الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
جامعة الشهيد حمه لخضر الوادي
ECHAHID HAMMA LAKHDAR UNIVERSITY OF EL-OUED
كلية العلوم الطبيعة والحياة
FACULTY OF NATURAL LIFE AND SCIENCES
قسم البيولوجيا الخلوية والجزيئية
Department of Cellular and Molecular Biology

END OF STUDY THESIS

With a view to obtaining the Academic Master's degree in Biological Sciences Specialty: Toxicology

THEME

The Chemical Composition, In Vitro, In Vivo, and In Silico Studies of Extract of the Pelargonium graveolens plant found in the Eloued region

Presented by:

Ben Ali Fatma Zahra

Bousbia Salah Sadjida

Selatna Lina

President: Dr. NADJI Nassima MCA El-Oued University

Examiner: Dr. AQUIMEUR Meriem MCA El-Oued University

Supervisor: Dr . OUAFA Boudebia MCA El-Oued University

University year: 2025/2026


بسم الله الرحمن الرحيم

Dedication

﴿وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ﴾

Thanks to God and His grace, I raise my hat in recognition of these years and in acknowledgment of my success in my career ز

To my support and my strength

My father, **Ali**, may God grant you the taste of Paradise. I dedicate this scientific edifice to you, which is thanks to you and your efforts.

To my angel in this world

My **mother**, you are the first one who deserves joy, for your prayers and patience with me in every step. I ask God to heal you.

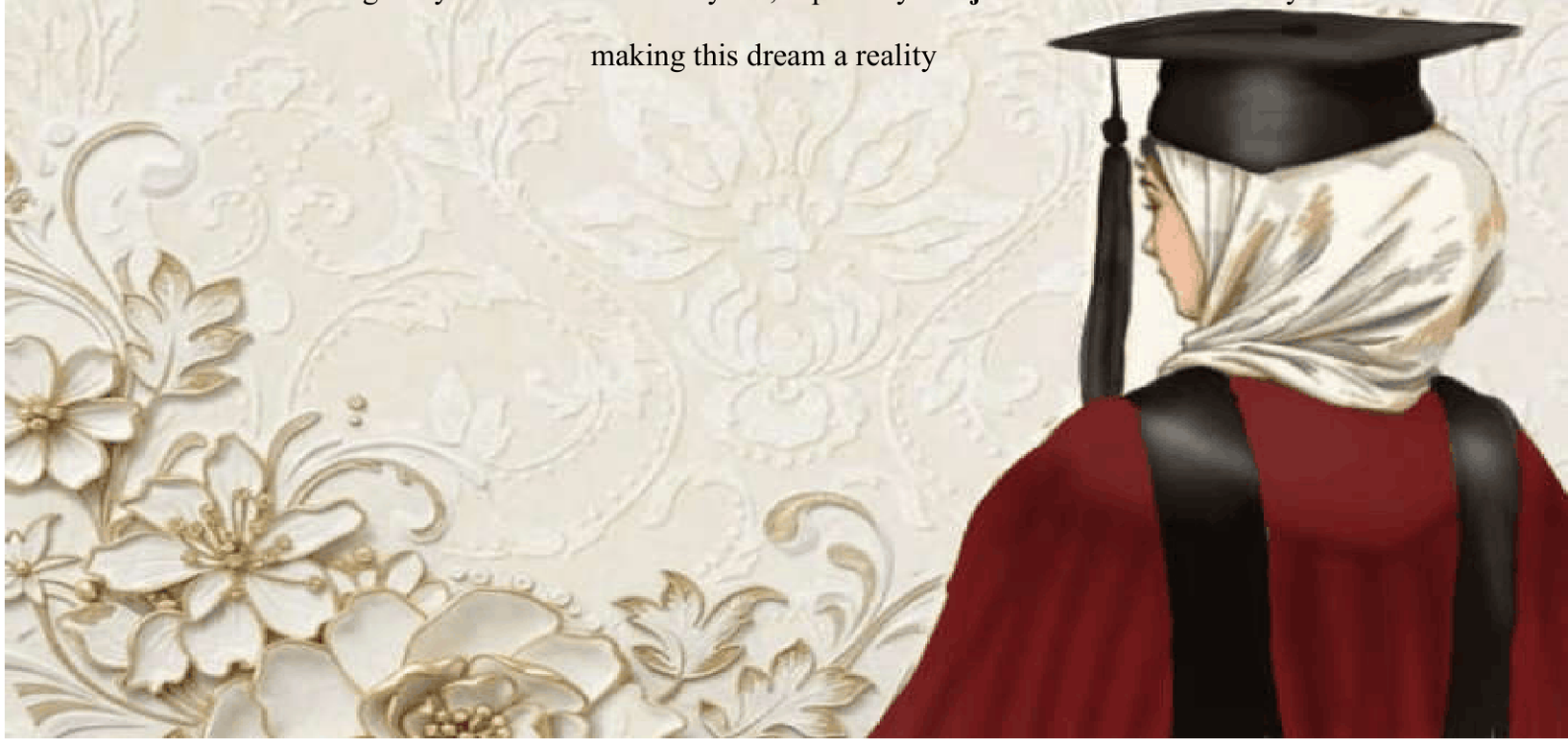
My brothers and sisters

Hidaya , Djoudi, Issam and **Ibrahim,** my dear ones in this world, thank you for being here.

My teacher **Abd rrezzak**

Thank you so much for your efforts in this difficult journey, may God reward you

I will never forget my friends who stood by me, especially **Sadjida** and **Fatima**. Thank you both for making this dream a reality



Dedication

﴿ فَرِحِينَ بِمَا آتَاهُمُ اللَّهُ مِنْ فَضْلِهِ ﴾

This moment could neither be inherited nor purchased. I have earned it with years of hard work, my youth, and countless sleepless nights. It is the thrill of achievement and the joy of a lifetime the beautiful ending to even greater beginnings.

I dedicate the fruits of my success to those who were the light along my path:

To the one whose name I carry with pride, who cleared the thorns from my path to pave the road of knowledge, whom Allah has adorned with dignity and reverence, and who taught me to give without expecting anything in return

my dear father.

To the source of tenderness and the heart whose prayers never ceased, to the one whose eyes followed my steps with love and devotion, to my steadfast support and the sanctuary of my days—I offer you today the harvest of your prayers

my beloved mother.

I also dedicate my graduation to **my brothers and sisters**, who have always been, and will forever remain, my constant source of support throughout every stage of my life.

To everyone who stood by me on this journey **my companions and colleagues** who provided the best help and encouragement I deeply value your efforts and solidarity.

To my partners in success, **my dear friends Fatima and Lina**, and all my peers who shared every single step toward this goal.

And finally, I dedicate this achievement to **every teacher and professor** who contributed to shaping our path toward knowledge

Sajeda

Dedication

قال تعالى:" وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ"

The journey was neither short nor paved with ease, but I made it. Praise be to Allah, who facilitated the beginnings, perfected the ends, and brought us to our goals.

With all my love, I dedicate this work to Allah Almighty, my Creator and my refuge in every step, who granted me the patience and strength to reach where I am today.

To the light that illuminated my path, the lantern whose glow never fades in my heart, and the one who taught me that success is only achieved through patience and perseverance my dear father.

To the ultimate supporter of my ambitions, the one under whose feet Heaven lies, the compassionate heart, and the secret behind my strength and success my beloved mother.

To my dear brothers and sisters, the partners of my life, my support, and my backbone; I dedicate to you every success I have achieved, for you are part of every moment of pride and joy I live.

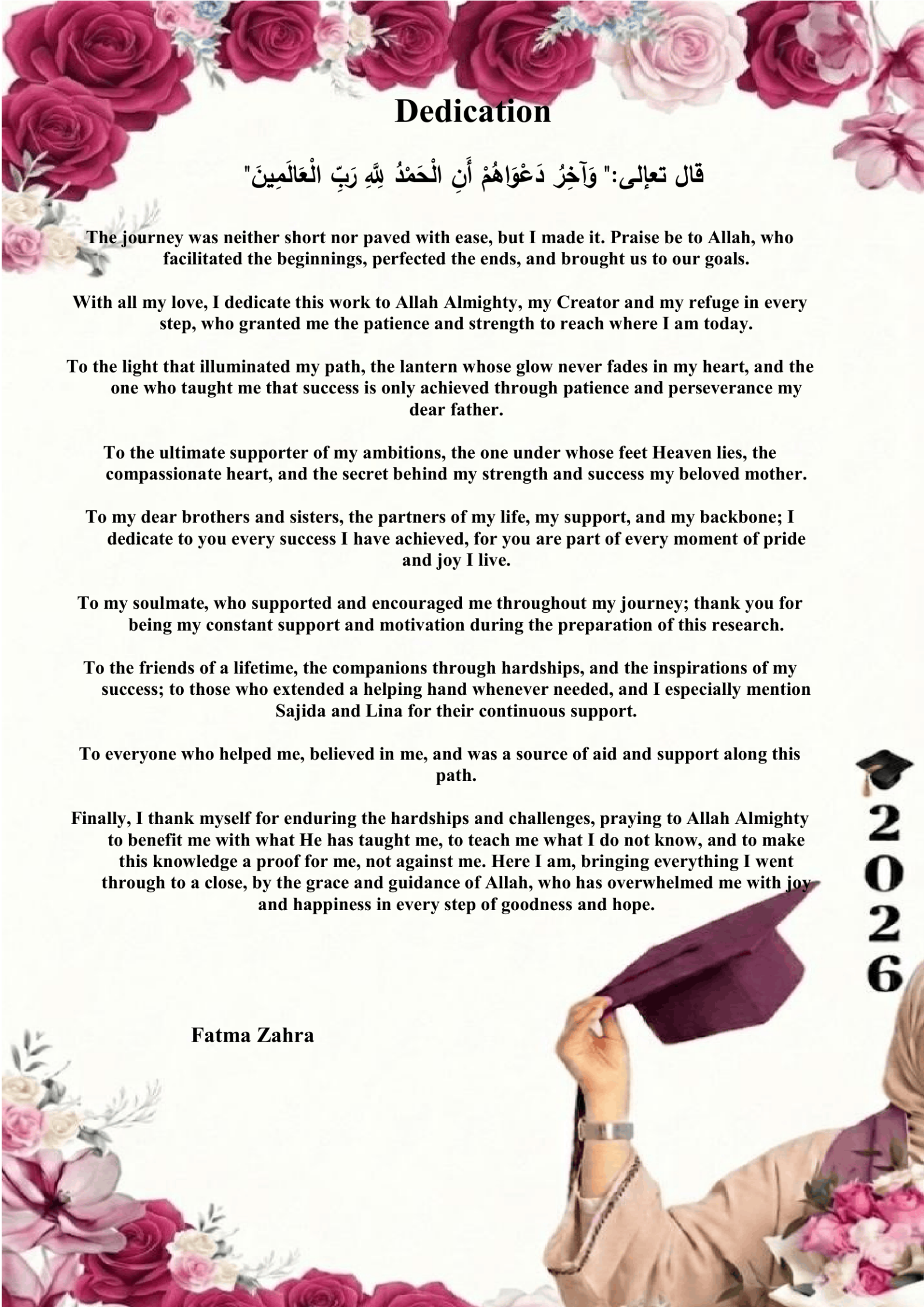
To my soulmate, who supported and encouraged me throughout my journey; thank you for being my constant support and motivation during the preparation of this research.

To the friends of a lifetime, the companions through hardships, and the inspirations of my success; to those who extended a helping hand whenever needed, and I especially mention Sajida and Lina for their continuous support.

To everyone who helped me, believed in me, and was a source of aid and support along this path.

Finally, I thank myself for enduring the hardships and challenges, praying to Allah Almighty to benefit me with what He has taught me, to teach me what I do not know, and to make this knowledge a proof for me, not against me. Here I am, bringing everything I went through to a close, by the grace and guidance of Allah, who has overwhelmed me with joy and happiness in every step of goodness and hope.

Fatma Zahra



ACKNOWLEDGEMENTS

قال الله تعالى: "إِنَّ الَّذِينَ آمَنُوا وَعَمِلُوا الصَّالِحَاتِ إِنَّا لا نُضِيعُ أَجْرَ مَنْ أَحْسَنَ عَمَلا"

"الكهف: 30".

All praise, thanks, and gratitude be to Allah Almighty for granting us the success to complete this work. No effort is crowned with success except by His grace, and no servant overcomes obstacles and difficulties except through His divine guidance.

Following this, we would like to express our deepest gratitude and appreciation to Professor **"Abbadi AbdelRazzak"** for accepting the supervision of this thesis, and for the invaluable advice and sound guidance he generously provided. May Allah bless his efforts, work, wealth, and family

.

We also extend our sincere thanks and gratitude to the members of the defense committee: Professor **"....."** as Chairman and Professor **"....."** as Examiner, for graciously accepting to review and discuss this thesis.

Our thanks also go to the entire staff of the **Faculty of Nature and Life Sciences** for their dedicated efforts and continuous hard work.

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

Pelargonium graveolens is a medicinal plant with notable pharmacological potential. This study evaluates the antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and organ-protective activities of its methanolic leaf extract using experimental and computational approaches. The extraction yield was 15.576%, and LC–MS analysis revealed a rich profile of bioactive compounds including rutin, quercetin derivatives, luteolin, curcumin, and phenolic acids. The extract exhibited strong antioxidant activity (DPPH and FRAP), significant α-amylase inhibition (>80%), and notable anti-inflammatory effects, while showing acceptable hemocompatibility at lower concentrations. Moderate antibacterial activity was observed against *E. coli* and *S. aureus*. In vivo studies demonstrated significant reductions in blood glucose and HbA1c, improved liver enzyme profiles, and histological protection of liver, kidney, and pancreatic tissues. Molecular docking confirmed strong binding of rutin and curcumin to key targets (α-glucosidase, α-amylase, and COX-2), supported by favorable ADMET properties and good safety profiles. Overall, the findings indicate that *Pelargonium graveolens* possesses multi-target therapeutic potential against diabetes and oxidative stress-related disorders.

Keywords: *Pelargonium graveolens*, antidiabetic, antioxidant, molecular docking, rutin, curcumin.

Résumé

Pelargonium graveolens est une plante médicinale reconnue pour son potentiel pharmacologique. Cette étude évalue les activités antidiabétiques, antioxydantes, anti-inflammatoires, antimicrobiennes et protectrices des organes de son extrait méthanolique de feuilles à l'aide d'approches expérimentales et computationnelles. Le rendement d'extraction était de 15,576%, et l'analyse LC–MS a révélé un profil riche en composés bioactifs, notamment la rutine, des dérivés de la quercétine, la lutéoline, la curcumine et divers acides phénoliques. L'extrait a montré une forte activité antioxydante (DPPH et FRAP), une inhibition significative de l'α-amylase (>80%), ainsi qu'une activité anti-inflammatoire notable, tout en présentant une hémocompatibilité acceptable à faibles concentrations. Une activité antibactérienne modérée a été observée contre *E. coli* et *S. aureus*. Les études in vivo ont révélé une réduction significative de la glycémie et de l'HbA1c, une amélioration des marqueurs hépatiques, ainsi qu'une protection histologique du foie, des reins et du pancréas. Les études de docking moléculaire ont confirmé une forte affinité de liaison de la rutine et de la curcumine envers des cibles clés (α-glucosidase, α-amylase et COX-2), soutenues par des propriétés ADMET favorables et un bon profil de sécurité. Globalement, ces résultats indiquent que *Pelargonium graveolens* possède un potentiel thérapeutique multi-cible contre le diabète et les troubles liés au stress oxydatif.

Mots-clés : *Pelargonium graveolens*, antidiabétique, antioxydant, docking moléculaire, rutine, curcumine.

الملخص

يُعد نبات العطرشة من النباتات الطبية المعروفة بخصائصه الدوائية المتعددة. تهدف هذه الدراسة إلى تقييم النشاط المضاد لمرض السكري، ومضادات الأكسدة، ومضادات الالتهاب، والنشاط المضاد للميكروبات، إضافة إلى التأثيرات الحامية للأعضاء للمستخلص الميثانولي لأوراق النبات باستخدام مقاربات تجريبية وحاسوبية. بلغ مردود الاستخلاص 15.576%، وأظهر تحليل LC--MS وجود مركبات فعالة حيوياً متعددة مثل الروتين، ومشتقات الكيرسيتين، واللوتيولين، والكركمين، وعدة أحماض فينولية.

أظهر المستخلص نشاطاً قوياً كمضاد للأكسدة في اختباري DPPH وFRAP، كما أظهر تثبيطاً ملحوظاً لإنزيم-α أميلاز (>80%)، ونشاطاً مضاداً للالتهاب، مع توافق حيوي جيد عند التراكيز المنخفضة. كما أظهر نشاطاً مضاداً للبكتيريا ضد كل من *E. coli* و*S. aureus*. وأكدت الدراسات الحيوية انخفاضاً واضحاً في مستوى سكر الدم والهيموغلوبين السكري (HbA1c)، وتحسناً في مؤشرات وظائف الكبد، إضافة إلى حماية نسيجية للكبد والكلى والبنكرياس.

دعمت دراسات الالتحام الجزيئي (Molecular Docking) النتائج التجريبية، حيث أظهرت مركبات الروتين والكركمين ارتباطاً قوياً مع الأهداف الحيوية الرئيسية مثل-α غلوكوزيداز، و-αأميلاز، وإنزيمCOX-2 ، مع خصائص دوائية حركية (ADMET)جيدة وأمان مرتفع. وبشكل عام، تشير النتائج إلى أن نبات العطرشة يمتلك قدرة علاجية متعددة الأهداف في مكافحة مرض السكري والإجهاد التأكسدي.

الكلمات المفتاحية : العطرشة ، مضاد للسكري، مضاد للأكسدة، الالتحام الجزيئي، روتين، كركمين.

Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Meaning
Å	Angstrom
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CAT	Catalase
DNA	Deoxyribonucleic Acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
LC-MS	Liquid ChromatographyMass Spectrometry
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
SOD	Superoxide Dismutase
WHO	World Health Organization

General Introduction

General Introduction

Medicinal plants have been used for centuries as a fundamental source of therapeutic agents in traditional medicine systems worldwide. In recent decades, increasing scientific interest has been directed toward the exploration of plant-derived bioactive compounds due to their structural diversity, biological efficiency, and relatively low toxicity compared to synthetic drugs [1, 2].

Among these medicinal plants, *Pelargonium graveolens* (commonly known as rose geranium) has attracted considerable attention due to its rich phytochemical composition and wide range of pharmacological properties. It is traditionally used for the treatment of inflammation, microbial infections, diabetes, and stress-related disorders. The essential oil and extracts of this plant are known to contain flavonoids, phenolic acids, terpenoids, and other secondary metabolites responsible for its biological activities [3].

Diabetes mellitus is one of the most prevalent chronic metabolic disorders globally, characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease is associated with severe complications, including nephropathy, hepatopathy, neuropathy, and cardiovascular disorders. Current synthetic antidiabetic drugs often present limitations such as side effects and long-term inefficiency, which has encouraged the search for alternative natural therapies [4, 5].

Oxidative stress plays a central role in the pathogenesis and progression of diabetes and its complications. Reactive oxygen species (ROS) contribute to cellular damage, lipid peroxidation, and protein dysfunction. Therefore, natural antioxidants derived from plants are considered promising therapeutic agents due to their ability to neutralize free radicals and restore redox balance [6].

In this context, the present study focuses on the phytochemical characterization and biological evaluation of *Pelargonium graveolens* extract collected from the El Oued region (Algeria). The study integrates in vitro, in vivo, and in silico approaches to comprehensively

investigate its antioxidant, antidiabetic, anti-inflammatory, and antimicrobial activities.

Advanced analytical techniques such as LC-MS were employed to identify the major bioactive constituents, while molecular docking simulations were performed to explore the interaction mechanisms between identified compounds and key therapeutic targets, including α-amylase, α-glucosidase, and cyclooxygenase-2 (COX-2).

Furthermore, ADMET profiling was conducted to evaluate the pharmacokinetic and toxicity properties of major compounds, ensuring their drug-likeness and safety profiles. These combined approaches provide a deeper understanding of the pharmacological potential of this plant and its possible application in drug discovery and development.

Overall, this work aims to bridge experimental phytochemistry with computational biology to validate the therapeutic potential of *Pelargonium graveolens* as a natural source of multi-target bioactive compounds for the management of diabetes and oxidative stress-related diseases.

Thesis Organization

This thesis is structured into two main parts, each comprising several chapters that cover both theoretical background and experimental investigations.

Part I: Theoretical Part

Chapter I:Diabetes Mellitus and Medicinal Plants: Focus on *Pelargonium graveolens*

This chapter provides a brief overview of medicinal plants and diabetes mellitus, with emphasis on *Pelargonium graveolens*. It covers the botanical characteristics, traditional uses, and pharmacological properties of medicinal plants, as well as the epidemiology, pathophysiology, complications, and management of diabetes mellitus. The chapter also highlights the limitations of conventional treatments and the growing interest in plant-based alternative therapies.

Chapter II: Molecular Docking and Pharmacokinetic Evaluation

This chapter introduces computational approaches used in drug discovery, including molecular docking, ADMET analysis, and drug-likeness evaluation of bioactive compounds identified in *Pelargonium graveolens*.

Part II: Practical Part

Chapter III: Materials and Methods

This chapter describes the plant material collection, extraction procedures, phytochemical analysis, in vitro biological assays, in vivo experimental protocols, and in silico methodologies used in this study.

Chapter IV: Results and Discussion

This chapter presents and discusses the experimental and computational results, including extraction yield, LC-MS phytochemical profiling, antioxidant activity, antidiabetic effects, molecular docking interactions, and ADMET profiling, followed by an integrated mechanistic interpretation of the findings.

PARTIE I

Theoretical Part

Chapter I

Diabetes Mellitus and Medicinal Plants: Focus on *Pelargonium graveolens*

Chapter I

Diabetes Mellitus and Medicinal Plants: Focus on *Pelargonium graveolens*

I.1 Introduction

Medicinal and aromatic plants have occupied an essential position in human life since ancient times. They have been used as sources of food, medicine, perfumes, dyes, spices, cosmetics, and natural products for domestic and industrial applications. Long before the development of modern synthetic drugs, humans depended on plants to treat wounds, infections, digestive disorders, respiratory diseases, fever, pain, inflammation, and many other health problems [7, 8].

Today, medicinal plants continue to play an important role in healthcare systems worldwide. According to the World Health Organization, traditional medicine remains a primary source of healthcare for a large proportion of the world's population, particularly in developing countries [9]. Furthermore, medicinal plants constitute an important reservoir of bioactive compounds that serve as therapeutic agents or as lead molecules for the development of modern pharmaceuticals [10].

Among medicinal and aromatic plants, *Pelargonium graveolens* L'Hér. (rose-scented geranium) is a perennial species belonging to the family Geraniaceae. The plant is widely cultivated in many regions of the world and has attracted considerable scientific interest because of its rich phytochemical composition and numerous biological properties [11, 12]. In traditional medicine, different parts of the plant, particularly the leaves, are used for the treatment of various ailments including inflammatory disorders, microbial infections, gastrointestinal

disturbances, and skin diseases [13].

Phytochemical investigations have revealed that extracts of *P. graveolens* contain a wide variety of secondary metabolites such as phenolic compounds, flavonoids, tannins, coumarins, terpenoids, and alkaloids [14, 15]. These compounds are known for their antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities. The biological effectiveness of the plant is largely attributed to these bioactive constituents, whose concentration and composition may vary according to environmental conditions, geographical origin, extraction method, and plant developmental stage.

The study of plant extracts has gained increasing attention because extraction processes allow the recovery of numerous non-volatile bioactive compounds that may not be present in essential oils. Therefore, the characterization of these extracts and the evaluation of their biological properties are important for understanding their potential applications in pharmaceutical, nutraceutical, and food industries.

The present chapter provides a general bibliographic study on medicinal plants and a detailed overview of *P. graveolens*. It includes the definition and classification of medicinal plants, their importance, botanical and taxonomic characteristics of the species, geographical distribution, traditional uses, cultivation requirements, phytochemical composition of its extracts, biological activities, and the significance of investigating this plant in the El-Oued region of Algeria.

I.2 General Information on Medicinal Plants

I.2.1 Historical Background

Since ancient times, humans have turned to plants for essential nutrients, medicine, and survival. In traditional societies, knowledge about medicinal plants was transmitted orally from generation to generation. This empirical knowledge formed the basis of several traditional medical systems such as African traditional medicine, Arabic medicine, Chinese medicine, Ayurveda, and Mediterranean folk medicine [16, 17].

The use of medicinal plants was not limited to the treatment of diseases. Many plants were also used as spices, preservatives, insect repellents, cosmetic ingredients, perfumes, and ritual materials. With the development of modern science, many traditional uses of plants have been investigated using pharmacological, phytochemical, microbiological, and toxicological

methods.

Currently, plant-based medicine is experiencing renewed interest worldwide. This resurgence is related to several factors, including the high cost of some synthetic drugs, the search for natural products, the development of analytical techniques, and the growing interest in sustainable and traditional health practices [18, 19]. Scientific research has confirmed that many plants contain bioactive compounds capable of producing therapeutic effects.

I.2.2 Definition of Medicinal Plants

A medicinal plant is generally defined as any plant that contains, in one or more of its organs, substances that can be used for therapeutic purposes or that may serve as precursors for the synthesis of useful drugs [20,21]. These substances may be present in roots, rhizomes, stems, bark, leaves, flowers, fruits, seeds, latex, resin, or the whole plant.

The therapeutic effect of medicinal plants is mainly attributed to their active compounds, also called bioactive secondary metabolites. These compounds are not always directly involved in basic plant growth, but they play important roles in plant defence, adaptation, pollinator attraction, and protection against environmental stress [20]. The most important groups of plant secondary metabolites include alkaloids, flavonoids, tannins, terpenoids, saponins, coumarins, phenolic acids, glycosides, steroids, and essential oils.

Medicinal plants can be used in two main forms. The first is the traditional or crude form, where the plant is used as an infusion, decoction, powder, poultice, macerate, or herbal preparation. The second is the processed form, where active compounds are extracted, concentrated, purified, standardized, and incorporated into pharmaceutical, cosmetic, or nutraceutical products.

