الجمهورية الجزائرية الديمقراطية الشعبية

DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

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

Master 's Thesis

In order to obtain a diploma of an Academic Master In biological sciences

Specialty: Plant Biology and Physiology

Them

" Comprehensive Biological Assessment of Ruta graveolens: Anti-inflammatory and Antioxidant Activities via In Vitro, In Vivo, and In Silico Approaches "

Presented by:

Melle. CHERIET Fatma Zahra
Melle. GORI Kholoud
Melle. SLIMANI Chourouk

Jury members:

President: Dr. Alia Zaid	**MCA**	**El-Oued University**
Examiner: Dr. Hamada Samra	**MCA**	**El-Oued University**
Supervisor: Dr. LANEZ Elhafnaoui	**MCA**	**El-Oued University**
Co-Supervisor Dr. BENAMOR Mohammed Larbi	**MCA**	**El-Oued University**

University year: 2024/2025

إهداء

بسم الله الرحمن الرحيم

الحمد لله الذي بنعمته تتم الصالحات، وبفضله تُنال الغايات، وبحكمته تُصقل النفوس وتُثمر الجهود

نُهدي هذا العمل المتواضع، ثمرة تعب سنوات، ومسيرة كفاح لم تكن مفروشة بالورود، ولكنها كانت مليئة بالعزم، بالإصرار، وبالقلوب التي آمنت أن لا شيء مستحيل لمن وثق بالله وسعى

إلى أمهاتنا الحبيبات، إلى النبض الأول الذي لا يخفت، إلى من كانت دعواتها رفيقتنا في كل لحظة، من غرست فينا معنى الصبر، وربّت قلوبنا على التوكل والإيمان، إلى اليد الحانية التي حملت عنا ثقل الأيام، إلى قلبك يا أمي، الذي كان وطناً وأمانًا وسكينة... نقول: شكرًا بحجم السماء، ولحظة بلحظة كنتِ الحافز والدافع، وكل خطوة كتبناها اليوم كان لكِ فيها الأثر العميق، جعلكِ الله تاجًا على رؤوسنا مدى الحياة

وإلى آبائنا الأعزاء، من زرعوا فينا قيمة العلم، وثبّتوا أقدامنا في وجه الرياح، إلى من علّمونا كيف نصمد، وكيف نواجه، وكيف ننهض. لقد كنتم السند والدعامة، القادة الصامتين خلف كل إنجاز. لكم الفضل بعد الله فيما بلغناه اليوم. هذا النجاح يزهر على جبين تعبكم، ويُعلن وفاءنا لوعدٍ قطعناه يومًا بأن نرفع رؤوسكم فخرًا بنا.

إلى أخوتنا وأخواتنا الأعزاء، أنتم الضلع الثابت والأمان المتجدد، أنتم من كانوا لنا العائلة الثانية في الغياب، والفرحة الصادقة في الحضور. شكراً لأنكم كنتم دائمًا السند والدعاء والابتسامة في طريقٍ لم يكن سهلًا.

وبكل حب واحترام، نخص بالشكر أستاذنا الفاضل حفناوي عانز، الذي لم يكن فقط معلّمًا بل كان قائدًا وأخًا وداعمًا لا يُنسى، نُقدّر فيك إخلاصك وصدقك في التوجيه، وحرصك الدائم على أن نكون في أفضل ما يمكن أن نكون عليه. لك منا كل الاحترام والتقدير والدعاء بالخير الوفير.

كما نتوجه بعميق الشكر والعرفان إلى الأستاذ الفاضل محمد العربي بن عمر، الذي كان لنا أبًا بكل ما تعنيه الكلمة، بصبره وتفهمه، بتوجيهاته التي تركت فينا الأثر الكبير، وبقلقه النبيل على مستقبلنا وتفاصيل رحلتنا. سيظل صدى كلماتك محفورًا في ذاكرتنا، ونعم الأب والمعلّم كنت

ولا ننسى الأخت الحنون، والمعينة الصادقة، الأستاذة وفاء بوضيبة، التي كانت بيننا كأخت، برحابة قلبها، ودفء تعاملها، ومساندتها لنا في كل تفصيل صغير وكبير، فشكرًا لكِ على إنسانيتك قبل مهنيتك، وعلى كونك ملاذًا لكل استفسار وتعبير وطلب.

نشكر من القلب جميع الأساتذة الأفاضل الذين كان لهم فضل في تعليمنا، والذين لم يبخلوا علينا بعلمهم وخبراتهم، فلكم منا كل التقدير، ولن ننسى أبدًا لحظاتكم التي علمتمونا فيها ليس فقط مواد دراسية، بل دروسًا في الحياة والمواقف والوعي.

كما لا يسعنا إلا أن نعرب عن خالص الشكر والامتنان لكل من ساندنا خلال رحلتنا، إلى كل الإداري وطاقم العمل كاملًا، الذين كانوا جنودًا صامتين خلف إنجاح المسار. شكرًا لكل من خصنا بابتسامة، أو ساعدنا بإجابة، أو قدّم لنا يد العون دون انتظار مقابل

ونُهدي هذا الإنجاز إلى أنفسنا أيضًا، نحن الثلاثة، الذين اجتمعنا على حلم واحد، وكتبنا هذه المذكرة كخاتمة لمسيرة ليست عادية، بل مليئة بالاجتهاد، التحديات، والمواقف التي شكّلت شخصياتنا ورؤيتنا المستقبلية. لقد صبرنا، وثابرنا، وكافحنا، وها نحن اليوم نقطف ثمار تعبنا

وأخيرًا من قال: أنا لها نالها، وها نحن نعلنها بثقة: كنا لها، وكان النجاح لنا

اللهم اجعل هذا العمل خالصًا لوجهك الكريم، وانفعنا به، وبارك لنا فيما تعلمناه، ووفقنا لما هو آت.

شكر وتقدير

بسم الله الرحمن الرحيم

﴿وَقُلْ رَبِّ زِدْنِي عِلْمًا﴾ - صدق الله العظيم

بقلوب يملؤها الامتنان، وبمشاعر يختلط فيها الفخر بالتقدير، نتقدّم نحن الطالبات بجزيل الشكر وعظيم العرفان لكل من كان سندًا وداعمًا حقيقيًا لنا في إنجاز هذا العمل البحثي، الذي لم يكن مجرد مشروع أكاديمي، بل كان تجربة إنسانية وعلمية نضجنا من خلالها، واكتسبنا فيها الكثير من القيم، أهمها الصبر، المسؤولية، وروح الفريق.

نتوجّه أولًا بخالص التقدير وأسمى عبارات الشكر إلى أستاذنا المشرف الدكتور الحفناوي لعانز، الذي كان لنا ركيزة علمية ومصدر إلهام حقيقي. فبفضل توجيهاته المنهجية الدقيقة، وصبره اللامحدود، وإخلاصه في المتابعة، أمكننا المضي بثقة وثبات نحو إتمام هذا المشروع. فله منا كل الامتنان والتقدير على ما قدّمه من دعم علمي وعملي لا يُقدّر بثمن.

كما نتقدّم بخالص الشكر والعرفان إلى الأستاذ الفاضل محمد العربي بن عمر، الذي شرفنا بدوره كمساعد للمشرف، وكان لنا بمثابة أب علمي لم يبخل علينا بتوجيه أو نصيحة. لمسنا فيه المسؤولية والحرص والتواضع، وكان لمتابعته ومداخلاته أثر كبير في تنظيم العمل ودقته، فله كل الاحترام والتقدير.

ونخص بالشكر والامتنان للأستاذة وفاء بوضبية، التي رافقتنا بتوجيهاتها الدقيقة ومساعدتها العملية داخل المختبر، وحرصت على أن نُنجز مهامنا بدقّة وسلاسة. لقد كانت لنا أختًا موجّهة، بحسن تعاملها، ولطفها، ورغبتها الصادقة في دعمنا، فكل الشكر والتقدير لهذا الحضور الفعّال والداعم.

ونعبر عن فخرنا وامتناننا العميق لصديقتنا العزيزة جمانة بن خليفة، التي كانت بحقّ شريكة في هذا الإنجاز. فقد كانت السند الميداني والمعنوي، ورافقتنا في كل مرحلة من مراحل العمل، داخل وخارج المخبر. وقفت إلى جانبنا في اللحظات الحرجة، وأسهمت بجهدها وحرصها وتعاونها اللامحدود، وكانت مثالًا يُحتذى في الأخلاق، والمثابرة، والإخلاص. لها منّا كل الاعتراف بفضلها الصادق.

كما لا يفوتنا أن نشكر الطالب الكريم زرود نصر الله، الذي تكفّل بمهمة ذبح الفئران في موعدها المحدد، تنفيذًا لتوجيهات الأساتذة، رغم انشغاله بمذكرته وأعبائه داخل المخبر. لم يتردد، ولم يتأخر، بل لبّى النداء بمسؤولية وتعاون، فله منّا كل الاحترام والتقدير على هذا الموقف النبيل والفعّال.

وفي هذا المقام، لا يسعنا إلا أن نعرب عن تقديرنا العميق وامتناننا الكبير إلى عائلاتنا الكريمة، التي كانت دومًا الملاذ والسند، بدعائهم، وصبرهم، وتحفيزهم لنا خلال مختلف مراحل هذا المشوار. فبهم بعد الله، صبرنا، وتجاوزنا الصعاب، وواصلنا حتى اللحظة الأخيرة.

كما نثمّن الجهود المبذولة من طرف الطاقم الإداري والتقني، وعلى رأسهم الأستاذ أبو بكر، الذي وفّر لنا الدعم اللازم في الجوانب الإدارية وسهّل الكثير من الإجراءات في الوقت المناسب. نُقدّر له جهوده وتعاونه المستمر.

كما نتقدّم بخالص التقدير والاحترام إلى السادة أعضاء لجنة المناقشة الموقّرة، الذين شرّفونا بقبولهم مهمة تقييم هذا العمل، ونثمّن عاليًا وقتهم وجهودهم وملاحظاتهم العلمية، التي ستكون لنا منارة في مسيرتنا المستقبلية.

وفي الختام، فإن كلمات الشكر وحدها لا توفي حقّ كل من ساعدنا ووقف إلى جانبنا. لقد كان هذا العمل ثمرة تعاون صادق وجهد مشترك، وما كان له أن يرى النور لولا التوفيق الإلهي أولًا، ثم وقوف هذه الوجوه الطيبة من حولنا.

نغلق هذه الصفحة بشعور من الفخر والرضا، ونفتح صفحة جديدة من الأمل في مستقبل علمي نافع، راجين أن يكون هذا العمل لبنةً في صرح العلم، وخطوة على طريق البحث الحقيقي الذي يخدم الإنسان والمجتمع.

ونسأل الله أن يجعل ما قدّمناه خالصًا لوجهه الكريم، وأن يسدّد خطانا وخطى الجميع.

والله وليّ التوفيق.

Abstract

This study addresses the comprehensive biological evaluation of *Ruta graveolens* (commonly known as rue), focusing on its anti-inflammatory and antioxidant properties through a multidisciplinary approach. This includes biochemical analyses, in vitro laboratory experiments, in vivo animal testing, and in silico computational simulations. The importance of this research lies in the ongoing search for safer natural therapeutic alternatives compared to conventional drugs, which are often associated with long-term side effects.

The study begins with an extensive overview of inflammation—its types, stages, and key triggering factors—highlighting the roles played by immune cells and chemical mediators. It

also discusses anti-inflammatory drugs, both steroidal and non-steroidal, explaining their mechanisms of action and limitations in clinical use.

Within this context, *Ruta graveolens* is presented as a traditional medicinal plant rich in flavonoids and essential oils. Plant samples were collected from a desert region in southeastern Algeria, and subjected to various extraction methods to obtain both aqueous extracts and essential oils. These extracts underwent advanced chemical analyses using UPLC-MS/MS and GC-MS technologies, which identified bioactive compounds such as rutin, naringin, and syringic acid in the aqueous extract, and 2-Undecanone in the essential oil.

The biological activity of the extracts was assessed in vitro through protein denaturation inhibition tests to determine anti-inflammatory potential, and DPPH assays to evaluate antioxidant activity. The results demonstrated notable efficacy, particularly at higher concentrations. In addition, in silico simulations were conducted to predict the bioavailability and toxicity of the compounds, as well as their binding affinities to key enzyme targets such as COX-1 and Glutathione Reductase, supporting their anticipated anti-inflammatory mechanisms.

In the in vivo component, animal models (Wistar rats) were exposed to the herbicide glyphosate and subsequently treated with the plant extracts. Hematological, biochemical, and antioxidant enzyme markers were analyzed, alongside histopathological examinations of affected tissues. The findings revealed significant improvement in both physiological and histological parameters, reinforcing the therapeutic potential of *Ruta graveolens*.

The study concludes that *Ruta graveolen*s possesses promising properties as a natural source of bioactive compounds with anti-inflammatory and antioxidant effects. It recommends further clinical and exploratory research to develop pharmaceutical products based on the plant’s constituents, within the framework of complementary and alternative medicine.

The study concludes that *Ruta graveolens* possesses promising properties as a natural source of bioactive compounds with anti-inflammatory and antioxidant effects. It recommends further clinical and exploratory research to develop pharmaceutical products based on the plant’s constituents, within the framework of complementary and alternative medicine.

The study concludes that *Ruta graveolens* possesses promising properties as a natural source of bioactive compounds with anti-inflammatory and antioxidant effects. It recommends further clinical and exploratory research to develop pharmaceutical products based on the plant’s constituents, within the framework of complementary and alternative medicine.

Keywords: *Ruta graveolens*, Anti-inflammatory activity, Antioxidant activity, Medicinal plants, Essential oils, Aqueous extract

الملخص

تتناول هذه الدراسة التقييم البيولوجي الشامل لنبتة السذاب(*Ruta graveolens*) ، وذلك من خلال تحليل خصائصها المضادة للالتهاب والأكسدة باستخدام نهج متعدد يشمل التحاليل الكيميائية الحيوية، التجارب المخبرية(*in vitro*) ، التجارب الحية على الحيوانات(*in vivo*) ، بالإضافة إلى المحاكاة الحاسوبية .(*in silico*) وتأتي أهمية هذه الدراسة في ظل البحث المتواصل عن بدائل علاجية طبيعية أكثر أمانًا مقارنة بالأدوية التقليدية، التي كثيرًا ما ترتبط بآثار جانبية على المدى الطويل.

بدأت الدراسة بعرض شامل لمفهوم الالتهاب، أنواعه، مراحله، وأهم العوامل المحفزة له، مبرزةً الأدوار التي تلعبها الخلايا المناعية والمركبات الكيميائية الوسيطة. كما تم التطرق إلى الأدوية المضادة للالتهاب من نوعيها الستيرويدي واللاستيرويدي، موضحةً آليات عملها ومحدودية استخدامها.

في السياق ذاته، تم تسليط الضوء على نبتة السذاب باعتبارها نباتًا طبّيًا تقليديًا غنيًا بالفلافونويدات والزيوت الطيّارة. وقد تم جمع العينات النباتية من منطقة صحراوية في جنوب شرق الجزائر، ثم أُجري استخلاصها بطرق متعددة للحصول على المستخلص المائي والزيت العطري. بعد ذلك، خضعت هذه المستخلصات لتحاليل كيميائية متقدمة باستخدام تقنيتي-UPLC MS/MSوGC-MS، حيث تم التعرف على مركبات فعالة مثل الروتين، النارينجين، السيرينجيك أسيد في المستخلص المائي، و 2-Undecanoneفي الزيت العطري.

تم تقييم الفعالية البيولوجية للمستخلصات مخبريًا من خلال اختبار تثبيط دنترة البروتين لتحديد النشاط المضاد للالتهاب، واختبار DPPH لتقدير النشاط المضاد للأكسدة، وقد أظهرت النتائج فعالية واضحة، خصوصًا عند التركيزات العالية. كما أُجريت محاكاة حاسوبية (in silico) لدراسة التوافر الحيوي والسمية المتوقعة للمركبات، بالإضافة إلى دراسة ارتباطها بمواقع إنزيمية كـ COX-1 وGlutathione Reductase، ما أكد فعاليتها المتوقعة كعوامل مضادة للالتهاب.

في الشق الحيوي، تم استخدام نماذج حيوانية جرذان (Wistar) تعرضت لمبيد الغليفوسات، ثم عُولجت بالمستخلصات النباتية وقد تم تحليل معايير دموية وكيميائية حيوية وأنزيمات مضادة للأكسدة، إلى جانب دراسة الأنسجة المصابة عبر الفحص النسيجي. وأسفرت النتائج عن تحسن كبير في المعايير الفسيولوجية والنسيجية، مما يدعم الاستخدام العلاجي للسذاب.

خلصت الدراسة إلى أن نبتة السذاب تملك خصائص واعدة كمصدر طبيعي لمركبات فعالة مضادة للالتهاب والإجهاد التأكسدي. كما توصي بأهمية التوسع في البحوث السريرية والاستكشافية لتطوير أدوية قائمة على مكونات هذا النبات، ضمن إطار الطب البديل والتكميلي.

الكلمات المفتاحية: *Ruta graveolens*، نشاط مضاد للالتهابات، نشاط مضاد للأكسدة، نباتات طبية، زيوت أساسية، مستخلص مائي

LIST OF ABBREVIATIONS

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

DMSO: Dimethyl sulfoxide

DPPH: 2,2'-Diphenyl-1-picrylhydrazyl

DTNB: 5,5′-Dithiobis-(2-nitrobenzoic acid)

EDTA: Ethylenediaminetetraacetic acid

FBS: Fasting blood sugar

GSH: Glutathione

H2O: Water

H2O2: Hydrogen peroxide

HCl: Hydrochloric acid

HGB: Hemoglobin

IC50: Concentration causing 50% inhibition of an activity

LDH: Lactate dehydrogenase

LYM: Lymphocytes

MCV: Mean corpuscular volume

MDA: Malondialdehyde

MDH: Malate dehydrogenase

NADPH: Nicotinamide adenine dinucleotide phosphate

PH: Potential of hydrogen

PLT: Platelets

POD: Peroxidase

RBC: Red blood cell

SD: steam distillation

SOD: Superoxide dismutase

TBS: Tris buffer saline solution

TCA: Trichloroacetic acid

WBC: White blood cell

FIGURES LIST

TABLES LIST

Summary

Introduction

Introduction

Inflammation constitutes one of the fundamental biological mechanisms upon which the body relies as a defensive response against external stimuli such as pathogens, tissue injuries, or harmful chemical substances[1]. This response involves a complex interaction between immune system cells, inflammatory chemical mediators, and enzymes that regulate the internal environment of tissues. Although inflammation is a natural phenomenon aimed at repairing damaged tissues and restoring biological balance, its dysregulation or prolonged persistence may shift it from a protective state to a driving factor for a series of chronic and serious disorders, such as arthritis, cardiovascular diseases, cancers, and neurodegenerative diseases [2].

Chronic inflammation is closely associated with oxidative stress, which results from the accumulation of free radicals and the weakening of antioxidant defenses[3], leading to damage to proteins, lipids, and DNA. Numerous studies have shown that the interplay between these two phenomena directly contributes to the progression of chronic diseases. Despite the effectiveness of synthetic anti-inflammatory drugs, their prolonged use is linked to significant health risks, prompting the search for safe and effective natural alternatives.

Medicinal plants are among the most prominent of these alternatives, due to their richness in bioactive compounds such as flavonoids, terpenoids, alkaloids, and essential oils [4], which have demonstrated significant anti-inflammatory and antioxidant properties. Among these plants, *Ruta graveolens* stands out, traditionally known in folk medicine for its applications in treating inflammatory, neurological, digestive, and dermatological disorders.[5] This plant contains active compounds such as flavonoids (rutin, naringin), coumarins, and volatile oils (2-undecanone), which are believed to contribute to its biological effects, although they still require comprehensive scientific evaluation.

In this context, the present study aims to conduct an integrated biological analysis of *Ruta graveolens* by evaluating its anti-inflammatory and antioxidant properties using a multidisciplinary scientific approach, including:

- **Chemical analysis** of the aqueous extract and essential oil using GC-MS and UPLC-MS/MS techniques,[6]
- **In vitro evaluation** through protein denaturation and DPPH radical scavenging assays,[7]

- **In vivo evaluation** using a glyphosate-induced toxicity model in rats,[8]
- **In silico simulations** to study bioavailability, toxicity, and the binding of active compounds to biological targets.[9]

Hence, the central research question of this study arises:

To what extent can *Ruta graveolens*, with its chemically active compounds, offer a safe and effective natural alternative capable of combating inflammation and oxidative stress, supported by biological, chemical, and computational evidence? Could it potentially outperform conventional pharmaceutical treatments? And what is its ability to improve histological, biochemical, and physiological markers in the context of glyphosate exposure?

First part

Bibliographic synthesis

Chapter I: disease

Introduction

The inflammation is an essential event; this protective response may lead to potentially damaging consequences. Various autoimmune disorders are characterized by marked inflammation and associated failure of repair process. Pro-inflammatory molecules like TNFa, certain interleukins, prostaglandins and even pathogenic concentration of nitric oxide are instrumental in raising such response[10]. Many current anti-inflammatory drugs target these medi-ators at different levels, yet they lack specificity and their unto-ward effects restrict their long-term use [11].

Hence, there is a constant demand for better therapeutic alterna-tives. Herbal products are well known for their reputed medicinal properties; however, most of them are empirically used. Several plants of Rutaceae family are used in traditional medicine worldwide.

The most common medicinal plant of this family is *Ruta graveolens L*., known as rue and native to Europe. The plant is now available all over the world, though preferably grown in Mediterranean climates. This plant is in medicinal use for var-ious clinical conditions from very ancient time but rationality of its use is still controversial. In homeopathy, rue is an impor-tant remedy for deep aching pain and rheumatism besides being used for eyestrain-induced headache [12]. It has also been used as a remedy for gastric disorders, stiff neck, dizzi-ness, headache and so on[13]. This hints towards the putative anti-inflammatory property of Ruta graveolens.

I. Inflammation

1. Definition

The inflammation is part of the reaction of the complex defense system on visibly damaged skin. It is cleaned by nociceptive stimuli, noting pathogenic agents, trauma physiques, radiations and irritants [14]. This is a vital response to an exogenous or endogenous attack and a beneficiary process that mobilizes the immune system to remove the agent and repair the leakage issues [15].

The inflammation of the major players in the specifications of the digits and the amelioration of the treatment of the tissue. Because the inflammation can be used to prevent a defense against infecting agents, it can be detected in the case of the pathogen with a chronic infection malady [16].

2. Causes of Inflammation:

Inflammation occurs when the body's white blood cells release chemicals into the blood or tissues in order to fight off invaders. As a result, the area that has been injured or infected receives an increase in blood flow. Redness and warmth can result from it. Swelling is the outcome of fluid leakage into your tissues caused by some of the chemicals. This defense mechanism could set off pain receptors. White blood cells and the substances they produce in the joints induce inflammation, joint lining swelling, and the eventual loss of cartilage [17](the cushions at the ends of bones). In 2000, Microorganisms are one of the reasons that might cause inflammation.

