Oxide ($NO^{\bullet}$) [35].

1-3-3. Sources:

- **Internal:** Immune cell activation and inflammation [37].
- **External:** Smoking, UV radiation, medications, and pesticides [36].

1-4. Antioxidants:

1-4-1. Definition:

Antioxidants are substances that delay, prevent, or remove oxidative damage to a target molecule [38]. They function either by directly scavenging oxidants or by indirectly

upregulating antioxidant defenses. A key characteristic of an effective antioxidant is its ability to form a stable new radical after scavenging, preventing further chain reactions [37].

1-4-2. Classification of Antioxidants:

Antioxidants are classified by their source [39]:

- **Natural Antioxidants:**
    - **Endogenous (Enzymatic):** The body's first line of defense, including Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) [35].
    - **Exogenous (Dietary):** Derived from food, such as Vitamin C, Vitamin E, Beta-carotene, and Zinc [37].
- **Synthetic Antioxidants:** Used in the food industry for cost-effectiveness, such as BHA (Butylated Hydroxyanisole), BHT (Butylated Hydroxytoluene), and Gallic Acid [39].

1-5. Evaluation of Antioxidant Activity:

There are three main types of methods for measuring antioxidant activity, which measure:

- Oxygen consumption [40].
- The resulting oxidized substance [41].
- Free radical absorbance or binding [42].

Among these methods or tests used for certain plant extracts, as well as natural and synthetic compounds, we mention:

- **DPPH assay** (Diphenyl picrylhydrazyl)

1-5-1. DPPH Assay (Diphenylpicrylhydrazyl):

This is one of the most widely used methods for testing antioxidant activity in plant samples [43]. This method relies on monitoring the ability of added substances (extracts) to scavenge or inhibit the formation of free radicals [42]. The DPPH radical absorbs at a specific wavelength around 517 nm, and through this, antioxidant activity can be determined by the decrease in this absorbance[40].

Results are reported as IC_{50} (or EC_{50}), which represents the amount of antioxidant required to reduce the initial DPPH concentration by 50% [42, 43].

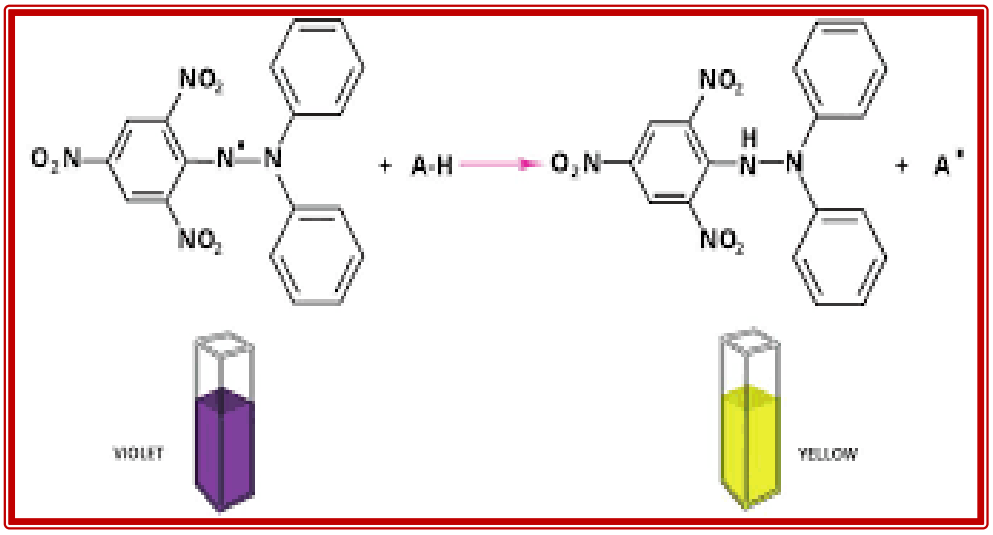

Figure II-1: Principles of the DPPH antioxidant activity test

2. Anti-denaturation Activity:

2-1. Protein Denaturation and Inflammation:

Denaturation of tissue proteins is one of the main causes of inflammation, which is a complex biological response in response to damaging stimulation. The loss of a protein's functional three-dimensional structure as an effect of heat, chemicals, or oxidative stress is known as denaturation [44]. Chronic inflammatory diseases result from the body's immune system attacking "auto-antigens," which are exposed throughout this process [45].

2-2. Mechanism of Protein Stabilization:

Potential anti-inflammatory agents are substances that can stop or slow down the denaturation of proteins [44]. The hydrogen, hydrophobic, disulfide, and electrostatic bonds that keep proteins stable can interact with some plant metabolites, such as oxygenated monoterpenes [46]. The plant oil helps the protein resists chemical or thermal stress by fortifying these bonds [47].

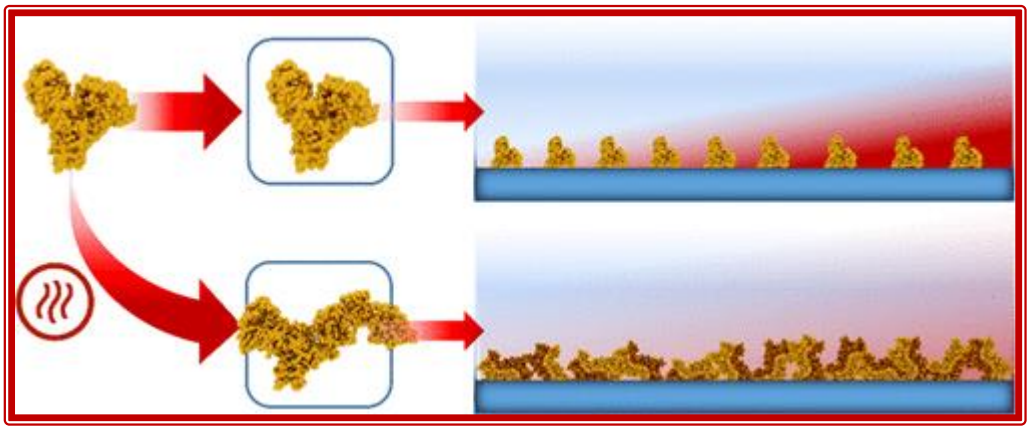

Figure II-2: BSA Response to Heat Stimulation [48]

2-3. Evaluation of Anti-Denaturation Activity:

2-3-1. BSA Heat-Induced Denaturation Assay:

Because it resembles human serum albumin, bovine serum albumin (BSA) is frequently employed as a model protein. In this experiment, BSA is heated under controlled conditions to cause denaturation, which results in a turbid (cloudy) solution. The effectiveness of the plant oil to prevent this turbidity is tested at different concentrations (50% to 1.56%).

An "*in vitro*" evaluation of the oil's anti-inflammatory capability is provided by calculating the percentage of inhibition by comparing the absorbance of the treated samples versus a control [49].

3. Hemolytic Activity:

3-1. Definition of Hemolysis and Biocompatibility:

Hemolysis is the process by which erythrocytes, commonly known as red blood cells, break down or are destroyed, releasing hemoglobin into the plasma [50]. A key variable in biocompatibility research is assessing a plant-derived product's hemolytic activity [51]. It makes sure that even while the bioactive compounds are efficient against pathogens, they will not cause toxic harm to the host's cellular components if they get into the systemic circulation [46].

3-2. Interaction with Erythrocyte Membranes:

Erythrocytes are frequently used as a model for cell membrane integrity. They are vulnerable to the volatile terpenes and surfactants present in essential oils due to the high lipid and protein content of their membranes. While low hemolytic activity indicates a high

safety profile, a high hemolytic percentage indicates that the oil might be too aggressive for some therapeutic uses [46].

3-3. Evaluation of Hemolytic Activity:

3-3-1. 96-Well Microplate Assay:

Using a Twofold Serial Dilution, this technique enables the quick screening of several concentrations [47]. Researchers can find the concentration at which 50% of the cells are lysed (HC50) by subjecting a suspension of erythrocytes to the plant oil (varying from 50% to 1.56%) [46, 52]. Spectrophotometry is used to assess the amount of liberated hemoglobin; the degree of cellular damage is directly correlated with the intensity of the red color in the supernatant [50].

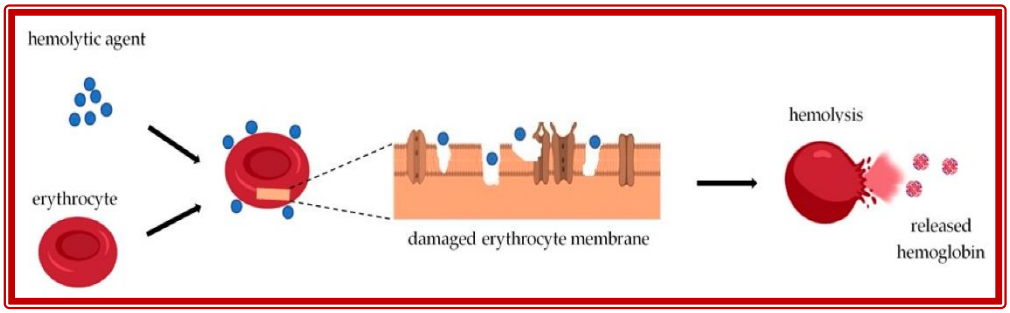

Figure II-3: Basic mechanism of hemolysis. [53]

4. Antibacterial Activity:

4-1. Definition of Antibacterial Activity:

The ability of a material to either kill bacteria (bactericidal) or prevent their growth and reproduction (bacteriostatic) is referred to as antibacterial activity. This activity is frequently a synergistic outcome of several secondary metabolites in the context of essential oils and plant-based ointments, especially oxygenated monoterpenes, which can pierce pathogens' lipid-rich cell walls [46].

4-2. Mechanisms of Action on Bacterial Cells:

The lipophilic trademark of plant oils is usually credited with their antibacterial effects. Such compounds pile up in the lipid bilayer of bacteria, resulting in:

Membrane Permeability: once the cell membrane becomes compromised, essential cytoplasmic constituents and ions like potassium will leak out [54].

Enzyme Interference: Disruption of the proton motive force and inhibition of membrane-bound enzymes [46].

Cell lysis is the ultimate collapse of the cell wall structure, especially in Gram-positive bacteria as they do not have the outer membrane that protects Gram-negative bacteria [54].

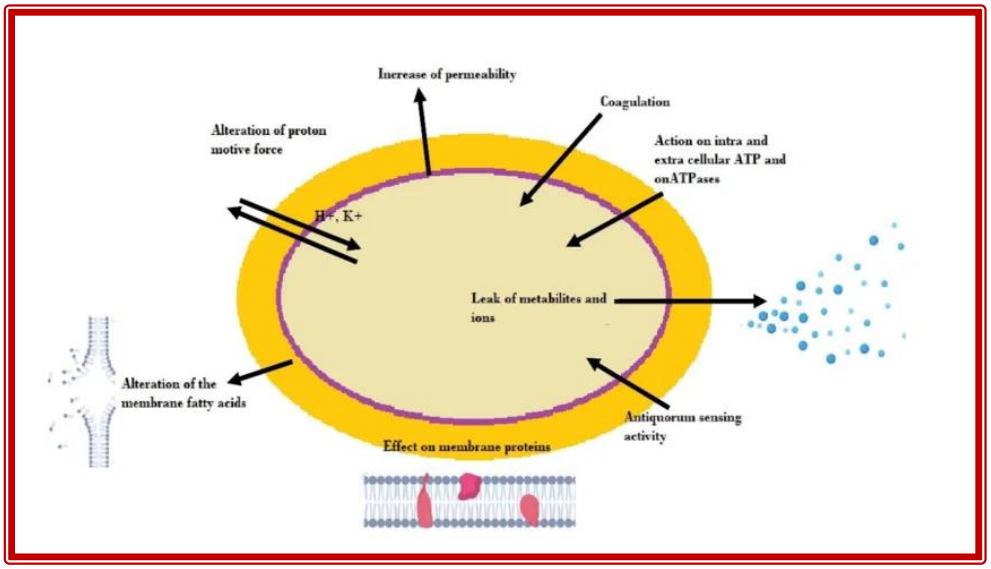

Figure II-4: Mechanisms of action of essential oil constituents on bacterial cells

[55]

4-3. Evaluation of Antibacterial Activity:

4-3-1. Agar Well Diffusion Method:

The agar well diffusion method is a standard "*in vitro*" screening tool [56]. An agar surface is covered with a standardized bacterial inoculum in this experiment. The test material (such as experimental ointment) is then poured into wells that have been punched into the agar [57]. A concentration gradient is produced as the active chemicals spread out radially throughout the gel [58]. The Zone of Inhibition (ZOI) is a circular region where bacterial growth is inhibited if the chemical is effective [59]. This zone's diameter, expressed in millimeters, provides a numerical representation of the antibacterial efficacy [60].

5. Anti-diabetic Activity:

5-1. Definition of Anti-diabetic Activity:

Anti-diabetic activity is a substance's ability to reduce blood glucose levels or increase insulin sensitivity [46]. Reducing postprandial (post-meal) hyperglycemia is one of the main methods for managing Type 2 diabetes [61]. This is frequently accomplished by inhibiting certain enzymes, such as alpha-amylase and alpha-glucosidase, which slows down the breakdown and absorption of carbohydrates in the digestive tract [62].

5-2. Mechanism of Enzyme Inhibition (alpha-amylase):

α-amylase is a significant enzyme present in both saliva and pancreatic juice, responsible for catalyzing the hydrolysis of starch into smaller oligosaccharides and maltose. Inhibiting this enzyme results in a delayed breakdown of complex carbohydrates, which consequently slows the rate of glucose absorption into the bloodstream.

Role of Plant Metabolites: Oxygenated monoterpenes and phenolic compounds have the ability to bind to either the active or allosteric sites of the alpha-amylase enzyme, thereby modifying its catalytic activity and hindering the processing of the substrate (starch).

5-3. Evaluation of Anti-diabetic Activity:

5-3-1. α-Amylase Inhibition Assay (Starch–Iodine Method):

A sensitive colorimetric technique for measuring the inhibitory potential of substances originating from plants, such as essential oils dissolved in DMSO. This technique is based on the unique chemical characteristic of iodine, which, when trapped inside the helical structure of amylose (starch), forms a deep blue/dark purple complex.

Principle of the Assay: The starch depletion is tracked by the test. The dark blue hue of the starch-iodine complex fades or vanishes as α-amylase hydrolyzes starch into smaller, non-complexing particles.

Mechanism of Inhibition: The α-amylase enzyme is bound by the bioactive components of the essential oil, such as oxygenated monoterpenes. Because of this inhibition, the starch cannot be broken down by the enzyme. As a result, the starch doesn't break down and can still combine with the iodine reagent to generate the dark blue complex.

Solubilization and Detection: Dimethyl sulfoxide (DMSO) is used as a co-solvent to provide a uniform mixture in the aqueous test environment because essential oils are hydrophobic. High-throughput screening across a concentration gradient (twofold dilution) is made possible by the reaction's execution in a 96-well microplate.

Interpretation: Since it demonstrates that the starch was shielded from enzymatic digestion, a stronger blue hue at the conclusion of the experiment suggests greater inhibitory efficacy. The results are usually computed as an IC_{50} value and presented as a percentage of inhibition.

6. *In vivo* Anti-diabetic Activity:

6-1. Mechanism of Alloxan-Induced Diabetes:

The glucose analogue alloxan (2,4,5,6-tetraoxypyrimidine) selectively targets and kills the pancreatic beta-cells that produce insulin [63]. There are two ways it works:

Selective Uptake: The GLUT2 glucose transporters in the plasma membrane of pancreatic β -cells effectively absorb alloxan because of its structural resemblance to glucose [64].

Redox Cycling and ROS Production: Once within the cell, alloxan goes through a "redox cycling" process with intracellular thiols (such as glutathione), producing a significant number of superoxide radicals and reactive oxygen species (ROS) [63], which then turn into hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). These are what destroys the DNA and cell membrane [64].

