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جامعة الشهيد حمة لخضر الوادي
University Echahid Hamma Lakhdar - EL OUED
كلية العلوم الطبيعة والحياة
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قسم البيولوجيا الخلوية والجزيئية
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THEME

Pharmacobiological study in vitro and in silico of the extract of the Hypericum perforatum plant

Presented By: **M^{iss} :**Amrani chahrazad

M^{iss} : Larouci aya

M^{iss}: Mekhieber soumia

Before the jury composed of:

Supervisor:	M^{m} .ADAIKA Aicha	Doctor, University of El Oued.
Co-Supervisor:	M^{m}.GUEDIRI Imane	MCB,University of El Oued.
Examinator:	M^{m} .MEDILA Ifriqya	Professor, University of El Oued.
President:	.GHANIA Ahmed	MAA, University of El Oued.

Incubator representative : ZAGHDI Zoubeir

Economic parenter : GANAMI Roukia

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Soumia

Abstract

الملخص

Abstract

This study aimed to evaluate the effectiveness of Hypericum perforatum extract, rich in bioactive compounds such as flavonoids, polyphenols, alkaloids, tannins, and cardiac glycosides, in inhibiting the cyclooxygenase-2 (COX-2) enzyme to reduce the formation of prostaglandins, key mediators in the development of fever and inflammatory responses. The anti-inflammatory activity of the extract was assessed through an in vitro assay examining the inhibition of bovine serum albumin (BSA) denaturation using UV-Vis spectroscopy, as well as via molecular docking simulations with the COX-2 enzyme.

Additionally, a range of other biological activities was investigated, including anti-complement, antioxidant, anti-diabetic, antibacterial, and phagocytic activities. The study also involved the preparation of a topical cream formulation incorporating the plant extract. The extract, rich in active compounds, was integrated into a suitable cream base, taking into account diffusion and stability properties to ensure the effectiveness of the final product. The physicochemical properties of the prepared cream, such as texture, viscosity, and homogeneity, were evaluated, alongside preliminary short-term stability studies.

This initial step aimed to explore the potential for transforming the promising extract into a user-friendly topical pharmaceutical product capable of providing localized relief from inflammation. The results demonstrated that the extracts possess potent anti-inflammatory activity, as evidenced by their IC50 values and negative ΔG values. The negative ΔG values indicated a strong binding affinity to the biological target, and the molecular docking results corroborated the in vitro findings. Furthermore, in simulations of the enzyme–extract interaction, the physicochemical properties, pharmacokinetics, and toxicity profiles of selected extracts were predicted using the SwissADME and ProTox servers.

In conclusion, our findings confirm the effectiveness of Hypericum perforatum extract as an anti-inflammatory agent and open avenues for the development of topical formulations based on it, making it a promising candidate for future topical anti-inflammatory therapeutics.

Keywords: Docking, SwissADME, Cyclooxygenase-2 (COX-2), Hypericum perforatum, Anti-inflammatory ointment, phagocytic activity.

ملخص

هدفت هذه الدراسة إلى تقييم فعالية مستخلص نبات *Hypericum perforatum* (عشبة القديس يوحنا)، الغني بالمركبات النشطة بيولوجيًا مثل الفلافونويدات والبوليفينولات والقلويدات والتانينات والغليكوسيدات القلبية، في تثبيط إنزيم سيكلوأوكسيجيناز-2 (COX-2) للحد من تكوين البروستاجلاندينات، وهي وسائط رئيسية في تطور الحمى والاستجابات الالتهابية.

تم تقييم النشاط المضاد للالتهابات للمستخلص من خلال فحص في المختبر لتثبيط تمسخ بروتين مصل الأبقار (BSA) باستخدام مطيافية الأشعة فوق البنفسجية والمرئية، وعبر محاكاة الإرساء الجزيئي مع إنزيم COX-2. بالإضافة إلى ذلك، تم التحقيق في مجموعة من الأنشطة الأخرى، بما في ذلك الأنشطة المضادة للمتممة، والمضادة للأكسدة، والمضادة لمرض السكري، والمضادة للبكتيريا، والبلعمية.

شملت الدراسة أيضًا تحضير تركيبة مرهم موضعي باستخدام المستخلص النباتي المحضر. تم دمج المستخلص، الغني بالمركبات النشطة، في قاعدة مرهم مناسبة، مع الأخذ في الاعتبار خصائص الانتشار والاستقرار لضمان فعالية المنتج النهائي. تم تقييم الخصائص الفيزيائية والكيميائية للمرهم المحضر، مثل الملمس واللزوجة والتجانس، إلى جانب دراسات استقرار أولية قصيرة الأجل. هدفت هذه الخطوة الأولية إلى استكشاف إمكانية تحويل المستخلص الواعد إلى منتج صيدلاني موضعي سهل الاستخدام يمكن أن يوفر راحة موضعية من الالتهابات.

أظهرت النتائج أن المستخلصات تمتلك نشاطًا قويًا جدًا مضادًا للالتهابات، كما يتضح من قيم IC50 و ΔG الخاصة بها. أشارت قيم ΔG السلبية أيضًا إلى تقارب ارتباط جيد بالهدف البيولوجي، وأكدت نتائج الإرساء الجزيئي النتائج التي تم الحصول عليها في المختبر. علاوة على ذلك، في محاكاة تفاعل الإنزيم مع المستخلص، تم التنبؤ بالخصائص الفيزيائية والكيميائية، والحرائك الدوائية، وسمية المستخلصات المختارة باستخدام خادمي swissADME و Protox.

في الختام، تؤكد نتائجنا فعالية مستخلص *Hypericum perforatum* هذا كمضاد للالتهابات، وتفتح آفاقًا لتطوير تركيبات موضعية منه، مما يجعله مرشحًا واعدًا لأدوية موضعية مضادة للالتهابات في المستقبل.

الكلمات المفتاحية:الإرساء الجزيئي، SwissADME، سيكلوأوكسيجيناز-2(COX-2) ، *Hypericum perforatum*، بومادة مضادة للالتهابات، نشاط بلعمي.

Résumé

Cette étude visait à évaluer l'efficacité d'un extrait de la plante *Hypericum perforatum* (millepertuis), riche en composés bioactifs tels que les flavonoïdes, les polyphénols, les alcaloïdes, les tanins et les glycosides cardiaques, dans l'inhibition de l'enzyme cyclooxygénase-2 (COX-2) afin de réduire la formation de prostaglandines, médiateurs clés dans le développement de la fièvre et des réponses inflammatoires.L'activité anti-inflammatoire de l'extrait a été évaluée par un test *in vitro* d'inhibition de la dénaturation des protéines de sérum bovin (BSA) à l'aide de la spectroscopie UV-Vis, et par une simulation d'amarrage moléculaire avec l'enzyme COX-2.

De plus, une série d'autres activités ont été étudiées, notamment les activités anti-complémentaires, antioxydantes, antidiabétiques, antibactériennes et phagocytaires.L'étude comprenait également la préparation d'une formulation de pommade topique utilisant l'extrait végétal préparé. L'extrait, riche en composés actifs, a été incorporé dans une base de pommade appropriée, en tenant compte des propriétés de diffusion et de stabilité pour garantir l'efficacité du produit final. Les propriétés physico-chimiques de la pommade préparée, telles que la texture, la viscosité et l'homogénéité, ont été évaluées, ainsi que des études de stabilité préliminaires à court terme.

Cette première étape visait à explorer le potentiel de transformer l'extrait prometteur en un produit pharmaceutique topique facile à utiliser qui pourrait procurer un soulagement local de l'inflammation.Les résultats ont montré que les extraits possèdent une activité anti-inflammatoire très puissante, comme en témoignent leurs valeurs IC50 et ΔG. Les valeurs ΔG négatives indiquent également une bonne affinité de liaison avec la cible biologique, et les résultats de l'amarrage moléculaire ont confirmé les résultats obtenus *in vitro*. De plus, dans la simulation de l'interaction de l'enzyme avec l'extrait, les propriétés physico-chimiques, la pharmacocinétique et la toxicité des extraits sélectionnés ont été prédites à l'aide des serveurs SwissADME et Protox.

En conclusion, nos résultats confirment l'efficacité de cet extrait d'*Hypericum perforatum* comme agent anti-inflammatoire et ouvrent des perspectives pour le développement de formulations topiques à partir de celui-ci, ce qui en fait un candidat prometteur pour de futurs médicaments anti-inflammatoires topiques.

Mots-clés: Amarrage moléculaire, SwissADME, Cyclooxygénase-2 (COX-2), *Hypericum perforatum*, Pommade anti-inflammatoire, Activité phagocytaire.

List of Tables

List of Figures

List of Figures

LIST OF ABBREVIATION

Å : Angström

A: Absorbance

AC: Absorbance of control

ADT: AutoDockTools

AG: Gallic acid

AINS: Non-steroidal anti-inflammatory drugs (NSAIDs)

AIS: Steroidal anti-inflammatory drugs

$AlCl_3$: Aluminum chloride

ADME: Absorption, Distribution, Metabolism, Excretion

At: Absorbance of the sample

C: Complement

COX: Cyclooxygenase

COX1: Cyclooxygenase type 1

COX2: Cyclooxygenase type 2

DMSO: Dimethyl sulfoxide

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EAG: Gallic acid equivalent

EGTA: Ethylene Glycol Tetraacetic Acid

EQ: Quercetin Equivalent

FC: Folin-Ciocalteu

g: Gram

H: Hydrogen

I%: Percentage of inhibition

IC50: Half maximal inhibitory concentration

Kg: Kilogram

mg: Milligram

mgEAG/gES: Milligrams of gallic acid equivalent per gram of dry extract

mgER/gES: Milligrams of rutin equivalent per gram of dry extract

Ml: Milliliter

mm: Millimeter

PBS: Phosphate-buffered saline

PDB: Protein Data Bank

pH: Potential of hydrogen

R: Yield

T°: Temperature

TFC: Total Flavonoid Content

TPC: Total Polyphenol Content

°C: Degrees Celsius

kJ: kilojoule

Summary

Chapter III :Materials and methods

Introduction

Introduction

The classical description of inflammation provides an explanation for the visible clinical changes observed during the inflammatory process. The sensation of heat is attributed to increased blood flow through dilated blood vessels in the affected areas, particularly the extremities, where the external environment cools them. This enhanced blood flow also leads to the appearance of redness, resulting from the elevated number of red blood cells passing through the inflamed tissue. Swelling (edema) occurs due to the passage of an increased amount of fluid from dilated and permeable blood vessels into the surrounding tissues, a process further intensified by the infiltration of inflammatory cells, chronic inflammatory reactions, and connective tissue deposition. Pain results directly from the action of inflammatory mediators, whether produced by the initial injury or secreted by the inflammatory cells themselves, as well as from the stretching of sensory nerve endings caused by edema. Finally, loss of function may occur either due to restricted joint mobility in the case of joint inflammation, severe pain, or as a consequence of the replacement of functional cells with scar tissue **(Yahiaoui & Ouldji, 2024).**

Currently, the treatment of inflammation primarily relies on the use of steroidal anti-inflammatory drugs (corticosteroids) and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen. Despite the proven efficacy of these agents in relieving inflammation symptoms, their long-term use is often limited by undesirable side effects. These include gastrointestinal disturbances, an increased risk of cardiovascular disease, and adverse effects on renal function **(Belkacemi, 2014).**

Medicinal plants represent a rich, diverse, and renewable source of biologically active natural compounds with broad therapeutic potential. According to estimates from the World Health Organization (WHO, 2002), more than 80% of the global population particularly in developing countries relies on herbal medicine and traditional healthcare practices to meet their primary health needs. Among plant-derived secondary metabolites, phenolic compounds (including flavonoids, tannins, and phenolic acids) have attracted considerable attention in in vivo and in vitro studies for their antioxidant, anti-inflammatory, and antimicrobial properties **(Alouche & Atik, 2014).**

This memorandum aims to evaluate the anti-inflammatory activity of a specific medicinal plant, Hypericum perforatum. This plant was selected due to its well-established traditional

reputation and widespread use as a natural remedy for inflammations and burns. The objective is to prepare natural ointments with anti-inflammatory properties derived from *Hypericum perforatum* extracts, offering a potential natural, safe, and effective therapeutic alternative to conventional pharmaceutical treatments currently used for inflammation. Through this research, we aim to contribute to the growing body of knowledge on the medicinal potential of this plant, particularly in managing inflammations and burns. The findings of this study may have significant implications in the field of traditional and complementary medicine, offering new perspectives for the development of alternative anti-inflammatory therapies.

Our work is structured into two main parts. The first part consists of a bibliographic study, divided into two chapters:

- **Chapter One** presents general bibliographic information about the medicinal plant under investigation.
- **Chapter Two** focuses on flavonoids and their associated biological activities.

The second part is dedicated to the experimental component of the study, which also includes two chapters:

- **Chapter One** outlines the materials and methods employed in this research.
- **Chapter Two** presents the results obtained and provides a discussion and analysis of these findings.

Finally, a general conclusion summarizes the overall work and proposes future research directions.

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Part I

Literature Review

Chapter I

Medicinal Plant

I. Generalities:

Medicinal plants, with their rich historical legacy and widespread use across various civilizations, continue to attract growing scientific interest for the identification and characterization of their bioactive compounds. Despite the remarkable progress achieved in modern medicine, these plants offer numerous advantages as natural, accessible, and cost-effective therapeutic alternatives particularly in regions where access to contemporary healthcare services remains limited. Globally, there is a noticeable trend towards re-evaluating the medicinal potential of these plants and integrating them into modern therapeutic practices. This approach emphasizes the necessity of rigorous scientific research and responsible use to guarantee both their efficacy and safety, thereby promoting their appropriate inclusion within evidence-based medical frameworks **(Salmerón and *al.*,2020).**

I.2.Studied Medicinal Plants:

I.2.1.Botanical study of Hypericum perfortum:

I.2.1.1. History:

Greece and Italy possess at least two dozen varieties of Hypericum perforatum, although no mention of them is found prior to the 1st century AD. It was during this period that Dioscorides described four distinct species under the names Hyperikon, Askyron, Androsaimon, and *Kor*ês. However, it remains impossible to determine whether our modern Hypericum perforatum was truly among those species **(Paul - Victor F, 2017).**

Frass (1845), however, believed he could identify it with *Askyron*, explaining the name by noting that the plant, when crushed between the fingers, releases a red juice resembling human blood. Pliny the Elder did little more than repeat the descriptions provided by Dioscorides. There is scarce mention of these plants in medieval texts. Saint Albert the Great referred to *Hypericum perforatum* as the "royal crown," and several collections of medicinal recipes from the period recommended its use in the treatment of gout **(Paul - Victor F, 2017).**

The name "Herb of Hypericum perforatum appears to have emerged around the 14th century, likely owing to the fact that the plant reaches its peak flowering period around Saint John’s Day (June 24th) **(Paul-Victor F., 2017; Daovy A., 2008).**

hroughout the Middle Ages, Hypericum perforatum was surrounded by numerous superstitions, particularly in Central Europe. Like many other balsamic plants, it was believed to possess the power to ward off evil spirits, which earned it names such as Fuga daemonum (flight of demons) and chasse-diable (devil chaser) **(Paul-Victor F., 2017).** In the 16th century, the plant gained considerable renown as a vulnerary (a wound-healing agent), with notable figures such as Matthiolus, Paracelsus, Camerarius, and Fallopius promoting its medicinal virtues a tradition later upheld by Scopoli and Geoffroy in the following century. It is possible that this enduring reputation, which Cazin argued should be dismissed as folklore, stemmed from the application of the doctrine of signatures, owing to the plant's reddish juice, previously described, symbolizing blood and thus believed to indicate its efficacy in treating wounds **(Paul-Victor F., 2017).**

By the mid-19 th century, *Hypericum perforatum* had fallen into near-complete disuse within official medicine, surviving primarily within the realm of folk remedies **(Paul-Victor F., 2017).** In 1854, Martin Lauzer remarked: Hypericum perforatum has fallen into such oblivion that it no longer finds a place in the modern formulary a fact that may one day be used against us as evidence of our ignorance **(Paul-Victor F., 2017).** It is worth while to recall this assessment today, particularly as Dr. H. Leclerc later reaffirmed the medicinal value of Hypericum perforatum, describing it as a highly useful antiseptic in the treatment of wounds, ulcers, and burns **(Paul-Victor F., 2017).**

I.2.1.2. Description:

St. John's Wort (Hypericum perforatum L) is a perennial herb belonging to the Hypericaceae family, native to China and widely distributed throughout the temperate regions of Eurasia and northwest Africa. Since the 1st century AD, H. perforatum has been extensively used in medicine due to its well-documented antidepressant, antibacterial, and anti-inflammatory properties. Despite becoming invasive in Oceania and the Americas, it has naturalized globally. Its rapid spread across continents is attributed to high genotypic plasticity and diverse facultative apomixis. Based morphological and geographical variation, the species is classified into four subspecies.

Clustered stamens and the presence of glands are common traits within the Hypericum genus, Hozever H. perforatum. is specifically characterized by translucent glands on its stems and leaves, dark glands on its flowers, and stamens arranged in three distinct bundles. The plant exhibits significant variability in morphology ,However, ploidy levels, and reproductive systems, ranging from sexual reproduction to apomixes. Further genetic resesarch is reauired to enhance our understanding of its , evolutionary history, and breeding potential**(liu and *al*., 2023).**

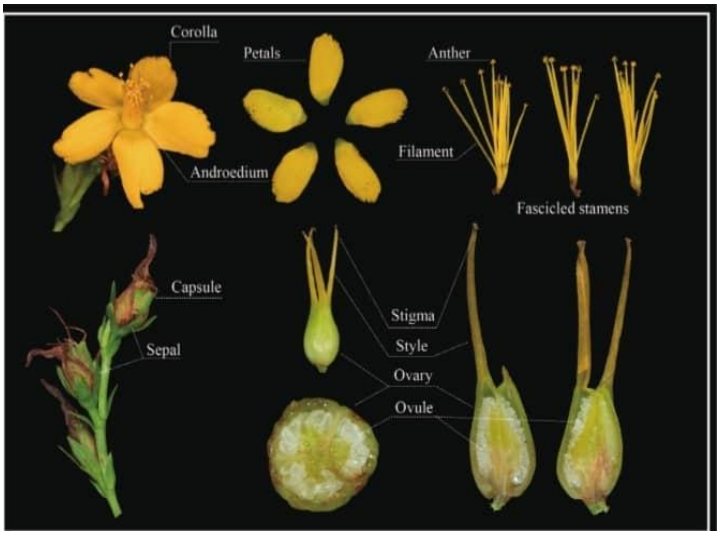


Figure 1: FloralanatomyofH.perforatum**(liu&*al*,2023)**.

he family Hypericaceae Jussieu comprises over 500 species distributed across nine genera worldwide. Generally, the Hypericaceae are classified into three tribes: Cratoxyleae J.D. Hooker & Bentham, Vismieae Choisy, and Hypericeae Choisy. Within the tribe Cratoxyleae, the smallest tribe of the family, two genera are recognized: Cratoxylum Blume and the monotypic genus *K*ielmeyera Cambess. Currently, at least two intraspecific taxa have been identified among three of the seven accepted species of Cratoxylum Blume (Hypericaceae, Malpighiales). **(Sudmoon&*al*., 2022).**

Figure 2: H ypericum perforatum plants in nature **(wikbedia).**

I.2.1.3. Names of the species:

Tableau 1: The different vernacular names of Hypericum perforatum **(Kaddour and Abed.,2023).**

	The names
Arabe classique	El-hamra-Mesmun-Meslikh نبتةالقديس يوحنا
Français	Millepertuis commun-Millepertuis perforé.- Herbe à mille trous - Herbe de la Saint-Jean.- Herbe percée - Herbe aux piqûres. Chasse-diable,trascalan perforé.Trucheran jaune.
Anglais	Saint John's wort. Klamath herb
Allemand	Johanniskraut-Hartheu.-TupfelHarheu. Jagdteufel.