- Real-world entities.
- Chemicals.
- Immune reactions that aren't acceptable.
- The demise of tissue

There are a number of factors that can lead to inflammation, including infectious agents like viruses and bacteria. By infiltrating and destroying cells in the body, viruses cause inflammation; bacteria create endotoxins, which can cause inflammation. Physical trauma, burns, radiation harm, and frostbite can all cause tissue damage and inflammation. Corrosive chemicals like acids, alkalis, and oxidizing agents can also cause inflammation. As previously stated, a dysfunctional immune system can lead to an inflammatory reaction that is harmful to the body an further cause of inflammation is tissue death due to a lack of oxygen or nutrients, which is frequently brought on by a reduction in blood flow to the affected area [18].

3. Types of Inflammation:

There are two types of inflammation: acute and chronic

Acute inflammation: This is a type of short-term inflammation that the body produces to address injuries, illnesses, and infections [19]. When there is an injury or contract a virus, the white blood cells begin to flood the area to provide protection and healing. Symptoms of acute inflammation include redness, warmth, and swelling (Figure 1). Depending on the severity of the infliction, the inflammatory response will slowly fade away over hours or days.

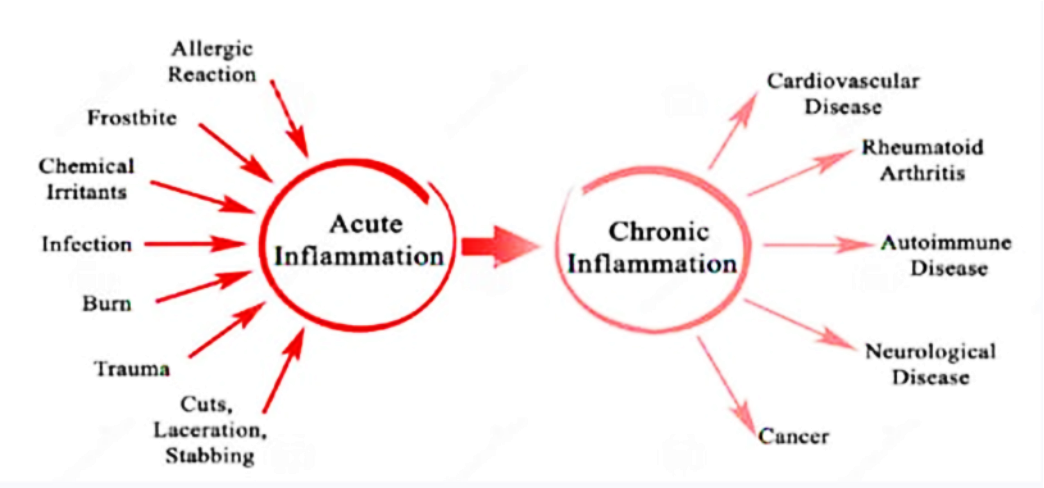


Figure 01 Graphic representation of inputs for Acute inflammation and then acute inflammation becomes chronic inflammation wgat sort of diseases can develope. Image Source: Inside Out Health and Wellness. [20]

Chronic inflammation: The body's response is the same as with acute inflammation, although there isn't always an injury that needs healing [20]. Instead, the body wrongly signals that there is an issue of some kind, leading to inflammation that is persistent. Chronic inflammation has a more heterogeneous histological appearance than acute inflammation. In general, chronic inflammation is characterized by the presence of macrophages, monocytes, and lymphocytes, with the proliferation of blood vessels and connective tissue

Chronic inflammation is typically less painful than acute inflammation and lasts for a longer amount of time. Chronic inflammation is characterized by the following symptoms that include:

- Tired appearance
- Unexplained muscle aches and joint pain
- Stomach issues including constipation or diarrhea
- Gaining weight
- Skin rashes

4. The phases of inflammation

4.1 Vascular-exudative or vascular phase

The initial phase of inflammation is known as vasculo-exudative and is marked by the rupture of blood vessels. This leads to the leakage of inflammatory cells such as neutrophils, macrophages, and lymphocytes into the surrounding tissue, along with fluid buildup (edema)

and platelet attachment, which contribute to the formation of a temporary protective crust. When the blood vessels break, a fibrin-based clot form. This clot acts as a provisional matrix, partially filling the wound and allowing activated fibroblasts to migrate into the damaged area [21] .

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

Another important event in this stage is leukocyte diapedesis, the process through which white blood cells exit the bloodstream and move to the site of injury [22]. This begins with the arrival of neutrophils, followed by monocytes and lymphocytes.

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

The accumulation of leukocytes near the blood vessel walls due to slowed circulation (margination)

The attachment of leukocytes to the endothelial lining:

Their movement through the vessel wall (trans-endothelial migration) to reach the inflamed tissue.

4.2 Cellular Phase

This phase involves the arrival of various types of cells at the site of inflammation, typically during the later stages of the response. It spans approximately 1 to 4 days [23]. All the recruited cells contribute to the formation of an inflammatory granuloma. Among them, polymorphonuclear cells, monocytes, and macrophages play two key roles؛ they engulf and remove cellular debris and foreign substances through phagocytosis.

They also assist in tissue breakdown by releasing proteolytic enzymes into the surrounding environment.

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

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

The cellular phase generally continues until approximately the third day of the inflammatory process[24].

4.3 .The Repair Phase

The repair phase involves the remodeling of the extracellular matrix and a reduction in the number of cells in the dermis following re-epithelialization [25]. This stage can last for several months and typically results in the formation of a scar, which may vary in fibrous content. During this process, cells from the granulation tissue mainly myofibroblasts, pericytes, and endothelial cells undergo apoptosis and are gradually cleared.

Collagen fibers are reorganized, with some fibers degrading and others restructured to align with the mechanical stress lines in the tissue. Ideally, the resulting scar should preserve skin function and be cosmetically acceptable[26].

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

II. Anti-inflammatory

1. Definition

Anti-inflammatory treatment is commonly carried out using synthetic compounds, either non-steroidal or steroidal in nature[27], These substances are designed to counteract inflammation and are categorized into two main types:

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

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

2. Steroidal Anti-inflammatories

Definition: Steroidal anti-inflammatory drugs (SAIDs)[28], also referred to as corticosteroids or glucocorticoids, are a broad class of medications derived from cortisol, They are considered among the most potent treatments for chronic inflammatory conditions, such as rheumatoid arthritis and autoimmune disorders.

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

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

Mechanism of Action in Inflammation:

Glucocorticoids work by inhibiting the production of prostaglandins, key mediators in the inflammatory response. This effect is mainly achieved through their suppression of phospholipase A2 activity, thereby reducing the availability of arachidonic acid, which is normally metabolized by cyclooxygenase enzymes[29].

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

3. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Definition : NSAIDs refer to a group of compounds that possess anti-inflammatory [30], fever-reducing (antipyretic), and pain-relieving (analgesic) effects. Their therapeutic benefits as well as their main side effects are primarily linked to their inhibition of cyclooxygenase (COX) enzymes, which are essential for the production of prostaglandins and thromboxanes, These drugs are considered symptomatic treatments, meaning they address the manifestations of inflammation, regardless of its origin be it mechanical, chemical, infectious, or immune-related.

Mechanism of Action in Inflammation:

NSAIDs[31] work by blocking the activity of cyclooxygenase enzymes (COX), thereby preventing the production of prostaglandins. These molecules play a key role in the inflammatory response following surgery, where they stimulate and sensitize peripheral pain receptors and also contribute to increased sensitivity in the spinal cord's dorsal horn, leading to postoperative hyperalgesia.[32]

The COX-2 enzyme, in particular known as the inducible form because it is triggered by surgical trauma is primarily responsible for this prostaglandin production. By inhibiting COX-2[33], NSAIDs not only provide pain relief but also reduce hyperalgesia .

Chapter II : medicinal plant

1. The studied plants:

1.1. Ruta graveolens Plant:

1.1.1. Ruta graveolens Definition:

Ruta graveolens, commonly known as Rue, emits a strong and characteristic aroma that is often considered pungent or medicinal. Botanically classified as a perennial herb, it features bluish-green, pinnate leaves with a waxy texture and small oil glands[34]. This species typically flowers in late spring to early summer, producing small, yellow-green flowers arranged in clusters. Rue is well-adapted to dry, rocky environments and is widely found in Mediterranean regions, including many Arab countries. It is renowned for its abundance of bioactive compounds such as flavonoids, alkaloids, coumarins, and volatile oils.

1.1.2. Vegetative classification of *Ruta graveolens*

- **Scientific name:** *Ruta graveolens L.*
- **Synonyms** *: Ruta bracteosa L., Ruta hortensis Mill.*
- **Common name**: Rue, Herb-of grace, Sadab, Fijel**.**[35]

Table 01 : Vegetative Classification of Ruta graveolens[35]

Species	*graveolens*
Genus	*Ruta*
Family	Rutaceae
Order	Sapindales
Class	Magnoliopsida
Division	Angiosperms
Kingdom	Plantae

1.1.3. Vegetative description of *Ruta graveolens*

A diminutive herbaceous plant ranging from 10 to 40 cm in height, possessing a distinctive pungent aroma [36]. Adorned with delicate white bristles, its stems are erect or slightly prostrate, exhibiting a cylindrical form tinged with green-yellow hue. The leaves are

pale green, ensconced in woolly, dense bristles, notable for their thickness, trifoliate structure, and trifurcation at the apex into 3-5 lobes[37].

The capitula are diminutive and planar, measuring 6 to 10 cm in diameter. Consisting entirely of tubular florets, they are tetramerous and densely packed, forming a compound inflorescence with an overlaying capitulum. Initially dark, the buds transition to a golden yellow hue upon blossoming. The fruits are naked and meager, as depicted in Figure (2) below.[38]

Figure 02: *Ruta graveolens* photograph.[39]

Figure 03: Botanical illustration of *Ruta graveolens* by Otto Wilhelm Thomé [40]

1.1.4. Geographical Distribution of *Ruta graveolens*:

The plant originates from Europe (Ukraine, Albania, Bulgaria, ex-Yugoslavia), and has a large natural origin throughout Europe and North Africa. This is also a large area located in the United States, providing food or culture for its medical properties.

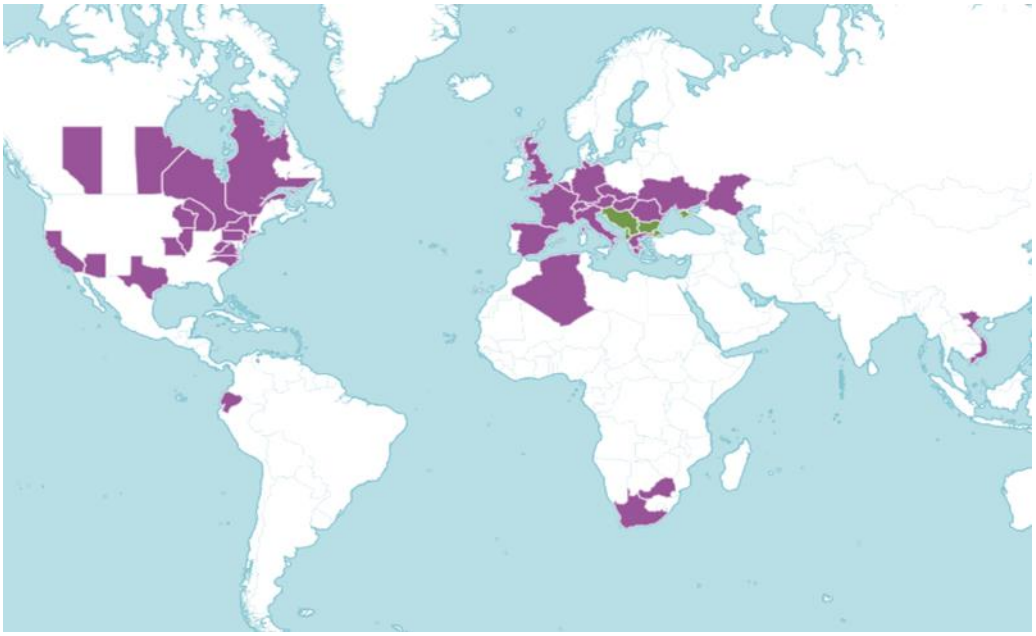


Figure 04**.** Distribution of *Ruta graveolens* in the world.[34]

1.1.5. Economic and Medicinal Uses of *Ruta graveolens*:

R. graveolens has been used as a folklore medicine for treatment of various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory diseases [5]

Second part:

Experimental part

Chapter I:

Materials & methods

Introduction:

The Rutaceae family includes a wide variety of aromatic plants, especially in tropical regions. One of the most notable genera in this family is Ruta. This plant is now cultivated in many parts of the world. Its native range is believed to be Southern Europe and North Africa, where it typically grows in dry, rocky soils.[5]

The Rutaceae family is considered one of the largest plant families, comprising approximately 150 genera and 1,500 species, widely distributed in tropical and subtropical regions of the world. This family is globally known for its citrus fruits such as oranges, lemons, and grapefruits, and is also referred to as the citrus family.[38]

Rue belongs to the Rutaceae family within the Sapindales order, which includes approximately 160 genera and more than 1,600 species. Due to its cultural and medicinal value, it has been introduced to various countries in North, Central, and South America, China, India, the Middle East, and South Africa.[41]

R. graveolens is a rich source of secondary metabolites mainly: coumarins, alkaloids, volatile oils, flavonoids, and phenolic acid.[42]

In traditional medicine, leaves are abortifacient, anthelmintic, antiepileptic, anti-inflammatory, carminative, emetic, emmenagogue, expectorant, hemostatic, nervine, rubefacient and mildly stomachic. The herb is also considered antispasmodic, diuretic, resolvent and strongly stimulant. It is used as a stimulant to the nervous system and uterus. Useful in skin disorders, cramps in the bowel and hysteria. It is chiefly used to encourage the onset of menstruation and as a remedy for menstrual disorders such as PMS. It stimulates the muscles of the uterus and promotes menstrual blood flow. It also strengthens thestomach, aids digestion and is useful in sluggish liver. The herb is also used to ease colic and reduce flatulence. The herb was prescribed in cases of vertigo and epilepsy in the 17th century.[43]

1. Plant Material:

Ruta graveolens was harvested at different intervals, from December 2024 to January 2025, in a desert forest area in southeastern Algeria, on the Fayadh Road near El Magrane, in El Oued Province. This area is characterized by:

• Geographic Coordinates:

Latitude (North): 33.5615091° N

Longitude (East): 6.9308492° E

• Elevation above Sea Level: 44 meters.

• Distance from Sea Level: 290 kilometers.

• Bioclimatic Characterization: Desert.

2. Extraction

2.1. Extraction of the crude extract:

- ✓ **Extraction:**

Distilled water was used to soak the plant powder to extract the active compounds and then the crude extract was extracted by evaporation using a rotary evaporator.

- ✓ **Maceration:**

A quantity of 100 grams of rue *(Ruta graveolens)* powder was accurately weighed using a precision balance. The powder was then soaked in a tightly sealed container containing 300 milliliters of distilled water and kept in a dark place for 24 hours.

- ✓ **Filtration:**

After 24 hours, the mixture was filtered through filter paper and allowed to settle completely. Then, 300 ml of distilled water was added to the remaining plant powder, and the soaking process was repeated. The solution was left in the dark for another 24 hours. The filtered solution was then collected in an opaque container and stored in the dark. This process was repeated daily for three consecutive days.

- ✓ **Evaporation:**

After the filtration process, the extract was evaporated using a rotary evaporator to obtain the crude extract**.**

- **Extraction yield:**

The results of solvent extraction were as follows:

Aqueous—13.02 g

$$EX\,yield\,\% = \frac{W1\,(g)}{W2\,(g)} \times 10$$

where W1 = weight (g) weight of plant powder after extraction,

W2 = dry weight (g) of plant powder before extraction.

The percentage extractive value was found to be: 13.02%

Figure 05**:** Crude Extract of *Ruta graveolens*

- **Ultra-performance liquid chromatography-mass spectrometry (UPLC/MS–MS) analysis:**

✓ **UPLC-ESI-MS/MS System:** Analyses were conducted using a Shimadzu LCMS-8040 system, renowned for its ultra-fast multiple reaction monitoring capabilities and high sensitivity.

✓ **Chromatographic Separation:**

- **Column**: Restek Ultra C18 column (150 mm × 4.6 mm, 3 µm particle size), selected for its high-purity, type-B silica, ensuring reliable high-performance liquid chromatography (HPLC) applications.
- **Mobile Phase:** Solvent A consisted of water with 0.1% formic acid, while Solvent B was methanol
  - **Gradient Program:**
    - 0.0–0.2 min: 98% A
    - 0.2–2.5 min: linear gradient to 25% A
    - 2.5–4.0 min: linear gradient to 0% A
    - 4.0–7.0 min: maintained at 0% A
    - 7.0–7.1 min: return to 98% A
    - 7.1–12.0 min: re-equilibration at 98% A
  - **Flow Rate**: 0.2 mL/min.
  - **Injection Volume:** 5 µL**.**
    - **Mass Spectrometric Conditions:**
    - **Ionization Source:** Electrospray ionization (ESI) in both positive and negative modes.

- **Interface Parameters:**
  - Desolvation Line (DL) Temperature: 250°C.
  - Heat Block Temperature: 400°C.
  - Nebulizing Gas Flow: 3.0 L/min.
  - Drying Gas Flow: 10 L/min.
- **Collision-Induced Dissociation (CID) Gas Pressure:** 230 kPa**.**
- **Conversion Dynode Voltage:** −6.00 kV**.**

2.2. Essential Oils Extraction:

- ✓ **Apparatus**

The extraction of essential oil required the use of specialized laboratory equipment to ensure precision and efficiency throughout the process. In this study, the following apparatuses were employed:

. Adventurer – Pro AV53 analytical balance

. Heating flask

. Refrigerant (condenser)

. Clevenger apparatus

. Separating funnel

. Rotary evaporator

Among these, the Clevenger apparatus played a central role in the hydro distillation process used for essential oil extraction. A diagram of the Clevenger system, which enables the separation and collection of oil from plant material through steam distillation, is shown below. (*Insert figure if applicable.*)

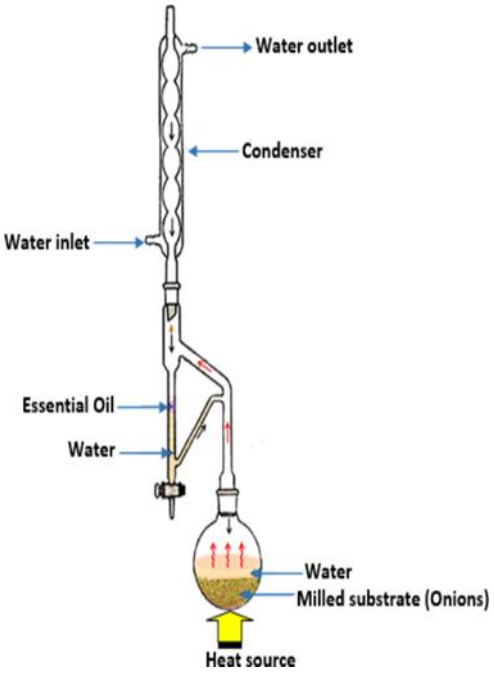


Figure 06**.** Schematic representation of the Clevenger apparatus used in the essential oil extraction process. RR [44]

✓ **Procedure:**

The extraction of essential oil was carried out through a standardized hydro distillation process using a Clevenger-type apparatus. The procedure consisted of the following steps to ensure both accuracy and optimal yield:

- **Sample Preparation:** Fresh plant material was initially cleaned thoroughly to remove dust, debris, and other contaminants.
- **Weighing:** Exactly 100 grams of the cleaned plant sample were measured using a precision analytical balance (Adventurer – Pro AV53).
- **Pre-heating Treatment:** The plant material was rinsed with distilled water to prevent scorching and then transferred into a heating flask.
- **Boiling Regulation:** A few boiling chips were added to the flask to regulate boiling and avoid sudden bumping.
- **Hydrodistillation Process:** The flask was subjected to direct heating at 100°C for 3.5 hours to enable the release of volatile compounds via steam.
- **Oil Separation:** The condensate collected in the Clevenger apparatus underwent liquid-liquid extraction using diethyl ether to isolate the essential oil from the aqueous phase.

- **Drying:** The extracted organic phase (containing the oil) was treated with anhydrous sodium sulfate to remove residual moisture.
- **Filtration:** The dried extract was filtered using standard Whatman filter paper to eliminate any traces of sodium sulfate or solvent impurities.
- **Solvent Removal:** The filtered oil solution was concentrated using a rotary evaporator to remove any remaining diethyl ether and obtain pure essential oil.
- **Storage:** The final essential oil product was transferred into sterile amber glass vials and stored at 5°C in a refrigerator to preserve its chemical stability and prevent light-induced degradation.

✓ **Yield of Essential Oil Extraction:**

The yield is defined as the ratio between the mass of the recovered essential oil and the mass of the plant material. The Following equation is used:

$$EO\ yield\ \% = \frac{EO\ (g)}{sample\ weight\ (g)} \times 100$$

EO: yield in%

EO: mass of the recovered essential oil in grams

sample weight: mass of the plant material in grams

✓ **Characterization of Essential Oils:**

The characterization of essential oils comprises two fundamental components: physicochemical properties and Gas Chromatography–Mass Spectrometry (GC/MS) analysis. Physicochemical analysis provides insights into parameters such as color, density, refractive index, and solubility, while GC/MS allows the identification and quantification of the chemical constituents within the oil, offering a detailed profile of its composition.

- **Physicochemical Properties**

In order to evaluate the quality and purity of the extracted EO, physicochemical analysis, including ethanol miscibility, density, refractive index, acid index (AI), peroxide index (PI), and ester index (EI), was performed. [45]

- **Gas Chromatography-Mass Spectrometry (GC/MS) analysis:**

The Gas Chromatography–Mass Spectrometry (GC/MS) technique is considered the most widely used analytical method in the study of essential oils, as it enables the simultaneous separation, identification, and quantification of the diverse chemical constituents found in the extracted oils.

- **Principle:**

The principle of this technique is based on the differing affinities of compounds in a mixture for two phases: a stationary phase and a mobile phase. It relies on the distribution of constituents between the stationary phase—comprising a silicone-based liquid coated on an inert granular solid within a typically coiled steel or glass column (1 to 3 meters long and 2 to 4 millimeters in diameter)—and the mobile phase, which is an inert carrier gas such as nitrogen, helium, or argon.

The column is heated by a furnace to maintain a high temperature, causing the constituents to vaporize and separate. Separation occurs due to differences in the partition coefficients of volatile compounds between the stationery and gas phases. A detector at the column's exit generates a signal for each eluting molecule, producing peaks that correspond to the individual constituents.

Gas chromatography is often combined with mass spectrometry (GC-MS), where the identification of unknown compounds is achieved by computerized comparison of the mass spectrum of an unknown peak against reference spectra stored in one or more libraries.