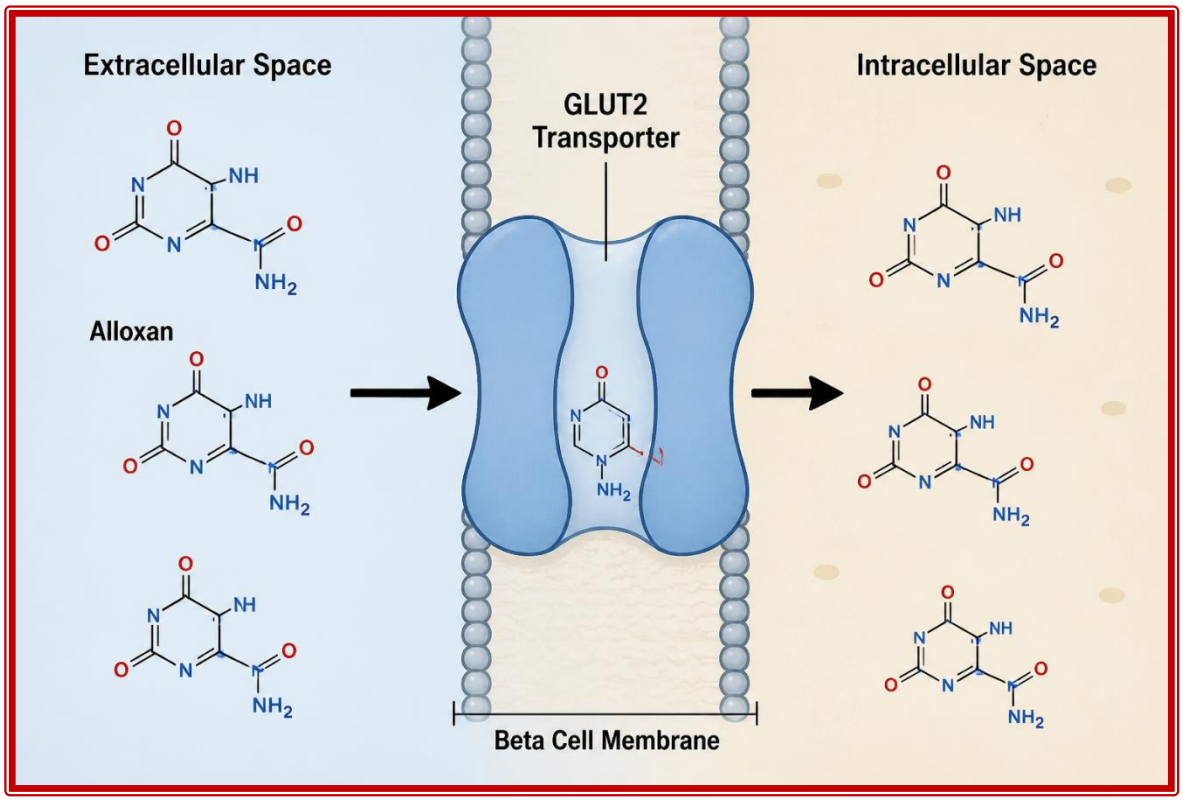

Figure II-1: Alloxan entry into pancreatic β-cells via the GLUT2 glucose transporter

6-2. Pancreatic β-cell Destruction:

The quick surge of ROS leads to DNA fragmentation and fast necrosis of β-cells [63]. The pancreas can no longer make and secrete insulin once these cells are damaged [64]. In the animal model, this results in a state of chronic hyperglycemia that resembles Type 1 Diabetes Mellitus [63, 65].

6-3. Physiological Indicators of Diabetes in the Model:

Theoretically, using alloxan to induce diabetes causes a number of noticeable physiological changes, including:

Hyperglycemia: High blood glucose levels [66, 67].

Polydipsia and Polyuria: Excessive thirst and frequent urination [67, 68].

Weight loss: When insulin-driven glucose absorption is absent, muscle and fat stores are broken down [66, 69].

6-4. Therapeutic Role of Essential Oils and Ointments:

The theory behind using the lavender-based products to treat diabetes brought on by alloxan is in two ways:

Hypoglycemic Activity: The potential for the components of the oil to enhance peripheral glucose consumption or stimulate residual β-cells [70].

Cytoprotective Effect: Since Alloxan acts through oxidative stress, the oil's antioxidant qualities may help shield the surviving pancreatic tissue from more oxidative damage [70].

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III. Chapter III:

Molecular docking and Pharmacokinetic Evaluation.

1. Principles of Molecular Docking:

1-1. Receptor-Ligand Interactions (Lock-and-Key vs. Induced Fit):

Molecular docking is an established bioinformatic modeling technique used to predict the preferred orientation of a small molecule (ligand) when bound to a biological macromolecule (receptor/protein) [71]. The fundamental objective of this computational tool is to optimize the spatial conformation and geometric fit of the ligand within the targeted binding pocket, thereby forming a stable complex.

The thermodynamic basis of this interaction relies on historical biochemical models. While Emil Fischer's classic "Lock-and-Key" model provides a foundational understanding of structural complementarity where the rigid ligand perfectly fits a rigid active site [72], modern docking applications utilize Daniel Koshland's "Induced Fit" theory. This principle recognizes that both the protein matrix and the chemical ligand possess structural flexibility. As a volatile constituent (such as a monoterpene from *Lavandula angustifolia*) approaches the catalytic site of a metabolic enzyme, minor conformational rearrangements occur in both entities to maximize favorable non-covalent interactions and minimize the overall free energy (ΔG) of the system [73].

1-2. Search Algorithms and the Lamarckian Genetic Approach:

To identify the optimal binding orientation (termed a "pose"), the docking software must explore the complex landscape of structural variables, including the ligand's translational, rotational, and internal torsional degrees of freedom. This multi-dimensional space is navigated using specialized computational routines known as Search Algorithms [74].

A highly effective and widely integrated search method in computational pharmacology is the Lamarckian Genetic Algorithm (LGA) [75]. This hybrid algorithm merges traditional Darwinian genetic principles with Lamarckian evolutionary theory, which asserts that characteristics acquired during an organism's lifetime can be passed down to its offspring.

- In a computational context, a "population" of diverse ligand conformations is generated.
- These conformations undergo simulated Darwinian evolutionary phases, including random mutations, structural crossovers, and rigorous selection based on fitness.

- Simultaneously, a localized Lamarckian "phenotypic" optimization (a local energy minimization search) is performed on each individual pose.

The optimized coordinates achieved via this local search are directly updated back into the ligand's genetic "genotype." This dual mechanism significantly accelerates the discovery of the global minimum energy state without trapping the simulation in unpromising local energy sub-basins.

1-3. Scoring Functions and Binding Affinity Thermodynamics:

Once the search algorithm populates a library of possible binding poses, the relative stability and biological relevance of each pose are calculated using mathematical equations called Scoring Functions. The primary metric yielded by these equations is the Binding Affinity [76], typically quantified in kilocalories per mole (kcal/mol).

The scoring function acts as a computational approximation of the physical free energy of binding ΔG_{bind} governed by standard laws of chemical thermodynamics:

$$\Delta G_{bind} = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{desolv} + \Delta G_{tors}$$

Where:

- ΔG_{vdw} represents steric van der Waals interactions.
- ΔG_{hbond} represents the directional stability of hydrogen bonds.
- ΔG_{elec} accounts for electrostatic Coulombic forces.
- ΔG_{desolv} calculates the thermodynamic gain when water molecules are displaced from the hydrophobic binding pocket.
- ΔG_{tors} applies an entropic penalty based on the number of rotatable bonds frozen during binding.

A more negative binding affinity value indicates a more spontaneous, stable, and highly favored interaction, implying a stronger prospective enzymatic inhibition *in vitro* [77].

1-4. Methodological Validation via Re-docking (RMSD Metric):

Because scoring functions and search grids rely on statistical approximations, a computational simulation cannot be trusted blindly without empirical validation. The gold standard for verifying the mathematical reliability of a specific docking environment is the Self-Docking (or Re-docking) Protocol.

In this validation process, an experimentally resolved 3D crystal structure containing a co-crystallized native drug/ligand is retrieved from the Protein Data Bank (PDB). The native ligand is computationally extracted from the active site, its conformational parameters are randomized, and it is docked back into the empty protein pocket using the exact algorithmic parameters intended for the experimental testing [78].

The spatial overlay between the newly predicted computational pose and the original crystallographic laboratory position is statistically measured using the Root-Mean-Square Deviation (RMSD) equation:

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

Where N is the total number of atoms and δ_i is the distance between atom i in the experimental structure and its corresponding position in the docked pose. An RMSD value below the standard threshold of 2.0 Å mathematically confirms that the scoring function and search algorithms can successfully reproduce authentic biological orientations [79].

2. Theoretical Principles of ADMET and Drug-Likeness:

2-1. The Concept of *In silico* ADMET Profiling:

In modern drug discovery and ethnopharmacological validation, evaluating therapeutic efficacy is insufficient without establishing a molecule's pharmacokinetic profile. Pharmacokinetics dictates how a biological system processes a foreign compound through four interconnected phases: Absorption, Distribution, Metabolism, and Excretion (ADME), alongside its overall Toxicity (T).

Historically, poor pharmacokinetic performance and unpredictable systemic toxicity were major causes of late-stage failures in drug development [80, 81]. *In silico* ADMET modeling utilizes computational algorithms, quantitative structure-activity relationships (QSAR), and machine learning topologies to predict these biological behaviors based entirely on a molecule’s structural and physicochemical descriptors [82]. This computational approach bypasses early, resource-intensive animal testing, allowing researchers to quickly screen volatile compounds such as those found in *Lavandula angustifolia* essential oil and assess their suitability for specialized delivery systems like topical ointments.

2-2. Physicochemical Descriptors and Lipinski's Rule of Five:

A molecule's capacity to act as an effective drug or active pharmaceutical ingredient (API) depends heavily on its fundamental structural parameters. To quantify this "drug-likeness," Christopher Lipinski established the Rule of Five (Ro5) [83], which sets empirical structural boundaries optimized for predicting oral bioavailability and membrane permeability. The rule dictates that a molecule is likely to exhibit good absorption and permeation if it meets the following criteria:

Molecular Weight (MW): Less than 500 g/mol. Smaller molecules possess lower steric hindrance, facilitating easier passive diffusion through cellular barriers.

Lipophilicity (Log P): An octanol-water partition coefficient of less than 5.

Hydrogen Bond Donors (HBD): Fewer than 5 (expressed as the total number of nitrogen-hydrogen and oxygen-hydrogen bonds).

Hydrogen Bond Acceptors (HBA): Fewer than 10 (expressed as the total number of nitrogen and oxygen atoms).

When a natural compound complies with Lipinski's boundaries, it indicates that the structural framework possesses favorable properties for crossing complex cell membranes.

2-3. Aqueous Solubility (Log S) and Partition Coefficient (Log P) Dynamics:

The therapeutic success of an active compound relies on a delicate thermodynamic balance between its solubility in water and its solubility in lipids.

Aqueous Solubility (Log S): Measures a compound's maximum dissolved concentration in an aqueous solvent at equilibrium. In biological environments, a compound must dissolve in fluid layers to reach target surfaces. Computational models like ESOL [84] and Ali [85] calculate solubility based on molecular topology and atom-type fragments. A low Log S indicates high hydrophobicity, which can entrap a molecule inside an ointment base, while a balanced Log S ensures proper release and dissolution.

Partition Coefficient (Log $P_{o/w}$): Quantifies the ratio of un-ionized compound concentrations between an organic phase (n-octanol) and an aqueous phase (water). Log P serves as a direct indicator of lipophilicity. High lipophilicity (Log P > 3) implies a strong affinity for lipid membranes, which is essential for penetrating lipid-rich biological tissue barriers, though excessively high values can result in systemic accumulation.

2-4. Topical Drug Delivery Barriers and Skin Permeability (K_p):

Because essential oils formulated into ointments are intended for topical applications, conventional oral absorption models must be supplemented with transdermal diffusion physics. The primary barrier to topical drug delivery is the stratum corneum, the lipid-dense, cornified outermost layer of the epidermis.

To model transdermal transport, the skin permeability coefficient (K_p, expressed in cm/s) is evaluated. The K_p mathematical model integrates a compound's molecular size and specific lipophilicity to predict the velocity of steady-state molecular flux across mammalian skin [86]. Highly lipophilic, low-molecular-weight volatile terpenes (such as monoterpenes) typically show favorable K_p coefficients, indicating they can cross the stratum corneum to interact with localized target enzymes or sub-dermal tissues.

2-5. Computational Toxicology and Safety Evaluation:

The final requirement of the ADMET profile is the evaluation of computational toxicological endpoints. *In silico* toxicology relies on pattern-recognition systems and structural alert libraries to identify molecular substructures associated with toxicity ("toxicophores") [87, 88]. Key predictive parameters include:

- *Ames Mutagenicity*: Predicts whether a molecule can cause genetic mutations by interacting with DNA [89].
- *Hepatotoxicity*: Evaluates potential disruption of hepatic functional pathways or metabolic cellular injury [90].
- *Skin Sensitization*: Evaluates the risk of inducing allergic contact dermatitis, an essential parameter for validating the safety of topical formulations [91].

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Part II:

Practical Part

IV. Chapter IV:

Materials and Methods.

1. Plant Material Collection and Preparation:

1-1. Collection:

The aerial flowering parts of *Lavandula angustifolia* were collected on April 12, 2025, in the region of El Oued, Algeria, during the peak flowering stage to ensure a high yield of bioactive secondary metabolites. To preserve the volatile profile, the plant material was gathered in the early morning hours (07:00-09:00 AM), before the increase in Saharan ambient temperature.

1-2. Preparation and Drying:

The collected flowers were cleaned and subjected to shade-drying at room temperature

(25 ± 2°C) for a period of 10 days. This controlled drying process was utilized to reduce moisture content while preventing the thermal degradation of the oxygenated monoterpenes identified in the oil. Once dried, the material was stored in paper bags in a cool, dark environment.

2. Extraction of the Essential Oil:

2-1. Hydro-distillation Protocol:

The essential oil (EO) was extracted by hydro-distillation using a Clevenger-type apparatus. A total of 50 g of dried flowering tops were immersed in 600 mL of distilled water in a 1L round-bottom flask.

2-2. Recovery and Storage:

The distillation process was conducted for 3 hours at a constant temperature. The obtained essential oil was separated from the aqueous phase and dried using Decantation where The distillate was allowed to stand until a clear interface formed between the upper organic layer (essential oil) and the lower aqueous layer, the bottom layer was disposed of in a beaker. The last layer meaning the oil was stored in amber glass vials at 4°C until its chemical characterization and biological evaluation.

2-3. Determination of Essential Oil Yield:

The yield was calculated based on the weight of the dry plant material (w/w) using the following formula:

$$Yield\ (\%) = \left(\frac{weight\ of\ the\ extracted\ oil\ (g)}{weight\ of\ dry\ plant\ material\ (g)}\right) \times 100$$

3. Chemical Characterization (GC-MS Analysis):

3-1. Instrumentation and Software:

The essential oils were analyzed using a Hewlett Packard 5973A gas chromatography system coupled to a mass spectrometer. The system was equipped with an apolar capillary column (HP-5MS) with dimensions of 30 m × 0.25 mm and a phase thickness of 0.25 µm

3-2. Chromatographic Conditions:

The analysis was conducted under the following optimized conditions:

Carrier Gas: Helium (He) was used as the mobile phase at a constant flow rate of 0.7 mL/min.

Oven Temperature Program: The initial temperature was held at 60°C for 8 minutes (isothermal). It was then increased at a rate of 2°C/min until reaching 280°C, where it remained for a final isothermal period of 15 minutes.

Injection: The injector temperature was maintained at 250°C. A volume of 1 µL of the diluted sample was injected using the split mode (1:20).