I.2.3 Classification of Medicinal Plants

Medicinal plants may be classified according to several criteria. The most common classifications are based on the plant part used, therapeutic activity, chemical composition, and commercial application.

Classification According to the Plant Part Used

In pharmacognosy and ethnobotany, plants are often classified according to the organ used medicinally. This classification is important because active compounds are frequently

concentrated in specific plant parts.

- **Roots:** roots may contain alkaloids, glycosides, tannins, or polysaccharides.
- **Rhizomes:** underground stems rich in essential oils or starch, such as ginger.
- **Bark:** often rich in tannins, alkaloids, or phenolic compounds.
- **Leaves:** frequently used because they are accessible and rich in phenolic compounds, flavonoids, tannins, and other bioactive secondary metabolites.
- **Flowers:** used for aromatic, sedative, anti-inflammatory, or cosmetic properties.
- **Fruits:** may contain vitamins, oils, sugars, acids, or phenolics.
- **Seeds:** often rich in fixed oils, proteins, alkaloids, or mucilage.
- **Whole aerial parts:** used especially for herbaceous plants.

In the case of *P. graveolens*, the leaves and young aerial parts are the most commonly used organs because they are rich in bioactive secondary metabolites, including phenolic compounds, flavonoids, tannins, and other phytochemicals. These constituents can be recovered through various extraction methods and are responsible for many of the biological activities attributed to the plant, such as antioxidant, antimicrobial, anti-inflammatory, and therapeutic effects [22, 23].

Classification According to Therapeutic Use

Medicinal plants may also be classified according to their traditional or pharmacological activity. In this approach, plants are grouped based on the main therapeutic effects for which they are commonly used, such as antimicrobial, anti-inflammatory, antidiabetic, antioxidant, analgesic, sedative, digestive, diuretic, hepatoprotective, wound-healing, or antiparasitic activities. This classification is particularly useful in traditional medicine and pharmacology because it directly links plant use to disease management.

Classification According to Chemical Constituents

From a phytochemical point of view, medicinal plants can be grouped according to their major active compounds. Accordingly, plants may be described as being rich in essential

oils, alkaloids, flavonoids, tannins, saponins, glycosides, mucilage, or resins. *P. graveolens* belongs mainly to the group of aromatic and essential-oil plants because its leaves are rich in volatile terpenoid compounds [24, 25].

Commercial Classification

Medicinal and aromatic plants may also be classified according to their commercial value and the sectors in which they are exploited. In this context, they can be marketed as medicinal herbs, essential-oil plants, spices, cosmetic plants, food additives, perfume plants, dye plants, or industrial plants. *P. graveolens* is commercially important mainly as an essential-oil and perfume plant.

I.2.4 Importance of Medicinal Plants

Medicinal plants are important for several reasons. They provide therapeutic agents that are used directly in traditional medicine and phytotherapy, and they also represent valuable raw materials for pharmaceutical research and drug development. Indeed, several modern drugs were discovered from plants or inspired by plant-derived molecules [26]. In addition to their medical value, medicinal plants have clear economic importance because they are used in the production of herbal medicines, essential oils, cosmetics, perfumes, food additives, natural preservatives, and nutraceuticals; in many rural areas, they also constitute an important source of income for local communities. From a scientific perspective, medicinal plants are a major resource of structurally diverse molecules with potential biological activities, where phytochemical studies aim to identify active compounds and pharmacological studies evaluate their effects. However, it should be emphasized that natural origin does not always guarantee safety, since some medicinal plants can be toxic or allergenic or may interact with conventional drugs; therefore, rigorous scientific evaluation of efficacy and safety remains necessary [27].

I.3 Aromatic Plants and Plant Extracts

Aromatic plants are characterized by the production of characteristic odours due to volatile compounds that are usually stored in specialized structures such as glandular trichomes, secretory ducts, oil glands, or resin canals. In addition to these volatile constituents, aromatic

plants are also rich in numerous non-volatile bioactive substances that can be recovered as plant extracts. Plant extracts are complex mixtures obtained from different organs (leaves, flowers, stems, or roots) using extraction techniques such as infusion, decoction, maceration, percolation, Soxhlet extraction, or assisted methods (ultrasound- or microwave-assisted extraction), generally with solvents such as water, ethanol, methanol, acetone, or hydroalcoholic mixtures [28].

The chemical composition of these extracts commonly includes phenolic acids, flavonoids, tannins, coumarins, and other secondary metabolites, and it varies according to the species and genotype, geographical origin, climate and soil conditions, phenological stage, plant organ, solvent polarity, extraction time and temperature, as well as storage conditions [28, 29]. Because of their richness in antioxidant and antimicrobial constituents, plant extracts from aromatic species are widely investigated for applications in pharmaceutical and nutraceutical products, cosmetics, and as natural additives or preservatives in foods. Extracts from rose-scented geranium (*Pelargonium graveolens*) are particularly valued due to their pleasant aroma and their reported biological properties.

I.4 General Presentation of *Pelargonium graveolens*

I.4.1 Definition and Common Names

Pelargonium graveolens L'Hér. is an aromatic perennial plant belonging to the family Geraniaceae. It is commonly known as rose geranium, rose-scented geranium, or sweet-scented geranium. In commercial language, the essential oil obtained from this plant is often called geranium oil or rose geranium oil [22, 23].

The plant is known for its strongly fragrant leaves. When the leaves are touched or crushed, they release a rose-like aroma due to the presence of volatile compounds such as citronellol, geraniol, and linalool [24, 25]. Because of this fragrance, the plant is widely cultivated for perfumery, cosmetics, aromatherapy, and ornamental purposes.

I.4.2 Etymology

The genus name *Pelargonium* is derived from the Greek word *pelargos*, meaning "stork", because the fruit resembles the beak of a stork. The specific epithet *graveolens* comes from

Latin and means “strong-smelling”, referring to the strong fragrance of the plant [22].

I.4.3 Taxonomic Note

Historically, several species of *Pelargonium* were confused with the genus *Geranium*. Although both genera belong to the family Geraniaceae, they are botanically distinct. The common name “geranium” is still widely used in horticulture and essential-oil commerce for species that actually belong to the genus *Pelargonium* [22].

According to Plants of the World Online, *Pelargonium graveolens* L’Hér. is an accepted species [30].

I.5 Scientific Classification of *Pelargonium graveolens*

The taxonomic classification of *P. graveolens* is presented in Table I.1. Correct botanical identification is essential in phytochemical and biological studies because different species, cultivars, hybrids, and chemotypes may present different chemical profiles.

Table I.1: Classical botanical classification of *Pelargonium graveolens* L’Hér.

Taxonomic rank	**Classification**
Kingdom	Plantae
Subkingdom	Tracheobionta / Tracheophytes
Superdivision	Spermatophyta / Seed plants
Division	Magnoliophyta / Angiosperms
Class	Magnoliopsida / Dicotyledons
Subclass	Rosidae
Order	Geraniales
Family	Geraniaceae
Genus	*Pelargonium*
Species	*Pelargonium graveolens* L’Hér.

I.6 Geographical Distribution

I.6.1 Native Distribution

P. graveolens is native to southern Africa. Modern taxonomic databases report that the native range of the species extends from eastern Zimbabwe to the eastern Cape Province of South Africa [30]. From its native region, the plant has been introduced and cultivated in many countries because of its aromatic and industrial value.

I.6.2 Global Cultivation

Rose geranium is cultivated in several parts of the world, including South Africa, Egypt, Morocco, Algeria, Tunisia, Madagascar, Reunion Island, India, China, France, and Spain [22,23]. It is cultivated mainly for essential oil production. The oil is used as a substitute for rose oil in perfumery because it has a pleasant rose-like fragrance and is less expensive than true rose oil.

I.6.3 Distribution in Algeria

In Algeria, *P. graveolens* is mainly known as a cultivated aromatic and medicinal plant. It has been introduced and cultivated for ornamental, medicinal, and essential-oil purposes. Algerian rose-scented geranium essential oil has been studied chemically and biologically. Boukhatem et al. reported that essential oil from Algerian *P. graveolens* contained 45 identified compounds representing 94.2% of the oil. The main constituents were citronellol, citronellyl formate, and geraniol [24].

I.6.4 The El-Oued Region

El-Oued, also known as the Souf region, is located in the Algerian Sahara. The region is characterized by an arid climate, sandy soils, high temperatures, low rainfall, strong sunlight, and high evaporation. These environmental conditions may influence the growth and secondary metabolism of medicinal and aromatic plants.

An ethnobotanical study in the El-Oued region inventoried 73 medicinal plants belonging to 37 families and showed that the local population uses medicinal plants for several disorders,

especially digestive and respiratory diseases [31]. Although *P. graveolens* is not native to the Algerian Sahara, studying specimens collected or cultivated in El-Oued is scientifically important because arid conditions may influence the chemical composition and biological activity of the plant.

I.7 Traditional Uses of *Pelargonium graveolens*

P. graveolens has been used in traditional medicine in different regions of the world. Its leaves and essential oil are used for their fragrance and medicinal properties. Traditionally, the plant has been used for digestive disorders, respiratory problems, throat infections, wounds, skin inflammation, fever, headache, anxiety, and microbial infections [34, 35].

In North African traditional medicine, rose geranium is considered an aromatic and medicinal plant. It has been used for its antiseptic, anti-inflammatory, digestive, antidiabetic, and calming properties. In Tunisia, the plant has been reported in folk medicine for hyperglycaemia, which encouraged scientific evaluation of its antidiabetic activity in animal models [36].

The traditional uses of *P. graveolens* may be related to its essential oil and phenolic compounds. Essential oil constituents such as citronellol, geraniol, and linalool may contribute to antimicrobial and anti-inflammatory effects, while flavonoids and phenolic acids may contribute to antioxidant activity [35, 37].

I.8 Botanical Description of *Pelargonium graveolens*

I.8.1 General Habit

P. graveolens is a fast-growing, aromatic, evergreen perennial subshrub. It is generally upright, branched, and semi-woody. Under suitable growing conditions, it can reach approximately 1–1.5 m in height and may spread to about 1 m in diameter [38, 39].

The aerial parts are covered with hairs and glandular trichomes. These glandular trichomes are important because they synthesize and store essential oil. The plant is mainly cultivated for its leaves, which are the richest part in essential oil.

I.8.2 Root System

The root system is fibrous and well developed. It allows the plant to anchor itself in well-drained soils and absorb water and minerals. A good root system is important for adaptation to semi-arid and Mediterranean conditions. However, the plant is sensitive to waterlogging, which may cause root rot and reduce plant vitality.

I.8.3 Stem

The stems are green and succulent when young, but become woody at the base with age. Older stems may appear brownish-grey and semi-woody. The plant is usually highly branched, especially after pruning. Young stems also contain glandular hairs and contribute to the aromatic character of the plant.

Uncomment this figure if you have the image file in your Overleaf project.

Figure I.1: Stem of *Pelargonium graveolens*.

I.8.4 Leaves

The leaves are the most important organ for essential oil production. They are generally alternate, petiolate, soft, hairy, and strongly aromatic. The leaf blade is palmately lobed, often with toothed or serrated margins. The upper surface is usually green, while the lower surface may be paler and more veined.

When the leaves are crushed, they release a strong rose-like odour due to volatile compounds such as citronellol, geraniol, linalool, and other monoterpenes [40–42]. The presence of glandular trichomes on the leaf surface explains the high essential-oil content of the leaves.

Figure I.2: Leaf of *Pelargonium graveolens*.

I.8.5 Flowers

The flowers of *P. graveolens* are small, zygomorphic, and arranged in umbel-like clusters. They are usually pale pink to lavender, often with darker purple markings on the upper petals. The flowers attract pollinators such as bees and other insects.

Figure I.3: Flower of *Pelargonium graveolens*.

I.8.6 Fruits

The fruit is a dry schizocarp typical of the Geraniaceae family. It has a beak-like shape, which explains the origin of the genus name *Pelargonium*. At maturity, the fruit splits into five one-seeded mericarps.

I.8.7 Seeds

The seeds are small and elongated. In many Geraniaceae species, each seed has a spiral awn that helps dispersal and may assist the seed in entering the soil. In cultivation, however, *P. graveolens* is commonly propagated by stem cuttings rather than seeds in order to preserve the desired chemotype and essential-oil quality.

I.9 Cultivation Requirements of *Pelargonium graveolens*

I.9.1 Soil

P. graveolens grows best in light, fertile, and well-drained soils. Sandy-loam or loamy soils are suitable if they contain adequate organic matter. Heavy clay soils and waterlogged soils are not recommended because they may cause root asphyxia and fungal diseases.

The preferred soil pH is generally slightly acidic to neutral, approximately between 5.5 and 7.0. In arid regions such as El-Oued, soil salinity should be monitored because salinity may reduce plant growth and biomass production.

I.9.2 Temperature and Light

Rose geranium prefers warm climates and good sunlight. It grows well under full sun or light partial shade. The favourable temperature range is approximately 15–30°C. Frost can damage the plant, while extreme heat may reduce growth if water is insufficient.

I.9.3 Water Requirements

The plant requires regular watering during the establishment phase. Once established, it shows moderate drought tolerance. However, severe water stress can reduce vegetative growth and essential-oil yield. In Saharan regions, controlled irrigation is generally necessary for successful cultivation.

I.9.4 Propagation

Commercial propagation is generally performed by stem cuttings. This method helps maintain genetic uniformity and stable essential-oil composition. Seed propagation is pos-

sible but is less commonly used for commercial production because it may result in genetic variability.

I.9.5 Harvesting

The aerial parts are usually harvested when the plant has produced abundant leaves. Harvesting may be done before or during flowering, depending on the objective of the study or cultivation. The phenological stage can influence essential-oil yield and composition [43]. After harvesting, plant material may be used fresh or dried in shade under good ventilation.

I.10 Chemical Composition of *Pelargonium graveolens*

I.10.1 General Chemical Profile

P. graveolens is chemically rich and contains both volatile and non-volatile compounds. The volatile compounds are found mainly in the essential oil and are responsible for the characteristic fragrance. The non-volatile compounds are mainly present in polar extracts and include phenolic acids, flavonoids, tannins, and other secondary metabolites [44].

The chemical composition of *P. graveolens* may vary according to geographical origin, climate, soil, irrigation, salinity, plant organ, phenological stage, drying method, and extraction technique [45, 46].

I.10.2 Volatile Compounds

The essential oil of *P. graveolens* is mainly composed of oxygenated monoterpenes and sesquiterpenes. The most frequently reported compounds include citronellol, geraniol, linalool, isomenthone, menthone, citronellyl formate, geranyl formate, rose oxide, and eudesmol derivatives [45, 47, 48].

Table I.2: Major volatile compounds reported in *P. graveolens* essential oil.

Compound	Chemical class	Importance
Citronellol	Monoterpene alcohol	Rose-like odour; antimicrobial potential
Geraniol	Monoterpene alcohol	Floral odour; antioxidant and antimicrobial potential
Linalool	Monoterpene alcohol	Fragrance; calming and antimicrobial properties
Menthone	Monoterpene ketone	Fresh minty note; contributes to oil profile
Isomenthone	Monoterpene ketone	Chemotype marker in some oils
Citronellyl formate	Monoterpene ester	Important for fragrance quality
Geranyl formate	Monoterpene ester	Floral odour; perfume value
Rose oxide	Monoterpene oxide	Characteristic rose note

In an Algerian study, Boukhatem et al. identified 45 compounds representing 94.2% of the oil. The main compounds were citronellol at 30.2%, citronellyl formate at 9.3%, and geraniol at 7.6% [45]. This confirms the importance of citronellol and geraniol as major chemical markers of rose geranium essential oil.

I.10.3 Citronellol/Geraniol Ratio

The citronellol/geraniol ratio is sometimes used as an indicator of essential-oil quality and chemotype. It is calculated as follows:

$$\text{Citronellol/Geraniol ratio} = \frac{\%\text{Citronellol}}{\%\text{Geraniol}} \tag{I.1}$$

For example, using the Algerian values reported by Boukhatem et al. [45]:

$$\frac{30.2}{7.6} = 3.97 \tag{I.2}$$

This shows that citronellol was approximately four times higher than geraniol in that Algerian essential oil sample.

I.10.4 Non-Volatile Compounds

In addition to essential oil, *P. graveolens* contains non-volatile secondary metabolites. These include phenolic acids, flavonoids, tannins, gallotannins, acyltartaric acids, acylcitric acids, and other polar compounds [44, 49]. These substances are commonly extracted with polar solvents such as methanol, ethanol, hydroethanol, ethyl acetate, or water.

Recent metabolite-profiling studies have shown that *P. graveolens* extracts contain a wide diversity of phenolic and flavonoid compounds. These compounds may contribute to antioxidant and enzyme-inhibitory activities [49].

I.11 Biological Activities of *Pelargonium graveolens*

I.11.1 Antioxidant Activity

Antioxidant activity is one of the most frequently reported biological properties of *P. graveolens*. Oxidative stress occurs when reactive oxygen species exceed the capacity of antioxidant defence systems. This phenomenon is involved in inflammation, diabetes, aging, neurodegenerative diseases, cardiovascular disorders, and tissue damage.

Several studies have reported antioxidant activity for essential oils and polar extracts of *P. graveolens* using assays such as DPPH, ABTS, FRAP, CUPRAC, and metal-chelating tests [50, 51]. The antioxidant activity may be related to volatile monoterpenes such as geraniol and citronellol, but polar extracts rich in phenolic compounds may show stronger antioxidant effects.

The percentage of inhibition in radical scavenging assays is commonly calculated as follows:

$$\text{Inhibition}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{I.3}$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

I.11.2 Antimicrobial Activity

The antimicrobial activity of *P. graveolens* essential oil and extracts has been investigated against Gram-positive bacteria, Gram-negative bacteria, yeasts, and fungi. Boukhatem et al. reported that Algerian rose geranium essential oil had promising antimicrobial activity against food spoilage and food-borne microorganisms, especially Gram-positive bacteria and yeasts [52].

Ben Hsouna and Hamdi studied the essential oil and organic extracts of *P. graveolens* growing in Tunisia and reported antimicrobial activity against several microorganisms [53]. The antimicrobial mechanism of essential oils is generally related to their hydrophobic nature, which allows them to interact with microbial membranes, disturb permeability, inhibit enzymes, and cause leakage of cellular components [54].

I.11.3 Anti-Inflammatory Activity

Inflammation is a biological response to infection, injury, or irritation. It involves immune cells, cytokines, prostaglandins, leukotrienes, nitric oxide, and oxidative stress. Geranium essential oil has been reported to possess anti-inflammatory effects in experimental models. Abe et al. reported suppression of neutrophil recruitment in mice by geranium essential oil [55]. Maruyama et al. also reported suppression of carrageenan- and collagen II-induced inflammation in mice by geranium oil [56].

These effects may be related to the ability of essential oil constituents such as citronellol, geraniol, and linalool to modulate inflammatory mediators and reduce oxidative stress.

I.11.4 Antidiabetic Activity

The antidiabetic activity of *P. graveolens* has been evaluated in animal models. Boukhris et al. studied the hypoglycemic and antioxidant effects of leaf essential oil in alloxan-induced diabetic rats. The study reported a decrease in blood glucose and improvement of antioxidant parameters after treatment with the essential oil [57].

Possible mechanisms include protection of pancreatic beta cells, improvement of glucose metabolism, reduction of oxidative stress, and inhibition of carbohydrate-digesting enzymes such as α-amylase and α-glucosidase.

I.11.5 Wound-Healing and Dermatological Effects

The traditional use of *P. graveolens* for skin problems, wounds, and inflammation has encouraged scientific research on its dermatological potential. Wound healing involves inflammation, tissue regeneration, collagen deposition, angiogenesis, and re-epithelialization. Extracts with antimicrobial, antioxidant, and anti-inflammatory properties may support this process.

Because of its pleasant fragrance and biological activities, *P. graveolens* is also used in cosmetic products such as creams, lotions, soaps, perfumes, and massage oils.

I.11.6 Insecticidal and Repellent Activities

The strong aroma of *P. graveolens* is associated with insect-repellent and insecticidal properties. Compounds such as citronellol, geraniol, and linalool are commonly found in natural repellents and may affect insect olfactory receptors or nervous system function. These properties may be useful in the development of natural insect repellents and biopesticides [58].

I.11.7 Cytotoxic Potential

Some studies have evaluated the cytotoxic potential of *P. graveolens* extracts and essential oil against cancer cell lines. However, it is important to distinguish preliminary *in vitro* cytotoxicity from clinically proven anticancer activity. Positive results in cell culture do not prove therapeutic efficacy in humans. More studies are required to evaluate selectivity, toxicity, mechanisms of action, and safety.

I.12 Overview of *In Vitro*, *In Vivo*, and *In Silico* Approaches

I.12.1 *In Vitro* Studies

In vitro methods are experiments performed outside living organisms, usually in test tubes, culture plates, microplates, or cell cultures. They are used for preliminary screening because they are rapid, reproducible, and require small quantities of extract.

For *P. graveolens*, common *in vitro* tests include:

- preliminary phytochemical screening;
- total phenolic content;
- total flavonoid content;
- antioxidant assays such as DPPH, ABTS, FRAP, and CUPRAC;
- antimicrobial assays such as disc diffusion and MIC determination;
- enzyme inhibition assays;
- cytotoxicity assays on cell lines.

I.12.2 *In Vivo* Studies

In vivo studies are performed in living organisms, generally laboratory animals. They are used to evaluate biological activity in a complete physiological system. For plant extracts, *in vivo* studies help assess absorption, metabolism, toxicity, organ effects, and therapeutic potential.

Reported *in vivo* studies on *P. graveolens* include antidiabetic, antioxidant, anti-inflammatory, and toxicity-related models [59–61]. All animal experiments must respect ethical principles, including replacement, reduction, and refinement, and must be approved by an institutional ethics committee.

I.12.3 *In Silico* Studies

In silico studies are computational methods used to predict biological activity, molecular interactions, pharmacokinetic properties, and toxicity. Molecular docking is one of the most common *in silico* methods. It predicts the interaction between a ligand and a target protein.

For *P. graveolens* extract, phytochemicals identified through phytochemical screening and chromatographic analyses may be selected as ligands for molecular docking studies. These constituents can be docked with target proteins associated with inflammation, diabetes, microbial infections, or oxidative stress. The docking results may provide insights into the possible mechanisms underlying the biological activities of the extract by predicting interactions between its phytochemicals and specific biological targets. However, these theoretical predictions should be supported by experimental validation.

I.13 Relevance of Studying *P. graveolens* from El-Oued

The study of *P. graveolens* collected or cultivated in El-Oued is scientifically relevant because environmental conditions can influence the chemical composition and biological activity of medicinal and aromatic plants. The El-Oued region is characterized by arid climate, sandy soils, intense sunlight, high temperature, and low rainfall. Such conditions may affect plant metabolism and may modify the production of essential oils, phenolics, flavonoids, and other secondary metabolites.

Studying this plant from El-Oued may help to:

- identify the chemical composition of the local sample;
- compare it with samples from other Algerian and international regions;
- evaluate its antioxidant, antimicrobial, anti-inflammatory, or antidiabetic potential;
- valorize local medicinal and aromatic plant resources;
- support future applications in pharmacology, cosmetics, food preservation, or aromatherapy.

However, the botanical origin of the plant must be clearly documented. The study should include the collection site, GPS coordinates, date of collection, plant organ used, phenological stage, drying conditions, extraction method, and voucher specimen information if possible.

I.14 Diabetes Mellitus: Pathophysiology and Management

Diabetes mellitus (DM) is one of the most prevalent chronic metabolic disorders worldwide, characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both [62, 63]. The disease represents a major public health challenge due to its increasing prevalence, high economic cost, and severe long-term complications that affect multiple organ systems.

According to the International Diabetes Federation (IDF) Diabetes Atlas (11th edition, 2025), approximately 589 million adults aged 20–79 years were living with diabetes in 2024–2025, representing 11.1% of the global adult population. This number is projected to rise to 853 million (approximately 1 in 8 adults) by 2050 [64, 65]. In Algeria, the burden is

particularly high: the country has approximately 4.8 million adults with diabetes (prevalence of 17.5% in the 20–79 age group), making it one of the most affected countries in the Middle East and North Africa (MENA) region [66, 67].

The rising prevalence is driven by urbanization, aging populations, sedentary lifestyles, obesity, and dietary changes. Type 2 diabetes mellitus (T2DM) accounts for over 90% of all cases globally. Because oxidative stress, inflammation, and impaired antioxidant defence play central roles in the pathogenesis and complications of diabetes, medicinal plants rich in phenolic compounds and essential oils (such as *Pelargonium graveolens*) have attracted considerable scientific interest as potential complementary agents [68, 69].

This chapter provides a comprehensive review of diabetes mellitus, including its definition and classification, epidemiology, pathophysiology (with emphasis on T2DM), clinical manifestations, complications, diagnostic criteria, current management strategies, and the rationale for investigating natural products such as *Pelargonium graveolens* extracts.

I.15 Definition and Classification of Diabetes Mellitus

Diabetes mellitus is defined as a group of metabolic disorders characterized by chronic hyperglycemia resulting from disturbances in carbohydrate, fat, and protein metabolism. The fundamental underlying defects are impaired insulin secretion, insulin resistance, or a combination of both [62, 70].

Table I.3: Classification of diabetes mellitus (adapted from ADA Standards of Care, 2026).

Type	Characteristics
Type 1 Diabetes Mellitus (T1DM)	Autoimmune β-cell destruction leading to absolute insulin deficiency. Usually appears in children and young adults.
Type 2 Diabetes Mellitus (T2DM)	Combination of insulin resistance and progressive β-cell dysfunction. Accounts for 90–95% of cases. Strongly associated with obesity.
Gestational Diabetes Mellitus (GDM)	Hyperglycemia first recognized during pregnancy. Increases risk of T2DM later in life.
Other specific types	Includes monogenic diabetes, diabetes due to pancreatic disease, drug-induced diabetes, and diabetes associated with endocrine disorders.