I.2.1.4. Botanical description :

The genus name Hypericum derives from the Greek words hyper (above) and eikon (icon), reflecting the traditional practice of hanging the plant above holy images in homes on St. John's Day to ward off evil spirits. The specific epithet perforatum refers to the presence of tiny oil glands in the leaves, which appear as small translucent "windows" when held up to the light.

The genus Hypericum includes shrubs and herbs characterized by copper or yellow flowers with four to five petals, a single pistil, and numerous stamens. Plants typically range in height from 40 to 80 cm. They feature densely leafed stems and branches, with oval-shaped leaves that have smooth margins, measuring between 1 to 3 cm in length and 0.3 to 1.0 cm in width. The leaves are interspersed with small translucent patches visible when held against the light.

Mature plants produce numerous yellow flowers, each with five petals, usually 1.0 to 2.0 cm in diameter, often exhibiting black spots along the petal margins. When crushed, the

yield a distinctive blood-red dye. By late summer, the flowers give way to cup-shaped fruit capsules containing dozens of tiny, dark brown seeds.Hypericum perforatum is commonly found in fields, meadows, abandoned areas, and roadsides.ines, and abandoned quarries, thriving even in poor soil **(Shrivastava and Dwivedi., 2015).**

I.2.1.5. Distribution by geography :

Hypericum perforatum is a plant native to Europe that has widely naturalized across various regions globally, including North and West Asia, Northern China, North Africa, and North America. This species typically does not grow at elevations above 1600 meters. In Algeria, it is particularly prevalent in hilly areas, thriving in both pastures and woodlands **(Benwie.,2011).**

Figure 3: Geographical distribution of Hypericum perforatum worldwide.

I.3.Phytoconstituents and photochemistry :

St. John's Wort (Hypericum perforatum) contains a wide variety of phytochemicals including phenolic compounds, flavonoids, tannins, alkaloids, hyperforin, and hypericin. These compounds are responsible for the plant's diverse biological activities. The major constituents such as hyperforin are known for their antidepressant effects, while hypericin contributes to antiviral and photodynamic properties **(Meghraj &*al*.,2024).**

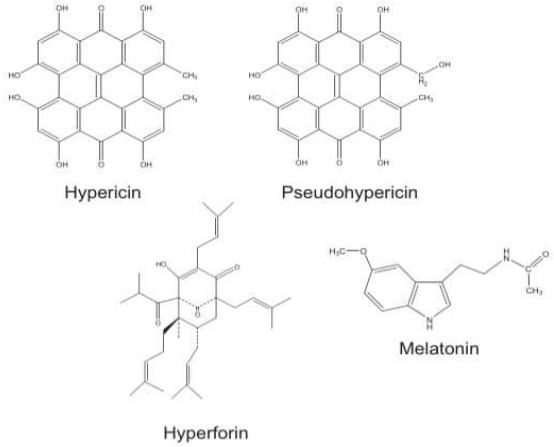


Figure 4 :known metabolites of hypericum perforatum.

I.3.1.Phenolic compounds :

I.3.1.1.Flavonoids :

Flavonoids are present in Hypericum perforatum at varying concentrations, ranging from 7% in the stems to 12% in the flowers and foliage. Examples of biogenetically occurring flavonoids include glycosides such as hyperoside, isoquercitrin, and rutin; biflavones such as biapigenin and amentoflavone; as well as myricetin, hyperin, oligomeric proanthocyanidins, miquelianin, and flavonols such as kaempferol and quercetin **(Shrivastava and Dwivedi., 2015).**

I.3.1.2. Biflavones :

Concentrated in the flowers:

- C-3–C-8" biapigenin (0.1 to 0.5% of the fresh plant) is a biflavonoid compound responsible for the plant's anticancer activity.

- C-3'–C-8" biapigenin, also known as amentoflavone (0.01 to 0.05% of the fresh plant), exhibits pharmacological effects including antidepressant, anti-inflammatory, and antipyretic (fever-reducing) properties **(Ross and *al*., 2013).**

I.3.2.Acylphlorolucionols :

I.3.2.1.Hyperforin :

Isolated from St. John's Wort (Hypericum perforatum, Hypericaceae), of which it is one of the principal constituents (2 to 4% in the leaves and flowers), hyperforin is a lipophilic molecule believed to contribute, predominantly in synergy with other compounds, to the mild antidepressant properties attributed to Hypericum perforatum. It has been proposed that hyperforin exerts its effects by non-specifically inhibiting the reuptake of neurotransmitters such as serotonin and dopamine **(Paul -Victor F.2017).**

I.3.3.Naphthodianthrones :

Naphthodianthrones represent the most extensively studied class of chemical compounds identified in Hypericum perforatum. This class includes isohypericin, protohypericin, hypericin, and pseudohypericin. Among these, hypericin, an anthraquinone-derived pigment, is primarily responsible for the characteristic red color of Hypericum perforatum oils. Hypericin is predominantly present in the flowers, where it appears as black spots along the petal margins. Its chemical structure renders hypericin highly photoreactive**(Shrivastava and Dwivedi., 2015).**

I.3.3.1.Hypericin :

Present in small quantities (<0.3%) in the leaves and flowers of *Hypericum perforatum*, the concentration of the active wound-healing compound hypericin varies according to altitude. It has been observed that hypericin content decreases from approximately 0.6% in plants growing in lowland areas to around 0.05% in those found in mountainous regions **(Kaddour and Abed.,2023).**

Its absorption and ability to cross the blood-brain barrier remain subjects of debate, and consequently, its direct involvement in the mild antidepressant properties attributed to Hypericum perforatum is considered questionable. Hypericin exhibits antibacterial and antiviral activities in vitro. Additionally, it functions as a photosensitizer a property that underlies one of the primary precautions associated with the use of Hypericum perforatum extracts**(Kaddour&Abed.,2023).**

Chapter II

Biological Activities

II.1.Introduction:

Secondary metabolites can be divided into three classes: **polyphenols**, **terpenoids**, and **alkaloids**.

II.1.1.Polyphenols:

Polyphenols, or phenolic compounds, represent a large class of chemical substances found in plants, primarily concentrated in superficial tissues. These polyhydroxylated phytochemicals are characterized by the presence of at least one aromatic ring containing six carbon atoms. Polyphenols are generally subdivided into major subclasses, including phenolic acids**,** flavonoids**,** lignins, and tannins **(Rezzik, 2016).** As these molecules constitute the foundation of the active principles within plants, they play a crucial role in plant physiology, particularly in defense mechanisms against pathogens, including molds and phytopathogenic bacteria. Moreover, they contribute to protection against ultraviolet (UV) radiation, as all phenolic compounds possess the capacity to absorb solar radiation. **(Sarni-Manchado, 2006).**

II.1.1.1.Flavonoids :

A large group of plant-based polyphenolic compounds**,** flavonoids are responsible for the yellow, orange, and red pigmentation observed in many plants. They are found either in their free form (aglycones) or bound to sugars (glycosides) throughout all parts of vascular plants. Flavonoids play an essential role in plant protection, contributing to defense against environmental stressors and pathogens. Additionally, they are important bioactive constituents in numerous medicinal plants traditionally used in various healthcare practices **(Youla and Latrous.,2017)**.

- **Structure of Flavonoids:**

A general term for a group of compounds with a basic carbon skeleton (C6-C3-C6), consisting of two phenyl rings (A and B) linked by a three-carbon unit that forms a central ring (C). These compounds are classified into subclasses based on the arrangement of atoms within ring C, while individual compounds within each subclass differ in the number, type, and position of chemical groups attached to rings A, B, and C **(Youla and Latrous.,2017)**.

II.1.1.2.Tannins:

tannins are bitter plant compounds that serve as a defense mechanism against pests. They cause tissue constriction and protein precipitation, and are utilized in leather tanning, hemostasis, and the treatment of certain skin conditions and diarrhea. Tannins are found abundantly in the bark of oak and acacia trees(**Hachemi and Rafas.,2023)**.

II.1.2.Alkaloids:

A diverse group of natural compounds derived from amino acids. They directly affect the nervous system, causing alterations in consciousness and movement, including inhibition, pupil dilation, anesthesia, and sedation. Alkaloids also possess various pharmacological properties such as antimalarial, antihypertensive, antitussive, central nervous system stimulant, cardiac depressant, and antitumor effects **(Lahcini and *al*., 2022).**

II.1.3.Terpenoids:

A large group of plant-based secondary metabolites that are natural hydrocarbons with cyclic or open-chain structures. Their basic structure is derived from the 5-carbon isoprene unit. They are found in essential oils, contribute to plant scent and taste, and act as pigments, hormones, and sterols **(Ounis &Boumaza., 2017)**.

II.1.3.1.Saponins:

Plant-based organic compounds that produce foam, composed of a water-loving sugar part and a fat-loving lipid part. They have various medicinal benefits as anti-inflammatory, antimicrobial, and anticancer agents, and help in lowering cholesterol (**Sati & *al*.,2014)**.

II.1.3.2.Steroids:

Steroidsare a type of triterpene characterized by their tetracyclic ring structure and contain fewer than 30 carbon atoms. Steroids are produced from acyclictriterpene compounds.**(Hachemi and Rafas.,2023).**

II.1.3.3.Cardiac glycosides:

Natural compounds that inhibit the sodium-potassium pump, historically used to treat heart conditions. They may act as hormones in mammals and influence various bodily functions

via nuclear receptors, including immunity and cancer. They are a basis for developing new drugs **(Melero,2015).**

II.1.4.Quinones:

Common compounds within the quinoid family, characterized by a specific structure and produced from the metabolism of aromatic compounds. They play a significant role in the side effects of drugs and chemicals and contribute to mutations and toxic reactions, but are difficult to detect **(Bolton and *al*.,2016).**

II.The biological activities of the plant Hypericum perforatum:

II.2.Anti-inflammatory activity:

II.2.1.Inflammation:

The body's response to defend and repair against damage or infection. Various cells and a network of chemicals are involved. It causes general symptoms (fever) and local symptoms (swelling). It's necessary for healing, but non-vascular and epithelial tissues have different roles in it **(chekirou and Zeroual., 2023**).

II.2.2.General Overview:

Cyclooxygenase (COX), also known as prostaglandin G/H synthase (PGHS), is a key enzyme involved in the metabolic cascade of arachidonic acid ($C_{20}H_{32}O_2$). It plays a central role in the production of several bioactive chemical mediators, most notably the prostanoids, which are crucial contributors to inflammatory responses [1]. COX was first identified in 1971 as the primary molecular target of nonsteroidal anti-inflammatory drugs (NSAIDs) [2], and it was subsequently isolated and purified in 1976 [3]. This enzyme exists in three distinct isoforms: COX-1, COX-2, and COX-3. Each isoform is characterized by unique patterns of expression and physiological functions within the body **(Gheddab, N. 2016).**

II.2.2.1.The COX-2 Enzyme:

COX-2 was first identified in 1991. In humans, this enzyme consists of 581 amino acids and has a molecular weight of approximately 74 kda. Its sequence shares about 60% homology with that of COX-1. COX-2 is encoded by a gene located on chromosome 1 and is primarily classified as an inducible enzyme, responding to inflammatory stimuli. However, it

is also constitutively expressed in certain tissues, such as the brain and kidneys**(Gheddab, N. 2016)**.

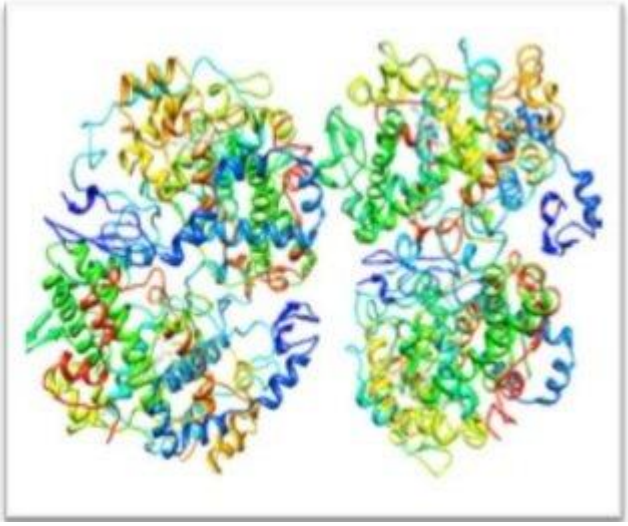


Figure 5 : structure of cycloogenase_2

II.2.3.Anti-inflammatory :

II.2.3.1.Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) :

A group of medications classified by their chemical structure. They inhibit the COX-1 and COX-2 enzymes, reducing the production of prostaglandin E2 and thromboxane A2, which are involved in inflammation. COX-1 is always present and protects the stomach and helps with blood clotting. COX-2 is activated during inflammation. Traditional NSAIDs inhibit both enzymes, while newer types selectively target COX-2 to reduce side effects **(Mekenza & Medjmedj.,2018).**

II.2.3.1.1. Diclofenac Metabolism:

II.2.3.1.1.1.Properties Pharmacokinetic:

- ✓ **Absorption:** Diclofenac is completely absorbed orally, but only about half of the dose becomes available in the body due to first-pass metabolism in the liver. Food does not significantly affect its absorption.

- ✓ **Distribution:** Diclofenac distributes in the body with an estimated volume of 1.4 L/kg and is highly bound (over 99%) to plasma proteins, mainly albumin. It diffuses into synovial fluid, but its significance for efficacy is unclear.
- ✓ **Metabolism:** Diclofenac is metabolized in the liver into five metabolites, one of which (4'-hydroxy-diclofenac) has weak pharmacological activity. These metabolites are excreted via bile.
- ✓ **Excretion:** A small amount of unchanged diclofenac is eliminated in the urine, while most of its metabolites (about 65%) are excreted via urine and the rest via feces. The elimination half-life of diclofenac from plasma ranges between 1.2 and 2 hours, and about 35% of the dose undergoes enterohepatic circulation **(Nagoudi & *al*., 2023).**

II.2.3.1.1.2. Pharmacodynamic Properties :

- ✓ **Mechanism of Action:** Its effect lasts longer than its half-life due to its concentration in synovial fluid. It inhibits the COX enzyme to reduce prostaglandin (less harmful to the stomach than some other NSAIDs). It may also inhibit LOX and affect phospholipase A2, contributing to its anti-inflammatory, antipyretic, and analgesic effects **(Nagoudi & *al*., 2023).**

II.2.3.2.Steroidal Anti-inflammatory Drugs (SAIDs) :

Medications derived from cortisol, highly effective for treating chronic inflammatory and autoimmune diseases. They are natural or synthetic hormonal substances, characterized by their steroid structure and potent anti-inflammatory properties. However, their use is associated with potential side effects that increase with treatment duration and dosage. **(Ferhaoui & *al*.,2023).**

II.2.3.2.1.Prednisone Metabolism :

II.2.3.2.1.1. Pharmacokinetic Properties :

- ✓ **Absorption:** Prednisone is absorbed rapidly (80%) from the upper small intestine and reaches peak plasma concentration within 1-2 hours. It is converted in the liver to the active prednisolone. Prednisone has better absorption than prednisolone metasulfobenzoate.

- ✓ **Protein Binding:** Prednisone, prednisolone, and methyl prednisolone are highly bound to plasma proteins (around 90% for prednisone and prednisolone, and 77% for methyl prednisolone) by two carrier proteins.
- ✓ **Metabolism:** Prednisone is primarily metabolized in the liver by enzymes such as "11β-hydroxysteroid dehydrogenase" and "20-keto-steroid reductase," but the precise pathways are not fully understood.
- ✓ **Excretion:** Prednisone and its metabolites are mainly excreted in the urine (80% as conjugated metabolites and 20% as unchanged prednisolone). The mean plasma elimination half-life of corticosteroids is 1.5-3.5 hours.

II.2.3.2.1.2. Pharmacodynamic Properties :

Mechanism of Action at the Tissue Level :

- **Effect of Steroidal Anti-inflammatory Drugs (SAIDs):** They work by reducing inflammatory cell movement, inhibiting the production of vasoactive substances, and modulating immune cell function.