3-3. Mass Spectrometry Settings:

The mass spectrometer operated in the electronic impact (EI) detection mode with an ionization energy of 70 eV. The interface temperature was set to 280°C, and the source pressure was maintained at 10^{-7} mbar.

3-4. Identification of Constituents:

The identification of the volatile components was performed based on a multi-step approach:

Retention Indices (RI): a homologous series of *n*-alkanes (C_5-C_{28}) was analyzed under identical operative conditions on the HP-5 column.

The retention indices of the sample constituents were calculated according to the formula by [92].

Spectral Libraries: The resulting mass spectra were compared with authentic compound data from the Wiley version 7.0 library and fragmentation patterns reported in established literature, such as [93].

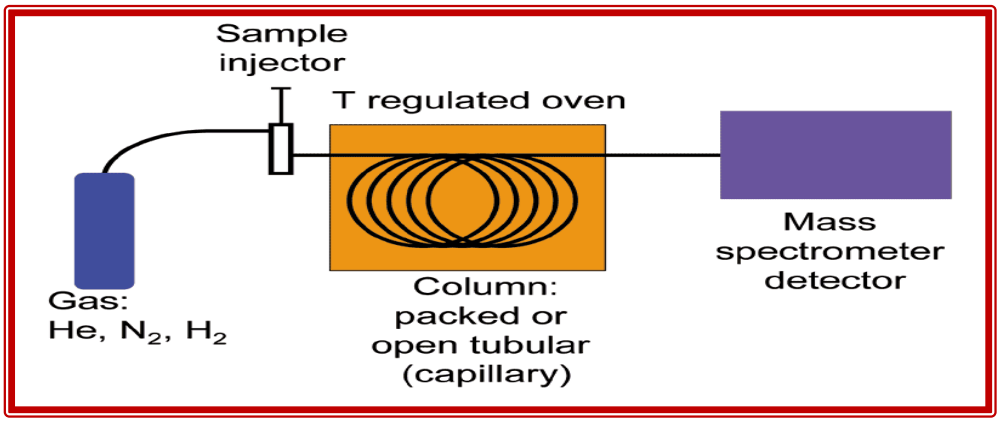

Figure IV-1: Schematic Diagram of a Gas Chromatography–Mass Spectrometry (GC–MS) System

4. *In vitro* Antioxidant Activity (DPPH Assay):

4-1. Reagents and Standards:

Radical Solution: A 0.02% (w/v) solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared using 99.5% ethanol.

Positive Controls: Butylated Hydroxytoluene (BHT) and Vitamin E (Vit. E) were employed as reference antioxidant standards.

Solvent: Analytical grade ethanol was used for all dilutions and as the blank.

4-2. Experimental Protocol:

The assay was performed following the method of [94] :

Sample Preparation: A volume of 500 µL of lavender essential oil (or reference standard) was mixed with 50 mL of ethanol and the prepared DPPH solution.

Incubation: The mixture was shaken vigorously and incubated in total darkness for 30 minutes at room temperature.

Measurement: After incubation, the absorbance was measured at 517 nm using a spectrophotometer.

Replication: All tests were conducted in triplicate (n=3), and results were expressed as the mean ± standard deviation.

4-3. Data Processing:

The percentage of DPPH radical-scavenging activity was determined using the following formula:

$$Radical\ Scavenging\ Activity\ (\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test sample. The IC_{50} values (the concentration required to inhibit 50% of the radicals) were calculated by projection on the concentration axis.

5. Anti-inflammatory Activity (BSA Anti-Denaturation Assay):

5-1. Reagent Preparation:

Protein Solution: A freshly prepared 0.3% (w/v) Bovine Serum Albumin (BSA) solution was used, dissolved in Tris-buffered saline (TBS, pH 6.4).

Sample Preparation: Due to the hydrophobic nature of the plant oil, it was first dissolved in hexane to ensure a homogeneous dispersion.

Serial Dilutions: A series of twofold dilutions were prepared to obtain six final concentrations: 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56% (v/v).

Controls: Aspirin served as the positive control. Distilled water mixed with BSA was used as a negative control, while hexane was used as a solvent control to ensure it did not interfere with protein stability.

5-2. Experimental Procedure:

The assay followed the methodology described by [49]:

Reaction Mixture: 1.0 mL of the 0.3% BSA solution was mixed with the appropriate volume of each oil dilution. The final volume was adjusted to 2.5 mL using distilled water.

Interaction Phase: The mixtures were initially incubated at 37°C for 15 minutes to allow for interaction between the protein and the oil components.

Thermal Stress: To induce controlled denaturation, the samples were heated at 70°C for 10 minutes.

Stabilization: The tubes were then cooled at room temperature for 20 minutes to stabilize turbidity.

5-3. Spectrophotometric Analysis:

Protein denaturation was quantified by measuring the absorbance at 660 nm. All measurements were performed in triplicate to ensure analytical reliability. The percentage of inhibition was calculated using the following equation:

$$\%\ inhibition\ = \left(\frac{A_0\ - A_s}{A_0}\right) \times 100$$

Where A_0 is the absorbance of the negative control and A_s is the absorbance in the presence of the plant oil.

6. *In vitro* Hemolytic Activity (96-Well Microplate Assay):

6-1. Preparation of Erythrocyte Suspension:

Collection: Fresh human blood was collected in heparinized tubes.

Isolation: Erythrocytes were isolated by centrifugation and washed three times with sterile phosphate-buffered saline (PBS 1X) at 1000 × g for 5 minutes to remove plasma and residual anticoagulants.

Suspension: The washed cells were resuspended in PBS to obtain a homogeneous suspension adjusted to a final hematocrit of 2%.

6-2. Sample Preparation and Serial Dilution:

Solubilization: The essential oil was first dissolved in a small volume of a compatible solvent (such as PBS containing a minimal percentage of DMSO or ethanol not exceeding 1%) to ensure proper dispersion.

Dilutions: Serial twofold dilutions were prepared to obtain six final concentrations (v/v): 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56%.

6-3. Experimental Procedure:

The assay was conducted following the method of [52]:

Reaction Mixture: 100 µL of the 2% erythrocyte suspension was added to each well of a sterile 96-well microplate, followed by 100 µL of the corresponding oil dilution.

Controls: Hydrogen peroxide (H_2O_2, 0.1%) was used as a positive control to induce complete hemolysis, while PBS 1X served as the negative control.

Incubation: The plate was incubated at 37 °C for 1 hour under gentle agitation.

Separation: After incubation, the plate was centrifuged at 1000 × g for 5 minutes to sediment intact erythrocytes.

6-4. Measurement and Calculation:

Detection: 100 µL of the supernatant from each well was transferred to a clean microplate, and the absorbance of released hemoglobin was measured at 540 nm using a microplate reader.

Equation: The percentage of hemolysis was calculated as follows:

$$Hemolysis\ (\%) = \left[\frac{A_s - A_n}{A_p - A_n}\right]$$

Where A_s, A_n, and A_p represent the absorbance of the sample, negative control, and positive control, respectively. All tests were performed in triplicate

7. *In vitro* Antibacterial Activity (Agar Well Diffusion Method):

7.1. Bacterial Strains and Inoculum Preparation:

The antibacterial efficacy of the experimental ointment was evaluated against two reference bacterial strains:

- Gram-negative: Escherichia coli (ATCC 25922).

- Gram-positive: Staphylococcus aureus (ATCC 25923).

Fresh bacterial cultures (18–24 hours old) were used to prepare inocula . The turbidity was adjusted to a 0.5 McFarland standard, corresponding to approximately 10^6 CFU/mL .

7.2. Media and Inoculation:

Mueller–Hinton agar plates were used for the assay. Sterile cotton swabs were employed to evenly spread the bacterial suspension across the agar surface to create a uniform bacterial lawn. The plates were allowed to dry under aseptic conditions before proceeding.

7.3. Experimental Procedure:

The assay followed the established agar well diffusion method [58, 95]:

Sample Preparation: The ointment was first dissolved in hexane to create a homogeneous stock solution, ensuring the active compounds could diffuse properly through the agar.

Dilutions: Four final concentrations were prepared: 50%, 25%, 10%, and 5% (v/v).

Well Application: Wells of 6 mm diameter were punched into the agar using a sterile cork borer. For each test, 50 µL of the prepared ointment solution was introduced into the corresponding wells.

Controls: Pure hexane was used as a negative control to ensure the solvent itself did not have intrinsic antibacterial effects.

Incubation: The plates were incubated at 37 °C for 24 hours under aerobic conditions to allow for bacterial growth and radial diffusion of the formulation.

7.4. Determination of Antibacterial Activity:

After the incubation period, the diameter of the Zones of Inhibition (ZOI) around each well was measured using a digital caliper or calibrated ruler. According to standard guidelines, zones greater than 6 mm (the diameter of the well) were considered indicative of antibacterial activity

8. *In vitro* Anti-diabetic Activity (α-Amylase Inhibition Assay):

8-1. Preparation of Reagents:

Phosphate Buffer: A 0.02 M sodium phosphate buffer (pH 6.9) containing 0.006 M NaCl was prepared to maintain physiological conditions for the enzyme.

Enzyme Solution: Porcine pancreatic α-amylase was dissolved in the phosphate buffer to achieve a final concentration of 2 U/mL .

Substrate Solution: A 1% (w/v) starch solution was prepared in the phosphate buffer.

Stop Reagent: A 1 M Hydrochloric acid (HCl} solution was used to terminate enzymatic activity.

Coloring Reagent: An Iodine/Potassium Iodide (I_2/KI} solution (prepared as 5 mM I_2 and 5 mM KI) was utilized for color development.

Samples and Standards: The lavender essential oil was prepared in serial dilutions using DMSO as the vehicle. Acarbose was prepared under identical conditions to serve as the positive reference standard

.8-2. Experimental Procedure :

The α-amylase inhibitory activity was evaluated using the starch–iodine colorimetric method where :

Pre-incubation of Enzyme and Sample: Twenty microliters (20 µL) of the sample solution (or Acarbose / DMSO vehicle control) was mixed with 20 µL of α-amylase enzyme solution. The reaction mixture was incubated at 37 °C for 10 minutes to allow interaction between the enzyme and the inhibitor.

Starch Addition and Incubation: After pre-incubation, 40 µL of 1% starch solution was added to each well to initiate the enzymatic reaction. The mixtures were further incubated at 37 °C for 30 minutes to allow starch hydrolysis.

Enzyme Inactivation: Following incubation, 80 µL of 1 M HCl was added to each well to terminate the enzymatic reaction.

Color Development: Finally, 100 µL of I_2/KI solution was added to each well for color development of the residual starch prior to absorbance measurement.

8.3. Spectrophotometric Measurement and Calculations:

The absorbance of each well was recorded at 580 nm using a UV-Vis spectrophotometer. The percentage of α-amylase enzyme inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \left[\frac{Abs\ Essential\ Oil - Abs\ Control}{Abs\ Essential\ Oil}\right] \times 100$$

Where:

Abs Essential Oil: is the absorbance of the reaction mixture containing lavender essential oil and α-amylase enzyme.

Abs Control: is the absorbance of the control reaction without inhibitor.

9. Molecular Docking Study:

9-1. Protein Preparation:

The crystal structures of the target proteins α-Amylase (4GQR) [96] and α- Glucosidase (3W37) were retrieved from the RCSB Protein Data Bank with a resolution of 1.20 Å and 1.70 Å respectively.

Protein preparation was conducted using AutoDockTools (v1.5.6) [97].it involved the removal of crystallographic water molecules, co-crystallized ligands, and heteroatoms. Polar hydrogen atoms were added, and Kollman charges were assigned to the protein residues. The ligands (Linalool and Linalyl Acetate) were sketched and optimized for 3D geometry, followed by the assignment of Gasteiger charges.

9-2. Docking Procedure:

Grid Box Generation: A grid box was centered on the active site of the enzyme (where the natural substrate, starch, usually binds).

Simulation: Each lavender component was "docked" into the enzyme to calculate the Binding Affinity (kcal/mol).

Analysis: The interaction types (Hydrogen bonds, Hydrophobic interactions, and Pi-stacking) were visualized to determine which compound had the strongest inhibitory potential.

9-3. Grid Box and Docking Parameters :

To target the active site of each enzyme, a grid box was defined with the following dimensions and coordinates:

Target Enzyme	PDB ID	Grid Center (X, Y, Z)	Grid Size (Points)
α-Amylase	4GQR	12.115 ; 14.906 ; 39.796	40×40×40
α- Glucosidase	3W37	0.31 ; -2.052 ; -21.786	60×60×60

Table IV-1: Docking Grid Configuration Parameters

The docking simulations were calculated using the Lamarckian Genetic Algorithm (LGA). For each ligand-protein complex, 15 independent docking runs were executed with a population size of 150 and a maximum of 2,500,000 energy evaluations. For α-glucosidase, 20 independent docking runs were executed to obtain a valid re-docking protocol validation value

9-4. Protocol Validation (Re-docking):

Before analyzing the lavender oil components, the docking protocol was validated via self-docking (re-docking). The native co-crystallized ligands Acarbose (3W37) and Myricetin (4GQR), were extracted and re-docked into their respective binding pockets.

The accuracy of the docking was assessed using the Root Mean Square Deviation (RMSD) between the predicted position and the original experimental crystal position.

10. Pharmacokinetic and ADMET Evaluation:

To ensure our lavender oil is safe and effective as a topical ointment, an ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile was generated for the major bioactive compounds.

10-1. Methodology:

The Simplified Molecular Input Line Entry System (SMILES) strings of the major compounds were entered into the SwissADME platform [98] and pkCSM web servers [99].

10.2. Evaluated Parameters:

Physicochemical Properties: Molecular weight, Lipophilicity LogP,and Solubility LogS.

Pharmacokinetics: * GI Absorption: High absorption is expected for small terpenes.

Skin Permeability (Kp): for validating the ointment's effectiveness through the skin.

BBB Permeability: To check if the oil has neuro-sedative potential.

The Brain Or IntestinaL EstimateD permeation method (BOILED-Egg model): : was used to predict gastrointestinal absorption, blood–brain barrier permeability, and P-glycoprotein substrate properties of the major lavender essential oil constituents.

Drug-likeness: Evaluated according to Lipinski’s Rule of Five (ensuring the molecules are "drug-like").

Toxicity Profile: Evaluation of Hepatotoxicity, Skin Sensitization, and LD50 values to ensure the ointment is non-toxic.

11. *In vivo* evaluation of the antidiabetic activity:

11-1. Experimental Animals :

Figure IV-2: Male Wistar rats (Rattus norvegicus) used in the experimental study

Adult male Wistar rats (95–175 g) were obtained from the institutional animal facility and acclimatized for one week before experimentation. Animals were maintained under standard laboratory conditions (22 ± 2 °C, relative humidity 50–60%, and a 12 h light/dark cycle) with free access to a standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals and complied with internationally accepted principles for animal research [100].

11-2. Induction of Diabetes :

Diabetes was induced in the experiment through a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight), freshly dissolved in cold sterile 0.9% saline. Alloxan causes diabetes by selectively destroying pancreatic β-cells via reactive oxygen species, resulting in insulin deficiency and high blood sugar. To avoid initial hypoglycemic shock, animals had free access to a 10% glucose solution for 24 hours after injection. Fasting blood glucose levels were measured after 72 hours with a portable glucometer (Accu-Chek®, Roche, Germany). Rats with fasting blood glucose levels of 250 mg/dL or higher (about 13.9 mmol/L) were deemed diabetic and included in the study.

Figure IV-3: Alloxan Injection for Diabetes Induction in Wistar Rat

11-3. Acute toxicity test :

Lavandula angustifolia essential oil was administered to rats orally at varying doses (25, 50, 75, and 100 mg/kg body weight). Then, the animals were monitored for 72 hours for any signs of abnormal behavior or mild allergic reactions, and subsequently observed daily for 21 days to assess delayed toxicological effects. No *mortality* or *behavioral abnormalities* were detected at any tested dose, including the highest dose tested, *100 mg/kg body weight*.