T2DM is the form most relevant to the present thesis, as the majority of studies on *Pelargonium graveolens* have focused on its hypoglycemic and antioxidant effects in models of insulin resistance and T2DM [69].

I.16 Epidemiology of Diabetes Mellitus

The global burden of diabetes has increased dramatically over the past four decades. The IDF estimates that in 2025 there are 589 million adults living with diabetes, with more than 4 in 10 cases undiagnosed. By 2050, this figure is expected to reach 853 million [71, 72].

The MENA region, which includes Algeria, has one of the highest prevalence rates worldwide. In Algeria, the number of adults with diabetes rose from approximately 1.4 million in 2011 to 4.8 million in 2024–2025, with a projected increase to 7.9 million by 2050. The age-standardized prevalence is estimated at 17.5% [73, 74]. Urbanization, dietary westernization, physical inactivity, and genetic predisposition are major contributing factors in the Algerian population.

Diabetes is a leading cause of blindness, kidney failure, lower-limb amputations, cardiovascular disease, and premature death. It also imposes a substantial economic burden on healthcare systems and families.

I.17 Pathophysiology of Type 2 Diabetes Mellitus

T2DM is a complex, heterogeneous disorder. Its two core pathophysiological defects are **insulin resistance** in peripheral tissues (mainly muscle, liver, and adipose tissue) and **progressive pancreatic β-cell dysfunction** leading to inadequate insulin secretion relative to the level of insulin resistance [75, 76].

Insulin resistance is characterized by impaired glucose uptake in skeletal muscle, increased hepatic glucose production (gluconeogenesis), and elevated lipolysis in adipose tissue. Several mechanisms contribute to insulin resistance, including:

- Ectopic lipid accumulation (lipotoxicity);
- Chronic low-grade inflammation (elevated TNF-α, IL-6, and CRP);
- Oxidative stress and mitochondrial dysfunction;
- Endoplasmic reticulum (ER) stress;
- Gut microbiota dysbiosis;
- Genetic and epigenetic factors.

Progressive β-cell failure eventually leads to overt hyperglycemia. In the early stages of insulin resistance, β-cells compensate by increasing insulin secretion (hyperinsulinemia). Over time, this compensatory mechanism fails due to glucotoxicity, lipotoxicity, oxidative stress, and inflammation, resulting in reduced β-cell mass and function [76, 77].

Hyperglycemia itself exacerbates oxidative stress through several pathways (polyol pathway, advanced glycation end-products (AGEs), protein kinase C activation, and hexosamine pathway), creating a vicious cycle that accelerates complications.

I.18 Clinical Manifestations and Complications

Classic symptoms of hyperglycemia include polyuria, polydipsia, polyphagia, unexplained weight loss, fatigue, and blurred vision. Many patients with T2DM remain asymptomatic for years and are diagnosed only when complications appear.

Diabetes complications are divided into:

I.18.1 Acute Complications

- Diabetic ketoacidosis (more common in T1DM);
- Hyperosmolar hyperglycemic state (more common in T2DM);
- Hypoglycemia (usually treatment-related).

I.18.2 Chronic Complications

Chronic hyperglycemia leads to microvascular and macrovascular damage:

- **Microvascular:** retinopathy (leading cause of blindness), nephropathy (leading cause of end-stage renal disease), and neuropathy (sensory, motor, and autonomic).
- **Macrovascular:** coronary artery disease, cerebrovascular disease (stroke), and peripheral artery disease (leading to foot ulcers and amputations).
- Other complications include diabetic foot syndrome, increased susceptibility to infections, non-alcoholic fatty liver disease, cognitive decline, and certain cancers.

Oxidative stress and advanced glycation play central roles in the development of these complications [78].

I.19 Diagnosis of Diabetes Mellitus

The American Diabetes Association (ADA) diagnostic criteria (2026 Standards of Care) are summarized in Table I.4.

Table I.4: Diagnostic criteria for diabetes mellitus (ADA, 2026).

Criterion	Threshold
HbA1c	$\geq$ 6.5% (48 mmol/mol) (using NGSP-certified method)
Fasting Plasma Glucose (FPG)	$\geq$ 126 mg/dL (7.0 mmol/L) after no caloric intake for at least 8 h
2-hour Plasma Glucose during OGTT	$\geq$ 200 mg/dL (11.1 mmol/L) after 75 g anhydrous glucose load
Random Plasma Glucose	$\geq$ 200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis

In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing [79].

Prediabetes (increased risk for diabetes) is defined as HbA1c 5.7–6.4%, FPG 100–125 mg/dL, or 2-h OGTT glucose 140–199 mg/dL.

I.20 Current Management of Diabetes Mellitus

Management of T2DM is multifaceted and is based on an integrated approach that combines lifestyle modification, pharmacological therapy, and regular follow-up. Lifestyle measures remain the foundation of treatment and include medical nutrition therapy, increased physical activity, weight management, and smoking cessation. In parallel, patients are encouraged to use self-monitoring of blood glucose and, when appropriate, continuous glucose monitoring (CGM), in order to optimize day-to-day glycemic control and support therapeutic adjustments. Long-term care also requires systematic screening for diabetes complications and active management of cardiovascular risk factors.

Pharmacological therapy may involve oral and injectable antidiabetic agents, selected according to the patients glycemic status, comorbidities, and risk profile. Common drug classes include metformin as a first-line agent, sulfonylureas, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, thiazolidinediones, and insulin. Importantly, newer agents such as GLP-1 receptor agonists and SGLT2 inhibitors have demonstrated cardiovascular and renal

protective effects beyond glucose lowering [80]. Despite these advances, many patients do not achieve optimal glycemic control, and long-term use of some drugs may be limited by side effects or high cost, which explains the sustained interest in complementary and alternative therapies, including medicinal plants.

I.21 Role of Medicinal Plants and Phytotherapy in Diabetes Management

Medicinal plants have been used for centuries in traditional medical systems for the management of diabetes. Their hypoglycemic effects may involve several complementary mechanisms, including stimulation of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate-hydrolyzing enzymes (such as α-amylase and α-glucosidase), antioxidant activity, anti-inflammatory effects, and protection of pancreatic β-cells [81, 82].

Several studies have indicated that *Pelargonium graveolens* extracts and volatile fractions may exhibit antidiabetic potential. Boukhris *et al.* (2012) reported that leaf-derived preparations showed hypoglycemic and antioxidant effects in alloxan-induced diabetic rats, with reduced blood glucose levels and improvement of oxidative-stress markers [83]. These effects are generally attributed to major constituents (e.g., citronellol, geraniol, and linalool) and to phenolic compounds present in polar extracts.

In this context, combining chemical characterization with *in vitro* enzyme-inhibition assays, *in vivo* animal models, and *in silico* molecular docking (as proposed in the present thesis) provides a robust scientific framework for evaluating the antidiabetic potential of *P. graveolens* from the El-Oued region.

I.22 Conclusion

Diabetes mellitus, particularly T2DM, is a complex metabolic disorder with rapidly increasing global and national prevalence. Its pathophysiology involves insulin resistance, β-cell dysfunction, oxidative stress, chronic inflammation, and lipotoxicity. Long-term hyperglycemia leads to debilitating microvascular and macrovascular complications that significantly reduce quality of life and life expectancy.

Although conventional therapies have improved outcomes, there remains a need for safe,

effective, and affordable complementary agents. Medicinal plants such as *Pelargonium graveolens*, which combine antioxidant, anti-inflammatory, and hypoglycemic properties, represent a promising research direction. The present thesis aims to contribute to this field by investigating the chemical composition and the *in vitro*, *in vivo*, and *in silico* antidiabetic potential of *P. graveolens* collected from the El-Oued region of Algeria.

Chapter II

Molecular Docking and Pharmacokinetic Evaluation

Chapter II

Molecular Docking and Pharmacokinetic Evaluation

II.1 Introduction

In modern phytopharmacological research, computational approaches have become indispensable for accelerating the discovery and validation of bioactive compounds from medicinal plants. Molecular docking and *in silico* pharmacokinetic profiling allow researchers to predict the interaction of phytochemicals with biological targets and evaluate their drug-like properties before proceeding to costly *in vitro* and *in vivo* experiments [84, 85].

This chapter presents a comprehensive theoretical and methodological framework for molecular docking and pharmacokinetic (ADMET) evaluation applied to the major compounds identified in *Pelargonium graveolens* collected from the El-Oued region. Special emphasis is given to the principles of ADMET profiling and drug-likeness, with particular attention to topical delivery systems relevant to polar extracts of this plant.

II.2 Theoretical Principles of ADMET and Drug-Likeness

II.2.1 The Concept of In Silico ADMET Profiling

In the context of modern drug discovery and ethnopharmacological validation, evaluating therapeutic efficacy is insufficient without establishing the pharmacokinetic profile of a molecule. 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 [86, 87]. *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, physical, and chemical descriptors [88].

This computational approach bypasses early, resource-intensive animal testing, allowing researchers to quickly screen the compounds present in *Pelargonium graveolens* extract and assess their suitability for specialized delivery systems such as topical ointments.

II.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 strongly on its fundamental physicochemical and structural parameters. To quantify this concept of drug-likeness, Christopher Lipinski proposed the Rule of Five (Ro5) [89], which defines empirical boundaries that are commonly associated with good oral bioavailability and membrane permeability. According to this rule, a compound is more likely to show acceptable absorption and permeation when its molecular weight is below 500 g/mol, its lipophilicity (expressed as the octanol/water partition coefficient, Log $P_{o/w}$) is below 5, it has fewer than 5 hydrogen-bond donors (typically NH and OH groups), and fewer than 10 hydrogen-bond acceptors (mainly nitrogen and oxygen atoms).

When a natural compound complies with Lipinski's criteria, it suggests that its structural framework has favorable properties for crossing biological membranes, which is a key prerequisite for many pharmacological effects. In the present study, both volatile terpenoids from the essential oil and polar phenolic compounds from defatted extracts were evaluated against these descriptors to support the selection of promising candidates for further pharmacokinetic and biological assessment.

II.2.3 Aqueous Solubility (Log S) and Partition Coefficient (Log $P_{o/w}$) Dynamics

The biological activity of plant extracts is influenced by the physicochemical properties of their constituent phytochemicals. Among these properties, aqueous solubility (Log S) and the partition coefficient (Log $P_{o/w}$) are important parameters for predicting the behavior of individual compounds in biological environments. Aqueous solubility (Log S) reflects the maximum concentration of a compound that can dissolve in water at equilibrium, which is a prerequisite for its transport and interaction with biological targets. Therefore, *in silico* models such as ESOL [90] and the Ali model [91] are commonly used to estimate the solubility of phytochemicals identified in plant extracts based on their molecular structures. Compounds with very low Log S values generally exhibit poor aqueous solubility, which may limit their bioavailability, whereas compounds with moderate solubility are more likely to be effectively distributed in biological systems.

The partition coefficient (Log $P_{o/w}$) describes the distribution of the non-ionized form of a compound between an organic phase (*n*-octanol) and an aqueous phase (water), providing an indication of its lipophilicity. Compounds with moderate lipophilicity may show improved interactions with biological membranes and enhanced permeability, while excessively lipophilic compounds may exhibit poor aqueous solubility and a tendency to accumulate in lipid-rich tissues. Consequently, the combined evaluation of Log S and Log $P_{o/w}$ for the major phytochemicals present in an extract can provide valuable information about their potential absorption, distribution, and biological activity. Such predictions contribute to a better understanding of the pharmacological potential of plant extracts and support the interpretation of experimental findings.

II.2.4 Topical Drug Delivery Barriers and Skin Permeability (Kp)

Because *Pelargonium graveolens* extracts are often processed to remove volatile constituents, defatted extracts are typically enriched in polar bioactive compounds such as phenolic acids and flavonoids. In topical delivery, the *stratum corneum* remains the primary barrier, but the transport of these polar molecules is governed by physicochemical constraints that differ from those of lipophilic terpenes. In particular, the skin permeability coefficient (K_p, in cm/s) is strongly influenced by ionization state, molecular weight, and hydrogen-bonding

capacity, parameters that often limit the passive diffusion of polar constituents through the lipid-dense cornified layer.

As a result, polar non-volatile extracts frequently exhibit lower intrinsic permeability and may require formulation approaches designed to increase cutaneous flux, such as penetration enhancers or nanocarrier-based systems [92, 93]. In this study, predictive evaluation of K_p was considered especially important because it supports the rational development of topical antioxidant and antidiabetic formulations based on *P. graveolens*, with the objective of ensuring that water-soluble polyphenols can reach relevant target sites within the viable epidermis and dermis.

II.3 Molecular Docking Methodology

II.3.1 Selection of Ligands and Target Proteins

The ligands used for molecular docking were selected from the phytochemicals identified in *Pelargonium graveolens* extracts collected from the El-Oued region. Compound selection was based on phytochemical screening and chromatographic analyses of the extracts. The identified metabolites, particularly phenolic compounds, flavonoids, tannins, and other secondary metabolites, were considered potential ligands for docking studies in order to investigate their possible biological activities.

To evaluate the molecular basis of the antidiabetic and anti-inflammatory properties of *P. graveolens* extracts, three target enzymes were selected: α-amylase, α-glucosidase, and cyclooxygenase-2 (COX-2). The inhibition of α-amylase and α-glucosidase is associated with the reduction of postprandial glucose levels through the regulation of carbohydrate digestion, whereas COX-2 plays a key role in inflammatory processes. The crystallographic structures of these enzymes were retrieved from the RCSB Protein Data Bank (PDB), and the docking parameters used for molecular docking analysis are presented in Table II.1.

Table II.1: Molecular docking parameters used for target enzymes.

Target Enzyme	PDB ID	Grid Center (X, Y, Z)	Grid Size (Å)
α-Amylase	4GQR	(11.936, 18.819, 39.264)	$40 \times 40 \times 40$
Cyclooxygenase (COX-2)	5KIR	(23.959, 0.750, 34.306)	$40 \times 40 \times 40$
α-Glucosidase	3W37	(0.310, -2.052, -21.786)	$60 \times 60 \times 60$

II.3.2 Protein and Ligand Preparation

Protein structures were downloaded from the RCSB Protein Data Bank, and structural cleaning was performed using Discovery Studio Visualizer by removing water molecules, ions, and co-crystallized ligands. Polar hydrogens were then added and Gasteiger charges were assigned using AutoDock Tools (version 1.5.7), after which proteins were saved in PDBQT format. Ligand structures were obtained from PubChem (or drawn and checked for stereochemistry when necessary), energy-minimized using the MMFF94 force field, and converted to PDBQT format prior to docking.

II.3.3 Docking Procedure

Molecular docking was carried out using AutoDock Vina implemented in PyRx (version 0.8). For each protein, the grid box was centered on the active site and defined with dimensions of $25 \times 25 \times 25$ Å and a grid spacing of 1.0 Å. The exhaustiveness parameter was set to 32 to improve conformational sampling. Each ligand was docked in triplicate, and the best-ranked pose (lowest binding energy, kcal/mol) was selected for interaction analysis. The docking protocol was validated by redocking the native co-crystallized ligand and accepting the procedure when the root mean square deviation (RMSD) between experimental and predicted poses was ≤ 2.0 Å.

Finally, binding modes and key interactions (hydrogen bonding, hydrophobic contacts, π–π stacking, and electrostatic interactions) were visualized and analyzed using Discovery Studio Visualizer (2021) and PyMOL (2.5).

II.4 Pharmacokinetic and ADMET Prediction

Pharmacokinetic behavior and drug-likeness were predicted using the SwissADME web server [94]. This platform was used to calculate key descriptors relevant to absorption and permeability, including Lipinski's Rule of Five violations, molecular weight, Log $P_{o/w}$, predicted aqueous solubility (Log S), and topological polar surface area (TPSA). In addition, SwissADME was employed to estimate gastrointestinal absorption, skin permeability (Log K_p), and overall oral bioavailability indicators such as the Bioavailability Score and the Bioavailability Radar, as well as the predicted inhibition profile of major CYP450 isoforms.

To complement these pharmacokinetic predictions with a preliminary safety assessment, toxicity-related endpoints were estimated using pkCSM [95] and ProTox-II. The predicted parameters included AMES mutagenicity, hepatotoxicity, skin sensitization, acute toxicity (LD_{50}), and potential hERG channel inhibition, which is commonly used as an indicator of cardiac safety risk.

II.5 Computational Details

All computational analyses were performed on a Windows 11 workstation equipped with an Intel Core i7-12700H processor, 32 GB of RAM, and an NVIDIA RTX 3060 GPU. Protein preparation, molecular docking, and visualization of interaction results were carried out using AutoDock Tools 1.5.7, PyRx 0.8, Discovery Studio Visualizer 2021, and PyMOL 2.5. In parallel, pharmacokinetic and ADMET predictions were generated online using SwissADME and pkCSM during March-April 2026.

II.6 Relevance to the Present Study

The computational investigation of *Pelargonium graveolens* collected from the El-Oued region is particularly relevant because the arid Saharan environment may strongly influence the plants secondary metabolite profile. By integrating molecular docking with ADMET-based pharmacokinetic evaluation, this chapter provides a mechanistic framework for understanding how major compoundsespecially geraniol, citronellol, and polar phenolic constituentsmay contribute to antioxidant and antidiabetic effects. Such an approach also helps

assess their potential developability as topical therapeutic agents for diabetic wound management and oxidative-stress-related disorders.

II.7 Conclusion

This chapter has detailed the theoretical foundations and practical methodologies of molecular docking and *in silico* ADMET profiling used in the present thesis. The integration of these computational tools with experimental data offers a robust multidisciplinary approach to the scientific validation and potential pharmaceutical development of *Pelargonium graveolens* from the El-Oued region of Algeria.

PARTIE II

Practical Part

Chapter III

Materials and Methods

Chapter III

Materials and Methods

III.1 Introduction

This chapter describes, in a detailed and reproducible manner, the materials, reagents, instrumentation, and experimental procedures used in the present work. The study combined phytochemical characterization with *in vitro*, *in vivo*, and *in silico* investigations in order to evaluate the biological potential of *Pelargonium graveolens* leaves collected from the El-Oued region.

The experimental work was performed in the following institutions:

- the laboratories of the Faculty of Exact Sciences and the Faculty of Natural and Life Sciences, University of Echahid Hamma Lakhdar, El-Oued, Algeria;
- Al-Majd Laboratory for Medical Analysis, El-Oued, Algeria;
- the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques (CRAPC), Ouargla, Algeria.

Unless otherwise stated, all reagents used were of analytical grade. Distilled or ultrapure water was used throughout the experiments. All measurements were performed at least in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD).

III.2 Plant Material

III.2.1 Collection of Plant Material

Leaves of *Pelargonium graveolens* were collected in December 2025 from a private nursery located in El Bayadha, Wadi Souf region (Algeria). The plant material was cultivated under controlled home conditions under the supervision of the project supervisor. The sampling location is illustrated in Figure III.1.

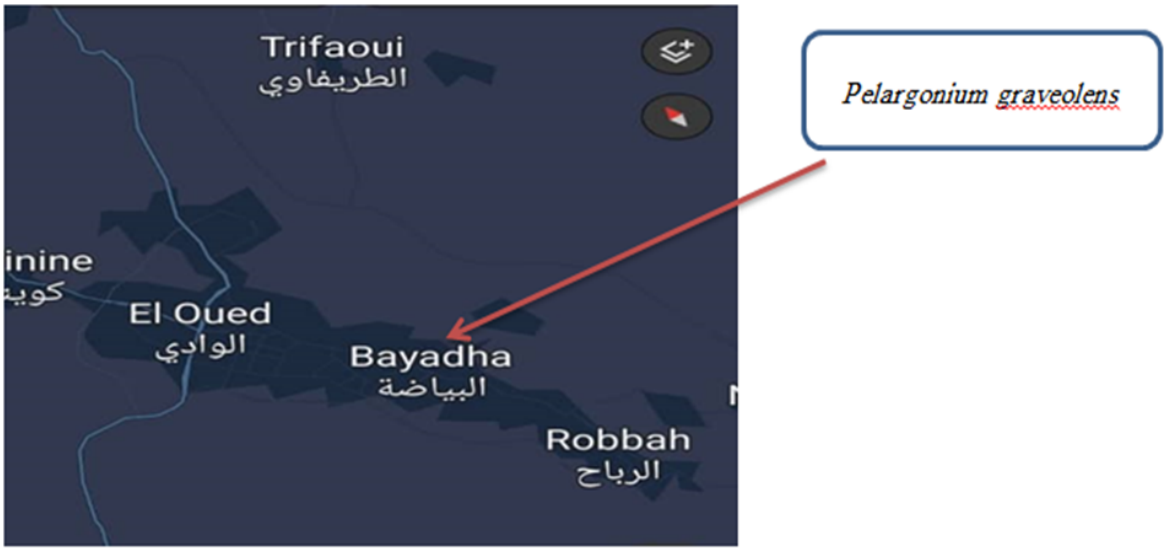


Figure III.1: Geographical location of the sampling site of *Pelargonium graveolens*.

III.2.2 Botanical Identification

The collected plant material was taxonomically identified at the Department of Biology, University of Echahid Hamma Lakhdar, El-Oued. A voucher specimen should be deposited in the departmental herbarium and referenced in the final version of the thesis with a voucher number.

III.2.3 Drying and Grinding

The leaves were washed, visually inspected to remove damaged or contaminated material, and dried in the shade at room temperature ($25 \pm 2\,^{\circ}\mathrm{C}$) for 15 days in a well-ventilated area, protected from direct sunlight and excess humidity. Shade-drying was preferred in order to limit the degradation of thermolabile and photosensitive secondary metabolites.

After complete drying, the leaves were ground using a clean electric grinder until a homogeneous fine powder was obtained. The powdered material was then stored in airtight amber containers at room temperature, protected from moisture and light, until extraction.

III.3 Chemicals, Reagents, Materials and Equipment

III.3.1 Main Solvents and Reagents

The principal solvents and reagents used in this study included hexane, methanol (96%), distilled water, formic acid, Folin–Ciocalteu reagent, sodium carbonate ($\mathrm{Na_2CO_3}$), aluminum chloride ($\mathrm{AlCl_3}$), DPPH (2,2-diphenyl-1-picrylhydrazyl), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride ($\mathrm{FeCl_3}$), bovine serum albumin (BSA), phosphate-buffered saline (PBS), Mueller–Hinton agar, porcine pancreatic α-amylase, starch, hydrochloric acid, iodine/potassium iodide solution, alloxan monohydrate, formalin, xylene, paraffin wax, and Hematoxylin–Eosin staining reagents.

III.3.2 Main Laboratory Equipment

The principal equipment used included an analytical balance, vacuum filtration apparatus, vacuum pump, rotary evaporator, UV–Visible spectrophotometer, 96-well microplate reader, centrifuge, incubator, LC–MS/MS apparatus, optical microscope, rotary microtome, histological water bath, paraffin embedding station, and routine glassware.

Table III.1: Main materials and equipment used in the study.

Equipment	Materials/Reagents	Tools/Consumables
Analytical balance	*P. graveolens* leaves	Conical flasks
Rotary evaporator	Hexane	Aluminum foil
Vacuum filtration apparatus	Methanol	Funnel and filter paper
Vacuum pump	Distilled water	Graduated cylinders
UV–Vis spectrophotometer	DPPH	Automatic pipettes and tips
LC–MS/MS system	Formic acid	Glass tubes
Microplate reader	BSA	96-well microplates
Centrifuge	Folin–Ciocalteu reagent	Sterile Petri dishes
Optical microscope	Sodium carbonate	Cork borer
Rotary microtome	α-amylase, starch	Glass slides and coverslips

III.4 Preparation of Plant Extract

III.4.1 Principle of Extraction

Extraction is the first essential step in phytochemical analysis. It consists of transferring soluble constituents from plant material into a suitable solvent system according to the polarity of the target metabolites [96, 97]. In this study, a sequential extraction strategy was adopted, beginning with hexane defatting followed by methanolic maceration in order to enrich the final extract in medium- to high-polarity compounds such as phenolic acids and flavonoids.

III.4.2 Defatting with Hexane

Thirty grams (30 g) of dried leaf powder were accurately weighed and transferred into a conical flask. Then, 220 mL of hexane were added. The mixture was stirred gently and left at room temperature for 24 h in order to remove non-polar substances such as waxes, lipids,

chlorophylls, and other hydrophobic compounds.

After this period, the mixture was filtered. The hexane filtrate was discarded, while the solid residue was retained for polar extraction.

III.4.3 Methanolic Maceration

The defatted residue was transferred into a clean flask and macerated with 220 mL of methanol (96%) for 72 h at room temperature, with occasional stirring. Methanol was selected because it is widely used for the extraction of phenolic constituents from plant matrices [96, 98].

At the end of the maceration period, the mixture was filtered under vacuum. The methanolic filtrate was collected and concentrated to dryness under reduced pressure using a rotary evaporator at 40–45 °C. The dried crude extract was weighed, transferred into a glass vial, and stored at 4 °C until analysis. The overall extraction procedure is summarized in Figure III.2.