- ✓ **Phytochemical Compounds:** Many possess anti-inflammatory properties and affect various stages of the inflammatory response, such as inhibiting cell activation and cytokine production. Some target specific proteins like COX-1/2, LOX, NO, and PLA2, potentially offering therapeutic benefits with fewer side effects than traditional drugs**(Nagoudi & *al.*, 2023).**

II.3.Antioxidant Activity :

II.3.1.Oxidative Stress and Antioxidants :

Oxidative stress is a condition that arises from an imbalance between free radicals and antioxidants in the body. In the context of metabolic processes, our bodies produce free radicals capable of initiating chain oxidation reactions. These reactions harm the body's cells and accelerate the signs of aging. Fortunately, the body possesses mechanisms to maintain this balance by producing antioxidants simultaneously with free radicals **(Dahel & Messaoudi.,2019).**

II.3.2.Definition of Oxidative Stress:

In biological systems, oxidative stress occurs as a result of an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms that work to eliminate them **(Ait Yahia & Zemmoura., 2014).**

Free Radicals: This is a comprehensive term referring to reactive oxygen species (ROS) and reactive nitrogen species (RNS). These are chemical entities (molecules or simple atoms) characterized by the presence of one or more unpaired electrons in their outer orbital, which gives them high chemical reactivity. Due to this unpaired electron, a free radical always seeks to regain stability by capturing another electron**(M'cili & melghid., 2020).**

II.3.3.Sources of Free Radicals :

ROS can be produced by physical agents such as radiation, chemical reactions, and especially enzymatic reactions. Indeed, any reaction involving O2 and an electron-transfer reducing system is likely to release ROS. This is how the respiratory chain causes a significant release of ROS. Other enzymatic activities also provide ROS, notably NADPH oxidases during inflammation and cytochromes P450 during the detoxification of xenobiotics. Thus, the mitochondria, the plasma membrane, and the endoplasmic reticulum are the main sites of ROS release. The endogenous and exogenous sources producing ROS are numerous **(M'cili & Melghid., 2020).**

II.3.4.Biochemical Consequences of Oxidative Stress :

Excessive production of free radicals leads to significant damage to essential biological molecules in the body, such as DNA, proteins, and lipids. As a consequence of this initial damage, secondary effects arise, including cytotoxicity and mutagenicity, due to the resulting substances, especially those generated during the process of lipid oxidation**(Boukerikaand *al*.,2019).**

II.3.5.Pathologies Related to Oxidative Stress :

Oxidative stress plays a pivotal role in the onset and progression of numerous serious diseases that significantly impact human health. It is considered a triggering factor for conditions such as cancer, neurodegenerative diseases (like Alzheimer's and Parkinson's), immune complications, and diabetes. Furthermore, oxidative stress is linked to pregnancy complications arising from placental issues and the development of rheumatic diseases. Notably, the risk of developing these diseases increases with age due to a decline in thebody's

antioxidant defense capacity and an increase in free radical production resulting from a progressive dysfunction of the mitochondrial **(Guillet-pichon & Verny, 2016).**

II.3.6.Definition of Antioxidants :

Antioxidants are chemical compounds characterized by their effective ability to minimize oxidation processes in food, such as preventing rancidity and delaying lipid peroxidation, without negatively affecting the sensory (like taste and smell) and nutritional properties of the food product. These compounds contribute to maintaining the quality of the product and extending its shelf life.

Furthermore, an ideal food antioxidant can be defined as a substance that is soluble in fats, highly effective at low doses, non-toxic, and does not cause any undesirable changes in the color, odor, or taste of the product. It also possesses the ability to withstand various technological processes and maintains its stability in the final product**(Hellal, 2011).**

II.3.6.1.Enzymatic Systems :

Antioxidant enzymatic proteins constitute the first line of defense in this antioxidant protection system. It consists of three essential metallo enzymes: superoxide dismutase, complementary and ensure the elimination of superoxide anions and hydrogen peroxide in all intracellular compartments **(Cano & *al.*, 2007)**.

II.3.6.1.1.superoxide Dismutase (SOD) :

Superoxide dismutases, the first line of defense against ROS, are present in almost all aerobic cells and in extracellular fluids. SODs catalyze the conversion of the superoxide anion into dioxygen and hydrogen peroxide **(Kabel, 2014).**

$$O2^{\bullet-} + 2\ H \rightarrow H2O2 + O2$$

II.3.6.1.2.Catalase (CAT):

is an intracellular enzyme found specifically in the peroxisomes of hepatocytes, erythrocytes, and renal cells **(Castaldo & *al.*, 2016).** This enzyme is characterized by its high capacity to convert hydrogen peroxide molecules into water and molecular oxygen atan astonishing rate of approximately 6 million molecules per minute for each catalase molecule. Consequently, catalase plays a vital role in catalyzing the detoxification reaction of hydrogen peroxide

(H2O2) **(Starlin & Gopalakrishnan.,2013)**. in catalyzing the detoxification reaction of hydrogen peroxide (H2O2) **(Starlin & Gopalakrishnan., 2013).**

$2H_2O_2 \rightarrow 2\ H_2O + O_2$

II.3.6.1.3.Glutathione Peroxidase (GPx):

constitutes a family of enzymes that is distributed in various locations inside and outside cells, including extracellular fluids, the cytoplasm, and cell membranes. Its primary function is to catalyze the transformation reaction of both hydrogen peroxides and lipid peroxides (ROOH) into less harmful compounds, namely water and alcohol (ROH).

During this reaction, two molecules of reduced glutathione (GSH) undergo oxidation to become glutathione disulfide (GSSG) **(Omodanisi & *al.*, 2017).**

$2H_2O_2 + 2\ GSH \rightarrow 2\ H_2O + GSSG$

$ROOH + 2\ GSH \rightarrow ROH + GSSG + H_2O$

II.3.6.1.4.Glutathione Reductase (GR):

Glutathione reductase is an NADPH-dependent oxidoreductase located in the cytosol and mitochondria. It catalyzes the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) **(Csiszár & *al*., 2016).**

$GSSG + NADPH \rightarrow 2\ GSH + NADP^+$

II.3.6.2.Non-Enzymatic Systems :

Unlike antioxidant enzymes, most of these components are not synthesized by the body and must be obtained through diet. There fore, they can be divided into endogenous and exogenous antioxidants.

II.3.6.2.1The Endogenous Non-Enzymatic Antioxidant System :

This group of antioxidant systems encompasses numerous endogenous substances, among which we can cite glutathione, uric acid, bilirubin, lipoic acid, and coenzyme Q.

- **Glutathione (GSH):** A tripeptide important in detoxification and protecting cells from oxidation **(Beaudeux & Geneviève., 2011).** Under normal conditions, most glutathione exists in its reduced form (GSH). During oxidative stress, it converts to its oxidized form (GSSG or GSSR). Glutathione plays a role as a co-factor for antioxidant enzymes and interacts synergistically with vitamins C and E in protecting the body from oxidation **(Stamler & *al.*, 1996).**
- **Uric Acid:** It accumulates in the human body due to the absence of an enzyme to convert it to allantoin. Despite this, it possesses strong antioxidant properties and acts to scavenge free radicals at the body's natural pH**(Laraba and *al.*,2016).**
- **Bilirubin:** A final product of heme breakdown, mainly resulting from the catabolism of hemoglobin. It's fat-soluble and protects albumin and fatty acids from free radical damage by scavenging them. It can be oxidized and recycled by a specific enzyme**(Laraba and *al.*, Ouassaa 2016).**
- **Lipoic Acid:** A potent antioxidant that can regenerate other antioxidants like vitamins C and E. It scavenges various free radicals, chelates heavy metals, reduces glycation, and may aid in DNA repair. The liver produces it in small amounts, and it's also found in certain foods. It exists in bothoxidized and reducedforms **(David, 2015).**
- **Coenzyme Q10 (Ubiquinone):** A fat-soluble compound found in all body cells, their membranes, and lipoproteins. The body synthesizes it from the same precursor as cholesterol. It is also present in some foods like nuts, liver, sardines, whole grains, and vegetables,It functions as a coenzyme for numerous enzymes in the electron transport chain within the mitochondria, which is essential for the production of energy (ATP) from carbohydrates and fats during cellular respiration. It also enhances the antioxidant effect ofvitamin E, and all energy-requiring physiological processes depend on it **(Canavyand *al.*, 2014).**

II.3.6.2.2.The Exogenous Antioxidant System :

The body possesses a second line of defense, "free radical scavengers, which are compounds mostly obtained through diet. Their essential role is to neutralize the toxic effects of Reactive Oxygen Species (ROS), thereby limiting any damage to cellular integrity.

Vitamin E (Tocopherols): A primary fat-soluble antioxidant in blood plasma and red blood cells. Alpha-tocopherol is the most active form. It scavenges singlet oxygen and hydroxyl

radicals, but its main role is reacting with peroxyl radicals to form a tocopherylradical**(Laraba and *al* .,2016).**

Vitamin C: A water-soluble antioxidant that protects cells by scavenging free radicals and regenerating vitamin E. It's important for the eyes and helps prevent fat oxidation, but it requires the absence of free transition metals to function **(Evans, 2000).**

Beta-carotene: Belongs to the carotenoid family found in fruits and vegetables, and it's a potent antioxidant. It scavenges hydroxyl and peroxyl radicals, inhibits lipid peroxidation, and neutralizes singlet oxygen. It also serves as a precursor to vitamin A **(Beaudeux & Geneviève., 2011).**

II.4.Antibacterial Activity :

II.4.1.Antibacterial Agents :

An antimicrobial agent is defined as any chemical, physical, or biological agent that inhibits the growth and/or survival of microorganisms .There are also chemotherapeutic agents, which inhibit the development of their target or kill it while being harmless to the host. This group includes antibiotics, antifungals, and antivirals **(Dahel & Messaoudi,2019).**

II.4.2.1.Antibiotics :

II.4.2.1.1.Definition :

An antibiotic (from the Greek anti: against, biotikos: concerning life) is a chemical substance produced by a microorganism that has the ability to selectively inhibit the growth of other microorganisms and even destroy them when present in a diluted solution. Healthcare professionals and laypeople frequently refer to substances used for therapeutic purposes in the treatment of bacterial diseases in humans and animals as "antibiotics"**(Bireche & Haderbache.,2023).**

II.4.2.1.2.Mode of Action of Antibiotics (Bacterial resistance and sensitivity) :

Antibiotics specifically block vital metabolic processes of susceptible bacteria, thus halting their development, most often only temporarily (bacteriostatic effect) but sometimes permanently (bactericidal effe).

There are different types of antibiotics capable of acting on bacteria through various mechanisms:

- ✓ Antibiotics that inhibit bacterial cell wall synthesis.
- ✓ Antibiotics that alter the permeability of the plasma membrane.
- ✓ Antibiotics that inhibit protein synthesis.
- ✓ Antibiotics active against the metabolism of nucleic acids and theirprecursors **(Brahima and *al*.,2016).**

II.5. anti-diabetic activity :

II.5. 1.Definition and Diagnosis of Diabetes :

Diabetes is a progressive and complex metabolic disorder primarily characterized by an abnormal increase in blood glucose levels, known as hyperglycemia. In most cases, diabetes is type 2. The enzyme α-D-glucosidase, found in the intestines, breaks down carbohydrates. Inhibiting α-D-glucosidase can slow down glucose production and absorption. Studies suggest that α-D-glucosidase inhibitors may help prevent diabetes and obesity.

Type 2 diabetes leads to disorders in insulin secretion from beta cells in the pancreas and resistance of peripheral tissues to the effects of insulin, resulting in elevated blood glucose levels. Diabetes is considered a major risk factor for cardiovascular diseases, as it increases the risk of coronary artery disease, myocardial infarction, in addition to strokes. It appears that maintaining blood sugar balance may prevent or delay the onset of vascular complications. The therapeutic approach to maintaining blood sugar control includes stimulating insulin secretion, increasing glucose utilization by peripheral tissues, and reducing its production in the liver or reducing carbohydrate absorption in the intestines **(Derbal and Benladjila.,2021).**

II.5.2. Alpha-amylase :

II.5.2.1.Definition :

Alpha-amylase is considered a large biomolecule belonging to the group of globular proteins, similar to all enzymes. This enzyme is characterized by its wide distribution, as it is produced in various living organisms. It is an endo-enzyme that randomly hydrolyzes the α 1-4 glycosidic bonds in molecules such as amylose, amylopectin, starch, and glycogen,

with the exception of the bonds found at the ends of these chains. As a result of this hydrolysis, glucose, maltose, and, particularly, a group of sugars known as alpha-dextrins are produced. Its molecular weight typically ranges between 40 and 70 kDa **(Sahraoui and Youmbai,.2021).**

II.5.2.2. Nomenclature :

- **Codified Name:** EC 3.2.1.1
- **Common Name:** alpha-amylase
- **Other Name(s):**glycogenase, α-amylase; endoamylase; Taka-amylase A, maxilase.
- **SystematicName:**1,4-alpha-D-glucan-4-glucanohydrolase

(Schamburg &Slzmann., 1991 ;Dauter & *al*., 1999 ;Nouadri., 2011).

II.5.2.3.The types Alpha-amylase :

The human body, the amylase enzyme is mainly produced in two locations: the salivary glands (where it is known as S-amylase) and the pancreas (where it is known as P-amylase). The production of these two types of amylase is controlled by two different genes, Amy1 and Amy2A, both of which are located on chromosome number 1. There are two main types of salivary S-amylase: type A, which is a glycosylated protein with a weight of 62 kDa, and type B, which is a non-glycosylated protein with a weight of 55 kDa**(Sahraoui and Youmbai.,2021).**

II.5.2.4.The role of alpha-amylase :

The digestion of starch, the main source of carbohydrates, begins in the mouth with the enzyme alpha-amylase found in saliva. This enzyme breaks down the α(1-4) bonds in starch into smaller fragments, but it cannot break the terminal bonds or those near branching points. When food reaches the acidic stomach, the action of salivary alpha-amylase stops. The digestion of starch is completed in the small intestine by the enzymes beta-amylase and pancreatic alpha-amylase. These enzymes further break down starch into disaccharides (maltose), trisaccharides (maltotriose), and oligosaccharides (dextrins with α(1-6) branches). Finally, specific enzymes located in the small intestine break down these oligosaccharides into monosaccharides for absorption **(Voet.D & Vo & .J., 2005).**

II.5.2.5The mechanism of action of alpha-amylase :

relies on three essential amino acids in its active site: aspartic acid (Asp231 as a nucleophile), glutamic acid (Glu261 as a proton donor), and another aspartic acid (Asp328as a catalyst assistant). The enzyme's activity is significantly affected by pH and temperature.

The hydrolysis process occurs in four steps:

1. **Formation of the enzyme/substrate complex:** The enzyme binds to the starch chain.
2. **Attack on the α(1-4) bond:** Glutamic acid (in its protonated form) donates a proton to break the glycosidic bond, while aspartic acid forms a temporary covalent bond with one end of the cleaved chain.
3. **Return of the enzyme to its original state:** This is achieved by a water molecule attacking the carbon atom bonded to aspartic acid, leading to the breaking of the bond and the release of the first part of the chain.
4. **Release of the second part of the chain:** After glutamic acid returns to its original state, the second part of the chain is freed.Glutamic acid acts as an acid catalyst, while aspartic acid acts as a basic catalyst in facilitating the breaking of the glycosidic bond. **(Kolli & *al*.,2015).**

II.6.immunomodulatory activities Immune reaction of the response to inflammation :

II.6.1.Immune system :

A complex system of organs and cells working in coordination to defend the body against infection. It has the remarkable ability to recognize and remember millions of threats and produce a specialized immune response to eliminate them **(Benlahoues & Marhaache., 2016).**

II.6.2.Types of the Immune System :

II.6.2.1. Innate immune :

Innate immunity is a primary, non-specific immune response that occurs when the body is exposed to pathogens.

This immunity utilizes cells such as phagocytes, which respond to stimuli in a way that grants them the ability to mount a faster and more effective response upon subsequent exposure to similar stimuli.

This response involves functional changes in cells after exposure to stimuli, leading to a state known as "trained immunity **(O'Farrell & *al*.,2025).**

The four main components of innate immunity include the following:

- Epithelial barriers of the skin.
- Phagocytic leukocytes (neutrophils and macrophages).
- Natural killer (NK) cells.
- Plasma proteins of the complement system.**(Benlahoues & Marhaache., 2016).**

The innate immune response serves as the first line of defense against infections and injuries, involving natural barriers like the skin and pH levels. When these barriers are breached, pathogens encounter immune cells such as macrophages and neutrophils that recognize them via TLRs and NLRs. These cells secrete inflammatory substances and enzymes to eliminate microbes. Macrophages are crucial resident inflammatory cells in tissues with diverse roles, and subtypes like M1 (for pathogen elimination) and M2 (for tissue repair) have been identified. M1 macrophages, activated by type 1 cytokines, are particularly important in recognizing microbes and infected/cancerous cells, as well as their role in antigen presentation to the adaptive immune system**(Bounihi, 2016)**.

Plasma Mediators :

- **Plasma kinins:** are peptide molecules present in the bloodstream. They exhibit vasodilatory properties and enhance vascular permeability, thereby facilitating the release of prostaglandins. Among them, bradykinin is the most biologically active. It
- plays a dual role by promoting vasodilation as well as contributing to intracapillary stasis through vasoconstrictive mechanisms **(Bounihi, 2016).**
- **The Complement System:**

The complement system is a sophisticated network of proteins that is highly conserved throughout evolution. As its name implies, it complements immune and inflammatory responses. It constitutes a critical component of the innate immune defense against microbial invasion. Beyond this, it plays essential roles in synaptic pruning, immune complex clearance,

complex clearance, angiogenesis, mobilization of hematopoietic stem cell progenitors, tissue regeneration, and lipid metabolism **(Millet, 2014).**

The complement system is activated via two main pathways:

- **The Classical Pathway:** This pathway is initiated when the C1q protein binds to antibodies already attached to an antigen on the surface of a pathogen or infected cell.
- It relies on antibodies and is considered part of the adaptive immunity. It leads to the formation of C3 convertase (C4b2a) which cleaves C3.
- **The Alternative Pathway:** This pathway is directly activated on the surface of a pathogen or foreign material by C3b. It does not depend on antibodies and is considered part of the innate immunity. It leads to the formation of C3 convertase (C3bBb) which amplifies the production of C3b.

Both pathways converge at the formation of the C3 convertase enzyme, which cleaves C3 into C3a and C3b, leading to functions such as opsonization, the formation of C5 convertase, and the terminal pathway resulting in the Membrane Attack Complex (MAC) **(Millet, 2014).**

II.6.2.2.Adaptive immunity

A specialized immune response relying on B and T cells to target specific pathogens. B cells produce antibodies and memory cells. T cells (cytotoxic and helper) recognize antigens presented by MHC, destroy infected cells, and coordinate the immune response. These cells form the basis of adaptive immunity, allowing for a better response to repeated exposures, but it faces challenges in chronic infections due to immune evasion**(Bizimana,2024).**

II.7. Formulation and quality control of the ointment :

II.7.1. Definition :

They are preparations with a soft consistency intended for application to the skin, with the aim of exerting a local action or achieving percutaneous absorption of the active substances**(Koné, 1994).**

II.7.2. Therapeutic benefits of ointments :

In addition to their moisturizing and protective effects on the skin, ointments work to regulate the skin's pH level back to normal. They have a systemic effect via the skin without passing through the liver. Besides their application to the skin, they can be applied to the rectal, vaginal, and conjunctival mucous membranes**(Koné, 1994).**

II.7.3. Classification of Ointments :

According to the classification by LEHIR, we have different types of ointments:

- Hydrophobic Ointments

They can normally absorb only small quantities of water. The most commonly used excipients for the manufacture of such ointments are: vaseline, paraffin, vegetable oils or animal fats, synthetic glycerides, waxes, and liquid polyalkyl siloxanes.