11-4. Experimental Design and Treatment :

After induction and confirmation of diabetes, rats were randomly assigned to four groups (n = 5 per group):

(i) Normal control (N): non-diabetic rats receiving vehicle (distilled water) orally (gavage) once daily for 28 days.

(ii) Diabetic control / Negative control (NC): diabetic rats receiving vehicle (distilled water) orally (gavage) once daily for 28 days.

(iii) Positive control (PC): diabetic rats treated with metformin (125 mg/kg/day, p.o.) once daily for 28 days.

(iv) Treatment (T): diabetic rats treated with water based *Lavandula angustifolia* essential oil solution (100 mg/kg/day, p.o.) once daily for 28 consecutive days.

The selection of metformin as the reference drug was based on the protocol described by [101], and the doses of *Lavandula angustifolia* essential oil were determined by the results of the acute toxicity test, which confirmed the safety of the selected concentrations.

Treatments were prepared fresh and given consistently at the same time each day. Throughout the study, body weight, food intake, and water consumption were recorded weekly to monitor metabolic health and potential treatment-related toxicity.

11-5. Biochemical analysis

At the end of the treatment period, animals were fasted overnight, and blood samples were collected via tail vein puncture under light anesthesia. Fasting blood glucose (FBG) was measured immediately with a calibrated glucometer. Glycated hemoglobin (HbA1c) was determined using a commercial diagnostic kit according to the manufacturer's instructions. Liver function biomarkers, including alanine aminotransferase

(ALAT) and aspartate aminotransferase (ASAT), were quantified using standard enzymatic assay kits to assess hepatic safety and the treatment's metabolic impact.

11-6. Histological preparation of liver, pancreas, and kidney

At the end of the treatment period, rats were euthanized under deep anesthesia in accordance with institutional animal care guidelines.

Figure IV-4: Blood and organ collection procedure in experimental rats, showing sample

The liver, pancreas, and kidneys were immediately excised, gently rinsed in cold normal saline to remove residual blood, and trimmed into representative tissue pieces (~3–5 mm thick). Specimens were fixed in 10% neutral buffered formalin for 24–48 h at room temperature.

Figure IV-5:Preparation of Tissue Samples for Histopathological Analysis

After fixation, tissues were processed by routine paraffin histology: dehydration through graded ethanol, clearing in xylene, and embedding in paraffin wax. Paraffin blocks were sectioned at 4–5 µm on a rotary microtome. Sections were mounted on glass slides, dried, deparaffinized in xylene, and rehydrated through descending ethanol concentrations to water. Slides were stained with hematoxylin and eosin (H&E), then dehydrated, cleared, and cover-slipped with a permanent mounting medium. Stained sections were examined by light microscopy for histoarchitecture and pathological alterations, and representative photomicrographs were obtained.

Figure IV-6: Histological processing of liver, pancreas, and kidney tissues including fixation, dehydration, embedding, sectioning, and H&E staining

11-7. Statistical analysis

All results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Tukey's multiple-comparison post hoc test to assess significant differences among groups. A p-value < 0.05 was considered statistically significant.

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V. Chapter V:

Results and Discussion.

1. Extraction of the Essential Oil:

Hydro-distillation of 50 g of dried flowering aerial parts of *Lavandula angustifolia* collected from El Oued, Algeria yielded 1.158 g of essential oil. The extraction yield was calculated according to the following equation:

$$Yield\ (\%) = \left(\frac{weight\ of\ the\ extracted\ oil\ (g)}{weight\ of\ dry\ plant\ material\ (g)}\right) \times 100$$

Using the obtained values:

$$Yield\ (\%) = \left(\frac{1.158}{50}\right) \times 100$$

$$Yield\ (\%) = 2.316\ \%$$

The obtained yield (2.316%) indicates an appreciable recovery of essential oil from Lavandula angustifolia. This yield falls within the range commonly reported for lavender essential oils obtained by hydro-distillation. Variations in essential oil yield are generally influenced by several factors, including geographical origin, climatic conditions, harvesting period, drying conditions, and extraction methodology.

The relatively favorable yield observed in the present study may be associated with the arid Saharan climate of El Oued, characterized by high solar exposure and elevated temperatures, which can stimulate the biosynthesis and accumulation of volatile secondary metabolites in aromatic plants.

2. Chemical Characterization (GC-MS):

Rank	Component	%	Rank	Component	%	Rank	Component	%
01	**Linalool**	39.7	14	Nerol oxide	1.4	27	Cubenol	0.2
02	**Linalyl acetate**	9.7	15	Hexyl tiglate	0.9	28	α-pinene	0.1
03	**α-terpineol**	8.7	16	cis-Geraniol	0.8	29	Hexyl isovalerate	0.1
04	**Camphor**	8.3	17	α-bisabolol	0.7	30	Estragole	0.1
05	**1,8-cineole**	5.4	18	Caryophyllene oxide	0.7	31	Hexyl caproate	0.1
06	**endo-Borneol**	5.0	19	β-caryophyllene	0.5	32	γ-muurolene	0.1
07	**Geranyl acetate**	3.3	20	Hexyl acetate	0.4	33	γ-eudesmol	0.1
08	**cis-Linalool oxide**	3.0	21	trans-Linalool oxide	0.4	34	tau-cadinol	0.1
09	**Nerol acetate**	2.1	22	cis-β-farnesene	0.3	35	α-bisabolol ox. B	0.1
10	**Lavandulol**	2.0	23	Lavandulol isovalerate	0.3	36	Humulene	0.0
11	β-myrcene	1.7	24	Camphene	0.2	37	Ledol	0.0
12	α-ocimene	1.5	25	Hexyl isobutyrate	0.2			
13	Hotrienol	1.5	26	L-bornyl acetate	0.2			
TOTAL							100,0	

Table V-1: Chemical Composition and Relative Percentage of *Lavandula angustifolia* Essential Oil by GC-MS

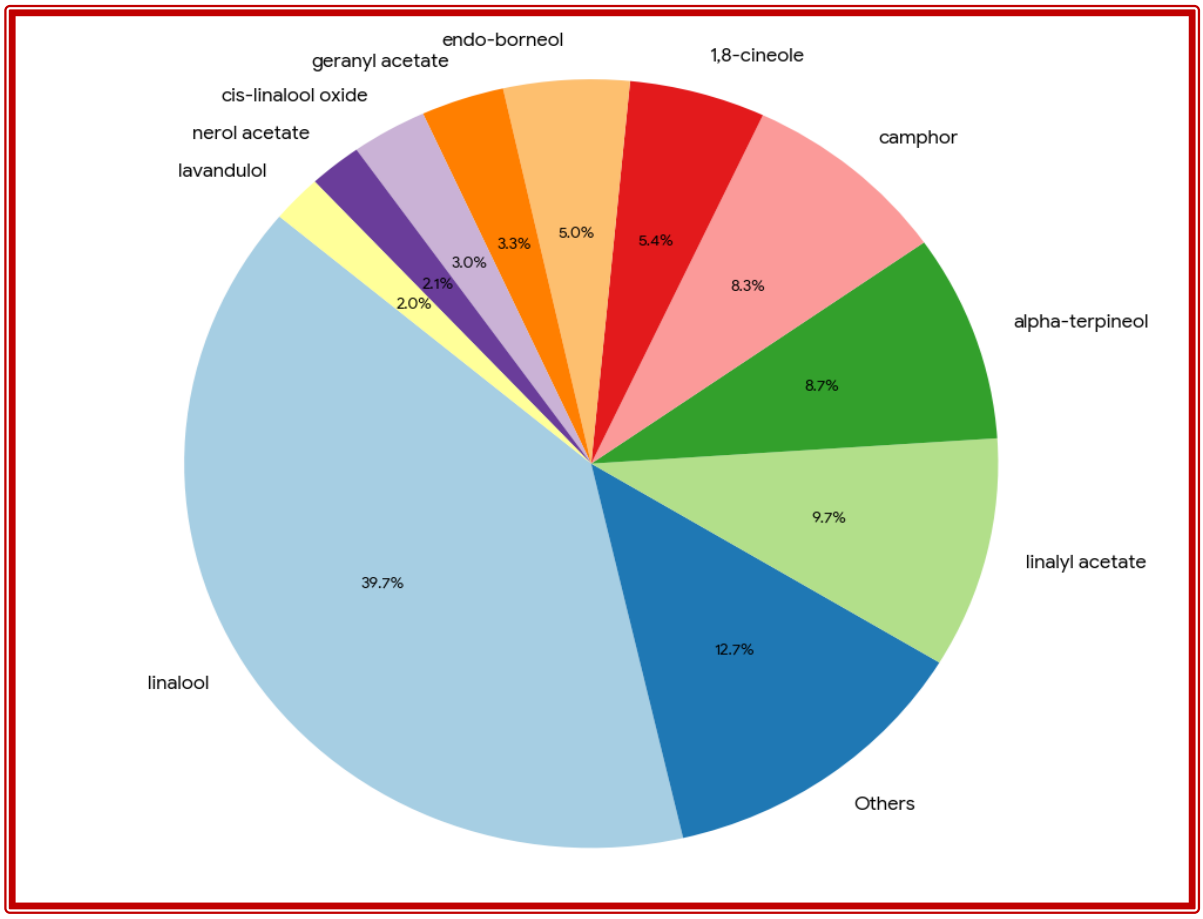

Figure V-1: Relative abundance (%) of chemical components in the Lavender essential oil sample.

GC–MS analysis of the essential oil extracted from *Lavandula angustifolia* revealed a complex phytochemical profile dominated primarily by oxygenated monoterpenes. A total of 37 volatile constituents were identified, representing 100% of the total oil composition.

The major compounds detected were linalool (39.7%), linalyl acetate (9.7%), α-terpineol (8.7%), camphor (8.3%), 1,8-cineole (5.4%), and endo-borneol (5.0%). Smaller amounts of geranyl acetate (3.3%), cis-linalool oxide (3.0%), nerol acetate (2.1%), and lavandulol (2.0%) were also identified. The predominance of linalool and linalyl acetate is considered a characteristic chemical marker of high-quality *Lavandula angustifolia* essential oil.

The obtained phytochemical composition is generally consistent with previous reports describing lavender essential oils rich in oxygenated monoterpenes, particularly linalool and linalyl acetate. These compounds are widely recognized for their

antimicrobial, antioxidant, anti-inflammatory, and enzyme inhibitory activities, which may explain the biological activities observed in the present study.

Linalool, the predominant constituent identified in the present oil sample, has been extensively reported to possess strong antimicrobial and anti-inflammatory properties. Likewise, linalyl acetate contributes significantly to the characteristic aroma and pharmacological activity of lavender essential oil. The relatively elevated percentages of α-terpineol, camphor, and 1,8-cineole may further enhance the biological effectiveness of the oil through synergistic interactions between volatile constituents.

Variations in essential oil composition are commonly influenced by several environmental and technical factors, including geographical origin, climatic conditions, harvesting period, drying conditions, plant developmental stage, and extraction methodology. In the present study, the lavender plants were collected from El Oued, Algeria, a Saharan region characterized by high temperatures, intense solar radiation, and low humidity. These abiotic conditions may stimulate terpene biosynthesis and accumulation, thereby influencing both the qualitative and quantitative composition of the essential oil.

Overall, the GC–MS profile obtained in this study confirms that the extracted lavender essential oil is particularly rich in bioactive oxygenated monoterpenes, which may contribute substantially to its observed antimicrobial and α-amylase inhibitory activities.

3. *In vitro* Antioxidant Activity (DPPH Assay):

We studied the antioxidant capacity of the *lavender* essential oil with two-components references, namely BHT and Vit. E, the study has been achieved via the method of free-radical DPPH trapping, which has been explained previously. The absorbance measurement of this radical was done at the length of 517nm wave of the ethanolic solution at different concentrations of the sample with a repetition of three times for each concentration yielding absorbance rate at the end. The results are described below.

Lavender	C(µg/ml)	500	1000	10000	70000	1400000	280000
	I (%)	4.22±0.025	5±0.03	7.6±0.045	34.2±0.2025	54.13±0.32	75±0.444

Table V-2: The changes of the free radical DPPH inhibitory rate in relation with the lavender concentration.

Reference compounds	C (µg/ml)	5	10	25	50	100	200	500
BHT	I (%)	0.5± 0.001	1± 0.003	3.4± 0.002	7.8± 0.008	18.5± 0.002	51.3± 0.004	67± 0.007
Vit. E		15.4± 0.016	31± 0.002	79.5± 0.008	89.9± 0.0014	93.1± 0.0014	91.1± 0.008	-

Table V-3: The changes of the free radical DPPH inhibitory rate in relation with the BHT and Vit. E concentration.

Where: C(µg/ml) equals the studied sample concentration in each test tube.

I (%): the inhibitory rate

From the two tables (VI-2, VI-3) we fix the inhibitory concentration of the rate 50% of the DPPH free radical for each studied sample by the projection on the concentration axis. The findings are shown as following.

HE	IC_{50} (µg/ml)	References	IC_{50} (µg/ml)
lavender	**120500**	BHT	180
		Vit. E	15

Table V-4: The values of the lowest inhibitory concentration IC50 for lavender and the two references BHT and Vit. E.

From the previous figures, it is shown that the inhibition ratio I (%) is increasingly growing with the increase of the inhibitory sample concentration. It is also noted that from the figures of the reference components that the inhibitory rate changes are high at low concentrations, which shows the antioxidant capacity of these materials that can be arranged according to their strength as follows Vit. E and BHT. However, we note that for the essential oil inhibition ratio increases when the changes in concentrations are significant, indicating the weakness of the antioxidant capacity of this essential oil for lavender

4. Anti-inflammatory Activity (BSA Anti-Denaturation Assay):

The anti-inflammatory activity of *Lavandula angustifolia* essential oil was evaluated using the BSA heat-induced protein denaturation assay. Protein denaturation is recognized as one of the major mechanisms involved in inflammatory disorders, and compounds capable of preventing thermal protein denaturation may possess significant anti-inflammatory potential.