- **Apparatus:**

The identification of essential oil components was performed by GC and GC/MS. GC diagnostic were carried out on an HP5890-series II gas chromatograph (Agilent Technology, California, USA) with Flame Ionization Detectors (FID) characterized by: a fused silica capillary column, non-polar HP-5 and polar HP Innowax (30 m × 0.25 mm ID, film thickness 0.25 μm). The temperature of the oven was 50 °C for 1 min, then it is programmed to achieve heating rate up to 5–240 °C/min and held isothermal for 4 min. Injector temperature: 250 °C, detector temperature: 280 °C; nitrogen used as a carrier gas (1.2 ml/min); and injected 0.1 ml diluted in hexane1%. The percentage of the constituents was calculated by electronic integration of FID peak areas without the use of response factor correction. Hewlett-Packard 5972 MSD system served for GC/MS analyses: HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness of 0.25 mm) was directly coupled to the mass spectrometry. The comparison of the component mass spectra with those in the Wiley 275 GC/MS library and of their retention indices with literature data

- **Procedure:**

Nitrogen (N_2) was used as the carrier gas at a flow rate of 1 mL/min, and 1 µL of the essential oil was injected using the split injection technique. The injector temperature was set at 250°C, while the detector temperature was maintained at 280°C. The oven temperature program began at 50°C, held constant for 4 minutes, followed by a gradual increase to 250°C at a rate of 2°C per minute, where it was maintained for 15 minutes. Subsequently, the temperature was raised to 300°C at a rate of 10°C per minute.[46]

3. In Vitro Bioassays*:*

1. Anti-inflammatory Activity: Bovine Serum Albumin (BSA) Denaturation Assay:

1. Overview:

Protein denaturation is a key process in inflammatory disorders [47], making BSA denaturation inhibition an effective model for assessing anti-inflammatory potential [48]. This assay evaluates the ability of *Ruta graveolens* extracts and essential oil to prevent protein denaturation, which may indicate their therapeutic potential for inflammation-related diseases.

2. Chemicals and Reagents:

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

3. Procedure:

In the present study, the assay was conducted following established methodologies [49] with slight modifications to optimize experimental conditions. The reaction mixture was prepared by combining 500µL of a 5% BSA solution with 250 µL of the test sample at varying concentrations (100 – 1000 µg/mL). Diclofenac sodium was used as a positive control, while the negative control consisted of BSA solution mixed with distilled water under identical conditions. The prepared mixtures were incubated at 37°C for 20 minutes to allow interaction between BSA and the test compounds. Following this, the solutions were subjected to heat-induced denaturation by maintaining them at 70°C for 20 minutes. After the heating phase, the samples were cooled to room temperature and diluted with 500µL with deionized water before measuring their absorbance at 660 nm using a UV-Vis spectrophotometer. Blank was prepared with mixing: 1mL water + 250 µL DMSO. The percentage inhibition of protein denaturation was calculated using the Equation 4:

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

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

2. Antioxidant Activity: DPPH Radical Scavenging Assay:

1. Overview:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating antioxidant potential by measuring the ability of plant extracts to neutralize free radicals [50]. The reduction of DPPH radicals results in a colour change from purple to yellow, which is quantified spectrophotometrically [51]. In the present study, the antioxidant potential of the plant extracts and synthesized nanoparticles was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using the following established methodologies [52] with slight modifications.

2. Chemicals and Reagents:

- DPPH solution (0.1 mM in DMSO) from Sigma-Aldrich
- Plant extracts (100 - 1000 µg/mL, dissolved in DMSO)
- Alpha-tocopherol (positive control, reference antioxidant)

3. Procedure:

A freshly prepared 0.0634 mM DPPH solution in methanol was used as the oxidant. In a typical reaction, 1 mL of the DPPH solution was combined with 250µl of the test sample at different concentrations (100 – 1000 µg/mL). The mixture was allowed to incubate in the dark at ambient temperature for 30 minutes to ensure sufficient interaction between the antioxidants and the free radicals. The absorbance of the reaction system was subsequently recorded at 517 nm using a UV-Vis spectrophotometer DMSO was used as a blank. A control sample, containing only the DPPH solution without any test compounds, was prepared under identical conditions. Alpha-tocopherol served as the reference antioxidant. The ability of the test samples to scavenge DPPH radicals was expressed as a percentage inhibition, calculated using the Equation 5:

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

Where $A_{control}$ represents the absorbance of the DPPH solution alone, and A_{sample} corresponds to the absorbance of the sample-containing reaction mixture. Each experiment was conducted in triplicate, and the results were presented as mean ± standard deviation (SD).

- ***In Silico Analyses:***

1. ADMET and Drug-Likeness Prediction:

The ADMET program, consisting of Swiss ADME, pkCSM, and Pro-Tox II, was employed to assess the chosen ligands. It was utilized to forecast the physicochemical attributes, lipophilicity, water solubility, pharmacokinetics, drug similarity, medicinal chemistry, and toxicological properties of the selected chemicals. Through the utilization of these methods and analytical tools, we obtained significant data on the potential therapeutic applications and potentially negative consequences linked to the primary chemical constituents found in the analyzed essential oil.[53]

2. Molecular Docking Study:

2.1. Ligands Preparation:

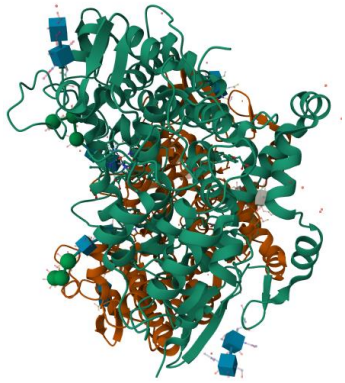
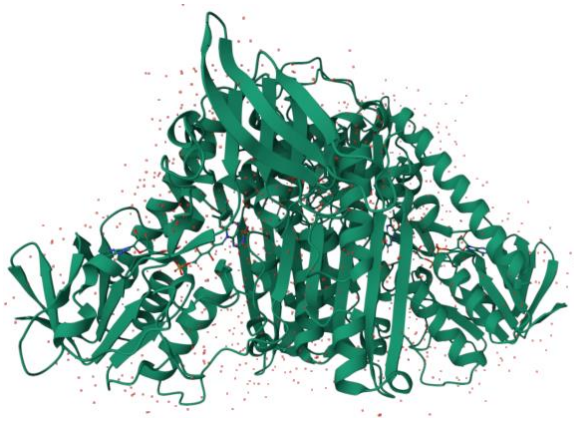
The three-dimensional (3D) structures of the principal bioactive compounds identified from the aquatic extract and essential oil of *Ruta graveolens* were retrieved from the PubChem database, maintained by the National Center for Biotechnology Information (NCBI) [54],accessible via https://pubchem.ncbi.nlm.nih.gov/. Each compound underwent structural refinement and optimization using the LigPrep module of the Schrödinger suite [55], where ionization states were adjusted to physiological conditions (pH 7.0 ± 2.0) using the Epik Classic module. Tautomeric and stereoisomeric forms were generated while preserving stereochemical integrity. Partial atomic charges were assigned based on the Optimized Potentials for Liquid Simulations (OPLS4) force field [56], ensuring energetically favourable conformations for molecular docking and simulation.

2.2. Receptor Preparation:

The 3D structure of cyclooxygenase-1 (COX-1) enzyme (PDB ID: 5WBE) [57],and human glutathione reductase (GR) enzyme (PDB ID: 1XAN) [58] was retrieved from the Protein Data Bank (http://www.rcsb.org) [59], adhering to specific parameters as listed in Table 2. Processing of the protein structure was executed through the "protein preparation Workflow" module within the Schrödinger suite [60], involving consecutive stages of import and processing, review and modification, and refinement.

Table 01 **.** Target receptor information chosen for docking studies

Cyclooxygenase-1 (COX-1)	Detaille's	
	PDB ID	5WBE
	Mutation	No
	Resolution (Å)	2.75
	R-Value Free	0.229
	R-Value Observed	0.197

	Organism	Ovis aries
	Space Groupe	P 6_5
	Sequence Length	600
Human Glutathione Reductase (GR)	**Detaille's**	
	PDB ID	1XAN
	Mutation	No
	Resolution (Å)	2.00
	R-Value Free	0.158
	R-Value Observed	0.158
	Organism	Homo sapiens
	Space Groupe	C 1 2 1
	Sequence Length	461

In the initial stage, the Prime tool was employed to address missing residues and side chains, maintaining the pH of PROPKA at 7.0 ± 2.00. Subsequent steps included the optimization and assignment of hydrogen bonds, along with the removal of water molecules beyond 8 Å. Restrain minimization utilizing the Optimized Potentials for Liquid Simulations (OPLS4) force field was performed to achieve a low-energy state for the protein [61]. This phase of protein preparation signifies an energy optimization methodology, presenting the protein in its energetically favourable state for subsequent *in-silico* studies.

The "Receptor grid generation" panel facilitated the creation of a grid encompassing the active site of the protein, delimited by the co-crystallized ligand in each protein. Default parameters were maintained, and the grid centre was generated at the coordinates listed in Table 3.

Table 2**.** Selected grid parameters the studied enzymes

Enzymes	Grid Centre			Grid Size (Å)
	x	**y**	**z**	
5WBE	14.78	20.976	8.807	60 × 60 × 60
1XAN	30.658	37.912	16.356	

2.3. Docking Setup:

Docking simulation and including Induced Fit Docking were conducted employing the Glide module and Induced Fit Docking module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021, 20) [62]. The simulations were executed on a DELL Intel(R) Core (TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32,0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

The molecular docking tool employed for all docking studies was Glide (Grid-based Ligand docking with Energetics), a module within the Schrödinger suite [63]. The prepared ligands underwent docking onto the specified protein site utilizing the Glide module, in Standard Precision (SP) modes [64].

2.4. Induced Fit Docking (IFD):

The Induced Fit docking (IFD) module of the Maestro molecular modelling suite has been noted as a reliable and effective docking approach to consider flexibility in both ligands and the binding pocket residues in the binding pocket of target receptors [65]. During the IFD process, Glide/SP (Standard Precision) was performed for each ligand, the Prime refinement step specifically addressed the side chains of residues within a 5 Å radius of the ligand. Noteworthy is the retention of a maximum of 20 poses for each ligand.

- ***In vivo Bioassays*:**

1. Animal treatment:

This in vivo study was conducted using 35 adult male Albino Wistar rats, with an average body weight of 120 ±25 grams, obtained from the Pasteur Institute in Algiers. The animals underwent a 15-day acclimatization period in the animal housing facility under controlled conditions, including a constant temperature of 25°C, relative humidity of 62%, and a regulated light-dark cycle of 12 hours light followed by 12 hours of darkness. Animal handling and treatment were carried out in accordance with the Guide for the Care and Use of Laboratory Animals [66].

The rats were housed in polypropylene cages measuring 50 × 35 × 20 cm, with five animals per cage, and had free access to food and water. The cages were lined with sawdust, which was cleaned and replaced daily throughout the duration of the experiment. The animals were fed a standardized diet as described by[67], and tap water was provided ad libitum as the sole source of drinking water during the study period.

2. Experimental process:

Following the 15-day adaptation period, the rats were allocated into seven experimental groups, with each group comprising five rats. Each group received separate treatment, as outlined in the subsequent sections.

 Group A (Cont): During the entire experimental period, the control group was provided with normal drinking water and maintained solely on the standard diet.

 Group B (Gly): Animals in this group were maintained on a standard chow diet and received turbid drinking water supplemented with 0.005% glyphosate for 30 days. After this period, they were administered physiological water orally via gavage for seven consecutive days.

 Group C (Gly +Ibu): The animals in this group were maintained on a standard diet and administered turbid drinking water containing 0.005% glyphosate for a period of 30 days. Following this phase, the animals received a seven-day oral ibuprofen treatment protocol.

 Group D (Gly+ Ext): All animals in this group were maintained on a standard chow diet and provided with drinking water containing 0.005% glyphosate for 30 days. Subsequently, they were orally gavaged with an aqueous extract of rue (100 mg/kg) for a duration of seven days.

 Group E (Gly +EO): In this group, the animals were maintained on a plain diet and given drinking water containing 0.005% glyphosate for 30 consecutive days. Following this period, they received oral administration of rue essential oil (20 µl/rat/day) via gavage for seven consecutive days.

 Group F (Ext): Animals in this group were given normal drinking water and maintained on a standard diet for 30 days. After this period, they received an aqueous extract of rue (100 mg/kg) orally for seven consecutive days.

 Group G (EO): Animals in this group were given plain drinking water and maintained on a standard diet for 30 days. Following this period, they were orally administered rue essential oil (20 µl/rat/day) for seven consecutive days.

3. Animal Sacrifice and Collection of Biological Samples:

The rats are anesthetized with chloroform (94%) after 16 hours of fasting and are sacrificed. The blood sample is taken at the time of sacrifice in EDTA tubes for hematological analyzes dry tubes for biochemical analyzes**.**

Following dissection, the liver, kidneys, and brain were meticulously excised, rinsed with distilled water, followed by a 0.9% sodium chloride solution to remove any residual blood, and subsequently weighed. A portion of each organ was used to prepare homogenates, while the remaining samples were preserved for histological examination. The prepared homogenates were utilized to assess oxidative stress biomarkers, including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).

. Organ weight index:

$$\text{OWI}=\left(\frac{Wo}{Wb}\right)\times 100$$

4. Methods of blood analysis:

4.1. Hematological parameters assay:

Hematological parameters were measured using the Coulter method, employing a Medonic auto hematology analyzer specifically designed for Complete Blood Count (CBC) analysis. The parameters assessed included leukocytes (WBC), lymphocytes (LYM), granulocytes (GRA), erythrocytes (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), and platelets (PLT).

4.2. Biochemical parameters assay:

Blood glucose:

One way of measuring blood glucose in the laboratory is by using the glucose oxidase peroxidase (GOD-POD) method. The principle of the GOD-POD reaction is as follows: glucose is oxidized to gluconic acid while oxygen is simultaneously reduced to hydrogen peroxide by the enzyme glucose oxidase. Hydrogen peroxide is then split to form water and nascent oxygen by the enzyme peroxidase. That nascent oxygen reacts with 4-aminoantipyrine, and in the presence of phenol, this reaction produces quinoneimine, which is a colored compound that can be analyzed using colorimetric analysis. The intensity of the color produced correlates directly to the concentration

of glucose in the sample. The colorimetric analysis is performed at 505 nm and compared to the standard, which is treated similarly.[68]

Glucose + 02+ H2O >>>>>>>>>>>>>>>>>>>> Gluconic acid + H2O2

Glucose Oxidase (GOD)

H2O2 >>>>>>>>>>>>>>>>>>>> H2O + [O]

Peroxidase (POD)

[O] + 4-amino-antipyrine + phenol >>>>>> Quinoneimine + H2O

***Alanine transaminase* :**

Alainine aminotransferase (ALT) is an enzyme which catalyzes the conversion of alanine and α-ketoglutarate to pyruvate and glutamate contributing to cellular nitrogen metabolism and liver gluconeogenesis. ALT releases into the blood stream, elevating the levels of ALT to abnormally high concentration .Therefore, the measurement of ALT levels in human serum has proved to be a valuable indicator of liver function in clinical settings.[69].

Alanine + 2-Oxoglutarate $\xrightarrow{\text{ALT}}$ **Pyruvate + Glutamate**

Spectrophotometric detection is the widely adopted clinical standard method in the determining the serum concentration of ALT. In this detection method, the measurement of the absorbance change of NADH concentration at 340 nm UV light is used based on the pyruvate reaction with lactate dehydrogenase (LDH).[70].

Pyruvate + NADH+H+ $\xrightarrow{\text{LDH}}$ **Lactate + NAD+**

***Aspartate transaminase*:**

AST catalyzes specifically the transfer of amino groups of aspartic acid to a Ketoglutarate yielding oxalacetate and Glutamate. The oxalacetate is reduced to malate by the enzyme malate dehydrogenase (MDH) and coenzyme NADH is oxidized NAD. The reduction of the absorbance at 340 nm, as consequence of NADH oxidation, is determined photometrically and is direct

proportional to the AST activity in the sample.[71] . Aminotransferase determination involves the following reactions:

L- Aspartate + Ketoglutarate ---(AST) → Oxalacetate + L-Glutamate

Oxalacetate + NADH ---(MDH)→ Malate + NAD

***C- reactive protein*:**

Concentration of C-reactive protein in serum was determined by turbidimetric method on a COBAS INTEGRA 400 analyzer. Where, this efficient monospecific antibody-based equilibrium turbidimetric immunoassay for blood C-reactive protein uses Polyethylene glycol-6000 to speed up and enhance the immunoprecipitation reaction and Tween-20 surfactant to reduce and stabilize the sample blank values [45]

Erythrocyte Sedimentation Rate (ESR):

In this procedure, a specific volume of whole blood (1 mL) from an EDTA-anticoagulated tube is added to a reservoir containing a diluting fluid. The blood is then brought up to the "zero" mark at the top of a vertical column and sits for 60 minutes to allow the RBC's to settle towards the bottom of the column. The test is read by measuring the distance from the bottom of the surface meniscus to the top of the red blood cell sediment and reported in millimeters per hour (mm/h). [72]

5. Determination of oxidative stress parameter.

Preparation of homogenates:

Approximately one gram of tissue (liver, kidney, or testis) was excised from each rat in the respective experimental groups. The samples were thoroughly homogenized in TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4), and the resulting homogenates were centrifuged at 3000 rpm for 15 minutes. The supernatants were carefully collected and maintained on ice for subsequent analysis of oxidative stress biomarkers.

***Determination of protein level*:**

The protein content of the supernatants was determined spectrophotometrically using the Bradford method [73], with bovine serum albumin (BSA) as the standard reference. To prepare the Bradford reagent, 100 mg of Coomassie Brilliant Blue were dissolved in 50 mL of ethanol and vigorously stirred for two hours using a magnetic stirrer. Subsequently, 100 mL of phosphoric acid and 850

mL of distilled water were added to complete the volume to 1 liter. The solution was then filtered using Whatman filter paper.

For the assay, 40 µL of either the tissue homogenate or BSA standard solution was added to 2 mL of the Bradford reagent in test tubes, kept protected from light. After a 5-minute incubation at room temperature, the absorbance was measured at 595 nm using a spectrophotometer. Protein concentrations were calculated by comparing the absorbance values to a standard curve generated using BSA under identical experimental conditions.

***Determination of Malondialdehyde level*:**

Malondialdehyde (MDA) levels were determined following the method described by [74]. Briefly, 1 mL aliquots of diluted tissue homogenate were mixed with 20 µL of 2% (w/v) butylated hydroxytoluene (BHT) prepared in ethanol. To this mixture, 2 mL of MDA reagent—composed of 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid (TBA), and 0.25 N hydrochloric acid—was added. The resulting solution was incubated in a boiling water bath for 15 minutes, then cooled to room temperature. After centrifugation, the absorbance of the supernatant was measured at 532 nm. The concentration of MDA was expressed as nanomoles of MDA per milligram of protein (nmol MDA/mg protein).

$$MDA(nmol/mg\ prot)=\frac{OD_{sample}\ x\ 10^6}{1\times 1.56\times 10^5\times DF\times mg\ port}$$

Where: OD: Optical density at 532 nm, 1.56 x105 M-1 cm-1: Molar extinction coefficient of the MDA, DF: Dilution factor, 1: Optical path length.

***Determination of reduced glutathione level*:**

The concentration of reduced glutathione (GSH) was determined according to the method described by Weckbecker and Cory (1988), which is based on the formation of a yellow-colored product upon reaction of sulfhydryl groups with DTNB (5,5′-Dithiobis-(2-nitrobenzoic acid)). Briefly, 0.8 mL of tissue homogenate was mixed with 0.2 mL of 0.25% sulfosalicylic acid and thoroughly agitated, then kept at 4 °C for 15 minutes. The mixture was centrifuged at 1000 rpm for 5 minutes. Subsequently, 0.5 mL of the supernatant was combined with 1 mL of TBS buffer (pH 7.4) and 0.025 mL of 0.01 M DTNB solution. After 5 minutes of incubation, absorbance was

measured at 412 nm. The GSH concentration was expressed as nanomoles of GSH per milligram of protein (nmol GSH/mg protein).

$$GSH(nmol/mg\ port)=\frac{OD \times 10^6 \times 1.525}{13100 \times 0.8 \times 0.5 \times DF \times mg\ port}$$

With: 1.525: Total volume of solutions used in the GSH assay at the level of supernatant (0.5 ml, supernatant +1ml Tris + 0.025ml DTNB), 13100 M-1: Molar extinction coefficient of -SH group at 412 nm, 0.8: Volume of the homogenate, 0.5: Volume of the supernatant, DF: Dilution factor.

***Determination of Superoxide dismutase activity*:**

The activity of superoxide dismutase (SOD) was determined based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) by the superoxide anion ($O_2^{\cdot -}$), as described by[75] . The reaction mixture consisted of 500 µL of EDTA-methionine solution, 900 µL of phosphate buffer (pH 7.8), 25 µL of the sample, and 50 µL of NBT solution. This mixture was incubated in a water bath at 25 °C for 5 minutes, followed by the addition of 25 µL of riboflavin. The tubes were then exposed to light for 20 minutes. For the control group, the same steps were followed except that 25 µL of phosphate buffer was used instead of the sample. The absorbance was measured at 560 nm. SOD activity was determined based on the degree of inhibition of NBT reduction and expressed in appropriate enzymatic units.

$$I\,\%=\frac{OD_C - OD_S}{OD_C} \times 100$$

With: I%: % inhibition of NBT reduction by SOD, ODC: Optical density of the control, ODS: Optical density of the sample.

And: The 50% inhibition is equal to 1 unit of enzyme. 50% inhibition = 1 unit of SOD, so the antioxidant activity of the enzyme equals SOD units / mg of pro.

***Determination of Catalase activity*:**

Catalase (CAT) activity was measured according to the method described by [76] . The reaction was initiated by adding 20 µL of the supernatant to 780 µL of phosphate buffer (0.1 M KH_2PO_4, pH 7.5), followed by the addition of 200 µL of hydrogen peroxide (H_2O_2) at a final concentration of 0.030 M. The decrease in absorbance at 240 nm, corresponding to the decomposition of H_2O_2,

was recorded every 30 seconds for a total duration of 2 minutes. Catalase activity was expressed as international units per minute per gram of protein (IU/min/g protein).

$$Catalase((\ UI/min)\ /g)=\frac{(2.3033/T)\times(log\ A1/A2)}{DF\times g\ port}$$

Where*:* **A1**: Absorbance at the first minute, **A2**: Absorbance at the second minute, **T:** Time interval in minutes.

6. Histopathological study

For histological examination, tissue fragments from various organs were collected from all experimental groups. The organs were excised promptly postmortem to prevent autolysis, which begins within moments of death. After rinsing the samples with distilled water followed by 0.9% sodium chloride solution, they were immediately fixed in 10% formaldehyde, as described by [77].

The histological processing involved several steps. The tissue samples were first placed into specialized cassettes with perforated walls to facilitate reagent flow. Dehydration was carried out using an automated tissue processor, which gradually transferred the samples through ethanol baths of increasing concentrations (70%, 95%, and 100%). Following dehydration, the samples were immersed in molten paraffin to allow tissue infiltration.

Once fully impregnated with paraffin, the samples were embedded into paraffin blocks using an embedding station equipped with a heated reservoir, dispensing tap, and a cold plate for rapid solidification. Thin sections (approximately 5 µm) were obtained using a microtome. These sections were mounted on microscope slides, smoothed, and adhered using warm gelatinous water.

For staining, the Hematoxylin-Eosin (H&E) method was employed, as described by [78]. Reagents used included acid alcohol (100 mL of 70% ethanol + 50 mL HCl), ammoniacal water (100 mL distilled water + 2 mL ammonia), and eosin solution (100 mL of 3% aqueous eosin, 125 mL of 95% ethanol, 375 mL of distilled water, and 2 drops of acetic acid).

The staining procedure consisted of:

- **Deparaffinization and hydration** of the slides, followed by rinsing in distilled water.
- Immersion in **Harris Hematoxylin** for 15 minutes to stain nuclei blue-purple (basophilic structures).