- Water-absorbing Ointments

These ointments can absorb larger quantities of water. Their excipients are those of a hydrophobic ointment in which water-in-oil (W/O) type emulsifiers such as wool fat, wool fat alcohols, sorbitan esters, monoglycerides, and fatty acids are incorporated.

- Hydrophilic Ointments

These are preparations whose excipients are miscible with water. These bases are usually composed of mixtures of liquid and solid polyethylene glycols. They may contain appropriate amounts of water**(Dembélé, 2011).**

II.8.Evaluate the anti-inflammatory activity in silico :

Introduction :

This research focuses on studying the active compound extracted from a plant to evaluate its anti-inflammatory activity under laboratory conditions (in vitro). The researchers will employ various analytical techniques, notably Ultraviolet-Visible (UV-Visible) spectroscopy. Furthermore, computational methods will be used to analyze the compound's properties and its potential interaction with certain drugs and the cyclooxygenase enzyme, aiming to theoretically support the experimental findings.

II.8.1. Principle of Ultraviolet-Visible Spectroscopy :

In a molecule, the electronic transitions in the ultraviolet-visible (UV-Vis) range involve the highest energies in chemistry (approximately 13,000 to 50,000 cm^{-1}, which is 160 to 665 $kJ.mol^{-1}$). The order of magnitude of the energies involved is the same as that of the bond energies of molecules, and these radiations can sometimes cause bond breakage.

More generally, they cause electronic transitions between the different energy levels of the molecules **(Baudouin, 2012).**

II.8.1.1.Beer-Lambert Law :

The absorbance of monochromatic radiation is directly proportional to the length of the absorption path (ℓ) and the concentration of the species in the medium (C). The concentration is generally expressed in moles per litter (mol L^{-1}) and the length in centimeters (cm), resulting in the following relationship**:**

$$A=\log(I0/I)=\varepsilon lC$$

Where:

A: Absorbance, formerly called optical density (O.D.) (unitless)

ε: Molar extinction coefficient ($mol^{-1}.cm^{-1}.L$)

C: Concentration (mol/L) l: Path length of the cuvette (cm)

I0: Intensity of the irradiation energy arriving at the sample (incident light)

I: Intensity of the radiation that has passed through the sample (transmitted light) **(Aouiche, and Beggas., 2021).**

II.8.2. Molecular docking :

Molecular docking is a computational technique that predicts how two molecules bind together by simulating the shape and orientation of a ligand (like a drug) when it connects with a target protein. This method helps in understanding the interactions between molecules and identifying which molecules can bind to a specific protein.**(Tao & *al.*, 2020; Taj & *al.*, 2021).**

II.8.2.1.Types of Molecular Docking :

he types of molecular docking vary in their complexity and how they handle the flexibility of the interacting molecules. Rigid docking is the simplest and fastest, treating the molecules as fixed entities, which is suitable for protein-protein interactions. Next is semi-flexible docking, which allows the ligand to change its shape while the protein remains fixed, common in protein-ligand interactions but potentially less accurate in some cases.

Finally, flexible docking, the most comprehensive but also the most computationally demanding, considers the flexibility of both molecules while imposing constraints toachieve a balance between speed and accuracy in predicting molecular interactions.**(Lebbad, 2016 ; El Hadji Said, 2016;Bouchagra,2018).**

II.8.2.2.Principle :

Molecular docking relies on simulating the process of two or more molecules coming together and forming a stable complex. Imagine trying to assemble two Lego pieces; molecular docking tries to find the best way for these pieces to fit together **(Asses,2011;Lanez,2016).**

II.8.2.3.Steps of Molecular Docking :

The docking process is iterative, and each calculation step is divided into two stages:

II.8.2.3.1.MolecularDocking with AutoDockTools :

In molecular docking, the ligand is placed in the target's active site, and multiple shapes, positions, and orientations are tested. Only the best ones are selected, making docking software a crucial tool in biology, pharmacy, and medicine for discovering drugs that interact with biological targets **(EL Hadj,2016;Lanez,2016).**

II.8.2.3.2.Modeling of Energy Potential :

To calculate the free energy of the ligand-receptor complex, AUTODOCK utilizes terms from a traditional force field to which two entropy-related terms are added. The energy is given by the following equation:

$$\Delta G=\Delta Gvdw+\Delta Ghbond+\Delta Gelec+\Delta Gconform+\Delta Gtor+\Delta Gsol$$

Where:

- ΔGvdw: Energy of atomic dispersion/repulsion

- ΔGhbond: Energy of hydrogen bonds
- ΔGelec: Energy of electrostatic interactions
- ΔGconform: Energy of deviations from covalent geometry
- ΔGtor: A term that reflects the increase in system energy due to the restriction of the ligand's free rotors and the restriction of the ligand's rotations and translations upon complexation with the receptor
- ΔGsol: Another entropy-related term that describes the energy variations of the system during ligand desolvation upon complexation with the receptor.

Passing through an empirical relationship of the QSAR type is necessary to relate the structure of the complexes and the free energy of binding. The empirical model used in AUTODOCK is a multiple linear regression of the different terms of the free energy equation. Each term is thus weighted by a coefficient derived from an extensive set of receptor-inhibitor complexes for which the inhibition constant Ki is known. The relationship between the inhibition constant and the 1 free energy of binding is given by the following equation:

ΔG=-RTlnK(Azeb & Azza.,2023).

II.8.3.SwissADME:

Figure 6: Homepage of the swiss ADME server**.**

SwissADME is a free and user-friendly web tool that offers rapid and reliable predictive models for evaluating crucial properties of molecules in drug discovery. These include physicochemical properties, pharmacokinetics (ADME), drug-likeness, and medicinal chemistry friendliness. Both experts and non-specialists can easily use it to quickly obtain predictions that support their research **(Daina & *al.*,2017).**

Figure 7: Homepage of the swiss ADME server**.**

II.8.4.Protox :

The free web server ProTox-II (http://tox.charite.de/protox_II) enables efficient prediction of chemical compound toxicity based on their 2D structure. It provides detailed results with confidence measures, a summary of overall toxicity, and identifies similar compounds with known acute toxicity.**(Banerjee & *al*., 2018).**

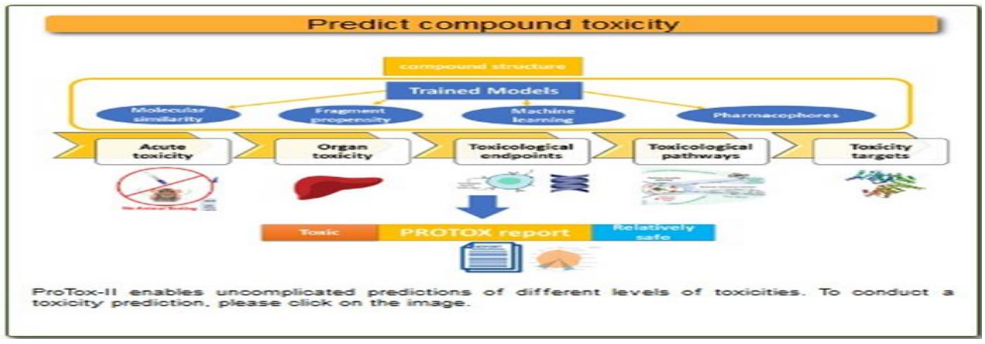


Figure 8: Homepage of the protox server**(Banerjeea & *al*.,2018).**

II.8.5.PubChem

PubChem is a major and free chemical database launched in 2004, offering comprehensive information on small and large molecules (like drugs and biomolecules) to scientists, students, and the general public through its website and programming services. This information includes chemical structures, physical and chemical properties, biological activities, toxicity data, and much more**(Trad, 2020).**

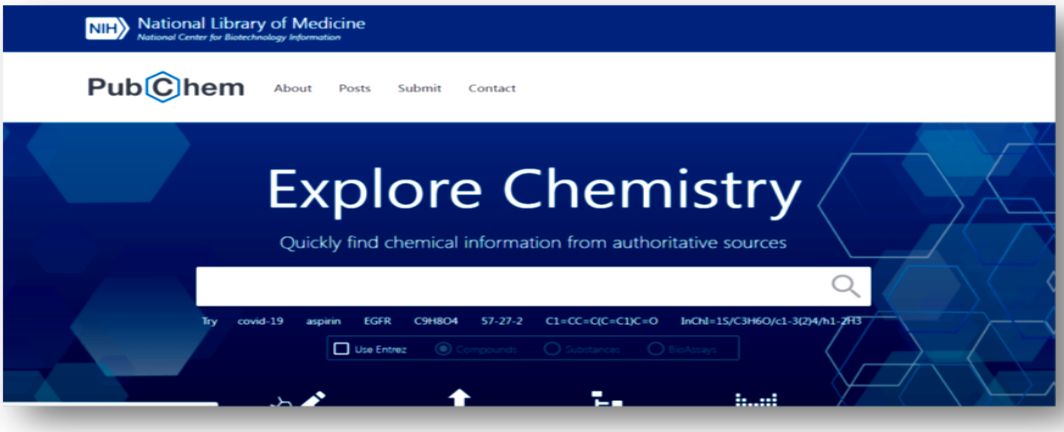


Figure 9:page d'accueil du serveur base des données Pubchem.

Chapter III

Materials

And methods

III.1. Research Problem :

What is the effect of Hypericum perforatum extract on inflammation and burns?

This research problem involves addressing several research axes, such as identifying the main active chemical groups in the plant, evaluating their anti-inflammatory activity, and their effect on inflammation and burns.Solving this problem can significantly contribute to scientific knowledge in the field of medicinal plants and pave the way for new treatments for burns.

III.1.1.Plant Material :

The plant material used consists of the leaves of Hypericum perforatum plant, a medicinal plant known for its therapeutic properties. This sample was purchased from an herbalist in Bouïra**(Yahiaoui & Ouldji.,2024).**

Figure 10: Dried Leaves of Hypericum perforatum Used in the Study**.**

III.2.Methods :

In vitro study :

III.2.1.Preparation of *Hypericum perforatum* extracts :

III.2.1.1.Preparation of Plant Powder

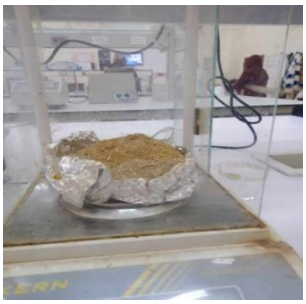
After harvesting, the plant parts (leaves and roots) were cleaned, and the leaves were dried

in an oven at 40°C until completely dry. The leaves were then ground into a fine powder and stored in airtight glass containers at room temperature until use **(Ghezal & Remili.,2024).**

Figure 11: Appearance of the leaf plant material (personal photo).

III.2.1.2.Preparation of the Extract :

To prepare hypericum perforatum extract, the process began by soaking 200 grams of the dried powder in 600 ml of distilled water and 300 ml of ethanol overnight in a dark place to ensure effective initial extraction of the compounds. After the initial filtration to remove solids, an additional 350 ml of distilled water and 150 ml of ethanol were added to the resulting liquid, and the mixture was left to macerate again overnight to optimize the extraction of the active compounds. The following day, a second filtration was performed to remove any remaining impurities. Finally, the resulting solution was concentrated by heating it to evaporate a portion of the solvent, yielding a more concentrated final extract ready for use with guaranteed effectiveness and purity.



weighing of samples

distilled water +plante+ethanol

filtration

cooling

Drying in an oven

Crude extract

Figure10:the method illustrates the extraction process**(Belksir & ferdi.,2021).**

III.2.1.3.Determination of the crude extract yield :

The crude extract yield is defined as the ratio between the mass of the dry extract obtained and the mass of the plant material. This yield is calculated using the following equation:

$$R\ (\%) = (Me / Mv) \times 100$$

Yield of extraction (%): Extraction yield expressed as a percentage (%).

Me: Mass of the resulting dry extract in g.

Mv: Mass of the plant material (powder) in g used for extraction.

III.2.2Phytochemical screening :

We used a plant extract to determine its chemical composition. This was done by performing simple tests on the extract, both neat and when mixed with water or other substances, to detect the presence of known chemical groups such as flavonoids, anthocyanins, tannins, coumarins, alkaloids, saponins, steroids, sterols, terpenes, and others. These tests help us to better understand the plant's chemical makeup**(Azeb & Azza.,2023).**

III.2.2.1.Test of polyphenols :

A few drops of HCl are added to 5 ml of the infusion. If polyphenols are present, the solution will turn red (**N'guessan & *al*.,2009).**

III.2.2.2.Test of Flavonoid :

Add 5 ml of hydrochloric acid (HCl) to 5 ml of the infusion. Add a small piece of magnesium (Mg) and 1 ml of iso-butanol alcohol. The reaction is considered positive when an orange-red coloration appears **(Brahimi,2023)**.

III.2.2.3.Teste of tannins :

To detect the presence of tannins, 1 mL of the extract is mixed with 10 mL of distilled water and then filtered. After that, 3 drops of $FeCl_3$ are added to the filtrate. The appearance of a blue-black or green precipitate indicates the presence of condensed tannins**(Karumi & *al*.,2004).**

III.2.2.4.Test of alkaloids :

2 ml of Wagner's reagent (2 g of potassium iodide (KI) + 1.27 g of iodine (I_2) + 100 ml of distilled water) were added to 2 ml of the infusion. The appearance of a white-yellow precipitate indicates the presence of alkaloids **(Chaouch, 2001).**

III.2.2.5.Test of Terpenoids :

2 ml of chloroform and 3 ml of concentrated sulfuric acid were added to 5 ml of each extract. The formation of two layers and the appearance of a brown color at the interface indicate the presence of terpenoids **(Edeoga & *al.*, 2005).**

III.2.2.6.Test of Saponins :

5 mL of the aqueous extract was thoroughly mixed with 10 mL of distilled water for 2 minutes. The formation of stable foam persisting for more than 15 minutes indicates the presence of saponins**(Karumi & *al.*,2004).**

III.2.2.7.Test of Steroids :

Add 2.5 ml of the infusion, 2.5 ml of acetic anhydride, then 2.5 ml of sulfuric acid (H_2SO_4). The appearance of a green color indicates the presence of steroids**(Brahimi,2023)**.

III.2.2.8.Test of Cardiac glycosides :

2 ml of each extract were dissolved in 2 ml of chloroform, then concentrated sulfuric acid was carefully added to form a separate layer. The appearance of a dark brown color at the interface indicates the presence of cardiac glycosides **(Edeoga & *al.*, 2005).**

III.2.2.9.Test of anthraquinones :

5g of the dried extract are mixed with benzene and then filtered. 5ml of 10% ammonia solution are added to the filtrate. After shaking, the appearance of a violet color in the lower layer indicates the presence of free hydroxyl anthraquinones**(Oloyede,2005).**

III.2.3.Dosage of phenolic compounds :

III.2.3.1.Dosage of Total Polyphenols:

The quantification of these metabolites is carried out using several analytical methods. The most widely used method is the Folin-Ciocalteu method **(Boukerika and *al.*,2019).**

a). Principle :

The Folin–Ciocalteu reagent consists of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40). The reaction of this reagent with oxidizable groups in phenolic coumpounds under alkaline conditions leads to the formation of a blue oxide complex. The intensity of th-is resulting blue coloration, which exhibits maximum

absorbance at 765 nm, is directly proportional to the amount of polyphenols present in the analyzedextract**(Georgéand *a l.*, 2005).**

b)Protocol :

Polyphenol content was determined using spectrophotometry, following the protocol described by Li et al. (2007). Briefly, 500 µL of Folin-Ciocalteu reagent (diluted to 10% with distilled water) were added to 100 µL of the extract at known concentrations. After a 4-minute reaction, 400 µL of Na2CO3 solution (75 mg/mL) were added to the mixture. Following a 30-minute incubation period at room temperature in the dark, the absorbance was measured at 765 nm. The concentration of total polyphenols was calculated based on the regression equation of a calibration curve, which was prepared using gallic acid standards at various concentrations under identical conditions and with the same reagent volumes. The results are expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g) **(Wong and *al.*, 2006).**

III.2.3.2.Dosage of Total Flavonoid :

The quantification of flavonoids in Hypericum perforatum extracts was performed using the aluminum chloride (AlCl3) method, following the protocol of **(Dahel and *al.*,2019).**

a)Principle :

The formation of a covalent bond between aluminum chloride (AlCl3) and the hydroxyl (OH) groups of flavonoids produces a yellow-colored complex with a maximum absorbance at 430 nm **(Ababsa, 2009).**

b). Protocol :

To 500 µL of sample, 500 µL of AlCl3 solution (2% in methanol) is added. After a 10-minute incubation at room temperature and in the dark, the absorbances are measured by spectrophotometer at 430 nm. The calibration curve using rutin allows the determination of the total flavonoid content of the extracts, and the results are expressed in milligrams of rutin equivalent per gram of dry extract (mg RE/g) **(Saba and *al.*, 2011).**

III.2.4.The biological activities of the plant :

In vitro study

III.2.4.1. Evaluation of the anti-inflammatory activity of Hypericum perforatum extracts :

Evaluation of the Anti-inflammatory Activity of Hypericum perforatum Extract According to the Williams et al. (2008) Protocol.Nine different concentrations of standard solutions were prepared using water as a solvent. A volume of 0.45 mL of BSA was added to 0.05 mL of the plant extract, followed by a first incubation in a water bath at 37 °C for 15 minutes. Subsequently, a second incubation was carried out at 71 °C for 5 minutes. To stop the reaction, 1.5 mL of a buffer solution with a pH of 6.9 was added after the incubation period. A control solution (blank) without the extract was prepared under the same conditions by replacing the extract with 50% ethanol. After cooling, the turbidity was measured at a wavelength of 660 nm. The results were compared with two standards: diclofenac and prednisolone.

The percentage of anti-inflammatory activity is calculated according to the following equation:

$$(\%) = [Ac - At/Ac] \times 100$$

Ac : Absorbance du contrôle.

At : Absorbance d'extrait ou standard.

III.2.4.2. Evaluation of the Antioxidant Activity un seul extract :

III.2.4.2. 1. Scavenging Effect of Extracts against the DPPH Radical :

a) Principle:

The anti-radical activity of the extracts obtained from *Hypericum perforatum* seeds is evaluated by measuring their ability to scavenge the DPPH radical. The reduction of the DPPH radical, which exhibits a dark violet color, by the hydroxyl groups of the antioxidants present in the extracts leads to the formation of a stable compound with a yellow color.