Concentration (% v/v)	Mean Absorbance (660 nm)	% Inhibition
50	2.22	14.20%
25	2.25	13.00%
12.5	2.32	10.30%
6.25	2.39	7.60%
3.125	2.38	8.00%
1.5625	2.53	2.20%
Positive control (Aspirin)	0.83	67.90%
Negative control	2.59	0%

Table V-5: Effect of *Lavandula angustifolia* Essential Oil on Heat-Induced BSA Denaturation at Various Concentrations

Concentration	Mean Abs (660 nm)	% Inhibition (Aspirin)
40 mg/mL	0.308	86.20%
20 mg/mL	0.369	83.40%
10 mg/mL	0.637	71.40%
5 mg/mL	1.064	52.20%
2.5 mg/mL	1.624	27.10%
1.25 mg/mL	1.809	18.80%

Table V-6: Effect of Aspirin on Heat-Induced BSA Denaturation at Various Concentrations

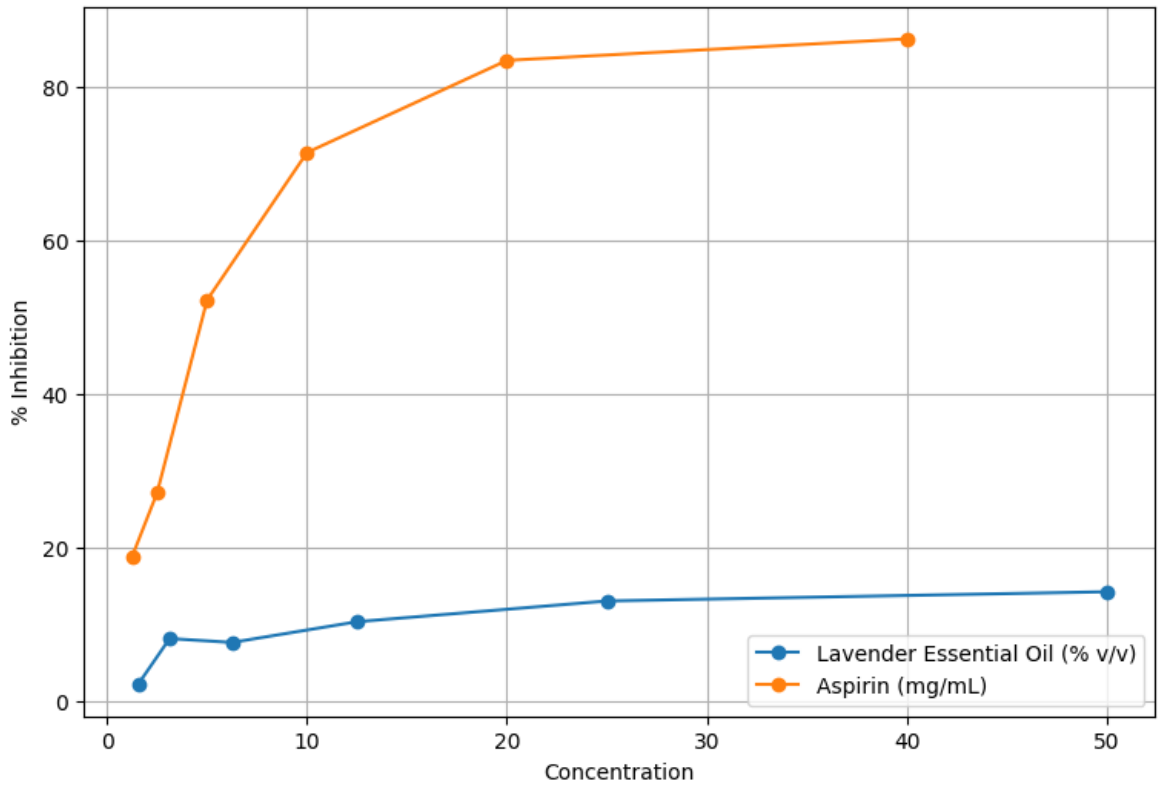

Figure V-2: In vitro Anti-Denaturation Activity of Lavender Essential Oil and Aspirin Using the BSA Heat-Induced Protein Denaturation Assay

The obtained results demonstrated that the lavender essential oil exhibited a concentration-dependent inhibitory effect against heat-induced BSA denaturation. The highest inhibition percentage was observed at 50% (v/v), reaching 14.2%, whereas the lowest activity was recorded at 1.56% (v/v) with an inhibition value of 2.2%. Intermediate concentrations showed moderate inhibitory effects ranging from 7.6% to 13.0%.

In comparison, the positive control aspirin exhibited substantially stronger anti-denaturation activity, with inhibition percentages ranging from 18.8% at 1.25 mg/mL to 86.2% at 40 mg/mL. This difference was expected because aspirin is a well-established nonsteroidal anti-inflammatory drug (NSAID) specifically designed to inhibit inflammatory pathways.

Although the inhibitory activity of the essential oil remained lower than that of aspirin, the observed effect still suggests the presence of bioactive volatile compounds capable of stabilizing protein structures under thermal stress conditions. The anti-inflammatory activity may be attributed mainly to the high abundance of oxygenated monoterpenes identified by GC–MS analysis, particularly linalool, linalyl acetate, α-terpineol, camphor, and 1,8-cineole. These compounds have previously been reported to

possess anti-inflammatory and antioxidant properties through membrane stabilization and modulation of inflammatory mediators.

The gradual increase in inhibition percentage with increasing oil concentration indicates a dose-dependent relationship, suggesting that higher concentrations of the essential oil provide greater protection against protein denaturation. However, the relatively moderate inhibition values compared with aspirin may be related to the hydrophobic nature of essential oils, limited solubility in aqueous media, or the lower potency of natural volatile compounds relative to synthetic anti-inflammatory drugs.

5. *In vitro* Hemolytic Activity (96-Well Microplate Assay):

The hemolytic activity assay was performed to evaluate the potential cytotoxic effect of *Lavandula angustifolia* essential oil on human erythrocyte membranes. Hemolysis reflects the disruption of red blood cell membrane integrity leading to the release of intracellular hemoglobin, and it is commonly used as an indicator of membrane toxicity and biocompatibility.

Concentration (mg/mL)	Hemolysis (%)
40	86.1 ± 2.8
20	75.6 ± 4.4
10	80.6 ± 4.8
5	9.6 ± 3.0
2.5	9.5 ± 3.9
1.25	8.2 ± 4.9
Positive control	100.0 ± 0.0
Negative control	0.0 ± 0.0

Table V-7: Hemolysis percentage of Eol (mean ± SD).

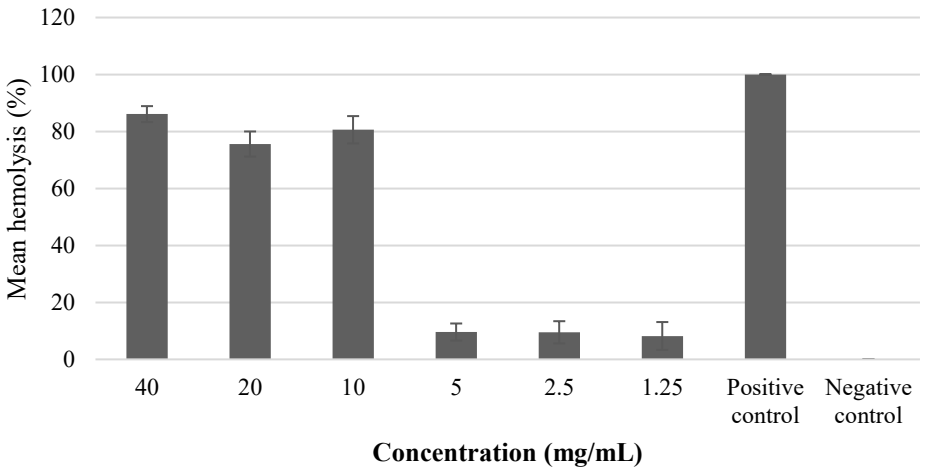

Figure V-3: Hemolytic activity of the Eol at different concentrations. Bars represent mean hemolysis percentage, and error bars indicate standard deviation (n = 3).

The obtained results demonstrated a concentration-dependent hemolytic effect of the essential oil. At high concentrations, the oil exhibited pronounced erythrocyte membrane disruption, with hemolysis percentages reaching 86.1 ± 2.8% at 40 mg/mL, 80.6 ± 4.8% at 10 mg/mL, and 75.6 ± 4.4% at 20 mg/mL. In contrast, lower concentrations (5, 2.5, and 1.25 mg/mL) produced markedly reduced hemolytic activity, with values below 10%.

The positive control (H_2O_2) induced complete hemolysis (100%), confirming the validity and sensitivity of the assay system, whereas the negative control (PBS) showed no detectable hemolysis. The relatively low hemolytic activity observed at concentrations ≤ 5 mg/mL suggests that diluted lavender essential oil may exhibit acceptable membrane compatibility under these experimental conditions.

The observed hemolytic activity at elevated concentrations may be associated with the lipophilic nature of essential oil constituents, particularly oxygenated monoterpenes such as linalool, camphor, α-terpineol, and 1,8-cineole identified by GC–MS analysis. These compounds can interact with phospholipid bilayers and alter membrane permeability, leading to erythrocyte destabilization and leakage of hemoglobin.

Interestingly, the results indicate a sharp reduction in hemolysis between 10 mg/mL and 5 mg/mL, suggesting the existence of a concentration threshold above which membrane disruption becomes significant. This behavior may indicate that membrane toxicity is strongly dose-dependent and related to the accumulation of hydrophobic terpenoid compounds within the erythrocyte membrane.

From a pharmacological perspective, the low hemolytic effect observed at lower concentrations supports the potential safe use of diluted *Lavandula angustifolia* essential oil in topical or biological applications. However, the high hemolytic activity detected at elevated concentrations highlights the importance of concentration optimization before therapeutic application.

6. *In vitro* Antibacterial Activity (Agar Well Diffusion Method):

The antibacterial activity of *Lavandula angustifolia* essential oil was evaluated using the agar well diffusion method against two reference bacterial strains: the Gram-

positive bacterium Staphylococcus aureus infection Staphylococcus aureus ATCC 25923 and the Gram-negative bacterium Escherichia coli infection Escherichia coli ATCC 25922.

Figure V-4: Antibacterial Activity of Lavender Essential Oil Against Staphylococcus aureus and Escherichia coli

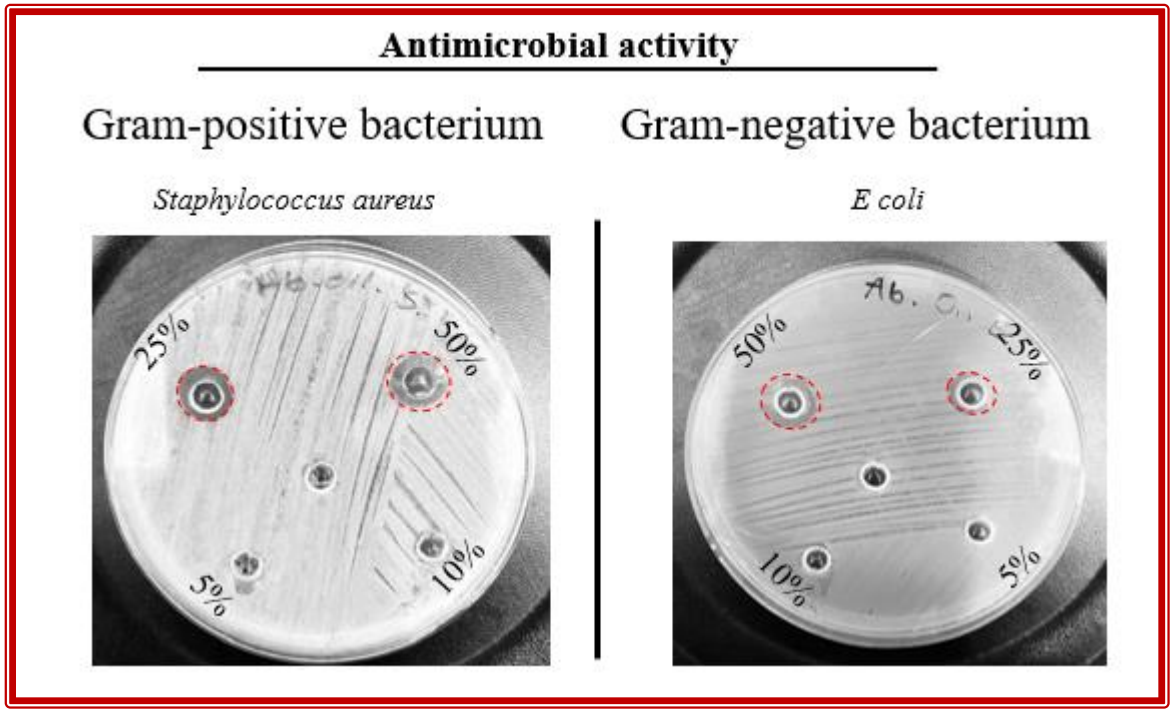

Table V-8: Inhibition zones (mm) produced by lavender essential oil

	Microbial inhibition (mm) OIL				Negative control.
Strains used	50%	25%	10%	5%	
Escherichia coli ATCC 25922	15	13	NI	NI	NI
Staphylococcus aureus ATCC 25923	16	11	NI	NI	NI

NI = No Inhibition

The obtained results demonstrated that the essential oil exhibited measurable antibacterial activity at higher concentrations (50% and 25%), while no inhibition was observed at lower concentrations (10% and 5%). The largest inhibition zone was recorded against Staphylococcus aureus at 50% concentration, with a diameter of 16 mm, followed by Escherichia coli with an inhibition zone of 15 mm. At 25%, the inhibition zones decreased to 11 mm and 13 mm for Staphylococcus aureus and Escherichia coli, respectively.

The absence of inhibition at lower concentrations indicates that the antibacterial effect of the essential oil is concentration-dependent. The stronger activity observed at

elevated concentrations may be attributed to the increased diffusion of active volatile constituents into the agar medium and their greater interaction with bacterial cell structures.

The antibacterial properties of the lavender essential oil are likely associated with its high content of oxygenated monoterpenes identified by GC–MS analysis, particularly linalool, linalyl acetate, α-terpineol, camphor, and 1,8-cineole. These compounds are known to interfere with bacterial membrane integrity, increase membrane permeability, and disrupt essential metabolic processes.

The slight difference in susceptibility between the two bacterial strains may be explained by structural differences in their cell envelopes. Gram-negative bacteria such as Escherichia coli possess an outer lipopolysaccharide membrane that can act as an additional permeability barrier against hydrophobic compounds. In contrast, Gram-positive bacteria possess a thicker peptidoglycan layer but lack the outer membrane, which may facilitate the penetration of volatile terpenoid compounds. Nevertheless, in the present study, both strains showed comparable sensitivity to the essential oil at higher concentrations.

The negative control (hexane) did not produce any inhibition zone, confirming that the observed antibacterial activity resulted exclusively from the bioactive constituents of the lavender essential oil rather than from the solvent itself.

7. *In vitro* Anti-diabetic Activity (α-Amylase Inhibition Assay):

The antidiabetic potential of *Lavandula angustifolia* essential oil was evaluated through its ability to inhibit α-amylase activity using the starch–iodine colorimetric assay. α-Amylase is a key digestive enzyme responsible for the hydrolysis of dietary starch into absorbable glucose units. Therefore, inhibition of this enzyme may contribute to the reduction of postprandial hyperglycemia and represents an important therapeutic strategy in the management of diabetes mellitus.

Concentration	Absorbance	Inhibition (%)
0.7	0.133	28.57
0.9	0.142	33.10
2	0.183	48.09
4	0.636	85.06
5	0.810	88.27
6	1.113	91.46
7	0.970	90.21
8	1.205	92.12
9	1.245	92.37
10	1.137	91.64

Table V-9: *In vitro* α-amylase inhibitory activity of lavender essential oil

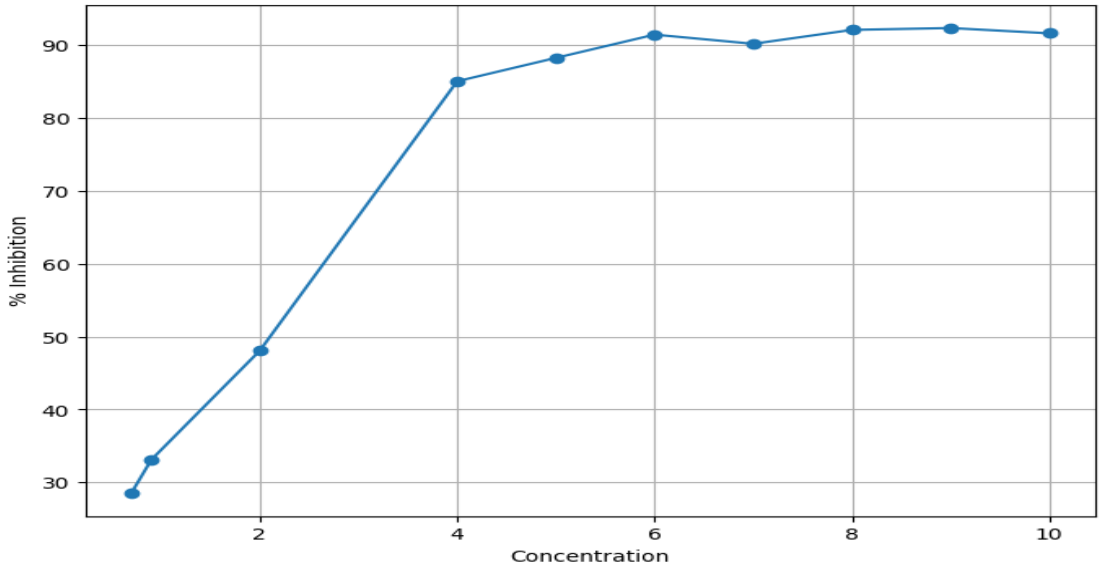

Figure V-5: In vitro α-amylase inhibitory activity of lavender essential oil

The obtained results demonstrated that the lavender essential oil exhibited strong α-amylase inhibitory activity in a concentration-dependent manner. The inhibition percentage increased progressively from 28.57% at the lowest tested concentration (0.7) to a maximum value of 92.37% at concentration 9. A slight stabilization of activity was

observed at higher concentrations, with inhibition values remaining above 90% between concentrations 6 and 10.