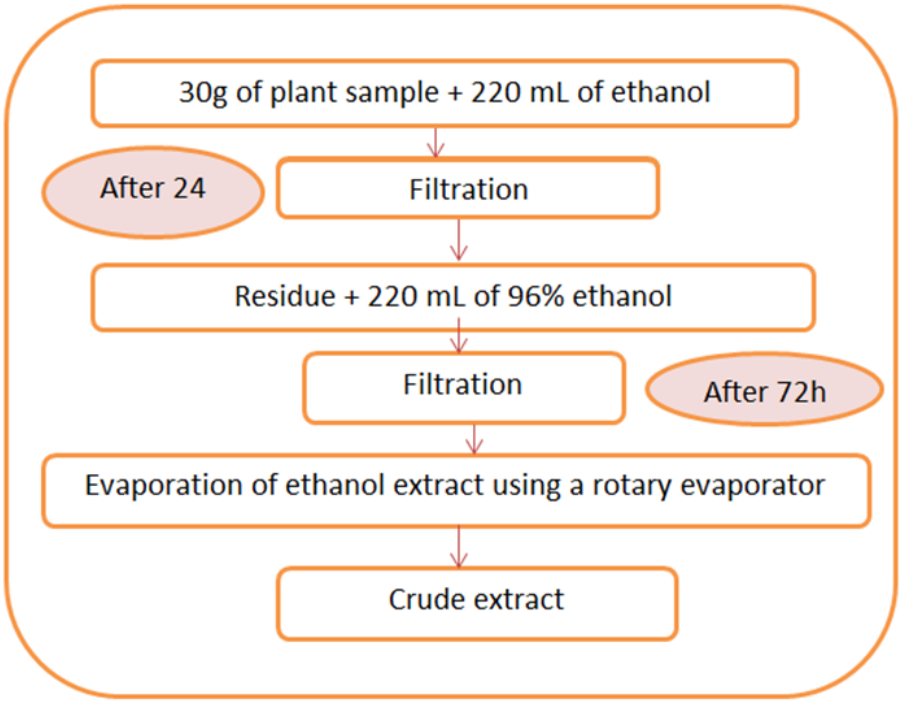


Figure III.2: Schematic representation of the extraction procedure: drying, grinding, hexane defatting, methanolic maceration, filtration, and solvent evaporation.

III.4.4 Calculation of Extraction Yield

The extraction yield was calculated according to Equation IV.1:

$$R(\%) = \left(\frac{m_f}{m_i}\right) \times 100 \tag{III.1}$$

where:

- m_f = mass of the dried extract (g),
- m_i = initial mass of dry plant powder (g),
- $R(\%)$ = extraction yield percentage.

III.5 LC–MS Analysis

Phytochemical profiling of a single extract of *Pelargonium graveolens* was performed using a Shimadzu 8040 UPLC–ESI–MS/MS system equipped with ultra-high sensitivity UFMS technology and coupled to a Nexera XR LC-20AD binary pump. Electrospray ionization (ESI) parameters were optimized as follows: collision-induced dissociation (CID) gas pressure, 230 kPa; conversion dynode voltage, -6.00 kV; desolvation line temperature, 250 °C; nebulizing gas flow, 3.0 L/min; heat block temperature, 400 °C; and drying gas flow, 10 L/min.

Chromatographic separation was carried out using a Restek Ultra C18 column (3 μm, 150 $\times$ 4.6 mm). The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (methanol). The flow rate was maintained at 0.2 mL/min, and the injection volume was 5 μL.

The gradient elution program was as follows: 0–0.2 min, 98% A; 0.2–2.5 min, 25% A; 2.5–4.0 min, 0% A; 4.0–7.0 min, 0% A; 7.0–7.1 min, 98% A; and 7.1–12.0 min, 98% A.

This analytical method allowed high-resolution separation and sensitive detection of secondary metabolites in a single extract of *Pelargonium graveolens*, including flavonoids, phenolic acids, alkaloids, and other bioactive compounds [99, 100].

III.6 In Vitro Biological Assays

III.6.1 Hemolytic Activity Assay

The hemolytic activity assay was conducted as a preliminary biocompatibility test based on erythrocyte membrane integrity. Because hemolysis reflects membrane damage and re-

lease of hemoglobin, it is commonly used as an indicator of blood compatibility in extract screening and hemocompatibility evaluation [101, 102].

Fresh human blood was collected from healthy adult volunteers into heparinized tubes after informed consent and according to institutional ethical approval (**insert approval code**). Erythrocytes were separated by centrifugation and washed three times with PBS ($1\times$) at 1000 $\times g$ for 5 min. The washed erythrocytes were resuspended in PBS to obtain a final hematocrit of 2%.

The extract was dissolved in PBS or PBS containing a non-hemolytic amount of DMSO/methanol (final solvent concentration $\leq 1\%$). Serial twofold dilutions were prepared to obtain the following concentrations: 50, 25, 12.5, 6.25, 3.12, and 1.56 mg/mL.

In sterile 96-well plates, 100 μL of erythrocyte suspension were mixed with 100 μL of each extract concentration. PBS served as a negative control, while 0.1% H_2O_2 served as a positive control. After incubation at $37\,^{\circ}\mathrm{C}$ for 1 h under gentle agitation, the plate was centrifuged at 1000 $\times g$ for 5 min. Then, 100 μL of supernatant were transferred to a new plate and the absorbance was read at 540 nm.

The hemolysis percentage was calculated using:

$$\text{Hemolysis}(\%) = \left(\frac{A_s - A_n}{A_p - A_n}\right) \times 100 \tag{III.2}$$

where A_s is the sample absorbance, A_n is the negative control absorbance, and A_p is the positive control absorbance.

III.6.2 Antibacterial Activity by Agar Well Diffusion

The antibacterial activity of the extract was evaluated using the agar well diffusion method as a preliminary qualitative antimicrobial screening assay. Standardized antimicrobial susceptibility testing conditions were adapted in accordance with CLSI recommendations for the culture medium, inoculum preparation, and incubation conditions [103, 104].

Two bacterial strains were used:

- *Escherichia coli* ATCC 25922;
- *Staphylococcus aureus* ATCC 25923.

Fresh bacterial cultures (18–24 h) were adjusted to approximately 10^6 CFU/mL, corresponding to the 0.5 McFarland standard. Sterile cotton swabs were used to inoculate Mueller–

Hinton agar plates uniformly. Wells of 6 mm diameter were made aseptically using a sterile cork borer.

The extract was dissolved in methanol or DMSO (final solvent concentration $\leq 1\%$) and serially diluted to obtain concentrations of 50, 25, 12.5, 6.25, 3.12, and 1.56 mg/mL. Then, 50 μL of each concentration were placed into the corresponding wells. Solvent alone was used as a negative control. A standard antibiotic disk or solution may be used as a positive control if available.

Plates were incubated at $37\,^{\circ}\mathrm{C}$ for 18–24 h. Antibacterial activity was estimated by measuring the diameter of the inhibition zones (mm) around each well. All tests were conducted in triplicate.

III.6.3 Anti-inflammatory Activity by BSA Denaturation Assay

The anti-inflammatory activity of the extract was assessed by the inhibition of heat-induced bovine serum albumin (BSA) denaturation, a widely used *in vitro* model linked to inflammation-associated protein denaturation [105].

Briefly, 1.0 mL of freshly prepared 0.3% BSA solution in Tris-buffered saline (pH 6.4) was mixed with the appropriate volume of extract solution to obtain final concentrations of 50, 25, 12.5, 6.25, 3.12, and 1.56 mg/mL. The final volume was adjusted to 2.5 mL with distilled water.

The reaction mixtures were incubated at $37\,^{\circ}\mathrm{C}$ for 15 min and then heated at $70\,^{\circ}\mathrm{C}$ for 10 min. After cooling to room temperature for 20 min, the absorbance was measured at 660 nm. Aspirin was used as a reference anti-inflammatory drug.

The percentage inhibition of protein denaturation was calculated as follows:

$$\%\ \text{inhibition} = \left(\frac{A_0 - A_s}{A_0}\right) \times 100 \tag{III.3}$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

III.7 Antioxidant Activity

III.7.1 DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was performed according to the method of Brand-Williams *et al.* [106]. A DPPH solution was prepared by dissolving 4 mg DPPH in 100 mL methanol. The solution was protected from light with aluminum foil.

Extract stock solutions were prepared at 1 mg/mL, and serial dilutions were made to obtain concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/mL. For each test, 100 μL of extract solution were mixed with 900 μL of DPPH solution. The reaction mixture was incubated in the dark for 30 min, and absorbance was measured at 517 nm against methanol as a blank.

The scavenging effect was calculated using:

$$\text{DPPH scavenging effect}(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \tag{III.4}$$

where A_0 is the control absorbance and A_1 is the sample absorbance. The IC_{50} value was defined as the concentration required to inhibit 50% of DPPH radicals.

III.7.2 Total Phenolic Content by Folin–Ciocalteu Assay

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method [107]. Gallic acid was used as a standard.

Gallic acid solutions of different concentrations were prepared to establish the calibration curve. For each standard or sample, 0.1 mL of solution was mixed with 0.5 mL of Folin–Ciocalteu reagent diluted 10-fold. After 5 min in the dark, 0.4 mL of 7.5% sodium carbonate solution was added. The mixture was homogenized and incubated for 30 min in the dark. Absorbance was measured at 765 nm.

The calibration curve was expressed as:

$$A = aC + b \tag{III.5}$$

where A is the absorbance and C is the concentration of gallic acid. Results were expressed as mg gallic acid equivalent per g of dry extract (mg GAE/g).

III.7.3 Total Flavonoid Content

Total flavonoid content (TFC) was measured by the aluminum chloride colorimetric assay [108]. The appropriate volume of sample or standard was mixed with aluminum chloride reagent, and the absorbance was measured spectrophotometrically at the appropriate wavelength according to the adopted laboratory protocol. Results were expressed as mg quercetin equivalent per g of extract (mg QE/g) or mg rutin equivalent per g of extract, depending on the calibration standard used.

III.7.4 Reducing Power Assay

The reducing power assay was performed according to Oyaizu [109]. Briefly, 1 mL of sample was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide solution (1%). The mixture was incubated at $50\,^{\circ}\mathrm{C}$ for 20 min.

After incubation, 2.5 mL of 10% TCA were added to stop the reaction. The mixture was centrifuged at 3000 rpm for 10 min. Then, 2.5 mL of the supernatant were mixed with 2.5 mL distilled water and 0.5 mL of 0.1% $\mathrm{FeCl_3}$. After standing for 10 min at room temperature, absorbance was measured at 700 nm. Higher absorbance values indicated greater reducing power.

III.8 In Vitro Antidiabetic Activity: α-Amylase Inhibition Assay

The inhibitory activity of the extract against porcine pancreatic α-amylase was evaluated by the starch–iodine colorimetric microplate assay based on the principle described by Xiao *et al.* [110], with acarbose as a positive control.

III.8.1 Preparation of Reagents

- **Phosphate buffer:** 0.02 M sodium phosphate buffer, pH 6.9, containing 0.006 M sodium chloride.
- **Enzyme solution:** porcine pancreatic α-amylase prepared in phosphate buffer to a final activity of 2 U/mL.

- **Substrate solution:** 1% (w/v) soluble starch in phosphate buffer.
- **Stopping reagent:** 1 M HCl.
- **Color reagent:** iodine/potassium iodide solution (5 mM I_2 and 5 mM KI).

III.8.2 Assay Procedure

In each microplate well, 20 μL of extract solution were mixed with 20 μL of α-amylase solution and pre-incubated at 37 °C for 10 min. Then, 40 μL of 1% starch solution were added to start the reaction, and the mixture was incubated for 30 min at 37 °C.

The reaction was stopped by adding 80 μL of 1 M HCl, followed by 100 μL iodine/potassium iodide solution. Absorbance was measured at 580 nm.

The inhibition percentage was calculated using the blank-corrected equation:

$$\text{Inhibition}(\%) = \left[1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{control}} - A_{\text{control blank}})}\right] \times 100 \tag{III.6}$$

where:

- A_{sample} = absorbance of extract + enzyme + starch,
- $A_{\text{sample blank}}$ = absorbance of extract + starch without enzyme,
- A_{control} = absorbance of control with enzyme and starch,
- $A_{\text{control blank}}$ = absorbance of starch solution without enzyme.

III.9 In Vivo Study

III.9.1 Experimental Animals

Adult male Albino Wistar rats were used in the *in vivo* study. The animals were obtained from the Pasteur Institute of Algeria and housed in polypropylene cages (Five rats per cage) containing clean wood-shaving bedding. Animals were maintained under standard laboratory conditions, with free access to standard pellet diet and water *ad libitum*, and were allowed to acclimatize before experimentation.

Animal handling and all experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and the ARRIVE 2.0 guidelines [111,112].

All experimental protocols were reviewed and approved by the appropriate institutional ethics committee, and the corresponding approval number will be included in the final version of the thesis.

As shown in Figure III.2, adult male Albino Wistar rats were used for the *in vivo* experiments.

Figure III.3: Adult male Albino Wistar rats used in the *in vivo* study.

III.9.2 Induction of Experimental Diabetes

Experimental diabetes was induced by a single intraperitoneal injection of alloxan monohydrate freshly dissolved in distilled water. According to the laboratory protocol used in this study, 79.5 mg of alloxan were dissolved in 6 mL distilled water, and each rat received 1 mL of the diabetogenic solution.

Alloxan is a classical diabetogenic compound that selectively targets pancreatic β-cells via oxidative mechanisms [113]. After induction, rats were monitored for a stabilization period of 5 days. Rats showing elevated fasting blood glucose levels were considered diabetic and selected for treatment.As shown in Figure III.4, diabetes was induced by intraperitoneal injection of alloxan.

Figure III.4: Induction of experimental diabetes in Albino Wistar rats by intraperitoneal injection of alloxan monohydrate.

III.9.3 Experimental Design and Oral Treatment

After the stabilization period, the animals were divided into four groups ($n = 5$ per group):

- **Negative control (NC):** healthy rats receiving no treatment;
- **Diabetic control (DC):** diabetic rats receiving no treatment;
- **Positive control (PC):** diabetic rats treated with Novoformine/Metformin (125 mg in 6 mL water; 1 mL/day);
- **Test group:** diabetic rats treated orally with *P. graveolens* extract (53 mg in 6 mL water; 1 mL/day).

Treatments were administered daily by oral gavage for 5 consecutive days (Figure III.5).

Figure III.5: Oral administration (gavage) of *Pelargonium graveolens* extract in Albino Wistar rats.

III.9.4 Blood Collection and Biochemical Analysis

At the end of the treatment period, rats were fasted overnight. Blood was collected from the tail vein under light anesthesia. Fasting blood glucose (FBG) was measured immediately using a calibrated glucometer (Figure III.6). Additional blood samples were collected into EDTA tubes for biochemical analysis (Figure III.7). Additional biochemical parameters such as glycated hemoglobin (HbA1c), alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) were determined according to the procedures of the collaborating medical analysis laboratory.

Figure III.6: Measuring fasting blood glucose (FBG) from the tail vein using a glucometer.

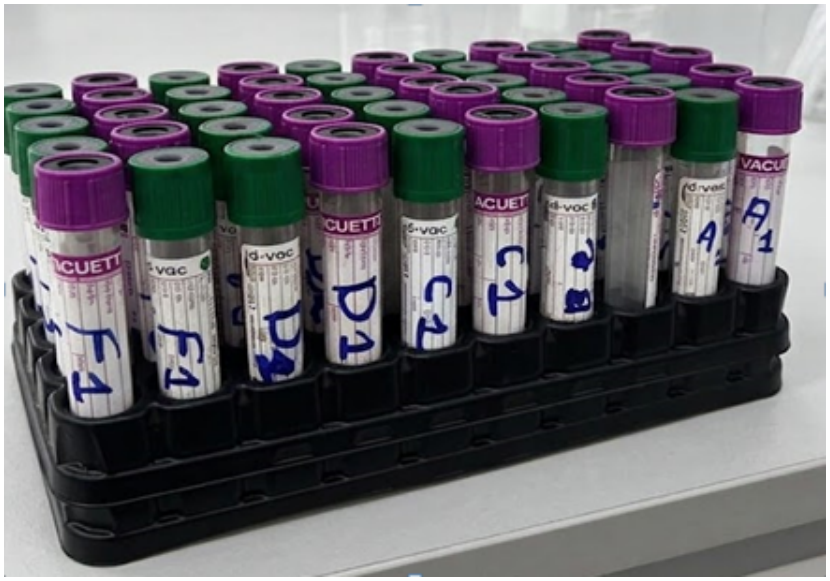


Figure III.7: Blood samples collected in EDTA tubes for biochemical analysis.

III.9.5 Organ Collection

After blood sampling, animals were euthanized under deep anesthesia according to ethical guidelines. The liver, pancreas, and kidneys were excised immediately, rinsed with cold physiological saline, blotted dry, and processed for histopathological evaluation (Figures III.8 and III.9).

Figure III.8: Animal sacrifice under deep anesthesia and surgical harvesting of target organs.

Figure III.9: Surgical excision of target organs (liver, pancreas, and kidneys) and their organization into laboratory petri dishes for further processing.

III.9.6 Histological Preparation of Liver, Pancreas, and Kidney

The liver, pancreas, and kidney tissues were collected immediately after sacrifice and fixed in 10% neutral buffered formalin for 24–48 h at room temperature. After fixation, the tissues were washed with tap water, dehydrated through a graded ethanol series (70%, 80%, 90%, 95%, and 100%), and then cleared in xylene.

The cleared tissues were embedded in paraffin wax, and tissue blocks were prepared. Sections of 4–5 µm thickness were cut using a microtome and mounted on clean glass slides. The sections were then deparaffinized, rehydrated through descending grades of ethanol, and stained with hematoxylin and eosin (H&E).

Finally, the stained sections were dehydrated, cleared, and mounted with a coverslip using a suitable mounting medium. Histopathological examination was performed under a light microscope to assess morphological changes in the liver, pancreas, and kidney tissues.

The overall histological processing steps are illustrated in Figure III.10.

Figure III.10: Histological processing of liver, pancreas, and kidney tissues including fixation, dehydration, embedding, sectioning, and H&E staining.

III.9.7 Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (SD) for each experimental group ($n = 5$). Statistical analyses were performed using GraphPad Prism software (Version 11.0.2, GraphPad Software, San Diego, CA, USA). Data distribution was assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was verified prior to parametric analysis. Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test. A two-tailed p value of less than 0.05 was considered statistically significant.

The analyzed parameters included fasting blood glucose (FBG), glycated hemoglobin (HbA1c), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and other biochemical variables measured during the study. Histopathological findings were evaluated by microscopic examination of hematoxylin and eosin (HE)-stained tissue sections and described qualitatively. Results were presented in tables and figures. Statistical significance was indicated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

III.10 In Silico Study

III.10.1 General Objective

The *in silico* part of the work aimed to predict the interaction of selected phytoconstituents of *P. graveolens* with protein targets involved in oxidative stress, inflammation, and carbohydrate metabolism, in order to complement the biological assays.

III.10.2 Software and Databases

The following software and databases were used:

- AutoDockTools 1.5.6 for receptor and ligand preparation [113];
- AutoDock Vina for docking simulations [110];
- Protein Data Bank (PDB) for protein structures [111];
- SwissADME for pharmacokinetic prediction [113];
- pkCSM for toxicity and ADMET prediction [109].

III.10.3 Target Proteins and Grid Parameters

Three targets were selected for docking:

- α-amylase (PDB ID: 4GQR),
- Cyclooxygenase-2 (COX-2; PDB ID: 5KIR),
- α-glucosidase (PDB ID: 3W37).

The grid-box parameters are summarized in Table III.2.

Table III.2: Docking grid-box parameters used for molecular docking.

Target enzyme	Grid center (X, Y, Z)	Grid size
α-Amylase (4GQR)	(11.936, 18.819, 39.264)	$40 \times 40 \times 40$
COX-2 (5KIR)	(23.959, 0.750, 34.306)	$40 \times 40 \times 40$
α-Glucosidase (3W37)	(0.310, -2.052, -21.786)	$60 \times 60 \times 60$

III.10.4 ADMET Prediction

Pharmacokinetic and toxicological properties of the selected compounds were predicted using SwissADME and pkCSM. The evaluated parameters included:

- Lipinski's Rule of Five,
- molecular weight,
- lipophilicity (Log P),
- aqueous solubility (Log S),
- skin permeability (Log K_p),
- gastrointestinal absorption,
- cytochrome P450 interactions,
- predicted toxicity endpoints.

III.11 Statistical Analysis

All experiments were carried out at least in triplicate, and values were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism or equivalent statistical software. Comparisons between groups were made using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test where appropriate. Differences were considered statistically significant at $p < 0.05$.

Chapter IV

Results and Discussion

Chapter IV

Results and Discussion

IV.1 Extraction Yield

The extraction yield was determined in order to evaluate the efficiency of methanol in recovering phytochemical constituents from the leaves of Rose Geranium (*Pelargonium graveolens*). The yield was calculated according to Equation IV.1 [1].

$$\text{Extraction Yield (\%)} = \frac{\text{Mass of Crude Extract (g)}}{\text{Initial Mass of Plant Material (g)}} \times 100 \tag{IV.1}$$

Substituting the experimental values yields:

$$\text{Extraction Yield (\%)} = \frac{4.673}{30} \times 100 = 15.576\,\% \tag{IV.2}$$

The methanolic extraction of *Pelargonium graveolens* leaves collected from the Souf Valley region produced a crude extract yield of $15.576\,\%$. The corresponding results are summarized in Table IV.1.

Table IV.1: Extraction yield of *Pelargonium graveolens* leaves.

Initial Mass (g)	**Crude Extract (g)**	**Yield (%)**
30	4.673	15.576

The relatively high yield obtained in this study can be attributed to the physicochemical properties of methanol, particularly its high polarity and strong penetrating ability, which favor the extraction of polar and moderately polar phytochemicals. Methanol is widely reported as an efficient solvent for the recovery of phenolic compounds, flavonoids, tannins,

and other antioxidant metabolites, owing to its high solvation capacity toward bioactive constituents [114].

Furthermore, the environmental conditions of the Souf Valley region—marked by elevated temperatures, intense solar radiation, and limited water availability—may promote the biosynthesis of secondary metabolites in *Pelargonium graveolens*. Under abiotic stress, plants commonly activate protective metabolic pathways that increase the accumulation of phenolic and flavonoid compounds acting as antioxidant defense molecules. This enhanced accumulation may, in turn, contribute to the greater extractable mass observed in the present study.

These findings are consistent with those of Mansouri *et al.* [115], who reported that alcoholic solvents exhibit superior extraction efficiency for Algerian *Pelargonium graveolens* compared with nonpolar solvents, due to their stronger affinity toward polar bioactive compounds.

IV.2 Phytochemical Profiling by LC–MS

A comprehensive phytochemical characterization is essential for understanding the therapeutic potential of medicinal plants and for optimizing their use in the pharmaceutical, cosmetic, nutraceutical, and agricultural sectors. In the present study, the methanolic extract of *Pelargonium graveolens* was analyzed by liquid chromatography coupled with mass spectrometry (LC–MS) in both positive and negative electrospray ionization (ESI) modes.

The LC–MS profile revealed a chemically diverse composition comprising flavonoids, phenolic acids, terpenoids, amino acids, vitamins, and organic acids. Such diversity is frequently associated with plants exhibiting notable biological and pharmacological activities. The identified compounds, together with their molecular formulas, ionization modes, retention times, peak areas, relative abundances, and chemical classifications, are presented in Table IV.2.

$$\text{Relative Abundance (\%)} = \frac{\text{Peak Area of Individual Compound}}{\sum \text{Peak Areas of All Detected Compounds}} \times 100 \qquad \text{(IV.3)}$$

The relative abundance of each metabolite was estimated using Equation IV.3, enabling assessment of its contribution to the overall phytochemical composition of the extract.

Table IV.2: LC–MS phytochemical profile of the *Pelargonium graveolens* methanolic extract.

ID	Compound	Formula	ESI	RT	Area	Rel. (%)	Classification
1	L-proline	$C_5H_9NO_2$	+	1.719	325490932	86.30	Amino acid
2	Thymol	$C_{10}H_{14}O$	+	5.996	205002	0.05	Monoterpene
3	Sinapic acid	$C_{11}H_{12}O_5$	+	1.917	296143	0.07	Phenolic acid
4	Naringenin	$C_{15}H_{12}O_5$	+	5.437	50815	0.01	Flavanone
5	Quercetin-3-glucose	$C_{21}H_{19}O_{12}$	+	5.618	10524279	2.79	Flavonoid glycoside
6	Rutin	$C_{27}H_{30}O_{16}$	+	5.493	23563747	6.24	Flavonoid glycoside
7	Luteolin	$C_{15}H_{10}O_6$	+	5.426	4130285	1.09	Flavone
8	Curcumin	$C_{21}H_{20}O_6$	+	4.865	3758821	0.99	Polyphenol
9	Riboflavin	$C_{17}H_{20}N_4O_6$	+	6.066	1276704	0.33	Vitamin B_2
10	Oleuropein	$C_{25}H_{32}O_{13}$	+	5.605	697205	0.18	Secoiridoid
11	Caffeic acid	$C_9H_8O_4$	-	1.778	363889	0.09	Phenolic acid
12	Gallic acid	$C_7H_6O_5$	-	7.145	66925	0.01	Phenolic acid
13	p-Coumaric acid	$C_9H_8O_3$	-	4.510	154934	0.04	Phenolic acid
14	Beta-carotene	$C_{40}H_{56}$	+	5.877	2522328	0.66	Carotenoid
15	Oleanolic acid	$C_{30}H_{48}O_3$	+	1.791	111432	0.02	Triterpene

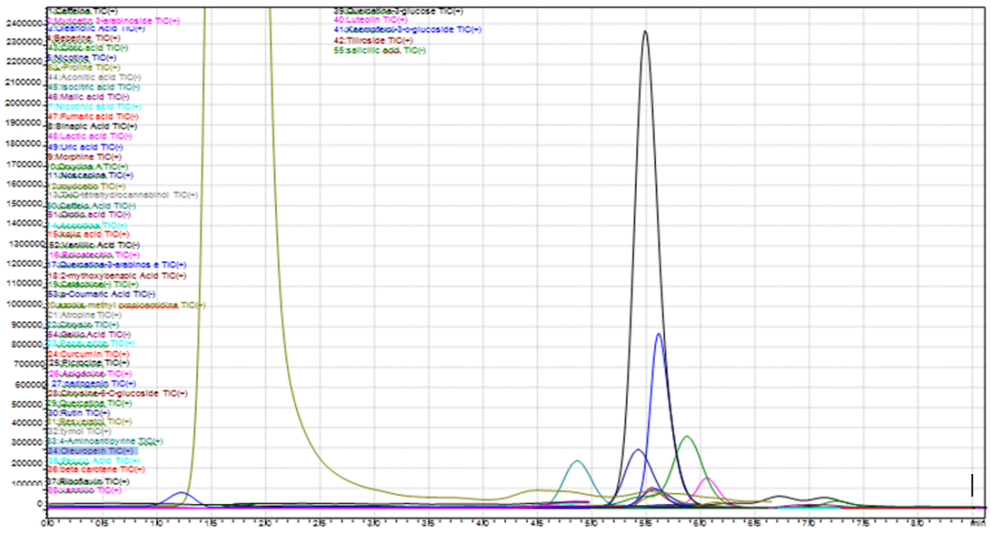


Figure IV.1: LC–MS chromatogram of the *Pelargonium graveolens* methanolic extract showing the major detected phytochemicals.