The intensity of the dark violet color, measurable at 517 nm, is inversely proportional to the anti-radical activity of the extract **(Locatelli and *al.*, 2010).**

b). Protocol:

Antioxidant activity of the samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay. Briefly, a 50 µL sample of the extract or standards (ascorbic acid, quercetin) was added to 50 µL of a DPPH solution dissolved in methanol, and the volume was made up to one milliliter using double distilled water. After incubation in the dark at room temperature for 30 minutes, the absorbance (Abs) was recorded at 515 nm **(Oubannin and *al*.,2024)**. The antioxidant activity (AA) was determined using the following equation:

Antiradical Activity (%) = [(A control - Asample) / A control] x 100

III.2.4.2. Evaluation of antibacterial activity:

III.2.4.2.1. Evaluation of in vitro antibacterial:

a). principle:

The principle of this method relies on the diffusion of the antimicrobial compound into a solid medium within a Petri dish, following a specific contact time between the product and the target microorganism. The effect of the antimicrobial product on the target is evaluated by measuring the inhibition zone. Based on the diameter of the inhibition zone, the strain will be classified as sensitive, very sensitive, highly sensitive, or resistant**(Azeb & Azza.,2023).**

- **Bacterial strain sused**:

The bacterial species used in our work are *Escherichiacoli ATCC*(25922), *Staphylococcus aureus ATCC*(25923), *Pseudomonas aeruginosa ATCC*(27853), *Enterococcusfaecalis ATCC* (29212), *Klebsiella pneumonia ATCC*(13883), and *Bacillus subtilis ATCC*(6051). These are reference strains and are available within our educational laboratory at the Faculty of Science of Nature and Life. En utilisant la gélose de Mueller-Hinton.

chelatingactivity(%) = [(Ac - At) / Ac] x100

- **The antibiotic discs used were**:
    - Amoxicillin

b). Experimental protocol :

- **Preparation of the discs**:

The discs are prepared from Whatman filter paper, with a diameter of 6 mm. Then, they are placed in a test tube, sterilized by autoclave, and stored until use.

- **Subculturing of microbial strains:**

The different microbial species to be tested were subcultured using the streak plate method onto Petri dishes containing nutrient agar, and then incubated at 37°C for 24 hours to obtain isolated colonies that will be used for inoculum preparation.

- **Pouring of Petri dishes:**

The Muller-Hinton culture medium was prepared and sterilized by autoclaving at 120°C for 20 minutes, after which it was poured into Petri dishes. These dishes must be dried at room temperature in the laboratory for 30 minutes before use.

- **Preparation of the inoculum:**

To prepare the inoculum, 4 to 5 well-isolated and perfectly identical colonies are scraped from a 24-hour culture grown on the isolation medium and then suspended in sterile physiological saline. The microbial suspension is then thoroughly homogenized (manual agitation), and its turbidity is adjusted to 0.5 McFarland standard, which corresponds to an optical density (OD) of 0.08 to 0.10; read at a wavelength of 625 nm, equivalent to 108 Colony Forming Units per milliliter (CFU/ml).

- **Inoculation:**

The previously poured Petri dishes, after a sterile swab is dipped into each inoculum, are spread with the Muller-Hinton agar. The swab should be passed over the entire surface 2 to 3 times to achieve a homogeneous distribution. The plates are then left to dry for 10 minutes before the discs are placed.

Using sterile forceps, filter paper discs impregnated with the test samples (10 µL) of extract are placed onto the surface of the agar.

- **Incubation**:

The Petri dishes are incubated in an oven at 37°C for 24 hours.

- **Reading of results**:

The diameter of the inhibition zone was measured in millimeters. A product is considered active if the diameter of the inhibition zone is greater than 9 mm.

III.2.4.3.anti-diabetic activity:

III.2.4.3.1.α-Amylase Inhibition Principle:

¬α-Amylase degrades starch into less complex compounds that cannot react with iodine. The inhibition of α-amylase by an inhibitor leads to the accumulation of starch, which subsequently reacts with iodine to form a blue-black complex whose absorbance is measured at 580 nm.

Protocol:

The sample preparation includes three types: the blank control, consisting of 100 µL of phosphate buffer and 100 µL of distilled water; the negative control, containing 100 µL of amylase enzyme and 100 µL of phosphate buffer; and the sample, composed of 100 µL of amylase enzyme and 100 µL hypericum perforatum extract diluted at different concentrations. Experimental Procedure:

The initial mixture is first incubated at 37°C for 10 minutes, followed by the addition of 100 µL of 1% starch solution, then incubated again at 37°C for 30 minutes. The reaction is then stopped by adding 50 µL of 1M HCl, followed by 100 µL of Lugol's reagent to detect the remaining starch. The sample is diluted with 1 mL of distilled water to prepare it for measurement. Finally, absorbance is measured at 580 nm using a spectrophotometer.

Glucor (Acarbose) was used as a standard (0 to 100 µg/mL) **(Gopinath and *al.*, 2013)**. The percentage of α-amylase inhibition is calculated using the following formula:

% d'inhibition=(Acontrol-A sample/Acontrol)*100

III.2.4.4. Evaluation of immunomodulatory activities:

III.2.4.4.1.Phagocytic activity:

a) Principe:

Peripheral blood, a primary source of monocytes in humans, plays a crucial role in the immune response through phagocytosis.This study utilized peripheral blood to evaluate the in vitro effect of *Hypericum perforatum* (St. John's Wort) extract on phagocytic activity against *Saccharomyces cerevisiae* (baker's yeast) **(Benaoun, 2017).**

Phagocytosis was assessed by evaluating the ability of leukocytes to ingest zinc particles, comparing the phagocytic activity across three groups:

- **Control group:** Not treated with any extract.
- **Treatment group:** Treated with *Hypericum perforatum* extract at various concentrations.
- **Positive control group:** Treated with opsonized particles (Zn) as a positive standard for phagocytosis.

The extent of phagocytic capacity is represented by the following equation:

% Phagocytic Capacity = (Number of phagocytosing cells / Total number of cells) × 100

Phagocytic activity for the extract and the positive control is calculated according to the method of**(Steven & *al*.,1984):**

Phagocytic Activity = (CP_test – CPblanc)

CP blanc: Phagocytic capacity in the absence of the extract or negative control.

CP test : Percentage of phagocytic capacity in the presence of the extract or positive control.

Protocol:

1)Blood sampling: Blood samples are taken from a healthy individual. Whole blood is collected tube containing sodium heparin (NH) as an anticoagulant. **(Oduah and *al*,2016).**

2)Preparation of buffer solutions: Buffer solutions are prepared according to the method of(Burnat et al. 2013) for the phosphate buffer solution, and according to**(Hanks,1975)** for Hanks' Balanced Salt Solution (HBSS).

3)Preparation of Brewer's yeast (Saccharomyces cerevisiae):

Preparation: A quantity of yeast is mixed with sugar, with the addition of a quantity of distilled water, and then it is heated

Measurement: The cell concentration is measured using a spectrophotometer at a wavelength of 540 nanometers **(Tavanti & *al*., 2006).**

4)Preparation of the extract: The stock solution is prepared as follows: In a test tube, 5 mg of the raw extract are added to 1 mL of the HBSS solution.

5)Zinc Preparation :

Zinc has indeed been used in numerous studies to stimulate immune cells, including macrophages, with the aim of evaluating its effect on their various functions, particularly phagocytic activity. 1 mg was dissolved in 1 ml of distilled water as a positive control for comparison(**Freire-Garabal & *al*., 1993).**

6)Exploration of phagocytosis and recovery of phagocytic cells:

40 microliters of plant extract are mixed with 200 microliters of heparinized blood, and then the mixture is incubated in a closed water bath with shaking (600 rpm) at 37 degrees Celsius for 30 minutes. To stop the reaction**(Harun and *al*., 2015).** Then the stimulated cells are washed twice using PBS.at 4 degrees Celsius, and then centrifuged at 2500 g for 5 mi40 microliters of brewer's yeast solution are added to the mixture at 0°C. The samples are incubated in a water bath with shaking at 37°C for 10 minutes. After incubation, phagocytosis is stopped by adding 2 mL of chilled PBS, and then the cells are washed three times using cold PBS**(Chena and *al*., 2014)** Extracellular yeasts are removed by nutes(**Rossiet*al*., 2013).**

centrifugation at 10000 g for 5 minutes at 4°C.

Analysis of red blood cells: Red blood cells are lysed to obtain pure granulocytes, which is an important step for isolating the cells being examined**. (Maqbool and *al*., 2011).**

Stopping the lysis process: After cell lysis, the process is stopped using another solution to prepare the cells for washing and centrifugation **(Harunand *al*., 2015).**

Preparation of smears: A drop of blood is taken and spread appropriately on a glass slide**(Pouletty, 2010).**

Cell fixation: The cells are fixed using methanol to ensure they do not move or change during staining **(Norumand *al*., 2005).**

Staining: The cells are stained using May-Grünwald and Giemsa solutions to visualize the fine details of the cells under a light microscope**(Norum and *al*., 2005).**

III.2.4.4.2. anti-complement activity:

III.2.4.4.2.1. Materials:

a) Apparatus and Small Equipment:

A range of specialized apparatus and small equipment were employed in the laboratory as part of our study, including: a centrifuge, an ultrasound device, two incubators (one set at 37°C and the other at 56°C), a water bath, a balance, a pH meter/temperature probe, a hot plate, micropipettes, graduated pipettes, a suction bulb, beakers, Erlenmeyer flasks, volumetric flasks, Eppendorf tubes, Petri dishes, graduated cylinders, a funnel, a spatula, parafilm, and other necessary laboratory consumables.

b)Chemicals, Reagents and Biological Material:

A range of buffer solutions were utilized in the study, including a neutral pH PBS/EGTA buffer and a PBS/EGTA/Mg++ buffer containing magnesium chloride, alongside agarose gel. Biological samples included chicken blood, from which erythrocytes were obtained and treated with a 20% (v/v) ACD (anticoagulant) solution before storage at 4°C, and a Normal Human Plasma (NHP) sample collected from a healthy adult donor. Furthermore,

Hypericum perforat*um* plant extract at varying concentrations was employed in the experiments.

III.2.4.4.2.2. Protocole :

- **Préparation du tampon EGTA/Mg++/pBs:**

Dans un tubestérilemettre9,8 ml de tampon EGTA/ Mg++/pBs

- **Preparation of Chicken Erythrocytes**:

This step aims to obtain chicken erythrocytes from the blood. Using a micropipette, take 200 µl of blood and place it at the bottom of a sterile tube as shown in figure13. Centrifuge the chicken blood at 2500 rpm for 10 minutes, then wash this quantity (the pellet obtained after centrifugation) three times with 2.1 ml of PBS washing buffer.

- **Agarose Gel Preparation**:

Using the balance, measure 0.2 g of agarose gel powder. Then add 13 ml of washing buffer (85°C). Lower the gel temperature to 45°C using the pH/temperature probe. Figure 14 illustrates this step.

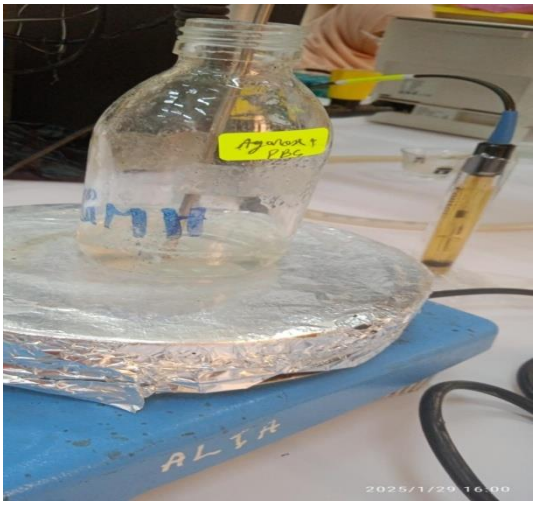


Figure .14: Depositing the pre-incubated mixture into the wells of the AP100 plate

- **Preparation of AP100 Plates**:

Mix the PBS/EGTA/Mg++ buffer (9.8 ml) with the previously prepared gel, then add the prepared chicken red blood cell suspension at 45°C, and mix everything gently. Pour the gel into Petri dishes and allow it to cool at 4°C for 10 minutes. Using a Pasteur pipette, carefully create wells in the plate, spaced 1.5 cm apart.

- **Preparation of the Positive Control**:

The heparin solution used in our study is at a concentration of 80ul, using 4ul of the buffer (PBS / EGTA/Mg++).

- **Preparation of the Negative Control**:

Contains 6 volumes of Acp with 1 volume of serum added.

- **Incubation**:

The investigation of the anti-complement activity of this plant extract is conducted using pre-prepared AP100 Petri dishes, following the previously described procedure. This involved introducing 25 microliters of each dilution of the extract into three separate wells on a single Petri dish for each dilution. Subsequently, 25 microliters of Normal Human Plasma (NHP) solution is added to each well.

III.3.Formulation and quality control of the ointment:

III.3.1. method:

III.3.1.1. Preparation Technique:

The preparation process involved three main phases. In Phase One (A), 60% distilled water was combined with 5% solubilizing humectant, 0.4% preservative, and 0.4% gelling agent, and the resulting mixture was heated to 75°C. In Phase Two (B), 3% nourishing agent, 6% emulsifier, 6% emollient, and 6% vegetable oil were incorporated in a separate container. Finally, Phase Three (C) involved preparing a third container with the plant extract. To complete the preparation, the contents of Phase (B) were added to

Phase (A) with thorough mixing, after which the mixture from Phase (C) was incorporated into the resulting blend, with continued agitation to obtain the final product**(Doctissimo, 2001).**

Figure 13: Depositing the pre- incubated mixture in to the wells of theAP100 plate

III.3.2.Quality Control of Ointments:

For the quality control of our ointments, we proceeded with the observation of macroscopic characteristics (color, consistency, odor), the verification of homogeneity, the measurement of the hydrogen potential (pH).

III.3.2.1.Macroscopic Characteristics:

The consistency, color, odor, and stability of each ointment were noted.

III.3.2.1.1.Homogeneity:

We verified the homogeneity of the ointments by spreading small quantities of each oexcipients.intment in a thin layer on a flat surface using a spatula, and in this way, we noted the excipients.

III.3.2.1.2.The Hydrogen Potential (pH) :

The hydrogen potential (pH) of each ointment was determined by spreading a small quantity of each ointment on a multiple pH paper. In the same way, the pH of each excipient was measured.

III.3.2.1.3.Stability:

The ointments are placed under various temperature conditions followed by an evaluation of the melting point.

III.3.2.1.4.Color:

A color chart is used to determine the colors of each ointment.

III.3.2. Packaging:

a. Materials:

Jars with a capacity of 50 g.

b. Jarring:

It is done manually. Each jar is weighed to verify its weight after filling.

c. **Labeling:**

The labels used for each ointment bear the following information:

- Name of the pharmaceuticalform
- Qualitative and quantitative composition
- Indication
- Manufacturing and expiration dates
- Batch number

I**II.4.In silico study using bioinformatics methods**:

III.4.1.Methods for Evaluating Pharmacokinetic and Physicochemical Properties using SWISS ADMET:

To evaluate the in silico anti-inflammatory activity, we first determined the physicochemical and pharmacokinetic parameters of the studied plant and standard drugs using the SwissADME tool available at (http://www.swissadme.ch). For this purpose, the structures of

the plant and standard drugs were obtained in SMILES format using the PubChem tool (Table 2, Table3).

The toxicity of these plants and drugs was subsequently studied using an online server (ProTox-II16) which provides the predicted values for toxicity, cytotoxicity, mutagenicity, carcinogenicity, and immunotoxicity, as well as Protox 2 (https://bioinformatics.charite.de/protox_II).

Tableau 2 : Smiles Structure of Hyperforin Compound

Compounds	Structure Smiles
Hyperforin	CC(C)C(-O)[C-12C(-O)C(-C-C-C-C-C)-C(C-H-(C-2(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C(C)C)O)CC-C(C)C(C)C(C)C(C)C(C)C)

Tableau 3: Smiles Structures of the Studied Standard Drugs

Drugs	**Structure smiles**
Diclofenac	C1=CC=C(C(=C1)CC(=O)[O-])NC2=C(C=CC=C2Cl)Cl.[K+]
Prednisolone	C[C@]12C[C@@H]([C@H]3[C@H]([C@@H]1CC[C@@]2(C(=O)CO)O)CCC4=CC(=O)C=C[C@]34C)O

III.4.2. General Properties of "Drug-like" Molecules:

For a compound to be considered "drug-like," it must possess specific characteristics that ensure its therapeutic effectiveness and safety. These properties include:

- Good bioavailability.
- Therapeutic effectiveness.
- Absence of toxicity

Drug-like" molecules are known as those that possess optimal ADME properties. Lipinski established the "Rule of Five" to evaluate these characteristics**(Lipinski C.A., 2000 ; Lipinski C.A. and *al.*, 1997).**These are as follows:

- MolecularWeight ≤ 500 Daltons.
- LogP value (a measure of lipophilicity) ≤ 5.
- Number of Hydrogen Bond Acceptors ≤ 10.
- Number of Hydrogen Bond Donors ≤ 5.

Subsequently, other complementary properties were added, such as**:**

- Number of Rotatable Bonds NROT: (0 < NROT < 10)
- Polarity TPSA: (20 < TPSA < 130)
- Csp3 Saturation: (0.25 < Fraction Csp3 < 1)
- Solubility (LogS): (-6 <LogS< 0)

III.4.3. General Overview of Physicochemical Properties:

he physicochemical properties of a drug significantly affect its pharmacokinetics. Measuring and calculating these properties can be used to predict ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties and also helps in classifying the selected compound **(Nagoudi and *al*., 2023).**

A. MolecularWeight (MW):

One of the essential physicochemical properties in the search for new drugs is the molecular weight (MW), which can have a significant differential impact on intracellular processes such as intestinal absorption, penetration of the blood-brain barrier, elimination rate, and interaction with molecular targets **(Gleeson & *al*., 2011).**

B. Lipophilicity (Fat-Loving):

Lipophilicity is a vital property for a drug, influencing its binding to the target, solubility, absorption, cell membrane permeability, binding to blood proteins, and distribution throughout the body (including the central nervous system and liver), ultimately determining how the body eliminates it.