The rapid increase in inhibitory activity between concentrations 2 and 4 suggests that the essential oil constituents interact efficiently with the catalytic site of the α-amylase enzyme once a threshold concentration is reached. The plateau observed at higher concentrations may indicate saturation of available enzyme-binding sites, resulting in maximal inhibitory efficiency.

The strong inhibitory activity observed in the present study may be attributed to the major oxygenated monoterpenes identified by GC–MS analysis, particularly linalool, linalyl acetate, α-terpineol, camphor, and 1,8-cineole. These volatile constituents have been reported to interact with digestive enzymes through hydrophobic interactions, hydrogen bonding, and conformational modification of the enzymatic active site.

Among the identified compounds, linalool is particularly recognized for its potential antidiabetic and antioxidant properties. In addition, synergistic interactions among multiple terpene constituents may enhance the overall inhibitory effect of the essential oil beyond the activity of individual compounds alone.

The high inhibition percentages obtained at elevated concentrations indicate that *Lavandula angustifolia* essential oil possesses promising *in vitro* antidiabetic potential. However, since essential oils are chemically complex mixtures with variable bioavailability and solubility, further investigations including enzyme kinetics, molecular docking analysis, and *in vivo* studies would be necessary to confirm the precise mechanism of inhibition and therapeutic applicability.

8. Molecular Docking Study:

Molecular docking analysis was performed to investigate the potential interactions between the major constituents of *Lavandula angustifolia* essential oil and two biologically relevant target proteins associated with diabetes. The selected targets are α-amylase (4GQR) and α-glucosidase (3W37).

8-1. Redocking validation:

Prior to the docking analysis, the computational protocol was validated through a self-docking (re-docking) procedure using the native co-crystallized ligands of each protein structure. The obtained RMSD values were below the generally accepted threshold of 2.0

Å for all complexes, the obtained RMSD values confirm the reliability and predictive accuracy of the docking protocol used in the present study.

Target protein (PDB ID)	Native Ligand	Binding Energy (kcal/mol)	Ref RMSD (Å)
α- Glucosidase (3W37)	Acarbose (chain D)	-8.97	1.49
α-Amylase (4GQR)	Myricetin (MYC)	-6.72	1.99

Table V-10: Molecular Redocking Validation Results

8-2. docking results of some lavender major compounds:

Ligand	Target protein	PDB ID	Binding Energy (kcal/mol)
Metformin	α-Amylase	4GQR	-5.9
Linalool	α- Glucosidase	3W37	-5.39
Linalyl Acetate	α-Amylase	4GQR	-4.48
Linalool	α-Amylase	4GQR	-4.46

Table V-11: Molecular docking Results

The molecular docking analysis revealed that both linalool and linalyl acetate interacted favorably with the active site of α-amylase (PDB ID: 4GQR), supporting the strong inhibitory activity observed experimentally in the α-amylase assay. Linalool established conventional hydrogen bonds with key amino acid residues including ASP197 and HIS299, in addition to multiple hydrophobic alkyl and π-alkyl interactions involving residues such as TYR62, TRP58, LEU165, and HIS101. These interactions may contribute to stabilization of the ligand within the catalytic pocket and interference with substrate binding.

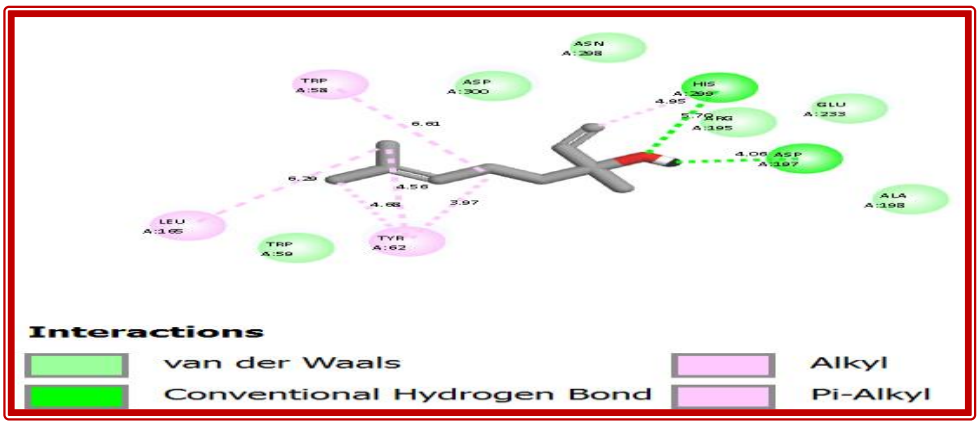

Figure V-6: 2D interaction map of Linalool within the active site of α-Amylase (PDB ID: 4GQR)

Similarly, linalyl acetate demonstrated important interactions with catalytic and surrounding residues of α-amylase. A conventional hydrogen bond was observed with ASP197, while additional hydrophobic interactions were formed with TYR62, HIS101, LEU165, and TRP58. The presence of hydrogen bonding with ASP197 is particularly significant because this residue belongs to the catalytic region of α-amylase and plays an essential role in starch hydrolysis. Therefore, interaction with this activated amino acid may contribute directly to enzyme inhibition.

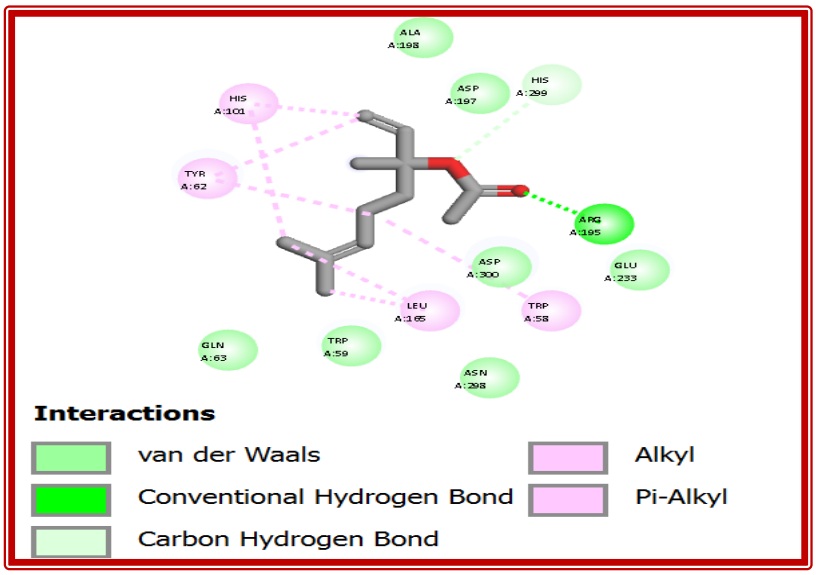

Figure V-7: 2D interaction map of Linalyl Acetate within the active site of α-Amylase (PDB ID: 4GQR)

The docking results suggest that the antidiabetic potential of *Lavandula angustifolia* essential oil may be attributed, at least partially, to the ability of its major oxygenated monoterpenes to bind within the α-amylase active site through hydrogen bonding, van der Waals forces, and hydrophobic interactions. These findings are consistent with the high *in vitro* α-amylase inhibition percentages obtained experimentally and support the hypothesis that lavender essential oil may reduce carbohydrate digestion and postprandial glucose absorption.

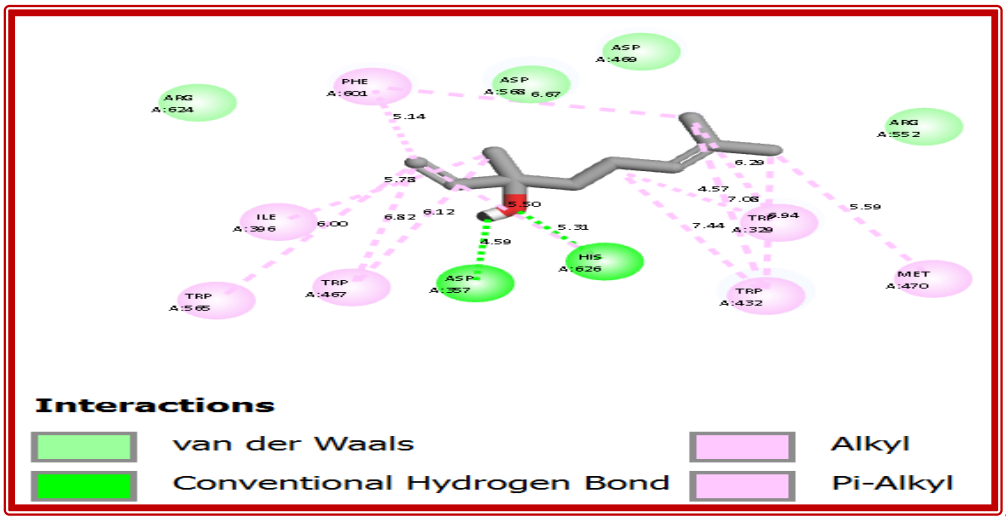

Figure V-8: 2D interaction map of Linalool within the active site of α-glucosidase (PDB ID: 3W37)

Although the docking scores obtained in this study were generally lower than those of reference pharmaceutical ligands, the results remain significant considering that natural volatile compounds typically possess simpler molecular structures and fewer functional groups available for strong polar interactions. Moreover, the biological activity of essential oils often results from synergistic interactions among multiple constituents rather than from a single highly potent molecule.

9. Pharmacokinetic and ADMET Evaluation:

9-1. BOILED-Egg model

The BOILED-Egg model generated by the SwissADME platform demonstrated that the major constituents of *Lavandula angustifolia* essential oil, namely linalool and linalyl acetate, are located within the physicochemical space associated with high gastrointestinal absorption and blood–brain barrier (BBB) permeability (Figure VI.9).

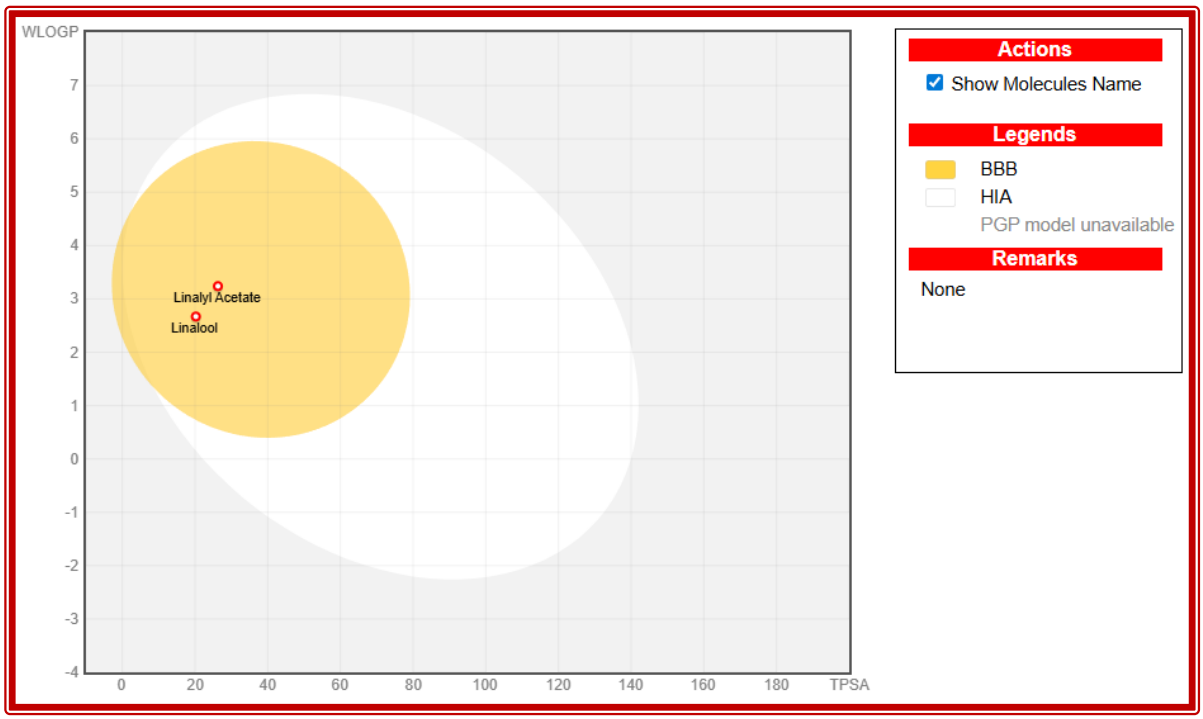

Figure V-9: BOILED-Egg model predicting gastrointestinal absorption and blood–brain barrier permeability of the major lavender essential oil constituents

♦ TPSA (X-axis) is the Topological Polar Surface Area, when its value is very low (below 30 Å²) it is a characteristic of molecules that easily slip through cell membranes.

♦ WLOGP (Y-axis) is the lipophilicity (Log P) its optimal range is between 2 and 4, allowing the molecules to dissolve in the fatty layers of the BBB.

♦ PGP- (The Red Circle) this shows that the molecules are not substrates for P-glycoprotein. which means once they enter the brain to inhibit Acetylcholinesterase, they stay there rather than being pumped back out by the body's natural defenses.

The low Topological Polar Surface Area (TPSA) values observed for the three compounds indicate favorable membrane permeability, facilitating passive diffusion across biological barriers. In addition, the WLOGP values ranged between 2 and 4, reflecting

balanced lipophilicity compatible with both intestinal absorption and penetration into lipid-rich tissues such as the central nervous system.

The BOILED-Egg analysis further revealed that all evaluated compounds were predicted to be non-substrates of P-glycoprotein (PGP−). This suggests that once absorbed, these terpenoid compounds are less likely to undergo active efflux transport, potentially enhancing their bioavailability and central nervous system retention.

These pharmacokinetic characteristics support the potential neuroactive, anti-inflammatory, and systemic biological activities previously associated with lavender essential oil constituents.

9-2. ADMET Profile:

Parameter	Linalool	Linalyl Acetate
Molecular Weight (g/mol)	154.25	196.29
TPSA (Å²)	20.23	26.30
Log $P_{o/w}$ (WLOGP)	2.67	3.24
LogS (ESOL)	-2.40	-3.14
GI Absorption	High	High
BBB Permeant	Yes	Yes
P-gp Substrate	No	No
Lipinski Violations	0	0
Bioavailability Score	0.55	0.55
Skin Permeation (log K_p) (cm/s)	-5.13	-4.71
Metabolism (CYP Inhibition)	None	None
Total Clearance (log ml/min/kg)	0.446	1.627
Hepatotoxicity (Toxicity)	No	No
AMES Toxicity	No	No
Skin Sensitization	Yes	Yes
Max. tolerated dose (human)	0.774	0.547
Oral Rat Acute Toxicity (LD50)	1.704	1.729

Table V-12: Predicted pharmacokinetic and ADMET properties of the major constituents identified in *Lavandula angustifolia* essential oil

The ADMET analysis revealed favorable drug-likeness and pharmacokinetic properties for the major lavender essential oil constituents. All compounds complied with Lipinski's Rule of Five without violations, suggesting good oral bioavailability and acceptable molecular characteristics for pharmaceutical development.