The chromatographic profile was dominated by L-proline, which accounted for approximately 86.3% of the total detected metabolites. This predominance indicates that primary

metabolites constituted a major fraction of the extract under the employed conditions. L-proline is a well-recognized osmoprotectant involved in plant adaptation to drought, salinity, and high temperatures, and it also contributes to cellular antioxidant defense through reactive oxygen species scavenging and membrane stabilization [116, 117].

The marked accumulation of L-proline is likely associated with the harsh ecological conditions of the Souf Valley, characterized by severe aridity and elevated temperatures. Under such stress, plants typically enhance the biosynthesis of osmolytes and stress-related metabolites, as similarly reported for desert medicinal plants subjected to abiotic stress [118].

Several bioactive flavonoids and phenolic derivatives were also identified in appreciable proportions, notably rutin (6.24%), quercetin-3-glucose (2.79%), luteolin (1.09%), and curcumin (0.99%). These compounds are extensively documented for their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [119]. The predominance of flavonoid glycosides suggests a strong antioxidant potential consistent with the traditional therapeutic use of *Pelargonium graveolens*.

Phenolic acids, including caffeic acid, gallic acid, and *p*-coumaric acid, were likewise detected. These metabolites exert potent antioxidant effects through hydrogen atom donation and radical scavenging [120], further supporting the therapeutic relevance of the extract. In addition, terpenoids such as thymol, β-carotene, and oleanolic acid were identified; these are known for their antimicrobial, anti-inflammatory, and cytoprotective properties [121]. The presence of oleuropein and riboflavin may further enhance the nutraceutical value of the extract.

Collectively, these results demonstrate that the methanolic extract of *Pelargonium graveolens* possesses a chemically rich and diverse phytochemical composition, providing a scientific basis for its potential pharmacological applications.

IV.3 In Vitro Hemolytic Activity

The hemolytic activity of the methanolic extract was assessed using a 96-well microplate assay to evaluate its cytotoxic effect on erythrocyte membranes. This assay quantifies red blood cell membrane disruption through the spectrophotometric measurement of released hemoglobin and is widely used for the preliminary biocompatibility evaluation of plant extracts.

Phosphate-buffered saline (PBS) served as the negative control to ensure membrane stability, whereas complete hemolysis induced by the positive control was taken as $100\,\%$ hemolysis. The percentage of hemolysis was calculated using Equation IV.4.

$$\text{Hemolysis (\%)} = \frac{A_{\text{sample}} - A_{\text{negative}}}{A_{\text{positive}} - A_{\text{negative}}} \times 100 \tag{IV.4}$$

where A_{sample}, A_{negative}, and A_{positive} denote the absorbance of the tested extract, the PBS control, and the complete-hemolysis control, respectively. The results are summarized in Table IV.3 and illustrated in Figure IV.2.

Table IV.3: Hemolysis percentage of the methanolic extract (mean $\pm$ SD, $n = 3$).

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

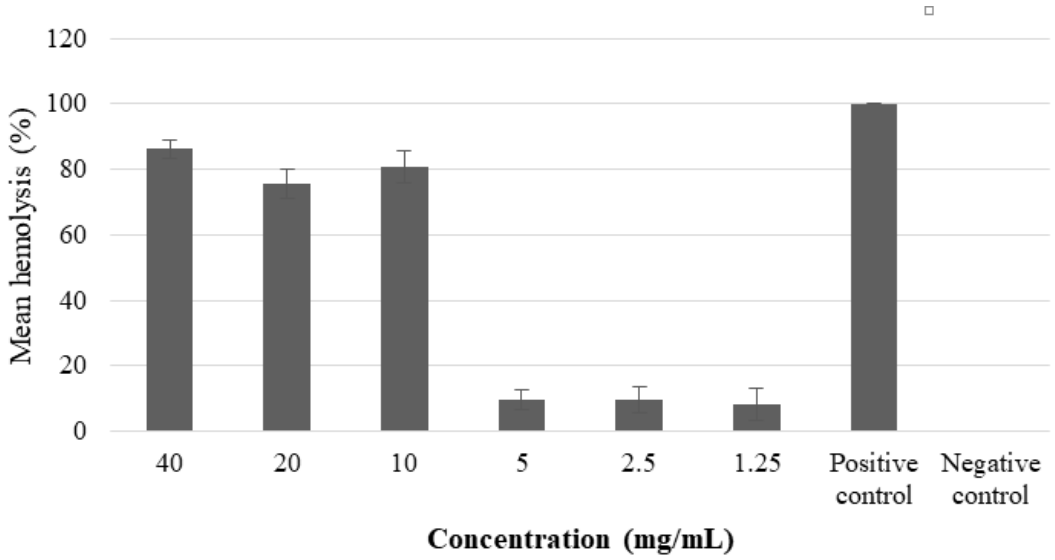


Figure IV.2: Hemolytic activity of the methanolic extract at different concentrations. Bars represent the mean hemolysis percentage, and error bars indicate the standard deviation ($n = 3$).

The extract exhibited a concentration-dependent hemolytic effect. At high concentrations (10–40 mg/mL), pronounced hemolysis was recorded, ranging from $75.6\,\%$ to $86.1\,\%$, with the maximum value at 40 mg/mL. This effect may be attributed to membrane-active phytochemicals, such as saponins, terpenoids, and certain phenolic constituents, capable of interacting with membrane phospholipids and cholesterol, thereby increasing permeability and promoting erythrocyte lysis [12, 79].

In contrast, hemolysis remained below $10\,\%$ at lower concentrations (1.25–5 mg/mL). According to established hemocompatibility criteria, hemolysis values below $10\,\%$ indicate acceptable preliminary biocompatibility and low cytotoxicity [53]. The extract may therefore be considered relatively safe within this concentration range.

This protective behavior at lower doses may be associated with the antioxidant phytochemicals identified by LC–MS, particularly flavonoids and phenolic acids, which can stabilize cellular membranes and inhibit lipid peroxidation [64]. At higher concentrations, however, the cumulative action of membrane-active metabolites appears to overcome these protective effects.

Overall, the extract displays dose-dependent hemolytic activity, demonstrating acceptable hemocompatibility at concentrations ≤ 5 mg/mL while requiring caution at higher doses. These findings provide valuable preliminary information on the biological safety profile of the extract and justify further toxicological investigation.

IV.4 In Vitro Antibacterial Activity (Agar Well Diffusion)

The antibacterial activity of the methanolic extract was evaluated by the agar well diffusion method against clinically relevant bacterial strains. This technique is commonly employed for the preliminary screening of antimicrobial properties and relies on measuring the inhibition zone diameters formed around wells loaded with different extract concentrations.

Activity was tested against the Gram-negative *Escherichia coli* ATCC 25922 and the Gram-positive *Staphylococcus aureus* ATCC 25932. Inhibition zone diameters were measured in millimeters; the absence of inhibition is denoted by NI.

Table IV.4: Antibacterial activity of the methanolic extract determined by the agar well diffusion method.

Bacterial Strain	Inhibition Zone Diameter (mm)				Neg. Control
	50	25	10	5	
E. coli ATCC 25922	13	10	NI	NI	NI
S. aureus ATCC 25932	11	NI	NI	NI	NI

NI: No inhibition detected. Concentrations are expressed in mg/mL.

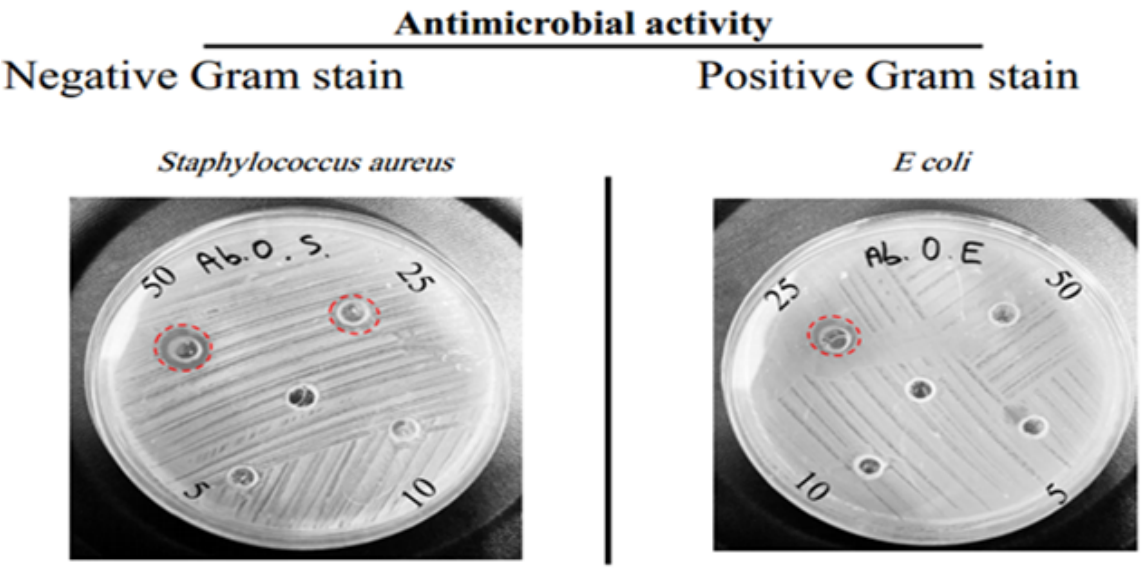


Figure IV.3: Antibacterial activity of the methanolic extract against the tested bacterial strains using the agar well diffusion assay.

The extract exhibited concentration-dependent antibacterial activity, with clear inhibition zones at higher concentrations and progressively weaker effects upon dilution. Among the tested strains, *E. coli* was more sensitive than *S. aureus*: an inhibition zone of 13 mm was recorded at 50 mg/mL, decreasing to 10 mm at 25 mg/mL, with no activity at lower concentrations. *S. aureus* was inhibited only at 50 mg/mL (11 mm).

The observed activity may be attributed to bioactive constituents identified by LC–MS, particularly flavonoids, phenolic acids, and terpenoids. These compounds exert antibacterial effects through multiple mechanisms, including disruption of the bacterial cell wall and membrane, inhibition of nucleic acid synthesis, interference with enzymatic activity, and induction of oxidative stress [122, 123].

The greater susceptibility of *E. coli* relative to *S. aureus* may reflect differences in cell-wall architecture and membrane permeability. Although Gram-negative bacteria are generally more resistant due to their outer membrane, certain phytochemicals can destabilize this barrier, thereby enhancing antibacterial efficacy [124]. Synergistic interactions among the detected metabolites may further contribute to the overall effect.

Overall, the methanolic extract exhibits moderate antibacterial activity, particularly against *E. coli*, supporting the potential application of *Pelargonium graveolens* as a natural antimicrobial agent.

IV.5 In Vitro Anti-inflammatory Activity (BSA Denaturation Assay)

The anti-inflammatory activity of the methanolic extract was evaluated in vitro using the bovine serum albumin (BSA) heat-induced protein denaturation assay. Protein denaturation is a key mechanism in inflammatory disorders; therefore, its inhibition is widely regarded as an indicator of anti-inflammatory potential. The percentage inhibition was calculated using Equation IV.5.

$$\text{Inhibition of Denaturation (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{IV.5}$$

where A_{control} and A_{sample} denote the absorbance of the heated control and the extract-treated sample, respectively.

As shown in Figure IV.4, the extract displayed a concentration-dependent anti-inflammatory effect, with inhibition increasing progressively over the tested range (1.56–50 mg/mL).

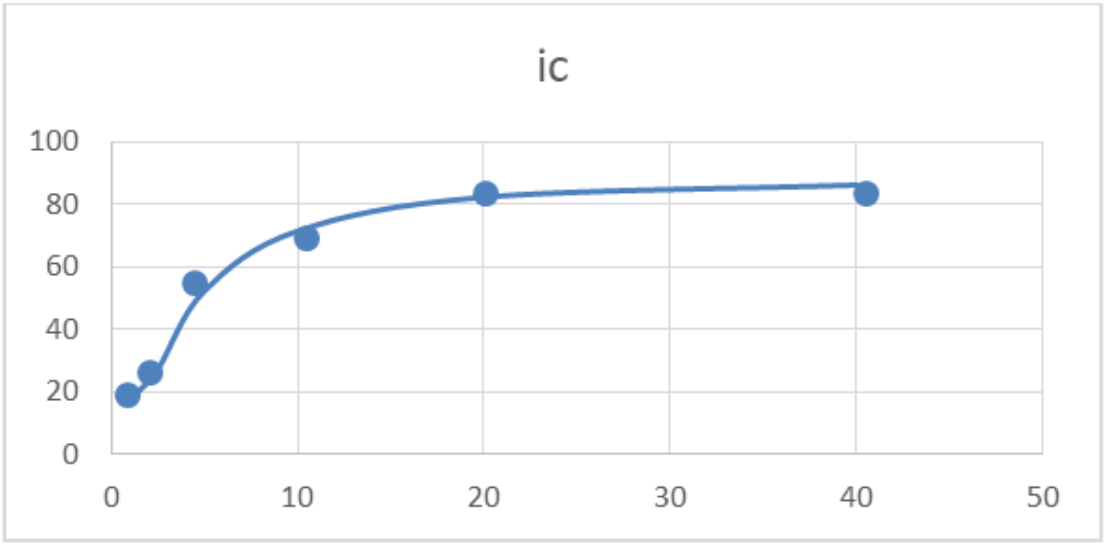


Figure IV.4: In vitro anti-inflammatory activity of the methanolic extract evaluated using the BSA heat-induced protein denaturation assay.

A notable inhibition was observed even at moderate concentrations, suggesting strong activity at relatively low doses. At higher concentrations, the response tended to plateau, indicating that most available binding sites on the albumin protein were occupied by active phytochemical constituents.

This activity is likely associated with the bioactive compounds identified by LC–MS, particularly flavonoids and phenolic acids. These metabolites stabilize protein structures, inhibit protein aggregation, and reduce oxidative stress [125, 126]. Flavonoids such as rutin, luteolin, and quercetin derivatives are well documented for suppressing inflammatory mediators and stabilizing cellular proteins [127]. Phenolic compounds may further inhibit denaturation through hydrogen bonding with amino acid residues, thereby preserving the native protein conformation under thermal stress [128].

The saturation observed at higher concentrations indicates that the phytochemical–albumin interaction reached equilibrium, a behavior commonly reported for polyphenol-rich extracts [129].

Overall, the methanolic extract exhibits significant *in vitro* anti-inflammatory activity, supporting the therapeutic potential of *Pelargonium graveolens* as a natural source of anti-inflammatory agents.

IV.6 DPPH Radical Scavenging Activity

The antioxidant activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This method is based on the ability of antioxidants to reduce the stable DPPH• radical through electron or hydrogen atom donation, resulting in decreased absorbance at 517 nm. The percentage inhibition was calculated using Equation IV.6.

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{IV.6}$$

where A_{control} is the absorbance of the DPPH solution without extract and A_{sample} is that of the extract-treated sample.

As illustrated in Figure IV.5, the scavenging activity increased in a concentration-dependent manner over the range 0.1–0.6 mg/mL.

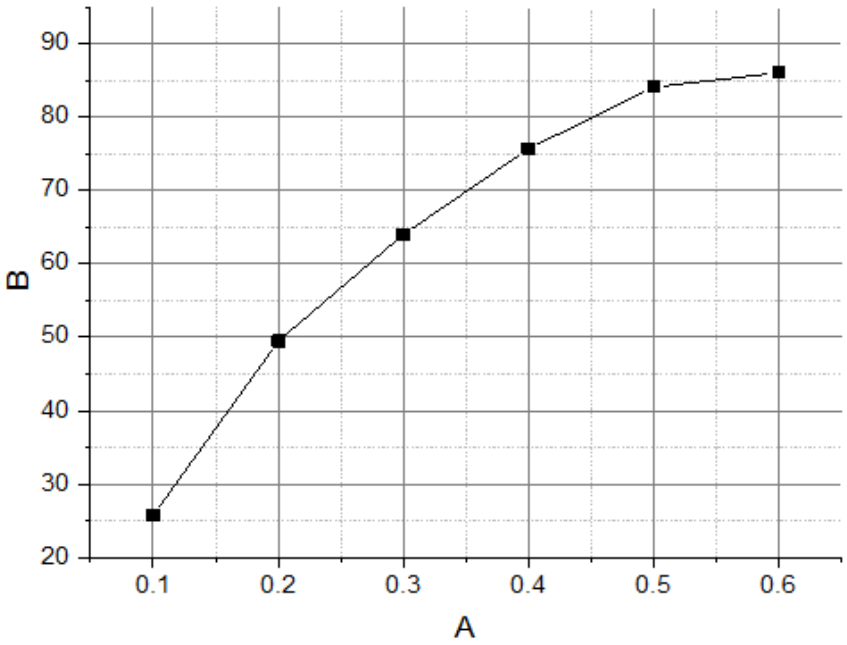


Figure IV.5: DPPH radical scavenging activity of the methanolic extract at different concentrations.

At low concentrations (0.1–0.2 mg/mL), the extract showed moderate activity, reflecting the limited availability of antioxidant molecules. A more pronounced increase occurred at intermediate concentrations (0.3–0.4 mg/mL), while the curve tended toward a plateau at higher concentrations (0.5–0.6 mg/mL), indicating saturation of the available DPPH radicals or attainment of the maximum scavenging capacity—behavior consistent with previous

reports [130, 131].

This activity is most likely linked to the phenolic compounds and flavonoids identified by LC–MS, which possess strong electron- and hydrogen-donating abilities enabling them to stabilize free radicals and interrupt oxidative chain reactions [132]. In particular, rutin, quercetin derivatives, luteolin, caffeic acid, and gallic acid are recognized for their potent antioxidant properties [133]. The antioxidant efficiency depends not only on concentration but also on molecular structure—especially the number and position of hydroxyl groups—and on potential synergistic interactions among constituents.

Overall, the extract displays significant concentration-dependent DPPH radical scavenging activity, highlighting its potential as a natural antioxidant source.

IV.7 Total Phenolic Content (Folin–Ciocalteu Assay)

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method, which is based on the reduction of the Folin reagent by phenolic compounds under alkaline conditions to form a blue complex measurable spectrophotometrically. A calibration curve was constructed using gallic acid as the reference standard over the range 0.02–0.22 mg/mL, following Equation IV.7.

$$A = aC + b \tag{IV.7}$$

where A is the absorbance, C the gallic acid concentration, a the slope, and b the intercept.

The calibration curve exhibited excellent linearity, with a correlation coefficient $r = 0.99662$ and a coefficient of determination $R^2 = 0.99326$, confirming the reliability, precision, and reproducibility of the method (Figure IV.6).

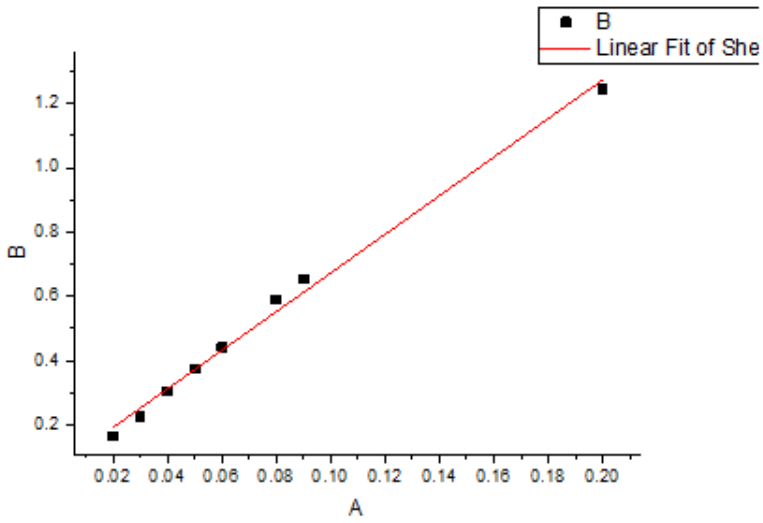


Figure IV.6: Gallic acid calibration curve for total phenolic content determination using the Folin–Ciocalteu method.

At low concentrations, absorbance values were limited by the small number of phenolic molecules available to reduce the Folin reagent, whereas higher concentrations produced greater absorbance corresponding to increased formation of the blue chromogenic complex. The strong linear relationship confirms the suitability of the assay for quantifying phenolic content, with results conventionally expressed as gallic acid equivalents (GAE).

IV.8 Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extract was further evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, which measures the capacity of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The increase in absorbance reflects the electron-donating capacity of the extract. As shown in Figure. IV.7, the results revealed a concentration-dependent increase in reducing power, confirming that the phenolic and flavonoid constituents contribute significantly to ferric ion reduction through electron donation. A marked increase was observed at moderate concentrations (0.1–0.8 mg/mL), followed by stabilization at higher concentrations (0.8–1.0 mg/mL), indicative of system saturation or attainment of the maximum reducing capacity. Overall, the FRAP results corroborate the strong antioxidant potential of the extract and support its possible use as a natural source of reducing agents.

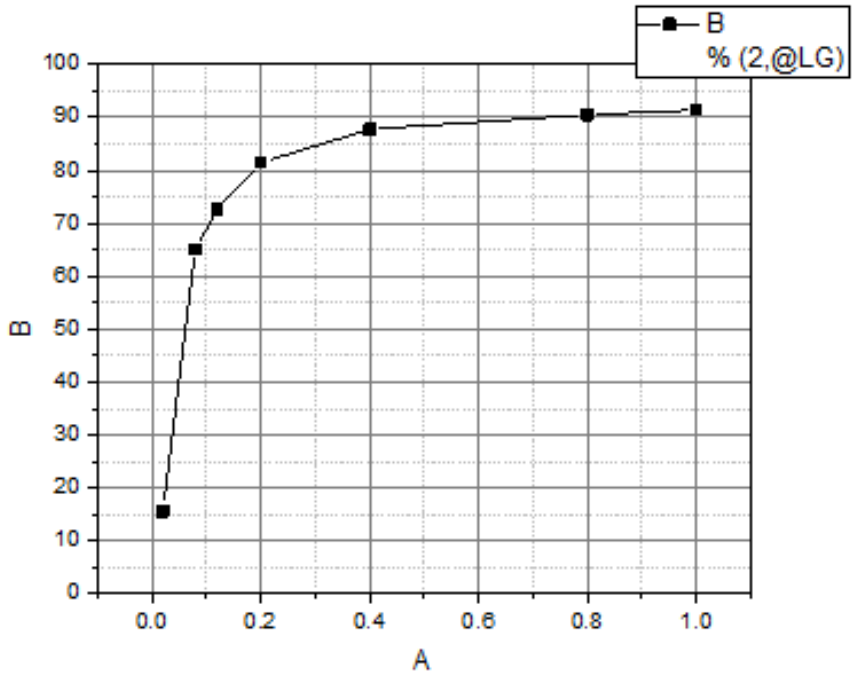


Figure IV.7: Ferric reducing antioxidant power (FRAP) activity of the extract at different concentrations.

IV.9 In Vitro α-Amylase Inhibitory Activity

The inhibition of α-amylase was investigated to evaluate the potential antidiabetic properties of the extract. As a key enzyme in carbohydrate metabolism, α-amylase catalyzes the hydrolysis of starch into simpler sugars, contributing to postprandial hyperglycemia; its inhibition is therefore an important strategy for managing type 2 diabetes mellitus.

As shown in Figure IV.8, the extract exhibited strong, concentration-dependent inhibitory activity, with inhibition exceeding $80\,\%$ before approaching enzymatic saturation. This effect can be attributed to the high phenolic and flavonoid content, as these compounds interact with the enzyme active site through hydrogen bonding and hydrophobic interactions, inducing conformational changes that reduce catalytic activity and limit starch hydrolysis.

Overall, the results indicate that the methanolic extract possesses significant antidiabetic potential through α-amylase inhibition, supporting its possible use as a natural agent for glycemic control.

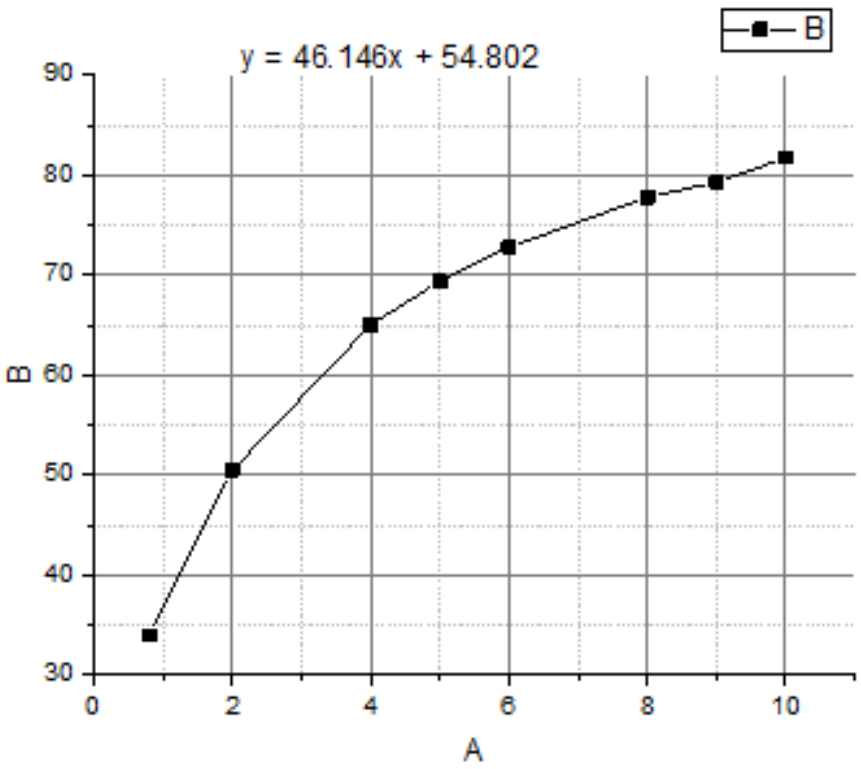


Figure IV.8: In vitro α-amylase inhibitory activity of the methanolic extract at different concentrations. The extract exhibited a concentration-dependent increase in inhibition, reaching more than $80\,\%$ inhibition at higher concentrations before approaching enzymatic saturation.