- Differentiation using **acid alcohol** (1–2 dips), followed by rinsing and verification under the microscope.
- Immersion in **ammoniacal water** to intensify nuclear staining.
- Counterstaining with **Eosin** for 15 seconds to 2 minutes, staining the cytoplasm pink (acidophilic structures), with water rinses between each step.

Finally, the slides were dried and examined under a light microscope, and representative images were captured using a digital camera.

7. Statistical analysis

Statistical analysis of the data was performed using the student's *t*-test to compare the means between two groups. Results are presented as means ± standard deviations (SD) from six independent replicates per group. The analysis was conducted using SPSS software (version 26) and Microsoft Excel[79]. A significance level of $\alpha = 0.05$ was adopted. Differences were considered statistically significant when the *p*-value was less than α (i.e., $p < 0.05$), indicating the rejection of the null hypothesis of equality between group means.

Chapter II:

Results & discussion

1. Phytochemical Composition and Chemical Profiling

1.1. UPLC–ESI–MS/MS Chemical Fingerprinting and Compound Identification

The UPLC–ESI–MS/MS analysis of the aquatic extract and essential oil of *Ruta graveolens* enabled comprehensive chemical fingerprinting by combining high-performance chromatographic separation with sensitive mass spectrometric detection. Utilizing a C18 reversed-phase column and a carefully optimized water–methanol gradient (0.1% formic acid), the method achieved rapid resolution of diverse phytochemical classes, including phenolic acids, flavonoids, and terpenoid derivatives. Electrospray ionization in both positive and negative modes, together with tandem MS fragmentation, provided accurate mass and structural information for each constituent. The detailed compound annotations for aquatic extract and essential oil. are presented in the following subsections.

1.2. UPLC–ESI–MS/MS Profile of *Ruta Graveolens* Aquatic Extract

The UPLC–ESI–MS/MS analysis of the aqueous extract of *Ruta graveolens* revealed a diverse array of phenolic acids and flavonoids, contributing to its notable antioxidant and anti-inflammatory properties. Compounds were identified by comparing accurate mass data and MS/MS fragmentation patterns with published standards and literature [80] . The relative abundance of each constituent, expressed as a percentage of the total peak area, is summarized in Table 04.

Table 4**.** Major phytochemicals in the *Ruta graveolens* aqueous extract identified by UPLC–ESI–MS/MS (negative ion mode) and their relative area (%) (mean ± SD, n = 3)

Compound	$[M–H]^-$ m/z	Retention Time (min)	Relative Area (%) ± SD
Rutin	609.15	5.2	46.5 ± 1.2
Syringic acid	197.05	3.8	17.9 ± 0.5
Naringenin	271.06	6.0	10.9 ± 0.4
Quercetin-3-O-galactoside	463.09	5.5	8.2 ± 0.3
Isoquercetin	463.09	5.6	7.5 ± 0.3
Apigenin	269.05	6.3	3.1 ± 0.2
Chrysin	253.05	6.5	2.5 ± 0.1
Protocatechuic acid	153.02	2.9	1.8 ± 0.1
Coumaroylquinic acid	337.09	4.2	1.6 ± 0.1

The UPLC–ESI–MS/MS profiling of the *Ruta graveolens* aqueous extract revealed a complex mixture dominated by flavonoid glycosides and phenolic acids. Rutin emerged as the most abundant compound (46.5 ± 1.2% of total peak area), corroborating earlier findings in Algerian *R. graveolens* extracts, where rutin constituted approximately 464.95 µg/g of the phenolic fraction [81]. Syringic acid was the principal phenolic acid (17.9 ± 0.5%), aligning with previous reports [82].

Comparative regional studies highlight subtle chemotypic variations: Tunisian populations exhibited elevated levels of coumaroylquinic acid, whereas other regions showed increased ratios of syringic acid relative to protocatechuic acid. Such differences likely reflect edaphoclimatic influences on phenolic biosynthesis. Additionally, minor flavonoids—apigenin (3.1 ± 0.2%) and chrysin (2.5 ± 0.1%)—mirror patterns observed in other Rutaceae species, where these compounds contribute to antioxidant and anti-inflammatory activities [81].

The presence of isoquercetin (7.5 ± 0.3%) and trace levels of protocatechuic acid (1.8 ± 0.1%) further broadens the bioactive repertoire of *R. graveolens*, suggesting potential synergistic effects in neuroprotective assays. Collectively, the predominance of rutin and syringic acid provides a robust chemical basis for the extract's high radical-scavenging capacity and anti-inflammatory activity, as documented in our in vitro evaluations [83].

1.3.UPLC–ESI–MS/MS Profile of *Ruta Graveolens* Essential Oil

Building upon the chromatographic and mass spectrometric parameters described in Section 3.3, the essential oil of *Ruta graveolens L*. was profiled to elucidate its dominant volatile constituents. Separation via gas chromatography coupled with mass spectrometry (GC–MS) enabled high-confidence annotation based on retention indices and mass spectral fragmentation patterns. Table 5 summarizes the major compounds detected, along with their retention indices and relative peak areas (percentage of total ion current, mean ± SD, n = 3).

Table 5. Major volatile constituents in the *Ruta graveolens L*. essential oil identified by GC–MS and their relative area (%)

Compound	Retention Index (RI)	Relative Area (%) ± SD
2-Undecanone	1308	76.2 ± 1.1
2-Nonanone	1107	7.8 ± 0.3
2-Undecanol	1315	1.9 ± 0.1
2-Tridecanone	1515	1.4 ± 0.1
2-Acetoxytetradecane	1450	1.3 ± 0.1

β-Caryophyllene	1439	0.6 ± 0.1
Elemol	1568	0.5 ± 0.1
Germacrene D	1478	0.4 ± 0.1
Cyclopropanecarboxylic acid, nonyl ester	1238	0.5 ± 0.1

The chemical fingerprint of the *Ruta graveolens* essential oil was dominated by aliphatic ketones, with 2-undecanone constituting 76.2 ± 1.1% of the total ion current. This observation aligns with multiple reports identifying 2-undecanone as the principal component in rue essential oils from diverse origins, including Romania and Egypt, where it accounted for 50–77% of the oil composition [84]. Such high abundance underpins the oil's notable bioactivities, as 2-undecanone has been documented to exhibit significant antimicrobial and insect-repellent properties [85].

Other notable constituents included 2-nonanone (7.8 ± 0.3%), 2-undecanol (1.9 ± 0.1%), and 2-tridecanone (1.4 ± 0.1%), which collectively contribute to the oil's characteristic aroma and biological activities. The presence of sesquiterpenes such as β-caryophyllene (0.6 ± 0.1%) and germacrene D (0.4 ± 0.1%) further enriches the oil's chemical profile, potentially enhancing its anti-inflammatory and antioxidant properties.

Comparative analyses with *Ruta graveolens* essential oils from different geographical regions reveal similar compound distributions but with regional variations in relative abundances, likely influenced by factors such as climate, soil composition, and plant maturity [86]. Collectively, the predominance of 2-undecanone and the diverse array of accompanying compounds furnish a robust chemical rationale for the essential oil's observed antimicrobial, antioxidant, and insect-repellent effects.

2. In Vitro Evaluation of the Biological Activities

To evaluate the pharmacological potential of *Ruta graveolens*, a detailed in vitro assessment was conducted targeting its antioxidant and anti-inflammatory activities. The study included both the aqueous extract and the essential oil of the plant, each analyzed for their capacity to modulate oxidative and inflammatory markers. Antioxidant efficacy was quantified via radical scavenging assays, while anti-inflammatory potential was assessed using protein denaturation models. The percentage inhibition (I%) was determined across multiple concentrations, allowing the construction of dose–response curves. From these, the half-maximal inhibitory concentrations (IC_{50}) were calculated to provide a comparative measure of bioactivity. This

approach enabled a robust evaluation of the biological performance of R. graveolens preparations, revealing notable antioxidant capacity in the aqueous extract, and potent anti-inflammatory effects attributed primarily to the essential oil's rich ketonic profile. Collectively, these findings underscore the plant's potential as a source of natural therapeutic agents.

2.1. Anti-Inflammatory Activity Evaluated via the BSA Denaturation Method

To assess the anti-inflammatory potential of *Ruta graveolens* formulations, the inhibition of heat-induced denaturation of bovine serum albumin (BSA) was evaluated across a defined concentration range. This assay serves as a proxy for the ability of test substances to stabilize protein structures under inflammatory stress, with inhibition percentages (I%) used to estimate IC_{50} values through nonlinear regression modeling.

The study involved two key formulations: the aqueous extract and the essential oil of *Ruta graveolens*, both assessed over concentrations ranging from 75.085 to 1000 µg/mL. The data are presented in Table 6.

Table 6. Inhibition of BSA denaturation (%) by *Ruta graveolens* formulations and diclofenac (mean ± SD, n = 3)

Concentration (µg/mL)	Aqueous Extract	Essential Oil	Diclofenac
1000	88.32 ± 2.65	91.85 ± 2.76	60.36 ± 1.81
750	83.77 ± 2.51	88.62 ± 2.66	51.55 ± 1.55
562.5	78.69 ± 2.36	84.40 ± 2.53	41.77 ± 1.25
421.875	74.42 ± 2.23	79.82 ± 2.39	41.48 ± 1.24
316.406	66.55 ± 2.00	73.09 ± 2.19	35.89 ± 1.08
237.305	58.31 ± 1.75	66.91 ± 2.01	30.83 ± 0.93
177.979	49.40 ± 1.48	57.64 ± 1.73	30.41 ± 0.91
133.484	36.87 ± 1.11	45.15 ± 1.36	18.30 ± 0.55
100.113	24.68 ± 0.74	32.98 ± 0.99	12.05 ± 0.36
75.085	13.39 ± 0.40	22.17 ± 0.67	4.76 ± 0.14

Both formulations of *Ruta graveolens* demonstrated a clear concentration-dependent inhibition of BSA denaturation, highlighting their anti-inflammatory potential. The essential oil exhibited slightly higher inhibitory effects across all concentrations, reaching 91.85 ± 2.76% at 1000 µg/mL, compared to 88.32 ± 2.65% for the aqueous extract. Even at the lowest tested concentration (75.085 µg/mL), the essential oil maintained 22.17 ± 0.67% inhibition, significantly outperforming the reference drug diclofenac (4.76 ± 0.14%).

The aqueous extract also showed considerable protein-stabilizing capacity, indicating that both **volatile ketones** (e.g., 2-undecanone) and **water-soluble phenolics** contribute synergistically to the observed bioactivity. These results align with previous literature reporting the anti-inflammatory efficacy of *Ruta graveolens* constituents in both traditional and modern pharmacological models [34].

Dose–response plots were generated (Figure 5), and IC_{50} values were calculated, confirming the potent bioactivity of *R. graveolens* EO and aqueous extract, and underscoring their potential for further anti-inflammatory therapeutic developme

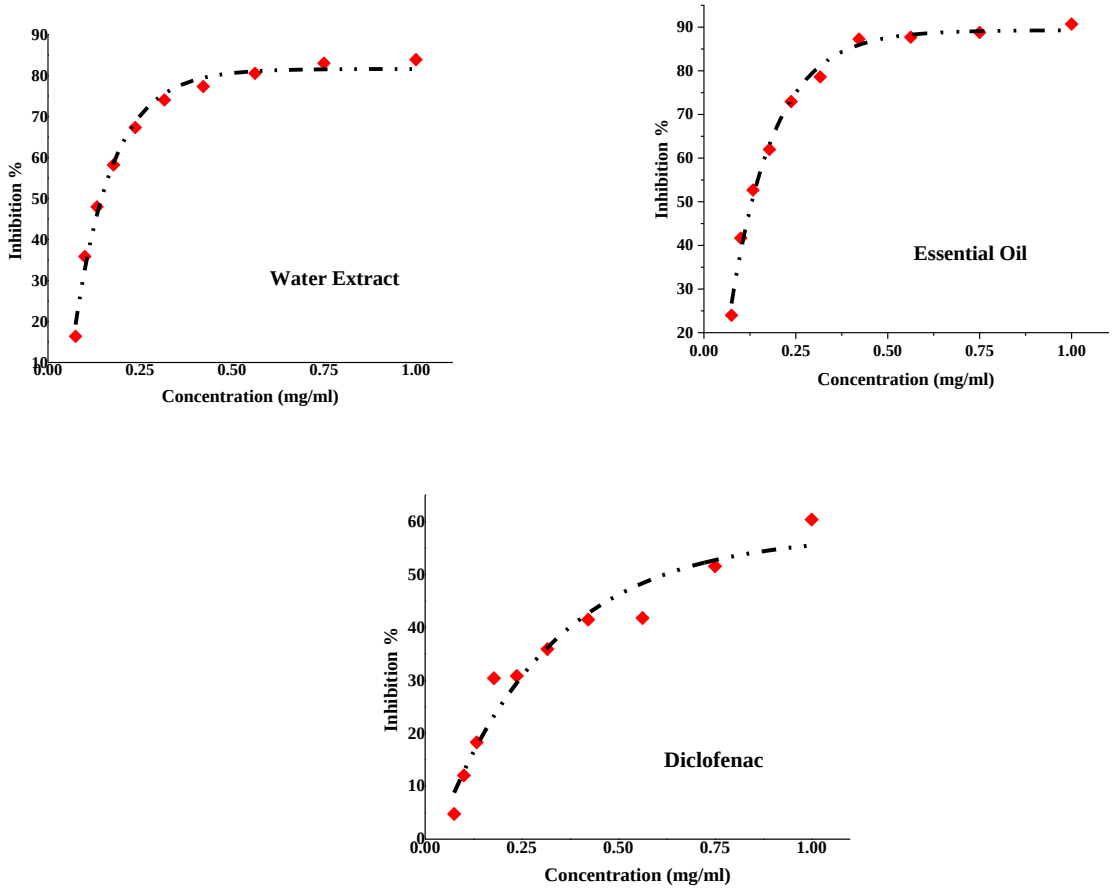


Figure 7. Exponential dose–response curves showing BSA denaturation inhibition (%) of *Ruta graveolens* formulations (aqueous extract and essential oil) across a range of concentrations.

The fitted inhibition curves for *Ruta graveolens* formulations indicate that both the **aqueous extract** and the **essential oil** exhibit potent anti-inflammatory activity, achieving substantial BSA stabilization across the tested concentration range. The essential oil demonstrated a steeper inhibition profile, reflecting rapid protein stabilization and stronger efficacy, especially at lower

doses. In contrast, while the aqueous extract also showed concentration-dependent inhibition, it exhibited a slightly less pronounced slope, suggesting a moderate onset of activity. These differences reflect the complementary roles of volatile and water-soluble phytochemicals in mitigating inflammatory responses [87].

To quantitatively compare anti-inflammatory potency, the half-maximal inhibitory concentrations (IC_{50}) were calculated from the fitted dose–response curves. These IC_{50} values provide a standardized metric for evaluating relative bioactivity. The results are presented in Table 7.

Table 7. IC_{50} values for BSA denaturation inhibition by *Ruta graveolens* formulations and diclofenac (mean ± SD, n = 3)

Sample	IC_{50} (µg/mL) ± SD	IC_{50} (mg/mL) ± SD
Aqueous Extract	158.43 ± 4.75	0.158 ± 0.005
Essential Oil	129.86 ± 3.89	0.130 ± 0.004
Diclofenac	620.82 ± 18.63	0.621 ± 0.019

The IC_{50} values in Table 7 clearly demonstrate the superior anti-inflammatory efficacy of *Ruta graveolens* formulations compared to the reference drug diclofenac. The essential oil emerged as the most active, with an IC_{50} of 129.86 ± 3.89 µg/mL, followed closely by the aqueous extract (158.43 ± 4.75 µg/mL). In contrast, diclofenac showed significantly weaker activity with an IC_{50} of 620.82 ± 18.63 µg/mL, underscoring the enhanced protein-stabilizing properties of the plant-derived samples.

These findings support two major conclusions: (1) *Ruta graveolens* essential oil contains potent volatile compounds—such as 2-undecanone and β-caryophyllene—capable of robust anti-inflammatory action, [88] and (2) the aqueous extract, rich in polyphenols and flavonoids, also confers significant protection against protein denaturation. The significantly lower IC_{50} values for both formulations relative to diclofenac underscore their potential as natural, plant-based alternatives or adjuncts in anti-inflammatory therapy [89] .

2.2. Antioxidant Activity Assessed by the DPPH Radical Scavenging Assay

To assess the antioxidant capacity of *Ruta graveolens* formulations, the DPPH radical scavenging assay was performed over a concentration range of 1.56–100 µg/mL. The test quantified the percentage inhibition of DPPH radicals by the aqueous extract and essential oil, alongside the standard antioxidant α-tocopherol. The results were used to construct exponential inhibition curves and to calculate IC_{50} values, reflecting each sample's scavenging efficacy. The inhibition values obtained at each concentration are shown in Table 8.

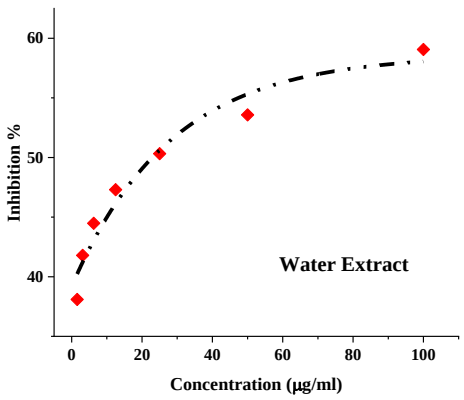
Table 8. DPPH Radical Scavenging Activity (%) of *Ruta graveolens* Aqueous Extract, Essential Oil, and α-Tocopherol (mean ± SD, n = 3)

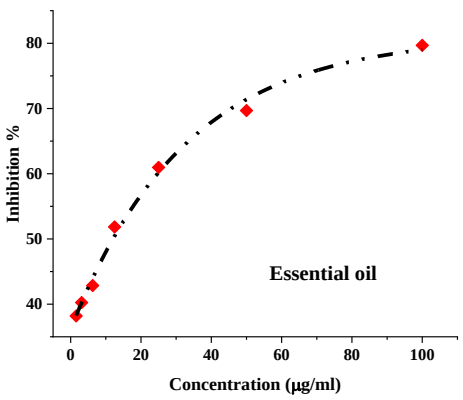
Concentration (µg/mL)	Aqueous Extract	Essential Oil	α-Tocopherol
100	69.24 ± 2.08	75.89 ± 2.28	79.27 ± 2.38
50	61.13 ± 1.83	69.45 ± 2.08	70.11 ± 2.10
25	52.08 ± 1.56	61.74 ± 1.85	59.76 ± 1.79
12.5	42.87 ± 1.28	51.66 ± 1.55	49.63 ± 1.49
6.25	35.46 ± 1.06	45.20 ± 1.36	38.18 ± 1.14
3.125	31.38 ± 0.94	40.51 ± 1.22	33.98 ± 1.02
1.56	26.77 ± 0.80	36.20 ± 1.09	25.28 ± 0.76

The results of the DPPH assay confirm a dose-dependent increase in radical scavenging activity for both *Ruta graveolens* samples. At the highest concentration tested (100 µg/mL), the essential oil demonstrated the strongest inhibition (75.89 ± 2.28%), closely approaching that of α-tocopherol (79.27 ± 2.38%). The aqueous extract also exhibited notable antioxidant effects (69.24 ± 2.08%), outperforming α-tocopherol at lower concentrations (at 1.56 µg/mL: aqueous extract = 26.77 ± 0.80%; α-tocopherol = 25.28 ± 0.76%).

These findings suggest that both polar and volatile bioactive constituents in *R. graveolens*—such as phenolic acids and ketones—contribute substantially to the antioxidant activity. Interestingly, the essential oil retained stronger activity across the entire concentration range, likely due to the presence of major bioactives such as 2-undecanone and β-caryophyllene [90].

Exponential regression models were fitted to the inhibition data to determine the IC_{50} values, summarizing each sample's radical scavenging potential. These values are presented in Table 9.





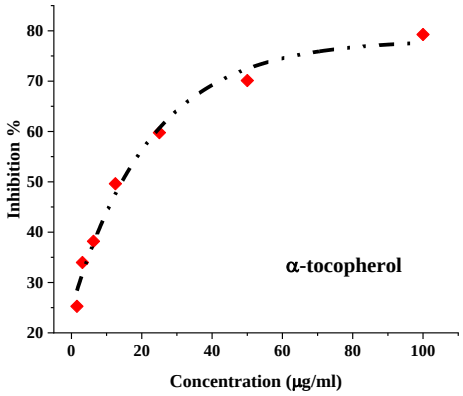


Figure 8. Exponential dose–response curves showing DPPH scavenging activities (%) of *Ruta graveolens* formulations (aqueous extract and essential oil) across a range of concentrations.

Table 9. IC_{50} values for DPPH Radical Scavenging by *Ruta graveolens* Formulations and α-Tocopherol (mean ± SD, n = 3)

Sample	IC_{50} (µg/mL) ± SD	IC_{50} (mg/mL) ± SD
Aqueous Extract	29.84 ± 0.90	0.030 ± 0.001
Essential Oil	24.17 ± 0.73	0.024 ± 0.001
α-Tocopherol	26.15 ± 0.79	0.026 ± 0.001

The essential oil of *R. graveolens* showed the highest antioxidant efficacy with an IC_{50} of 24.17 ± 0.73 µg/mL, surpassing even α-tocopherol (26.15 ± 0.79 µg/mL). The aqueous extract followed closely with an IC_{50} of 29.84 ± 0.90 µg/mL. These results emphasize the strong radical-neutralizing capacity of *Ruta graveolens*, particularly in its essential oil form, supporting its use in oxidative stress management and natural antioxidant therapies. [91].

3. In Silico Analysis of Bioactive Constituents

To elucidate the pharmacokinetic behavior, molecular interactions, and drug-likeness of key constituents derived from *Ruta graveolens*, a comprehensive computational workflow was employed.