Lipophilicity is the physicochemical property most widely used to predict the penetration of drugs into biological systems **(Testa B & *al.*, 1996).**LogP is defined as the logarithm of coefficient of a compound between the non-aqueous octanol phase and the aqueous phase. It is measured by the distribution of the neutral (non-ionized) form of the compound between these two phases **(Delaney JS., 2004).**

It is essential for a drug to possess a precise balance between its water-loving (hydrophilic) and fat-loving (hydrophobic) properties. This is because during the journey of drug molecules through the body, they must dissolve in both the aqueous environments of the cytoplasm and extracellular fluids, as well as in the non-polar environment of the cell membrane **(Rouahna., 2016).**

C.Solubility:

Aqueous solubility (expressed as LogS), a crucial physicochemical parameter for drug candidates, directly correlates with their oral bioavailability. The extent to which a drug dissolves in water significantly influences its biological activity. This parameter is critical for attaining precise drug concentrations within the systemic circulation, thereby ensuring the desired pharmacological response. Indeed, limited aqueous solubility impedes effective and complete oral absorption of the compound across the gastrointestinal tract **(Kararli T., 1989).**

D. Polarity TPSA (20 < TPSA < 130) :

The polarizability of a molecule refers to the ease with which its electronic system can be distorted by an external electric field and is considered a significant factor in understanding and predicting various molecular properties and biological activities. Highly polarizable molecules are capable of forming strong interactions with other molecules, and an increase in polarizability can contribute to the enhancement of a molecule's aqueous solubility **(Nagoudi and *al.*, 2023).**

E. Molar Refractivity MR (0 < MR < 10) :

Molar refractivity (MR) represents an important criterion for estimating the steric (volumetric) size of molecules. It is generally used as a simple measure of the volume

occupied by either an individual atom or a group of atoms within the molecule **(Youmbai ,Sahraoui., 2021).**

F. Number of Rotatable Bonds (NROT) :

The number of rotatable bonds refers to all single bonds within a molecule that are not part of a ring system and connect two non-hydrogen atoms. Amide C-N bonds are excluded from this count due to their high rotational energy barrier **(Nagoudi and *al*.,2023).**

III.4.4. General Overview of Drug-likeness:

Bioavailability refers to the rate and extent to which the active drug reaches the bloodstream and its site of action. Oral drugs face absorption challenges due to crossing the intestinal wall and undergoing first-pass metabolism in the liver, which can reduce the drug's concentration in the blood. Poor water solubility, slow absorption, and short residence time in the gastrointestinal tract decrease bioavailability. Also, slow dissolution or difficulty penetrating intestinal cells (especially for charged and polar drugs) reduces the available absorption time and causes significant variations in bioavailability **(Youmbai Sahraoui, 2021).**

III.4.5. General Overview of the Pharmacokinetic Profile (ADME) and Toxicity Prediction :

The optimization process of a drug candidate would greatly benefit from an in silico study of various ADME properties before *in vitro* experiments are conducted. Researchers have extensively studied ADME-related properties, including the inhibition of the P-glycoprotein transporter (ABCB1 or Pgp) or enzymes of the cytochrome P450 (CYP) family **(Launay-Vacher ., 2006)** ; One of the defining characteristics of drug metabolism is the initial oxidation phase (Phase I), in which a group of enzymes known as human cytochrome P450 (CYP) isoforms plays a key role. These isoforms include CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and most notably CYP3A4. It is worth noting that CYP2C9, CYP2C19, and CYP2D6 are responsible for approximately 80% of the known oxidation reactions in drug metabolism **(Williams., 2004).**

Due to the pivotal role of cytochrome P450 in drug metabolism, significant efforts have been invested in developing *in silico* tools to predict its substrates and inhibitors **(Ekins., 2001;**

Ekins., 2000). These tools are primarily based on the physicochemical properties of the compound or on our understanding of the structure and mechanism of these enzymes.

Inhibition of cytochrome P450 enzymes is undesirable due to the risk of serious side effects related to drug interactions. Furthermore, it is necessary to evaluate the membrane permeability of targets (e.g., hepatocyte membrane, gastrointestinal epithelial cells, blood capillary wall, glomerulus, restrictive organic barriers) and the volume of distribution or renal clearance **(Launay-Vacher ., 2006).**

The pharmacokinetic parameters evaluated in this study included the assessment of: Gastrointestinal absorption, the compounds' ability to penetrate the Blood-Brain Barrier, Skin Permeation, their ease of chemical synthesis (synthetic associability), in addition to predicting the interactions of ferrocenylmethyl-nucleobase molecules with cytochrome P450 (CYP) enzymes (interaction of molecules with cytochromes P450 (CYP), and estimating their bioavailability score.

The toxicity of a substance can be defined as its ability to produce harmful effects on a living organism. The degree of this toxicity varies depending on several factors, including the dose, frequency of exposure, duration of exposure, and the time required for clinical signs to appear. To assess the potential harm of any substance before it is marketed, whether it is a drug or a chemical product, it must undergo three main types of toxicity tests. Clinically, toxicity is divided into three essential forms: acute toxicity, short-term toxicity (also known as subacute or subchronic toxicity), and long-term toxicity (or chronic toxicity).

Determining the Lethal Dose 50 (LD50) value plays an important role in the legal framework, as it is used to classify chemical substances according to their degree of toxicity **:**

Tableau 4: Classification of chemical products according to their toxicities (Hodge and Sterner Scale) **(Bensakhria & Ayoub., 2018).**

Oral LD50 (Rat)	Toxicity Index
Up to 1 mg/kg	1 = Extremelytoxic
1 to 50 mg/kg	2 = Highlytoxic
50 to 500 mg/kg	3 = Moderatelytoxic
500 to5000 mg/kg	4 = Slightlytoxic
5000 to 15000 mg/kg	5 = Practically non-toxic
More than 15000 mg/kg	6 = Relativelyharmless

Determining the Lethal Dose 50 (LD50) is an important tool for assessing overdose risk, planning toxicity studies and clinical trials, quality control of chemicals, and calculating the therapeutic index (LD50/ED50). LD50 is commonly used to express the level of acute toxicity and to classify and compare toxic substances. However, its value is limited because it focuses only on mortality and provides no information about the mechanisms of toxicity or the nature of the damage, and it is a preliminary estimate influenced by various factors such as animal species, age, and sex **(Bensakhria & Ayoub., 2018).**

III.4.6. Molecular docking study :

III.4.6.1. Preparation of the active principles of the extracts and the drugs:

The molecular structures of the studied plant and standard drugs were prepared and optimized using the Gaussian 09 software, and subsequently saved in pdb format.

III.4.6.2. Macromolecule (Target Protein) :

A computational test of the anti-inflammatory effect of St. John's Wort extract was conducted on the COX-2 protein. Prostaglandins are important mediators of inflammation in the body. The enzyme prostaglandin synthase (also known as cyclooxygenase) is responsible for converting arachidonic acid into several products, including prostaglandin PGG2**(Nagoudiand *al* ,.2023).**

The structure with PDB ID: 1CX2, at a resolution of 2.80 Å, was downloaded in PDB format, and its three-dimensional structure was obtained through X-ray diffraction**.**

Tableau 5: Presentation of the 1COX2 Target

PDB ID Protein	**Determination method**	**Resolution**	**R-value**	**Number of residues**	**Co-crystallized Ligand**
1CX2	X-ray diffraction	2.80Å	0.220	587	SC-558

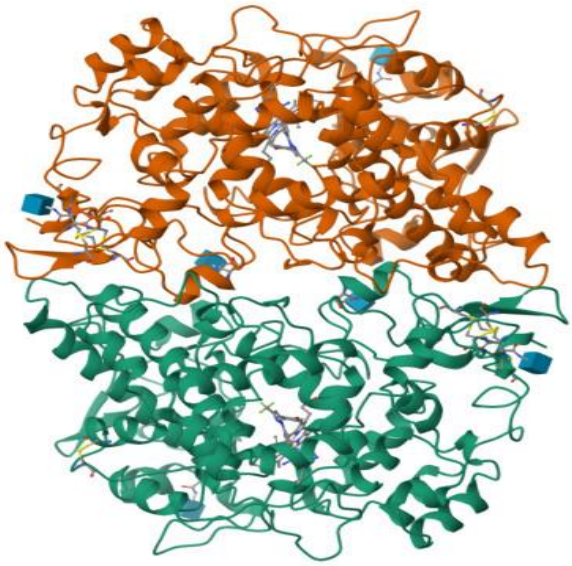


Figure 14: Three-dimensional structure of cyclooxygenase2(COX2).

III.4.6.3. Study of molecular interactions using AutoDockTools :

✓ **Operating Procedure:**

The docking procedure consists of the following two main steps:

- Generation of interaction maps of the DNA structure (receptor) using the AutoGrid tool.
- Docking of the ligand to the receptor with AutoDockTools. We will use the AutoDockTools (ADT) graphical interface to perform the docking and visualize the results. The steps are described below**:**

A. Receptor Preparation:

File → Read Molecule → select ADT-(molecule).pdb. Edit → Delete Water → Hydrogens → Add → polar only

→ Charges → Add Kollman Charges

B. Loading Receptor and Ligand:

- Ligand → Input → Open → ligand.pdb

→ Torsion Tree → Detect Root

→ Output → Save as pdbqt

C. Auto Grid Parameter Configuration:

Grid → Macromolecule → Choose → select ADT-(molecule) → write ADT-(molecule)pre.pdbqt → save

Grid → Set Map Types → Choose Ligand → select Ligand

Grid → Grid Box → Set number of points in x, y, and z dimensions to 60 and set Spacing (Angstrom) to 1.000.

Grid → Output → Save GPF → write grid.gpf → save.

- **D. Auto Grid Execution**

Run → AutoGrid.

- **E. Auto Dock Execution**

Docking → Macromolecule → Rigid Filename → select ADT-(molecule)pre.pdbqt.
Docking → Ligand → Choose → select Ligand.

Docking → Search Parameters → Genetic Algorithm → set Number of GA Runs.
Docking → Output → Lamarckian GA (4.2) → save output as dock.dpf

Run → AutoDock.

- **F. Results Analysis:**

This step is performed using the following commands: Analyze → Docking → Open → dock.dlg file.

Analyze → Macromolecule → Open

Analyze → Conformation → Play, Ranked by energy → &analyze interactions.

Tableau 6: Docking parameters selected for cyclooxygenase-2

Grid center			**Grid size (Å)**		
X	**Y**	**Z**			
23.665	23.312	47.826	50	50	50

CHAPTER IV

Results and discussion

IV.1. Results:

IV.1. 1.Extraction Yield:

The first quantification to be done is that of the crude extract yield from Hypericum perforatum leaves. The results obtained from the yield calculations of the extract are presented in the following table:

Tableau 7: Crude extract yield from Hypericum perforatum

Plante	**Hypericum perforatum**
Me (Masse de l'extrait sec (g))	40
M (Masse du matériel végétal (poudre) sec (g))	200
R (Rendement d'extraction (%))	20%

This text indicates that the aqueous extract of St. Hypericum perforatum showed a yield of 20%. This means that the percentage of the substance extracted from this plant using water as a solvent is 20% of the original weight of the plant.

It is important to emphasize that the variation in results from one extract to another is influenced by the method used (the choice of solvents), as well as the conditions under which the extraction is carried out (hot or cold**) (Celhay ,2013).**

IV.1.2.Screening phytochimique :

The results of the preliminary phytochemical screening tests are presented in the tables below.

Tableau 8: Highlighting certain secondary metabolites in crude extracts.

test	***Plant***
Polyphenols	+
Flavonoids	++
Tannins	++
Alkaloids	+++
Terpenoids	++
Saponins	+
Steroids	+
Cardiac Glycosides	+
Quinones	+++

(+**)**present**, (++)**mdiumpresent**, (**+++**)**strongly present

Given the attached table, a strong (+++) presence of both alkaloids and quinones was observed, indicating the richness of the plant in these two groups of compounds. In contrast, the plant also showed the presence of flavonoids, tannins, and terpenes. A low concentration of polyphenols, saponosides, sterols, and cardiac glycosides was detected.

IV.1.3. Quantitative analysis of phenolic compounds :

IV.1.3.1.Total Polyphenols Dosage :

The total polyphenol content in the extract was determined using the Folin-Ciocalteu reagent method. The curve shows a linearity of absorbance as a function of concentration. The corresponding polyphenol amounts in the extract were expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g), and were determined using the following linear equation: $y = 3.7143x - 0.0914$, with a coefficient of determination $R^2 = 0.9951$.

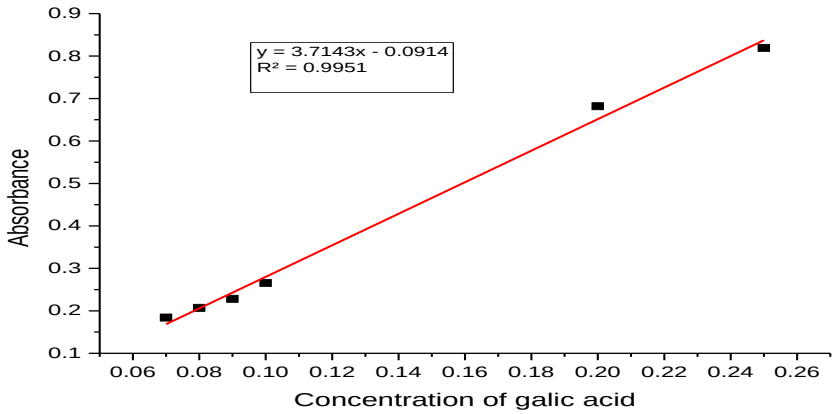


Figure 15: Calibration curve of polyphenol.

Tableau 9: Total polyphenol contents.

extract	**Total Polyphenols (mgEAG/g)**
hypericum perforatum	70±0.001

The analysis shows that the *Hypericum perforatum* extract contains 70mg of gallic acid equivalents of polyphenols per gram of extract, indicating a notable quantity of these compounds known for their benefits.

IV.1.3.2.Total Flavonoids Dosage:

The determination of flavonoids was carried out using the aluminium trichloride ($AlCl_3$) method and the standard is rutin. The flavonoid content is expressed in milligram$_{rutin}$ equivalent per gram of plant extract (mg RE/g). The flavonoid content of extracts was obtained from the calibration curve following a type equation: $y = 11.787x + 0.0991$, $R^2 = 0.9912$.

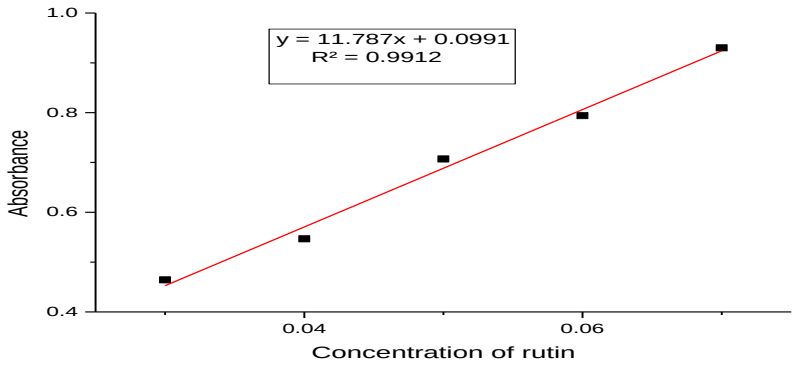


Figure 16: Calibration curve of flavanoid.

Tableau 10: Flavanoid contents.

extract	**Total Flavonoids (mgEAG/1g Ext)**
hypericum perforatum	40±0.005

The analysis shows that the *Hypericum perforatum* extract contains 40mg of gallic acid equivalents offlavonoids per gram of extract, indicating a notable quantity of these compounds known for their benefits.

IV.2. Evaluation of the anti-inflammatory activity of Hypericum perforatum :

IV.2.1.Comparison of the extract with Prednisolone and Diclofenac standards :

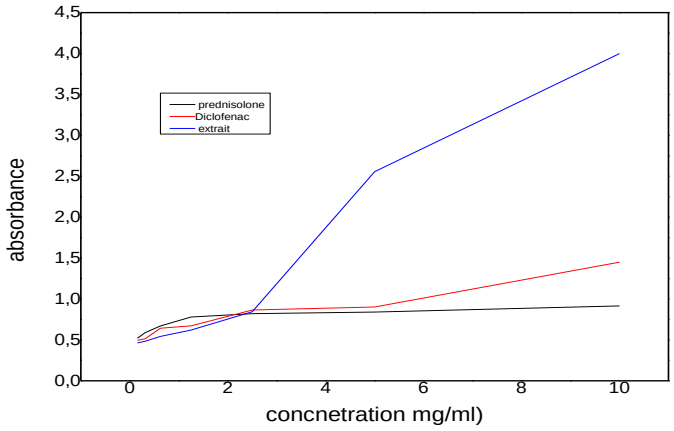


Figure 17: Curve of hypericum extract compared with the curves of Diclofenac and prednisolone.

The curve represents an extract from the studied plant, resulting from the interaction of different concentrations of the extract and standard drugs with bovine serum albumin (BSA). The results show that the extract yields results close to some of the studied standard drugs.

IV.2.2.Determination of Gibbs Free EnergyΔG and Binding Coefficient K:

The binding free energy ΔG of BSA with plants and drugs was determined by studying the pectroscopic behavior.

According to the absorbance results, it is possible to calculate the Gibbs free energy of binding (ΔG) using the formula**: A/A0-A**

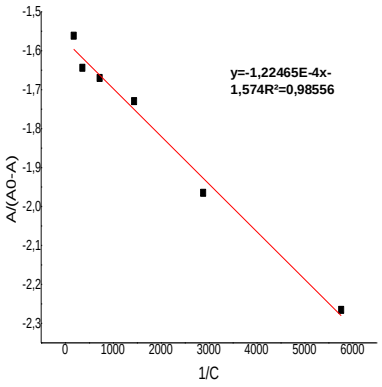


Figure18: : Plot of A/(A0 - A) as a function of 1/C[Prednisolone]**.**

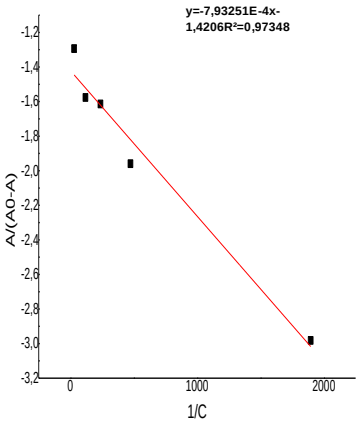


Figure 19: : Plot of A/(A0 - A) as a function of 1/C**[**Diclofenac**]**.

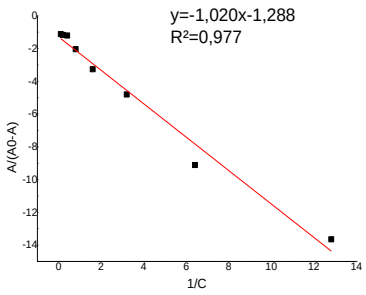


Figure 20:plot of A/(A0-A)as a function of 1/C[hypericum perforatum extract].