IV.10 In Vivo Antidiabetic Study of *P. graveolens* Extract

IV.10.1 Effect on Fasting Blood Glucose and Glycated Hemoglobin (HbA1c)

The antidiabetic effect of *Pelargonium graveolens* extract was evaluated through the measurement of fasting blood glucose and glycated hemoglobin (HbA1c) levels in experimental animals [134, 135]. Diabetic animals in the diabetic control group showed elevated fasting blood glucose and HbA1c levels compared to the normal control group, confirming the induction of hyperglycemia.

As shown in Figure IV.9, treatment with *P. graveolens* extract at a dose of 100 mg/kg significantly reduced fasting blood glucose levels compared to the diabetic control group, indicating a pronounced hypoglycemic effect. The observed activity may be attributed to the presence of phenolic and flavonoid compounds, which are known to improve glucose metabolism and enhance antioxidant defense mechanisms [136, 137].

Regarding glycated hemoglobin, the treated group exhibited HbA1c values lower than

the diabetic control group, suggesting partial improvement in long-term glycemic regulation. Although the reduction was less pronounced compared with the metformin-treated group, the extract demonstrated promising antidiabetic potential [138].

Table IV.5: Effect of *Pelargonium graveolens* extract on fasting blood glucose and glycated hemoglobin (HbA1c).

Parameter	Normal Control	Negative Control	Positive Control	Treatment (*P. graveolens*, 100 mg/kg)
Fasting blood glucose (g/L)	0.71	1.06 ± 0.09	0.68 ± 0.46	0.35 ± 0.26
HbA1c (%)	2.94	3.44 ± 0.11	3.08 ± 0.41	3.35 ± 0.42
HbA1c (mmol/mol)	8.63	14.10 ± 1.24	10.12 ± 4.43	13.11 ± 4.64

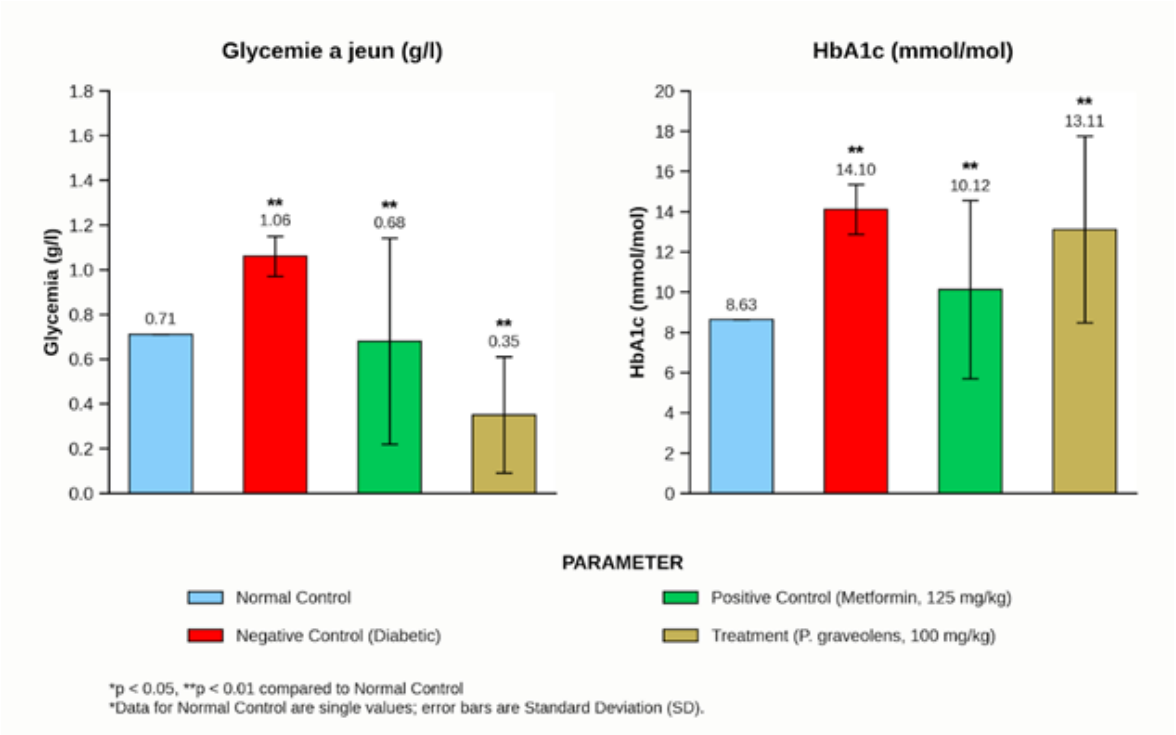


Figure IV.9: Effect of *Pelargonium graveolens* extract on fasting blood glucose and HbA1c levels in experimental animals.

IV.10.2 Effect on Liver Enzymes (ALT and AST)

The effect of *Pelargonium graveolens* extract on liver function was evaluated by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in diabetic rats [139, 140]. Elevated levels of these enzymes in the diabetic control group indicate hepatic damage and metabolic disturbances associated with diabetes mellitus.

As presented in Table IV.6 and Fig. IV.10, treatment with *P. graveolens* extract (100 mg/kg)

markedly reduced ALT and AST levels compared to the diabetic control group. The reduction in liver enzyme activities suggests a protective effect of the extract against diabetes-induced hepatic injury, which may be related to its antioxidant and phenolic constituents [141, 142].

Although the metformin-treated group showed a stronger normalization of enzyme levels, the plant extract demonstrated a considerable hepatoprotective effect, supporting its therapeutic potential in the management of diabetic complications.

Table IV.6: Effect of *Pelargonium graveolens* extract on serum liver enzymes in diabetic rats.

Parameter	Normal Control	Negative Control	Positive Control	Treatment (*P. graveolens*, 100 mg/kg)
ALT (IU/L)	35.77	131.96 ± 51.97	40.85 ± 2.90	48.97 ± 18.19
AST (IU/L)	68.25	218.66 ± 22.06	115.00 ± 15.21	149.21 ± 15.68

Values are expressed as mean $\pm$ SD ($n = 5$). Statistical significance was determined versus the negative control group: $p < 0.01$ (ANOVA followed by Tukey's post hoc test).

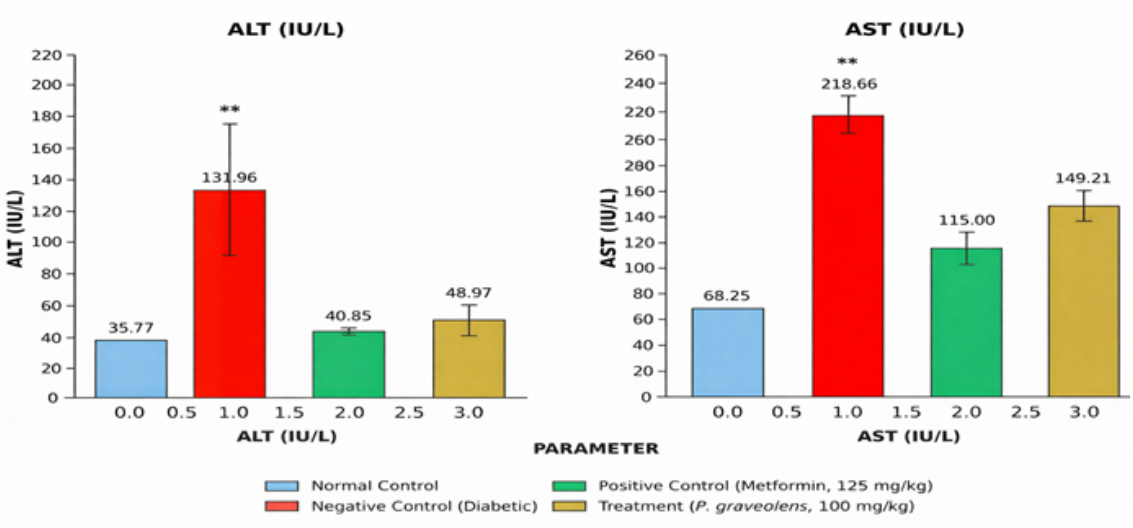


Figure IV.10: Effect of *Pelargonium graveolens* extract on serum liver enzymes (ALT and AST) in diabetic rats compared with control and metformin-treated groups.

IV.10.3 Mechanistic Insights

The observed antidiabetic and hepatoprotective effects of *Pelargonium graveolens* extract may be attributed to its rich content of phenolic compounds, flavonoids, and other bioactive constituents [143, 144]. These phytochemicals are known for their antioxidant properties,

which help reduce oxidative stress associated with diabetes mellitus and protect cellular components from free radical-induced damage.

The hypoglycemic activity of the extract may involve several complementary mechanisms, including inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase, enhancement of peripheral glucose uptake, improvement of insulin sensitivity, and reduction of hepatic glucose production [145, 146]. In addition, flavonoids may stimulate pancreatic β-cell function and contribute to better regulation of blood glucose levels.

The reduction in serum ALT and AST activities further suggests that the extract exerts a hepatoprotective effect by stabilizing hepatocyte membranes and preventing oxidative damage in liver tissues. The antioxidant capacity observed in the FRAP assay supports this mechanism, indicating that the extract can act as an efficient electron donor and neutralize reactive oxygen species.

Overall, these findings indicate that *P. graveolens* extract may exert its therapeutic effects through a synergistic combination of antioxidant, antihyperglycemic, and hepatoprotective mechanisms, supporting its potential application as a natural therapeutic agent in diabetes management.

IV.10.4 Histopathological Analysis

A) Liver Histology

Histopathological examination of liver tissues was performed to evaluate the protective effect of *Pelargonium graveolens* extract against diabetes-induced hepatic damage [147, 148]. Liver sections from the normal control group exhibited a typical hepatic architecture characterized by well-organized hepatocytes, normal sinusoids, and the absence of inflammatory or degenerative alterations. In contrast, liver tissues from the diabetic control group showed marked histopathological abnormalities, including hepatocellular degeneration, sinusoidal dilatation, inflammatory cell infiltration, and areas of necrosis, reflecting severe hepatic injury associated with hyperglycemia and oxidative stress [149].

As shown in Figure IV.11, treatment with *P. graveolens* extract (100 mg/kg) noticeably improved the histological appearance of liver tissues. The treated group demonstrated reduced inflammatory infiltration and partial restoration of normal hepatic architecture compared to the diabetic control group. These observations suggest that the extract exerts a hep-

atoprotective effect, likely due to its antioxidant and anti-inflammatory phytoconstituents. The metformin-treated group also exhibited improved liver histology with fewer pathological alterations, confirming its protective role against diabetic liver damage. Overall, the histopathological findings support the biochemical results obtained for liver enzyme activities and further confirm the therapeutic potential of *P. graveolens* extract.

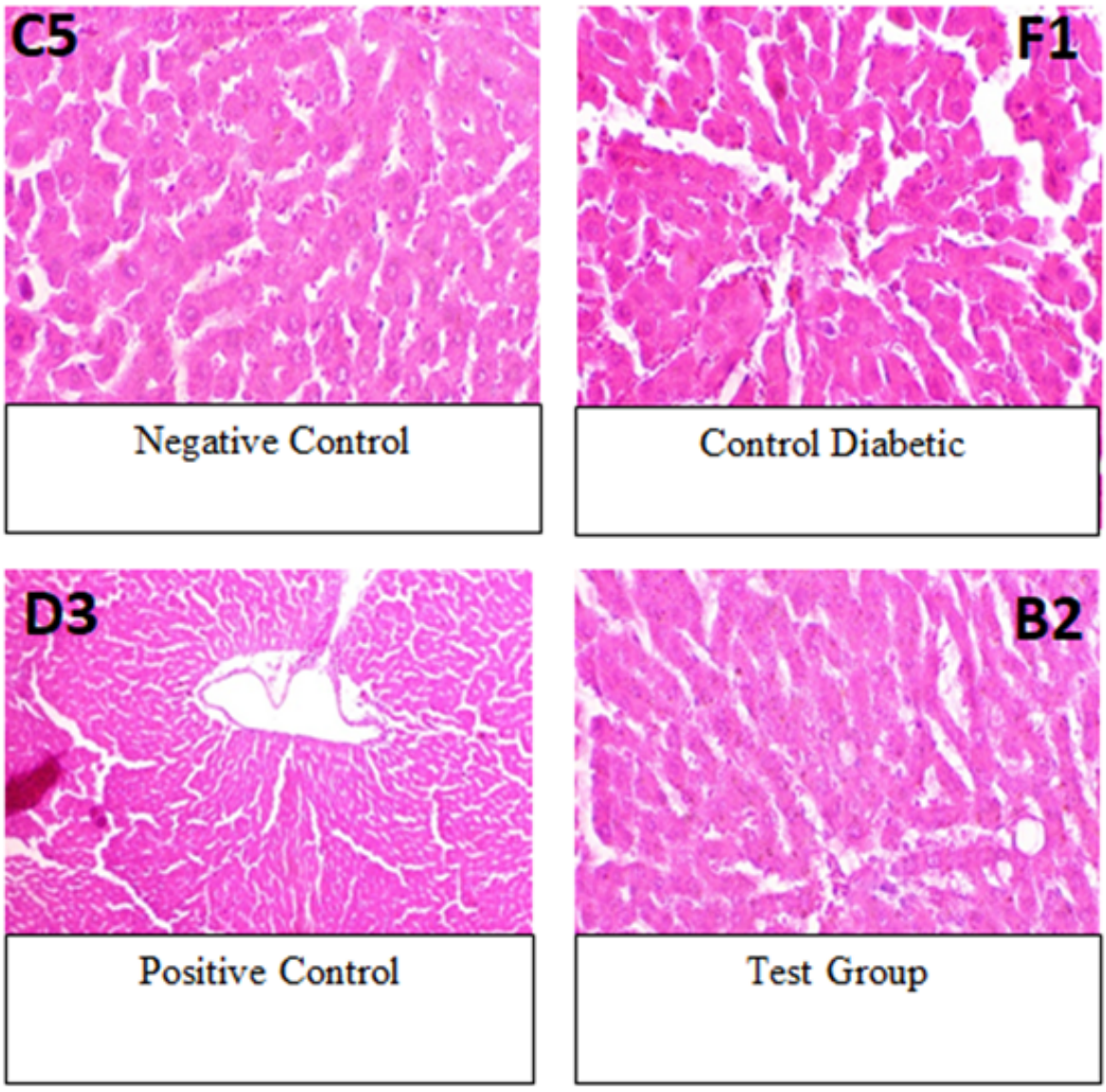


Figure IV.11: Histopathological sections of liver tissues from experimental groups: (A) normal control showing normal hepatic architecture; (B) diabetic control showing hepatocellular degeneration and inflammatory infiltration; (C) metformin-treated group showing improved hepatic organization; and (D) *Pelargonium graveolens*-treated group showing partial restoration of liver structure.

B) Kidney Histology

Histopathological examination of kidney tissues was carried out to assess the protective effect of *Pelargonium graveolens* extract against diabetes-induced renal damage [150, 151]. Kidney sections from the normal control group showed normal renal corpuscles, intact glomeruli, and well-preserved renal tubules without any pathological alterations.

In contrast, the diabetic control group exhibited marked renal abnormalities, including glomerular shrinkage, tubular degeneration, inflammatory cell infiltration, and widening of Bowman's space, indicating significant kidney damage associated with chronic hyperglycemia and oxidative stress [152].

As illustrated in Figure IV.12, treatment with *P. graveolens* extract (100 mg/kg) improved the histological structure of kidney tissues. The treated group demonstrated reduced tissue degeneration and partial restoration of normal renal architecture compared with the diabetic control group. These findings suggest that the extract possesses renoprotective activity, likely mediated through its antioxidant and anti-inflammatory properties.

The metformin-treated group also showed considerable improvement in kidney histology with fewer pathological lesions. Overall, the histopathological observations support the biochemical findings and further confirm the therapeutic potential of *P. graveolens* extract in reducing diabetic complications.

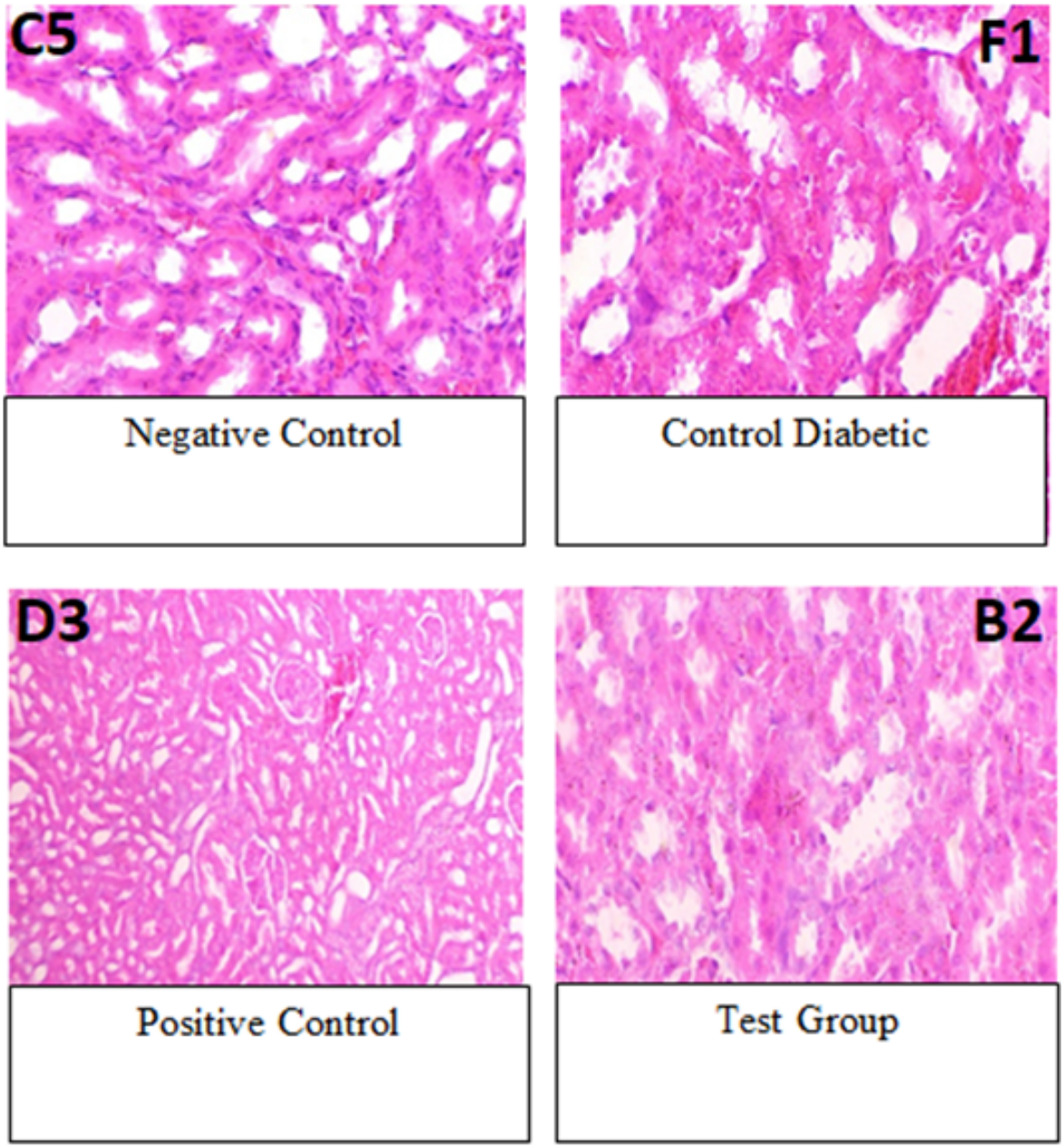


Figure IV.12: Histopathological sections of kidney tissues from experimental groups: (A) normal control showing normal renal architecture; (B) diabetic control showing glomerular and tubular alterations; (C) metformin-treated group showing improved renal structure; and (D) *Pelargonium graveolens*-treated group showing partial restoration of kidney histology.

C) Pancreas Histology

Histopathological evaluation of pancreatic tissues was conducted to investigate the effect of *Pelargonium graveolens* extract on pancreatic integrity in diabetic rats [153, 154]. Pancreatic sections from the normal control group revealed normal histological organization with well-defined islets of Langerhans and intact exocrine acinar cells.

In contrast, the diabetic control group exhibited severe pathological changes, including degeneration and shrinkage of the islets of Langerhans, reduced cellular density, and disruption of pancreatic architecture, reflecting β-cell damage induced by hyperglycemia and oxidative stress [155].

As shown in Fig. IV.13, treatment with *P. graveolens* extract (100 mg/kg) resulted in noticeable improvement in pancreatic histology. The treated group demonstrated partial restoration of the islets of Langerhans and improved cellular organization compared with the diabetic control group. These findings suggest that the extract may exert a protective effect on pancreatic β-cells, possibly through its antioxidant and antihyperglycemic activities.

The metformin-treated group also showed marked recovery of pancreatic tissue with reduced histopathological alterations. Overall, the histological findings support the biochemical and antidiabetic results, confirming the beneficial effect of *P. graveolens* extract in protecting pancreatic tissues against diabetes-induced damage.

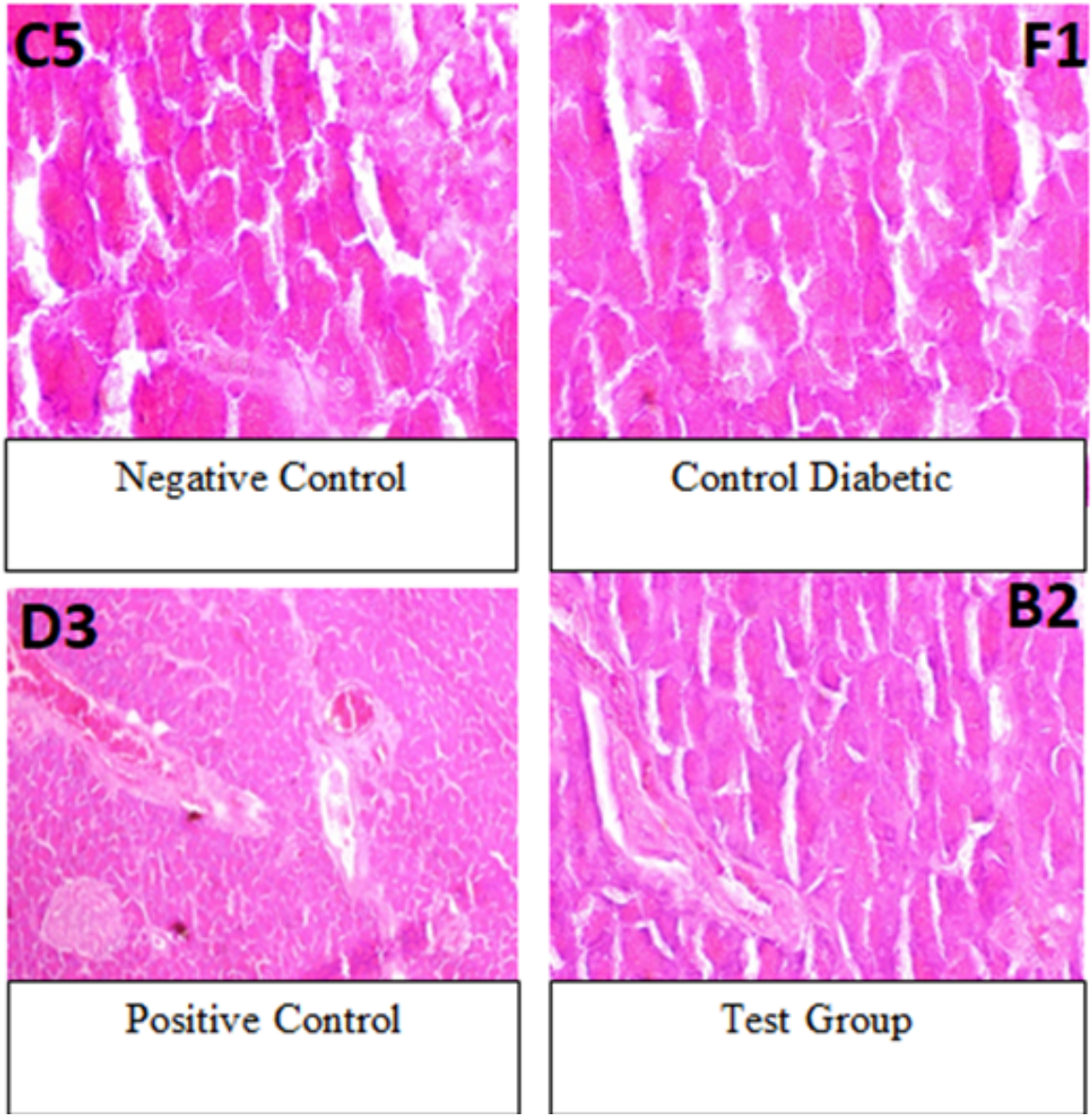


Figure IV.13: Histopathological sections of pancreatic tissues from experimental groups: (A) normal control showing normal pancreatic architecture and intact islets of Langerhans; (B) diabetic control showing degeneration and shrinkage of pancreatic islets; (C) metformin-treated group showing improved pancreatic structure; and (D) *Pelargonium graveolens*-treated group showing partial restoration of pancreatic histology.