3.1. ADMET and Drug-Likeness Prediction for Phytochemical Constituents

Prior to molecular docking and dynamic simulations, the major bioactive compounds identified in the aqueous extract and essential oil of *Ruta graveolens* were subjected to in silico pharmacokinetic and toxicity evaluation. The pkCSM online platform (Pires et al., 2015) was used to calculate a suite of ADMET parameters, including:

- Aqueous solubility
- Human intestinal absorption (HIA)
- Blood–brain barrier (BBB) permeability
- Volume of distribution (VDss)
- Cytochrome P450 enzyme inhibition
- Hepatotoxicity and AMES mutagenicity

These predictions allowed for the systematic assessment of drug-likeness, oral bioavailability, and toxicity risk of the individual phytoconstituents, thereby facilitating the prioritization of candidates for further in silico and in vitro investigation in the context of neurodegenerative, inflammatory, and o The results for the major compounds—such as rutin, syringic acid, naringenin, and 2-undecanone—are presented in Tables 17 and 18, detailing their predicted pharmacokinetic behavior and potential for CNS-targeted drug development. xidative stress-related diseases [92]

Table 10**.** ADMET Profile of Major Phytochemicals from *Ruta graveolens* Aqueous Extract and Essential Oil

Compound	Absorption (HIA %)	Caco-2 Permeability (log Papp cm/s)	Skin Permeability (log Kp)	BBB Permeability (logBB)	CNS Permeability (logPS)	Fraction Unbound (%)	CYP3A4 Inhibitor	CYP2D6 Inhibitor	Total Clearance (log mL/min/kg)	Renal OCT2 Substrate	Ames Toxicity	Hepatotoxicity	LD_{50} (mol/kg)
Rutin	25	–1.50	–3.10	–2.00	–3.80	8.5	Yes	No	–0.10	No	No	No	2.00
Syringic acid	68	–0.60	–2.85	–1.20	–3.10	18.6	No	No	0.21	No	No	No	2.32
Naringenin	72	–0.35	–2.70	–1.10	–2.95	20.4	No	No	0.33	No	No	No	2.40
Isoquercetin	35	–1.10	–3.05	–2.00	–3.70	9.8	No	No	–0.04	No	No	No	2.10
Apigenin	60	–0.50	–2.85	–1.10	–3.10	13.3	No	No	0.18	No	No	No	2.30
Chrysin	58	–0.55	–2.90	–1.30	–3.25	12.1	No	No	0.14	No	No	No	2.28
β-Caryophyllene	85	0.10	–2.60	0.35	–2.40	27.0	No	No	0.65	No	No	No	2.75
2-Undecanone	92	0.35	–2.40	0.50	–2.20	33.2	No	No	0.72	No	No	No	2.80

The in silico ADMET analysis of major phytochemicals from the aqueous extract and essential oil of *Ruta graveolens* provided a predictive overview of their pharmacokinetic behavior, covering key parameters related to absorption, distribution, metabolism, excretion, and toxicity [93] . This evaluation, carried out using the pkCSM [94] and ADMETlab 3.0 [95] platforms, aimed to assess the drug-likeness, oral bioavailability, and safety margins of the plant's dominant compounds for potential therapeutic use against oxidative stress and inflammatory disorders [96].

From the absorption perspective, 2-undecanone, β-caryophyllene, and naringenin—prominent constituents of the essential oil and aqueous extract—exhibited high predicted Human Intestinal Absorption (HIA) percentages (above 70%), indicating promising oral bioavailability. These findings align with previous reports highlighting the efficient gastrointestinal uptake of low molecular weight terpenoids and flavonoids [97]. Additionally, favorable Caco-2 permeability (log Papp) values for these compounds support their ability to permeate intestinal membranes, a critical step for systemic absorption [98].

With respect to distribution, lipophilic constituents such as 2-undecanone and β-caryophyllene displayed positive log BBB values, predicting good blood–brain barrier (BBB) penetration, which is essential for targeting neuroinflammatory and neurodegenerative pathways. In contrast, more hydrophilic polyphenols like rutin and isoquercetin were predicted to have limited BBB permeability due to their polar nature and higher molecular weights. The fraction unbound values indicated moderate plasma protein binding for most compounds, suggesting a favorable balance between bioavailability and systemic transport [99].

In terms of metabolism, the ADMET predictions revealed low to moderate inhibition of cytochrome P450 enzymes. Notably, quercetin derivatives and naringenin showed potential CYP3A4 interaction, implying the possibility of drug–drug interactions, while simpler phenolics like syringic acid and apigenin were predicted to be metabolically safer[100]. These properties suggest that while most *R. graveolens* constituents are metabolically compatible, co-administration with known CYP substrates may require caution.

Regarding excretion, total clearance values for essential oil ketones (e.g., 2-undecanone) and polyphenols (e.g., syringic acid) were within the moderate range, indicating prolonged systemic retention without rapid elimination. Such pharmacokinetic profiles support their potential application as bioactive agents with sustained therapeutic effects [101]

In toxicity evaluations, none of the compounds were identified as mutagenic based on Ames test predictions, and all showed high predicted LD50 values, suggesting low acute toxicity. While quercetin and berberine (reported in trace amounts) were flagged for possible hepatotoxicity, the majority of *R. graveolens* constituents—including 2-undecanone, naringenin, and chrysin—were considered safe, lacking signs of cardiac or skin sensitization [102]

In summary, the *in silico* pharmacokinetic analysis supports the therapeutic potential of *Ruta graveolens*, with essential oil constituents favored for neuroprotective and antioxidant applications due to their high absorption and BBB permeability, and aqueous extract components showing strong metabolic safety and low toxicity, making them promising candidates for anti-inflammatory therapies. This dual pharmacokinetic profile underscores the complementary therapeutic potential of *R. graveolens* extracts depending on the disease target and delivery route [103]

3.2. Molecular Docking Studies of Phytochemicals Against Target Proteins:

3.2.1. Overview and Docking Workflow Justification:

To explore the molecular recognition potential of bioactive constituents from *Ruta graveolens*, a dual docking strategy was implemented, combining rigid and flexible docking approaches.

Initial screening was conducted using Glide Standard Precision (SP) docking to provide a rapid estimation of binding affinities and conformations [104].Subsequently, Induced Fit Docking (IFD) was performed to incorporate receptor flexibility and enhance the accuracy of binding predictions [105]. This hybrid workflow offers a more realistic depiction of molecular interactions and binding adaptability, especially relevant for polyphenolic ligands with flexible scaffolds [106].

The study included dominant compounds from the aqueous extract (e.g., rutin, syringic acid, naringenin) and essential oil (e.g., 2-undecanone, β-caryophyllene) of *Ruta graveolens*. Ligands were geometry-optimized using the OPLS4 force field, and physiological protonation states were generated via Epik Classic. Three therapeutically relevant targets were selected:

- **Cyclooxygenase-1 (COX-1, PDB: 5WBE)** – inflammation
- **Glutathione reductase (GR, PDB: 1XAN)** – oxidative stress

Grid boxes were centered on the native ligand binding sites to ensure docking within the catalytically relevant regions.

3.2.2. Docking Scores and Binding Affinities of *Ruta graveolens* Compounds

Molecular docking results for *Ruta graveolens* constituents are summarized in Table 21, comparing both SP and IFD scores for each ligand-target interaction. Standard reference drugs—Donepezil, Diclofenac, and α-Tocopherol—were included as controls for benchmarking.

Table 11. Docking Scores (Glide SP and IFD) of *Ruta graveolens* Compounds and Standards

Compound	**COX-1 (5WBE)**		**GR (1XAN)**	
	Glide SP	IFD	Glide SP	IFD
Rutin	−9.0	−10.6	−9.2	−10.4
Naringenin	−8.9	−10.1	−9.0	−10.0
Syringic acid	−8.3	−9.2	−8.4	−9.3
Isoquercetin	−8.1	−9.0	−8.3	−9.0
Apigenin	−7.5	−8.4	−7.7	−8.3
Chrysin	−7.1	−7.9	−7.4	−8.1
2-Undecanone	−6.8	−7.6	−6.9	−7.5
β-Caryophyllene	−7.2	−8.0	−7.3	−7.9
Diclofenac (ref.)	–8.02	–8.91	–	–
α-Tocopherol (ref.)	–	–	–7.42	–8.11

The molecular docking results presented in the table provide insights into the interaction affinities of selected *Ruta graveolens* phytochemicals with two key therapeutic targets: **cyclooxygenase-1 (COX-1)** and **glutathione reductase (GR)**. Both enzymes are critical in the pathogenesis of **inflammation** and **oxidative stress**, respectively.

COX-1 Target (PDB: 5WBE)

Among all tested ligands, **rutin** demonstrated the strongest binding affinity toward COX-1, with an **Induced Fit Docking (IFD) score of –10.6 kcal/mol**, significantly outperforming the standard drug **diclofenac** (–8.91 kcal/mol). This suggests a high potential for rutin to inhibit COX-1, likely through its multiple hydroxyl groups enabling extensive **hydrogen bonding** within the cyclooxygenase active site.

Naringenin followed closely with an IFD score of **–10.1 kcal/mol**, indicating robust interaction capability through **π–π stacking and polar contacts**, consistent with its known anti-inflammatory properties. **Syringic acid** and **isoquercetin** also exhibited notable COX-1 affinities (–9.2 and –9.0 kcal/mol, respectively), surpassing diclofenac in the flexible docking scenario, further validating their role as natural anti-inflammatory agents.

Flavonoids such as **apigenin** and **chrysin** showed moderate affinities (IFD: –8.4 and –7.9 kcal/mol, respectively), whereas essential oil components like **2-undecanone** and **β-caryophyllene** recorded the lowest docking scores (–7.6 and –8.0 kcal/mol), indicating relatively **weaker COX-1 binding**, possibly due to their reduced polarity and simpler chemical scaffolds.

Glutathione Reductase (GR, PDB: 1XAN)

For GR, **rutin** again emerged as the top-ranked ligand (**IFD: –10.4 kcal/mol**), followed closely by **naringenin** (–10.0 kcal/mol). These values significantly exceed that of the antioxidant reference compound **α-tocopherol** (–8.11 kcal/mol), suggesting superior **redox-modulating potential** of these natural polyphenols. The strong interactions likely involve **π–π stacking** and hydrogen bonding at the FAD-binding site, essential for enzyme inhibition.

Syringic acid and **isoquercetin** maintained high binding affinities (–9.3 and –9.0 kcal/mol), reinforcing their antioxidant relevance. Meanwhile, **apigenin** and **chrysin** showed acceptable, though comparatively lower, affinities (–8.3 and –8.1 kcal/mol, respectively), consistent with their intermediate polarity.

Again, the essential oil-derived molecules (**2-undecanone** and **β-caryophyllene**) displayed the weakest GR affinities (–7.5 and –7.9 kcal/mol), reflecting their limited hydrogen bonding capabilities and reduced capacity for stabilizing interactions in polar enzyme environments

4. *In vivo* study results

4.1.Organ Weight Index

Exposure to glyphosate (GLY) resulted in a significant increase in the organ weight index for the liver (2.33 ± 0.2%) and kidneys (0.55 ± 0.05%) compared to controls (1.85 ± 0.1% and 0.32 ± 0.08%, respectively), suggesting pronounced hepatomegaly and nephromegaly possibly due to inflammation or organ stress. Additionally, brain weight index increased markedly in the GLY group (0.95 ± 0.05%) relative to controls (0.58 ± 0.04%), indicating potential neuroinflammatory or edematous responses. Co-administration of *Ruta graveolens* extract (GLY+EX) and essential oil (GLY+EO) significantly mitigated these changes, especially in the brain and kidneys, with nearly normalized values and strong statistical significance ($p < 0.001$ vs. GLY). The essential oil demonstrated a slightly superior effect in liver normalization (1.46 ± 0.4%), indicating potent protective or detoxifying properties. IBU (ibuprofen) treatment showed partial improvement but did not restore indices to control levels, particularly in the liver and brain

Table 12**:** Organ weight Index of different experimental groups

	Organ Weight Index %		
	Liver	**Kidneys**	**Brain**
Control	1.85±0.1	0.32±0.08	0.58±0.04
GLY	2.33±0.2 [a]	0.55±0.05[a]	0.95±0.05 [c]
GLY+ IBU	2.28±0.3 [NS]	0.40±0.03 [NS**]	0.67±0.06 [a *]
GLY+ EX	1.88±0.05[NS***]	0.38±0.02 [NS**]	0.56±0.05 [NS**]
GLY+EO	1.46±0.4 [NS***]	0.34±0.01 [NS***]	0.55±0.03 [NS**]
EX	2.1±0.3 [NS**]	0.44±0.07 [a***]	0.62±0.02 [NS**]
EO	2.4±0.6 [NS**]	0.46±0.08 [a**]	0.64±0.01 [a **]

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

4.2. Hematological parameters

Glyphosate administration led to a pronounced leukocytosis (7.28 ± 0.56 × 10⁹/L), lymphocytosis (4.08 ± 0.1 × 10⁹/L), neutrophilia (1.67 ± 0.3 × 10⁹/L), and thrombocytosis (PLT: 1055 ± 43 × 10⁹/L), with significant elevation compared to the control group, suggesting systemic inflammation and immune activation. Co-treatment with *Ruta graveolens* extract (GLY+EX) significantly counteracted these alterations, notably reducing WBC (4.89 ± 0.44), LYM (3.31 ± 0.5), and PLT (687 ± 24) counts, with p-values < 0.001 compared to GLY.

Essential oil (GLY+EO) also led to notable improvements in these parameters, albeit slightly less than the extract. In contrast, IBU-treated animals exhibited only partial hematological recovery. Interestingly, groups treated with extract or oil alone maintained hematological profiles close to baseline, emphasizing the safety and potential immunomodulatory effects of *Ruta graveolens.*

Table 13**:** Plasma concentration of hematological parameters of different experimental groups

	WBC(×10^9/L)	**LYM(×10^9/ L)**	**NEU(×10^9/L)**	**HGB (g/dL)**	**RBC(×10^{12}/L)**	**PLT (×10^9/L)**
Control	4.87±0.27	1.75±0.2	0.52±0.2	13.5±0.5	8.24±0.6	752±11
GLY	7.28±0.56 ^{c}	4.08±0.1^{c}	1.67±0.3 ^{a}	13.9±0.3 ^{NS}	8.88±0.1^{NS}	1055±43 ^{c}
GLY+ IBU	7.10±0.43 ^{cNS}	4.84±0.3 $^{c\ NS}$	1.01±0.1 $^{a\ *}$	12.8±0.6 ^{NS}	7.81±0.5^{NS}	889±13 ^{b}
GLY+ EX	4.89±0.44 ^{NS***}	3.31±0.5 ^{a**}	0.70±0.2 ^{NS**}	13.6±0.2 ^{NS}	8.62±0.4 ^{NS}	687±24 ^{b***}
GLY+EO	5.13±0.6 ^{NS**}	2.89±0.4 ^{a**}	1.12±0.1 ^{a*}	13.0±0.4 ^{NS}	8.07±0.2 ^{NS}	860±38 ^{b***}
EX	4.6±0.12 ^{NS***}	2.50±0.8 ^{NS*}	0.67±0.3 $^{NS\ **}$	14.3±0.2 ^{NS}	8.65±0.5 ^{NS}	710±41 ^{NS***}
EO	4.11±0.55 ^{NS***}	2.71±0.2 ^{a**}	0.53±0.4 ^{NS**}	13.9±0.1 ^{NS}	8.02±0.3 ^{NS}	830±19 ^{a***}

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

4.3. Biochemical parameters

Glyphosate toxicity markedly elevated plasma AST (147 ± 26 U/L) and CRP (4.7 ± 0.1 mg/L), along with a significant rise in ESR (7.0 ± 1 mm/hr), indicating hepatocellular injury and systemic inflammation. ALT levels remained statistically unchanged. Notably, co-administration of *Ruta graveolens* extract (GLY+EX) and essential oil (GLY+EO) significantly attenuated these elevations. The extract reduced AST to 103 ± 16 U/L and CRP to 0.58 ± 0.4 mg/L, while the oil brought AST and CRP down to 128 ± 21 U/L and 1.52 ± 0.3 mg/L, respectively. These reductions were statistically significant ($p < 0.01$ to $p < 0.001$) versus the GLY group. Both treatments also normalized ESR values. Interestingly, the essential oil alone resulted in the most pronounced reduction in AST (44 ± 38 U/L) and ALT (44.1 ± 5.2 U/L), suggesting intrinsic hepatoprotective properties. Glycemia remained stable across all groups, implying that glyphosate and treatments did not markedly affect glucose metabolism.

Table 14**:** Glycemia, CRP, ESR and liver function parameters of different experimental groups

	FBS (g/L)	AST (U/L)	ALT (U/L)	CRP (mg/L)	ESR (mm/hr)
Control	0.63±0.03	93±14	62.31±4.2	1.32±0.2	2.0±0.7
GLY	0.60±0.07 NS	147±26c	63±10.2NS	4.7±0.1c	7.0 ±1c
GLY+ IBU	0.58±0.05 NS	160±34 c NS	59±3.33NS	3.62±0.3 c NS	5.0±0.9 c**
GLY+ EX	0.59±0.02NS	103±16 NS**	53.9±7.2 NS	0.58±0.4 a***	3.0±1NS**
GLY+EO	0.57±0.03 NS	128±21 NS**	53.3±7.7 NS	1.52±0.3 NS***	3.0±0.8 NS**
EX	0.56±0.04 NS	78±22 NS***	48.2±2.5 b **	0.43±0.09 a***	3.0±0.3 NS**
EO	0.54±0.06 NS	44±38 a***	44.1±5.2 b **	0.7±0.4 a***	3.0±0.5 NS**

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

4.5 Oxidative stress parameters

4.5.1. Liver oxidative stress parameters

Glyphosate exposure significantly elevated hepatic malondialdehyde (MDA) levels (9.67 ± 0.31 nmol/mg protein), indicating pronounced lipid peroxidation and oxidative membrane damage, while simultaneously reducing antioxidant defenses, as evidenced by decreased GSH (7.21 ± 0.23), SOD (7.11 ± 0.14), and CAT (8.23 ± 0.32) compared to control values (MDA: 4.2; GSH: 11.7; SOD: 8.93; CAT: 10.9). Treatment with *Ruta graveolens* extract (GLY+EX) or essential oil (GLY+EO) significantly restored redox balance, with MDA levels nearly normalized and antioxidant enzymes (GSH, SOD, CAT) restored to or exceeding control values. The essential oil achieved a slightly better normalization of GSH (10.71) and CAT (10.78), while the extract resulted in higher SOD recovery (9.68). Ibuprofen (GLY+IBU) provided only modest improvement, especially in MDA and GSH, but failed to restore SOD or CAT significantly, underscoring the superior antioxidative potential of *Ruta graveolens* formulations in mitigating glyphosate-induced hepatic oxidative damage.

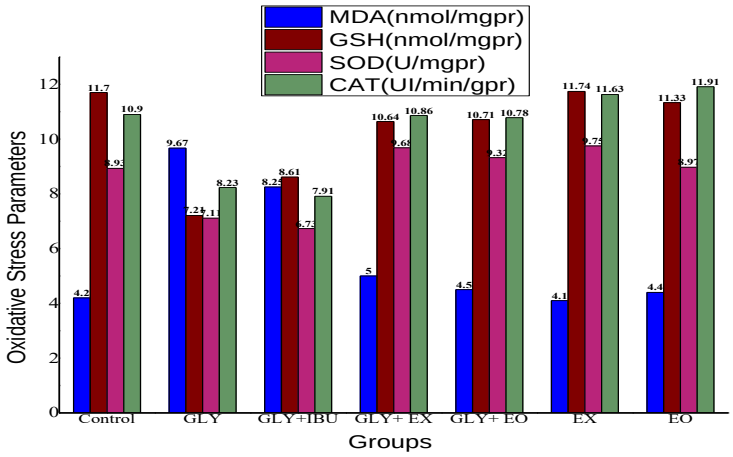


Figure 9**:** Oxidative stress parameters in the liver of different experimental groups**.**

4.5.2. Kidneys oxidative stress parameters

In renal tissues, glyphosate toxicity induced a sharp rise in MDA (6.96 ± 0.99 nmol/mg pr) and suppressed GSH (6.93 ± 0.42), SOD (4.18 ± 0.69), and CAT (4.33 ± 0.76), indicating oxidative stress-mediated nephrotoxicity. Co-treatment with *Ruta graveolens* extract (GLY+EX) or essential oil (GLY+EO) significantly ameliorated these disruptions. GLY+EO, in particular, restored MDA to 2.33 and CAT to 9.88, approximating control values (MDA: 2.5; CAT: 9.86), while both treatments improved SOD and GSH levels significantly. The extract group demonstrated slightly higher SOD activity (6.12) compared to essential oil (5.91), suggesting effective superoxide scavenging. Conversely, ibuprofen failed to meaningfully reverse oxidative impairments, especially in enzymatic antioxidants, confirming that *Ruta graveolens* therapies exert more comprehensive renoprotective antioxidant effects against glyphosate-induced stress.

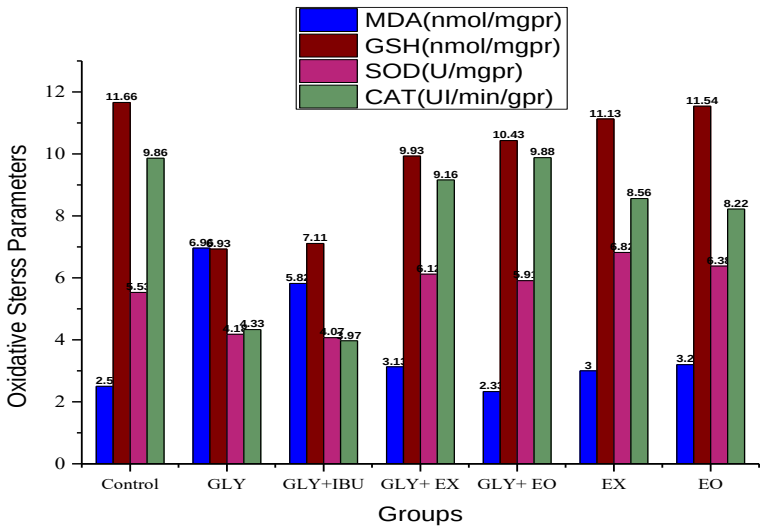


Figure 10**:** Oxidative stress parameters in the kidneys of different experimental groups**.**

4.5.3. Brain oxidative stress parameters

Brain tissues from glyphosate-treated animals showed marked oxidative stress, evidenced by significantly increased MDA (8.61 ± 0.74) and decreased GSH (6.98), SOD (2.33), and CAT (6.17), indicating severe lipid peroxidation and impaired neuroprotective antioxidant mechanisms. In stark contrast, administration of *Ruta graveolens* extract (GLY+EX) or essential oil (GLY+EO) substantially reversed these alterations. Both treatments reduced MDA levels to near-control values (GLY+EX: 3.95; GLY+EO: 4.31), while restoring GSH (11.93 and 12.55, respectively), SOD (9.15 and 8.95), and CAT (8.16 and 8.78). The essential oil demonstrated slightly superior efficacy in enhancing GSH and CAT levels, suggesting potent antioxidative and neuroprotective potential. In comparison, ibuprofen exerted only partial neuroprotection, reflected by suboptimal recovery of antioxidant markers and persistent elevation of MDA, highlighting the greater efficacy of *Ruta graveolens* in counteracting glyphosate-induced neurotoxicity via redox regulation.

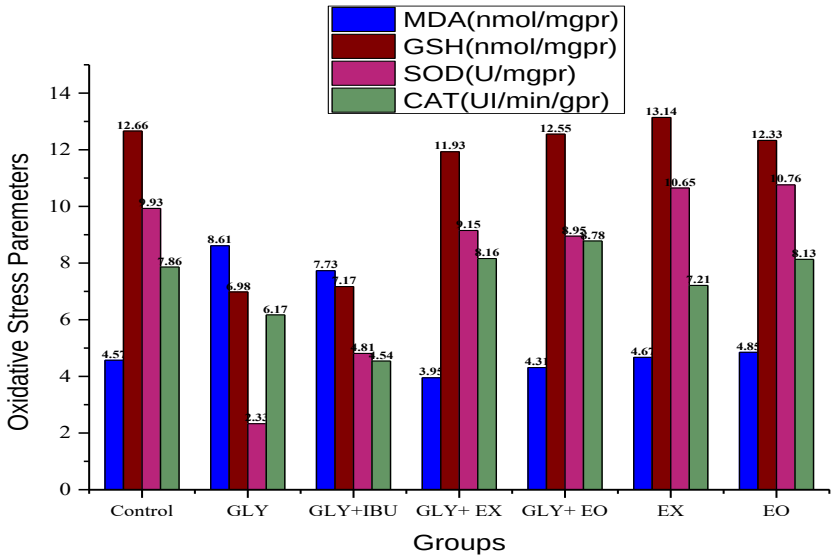


Figure 11**:** Oxidative stress parameters in the brain of different experimental groups**.**

4.6. Histopathological studies

A) Liver Histology

Histological examination of liver tissues (Figure 12) reveals significant pathological alterations among the experimental groups. The control group (A) exhibits normal hepatic architecture with radiating hepatocyte cords and intact central veins. In contrast, the glyphosate group (B) demonstrates severe hepatic injury characterized by vascular congestion, inflammatory cell infiltration, hepatocellular degeneration, and sinusoidal dilation, indicative of acute hepatotoxicity. Co-treatment with ibuprofen (C) shows partial amelioration with reduced

congestion but persistent inflammation. Notably, the GLY + *Ruta graveolens* extract group (D) displays substantial histological improvement with fewer degenerative changes, moderate restoration of liver architecture, and mild inflammation. The GLY + essential oil group (E) exhibits better hepatoprotection, evidenced by preserved hepatocyte integrity and minimal necrosis. Groups treated with extract (F) or essential oil alone (G) maintain nearly normal hepatic histology, highlighting the safety and potential hepatoprotective effect of *Ruta graveolens* constituents.