The obtained values of the binding free energy for the interaction of plant extracts and drugs

with Bovine Serum Albumin (BSA) are compiled in the following table:

Tableau 11:K and ΔG values of the studied compounds obtained from UV-Vis spectroscopic data.

Plant extracts and drugs	**K(mol-1)**	**ΔG(kj.mol-1)**
Hypericum perforatum	1.25×10^3	-17.70
Prednisolone	1.2853×10^4	-23.45
Diclofenac	1.790×10^3	-18.57

Based on the Gibbs free energy of binding (ΔG) values, it is noticeable that the extract has a slightly closer binding strength to the non-steroidal drug (Diclofenac) compared to the steroidal drug (Prednisolone).

IV.2.3.Determination of IC50:

Based on the absorbance results, it is possible to calculate the percentage of inhibition using the formula:

%inhibition= 100[(OD de test solution OD decontrol/ OD de test control)]x100 Where:

OD Control: Optical density of the negative control.

OD Solution: Optical density of different concentrations of the extracts.

Tableau 12: Study of the inhibitory activity of Bovine Serum Albumin (BSA) and the studied extract.

absarbance	Pourcentage d'inhibition
4	85,176
2,556	83,737
3,015	49,052
0,844	30,645
0,62	20,81
0,543	10,379
0,483	7,327
0,464	1,826

Tableau 13: Study of the inhibitory activity of BSA and different standard drugs.

	Pourcentage d'inhibition	
Concentration	**Diclofenac**	**prednizolone**
0.0078125	33.535	9.863
0.015625	35.992	36.730
0.03125	48.833	44.142
0.0625	51.041	50.895
0.125	61.965	57.820
0.25	63.444	59.878
0.5	63.525	60.833
1	77.310	64.043

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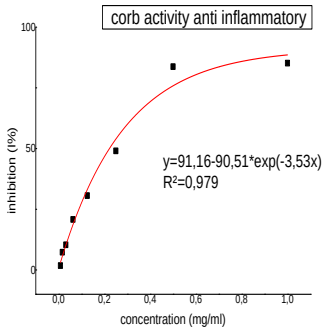

Figure 21: Regression curve of the percentage of inhibition as a function of the concentration of hypericum perforatum extract.

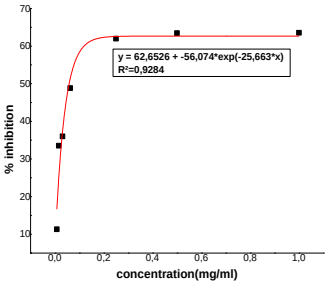

Figure23: Regression curve of the percentage of inhibition as a function of Diclofenac concentration.

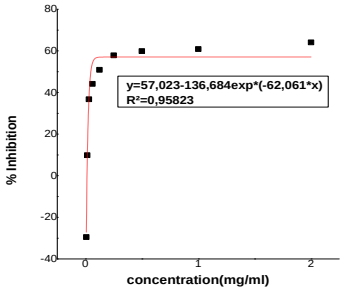

Figure 22: Regression curve of the percentage of inhibition as a function of Prednisolone concentration.

The obtained values for the half maximal inhibitory concentration (IC50) of the interaction of the plant extract and drugs with Bovine Serum Albumin (BSA) are compiled in the table below.

Tableau 14:IC50 values of the studied extract and drugs, obtained from UV-Vis spectroscopic data.

Plant extracts / drugs	IC50(mg/ ml)
Hypericum perforatum	0.22
Prednisolone	0.04
Diclofenac	0.05

The IC50 value represents the concentration of a substance required to inhibit a specific biological process by 50%. The smaller the IC50 value, the more effective the substance is at inhibition.

IV.3. Antioxidant Activity :

The antioxidant activity of plant extracts depends on the composition and structure of their bioactive compounds, such as phenolic acids and flavonoids, which have the ability to neutralize Reactive Oxygen Species (ROS) and other free radicals. These compounds act either by chelating metals to prevent harmful oxidation reactions or by scavenging free radicals and neutralizing their effects, giving them antioxidant properties **(Carochoand *al*., 2013).**

IV.3.1.Antiradical Activity using DPPH :

To evaluate the antioxidant activity of plant extracts, the DPPH assay is considered one of the fundamental and most widely used methods. This test relies on the use of DPPH as a free radical scavenger, which allows us to determine the effectiveness of our extracts in neutralizing these radicals, even when active components are present in minute concentrations. The ability of the extracts to counteract free radicals is quantified by measuring the decrease in DPPH absorbance at 517 nanometers using a spectrophotometer, where this reduction indicates the presence of compounds in the extracts capable of reacting with and quenching DPPH free radicals**(Guediri,2022).**

In this test ascorbic acid is used as the standard, the obtained results (percentage of inhibition I %) are represented in the calibration curve (Figure23), having the equation: y=11274x-28.616 with a correlation coefficient $R^2 = 0.9915$

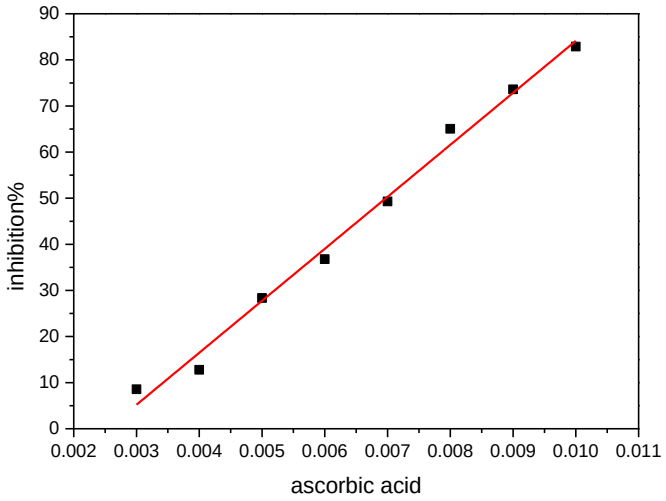

Figure 24: Calibration curve of ascorbic acid.

To characterize the antioxidant power, we introduced the IC50 parameter.

IC50: It defines the effective concentration of the substrate that causes a 50% reduction of DPPH in solution.

The IC50 values of the extract were estimated using the linear regression curve:

y = ax + b. The IC50 values are presented in Table15.

- **Calculation of IC50 :**

To calculate the effectiveness of antioxidants, we use the IC50 parameter, which represents the concentration of the extract necessary to reduce the activity of DPPH free radicals by 50%. A low IC50 value indicates a stronger antioxidant capacity of the extract. These values are determined based on the graphs shown in the following figure.

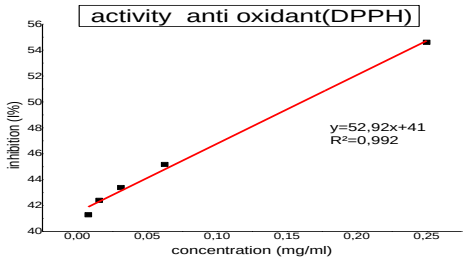

Figure 25: Change in inhibition strength as a function of extract concentration.

Tableau 15:IC50 values of the extract using DPPH.

Sample	DPPH method		
	Equation	**R^2values**	**IC50 (mg/mL)**
Extract	Y=52.92x+41	0.992	0.17
Ascorbic acid	y=11274x-28.616	0.9915	0.0069

These results indicate that the extract possesses significant antioxidant activity.

IV.4.Evaluation of antibacterial activity :

In vitro assessment of antibacterial activity :

In the following study, we investigated in vitro the antimicrobial activity of Hypericum perforatum extracts using an experimental technique. The objective of this work is to evaluate the ability of these extracts to produce bioactive compounds that may exhibit antibacterial therapeutic effects.

IV.4.1. Evaluation of antibacterial activity by inhibition diameter :

The table presents the diameters of the inhibition zones (in mm) for each bacterial strain and each concentration (mg/ml).

Tableau 16: Diameters of inhibition zones for bacterial strains.

	bacteria					
C(mg/ml)	P. aeruginosa	S. aureus	E,coli	B,subtilis	K, pneumoniae	E,faecalis
40	1	2	ND	4	5	1
20	1	2	ND	4	5	4
10	4	4	ND	5	8	4
5	2	5	4	4	7	5
2,5	5	4	2	5	7	4
1,25	4	4	4	4	8	4
0,625	5	6	5	6	9	5
0,312	5	8	7	7	ND	6
Control + (Amoxicillin)	24	24	24	24	24	24

ND: Not defined.

Figure 26: Inhibition Zones Studiedfor the Tested Bacterial Strain Enterococcus faecalis.

Figure 27: Inhibition Zones Studied for the Tested Bacterial Strain P. Aeruginosa.

Figure 28:: Inhibition Zones Studied for the Tested Bacterial Strain Escherichia coli.

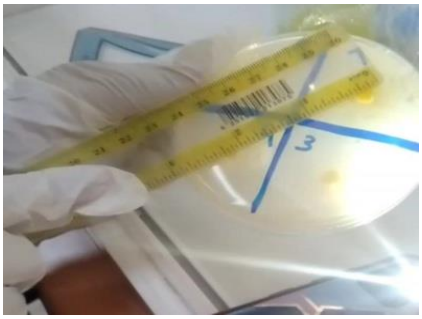

Figure 29: Inhibition Zones Studied for theTested Bacterial Bacillus subtillis.

Tableau 18: The inhibition values for the growth medium of bacterial strains extracted from inhibition zone data.

C(mg/ml)	B,subtilis	I%	K, pneumoniae	I%	E,faecalis	I%
40	4	83.33	5	79,166	1	95.83
20	4	83.33	5	79,166	4	83.33
10	5	79.16	8	66,666	4	83,33
5	4	83.33	7	70.83	5	79,166
2,5	5	79,166	7	70.83	4	83.33
1,25	4	83,333	8	66,666	4	83.33
0,625	6	79,166	9	62.5	5	79.166
0,312	7	70.83	/	/	6	75
Control + (Amoxicillin)	24	-	24	-	24	-

C(mg/ml)	P. aeruginosa	I%	S,aureus	I%	E,coli	I%
40	1	95.83	2	91.66	ND	/
20	1	95.83	2	91.66	ND	/
10	4	83.33	4	83,333	ND	/
5	2	91.66	5	79.16	4	71
2,5	5	79,166	4	83,333	2	75
1,25	4	83.333	4	83,333	4	83.333
0,625	5	79,166	6	79,166	5	79,166
0,312	5	79,166	8	66.66	7	70.83
Control + (Amoxicillin)	24	-	24	-	24	-

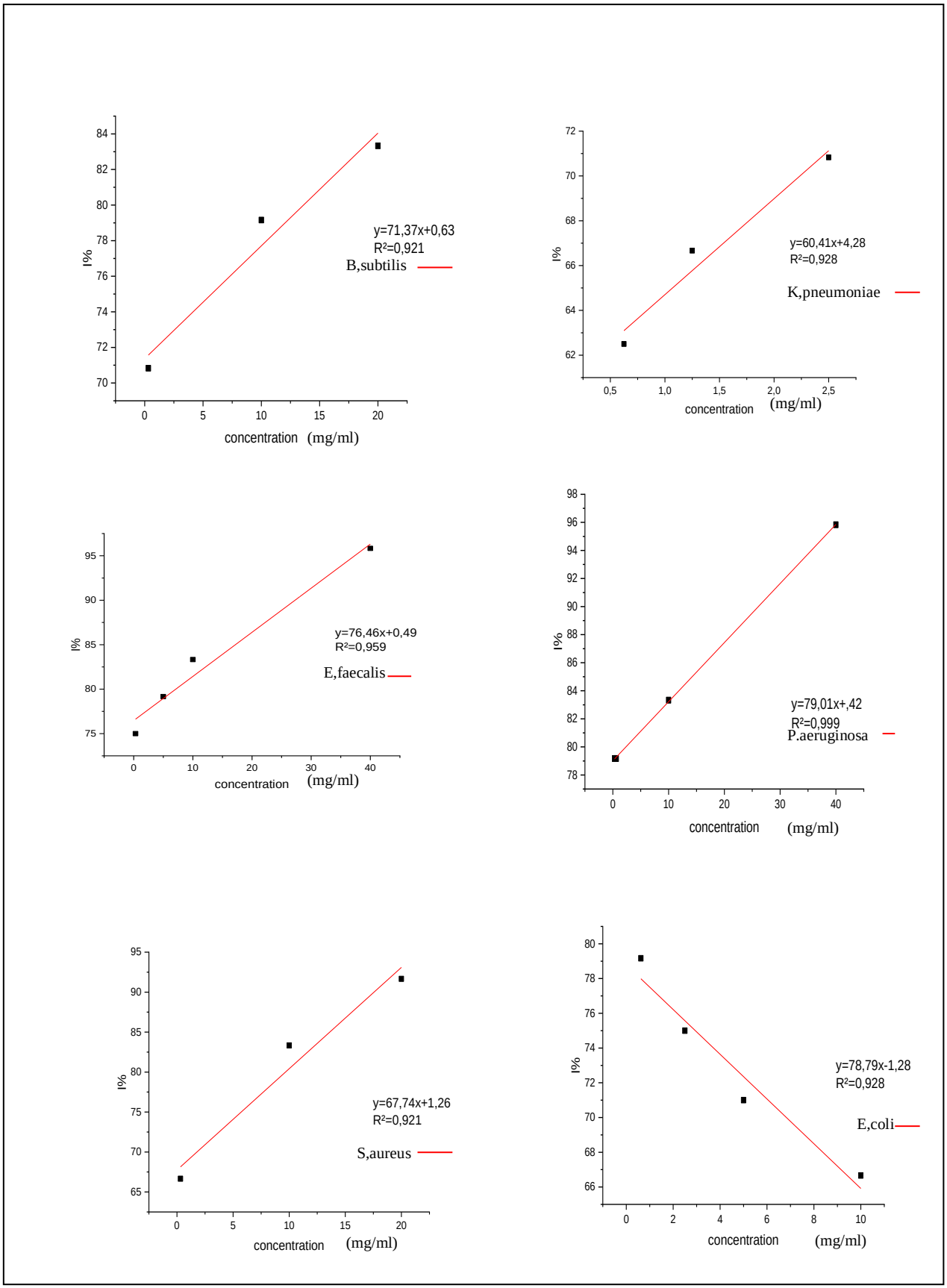

Figure30: Figure Regression lines of inhibition percentages obtained for the studied bacteria.

We can observe a linear relationship between the percentage of bacterial inhibition and the concentration of the studied extract. The IC50 values obtained for the interaction of the studied extract with the bacteria are summarized in the table below.

Tableau 19:IC50 values.

bacteria	**IC50 (mg/mL)**
B,subtilis	0.70
K, pneumoniae	0.75
E,faecalis	0.64
P. aeruginosa	0.62
S,aureus	0.71
E,coli	0.65

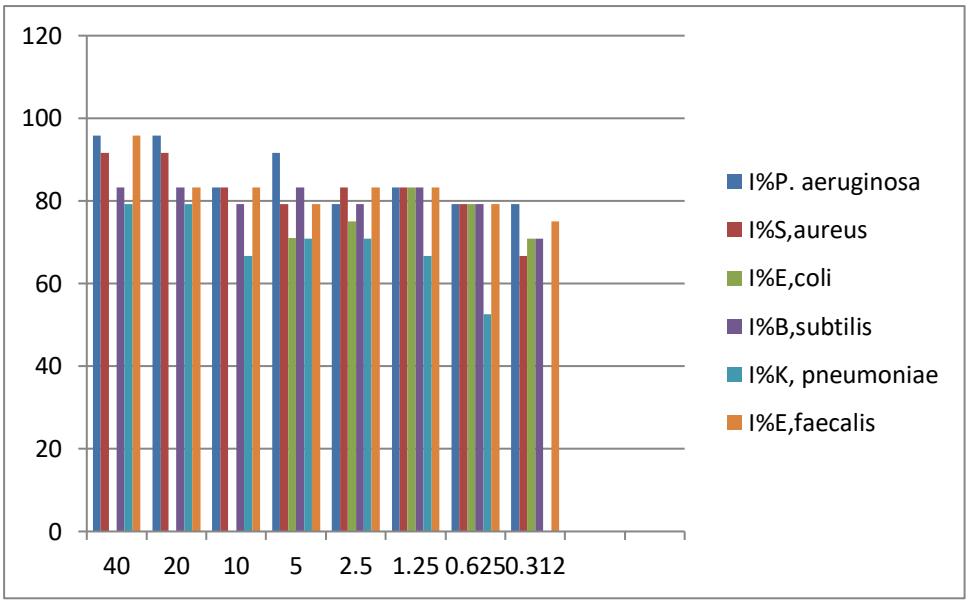

Figure 31: The inhibition percentages of different bacterial strains at gradedconcentrations of the extract.

IV.5.In vitro antidiabetic activity :

The enzyme α-amylase was used to evaluate the in vitro antidiabetic activity of the extract. In this section, the inhibitory potential of the *Hypericum perforatum* (St. John's Wort) extract is studied.

IV.5.1.Inhibitory effect of α-amylase :

The inhibitory capacities of *Hypericum perforatum* (St. John's Wort) extract and acarbose on α-amylase were evaluated, and the results obtained are presented in Figures 30 and31.

The inhibitory capacities of Hypericun perforatum extract on α-amylase were evaluated, and the obtained results are presented in the figure.

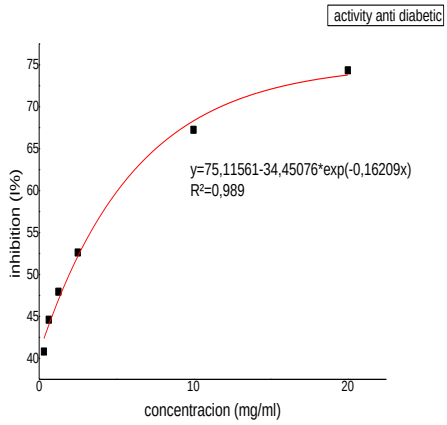

Figure32: Percentages of αamylase inhibition by byextract *perforatum Hypericum*

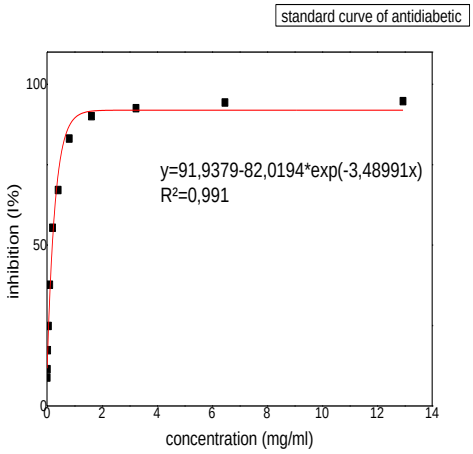

Figure 33: Percentages of α-amylase inhibition by acarbose.