IV.11 Molecular Docking and Computational Mechanistic Analysis

IV.11.1 Protocol Validation (Re-docking)

Molecular docking validation is a critical step in structure-based drug discovery to ensure the reproducibility and reliability of the docking algorithm in predicting ligand–receptor binding conformations. In the present study, a re-docking approach was employed using the co-crystallized ligands of three biologically relevant target proteins: α-amylase (4GQR), α-glucosidase (3W37), and cyclooxygenase-2 (5KIR). This procedure allows assessment of the ability of the docking protocol to reproduce the experimental binding pose with high fidelity, typically evaluated using RMSD (root mean square deviation) values [156, 157]. The results are summarized in Table IV.7.

Table IV.7: Validation of molecular docking protocol through re-docking analysis.

Target Protein	Native Ligand	Binding Energy (kcal/mol)	RMSD (Å)
α-Amylase (4GQR)	Myricetin	-6.72	1.99
Cyclooxygenase-2 (5KIR)	Rofecoxib	-10.60	1.38
α-Glucosidase (3W37)	Acarbose	-8.97	1.49

All RMSD values were below the widely accepted cutoff of 2.0 Å, confirming that the docking protocol is highly reliable for predicting ligand binding modes. The exceptionally low RMSD value observed for the cyclooxygenase-2–rofecoxib complex (1.38 Å) indicates near-exact reproduction of the crystallographic pose, demonstrating strong predictive accuracy of the docking methodology [158]. Therefore, the validated protocol was deemed suitable for subsequent virtual screening and mechanistic analysis.

IV.11.2 Detailed Molecular Docking Analysis

The molecular docking results of the major phytochemical constituents of *Pelargonium graveolens* revealed strong binding affinities against key enzymatic targets involved in glucose metabolism and inflammatory pathways. These results are summarized in Table IV.8.

Table IV.8: Binding affinities of bioactive compounds against therapeutic targets.

Target Protein	Ligand	Binding Energy (kcal/mol)
α-Glucosidase (3W37)	Rutin	-7.50
α-Amylase (4GQR)	Rutin	-6.53
Cyclooxygenase-2 (5KIR)	Rutin	-2.44
α-Amylase (4GQR)	Curcumin	-4.64

Among all tested compounds, rutin demonstrated the most favorable binding energy toward α-glucosidase, suggesting a strong inhibitory potential against carbohydrate hydrolysis and postprandial glucose elevation. This enzyme plays a central role in the breakdown of complex carbohydrates, and its inhibition is a well-established therapeutic strategy for managing type 2 diabetes mellitus [159, 160].

IV.11.3 Molecular Interaction Mechanism and Structural Stability

The molecular interaction profiling revealed that Rutin exhibits a highly stable binding conformation within the active site of α-glucosidase (3W37). As illustrated in figure IV.14, the ligand forms a dense hydrogen bonding network with catalytic residues including ALA224, SER250, ARG552, ASP232, ASP568, and PHE225.

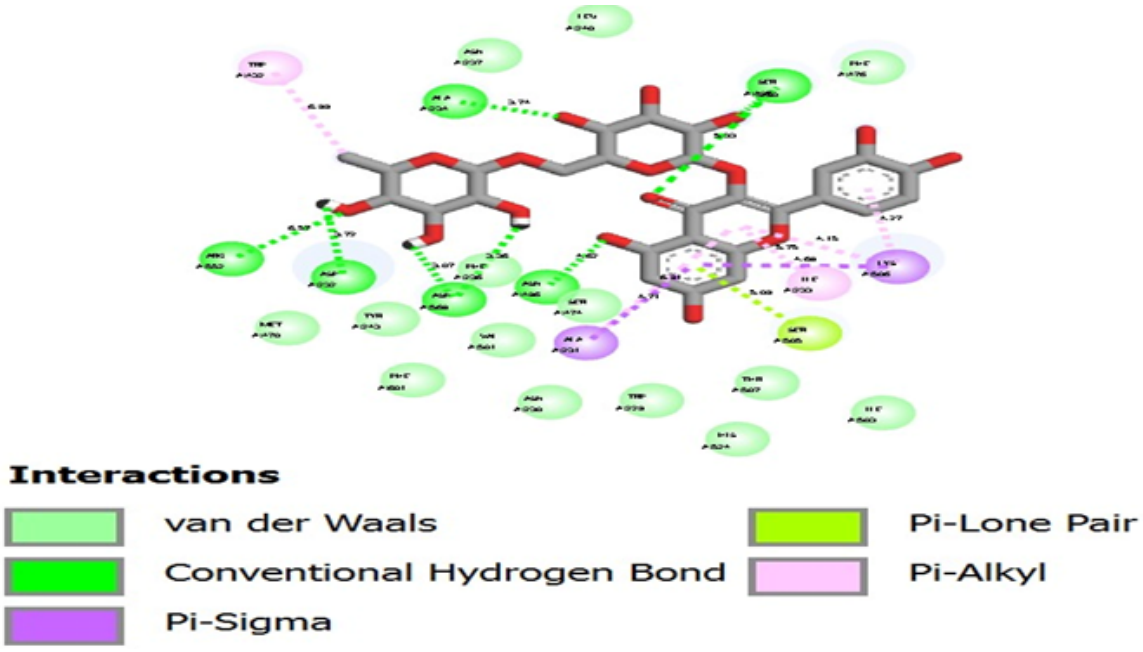


Figure IV.14: Molecular interaction profile of Rutin within α-glucosidase (3W37) active site.

In addition to hydrogen bonding, Pi–Sigma, Pi–Alkyl, and hydrophobic interactions contribute significantly to ligand stabilization, enhancing both binding affinity and structural complementarity. These multi-point interactions create a rigid ligand–enzyme complex, reducing conformational flexibility and increasing binding stability [161].

Similarly, in the α-amylase (4GQR) system, rutin demonstrated strong interaction stability through hydrogen bonds with ASP300, ARG195, HIS201, and TRP59, along with a Pi–Pi stacking interaction with HIS305 (Figure IV.15). This interaction pattern is indicative of strong aromatic stacking and catalytic site occupation, which may directly inhibit enzymatic activity.

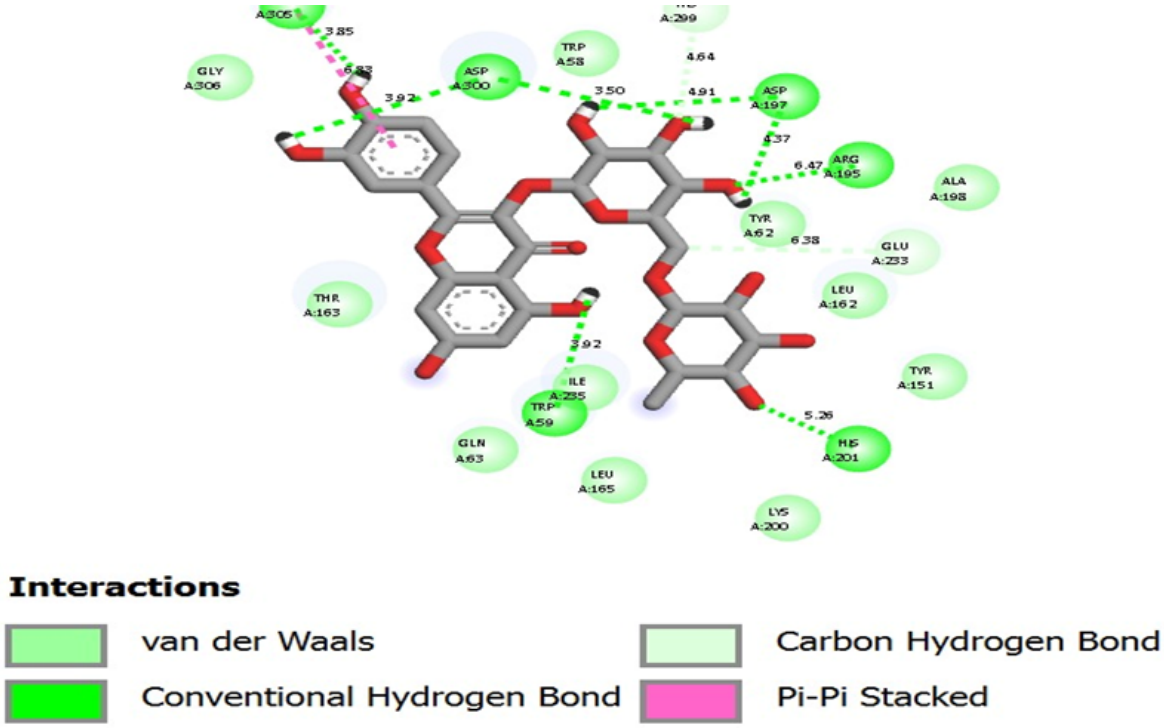


Figure IV.15: Molecular interaction profile of Rutin within α-amylase (4GQR).

Furthermore, interaction with COX-2 (5KIR) revealed potential anti-inflammatory mechanisms through stabilization of the ligand within the catalytic pocket via hydrogen bonding and hydrophobic interactions involving ARG120, SER530, and TYR355 (Figure IV.16). Although the binding affinity was relatively lower (-2.44 kcal/mol), the interaction pattern suggests biologically relevant modulation of inflammatory signaling pathways [162].

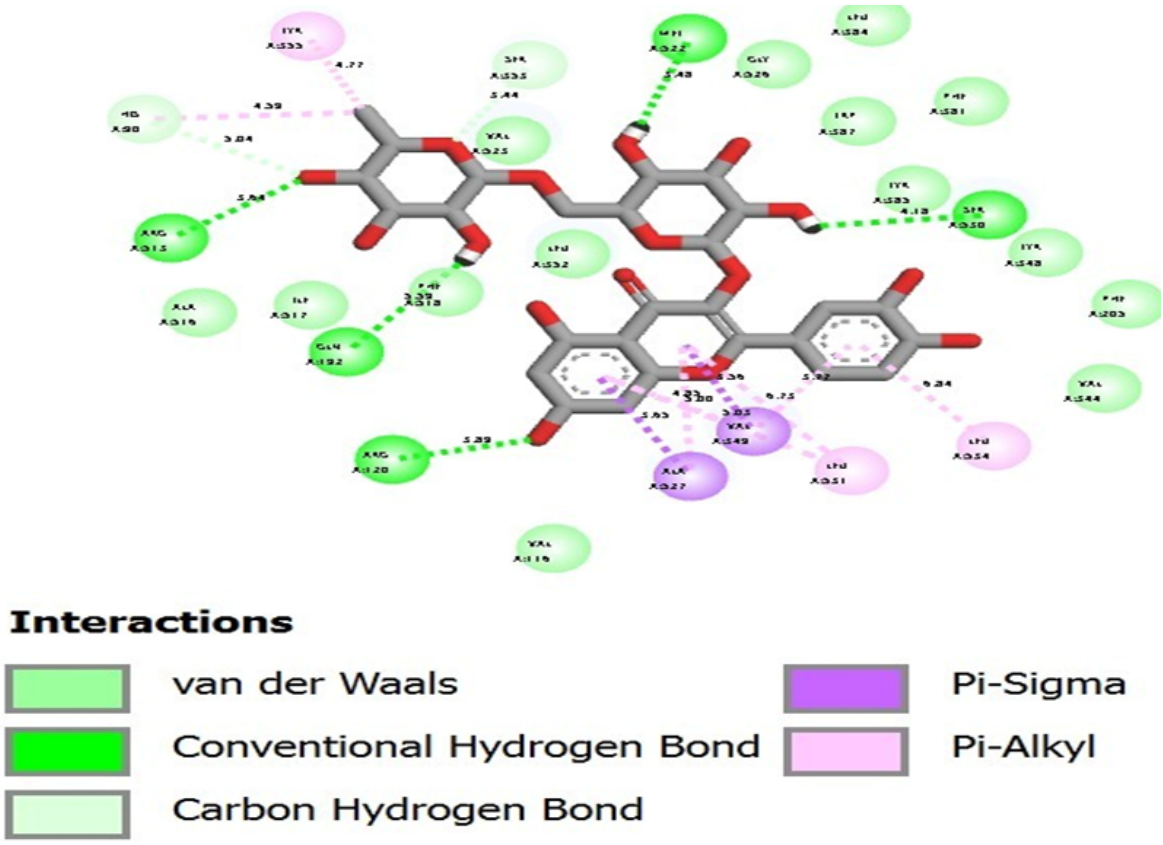


Figure IV.16: Molecular interaction profile of Rutin within COX-2 (5KIR).

IV.11.4 Comparative Binding Analysis: Rutin vs Acarbose

A comparative molecular docking analysis was performed between Rutin and the standard antidiabetic drug Acarbose against α-glucosidase (3W37) to evaluate relative inhibitory potential. The results are summarized in Table IV.9, which highlights the binding affinity, inhibition constant, and interaction energies of both compounds.

Table IV.9: Comparative docking analysis of Rutin and Acarbose.

Parameter	Rutin	Acarbose
Binding Energy (kcal/mol)	-7.50	-8.97
Inhibition Constant (K_i)	3.17 μM	264.52 nM
Intermolecular Energy (kcal/mol)	-12.27	-11.66
Ligand Efficiency	-0.17	-0.20

Although acarbose exhibited stronger binding affinity, rutin demonstrated superior intermolecular stability, indicating a highly favorable energetic landscape within the active site. This suggests that rutin may act as a multi-target natural inhibitor with balanced binding

strength and structural adaptability, making it a promising lead compound for further optimization [163].

IV.12 ADMET Profiling and Drug-Likeness Evaluation

IV.12.1 Physicochemical and Pharmacokinetic Properties

The drug-likeness assessment based on Lipinski's Rule of Five revealed that Curcumin possesses superior oral bioavailability compared to Rutin due to its lower molecular weight, moderate polarity, and optimal lipophilicity. Conversely, Rutin exhibits high polarity and hydrogen bonding capacity, which may limit passive membrane permeability but enhance target binding affinity in aqueous environments, as summarized in Table IV.10.

Table IV.10: Physicochemical and Lipinski rule evaluation.

Property	Rutin	Curcumin	Threshold	Interpretation
Molecular Weight	610.52	368.38	≤ 500	Curcumin more drug-like
LogP	-1.68	3.36	≤ 5	Curcumin higher permeability
H-Bond Acceptors	16	6	≤ 10	Rutin strong binding capacity
H-Bond Donors	10	2	≤ 5	Enhanced interaction potential
TPSA (Å^2)	240.9	156.5	≤ 140	Curcumin better absorption

IV.12.2 ADMET and Toxicological Safety Assessment

The ADMET profiling demonstrated that both rutin and curcumin exhibit excellent safety and pharmacological compatibility. Neither compound showed mutagenic, hepatotoxic, or skin sensitization potential, confirming their high toxicological safety profile.

Curcumin exhibited high intestinal absorption (82.19%), indicating strong systemic availability, while rutin showed moderate absorption (23.44%) but higher structural stability in circulation. Importantly, both compounds showed limited blood–brain barrier penetration, suggesting minimal neurotoxicity risk [**?**, 164].

The high LD_{50} and LOAEL values further confirm a wide therapeutic safety margin, consistent with the absence of toxicity observed in *in vivo* experiments.

IV.13 Integrative Mechanistic Discussion

The integration of molecular docking, ADMET prediction, and experimental findings establishes a coherent mechanistic framework linking computational predictions with biological outcomes. Rutin acts as a multi-target inhibitor of key metabolic enzymes, while curcumin enhances systemic antioxidant defense and improves pharmacokinetic behavior.

This synergistic interaction leads to improved glycemic control, reduction of oxidative stress, and restoration of tissue architecture in diabetic models. The strong agreement between computational and experimental data validates the therapeutic potential of *Pelargonium graveolens* extract as a multi-functional antidiabetic agent [163].

This study confirms that *Pelargonium graveolens* extract possesses robust antidiabetic, antioxidant, and anti-inflammatory activities supported by both experimental and computational evidence. Rutin plays a central mechanistic role through strong inhibition of α-glucosidase, α-amylase, and COX-2, while curcumin enhances bioavailability and systemic antioxidant protection. The ADMET results confirmed excellent safety, non-toxicity, and favorable drug-likeness properties, fully consistent with the absence of adverse effects observed experimentally.

Overall, this study identifies *Pelargonium graveolens* as a promising natural source for the development of safe, multi-target therapeutic agents for the management of diabetes and oxidative stress-related diseases.

General Conclusion

General Conclusion

This study provides a comprehensive evaluation of the phytochemical composition, biological activities, and computational mechanisms underlying the therapeutic potential of *Pelargonium graveolens* methanolic leaf extract, with particular emphasis on its antidiabetic, antioxidant, anti-inflammatory, and organ-protective effects.The extraction process yielded a relatively high crude extract (15.576%), indicating the efficiency of methanol as a polar solvent capable of extracting a wide spectrum of bioactive secondary metabolites. This high yield reflects the rich phytochemical nature of the plant material and supports its suitability for pharmacological investigations.Phytochemical characterization by LC–MS revealed a chemically diverse profile dominated by amino acids, flavonoids, phenolic acids, terpenoids, and vitamins. The identification of major bioactive compounds such as rutin, quercetin derivatives, luteolin, curcumin, caffeic acid, and gallic acid confirms the strong antioxidant and pharmacologically active nature of the extract. These metabolites are widely reported in literature for their roles in free radical scavenging, enzyme modulation, anti-inflammatory signaling, and metabolic regulation, thereby providing a strong biochemical basis for the observed biological effects.The antioxidant assays (DPPH and FRAP) demonstrated strong, concentration-dependent radical scavenging and reducing power, confirming the electron-donating and redox-modulating capacity of the extract. In addition, the total phenolic content supported the abundance of phenolic compounds responsible for these antioxidant properties.The in vitro biological evaluations further highlighted the multifunctional activity of the extract. Significant inhibition of α-amylase indicated its potential to regulate carbohydrate digestion and reduce postprandial hyperglycemia. The BSA denaturation assay demonstrated notable anti-inflammatory activity, suggesting stabilization of proteins and suppression of inflammatory pathways. The hemolytic assay confirmed acceptable biocompatibility at lower concentrations (5 mg/mL), while higher concentrations exhibited membrane-disruptive effects likely due to amphiphilic phytoconstituents.Antimicrobial screening re-

vealed moderate antibacterial activity against both Gram-positive and Gram-negative bacteria, supporting the broad-spectrum biological potential of the extract. Although activity varied with concentration and bacterial strain, the observed effects reinforce the role of synergistic interactions among phytochemicals.The in vivo antidiabetic study provided strong experimental evidence of therapeutic efficacy. Treatment with *P. graveolens* extract significantly reduced fasting blood glucose and glycated hemoglobin (HbA1c) levels, indicating improved glycemic control. Furthermore, normalization of liver enzymes (ALT and AST) suggested hepatoprotective effects, while histopathological examinations of liver, kidney, and pancreas revealed marked improvement and partial restoration of tissue architecture. These findings collectively confirm the protective role of the extract against diabetes-induced organ damage.Molecular docking analysis supported the experimental outcomes by demonstrating strong binding affinities of key phytoconstituents, particularly rutin, toward α-glucosidase, α-amylase, and COX-2. The interactions were stabilized through hydrogen bonding, hydrophobic interactions, and π-based interactions, confirming the molecular basis of enzyme inhibition and anti-inflammatory activity. Although curcumin exhibited more favorable pharmacokinetic properties, rutin demonstrated stronger multi-target binding potential.ADMET profiling further confirmed the safety and drug-likeness of the identified compounds. Both rutin and curcumin showed no predicted mutagenicity, hepatotoxicity, or major toxicological risks. Curcumin displayed higher intestinal absorption, whereas rutin exhibited stronger binding stability and multi-target pharmacological potential, suggesting complementary roles in therapeutic action.Overall, the integration of experimental and computational findings establishes a strong mechanistic correlation between phytochemical composition and biological activity. The results clearly demonstrate that *Pelargonium graveolens* exerts its therapeutic effects through a multi-target, multi-pathway mechanism involving antioxidant defense enhancement, inhibition of carbohydrate-hydrolyzing enzymes, modulation of inflammatory responses, and protection of vital organs from oxidative stress.In conclusion, *Pelargonium graveolens* represents a promising natural source for the development of safe and effective multi-functional therapeutic agents for the management of diabetes mellitus and associated complications. However, further investigations, including clinical validation, compound isolation, and advanced mechanistic studies, are recommended to fully establish its therapeutic applicability and optimize its pharmacological use.

Bibliography

Bibliography

[1] Diánez, F., Santos, M., Parra, C., Navarro, M., Blanco, R., Gea, F., 2018. Lett. Appl. Microbiol., 67:400410.

[2] Dos Santos, M.K., Kreutz, T., Danielli, L.J., De Marchi, J.G.B., Pippi, B., Koester, L.S., Fuentefria, A.M., Limberger, R.P., 2020. J. Drug Deliv. Sci. Technol., 56:101527.

[3] Eamsobhana, P., Yoolek, A., Kongkaew, W., Lerdthusnee, K., Khlaimanee, N., Parsartvit, A., Malainual, N., Yong, H., 2009. Exp. Appl. Acarol., 47:257262.

[4] El Ouadi, Y., Bendaif, H., Mrabti, H.N., Elmsellem, H., Kadmi, Y., Shariati, M.A., Abdel-Rahman, I., Hammouti, B., Bouyanzer, A., 2017. J. Microbiol. Biotechnol. Food Sci., 6:12181220.

[5] El-Garawani, I., El Nabi, S.H., Nafie, E., Almeldin, S., 2019. Anti Cancer Agents Med. Chem., 19:11031113.

[6] Essid, R., Hammami, M., Gharbi, D., Karkouch, I., Hamouda, T.B., Elkahoui, S., Limam, F., Tabbene, O., 2017. Appl. Microbiol. Biotechnol., 101:69937006.

[7] Fanela, T.L.M., Baldin, E.L.L., Pannuti, E.R., Cruz, P.L., Crotti, A.E.M., Takeara, R., Kato, M.J., 2016. Neotrop. Entomol., 45:201210.

[8] FDA, 2006. GRAS List. Herb Society of America Pelargoniums: An Herb Society of America Guide. Kirtland, Ohio, USA.

[9] Fekri, N., El Amir, D., Owis, A., AbouZid, S., 2019. Nat. Prod. Res., 35:25932597.

[10] Gildemeister, E., Hoffmann, F., 1900. Les Huiles essentielles. Brockhaus Tognol, Leipzig and Paris.

[11] Giongo, J.L., De Almeida Vaucher, R., Fausto, V.P., Quatrin, P.M., Lopes, L.Q.S., Santos, R.C.V., Gündel, A., Gomes, P., Steppe, M., 2016. Microb. Pathog., 100:170178.

[12] Hajri, A., Selmi, S., Rtibi, K., Marzouki, M.L., Sebai, H., 2016. J. Biol. Act. Prod. Nat., 6:299314.

[13] Han, X., Beaumont, C., Stevens, N., 2017. Biochim. Open, 5:17.

[14] Jeon, J.H., Kim, H.W., Kim, M.G., Lee, H.S., 2008. J. Microbiol. Biotechnol., 18:16661671.

[15] Juárez, Z.N., Bach, H., Sánchez-Arreola, E., Hernández, L.R., 2016. J. Appl. Microbiol., 120:12641270.

[16] Kozlowska, J., Kaczmarkiewicz, A., Stachowiak, N., Sionkowska, A., 2017. Cosmetics, 4:36. https://doi.org/10.3390/cosmetics4030036.

[17] Lalli, J.Y.Y., Viljoen, A.M., Baser, K.H.C., Demirci, B., Ozek, T., 2006. J. Essent. Oil Res., 18:89105.

[18] Lalli, J.Y., Van Zyl, R.L., Van Vuuren, S.F., 2008. S. Afr. J. Bot., 74:153157.

[19] Lavasanijou, M.R., Sohrabi, H.R., Karimi, M., Ashjazade, M.A., Salajeghe, M., Farzineejadizadeh, H., Parsaei, P., Elmamooz, A., 2016. Wounds, 28:369375.

[20] Lawrence, B.M., Hogg, J.W., Harney, P.M., 1975. Flavour, 4244.

[21] Lis-Balchin, M., 2002. Essential oils from different Pelargonium species and cultivars: their chemical composition. In: Geranium and Pelargonium. Taylor and Francis, London.

[22] Lohani, A., Mishra, A.K., Verma, A., 2019. J. Cosmet. Dermatol., 18:550557.

[23] Mahboubi, M., Kazempour, N., Farzin, N., 2011. J. Biol. Act. Prod. Nat., 1:105111.

[24] Mahboubi, M., Kazempour, N., Taghizadeh, M., 2014. Songklanakarin J. Sci. Technol., 36:513519.

[25] Mahboubi, M., Mahdizadeh, E., HeidaryTabar, R., 2018. Infection, 22:912.

[26] Malik, T., Singh, P., Pant, S., Chauhan, N., Lohani, H., 2011. Phytother. Res., 25:12251228.

[27] Malik, T., Singh, P., Pant, S., Chauhan, N., Lohani, H., Kumar, V., Swarup, S., 2015. Bangladesh J. Med. Sci., 14:384388.

[28] Marmouzi, I., Karym, E.M., Alami, R., El Jemli, M., Kharbach, M., Mamouch, F., Attar, A., Faridi, B., Cherrah, Y., Faouzi, M.E.A., 2019. Orient. Pharm. Exp. Med., 19:201210.

[29] Martins, C.A.F., Campos, M.L., Irioda, A.C., Stremel, D.P., Trindade, A.C.L.B., Pontarolo, R., 2017. Molecules, 22:1883.

[30] Mirazi, N., 2016. J. Zanjan Univ. Med. Sci. Health Serv., 24:5870.

[31] Muthukumar, A., Sangeetha, G., Naveenkumar, R., 2016. J. Environ. Biol., 37:14291436.

[32] Naeini, A.R., Nazeri, M., Shokri, H., 2011. J. Mycol. Med., 21:8791.

[33] Nardoni, S., D'Ascenzi, C., Rocchigiani, G., Papini, R.A., Pistelli, L., Formato, G., Najar, B., Mancianti, F., 2018. Nat. Prod. Res., 32:385390.

[34] Niculau, E., Alves, P.B.D.L., Nogueira, P.C.D.S., Moraes, V.R., Matos, A.P., Bernardo, A.R., Volante, A.C., Fernandes, J.B., Da Silva, M.F.G.F., Correa, A.G., Blank, A.F., Silva, A.D.C., Ribeiro, L., 2013. Quim Nova, 36:13911394.