The evaluated compounds exhibited high predicted gastrointestinal absorption and positive blood–brain barrier permeability, which may contribute to the neuropharmacological effects commonly reported for lavender essential oil, particularly its anxiolytic and sedative activities.

Skin permeation values (log Kp) indicated adequate transdermal diffusion potential, supporting the suitability of these compounds for topical formulations such as ointments or dermal applications.

From a toxicological perspective, none of the investigated compounds showed predicted hepatotoxicity, Ames mutagenicity, or cytochrome P450 inhibitory activity, indicating a relatively safe pharmacokinetic profile. However, all compounds were predicted to possess potential skin sensitization properties, which is consistent with the known allergenic potential of certain monoterpenes and should therefore be considered during formulation development.

10. *In vivo* evaluation of the antidiabetic activity:

10-1. Antihyperglycemic effect and glycemic control:

The present study evaluated the antihyperglycemic effect of *Lavandula angustifolia* essential oil (100 mg/kg) in alloxan-induced diabetic rats through the assessment of fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) levels (Table VI-13). Diabetic animals showed elevated fasting blood glucose levels (1.06 ± 0.09 g/L) compared with the normal control group (0.71 g/L), confirming the establishment of hyperglycemia following alloxan administration.

Treatment with *L. angustifolia* essential oil reduced fasting blood glucose to 0.84 ± 0.38 g/L, approaching the normal control values and showing improvement in glycemic regulation. The antihyperglycemic effect was comparable, although less pronounced, than that observed with metformin treatment (0.68 ± 0.46 g/L), which served as the standard antidiabetic reference drug.

Similarly, HbA1c values were slightly reduced in the lavender-treated group (3.34 ± 0.37%) compared with the diabetic control group (3.44 ± 0.11%). HbA1c reflects long-

term glycemic status and protein glycation; therefore, the observed reduction suggests a moderate improvement in chronic glucose control. The corresponding HbA1c values expressed in mmol/mol also showed a reduction from 14.10 ± 1.24 mmol/mol in diabetic rats to 13.00 ± 4.08 mmol/mol following treatment.

The antidiabetic activity of *L. angustifolia* essential oil may be attributed to its high content of oxygenated monoterpenes, particularly linalool, linalyl acetate, and 1,8-cineole, identified through GC–MS analysis. These compounds are known to possess antioxidant and anti-inflammatory properties that may reduce oxidative stress associated with β-cell dysfunction. In addition, the *in vitro* α-amylase inhibitory activity observed in the present study suggests that lavender essential oil may contribute to improved glycemic control by reducing carbohydrate digestion and glucose absorption.

Parameter	Normal Control	Negative Control (Diabetic)	Positive Control (Metformin, 125 mg/kg)	Treatment (*Lavandula angustifolia*, 100 mg/kg)
Fasting blood glucose (g/l)	0.71	1.06±0.09	0.68±0.46	0.84±0.38
HbA1c (%)	2.94	3.44±0.11	3.08±0.41	3.34±0.37
HbA1c (mmol/mol)	8.63	14.10±1.24	10.12±4.43	13.00±4.08

*Values are expressed as mean ± SD (n = 5). Statistical significance versus negative control: *p < 0.05, **p < 0.01, **p < 0.001 (one-way ANOVA followed by Tukey's post hoc test).

Table V-13: Effect of *Lavandula angustifolia* essential oil (100 mg/kg) on fasting blood glucose and HbA1c in diabetic rats

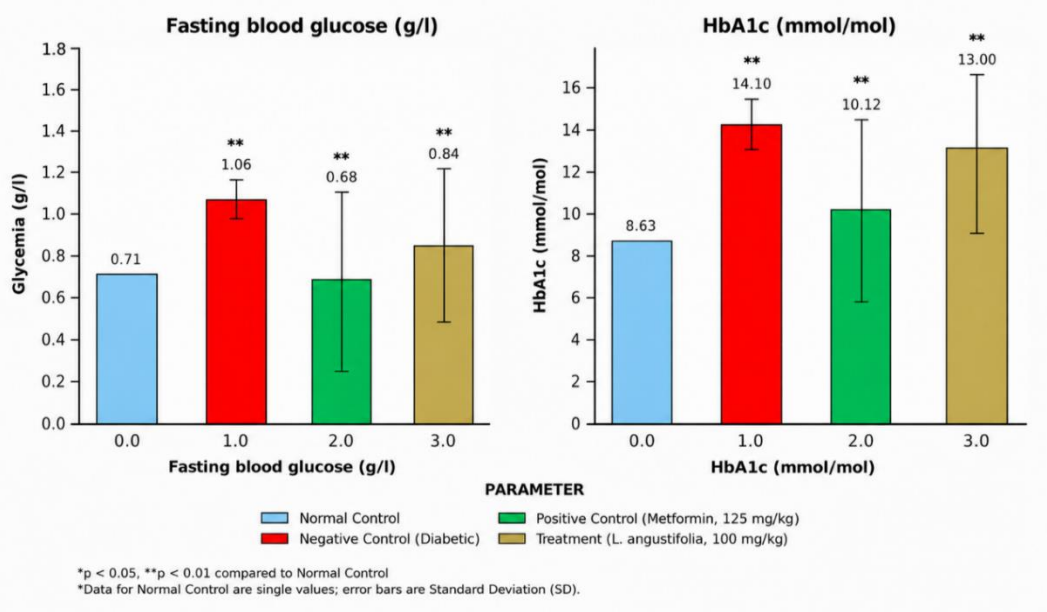

Figure V-10: Effect of Lavandula angustifolia essential oil (100 mg/kg) on fasting blood glucose and HbA1c levels in alloxan-induced diabetic rats

10-2. Hepatoprotective Activity

The hepatoprotective potential of *Lavandula angustifolia* essential oil was evaluated by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in alloxan-induced diabetic rats (Table VI-14). The diabetic control group exhibited markedly elevated ALT (131.96 ± 51.97 IU/L) and AST (218.66 ± 22.06 IU/L) levels compared with the normal control group, indicating significant hepatic dysfunction and hepatocellular damage associated with diabetic oxidative stress.

Treatment with *L. angustifolia* essential oil substantially reduced serum ALT levels to 35.12 ± 3.72 IU/L, restoring values close to those of the normal control group (35.77 IU/L) and slightly improving upon the metformin-treated group (40.85 ± 2.90 IU/L). Similarly, AST levels decreased to 131.89 ± 9.99 IU/L following lavender treatment, indicating attenuation of hepatic injury and partial restoration of liver function.

The hepatoprotective effect of *L. angustifolia* essential oil may be related to the antioxidant activity of its major volatile constituents, particularly linalool and linalyl acetate. These compounds have been reported to reduce lipid peroxidation, scavenge reactive oxygen species, and stabilize cellular membranes, thereby protecting hepatocytes from oxidative injury induced by hyperglycemia and alloxan toxicity.

Moreover, improved glycemic regulation may indirectly contribute to hepatic protection by reducing glucose-mediated oxidative stress and metabolic burden on liver

tissues. The normalization trend observed for ALT and AST strongly suggests preservation of hepatocellular integrity and improved hepatic metabolic function.

Parameter	Normal Control	Negative Control (Diabetic)	Positive Control (Metformin, 125 mg/kg)	Treatment 1 (Lavandula angustifolia, 100 mg/kg)
ALT (IU/L)	35.77	131.96±51.97	40.85±2.90	35.12±3.72
AST (IU/L)	68.25	218.66±22.06	115.00±15.21	131.89±9.99

*Values are expressed as mean ± SD (n = 5). Statistical significance versus negative control: *p < 0.01 (ANOVA, Tukey's post hoc test).

Table V-14: Effect of *Lavandula angustifolia* essential oil (100 mg/kg) on serum liver enzymes in diabetic rats

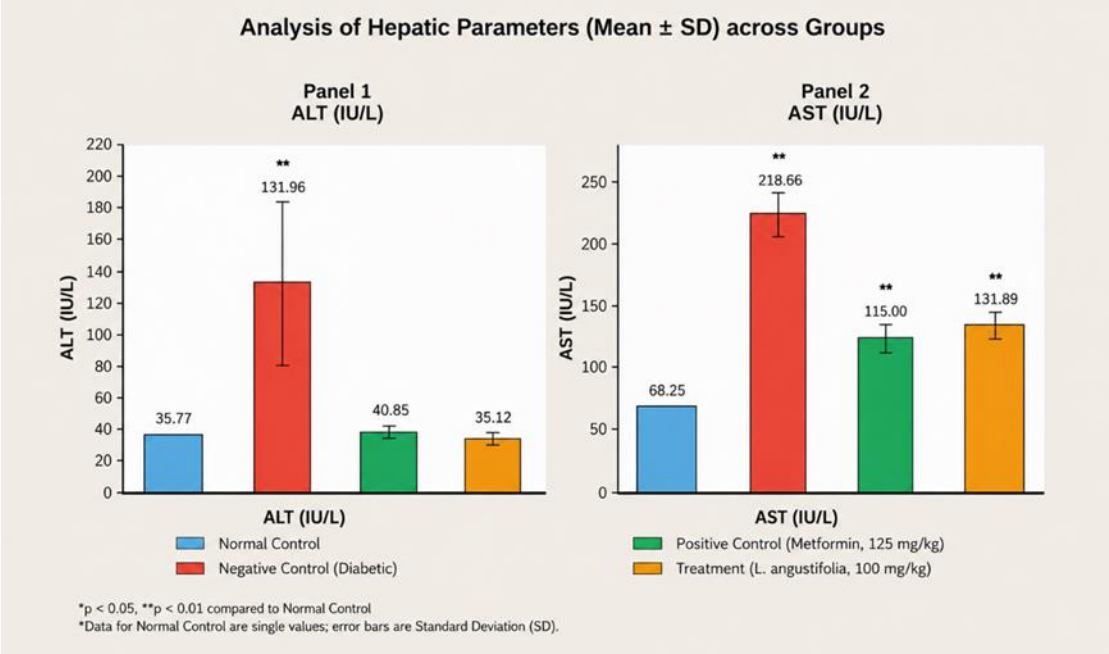

Figure V-11: Effect of Lavandula angustifolia essential oil on serum liver enzymes (ALT and AST) in diabetic rats compared with control and metformin groups

10-3. Mechanistic Basis

The antihyperglycemic and hepatoprotective effects of *Lavandula angustifolia* essential oil observed in alloxan-induced diabetic rats may be explained by the synergistic activity of its major bioactive monoterpenes and oxygenated volatile constituents. Alloxan-induced diabetes is characterized by oxidative destruction of pancreatic β-cells, leading to impaired insulin secretion and chronic hyperglycemia. Therefore, the moderate reductions observed in fasting blood glucose and HbA1c levels following lavender treatment may indicate partial protection of pancreatic tissue and improvement in glucose homeostasis.

The GC–MS analysis revealed that the essential oil was predominantly composed of linalool, linalyl acetate, α-terpineol, camphor, and 1,8-cineole. These compounds are widely recognized for their antioxidant, anti-inflammatory, and membrane-protective activities. Their ability to scavenge reactive oxygen species and reduce oxidative stress may contribute to preservation of β-cell integrity and enhancement of insulin sensitivity in peripheral tissues.

Additionally, the *in vitro* α-amylase inhibition assay demonstrated that lavender essential oil possesses carbohydrate-hydrolyzing enzyme inhibitory activity, suggesting a possible reduction in intestinal glucose absorption and postprandial hyperglycemia. Molecular docking studies further supported these findings by demonstrating favorable interactions between major lavender constituents and α-amylase, α-glucosidase, and COX-2 target proteins.

The reduction in serum ALT and AST levels indicates a partial hepatoprotective effect, particularly evident for ALT normalization, likely mediated through attenuation of lipid peroxidation, stabilization of hepatocyte membranes, and reinforcement of endogenous antioxidant defense systems. Improved glycemic control may also indirectly reduce hepatic oxidative burden and metabolic stress.

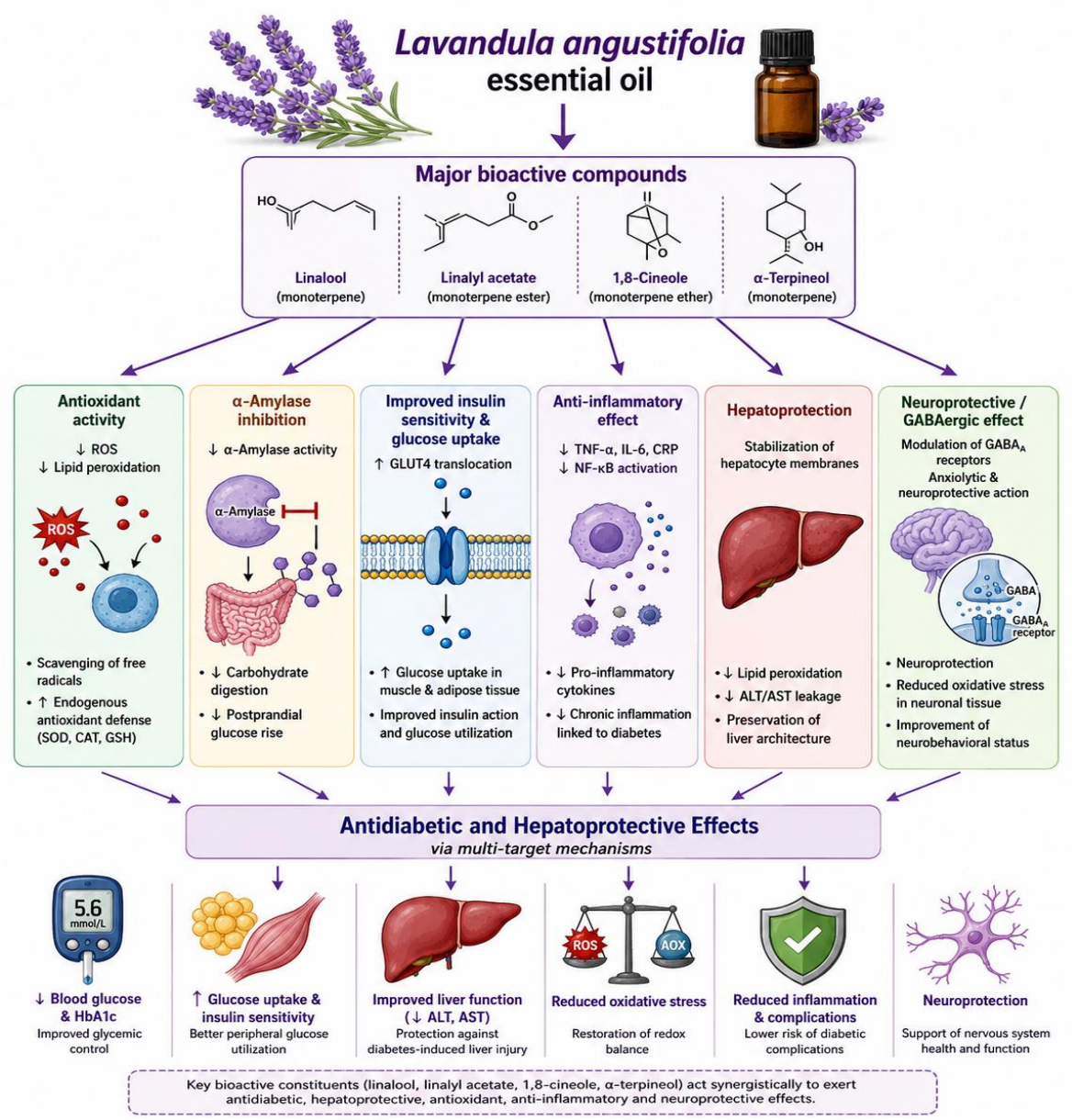

Figure V-12: Proposed multi-target mechanistic pathways underlying the antidiabetic and hepatoprotective effects of Lavandula angustifolia essential oil in alloxan-induced diabetic rats.