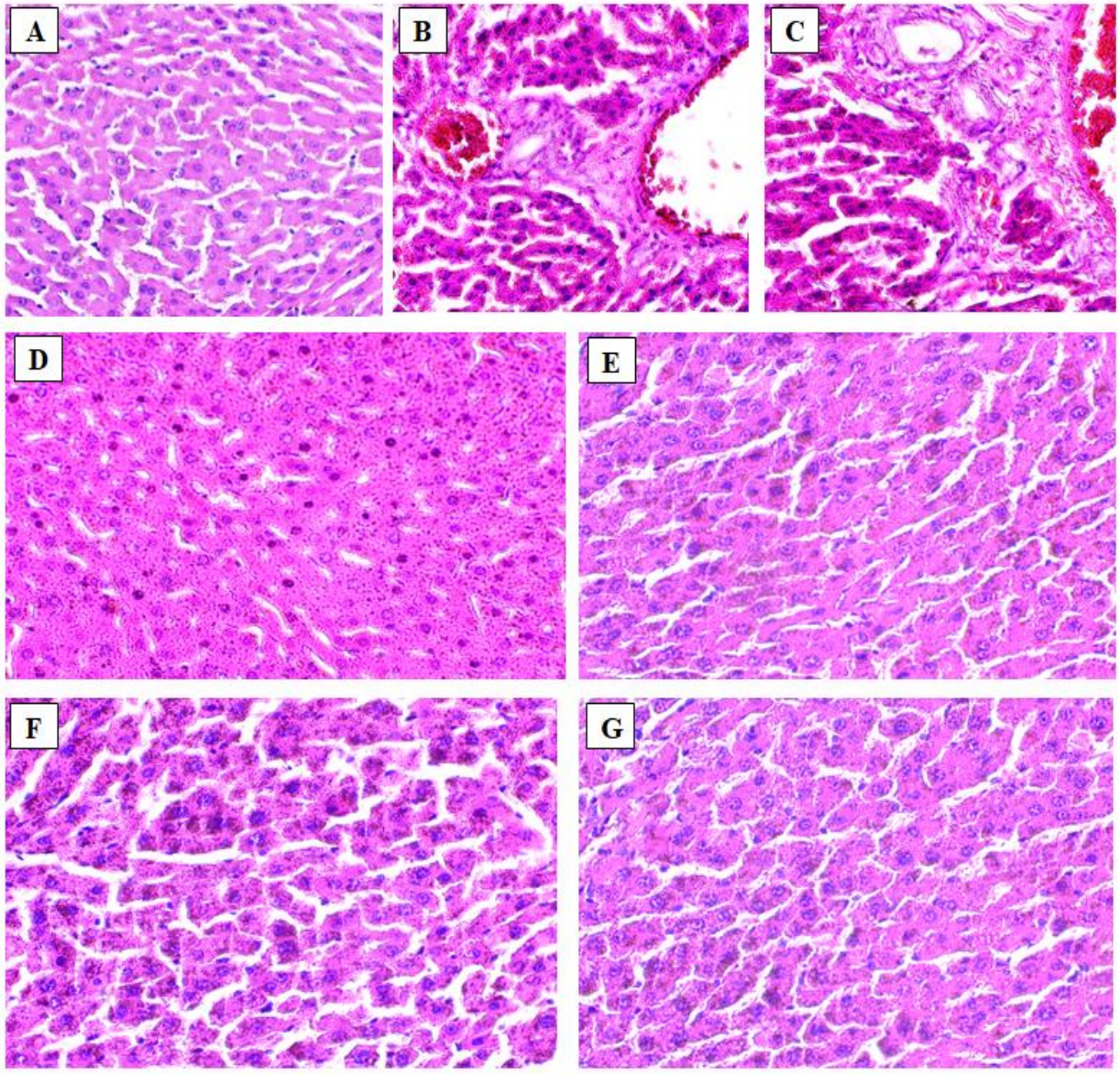


Figure 12**:** Microscopic observation of Liver histological sections from different experimental groups, (A) Control group, (B) Glyphosate group, (C) GLY+ Ibuprofen group, (D) GLY+ Ex group, (E) GLY+ EO group, (F) Ex group, (G) EO group, Magnification ×40.

B) Kidneys Histology

Renal tissue sections (Figure 13) show a similar trend of glyphosate-induced nephrotoxicity and the protective influence of *Ruta graveolens*. The control group (A) reveals normal renal histology with well-defined glomeruli and tubules. The glyphosate group (B) presents marked pathological lesions including glomerular atrophy, tubular necrosis, hemorrhage, and inflammatory infiltration. The GLY + ibuprofen group (C) shows slight improvement but persistent tubular damage and congestion. Treatment with *Ruta graveolens* extract (D) leads to noticeable attenuation of injury, reflected in more organized glomeruli and reduced necrosis. The GLY + essential oil group (E) demonstrates superior nephroprotection, with nearly intact renal architecture and mild pathological signs. Extract-only (F) and essential oil-only (G) groups show normal renal histology, suggesting the non-toxic nature and renoprotective potential of *Ruta graveolens* components.

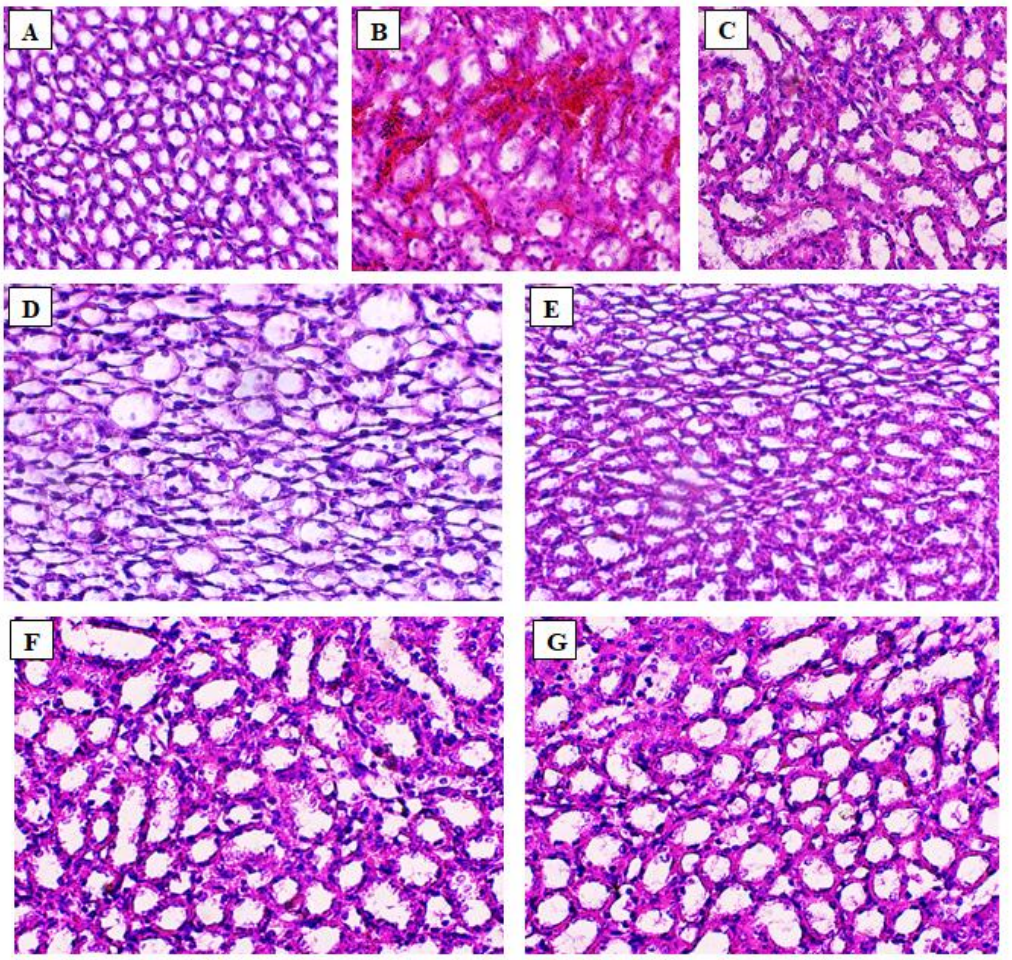


Figure 13**:** Microscopic observation of Kidneys histological sections from different experimental groups, (A) Control group, (B) Glyphosate group, (C) GLY+ Ibuprofen group, (D) GLY+ Ex group, (E) GLY+ EO group, (F) Ex group, (G) EO group, Magnification ×40.

C) Brain Histology

Microscopic observation of brain sections (Figure 14) highlights neurotoxic effects of glyphosate and the therapeutic potential of *Ruta graveolens*. The control group (A) presents with normal cortical structure, healthy neurons, and no signs of gliosis. Glyphosate exposure (B) causes severe neurodegeneration, characterized by neuronal shrinkage, pyknotic nuclei, vacuolation, and gliosis, indicating pronounced neurotoxicity. Co-administration with ibuprofen (C) shows mild neuroprotective effects with partial restoration of neuronal morphology. The GLY + extract group (D) displays significant neuroprotection, with fewer degenerated neurons and moderate preservation of neural architecture. The GLY + essential oil group (E) shows even greater improvement, with minimal pathological alterations and better neuronal survival. Groups F and G show normal histology, reinforcing the safety profile of the extract and essential oil and their potential neuroprotective roles.

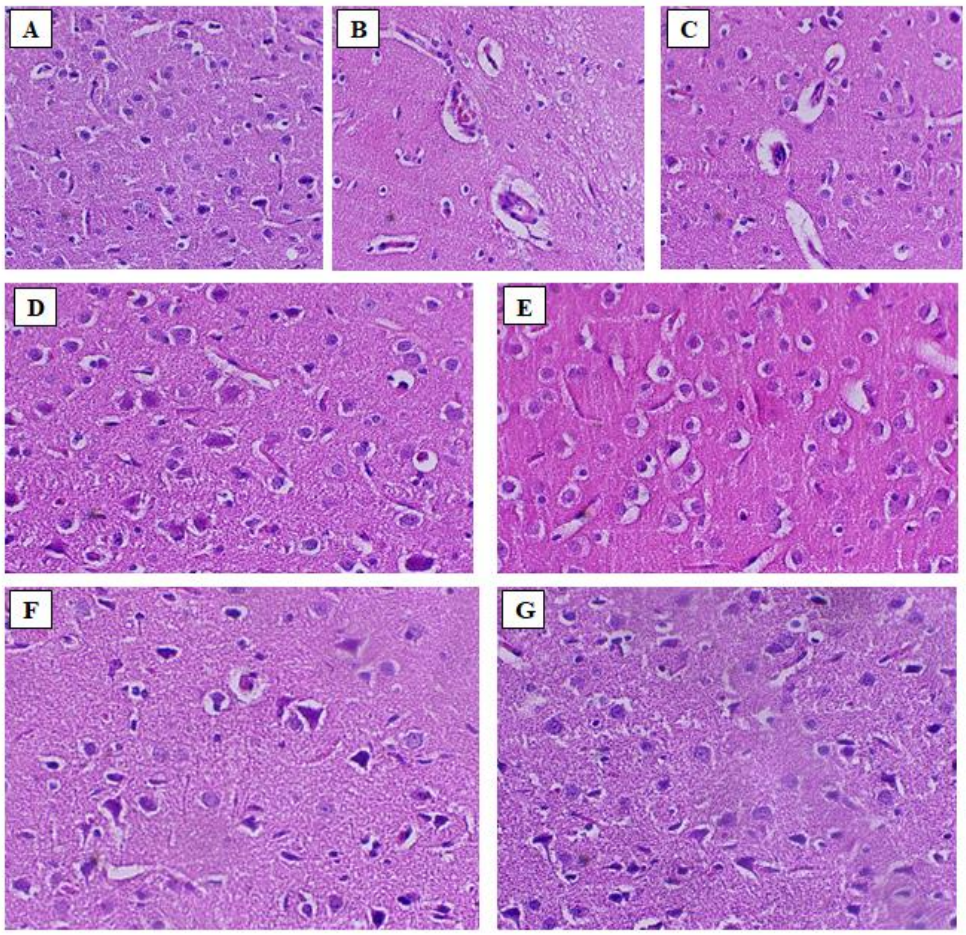


Figure 14**:** Microscopic observation of Brain histological sections from different experimental groups, (A) Control group, (B) Glyphosate group, (C) GLY+ Ibuprofen group, (D) GLY+ Ex group, (E) GLY+ EO group, (F) Ex group, (G) EO group, Magnification ×40.

Table 15**:** Histopathological Comparison of Liver, Kidney, and Brain Across Treatment Groups.

Description	Organ	Degeneration	Hemorrhage	Inflammatory Infiltrate	Necrosis	Vacuolated Cytoplasm
Control	Liver	–	–	–	–	–
	Kidney	–	–	–	–	–
	Brain	–	–	–	–	–
Glyphosate (GLY)	Liver	+++	++	+++	+++	+++
	Kidney	+++	++	++	+++	++
	Brain	+++	+	+++	+++	+++
GLY + Ibuprofen	Liver	++	+	++	++	++
	Kidney	++	+	+	++	+
	Brain	++	±	++	++	++
GLY + Ruta Extract	Liver	+	±	+	+	+
	Kidney	+	±	+	+	±
	Brain	+	–	+	+	+
GLY + Ruta Oil	Liver	±	–	±	±	±
	Kidney	±	–	±	±	–
	Brain	±	–	±	±	±
Ruta Extract only	Liver	–	–	–	–	–
	Kidney	–	–	–	–	–
	Brain	–	–	–	–	–
Ruta Oil only	Liver	–	–	–	–	–
	Kidney	–	–	–	–	–
	Brain	–	–	–	–	–

(–) Absent, (±) Minimal, (+) Mild, (++) Moderate, (+++) Severe

Conclusion

Conclusion

This study was conducted to contribute to the enrichment of scientific knowledge regarding the therapeutic potential of medicinal plants, with a focus on *Ruta graveolens*, one of the most widely used plants in traditional medicine due to its rich and diverse chemical composition. This work adopted a multidisciplinary approach combining laboratory techniques (in vitro), biological experiments (in vivo), and computational studies (in silico) to provide a comprehensive evaluation of the plant's anti-inflammatory and antioxidant properties.

The chemical analysis of both the aqueous extract and essential oil revealed the presence of biologically active compounds, notably rutin, naringin, syringic acid, and 2-undecanone—compounds known for their anti-inflammatory and free radical-scavenging effects. The biological assays demonstrated the extracts' effective capacity to inhibit protein denaturation and neutralize free radicals, confirming their promising therapeutic potential in managing both acute and chronic inflammation, as well as their role in reducing cellular oxidative stress.

In animal models, the use of *Ruta graveolens* extracts was shown to restore physiological balance in affected rats by improving hematological and biochemical markers and reducing tissue damage in the liver, kidneys, and brain. The study also revealed the superior performance of the natural extracts compared to certain synthetic drugs, in terms of both efficacy and lower toxicity, emphasizing the value of medicinal plants as safer and effective alternative therapeutic sources.

Moreover, in silico studies demonstrated good compliance of the major compounds with ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) criteria. Molecular docking results revealed strong binding affinities to active sites of key enzymes such as COX-1 and Glutathione Reductase, reinforcing the therapeutic hypothesis and confirming their candidacy for natural drug development.

Based on these findings, it can be concluded that *Ruta graveolens* is a promising botanical source with high potential for pharmaceutical and therapeutic applications—particularly in the development of natural anti-inflammatory and antioxidant agents with greater safety and lower toxicity than conventional medications. Despite the encouraging results, further investigations are recommended, including human clinical trials, long-term toxicity assessments, expanded molecular modeling studies, and deeper exploration of other active compounds within this plant.

This work paves the way for future research to enhance the systematic medical use of *Ruta graveolens* and reaffirms the importance of medicinal plants as a scientific foundation for promoting human and public health.

References

References

[1] G. Harvanová and S. Duranková, “Inflammatory process: Factors inducing inflammation, forms and manifestations of inflammation, immunological significance of the inflammatory reaction,” *Alergol. Pol. J. Allergol.*, vol. 12, no. 1, pp. 54–61, 2025.

[2] T. A. Butterfield, T. M. Best, and M. A. Merrick, “The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair,” *J. Athl. Train.*, vol. 41, no. 4, p. 457, 2006.

[3] M. Sharifi-Rad *et al.*, “Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases,” *Front. Physiol.*, vol. 11, p. 552535, 2020.

[4] A. Roy *et al.*, “Flavonoids a bioactive compound from medicinal plants and its therapeutic applications,” *Biomed Res. Int.*, vol. 2022, no. 1, p. 5445291, 2022.

[5] M. H. Shahrajabian, “A Candidate for Health Promotion, Disease Prevention and Treatment: Common Rue (Ruta graveolens L.), an Important Medicinal Plant in Traditional Medicine.,” *Curr. Rev. Clin. Exp. Pharmacol.*, vol. 19, no. 1, pp. 2–11, 2023, doi: 10.2174/2772432817666220510143902.

[6] M. Pan, Q. Lei, N. Zang, and H. Zhang, “A strategy based on GC-MS/MS, UPLC-MS/MS and virtual molecular docking for analysis and prediction of bioactive compounds in Eucalyptus globulus leaves,” *Int. J. Mol. Sci.*, vol. 20, no. 16, p. 3875, 2019.

[7] R. Murugan and T. Parimelazhagan, “Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from Osbeckia parvifolia Arn.–An in vitro approach,” *J. King Saud Univ.*, vol. 26, no. 4, pp. 267–275, 2014.

[8] R. Lacroix and D. M. Kurrasch, “Glyphosate toxicity: in vivo, in vitro, and epidemiological evidence,” *Toxicol. Sci.*, vol. 192, no. 2, pp. 131–140, 2023.

[9] F. E. Agamah *et al.*, “Computational/in silico methods in drug target and lead prediction,” *Brief. Bioinform.*, vol. 21, no. 5, pp. 1663–1675, 2020.

[10] V. Chiurchiu and M. Maccarrone, “Chronic inflammatory disorders and their redox control: from molecular mechanisms to therapeutic opportunities,” *Antioxid. Redox Signal.*, vol. 15, no. 9, pp. 2605–2641, 2011.

[11] A. L. De Weck, P. M. Gamboa, R. Esparza, and M. L. Sanz, “Hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs),” *Curr. Pharm. Des.*, vol. 12, no. 26, pp. 3347–3358, 2006.

[12] S. Khan, K. J. Mirza, M. R. Tyagi, and M. Z. Abdin, “Development of RAPD markers for authentication of Ruta graveolens (L) and its adulterant,” *MAPSB*, vol. 5, no. 1, pp. 58–61, 2011.

[13] M. Thambyayah, *Integrative Healthcare Remedies for Everyday Life-E-Book: Integrative Healthcare Remedies for Everyday Life-E-Book*. Elsevier Health Sciences, 2022.

[14] L. Marek-Jozefowicz *et al.*, “Molecular mechanisms of neurogenic inflammation of the skin,” *Int. J. Mol. Sci.*, vol. 24, no. 5, p. 5001, 2023.

[15] E. Cumming and C. Peters, “Immune response to infection,” *Anaesth. Intensive Care Med.*, 2024.

[16] M. J. Saldana, “Trigger digits: diagnosis and treatment,” *JAAOS-Journal Am. Acad. Orthop. Surg.*, vol. 9, no. 4, pp. 246–252, 2001.

[17] S. Omoigui, “The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3–inflammatory profile of pain syndromes,” *Med. Hypotheses*, vol. 69, no. 6, pp. 1169–1178, 2007.

[18] S. V Stankov, “Definition of inflammation, causes of inflammation and possible anti-inflammatory strategies,” *Open Inflamm J*, vol. 5, no. 1, pp. 1–9, 2012.

[19] P. A. Ward, “Acute and chronic inflammation,” *Fundam. Inflamm.*, vol. 3, pp. 1–16, 2010.

[20] P. D. Gupta, “Pathogenesis Due to Inflammation,” *J. Vet. Med. Res.*, vol. 8, no. Figure 3, pp. 3–5, 2021.

[21] R. J. Snyder, G. Schultz, C. Wachuku, A. M. Rashid, and J. K. Ead, “Proposed Mechanism of Action of Topically Applied Autologous Blood Clot Tissue: A Quintessential Cellular and Tissue-Based Therapy,” *J. Am. Podiatr. Med. Assoc.*, vol. 113, no. 3, 2023.

[22] M.-D. Filippi, “Mechanism of diapedesis: importance of the transcellular route,” *Adv. Immunol.*, vol. 129, pp. 25–53, 2016.

[23] D. S. Pearlman, “Pathophysiology of the inflammatory response,” *J. Allergy Clin. Immunol.*, vol. 104, no. 4, pp. s132–s137, 1999.

[24] F. Ceciliani, A. Giordano, and V. Spagnolo, “The systemic reaction during inflammation: the acute-phase proteins,” *Protein Pept. Lett.*, vol. 9, no. 3, pp. 211–223, 2002.

[25] A. Michopoulou and P. Rousselle, “How do epidermal matrix metalloproteinases support re-epithelialization during skin healing?,” *Eur. J. Dermatology*, vol. 25, pp. 33–42, 2015.

[26] P. Chavoshnejad, A. H. Foroughi, N. Dhandapani, G. K. German, and M. J. Razavi, “Effect of collagen degradation on the mechanical behavior and wrinkling of skin,” *Phys. Rev. E*, vol. 104, no. 3, p. 34406, 2021.

[27] K. A. Ali, A. Maity, S. D. Roy, S. Das Pramanik, P. P. Das, and M. A. Shaharyar, “Insight into the mechanism of steroidal and non-steroidal anti-inflammatory drugs,” in *How Synthetic Drugs Work*, Elsevier, 2023, pp. 61–94.

[28] M. D. G. Paglia *et al.*, “Use of corticoids and non-steroidal anti-inflammatories in the treatment of rheumatoid arthritis: Systematic review and network meta-analysis,” *PLoS One*, vol. 16, no. 4, p. e0248866, 2021.