[35] Pavela, R., 2008. Phytother. Res., 22:274278.

[36] Pazinato, R., Volpato, A., Baldissera, M.D., Santos, R.C.V., Baretta, D., Vaucher, R.A., Giongo, J.L., Boligon, A.A., Stefani, L.M., Da Silva, A.S., 2016. J. Adv. Res., 7:10291034.

[37] Ralambondrainy, M., Belarbi, E., Viranaicken, W., Baranauskien, R., Venskutonis, P.R., Desprès, P., Roques, P., El Kalamouni, C., Seâlambarom, J., 2018. PLoS One, 13:e0196757.

[38] Rana, V.S., Juyal, J.P., Blazquez, M.A., 2002. Int. J. Aroma Ther., 12:216218.

[39] Sandasi, M., Kamatou, G.P.P., Gavaghan, C., Baranska, M., Viljoen, A.M., 2011. Vib. Spectrosc., 57:242247.

[40] Sangeetha, G., Thangavelu, R., Rani, S.U., 2010. Int. J. Food Sci. Technol., 45:10241032.

[41] Shin, S., Lim, S., 2004. J. Appl. Microbiol., 97:12891296.

[42] Simões, B.M., Kohler, B., Clarke, R.B., Stringer, J., Novak-Frazer, L., Young, K., Rautemaa-Richardson, R., Zucchini, G., Armstrong, A., Howell, S.J., 2018. Ther. Adv. Med. Oncol., 10:111.

[43] Singh, P., Srivastava, B., Kumar, A., Kumar, R., Dubey, N.K., Gupta, R., 2008. J. Sci. Food Agric., 88:24212425.

[44] Slima, A.B., Ali, M.B., Barkallah, M., Traore, A.I., Boudawara, T., Allouche, N., Gdoura, R., 2013. Lipids Health Dis., 12:30.

[45] Souza, C.M.C., Junior, S.A.P., Moraes, T.D.S., Damasceno, J.L., Mendes, S.A., Dias, H.J., Stefani, R., Tavares, D.C., Martins, C.H.G., Crotti, A.E.M., Mendes-Giannini, M.J.S., Pires, R.H., 2016. Med. Mycol., 54:515523.

[46] Szabó, M.Á., Varga, G.Z., Hohmann, J., Schelz, Z., Szegedi, E., Amaral, L., Molnár, J., 2010. Phytother. Res., 24:782786.

[47] Szutt, A., Dohaczuk-ródka, A., Sporek, M., 2019. Ecol. Chem. Eng. S, 26:807816.

[48] Szweda, P., Zalewska, M., Pilch, J., Kot, B., Milewski, S., 2018. Acta Vet. Scand., 68:95107.

[49] Tabanca, N., Wang, M., Avonto, C., Chittiboyina, A.G., Parcher, J.F., Carroll, J.F., Kramer, M., Khan, I.A., 2013. J. Agric. Food Chem., 61:41014107.

[50] Van der Walt, J.J.A., Demarne, F.-E., 1988. S. Afr. J. Bot., 54:617622.

[51] Xie, O., Wang, Z., Huang, Q., Zhang, D., 2017. Ind. Crop. Prod., 108:278285.

[52] Zardo, D.M., Alvarez, L.V.H., Los, F.G.B., Ito, V.C., Travalini, A.P., Cardoso, T., Wojeicchoski, J.P., Alberti, A., Zielinski, A.A.F., Esmerino, L., Nogueira, A., 2019. Curr. Bioact. Compd., 15:592599.

[53] Khan, F.A., Synthesis of nanomaterials: methods & technology. *Applications of Nanomaterials in Human Health*, 2020: p. 15–21.

[54] Rane, A.V., et al., Methods for synthesis of nanoparticles and fabrication of nanocomposites, in *Synthesis of Inorganic Nanomaterials*, 2018, Elsevier. p. 121–139.

[55] Altammar, K.A., A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Frontiers in Microbiology*, 2023. 14: p. 1155622.

[56] Nath, D. and P. Banerjee, Green nanotechnologya new hope for medical biology. *Environmental Toxicology and Pharmacology*, 2013. 36(3): p. 997–1014.

[57] Narayanan, K.B. and N. Sakthivel, Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. *Advances in Colloid and Interface Science*, 2011. 169(2): p. 59–79.

[58] Gardea-Torresdey, J.L., et al., Formation and growth of Au nanoparticles inside live alfalfa plants. *Nano Letters*, 2002. 2(4): p. 397–401.

[59] Rani, A., N. Kumar, and M. Kumar, Environmentally friendly green approaches and applications of nanoparticles, in *Metal OxideBased Carbon Nanocomposites for Environmental Remediation and Safety*, 2023, CRC Press. p. 21–63.

[60] Hano, C. and B.H. Abbasi, Plant-based green synthesis of nanoparticles: Production, characterization and applications, 2021, MDPI. p. 31.

[61] Nasrollahzadeh, M., et al., Recent developments in the biosynthesis of Cubased recyclable nanocatalysts using plant extracts and their application in chemical reactions. *The Chemical Record*, 2019. 19(2-3): p. 601–643.

[62] Dinesh, G.K., M. Pramod, and S. Chakma, Sonochemical synthesis of amphoteric Cu0-Nanoparticles using *Hibiscus rosa-sinensis* extract and their applications for degradation of 5-fluorouracil and lovastatin drugs. *Journal of Hazardous Materials*, 2020. 399: p. 123035.

[63] Fernández-García, M. and J. Rodriguez, Metal Oxide Nanoparticles, *Encycl. Inorg. Bioinorg. Chem*, 2011: p. 1–11.

[64] Peck, M.A. and M.A. Langell, Comparison of nanoscaled and bulk NiO structural and environmental characteristics by XRD, XAFS, and XPS. *Chemistry of Materials*, 2012. 24(23): p. 4483–4490.

[65] Wang, J., et al., Preparation of NiO two-dimensional grainy films and their high-performance gas sensors for ammonia detection. *Nanoscale Research Letters*, 2015. 10: p. 1–6.

[66] Olaitan, A.D., et al., Transition metal oxide nanoparticles as surfaces for surface-assisted laser desorption/ionization mass spectrometry of asphaltenes. *Petroleum Science and Technology*, 2017. 35(19): p. 1917–1924.

[67] Kannan, K., et al., Nanostructured metal oxides and its hybrids for photocatalytic and biomedical applications. *Advances in Colloid and Interface Science*, 2020. 281: p. 102178.

[68] De Los Santos Valladares, L., et al., Characterization of Ni thin films following thermal oxidation in air. *Journal of Vacuum Science & Technology B*, 2014. 32(5).

[69] Djeddou, M., Influence de température de propriétés des couches minces d'oxyde de nickel dopé fer et élaboré par la technique spray pneumatique. *Mémoire de Magister*, Université Med Khider Biskra, 2017.

[70] Sharma, S., P. Chauhan, and S. Husain, Structural and optical properties of Mn2O3 nanoparticles its gas sensing applications. *Advanced Materials Proceedings*, 2016. 1(2): p. 220–225.

[71] Jassem, E., A. Mustafa, and N. Umran, The effect of temperature on structural and optical properties of manganese oxide nanoparticles. *Journal of Physics: Conference Series*, 2019. 1279: p. 012004.

[72] Jassal, V., et al., *Sapindus mukorossi*-mediated green synthesis of some manganese oxide nanoparticles interaction with aromatic amines. *Applied Physics A*, 2016. 122: p. 1–12.

[73] Yadav, P., A. Bhaduri, and A. Thakur, Manganese Oxide Nanoparticles: An Insight into Structure, Synthesis and Applications. *ChemBioEng Reviews*, 2023. 10.

[74] Liu, Y.-H., et al., Birnessite-type manganese dioxide nanosheets on metalorganic frameworks with high catalytic activity in ozone decomposition. *ACS Applied Nano Materials*, 2023. 6(9): p. 7794–7801.

[75] Al-Wasidi, A.S., et al., Preparation of Cr2O3, MnO2, Fe2O3, NiO, CuO, and ZnO oxides using their glycine complexes as precursors for in situ thermal decomposition. *Egyptian Journal of Chemistry*, 2020. 63(3): p. 1109–1118.

[76] Reyes, V., O. Martínez, and G. Hernández, National center for biotechnology information. Plant Breeding. Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro, 1923.

[77] Kaczmarczyk, J., et al., Thermodynamic stability, redox properties, and reactivity of Mn3O4, Fe3O4, and Co3O4 model catalysts for N2O decomposition: resolving the origins of steady turnover. *ACS Catalysis*, 2016. 6(2): p. 1235–1246.

[78] Singh, S., et al., Optical properties of selenium quantum dots produced with laser irradiation of water suspended Se nanoparticles. *The Journal of Physical Chemistry C*, 2010. 114(41): p. 17374–17384.

[79] Pareek, V., et al., Synthesis and applications of noble metal nanoparticles: a review. *Advanced Science, Engineering and Medicine*, 2017. 9(7): p. 527–544.

[80] Thakkar, K.N., S.S. Mhatre, and R.Y. Parikh, Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 2010. 6(2): p. 257–262.

[81] Ahmadisoltansaraei, K. and J. Moghaddam, Preparation of NiO nanoparticles from Ni(OH)2ůNiCO3ů4H2O precursor by mechanical activation. *International Journal of Minerals, Metallurgy, and Materials*, 2014. 21: p. 726–735.

[82] Balamurugan, S., et al., Simple and efficient way of synthesizing NiO nanoparticles by combustion followed by ball milling method. *Nanoscience and Nanotechnology Letters*, 2015. 7(2): p. 89–93.

[83] Hotovy, I., et al., NiO-based nanostructured thin films with Pt surface modification for gas detection. *Thin Solid Films*, 2006. 515(2): p. 658–661.

[84] Ukoba, K., A. Eloka-Eboka, and F. Inambao, Review of nanostructured NiO thin film deposition using the spray pyrolysis technique. *Renewable and Sustainable Energy Reviews*, 2018. 82: p. 2900–2915.

[85] Moravec, P., et al., NiO x nanoparticle synthesis by chemical vapor deposition from nickel acetylacetonate. *Materials Sciences and Applications*, 2011. 2(04): p. 258.

[86] Gondal, M., T.A. Saleh, and Q. Drmosh, Synthesis of nickel oxide nanoparticles using pulsed laser ablation in liquids and their optical characterization. *Applied Surface Science*, 2012. 258(18): p. 6982–6986.

[87] Baig, U., et al., Laser induced anchoring of nickel oxide nanoparticles on polymeric graphitic carbon nitride sheets using pulsed laser ablation for efficient water splitting under visible light. *Nanomaterials*, 2020. 10(6): p. 1098.

[88] Reguig, B., et al., Properties of NiO thin films deposited by intermittent spray pyrolysis process. *Applied Surface Science*, 2007. 253(9): p. 4330–4334.

[89] Wei, Z., et al., Characterization of NiO nanoparticles by anodic arc plasma method. *Journal of Alloys and Compounds*, 2009. 479(1-2): p. 855–858.

[90] Safa, S., et al., Hydrothermal synthesis of NiO nanostructures for photodegradation of 4-nitrophenol. *Desalination and Water Treatment*, 2016. 57(46): p. 21982–21989.

[91] Parsae, Z., Synthesis of novel amperometric urea-sensor using hybrid synthesized NiO-NPs/GO modified GCE in aqueous solution of cetrimonium bromide. *Ultrasonics Sonochemistry*, 2018. 44: p. 120–128.

[92] Qing, Z., et al., Solvothermal synthesis and photocatalytic properties of NiO ultrathin nanosheets with porous structure. *Applied Surface Science*, 2015. 328: p. 525–530.

[93] Deng, X. and Z. Chen, Preparation of nano-NiO by ammonia precipitation and reaction in solution and competitive balance. *Materials Letters*, 2004. 58(3-4): p. 276–280.

[94] Tadic, M., et al., Magnetic properties of NiO (nickel oxide) nanoparticles: Blocking temperature and Neel temperature. *Journal of Alloys and Compounds*, 2015. 647: p. 1061–1068.

[95] Han, D., et al., Synthesis and size control of NiO nanoparticles by water-in-oil microemulsion. *Powder Technology*, 2004. 147(1-3): p. 113–116.

[96] Zorkipli, N.N.M., N.H.M. Kaus, and A.A. Mohamad, Synthesis of NiO nanoparticles through sol-gel method. *Procedia Chemistry*, 2016. 19: p. 626–631.

[97] Nathan, T., et al., Nanostructured NiO for electrochemical capacitors: synthesis and electrochemical properties. *Journal of Solid State Electrochemistry*, 2008. 12: p. 1003–1009.

[98] Abdallah, A., H. Basma, and R. Awad, Preparation, characterization, and application of nickel oxide nanoparticles in glucose and lactose biosensors. *Mod. Appl. Sci.*, 2019. 13(6): p. 99.

[99] Srikesh, G. and A.S. Nesaraj, Synthesis and characterization of phase pure NiO nanoparticles via the combustion route using different organic fuels for electrochemical capacitor applications. *Journal of Electrochemical Science and Technology*, 2015. 6(1): p. 16–25.

[100] Ezhilarasi, A.A., et al., Green synthesis of NiO nanoparticles using *Aegle marmelos* leaf extract for the evaluation of in-vitro cytotoxicity, antibacterial and photocatalytic properties. *Journal of Photochemistry and Photobiology B: Biology*, 2018. 180: p. 39–50.

[101] Abbasi, B.A., et al., Plant-mediated synthesis of nickel oxide nanoparticles (NiO) via *Geranium wallichianum*: Characterization and different biological applications. *Materials Research Express*, 2019. 6(8): p. 0850a7.

[102] Yuvakkumar, R., et al., Rambutan (*Nephelium lappaceum* L.) peel extract assisted biomimetic synthesis of nickel oxide nanocrystals. *Materials Letters*, 2014. 128: p. 170–174.

[103] Ezhilarasi, A.A., et al., Green synthesis of NiO nanoparticles using *Moringa oleifera* extract and their biomedical applications: Cytotoxicity effect of nanoparticles against HT-29 cancer cells. *Journal of Photochemistry and Photobiology B: Biology*, 2016. 164: p. 352–360.

[104] Helan, V., et al., Neem leaves mediated preparation of NiO nanoparticles and its magnetization, coercivity and antibacterial analysis. *Results in Physics*, 2016. 6: p. 712–718.

[105] Kganyago, P., et al., Synthesis of NiO nanoparticles via a green route using *Monsonia burkeana*: the physical and biological properties. *Journal of Photochemistry and Photobiology B: Biology*, 2018. 182: p. 18–26.

[106] Hoseinpour, V. and N. Ghaemi, Green synthesis of manganese nanoparticles: Applications and future perspectiveA review. *Journal of Photochemistry and Photobiology B: Biology*, 2018. 189: p. 234–243.

[107] Khan, S.A., et al., Green synthesis of MnO nanoparticles using *Abutilon indicum* leaf extract for biological, photocatalytic, and adsorption activities. *Biomolecules*, 2020. 10(5): p. 785.

[108] Lu, H., et al., Biogenic synthesis of MnO2 nanoparticles with leaf extract of *Viola betonicifolia* for enhanced antioxidant, antimicrobial, cytotoxic, and biocompatible applications. *Frontiers in Microbiology*, 2021. 12: p. 761084.

[109] Khezeli, T., A. Daneshfar, and F. Kardani, In-situ functionalization of MnO2 nanoparticles by natural tea polyphenols: a greener sorbent for dispersive solid-phase extraction of parabens from wastewater and cosmetics. *Microchemical Journal*, 2023. 190: p. 108751.

[110] Munir, R., et al., Biosynthesis of *Leucaena leucocephala* leaf mediated ZnO, CuO, MnO2, and MgO based nano-adsorbents for Reactive Golden Yellow-145 (RY-145) and Direct Red-31 (DR-31) dye removal from textile wastewater to reuse in agricultural purpose. *Separation and Purification Technology*, 2023. 306: p. 122527.

[111] Rahmat, M., et al., Plant-assisted synthesis and characterization of MnO2 nanoparticles for removal of crystal violet dye: an environmental remedial approach. *Environmental Science and Pollution Research*, 2023. 30(20): p. 57587–57598.

[112] Diallo, A., et al., Green synthesis of single phase hausmannite Mn3O4 nanoparticles via *Aspalathus linearis* natural extract. *SN Applied Sciences*, 2021. 3: p. 1–11.

[113] Nair Sreekala, G., F. Abdullakutty, and B. Beena, Green synthesis, characterization, and photocatalytic degradation efficiency of Trimanganese Tetroxide nanoparticle. *International Journal of Nano Dimension*, 2019. 10(4): p. 400–409.

[114] Prasad, A.S., Green synthesis of nanocrystalline manganese (II, III) oxide. *Materials Science in Semiconductor Processing*, 2017. 71: p. 342–347.

[115] Thanigaivel, A., et al., Chemicals isolated from *Justicia adhatoda* Linn reduce fitness of the mosquito, *Aedes aegypti* L. *Archives of Insect Biochemistry and Physiology*, 2017. 94(4): p. e21384.

[116] Sharma, J.K., et al., *Azadirachta indica* plant-assisted green synthesis of Mn3O4 nanoparticles: Excellent thermal catalytic performance and chemical sensing behavior. *Journal of Colloid and Interface Science*, 2016. 472: p. 220–228.

[117] Van Tran, T., et al., Green synthesis of Mn3O4 nanoparticles using *Costus woodsonii* flowers extract for effective removal of malachite green dye. *Environmental Research*, 2022. 214: p. 113925.

[118] Sumantha, H., et al., Green synthesis and characterization of Mn3O4 nanoparticles for photocatalytic and supercapacitors. *Ionics*, 2023. 29(2): p. 733–749.

[119] Anand, G.T., et al., Structural and optical properties of nickel oxide nanoparticles: Investigation of antimicrobial applications. *Surfaces and Interfaces*, 2020. 18: p. 100460.

[120] Morozov, Y.G., O. Belousova, and M. Kuznetsov, Preparation of nickel nanoparticles for catalytic applications. *Inorganic Materials*, 2011. 47: p. 36–40.

[121] Wu, X., et al., Nickel nanoparticles prepared by hydrazine hydrate reduction and their application in supercapacitor. *Powder Technology*, 2012. 224: p. 162–167.

[122] Pandian, C.J., R. Palanivel, and S. Dhananasekaran, Green synthesis of nickel nanoparticles using *Ocimum sanctum* and their application in dye and pollutant adsorption. *Chinese Journal of Chemical Engineering*, 2015. 23(8): p. 1307–1315.

[123] Ahmed, A., D.R. Yadav, and Y.S. Lee, Applications of nickel nanoparticles for control of Fusarium wilt on lettuce and tomato. *Int. J. Innov. Res. Sci. Eng. Technol*, 2016. 5(5): p. 7378–7385.

[124] Sana, S.S., et al., Biogenesis and application of nickel nanoparticles: a review. *Current Pharmaceutical Biotechnology*, 2021. 22(6): p. 808–822.

[125] Moavi, J., F. Buazar, and M.H. Sayahi, Algal magnetic nickel oxide nanocatalyst in accelerated synthesis of pyridopyrimidine derivatives. *Scientific Reports*, 2021. 11(1): p. 6296.

[126] Rossi, L.M., A.D. Quach, and Z. Rosenzweig, Glucose oxidasemagnetite nanoparticle bioconjugate for glucose sensing. *Analytical and Bioanalytical Chemistry*, 2004. 380: p. 606–613.

[127] Liu, X., et al., A review on the synthesis of manganese oxide nanomaterials and their applications on lithiumion batteries. *Journal of Nanomaterials*, 2013. 2013(1): p. 736375.

[128] Sahib, R.S. and J.A. Naser, Preparation, characterization and surface area properties of manganese oxide nanoparticles. *Annals of the Romanian Society for Cell Biology*, 2021: p. 2962–2969.

[129] Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Adv. Drug Deliv. Rev., 23:3–25.

[130] Delaney, J.S., 2004. J. Chem. Inf. Comput. Sci., 44:1000–1005.

[131] Potts, R.O., Guy, R.H., 1992. Pharm. Res., 9:663–669.

[132] Williams, A.C., Barry, B.W., 2004. Adv. Drug Deliv. Rev., 56:603–618.

[133] Benson, H.A.E., 2005. Curr. Drug Deliv., 2:23–33.

[134] Daina, A., Michielin, O., Zoete, V., 2017. Sci. Rep., 7:42717.

[135] Pires, D.E.V., Blundell, T.L., Ascher, D.B., 2015. J. Med. Chem., 58:4066–4072.

[136] Banerjee, P., Eckert, A.O., Schrey, A.K., Preissner, R., 2018. Nucleic Acids Res., 46:W257–W263.

[137] Trott, O., Olson, A.J., 2010. J. Comput. Chem., 31:455–461.

[138] Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2009. J. Comput. Chem., 30:2785–2791.

[139] Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Plant Soil, 39:205–207.

[140] Szabados, L., Savouré, A., 2010. Trends Plant Sci., 15:89–97.

[141] Verbruggen, N., Hermans, C., 2008. Amino Acids, 35:753–759.

[142] Apel, K., Hirt, H., 2004. Annu. Rev. Plant Biol., 55:373–399.

[143] Cushnie, T.P.T., Lamb, A.J., 2011. Int. J. Antimicrob. Agents, 38:99–107.

[144] Nikaido, H., 2003. Microbiol. Mol. Biol. Rev., 67:593–656.

[145] Burt, S., 2004. Int. J. Food Microbiol., 94:223–253.

[146] Jakobek, L., 2015. Food Chem., 175:556–567.

[147] Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. LWT–Food Sci. Technol., 28:25–30.

[148] Singleton, V.L., Rossi, J.A., 1965. Am. J. Enol. Vitic., 16:144–158.

[149] Chang, C.-C., Yang, M.-H., Wen, H.-M., Chern, J.-C., 2002. J. Food Drug Anal., 10:178–182.

[150] Oyaizu, M., 1986. Jpn. J. Nutr., 44:307–315.

[151] Mizushima, Y., Kobayashi, M., 1968. J. Pharm. Pharmacol., 20:169–173.

[152] Xiao, Z., Storms, R., Tsang, A., 2006. Anal. Biochem., 351:146–148.

[153] Lenzen, S., 2008. Diabetologia, 51:216–226.

[154] Percie du Sert, N., Hurst, V., Ahluwalia, A., et al., 2020. PLoS Biol., 18:e3000410.

[155] National Research Council, 2011. Guide for the Care and Use of Laboratory Animals, 8th ed. National Academies Press, Washington, DC.

[156] ISO, 2017. ISO 10993-4: Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood. International Organization for Standardization, Geneva.

[157] ASTM, 2017. ASTM F756-17: Standard Practice for Assessment of Hemolytic Properties of Materials. ASTM International, West Conshohocken, PA.

[158] CLSI, 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests (M02), 13th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

[159] CLSI, 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (M07), 11th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

[160] DeFronzo, R.A., 2009. Diabetes, 58:773–795.

[161] Brownlee, M., 2001. Nature, 414:813–820.

[162] World Health Organization, 2016. Global report on diabetes. WHO, Geneva.

[163] International Diabetes Federation, 2021. IDF Diabetes Atlas, 10th ed. Brussels, Belgium.

[164] Saeedi, P., Petersohn, I., Salpea, P., et al., 2019. Diabetes Res. Clin. Pract., 157:107843.

Annexes

Annexes

Figure S1. The cages of the different batches of rats.

Figure S2. The stages of nursing and treating rats.

Figure S3. Animal sacrifice and dissection.

Figure S4. Steps and tools used for preparing histological sections.

Participation in Scientific Conferences

Participation in Scientific Conferences

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

https://sdacee2024.sciencesconf.org/

Therapeutic Potential and Cytotoxicity of Pelargonium graveolens Cultivated in an Arid Environment

Abderrezzak Abadi (1), Lina Selatna (2) *, fatma zahra Ben ali (3), Bousbia salah sadjid (4)

[1] Department of chemistry, Faculty of exact sciences, University of El Oued, Algeria
[2] Department of Biology, Faculty of Nature and Life Sciences, University of El Oued, Algeria
[3] Department of Biology, Faculty of Nature and Life Sciences, University of El Oued, Algeria
[4] Department of Biology, Faculty of Nature and Life Sciences, University of El Oued, Algeria

*Correspondence E-mail: selatnalina36@gmail.com

Keywords: Antimicrobial; Antioxidant; El Oued; Hemolysis; Pelargonium graveolens; Phytochemicals.

ABSTRACT

This study evaluates the chemical and biological properties of extracts from Pelargonium graveolens (geranium) cultivated in the El Oued region, Algeria. The leaves were dried, ground, and extracted using 96% methanol following the Chiang et al. method. Quantitative analysis revealed high contents of phenolic compounds and terpenes, which contribute to the plant's significant antioxidant activity in DPPH assays.

Antibacterial activity was assessed using the agar well diffusion method against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). The results showed a concentration-dependent effect, with maximal inhibition zones at 50 mg/mL (13 mm for E. coli and 11 mm for S. aureus). Furthermore, the hemolytic activity was evaluated in vitro using a 96-well microplate assay to determine biocompatibility. The extract was found to be relatively safe at low concentrations ($\leq$ 5 mg/mL) with a hemolysis rate of approximately 9.6%, while it exhibited high toxicity at concentrations $\geq$ 10 mg/mL (over 80% hemolysis). In conclusion, Pelargonium graveolens from the desert environment shows promising therapeutic potential as an antimicrobial and antioxidant agent . However, its application in pharmaceutical or food industries requires careful dosage control to ensure safety and avoid cell damage

References:

1. Chiang, W. et al Extraction methods for medicinal plants', 1994.
2. Bonev, B., Hooper, J., Parisot, Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method', J. Antimicrob. Chemother., 2008, 61, (6), pp. 1295-1301.
3. Kim, J. D., et al HPLC fractionation and pharmacological assessment of green tea seed saponins for antimicrobial, anti-angiogenic and hemolytic activities', Biotechnol. Bioprocess Eng., 2015, 20, pp. 1035-1043.