10-4. Histopathological Analysis

A) Liver Histology:

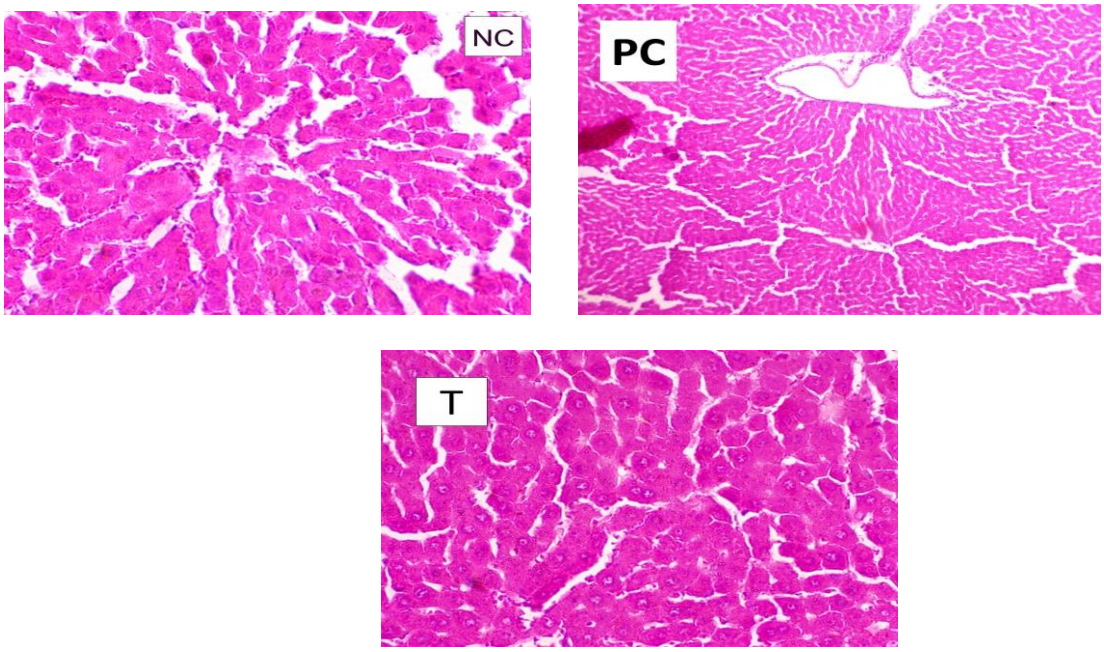

Figure V-13: Comparative histopathological evaluation of liver tissues (H&E staining) showing negative control (NC), positive control (PC), and Lavandula angustifolia extract (T)-treated groups.

Histopathological examination of liver tissues stained with hematoxylin and eosin (H&E) revealed clear structural differences among the experimental groups (Figure VI-15). The negative control (NC) group exhibited marked hepatic alterations characterized by disorganization of hepatic cords, sinusoidal dilation, cellular degeneration, and disruption of the normal hepatic architecture, reflecting severe alloxan-induced hepatic injury associated with oxidative stress and diabetic metabolic disturbances.

In contrast, the positive control (PC) group treated with metformin demonstrated a near-normal hepatic organization with preserved hepatocyte arrangement and reduced pathological alterations, indicating effective protection against diabetes-induced liver damage.

Similarly, the *Lavandula angustifolia* treated group (T) showed noticeable improvement in liver histoarchitecture compared with the diabetic control group. Hepatic cells appeared more organized with reduced cellular degeneration and partial restoration of sinusoidal integrity. These observations support the biochemical findings showing reductions in ALT and AST levels and suggest a moderate hepatoprotective effect of

Lavandula angustifolia essential oil, likely mediated through its antioxidant and anti-inflammatory bioactive constituents such as linalool and linalyl acetate.

B) Kidney Histology:

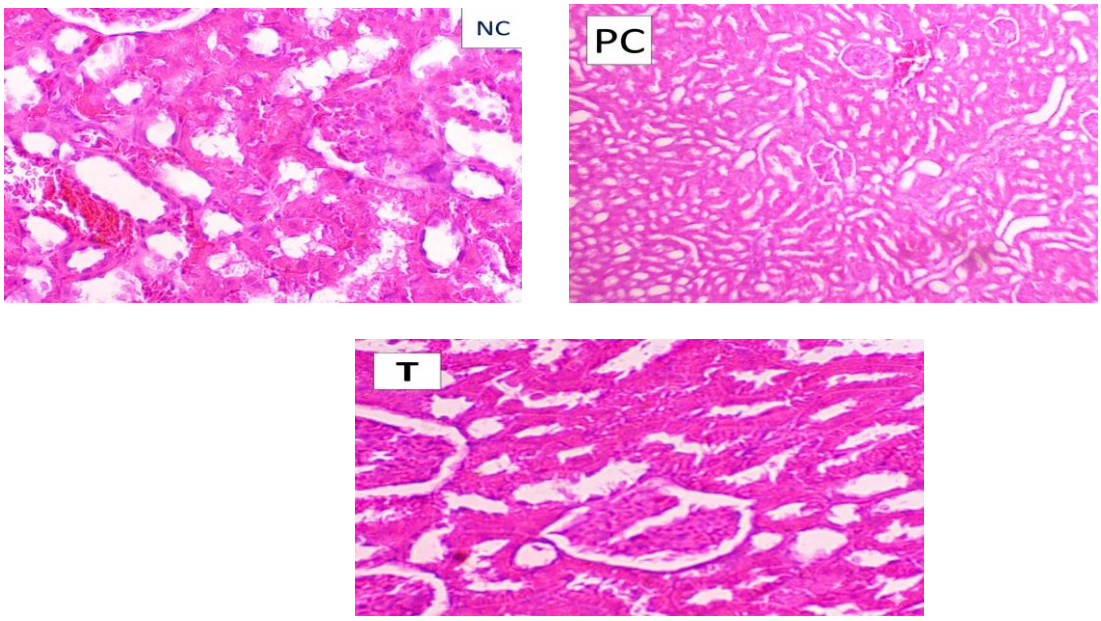

Figure V-14: Comparative histopathological evaluation of kidney tissues (H&E staining) showing negative control (NC), positive control (PC), and *Lavandula angustifolia* extract (T)-treated groups.

Microscopic examination of renal tissues (Figure VI-16) demonstrated substantial histopathological alterations in the diabetic negative control group. Kidney sections showed tubular disorganization, enlargement of Bowman's spaces, and structural degeneration of renal tubules, indicating diabetic nephrotoxic effects induced by chronic hyperglycemia and oxidative stress.

The positive control group displayed a marked preservation of renal architecture with normal glomerular and tubular organization, confirming the nephroprotective effect of metformin treatment.

The *Lavandula angustifolia* treated group exhibited moderate improvement in renal histology compared with the diabetic control group. The renal corpuscles and tubular structures appeared relatively preserved with reduced tissue degeneration and improved structural integrity. These findings suggest that the essential oil may exert a protective effect against diabetes-associated renal damage, potentially through reduction of oxidative stress and stabilization of cellular membranes.

C) Pancreas Histology

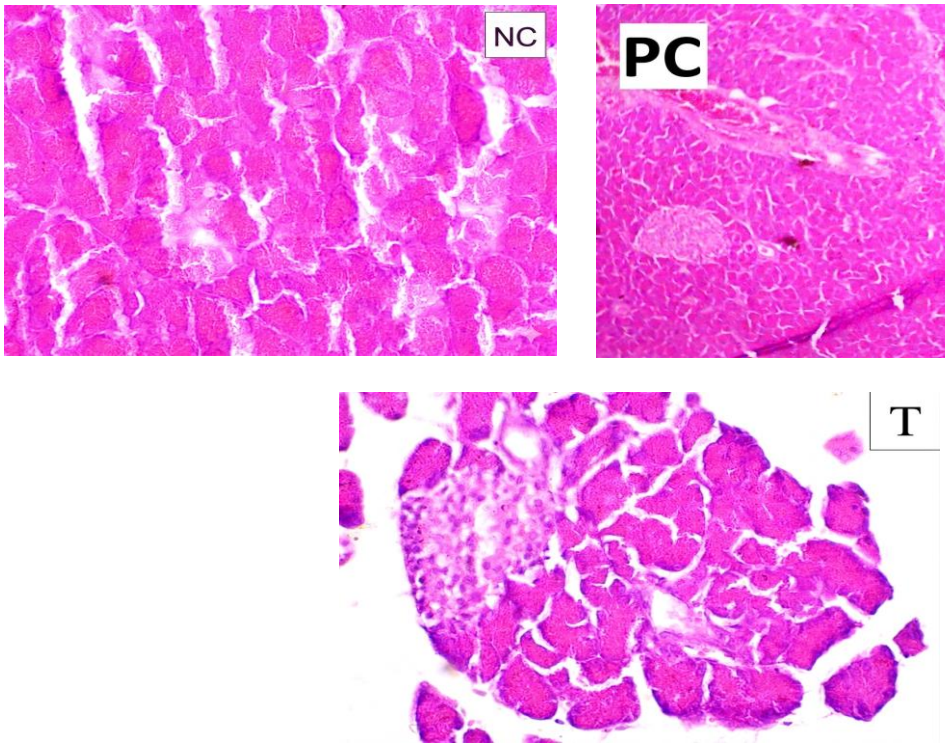

Figure V-15: Comparative histopathological evaluation of pancreas tissues (H&E staining) showing negative control (NC), positive control (PC), and Lavandula angustifolia extract (T)-treated groups.

Histological analysis of pancreatic tissues (Figure VI-17) revealed significant pathological alterations in the diabetic negative control group, including disruption of pancreatic architecture, degeneration of islet cells, and reduction in the integrity of the endocrine regions. These alterations are consistent with the destructive action of alloxan on pancreatic β-cells.

In the positive control group, pancreatic tissues exhibited improved morphology with better preservation of the islets of Langerhans and surrounding acinar cells, indicating partial recovery of pancreatic structure following metformin treatment.

The *Lavandula angustifolia* treated group demonstrated moderate restoration of pancreatic histoarchitecture compared with the diabetic control group. The pancreatic tissue showed improved cellular organization and partial preservation of the islet structures, suggesting that the essential oil may contribute to attenuation of β-cell damage and oxidative injury. This protective effect may be associated with the antioxidant properties of lavender monoterpenes, which could help limit alloxan-induced pancreatic degeneration and support improved glycemic regulation.

General Conclusion

General Conclusion:

The present study provided a comprehensive investigation into the chemical composition and biological properties of the essential oil extracted from *Lavandula angustifolia* Mill. collected from regions of Algeria. Through the combination of phytochemical characterization, *in vitro* biological assays, *in silico* molecular modeling, and *in vivo* experimental evaluation, this work highlighted the considerable therapeutic potential of lavender essential oil as a natural bioactive product.

The chromatographic analysis confirmed that the essential oil is rich in oxygenated monoterpenes and ester compounds, particularly linalool and linalyl acetate, which are recognized as the principal constituents responsible for the characteristic aroma and pharmacological properties of lavender. The chemical profile obtained supports the high biological value of Algerian *Lavandula angustifolia* and demonstrates its similarity to high-quality lavender oils reported internationally.

The *in vitro* assays demonstrated that the essential oil possesses significant antioxidant activity through its capacity to neutralize free radicals, in addition to notable anti-inflammatory effects through the inhibition of protein denaturation. Furthermore, the oil exhibited antibacterial activity against tested microbial strains, confirming its potential as a natural antimicrobial agent. The hemolytic assay also indicated that the oil maintained acceptable biocompatibility at controlled concentrations, suggesting relative safety for therapeutic applications. Moreover, the α-amylase inhibition assay revealed promising anti-diabetic activity, supporting the potential role of lavender essential oil in the management of hyperglycemia.

The *in silico* molecular docking study further strengthened the experimental findings by demonstrating favorable interactions between major lavender constituents and selected biological targets. The obtained binding affinities suggested that compounds such as linalool and related terpenoids may contribute directly to the observed biological activities. In addition, ADMET and drug-likeness evaluations revealed encouraging pharmacokinetic characteristics and acceptable theoretical safety profiles, supporting the pharmaceutical relevance of these natural compounds.

The *in vivo* investigation using alloxan-induced diabetic models demonstrated the ability of lavender essential oil to reduce blood glucose levels and mitigate oxidative damage associated with diabetes. Histopathological analyses of pancreatic, hepatic, and

renal tissues suggested a protective effect against tissue degeneration induced by oxidative stress and hyperglycemia.

Taken together, the results obtained throughout this thesis confirm that *Lavandula angustifolia* essential oil from Algerian regions constitutes a promising natural source of biologically active compounds with antioxidant, anti-inflammatory, antibacterial, and anti-diabetic activities. This study contributes to the scientific valorization of Algerian medicinal flora and supports the future development of lavender-based pharmaceutical, cosmetic, and nutraceutical products.

Nevertheless, further investigations remain necessary to isolate individual active compounds, clarify their precise molecular mechanisms, evaluate long-term toxicity, and validate their efficacy through advanced clinical studies. Such future research could contribute significantly to the development of safer and more effective natural therapeutic alternatives.

Participation in Scientific Conferences

The third Study Day on Applied Chemistry& Environmental Engineering (SDACEE'3)

As part of their academic and scientific training, the authors actively participated in The third Study Day on Applied Chemistry& Environmental Engineering (SDACEE'3) organized by the University of El-Oued, This national scientific event brought together researchers, academics, doctoral candidates, and graduate students from various Algerian universities to discuss recent advances and emerging perspectives in chemical sciences.

The seminar covered several important fields, including organic chemistry, analytical

chemistry, environmental chemistry, materials chemistry, and green chemistry. Special attention was devoted to sustainable chemical processes, the valorization of natural bioactive compounds, and the integration of modern analytical and computational techniques in scientific research.

During this scientific event, the authors made a poster and delivered an oral presentation related to their research work on the phytochemical composition and biological activities of medicinal plants from southeastern Algeria. The presentations highlighted the importance of natural bioactive compounds in the development of sustainable therapeutic and pharmacological applications.

Participation in this seminar provided a valuable opportunity to strengthen scientific knowledge, exchange ideas with researchers from different institutions, improve scientific communication skills, and gain deeper insight into current research trends and innovations in chemistry. It also encouraged interdisciplinary collaboration and academic exchange among young researchers.

3rd Study Day on Applied Chemistry and Environmental Engineering – SDACEE'3

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In Vitro Evaluation of Anti-inflammatory, Anti-hemolytic, and Antibacterial Properties of Lavandula angustifolia Essential Oil

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Keywords: *BSA Denaturation; E. coli; Hemolysis; Lavandula angustifolia; Ointment; S. aureus*

ABSTRACT:

This study takes a closer look at the pharmacological profile of Lavandula angustifolia essential oil (EO) and its experimental ointment formulation. To evaluate its anti-inflammatory potential, the Bovine Serum Albumin (BSA) heat-induced protein denaturation assay was used, which involved a 0.3% w/v BSA solution in TBS at pH 6.4. The EO showed a dose-dependent stabilization effect, achieving a peak inhibition of 14.20% at a 50% concentration, while the positive control, Aspirin, reached 67.90%. For cytocompatibility, a hemolytic activity assay was performed using a red blood cell suspension, and the results indicated a strong safety profile at concentrations of 5 mg/mL or less (with hemolysis under 10%). However, at 40 mg/mL, membrane disruption rose to 86.1%. Additionally, the antibacterial effectiveness of the EO-based ointment was confirmed through the agar well diffusion method, showing significant inhibition zones for both Staphylococcus aureus (16 mm) and Escherichia coli (15 mm) at a 50% concentration, with activity sustained across twofold dilutions. These findings suggest that while the oil offers some protein stabilization, its main therapeutic benefits are its strong antimicrobial properties and cellular safety at therapeutic doses.

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