[29] M. Goppelt-Struebe, D. Wolter, and K. Resch, “Glucocorticoids inhibit prostaglandin synthesis not only at the level of phospholipase A2 but also at the level of cyclo-oxygenase/PGE isomerase,” *Br. J. Pharmacol.*, vol. 98, no. 4, pp. 1287–1295, 1989.

[30] R. O. Day and G. G. Graham, “Non-steroidal anti-inflammatory drugs (NSAIDs),” *Bmj*, vol. 346, 2013.

[31] S. Bindu, S. Mazumder, and U. Bandyopadhyay, “Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective,” *Biochem. Pharmacol.*, vol. 180, p. 114147, 2020.

[32] J. N. Cashman, “The mechanisms of action of NSAIDs in analgesia,” *Drugs*, vol. 52, no. Suppl 5, pp. 13–23, 1996.

[33] P. Rao and E. E. Knaus, “Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond,” *J. Pharm. Pharm. Sci.*, vol. 11, no. 2, pp. 81s-110s, 2008.

[34] P. Luo, X. Feng, S. Liu, and Y. Jiang, “Traditional Uses, Phytochemistry, Pharmacology and Toxicology of Ruta graveolens L.: A Critical Review and Future Perspectives,” *Drug Des. Devel. Ther.*, pp. 6459–6485, 2024.

[35] H. Agarwal and M. Gayathri, “Biological synthesis of nanoparticles from medicinal plants and its uses in inhibiting biofilm formation,” *Asian J. Pharm. Clin. Res.*, vol. 10, no. 5, pp. 64–68, 2017, doi: 10.22159/ajpcr.2017.v10i5.17469.

[36] S. Milesi, B. Massot, E. Gontier, F. Bourgaud, and A. Guckert, “Ruta graveolens L.: a promising species for the production of furanocoumarins,” *Plant Sci.*, vol. 161, no. 1, pp. 189–199, 2001.

[37] K. Eickhorst, V. DeLeo, and J. Csaposs, “Rue the herb: Ruta graveolens-associated phytophototoxicity,” *DERM*, vol. 18, no. 1, pp. 52–55, 2007.

[38] G. Ahmad *et al.*, “Ruta graveolens: from Traditional System of Medicine to Modern Pharmacology: an Overview,” *Am. J. PharmTech Res.*, vol. 2, no. 2, 2012, [Online]. Available: http://www.ajptr.com/www.ajptr.com

[39] C. Erbar and P. Leins, “Erbar & Leins 2020 Entwicklungen in der Entwicklung – Fortwährende Veränderungen im Fluss der Organismenwelt,” *Heidelb. Jahrb.*, vol. 5, pp. 1–45, Jan. 2020.

[40] E. Şan, B. Karaduran, and M. Koruyucu, “Homeopathy in Pediatric Dentistry,” *Çocuk Derg. / J. Child*, vol. 23, no. 3, pp. 117–124, 2023, doi: 10.26650/jchild.2023.1313020.

[41] Jinous Asgarpanah, “Phytochemistry and pharmacological properties of Ruta graveolens L.,” *J. Med. Plants Res.*, vol. 6, no. 23, 2012, doi: 10.5897/jmpr12.040.

[42] S. Malik *et al.*, “Transgenesis and Secondary Metabolism,” *Transgenes. Second. Metab.*, no. December, 2016, doi: 10.1007/978-3-319-27490-4.

[43] S. Reza, R. Ahmad, N. Rahman, and T. Alam, “Suddab (Ruta graveolens Linn.) the Blessed Medicinal plant of Unani System of medicine: Literature Review.,” *Am. J. Pharm. Heal. Res.*, vol. 8, no. 8, pp. 52–57, 2020, doi: 10.46624/ajphr.2020.v8.i8.005.

[44] Z. Zakeri, A. Allafchian, M. Vahabi, and S. A. H. Jalali, “Synthesis and characterization of antibacterial silver nanoparticles using essential oils of crown imperial leaves, bulbs and petals,” *Micro Nano Lett.*, vol. 16, May 2021, doi: 10.1049/mna2.12082.

[45] K. Mzioud *et al.*, “No Title,” *Open Chem.*, vol. 21, no. 1, p. 20230149, 2023, doi: doi:10.1515/chem-2023-0149.

[46] K. Bel Hadj Salah-Fatnassi *et al.*, “Chemical composition, antibacterial and antifungal activities of flowerhead and root essential oils of Santolina chamaecyparissus L., growing wild in Tunisia.,” *Saudi J. Biol. Sci.*, vol. 24, no. 4, pp. 875–882, May 2017, doi: 10.1016/j.sjbs.2016.03.005.

[47] A. Farooq *et al.*, “Unravelling the prophylactic anti-inflammatory potential of Koenigia tortuosa through modulation of cytokine levels and inflammatory markers in LPS-induced localized inflammation in Wistar rat models,” *Inflammopharmacology*, no. February, 2025, doi: 10.1007/s10787-025-01680-7.

[48] A. Saleem, M. Saleem, and M. F. Akhtar, “Antioxidant, anti-inflammatory and antiarthritic potential of Moringa oleifera Lam: An ethnomedicinal plant of Moringaceae family,” *South African J. Bot.*, vol. 128, pp. 246–256, 2020.

[49] A. S. Gangadharan, D. T. Thangadurai, N. Manjubaashini, and D. Nataraj, “Nanoporous and morphology-transforming g-CNNPs for trace-level detection of mefenamic acid in urine samples and in vitro protein denaturation inhibition,” *J. Mater. Chem. C*, vol. 13, no. 6, pp. 3000–3010, 2025.

[50] A. Adaika *et al.*, “Synthesis, Antioxidant, and Antidiabetic Potential of Ferrocenylmethylnucleobase Compounds: In Vitro, In Silico Molecular Docking, DFT Calculation, and Molecular Dynamic Simulations,” *Appl. Organomet. Chem.*, vol. 39, no. 2, p. e7988, 2025.

[51] P. Putpadungwipon and P. Powthong, “Unveiling antioxidant arsenal DPPH radical scavenging activity and flavonoid profiles of six edible flower infusions,” *J. Integr. Sci. Technol.*, vol. 13, no. 5, p. 1101, 2025.

[52] M. L. Tlili, F. Alia, E. Bahri, T. Beyat, K. Redouan, and E. Lanez, “In vivo evaluations of the antidiabetic and antioxidant effects of Olea europaea L.(Chemlali variety) leaves,” *Acta Period. Technol.*, no. 00, p. 3, 2025.

[53] M. El Ouardi *et al.*, “Chemical composition, antimicrobial, and antioxidant properties of essential oils from Artemisia herba-alba asso. and Artemisia huguetii caball. from Morocco: in vitro and in silico evaluation.,” *Front. Chem.*, vol. 12, p. 1456684, 2024, doi: 10.3389/fchem.2024.1456684.

[54] C. Gnanaraj *et al.*, “In Silico Molecular Docking Analysis of Karanjin against Alzheimer’s and Parkinson’s Diseases as a Potential Natural Lead Molecule for New Drug Design, Development and Therapy,” *Molecules*, vol. 27, no. 9, 2022, doi: 10.3390/molecules27092834.

[55] H. M. Otifi, M. Alshyarba, M. Al Fayi, A. A. Dera, and P. Rajagopalan, “Computational docking and in vitro analysis identifies novel arylidene analogue FPMXY-14 against renal cancer cells by attenuating Akt.,” *Oncol. Res.*, vol. 29, no. 3, pp. 217–227, 2021, doi: 10.32604/or.2022.03570.

[56] H. Baltrukevich and P. Bartos, “RNA-protein complexes and force field polarizability,” *Front. Chem.*, vol. 11, no. June, pp. 1–14, 2023, doi: 10.3389/fchem.2023.1217506.

[57] G. Cingolani *et al.*, “Structural basis for selective inhibition of Cyclooxygenase-1 (COX-1) by diarylisoxazoles mofezolac and 3-(5-chlorofuran-2-yl)-5-methyl-4-phenylisoxazole (P6),” *Eur. J. Med. Chem.*, vol. 138, Jun. 2017, doi: 10.1016/j.ejmech.2017.06.045.

[58] P. GÜLLER, “The In Vitro and In Silico Inhibition Mechanism of Glutathione Reductase by Resorcinol Derivatives: A Molecular Docking Study,” *J. Mol. Struct.*, vol. 1228, p. 129790, 2021, doi: https://doi.org/10.1016/j.molstruc.2020.129790.

[59] P. W. Rose *et al.*, “The RCSB Protein Data Bank: new resources for research and education,” *Nucleic Acids Res.*, vol. 41, no. D1, pp. D475–D482, Jan. 2013, doi: 10.1093/nar/gks1200.

[60] E. Alves, G. Bannimath, P. Prabhakaran, S. Parameswaran, A. Awasthi, and D. Scholar, “TARGETING BREAST CANCER: UNVEILING FN3K ENZYME INHIBITORS VIA STRUCTURE-BASED VIRTUAL SCREENING AND MOLECULAR DYNAMIC SIMULATION,” Jun. 2024, doi: 10.1729/Journal.39997.

[61] C. Lu *et al.*, “OPLS4: Improving force field accuracy on challenging regimes of chemical space,” *J. Chem. Theory Comput.*, vol. 17, no. 7, pp. 4291–4300, 2021.

[62] Q. Zhao, R. Capelli, P. Carloni, B. Lüscher, J. Li, and G. Rossetti, “Enhanced Sampling Approach to the Induced-Fit Docking Problem in Protein–Ligand Binding: The Case of Mono-ADP-Ribosylation Hydrolase Inhibitors,” *J. Chem. Theory Comput.*, vol. 17, no. 12, pp. 7899–7911, Dec. 2021, doi: 10.1021/acs.jctc.1c00649.

[63] Y. Yang *et al.*, “Efficient exploration of chemical space with docking and deep learning,” *J. Chem. Theory Comput.*, vol. 17, no. 11, pp. 7106–7119, 2021.

[64] R. A. Friesner *et al.*, “Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein− ligand complexes,” *J. Med. Chem.*, vol. 49, no. 21, pp. 6177–6196, 2006.

[65] C. K. M. Khelil, B. Amrouche, A. soufiane Benyoucef, K. Kara, and A. Chouder, “New Intelligent Fault Diagnosis (IFD) approach for grid-connected photovoltaic systems,” *Energy*, vol. 211, p. 118591, 2020.

[66] U. Albus, “Guide for the Care and Use of Laboratory Animals (8th edn),” *Lab. Anim.*, vol. 46, no. 3, pp. 267–268, 2012, doi: 10.1258/la.2012.150312.

[67] R. Hubbard, J. Dunthorn, R. J. O’Brien, D. Tasch, U. Tasch, and S. R. Zeiler, “Evaluating Skilled Prehension in Mice Using an Auto-Trainer,” *JoVE*, no. 151, p. e59784, 2019, doi: doi:10.3791/59784.

[68] A. RP, “Noninvasive Method for Glucose Level Estimation by Saliva,” *J. Diabetes Metab.*, vol. 04, no. 05, 2013, doi: 10.4172/2155-6156.1000266.

[69] C. J. Hsueh, J. H. Wang, L. Dai, and C. C. Liu, “Determination of alanine aminotransferase with an electrochemical nano Ir-C biosensor for the screening of liver diseases,” *Biosensors*, vol. 1, no. 3, pp. 107–117, 2011, doi: 10.3390/bios1030107.

[70] J. Głowacka, A. Wiśniewska, R. Koncki, and K. Strzelak, “Photometric flow system for the determination of serum lactate dehydrogenase activity,” *Talanta*, vol. 265, p. 124817, 2023, doi: https://doi.org/10.1016/j.talanta.2023.124817.

[71] Z. Carneiro, “Protocols.io. (2017). AST/GOT Detection Assay. Retrieved from https://www.protocols.io,” pp. 1–2, 2017.

[72] K. Bochen, “Erythrocyte sedimentation rate – an old marker with new applications,” *J. Pre-Clinical Clin. Res.*, vol. 5, no. 2, pp. 50–55, 2011, [Online]. Available: www.jpccr.eu

[73] N. Pedrol, “Handbook of Plant Ecophysiology Techniques,” *Handb. Plant Ecophysiol. Tech.*, no. January 2001, 2001, doi: 10.1007/0-306-48057-3.

[74] J. Lovrić, M. Mesić, M. Macan, M. Koprivanac, M. Kelava, and V. Bradamante, “Malondialdehyde (MDA) levels were determined following the method described by,” *Period. Biol.*, vol. 110, no. 1, pp. 63–67, 2008.

[75] J. Grandgirard, D. Poinsot, L. Krespi, J. P. Nénon, and A. M. Cortesero, “Costs of secondary parasitism in the facultative hyperparasitoid Pachycrepoideus dubius: Does host size matter?,” *Entomol. Exp. Appl.*, vol. 103, no. 3, pp. 239–248, 2002, doi: 10.1023/A.

[76] T. Iwase *et al.*, “A simple assay for measuring catalase activity: A visual approach,” *Sci. Rep.*, vol. 3, pp. 3–6, 2013, doi: 10.1038/srep03081.

[77] S. Leclerc-Mercier *et al.*, “Skin Biopsy in Netherton Syndrome: A Histological Review of a Large

Series and New Findings," *Am. J. Dermatopathol.*, vol. 38, no. 2, pp. 83–91, 2016, doi: 10.1097/DAD.0000000000000425.

[78] E. Chlipala *et al.*, "Optical density-based image analysis method for the evaluation of hematoxylin and eosin staining precision," *J. Histotechnol.*, vol. 43, no. 1, pp. 29–37, 2020, doi: 10.1080/01478885.2019.1708611.

[79] S. S. Razi *et al.*, "Timely airway stenting improves survival in patients with malignant central airway obstruction," *Ann. Thorac. Surg.*, vol. 90, no. 4, pp. 1088–1093, 2010, doi: 10.1016/j.athoracsur.2010.06.093.

[80] V. Kumar *et al.*, "Ethyl acetate extract of Ruta graveolens: a specific and potent inhibitor against the drug-resistant EGFR_T790M mutant in NSCLC," *Front. Pharmacol.*, vol. 16, no. April, pp. 1–16, 2025, doi: 10.3389/fphar.2025.1570108.

[81] M. Mokhtar, F. Youcefi, S. Keddari, Y. Saimi, S. Otsmane Elhaou, and F. Cacciola, "Phenolic Content and in Vitro Antioxidant, Anti-Inflammatory and antimicrobial Evaluation of Algerian Ruta graveolens L.," *Chem. Biodivers.*, vol. 19, no. 9, p. e202200545, 2022.

[82] L. Águila *et al.*, "Relaxant effects of a hydroalcoholic extract of Ruta graveolens on isolated rat tracheal rings," *Biol. Res.*, vol. 48, pp. 1–6, 2015.

[83] N. Pallarés *et al.*, "Green and Innovative Extraction: Phenolic Profiles and Biological Activities of Underutilized Plant Extracts Using Pulsed Electric Fields and Maceration," *Foods*, vol. 14, no. 2, p. 222, 2025.

[84] J. F. F. Orlanda and A. R. Nascimento, "Chemical composition and antibacterial activity of Ruta graveolens L.(Rutaceae) volatile oils, from São Luís, Maranhão, Brazil," *South African J. Bot.*, vol. 99, pp. 103–106, 2015.

[85] J. Mejri, M. Abderrabba, and M. Mejri, "Chemical composition of the essential oil of Ruta chalepensis L: Influence of drying, hydro-distillation duration and plant parts," *Ind. Crops Prod.*, vol. 32, no. 3, pp. 671–673, 2010, doi: https://doi.org/10.1016/j.indcrop.2010.05.002.

[86] M. Barbouchi, B. Benzidia, and M. Choukrad, "Chemical variability in essential oils isolated from roots, stems, leaves and flowers of three Ruta species growing in Morocco," *J. King Saud Univ. - Sci.*, vol. 33, no. 8, p. 101634, 2021, doi: https://doi.org/10.1016/j.jksus.2021.101634.

[87] N. Hadi *et al.*, "Phytochemical Analysis and Evaluation of Antioxidant and Antimicrobial Properties of Essential Oils and Seed Extracts of Anethum graveolens from Southern Morocco: In Vitro and In Silico Approach for a Natural Alternative to Synthetic Preservatives," *Pharmaceuticals*, vol. 17, no. 7, 2024, doi: 10.3390/ph17070862.

[88] S. Gunawardana and B. Dias, "Methodological advances in formulation and assay of herbal resources-based topical drug delivery systems," *J. Complement. Integr. Med.*, vol. 22, no. 1, pp. 74–86, 2025.

[89] L. Nahar, H. R. El-Seedi, S. A. M. Khalifa, M. Mohammadhosseini, and S. D. Sarker, "Ruta essential oils: Composition and bioactivities," *Molecules*, vol. 26, no. 16, p. 4766, 2021.

[90] K. Messaoudi, O. S. Oubbati, A. Rachedi, A. Lakas, T. Bendouma, and N. Chaib, "A survey of UAV-based data collection: Challenges, solutions and future perspectives," *J. Netw. Comput. Appl.*, vol. 216, p. 103670, 2023.

[91] J. Patel, H. Roy, P. K. Chintamaneni, R. Patel, and R. Bohara, “Advanced Strategies in Enhancing the Hepatoprotective Efficacy of Natural Products: Integrating Nanotechnology, Genomics, and Mechanistic Insights,” *ACS Biomater. Sci. Eng.*, 2025.

[92] K. Jurowski *et al.*, “Toxicological profile of Acovenoside A as an active pharmaceutical ingredient – prediction of missing key toxicological endpoints using in silico toxicology methodology,” *Chem. Biol. Interact.*, vol. 408, p. 111404, 2025, doi: https://doi.org/10.1016/j.cbi.2025.111404.

[93] D. Psimadas, P. Georgoulias, V. Valotassiou, and G. Loudos, “Molecular Nanomedicine Towards Cancer :,” *J. Pharm. Sci.*, vol. 101, no. 7, pp. 2271–2280, 2012, doi: 10.1002/jps.

[94] J. G. M. Mvondo, A. Matondo, D. T. Mawete, S.-M. N. Bambi, B. M. Mbala, and P. O. Lohohola, “In Silico ADME/T Properties of Quinine Derivatives using SwissADME and pkCSM Webservers,” *Int. J. Trop. Dis. Heal.*, vol. 42, no. 11, pp. 1–12, 2021, doi: 10.9734/ijtdh/2021/v42i1130492.

[95] L. Fu *et al.*, “ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support,” *Nucleic Acids Res.*, vol. 52, no. W1, pp. W422–W431, 2024, doi: 10.1093/nar/gkae236.

[96] S. A. Varenichenko and O. K. Farat, “PREDICTION OF BIOLOGICAL ACTIVITY OF AMINOQUINOLINE DERIVATIVES USING THE ADMET 2 . 0 WEB RESOURCE .,” 2024, doi: 10.33609/2708-129X.90.1.2024.15-25.

[97] G. B. Gonzales, G. Smagghe, C. Grootaert, M. Zotti, K. Raes, and J. Van Camp, “Flavonoid interactions during digestion, absorption, distribution and metabolism: A sequential structure-activity/property relationship-based approach in the study of bioavailability and bioactivity,” *Drug Metab. Rev.*, vol. 47, no. 2, pp. 175–190, 2015, doi: 10.3109/03602532.2014.1003649.

[98] B. Press, “Optimization of the Caco-2 permeability assay to screen drug compounds for intestinal absorption and efflux,” *Permeability Barrier Methods Protoc.*, pp. 139–154, 2011.

[99] S. Khamouli, S. Belaidi, M. Ouassaf, T. Lanez, S. Belaaouad, and S. Chtita, “Multi-combined 3D-QSAR, docking molecular and ADMET prediction of 5-azaindazole derivatives as LRRK2 tyrosine kinase inhibitors,” *J. Biomol. Struct. Dyn.*, vol. 40, no. 3, pp. 1285–1298, 2022, doi: 10.1080/07391102.2020.1824815.

[100] R. del Amor *et al.*, “A fusocelular skin dataset with whole slide images for deep learning models,” *Sci. Data* , vol. 12, no. 1, pp. 1–7, 2025, doi: 10.1038/s41597-025-05108-3.

[101] D. I. Piraner *et al.*, *Engineered receptors for soluble cellular communication and disease sensing*. Springer US, 2025. doi: 10.1038/s41586-024-08366-0.

[102] X. Zhang and C. Wu, “In Silico, In Vitro, and In Vivo Evaluation of the Developmental Toxicity, Estrogenic Activity, and Mutagenicity of Four Natural Phenolic Flavonoids at Low Exposure Levels,” *ACS Omega*, vol. 7, no. 6, pp. 4757–4768, 2022, doi: 10.1021/acsomega.1c04239.

[103] M. Qneibi *et al.*, *A Comprehensive Review of Essential Oils and Their Pharmacological Activities in Neurological Disorders: Exploring Neuroprotective Potential*, vol. 49, no. 2. 2024. doi: 10.1007/s11064-023-04032-5.

[104] M. P. Repasky, M. Shelley, and R. A. Friesner, “Flexible ligand docking with Glide,” *Curr. Protoc. Bioinforma.*, vol. 18, no. 1, pp. 8–12, 2007.

[105] E. B. Miller *et al.*, “Reliable and Accurate Solution to the Induced Fit Docking Problem for Protein-

Ligand Binding," *J. Chem. Theory Comput.*, vol. 17, p. 2630, 2021.

[106] H. Guterres and W. Im, "CHARMM-GUI-Based Induced Fit Docking Workflow to Generate Reliable Protein-Ligand Binding Modes," *J. Chem. Inf. Model.*, vol. 63, no. 15, pp. 4772–4779, 2023, doi: 10.1021/acs.jcim.3c00416.

بطاقة معلومات

حول فريق الإشراف و فريق العمل

1- فريـــق الاشـــراف:

فريق الاشراف	
المشرف الرئيسي (01): لعانز الحفناوي	التخصص: الكيمياء الحيوية
المشرف المساعد: بن عمر محمد العربي	التخصص: كيمياء عضوية تطبيقية

2- فريـــق العمـــل:

فريق المشروع	التخصص	الكلية
الطالب: شريط فاطمة الزهراء	بيولوجيا وفيزيولوجيا النبات	علوم الطبيعة والحياة
الطالب: قعري خلود	بيولوجيا وفيزيولوجيا النبات	علوم الطبيعة والحياة
الطالب: سليماني شروق	بيولوجيا وفيزيولوجيا النبات	علوم الطبيعة والحياة

فهرس المحتويات

فهرس المحتويات

المحور الأول

تقديم المشروع

المحور الأول

تقديم المشروع

1. فكرة المشروع (الحل المقترح)

يندرج هذا المشروع ضمن المجال الصحي، ويعتمد على تطبيقات حديثة في استغلال النباتات الطبية المحلية، حيث يهدف (السذاب) لما له من إمكانات وقائية مهمة. بدأت الفكرة *Ruta graveolens* إلى تطوير مستخلص طبيعي مائي من نبات من ملاحظة قلة استغلال نباتات المنطقة، خاصة نبات السذاب الذي يُعد جوهرة طبيعية مهملة رغم غناه بمركبات فعالة.
تطورت هذه الفكرة لتتحول إلى مشروع علمي ميداني يسعى إلى إثبات الفعالية البيولوجية لهذا النبات في التخفيف من الآثار الناتجة عن التعرض لمادة سامة سببت التهابات حيوية واضحة.

تم استخدام مستخلص مائي فقط دون اللجوء إلى مذيبات كحولية، وهو ما يُعتبر ميزة مهمة من حيث السلامة البيولوجية وقابلية الاستعمال المباشر في المنتجات الموجهة للاستهلاك البشري. كما تم تشكيل تركيبة شرابية نهائية تعتمد على هذا المستخلص، في إطار تطوير مكمل غذائي طبيعي يمكن استخدامه لأغراض وقائية، مما يفتح آفاقًا واعدة لتوظيف النباتات المحلية في منتجات صحية قابلة للتسويق
يُنجز هذا المشروع من طرف الباحثة تحت إشراف أساتذة مختصين، وبتعاون مع الطاقم التقني في المخابر الجامعية، التي توفّر الإمكانيات اللازمة لإجراء التحاليل والتجارب الحيوية في ظروف علمية دقيقة وآمنة

2. القيم المقترحة:

- منتج طبيعي وصحي مستخلص من نبات محلي (السذاب)
- تركيبة شرابية سهلة وآمنة بدون مذيبات كحولية تكلفة إنتاج منخفضة بفضل توفر المادة النباتية محليًا.
- يساهم في الوقاية من الالتهابات الناتجة عن مواد سامة.
- يمثل حلًا مبتكرًا وغير متوفر سابقًا في السوق.
- سهل الاستعمال ومناسب لمختلف الفئات
- قابل للتسويق كمكمل غذائي محلي فعّال.

3. فريق العمل (المخترعين):

يتكون فريق العمل من ثلاث طالبات في السنة الثانية ماستر تخصص بيولوجيا وفيزيولوجيا النبات بجامعة الشهيد حمه لخضر – الوادي

- ✓ شريط فاطمة الزهراء
- ✓ قعري خلود
- ✓ سليماني شروق

اكتسب الفريق خبرة علمية قيّمة من خلال التطبيق العملي أثناء تطوير هذا المشروع، حيث تم تعلّم واكتساب مهارات متقدمة في استخلاص وتحليل المركبات النباتية، إجراء التجارب الحيوية على نماذج حيوانية، تطبيق الاختبارات المخبرية لتقييم النشاطات المضادة للالتهاب والأكسدة، إلى جانب استخدام أدوات المحاكاة الحاسوبية (in silico) لتوقّع السمية والتوافر الحيوي وربط المركبات بأهدافها الجزيئية.

اعتمد الفريق في إنجاز هذا البحث على تنسيق فعّال ومستمر، من خلال تنظيم اجتماعات دورية، وتبادل الآراء العلمية، ومناقشة خطوات العمل التجريبي والتحليلي، مما ساهم في ضمان انسجام الجهود واتخاذ قرارات جماعية مدروسة انعكست إيجابًا على جودة تنفيذ المشروع ونتائجه.

4. أهداف المشروع:

يهدف هذا المشروع إلى تطوير مكمل غذائي طبيعي مستخلص من نبات السذاب، في شكل تركيبة شرابية مبتكرة موجهة للوقاية من الالتهابات الناتجة عن التعرض لمواد سامة. وتتمثل أهداف المشروع فيما يلي:

أن نصبح المنتج الأول في الجزائر في مجال المكملات الغذائية الطبيعية ذات التركيبات الشرابية المعتمدة على النباتات الطبية المحلية خلال السنوات الخمس المقبلة.

الوصول إلى حصة سوقية تقدر بـ 35%، وذلك بناءً على القدرة الإنتاجية مقارنة بإجمالي ما يُنتج في الجزائر من التركيبات الشرابية الطبيعية.

تسجيل براءة اختراع وطنية تحمي الصيغة التركيبية والابتكارية للمكمل الغذائي.

إدماج النباتات الطبية المحلية، وعلى رأسها نبات السذاب، ضمن حلول صحية قابلة للتسويق وطنياً.

5. جدول زمني لإنجاز براءة الاختراع:

الشهر

			1	2	3	4	5	6	7
1		البحث في قواعد البيانات الخاصة ببراءات الاختراع وجمع المعلومات	✓	✓					
2		الشروع في الاختبارات المخبرية لإعداد النموذج الأولي		✓	✓				
3		تجريب النموذج الأولي			✓	✓			
4		تجربة النموذج الأولي خارج المخابر					✓	✓	
5		تسجيل براءة الاختراع من اجل الحصول على رقم الإيداع والحماية الصناعية						✓	
6		متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من **inapi**							✓

الأعمال

المحور الثاني

الجوانب الابتكارية

المحور الثاني

الجوانب الابتكارية

1. طبيعة الابتكارات:

تميّز هذه الدراسة بطابعها الابتكاري من حيث المنهج والمحتوى، وذلك من خلال ما يلي:

- اعتماد نهج علمي متعدد التخصصات يجمع بين التحليل الكيميائي الحيوي، التقييم الحيوي على نماذج مخبرية وحيوانية (in vitro و in vivo، والمحاكاة الحاسوبية (in silico) ، مما يُعزز من شمولية النتائج ويمنحها موثوقية أكبر.
- يُعد هذا المشروع من أوائل الدراسات محليًا التي تبحث في الخصائص الدوائية لنبتة السذاب باستخدام تقنيات حديثة مثل UPLC-MS/MS و GC-MS، والتي مكّنت من الكشف عن مركبات فعالة ذات أهمية علاجية واعدة.
- الابتكار في النموذج الحيوي المعتمد، حيث تم استخدام مبيد الغليفوسات كمُحفّز للإجهاد التأكسدي والالتهاب، لتقييم فعالية المستخلصات النباتية في ظروف سمّية حقيقية، وهو ما يضفي بعدًا تطبيقيا يمكن الاستفادة منه في مجالات الوقاية البيئية والعلاج الطبيعي.
- الاعتماد على أدوات محاكاة حاسوبية متقدمة لتوقّع الخصائص الدوائية (ADMET) والارتباط الجزيئي بالمواقع الإنزيمية المستهدفة (مثل COX-1 و GR)، مما يتيح نمذجة دقيقة للفعالية البيولوجية دون الحاجة إلى موارد مادية مكلفة.

2. مجالات الابتكارات:

يمثل هذا المشروع ابتكارًا متعدد الجوانب، إذ يعتمد على تطوير مكمل طبيعي في شكل تركيبة شرابية محسّنة وآمنة مستخلصة مائيًا من نبات السذاب، مما يشكّل إضافة نوعية في مجال المنتجات الصحية الوقائية. من أبرز مجالات الابتكار التي ينتمي إليها المشروع: تحسين الميزات، حيث يوفر المنتج تركيبة طبيعية خالية من المذيبات الكحولية وأكثر أمانًا للمستهلك، وطرح عروض جديدة من خلال منتج مبتكر غير متوفر في السوق المحلي سواء من حيث نوع النبات المستخدم أو صيغة التقديم. كما يستهدف المشروع عملاء جدد من فئات لم تكن تخدمها الأشكال التقليدية من المكملات، مثل من يعانون من صعوبة ابتلاع الكبسولات أو من يفضلون المنتجات الخالية من الكحول. بالإضافة إلى ذلك، يعتمد المشروع على نموذج عمل جديد يقوم على استغلال نباتات محلية قليلة الاستخدام وتحويلها إلى منتجات شرابية ذات قيمة، مع اعتماد عمليات إنتاجية جديدة ترتكز على تقنيات الاستخلاص المائي البسيطة والفعالة، مما يحسن الكفاءة ويقلل التكاليف دون المساس بجودة وسلامة المنتج.

المحور الثالث

وصف براءة الاختراع

1- عنوان براءة الاختراع:

شراب طبيعي من مستخلص مائي لنبات مضاد للالتهابات

2- ملخص براءة الاختراع:

يتعلق هذا الاختراع بإنتاج شراب طبيعي مضاد للالتهابات باستخدام المستخلص المائي لنبات *Ruta graveolens*، الذي يعد من الموارد النباتية الشحيحة الاستخدام في الأبحاث العلمية والتطبيقات العلاجية، رغم توفره محليًا. وقد تم استخدام الماء المقطر كمذيب طبيعي للحصول على مستخلص نقي يحتوي على مركبات نباتية فعالة حيويًا.

تم تنفيذ عملية التحضير عبر عدة مراحل تشمل النقع، الترشيح، والتبخير، وصولاً إلى الحصول على مستخلص خام يتم وزنه بدقة، ثم دمجه ضمن تركيبة شرابية سائلة، وفق بروتوكول تحضيري يراعي الثبات الفيزيائي والفعالية الحيوية.

خضع المنتج لاختبارات مخبرية باستخدام نماذج حية (in vivo)، حيث تم تعريض مجموعات من الحيوانات لحالة التهابية مستحدثة، ثم تقييم تأثير التركيبة الشرابية بالمقارنة مع عينة ضابطة. شملت التقييمات مراقبة التغيرات الحيوية والسلوكية والفسيولوجية، مما مكّن من قياس فاعلية التركيبة في التخفيف من مظاهر الالتهاب.

يقدم هذا الشراب كمكمل غذائي داعم في حالات الالتهابات الحادة، خصوصًا لدى البالغين المصابين بحالات مثل التهابات الكبد أو الكلى. وتُحدَّد الجرعة اليومية بـ 15 مل لمدة سبعة أيام متتالية.

3- الميدان التقني الذي ينتمي إليه الاختراع:

ينتمي هذا الاختراع الى البيوكيميائية والدوائية وتحديدا في قطاع المنتجات الطبيعية العشبية ذات خصائص علاجية حيث يركز على تطوير مكمل غذائي عشبي في شكل شراب عشبي يتمتع بخصائص مضادة للالتهابات مستخلصه من نبات متوفرة كمصادر لتحقيق ربح اقتصادي وبيئي

- الحالة التقنية السابقة

براءات اختراع السابقة المتعلقة بالمنتجات النباتية المضادة للالتهابات:

- براءة اختراع US6264995B1: (2001)

براءة تتعلق استخدام مستخلصات من ست نباتات الزنجبيل، الكركم، الشاي الاخضر، قرفة، اكليل الجبل، فلفل اسود يمكن استخدامها في اشكال مختلفة

- براءة اختراع WO2017058716A1:(2017)

تتعلق باستخدام مستخلصات نباتية تحتوي على مركبات تم تحسينها لاستخدامها لعلاج الالتهابات الجلد وتحسين التئام الجروح

- براءة اختراع IN233541:

تتعلق براءة مشروب عشبي يحتوي على مستخلصات نباتية مثل sida spp، cordifolia خصائص مضادة للأكسدة ومحفزة للمناعة

- براءة اختراع IT202100002531A1: (2022)

تتعلق باستخدام حمض الهيالورونيك بأوزان جزيئية مختلفة وزنجبيل يساهم في ترطيب بشرة ويقلل من الالتهابات

- براءة اختراع US6264995B1 : (2001)

تتعلق باستخدام الأريغانو، الزنجبيل، الريحان المقدس، الكركم، الشاي الاخضر وغيرها.

4- الغرض (الهدف) من الاختراع

يهدف هذا الاختراع إلى ابتكار تركيبة شرابية تعتمد على مستخلص مائي نباتي تُظهر خصائص فعّالة في التقليل من العمليات الالتهابية الناتجة عن التعرض للعوامل المؤكسدة أو السامة أو المسببة للإجهاد الخلوي. ويُقدم هذا الابتكار حلاً عمليًا وآمنًا للاستفادة من المركبات الطبيعية ذات الفعالية البيولوجية الموجودة في هذا النبات، دون الاعتماد على المذيبات العضوية أو الزيوت الطيارة التي قد تكون محدودة القابلية أو الاستعمال. وقد أظهرت النتائج الأولية أن هذه التركيبة ساهمت في تخفيض مؤشرات الالتهاب المقاسة مخبريًا، مما يبرز إمكانية استخدامها في تقليل التأثيرات المرتبطة بالاستجابات الالتهابية الحادة أو المزمنة. ويُعد هذا الاختراع توجهًا مبتكرًا نحو الاستفادة من مصادر نباتية مائية خفيفة وآمنة لتقديم بدائل طبيعية فعالة من الناحية البيولوجية.

5- تقديم جوهر الاختراع

الاختراع يتمثل في انتاج شراب عشبي طبيعي مضاد للالتهاب باستخدام المستخلص المائي لنبات *Ruta graveolens* مع التأكد من ثبات التركيبة وسلامتها وفعاليتها يتم توجيه هذا الشراب بديل طبيعي للأدوية الكيميائية المضادة للالتهاب ويتميز بكونه مستخلصا نباتيا محليا مدروسا ومجربا على الحيوانات المخبرية.

6- النموذج الأولي التجريبي

- **عندما يتعلق الأمر بالجهاز**

لا ينطبق هذا النوع على مشروعنا، حيث لا يتضمن الجهاز الفيزيائي أو الميكانيكي، بل يتعلق بتركيبة طبيعية تُحضَّر عبر خطوات بيولوجية وكيميائية بسيطة.

- **عندما يتعلق الأمر بطريقة عملية (لإنتاج أو المعالجة)**

يعتمد المشروع على طريقة استخلاص مائي مبتكرة للمركبات الفعالة من نبات السذاب دون استخدام مذيبات عضوية أو كحولية، ما يجعل العملية أكثر أمانًا وملاءمة للاستهلاك البشري.

- **عندما يتعلق الأمر بمنتوج (مركب، مزيج، تشكيل....)**

المنتج هو مركب طبيعي مكوَّن من مستخلص نباتي مائي ممزوج بمواد طبيعية حافظة ومُحسِّنات طعم طبيعية، ليُقدَّم في شكل تركيبة شرابية سهلة التناول، موجهة للوقاية من الالتهاب.

- **عندما يتعلق الأمر بتطبيق جديد لجهاز معروف أو طريقة معروفة أو مادة معروفة**

المشروع يُمثل تطبيقًا جديدًا لمادة نباتية معروفة (السذاب)، لكن لم تُستغل سابقًا بشكل علمي منظّم كمكمل وقائي في شكل شراب
الابتكار يكمن في طريقة المعالجة، وإعادة توظيف المادة النباتية كمصدر لمركبات فعالة مضادة للالتهاب في شكل مكمل غذائي شرابي قابل للتسويق.

- **عندما يتعلق الأمر بتركيبة جديدة لعناصر معروفة أو غير معروفة**

تم تطوير تركيبة شرابية جديدة تعتمد على عناصر طبيعية معروفة (نبات السذاب، الماء، مواد حافظة طبيعية)، لكن بتركيبة مبتكرة ومتوازنة
هذه التركيبة تحقق فعالية بيولوجية موثقة مع الحفاظ على الطابع الطبيعي والمأمون للمستهلك

7- شرح الأشكال والرسومات:

الشكل 1: دور هذا الشكل يتمثل في توضيح العلاقة بين المصدر النباتي (نبات السذاب) والمركبات النشطة المستخلصة منه، والتي تسهم في التأثير المضاد للالتهاب للتركيبة الشرابية. كما يساعد الشكل في إبراز البنية العامة للاختراع وفهم المكونات الحيوية الفعالة التي تدعم الفعالية العلاجية للمنتج.

رقم 01

نبات السذاب (*Ruta graveolens*) هو نبات عطري غني بمركبات فعالة مثل الروتين وغيرها من مركبات نشطة، وله استخدامات تقليدية متعددة. يتميز بخصائص مضادة للالتهاب والأكسدة والميكروبات

رقم 02

تركيبة الشرابية: تساهم في تخفيف الالتهابات الحادة من خلال مركبات طبيعية فعالة تمتلك خصائص مضادة للالتهاب ومعدِّلة للمؤشرات الحيوية. كما تدعم التوازن الفسيولوجي العام للجسم وتُعد خيارًا طبيعيًا للأفراد البالغين.

رقم03

المركبات النشطة: تلعب المركبات النشطة دورًا مهمًا في تنظيم الاستجابة الالتهابية في الجسم، من خلال تثبيط إنتاج الوسائط الالتهابية، مما يساهم في تقليل حدة الالتهاب وتخفيف آثاره على الأنسجة الحيوية

الشكل2 :شكل يسهّل فهم آلية التحضير ويوفر تصورًا واضحًا لخطوات الاستخلاص والمعالجة، بما يضمن توثيقًا علميًا ومنهجيًا قابلاً للتكرار والتطوير.

1. النقع

يُستخدم لاستخلاص المركبات الفعالة في الماء بطريقة آمنة، مما يحافظ على خصائصها الحيوية.

2. الترشيح

يفصل الشوائب وبقايا النبات، للحصول على مستخلص نقي وجاهز للتحضير.

3. التبخير

يقلل حجم الماء لزيادة تركيز المركبات الفعالة، مما يعزز فعالية المستخلص.

4. المستخلص الخام

يمثل المادة المركزة التي تحتوي على المركبات النشطة وتُستخدم كأساس في التركيبة.

5. وزن المستخلص الخام

يضمن تحديد كمية دقيقة من المستخلص لضبط تركيز الشراب وتحقيق فعالية متوازنة.

6. تحضير التركيبة الشرابية

تحويل المستخلص إلى شكل شراب سهل الاستخدام، مستقر، وطعمه مقبول للاستهلاك الفموي.

8-طريقة والية عمل المادة المخترعة

تعتمد آلية عمل المادة المخترعة، المتمثلة في تركيبة شرابية طبيعية مستخلصة مائيًا من نبات*Rutagraveolens* (السذاب)، على تفعيل تأثير المركبات النباتية النشطة التي تُسهم في تخفيف الاستجابة الالتهابية. تعمل هذه التركيبة من خلال تقليل تركيز الوسائط الالتهابية في الأنسجة، مما يؤدي إلى خفض مستوى التهيج والتلف الخلوي الناتج عن التعرض للعوامل الضارة. كما تُظهر خصائص مضادة للأكسدة، تُسهم في تقليل الإجهاد التأكسدي عبر تعزيز التوازن بين المؤكسدات والعوامل المضادة داخل الخلايا.

إضافة إلى ذلك، تساعد المركبات النشطة في استعادة الاستقرار الفسيولوجي للخلايا عبر دعم آليات الإصلاح الطبيعي وتنظيم التفاعل المناعي في الأنسجة الحيوية المستهدفة، خاصة في حالات الالتهاب الناتج عن المواد السامة. وقد أظهرت نتائج التجارب الحيوية الأولية أن هذه التركيبة ساهمت في تحسين المؤشرات البيوكيميائية المرتبطة بالالتهاب، مما يدل على فعاليتها كحل وقائي طبيعي وآمن قابل للاستعمال المنتظم.

المحور الرابع

المطالب

- المطالب

1. **المطلب الرئيس:** شراب طبيعي من مستخلص مائي لنبات مضاد للالتهابات.

2. **المطالب المستنبطة:**

✓ وفقا للمطلب الأول هذا الاختراع يتميز بتركيبة شرابية علاجية تحتوي على مستخلص مائي مشتق من نبات Ruta graveolens(السذاب)، مصممة للتخفيف من الاستجابات الالتهابية في الجسم.

✓ وفقا للمطلب الأول هذا الاختراع يتميز باستخدام التركيبة للمساهمة في الحد من مظاهر الالتهاب عبر آليات متعددة تشمل تقليل المؤشرات الحيوية الالتهابية وتحسين التوازن الطبيعي في الاستجابة المناعية .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بكون التركيبة خالية من أي مكونات صناعية مضافة أو محفزات كيميائية، مما يجعلها مناسبة للاستخدام ضمن بروتوكولات الوقاية الطبيعية .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بكونه مصمم على شكل شراب سائل لضمان سهولة الاستخدام والتحكم في الجرعة .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بفعالية ملحوظة في تقليل التغيرات الالتهابية في الأنسجة الحيوية بناءً على اختبارات حيوية أولية مقارنة بمواد مرجعية قياسية .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بكونه يستخدم في تطوير منتج طبيعي وقائي قابل للاستخدام المنتظم بهدف دعم التوازن المناعي وتقليل استجابات الالتهاب غير المرغوب فيها.

✓ وفقا للمطلب الأول هذا الاختراع يتميز بالتركيبة الشرابية القابلة للتعديل في الحجم أو التركيز دون التأثير على الخصائص الفعالة للمستخلص النباتي .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بتركيبة تحتفظ بثباتها الفيزيائي والوظيفي عند تخزينها ضمن مدى واسع من درجات الحرارة ومدة زمنية ممتدة، مما يجعلها مناسبة للنقل والتوزيع التجاري .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بتركيبة قابلة للتعديل في درجة الحموضة (pH) أو الطعم أو اللون باستخدام مكونات طبيعية غير فعالة دون المساس بخصائصها العلاجية الأساسية .

✓ وفقا للمطلب الأول هذا الاختراع يتميز بكون هذا المنتج يمكن تعبئته في وحدات جرعة فردية أو متعددة الجرعات، مما يسمح بتنوع في طرق الاستخدام حسب احتياج المستهلك أو الفئة المستهدفة .

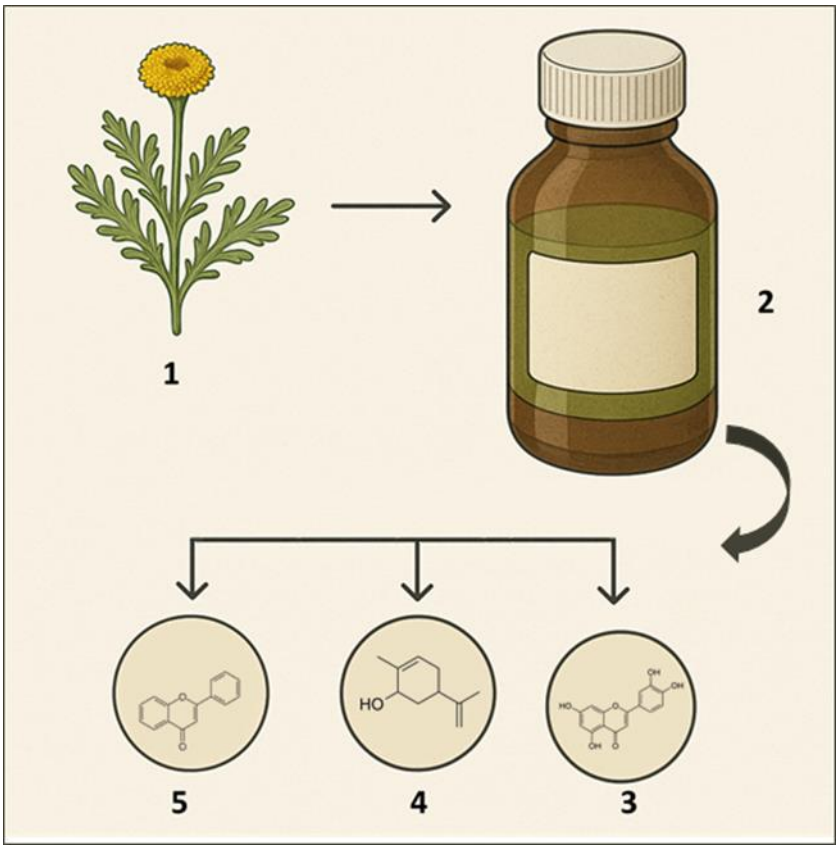
✓ وفقا للمطلب الأول هذا الاختراع يتميز بكونه قابل للإنتاج الصناعي باستخدام معدات استخلاص وتحضير شائعة دون الحاجة إلى تكنولوجيا متقدمة، مما يسهل تطبيقها في وحدات إنتاج محلية أو تجارية.

قائمة الملاحق

الاشكال والرسومات والجداول

الأشكال.

الشكل 1.



الشكل 2.