The two figures show that the effectiveness of the inhibition increases with the rise in concentration for both the extract and acarbose. The maximum inhibition rate reached approximately 75% for the extract and 91% for acarbose, with good agreement of the results (R2 close to 1). This indicates that the extract has acceptable inhibitory activity compared to the pharmaceutical reference.

Tableau 20: Alpha-amylase enzyme inhibition for hypericum perforatum extract

concentration	20	10	5	2,5	1,25	0,625	0,312	0,156	IC50(mg/ml)
inhibition %	74,32	67,24	54,76	52,61	47,94	44,6	40,8	3,55	1

Tableau 21: Alpha-amylase enzyme inhibition for acarbose.

concentration	12,94	6,47	3,235	1,6175	0,808	0,404	0,202	0,101	IC50(mg/ml)
inhibition %	94,73	94,31	92,55	90,05	83,1	67,1	55,39	37,68	0,19

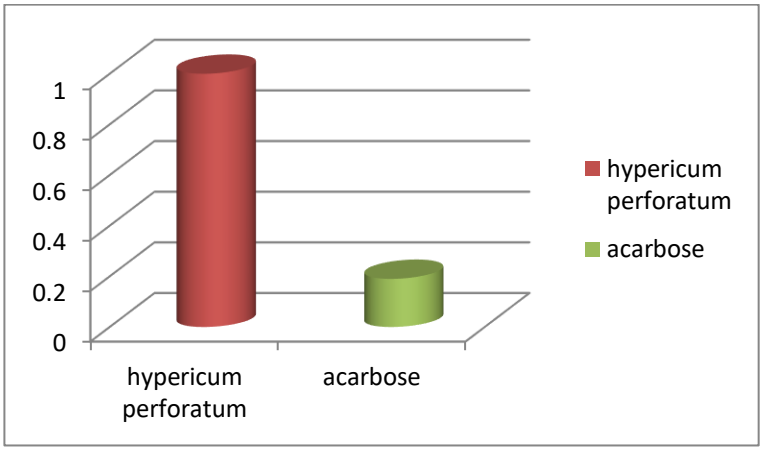

Figure 34: IC50 values for the extract and the standard.

Figure (50) illustrates the IC50 values for both the Hypericum perforatum extract and the standard substance Acarbose in the enzyme inhibition assay. The *Hypericum perforatum* extract demonstrated good inhibitory activity, confirming its possession of effective biological properties that warrant further study and development. These results highlight the importance of medicinal plants as promising sources that can be utilized to support therapeutic applications, alongside the standard substances used.

IV.6.Phagocytic activity :

This work focuses on studying the phagocytic activity of immune cells derived from whole blood against brewer's yeast. The crude extract of the Hypericum plant was tested, with zinc considered as a positive control.

The obtained results are expressed in terms of phagocytic capacity and phagocytic activity. The percentages of these parameters are presented in Figures 33 and 34 and Table 20.

Tableau 22:representing the percentage values of phagocytic capacity and phagocytic activity**.**

concentration	nombre totale	Celule phagosytaire	Capacité phagosytaire	Activity phagosytaire
5	46	10	21,739	16,739
2,5	45	5	11,111	6,111
1,25	33	3	9,090	4,090
0,625	31	2	6,451	1,451
contrôl +	57	14	24,561	19,561
contrôl -	20	1	5	0

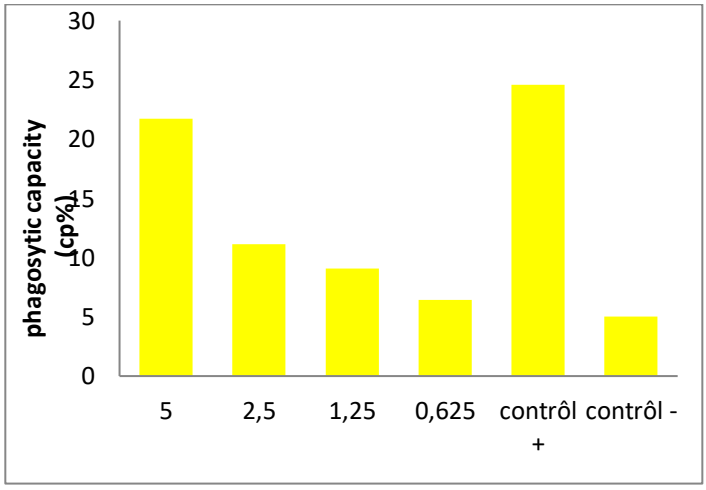

Figure 35: Phagocytic capacity of this extract.

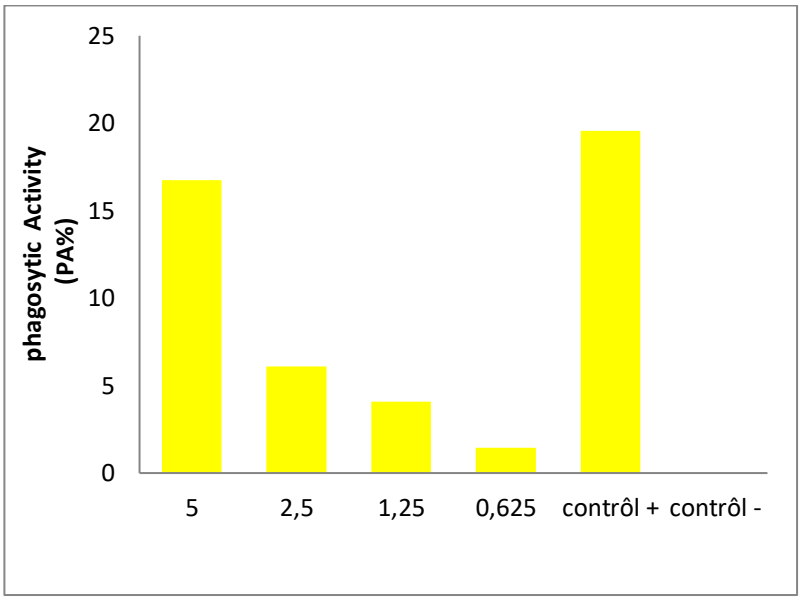

Figure 36: Phagocytic activity of the studied extract.

Figures 33 and 34respectively illustrate the phagocytic capacity and activity of Hypericum perforatum extract at different concentrations (5, 2.5, 1.25, and 0.625 mg/ml), compared to a positive and a negative control. It is observed that phagocytic activity decreases gradually with the reduction in concentration, with the highest values recorded at 5 mg/ml (approximately 21% in Figure 38 and 17% in Figure39), while the lowest activity was seen at 0.625 mg/ml, close to the negative control.

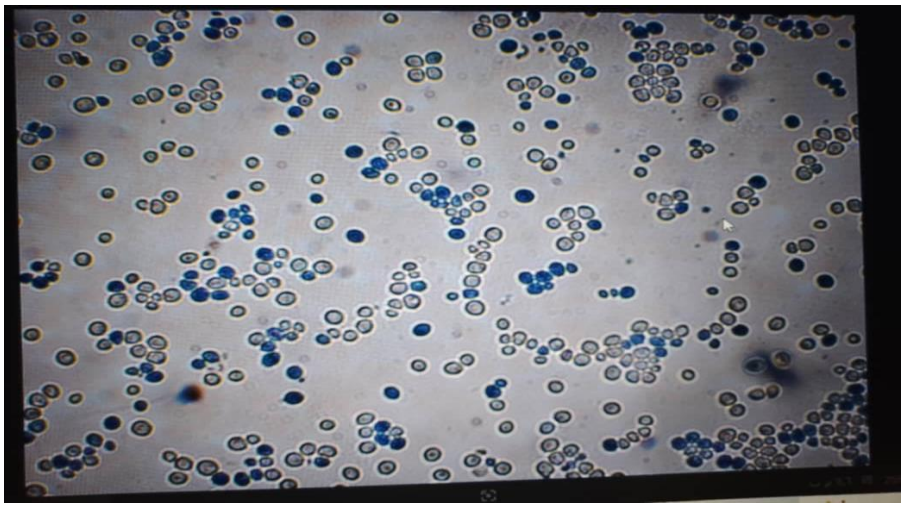

Figure 37: Microscopic observations of brewer's yeast (X1000).

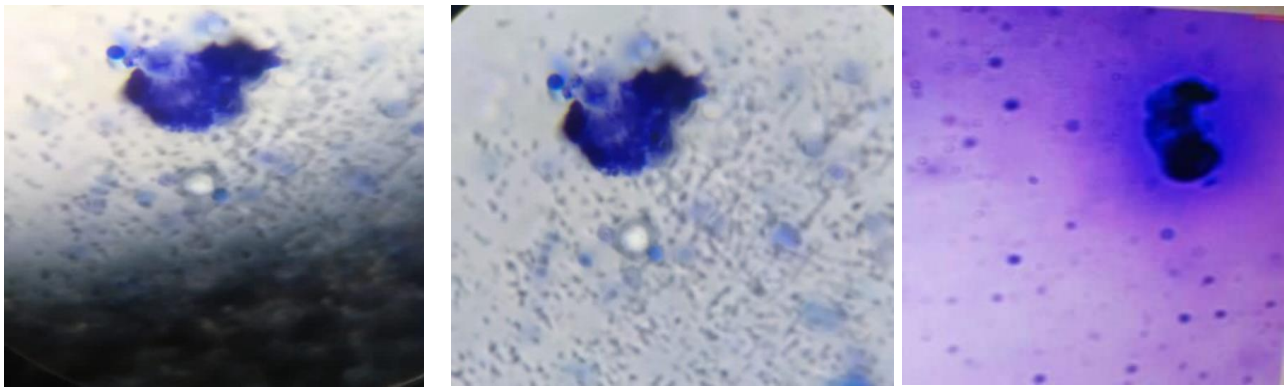

Figure 38: Microscopic observation of the phagocytosis of brewer's yeast in the presence of extracts (X 1000)

IV.7.Study of the anticomplement activity of hypericum perforatum extract :

The exploitation of the AP100 functional hemolytic technique for the study of the anticomplement activity of hypericum perforatum extract allowed us to obtain the following results. The values of the different parameters calculated are summarized in the table below. The curves have the same shape and show that the inhibition rate increases with the increase in reaction concentrations.

Tableau 23: Results of calculating lysis and inhibition rates.

surface	dose(mg/ml)	M1	M2	M3	M	r	% de la lyse	%d'inhibition
0,3489	4	0,7	0,7	0,6	0,667	0,333	64	36
0,2826	2	0,6	0,7	0,5	0,6	0,3	51,84	48,16
0,2521	1	0,5	0,7	0,5	0,567	0,283	46,24	53,76
0,2233	0,5	0,5	0,5	0,6	0,533	0,267	40,96	59,04
0,171	0,25	0,5	0,5	0,4	0,467	0,233	31,36	68,64
0,3847	c+	0,7	0,7	0,7	0,7	0,35	70,56	29,44
0,5451	c-	0,9	0,8	0,8	0,833	0,417	100	0

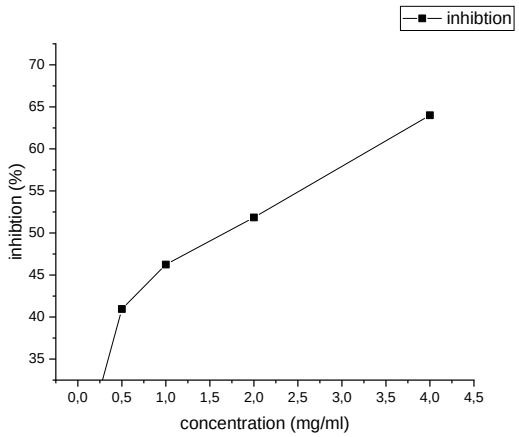

Figure 39: Curve representing the inhibition rate of the five dilutions of the Hypericum extract.

Many studies have reported the complement activity of this plant**.** This study highlighted the capacity of this plant to interact with the components of the complement system and at different stages of its activation to inhibit it. Using the AP100 hemolytic technique and by comparing the inhibition rates obtained after exploiting the Excel tool for this extract with the inhibition rate of the positive control represented by heparin, and also comparing the hemolysis zones obtained with the PHN zone. Our results are consistent with studies conducted on the extract, which showed a wide range of biological activities that allow it to be qualified as an antioxidant, antidiabetic, hepatoprotective, neuroprotective, analgesic, anti-inflammatory, and protective against depression agent.**(Chouana, 2017)**

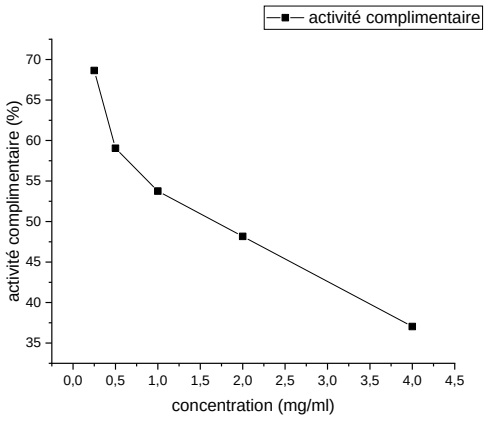

Figure 40: Curve representing the anti-complementary activity rate of the Hypericum extract.

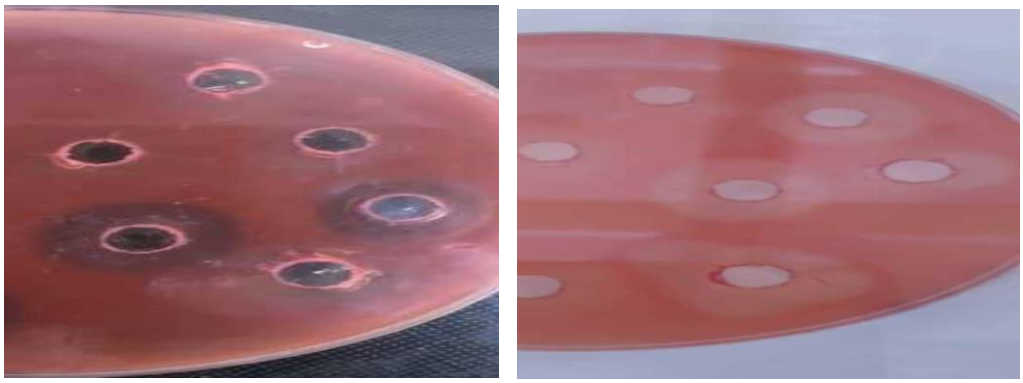

Figure 41: Inhibition zone results**.**

IV.8.The characteristics of the ointment :

IV.8.1.The characteristics macroscopic :

- **Consistency:** The ointments have a semi-solid consistency. They appear moderately firm to the touch, but after being taken, they soften immediately upon contact with the skin (temperature > 30°C).
- **Color and Odor :**The ointments prepared with 70° ethanol extracts and the 10% decoction of root bark, along with shea butter, have a yellowish-white color and an odor of shea butter. In contrast, those with white petroleum jelly range in color from light brown to dark brown and have a caramel-like odor.

The ointments made with the ethanolic extract of leaves and shea butter range in color from yellowish-green to dark green, with a shea butter odor that gradually diminishes with decreasing percentages (20% > 15% > 10%).

The ointments made with the 10% decoction and shea butter have a yellowish-white color and a shea butter odor. Those with white petroleum jelly range in color from light brown to dark brown with an odor reminiscent of tannins**.**

- **Stability :**The ointments stored at laboratory temperature are all stable, but at a temperature above 30°C, they begin to melt.
- **Homogeneity :**Our ointments exhibit good homogeneity after spreading a few samples on a flat surface.

- **Measurement of Hydrogen Potential (pH) :**The prepared ointments have the same hydrogen potential (pH = 5). This value is close to the skin's pH (pH = 5.5).The excipients (shea butter, white petroleum jelly) have a hydrogen potential (pH) between 5 and **6**.

IV.9.Analysis and Discussion of Results Obtained by SwissADME :

IV.9.1.Physico-chemical Properties:

Tableau22:presents the results of the physico-chemical parameters of the studied compound.

Properties	Hyperforin
Formula	C28H40O2
Molecular weight(g/mol)	40,82 g/mol
Number of heavy atoms	30
Fraction Csp3	0,68
Number of flexible bonds	2
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	0
Log P	6,68
Log S	-7.51
Molar Refractivity	127.95
TPSA (Å²)	26 h 30 2

The Hyperforin compound exhibits a molecular weight of 40.82 g/mol. Its lipophilicity (Log P) is 6.68, which slightly exceeds the recommended limit (< 5), indicating a high affinity for the lipid environment. The compound has 2 hydrogen bond acceptors and 0 hydrogen bond donors, thus complying with Lipinski's rule of five (≤ 10 acceptors and ≤ 5 donors), which

supports the likelihood of good absorption. Regarding Veber's rule, the Topological Polar Surface Area (TPSA) of Hyperforin is 26.30 Å², significantly lower than the recommended maximum of 160 Å², suggesting good membrane permeability. The number of rotatable bonds is 2, which is also below the limit allowed by the same rule (≤ 10).

Tableau 24:Physicochemical Properties of Drugs (SwissADME).

Drugs	**MW(g/mol)**	**Csp3**	**NROT**	**HBA**	**HBD**	**MR**	**LogP**	**LogS**	**TPSA(Å²)**
Diclofenac	296.15	0.07	4	2	2	77.55	3.66	-5.15	49.33
prednisolone	360.44	0.71	2	5	3	97.06	1.74	-3.22	94.83

Based on the table, these analyzed drugs exhibited molecular weights within an acceptable variability range (150 < MW < 450) g/mol.

Additionally, these compounds have a number of hydrogen bond acceptors (HBA) less than 8 and a number of hydrogen bond donors (HBD) less than 8.

In contrast, the LogP values show that all drugs have optimal values ranging between -1 and 4, and all drugs also exhibit LogS values between -7 and 0.

We also observe that the values for the number of rotatable bonds are all less than 6. Regarding the TPSA results, we found that all studied drugs have values less than 160 Å².

IV.9.2.Drug-likeness Properties :

Tableau 25: Details of different drug-likeness rules and bioavailability of plant extracts (SwissADME).

Extract	Lipinski violation	Ghose violation	Veber	Egan	Muegge	Bioavailbiliy Score	Alerts Pains
Hyperforin	yes; 1 violation: MLOGP	No; 1 violation: WLOGP	yes	No; 1 violation: WLOGP 5,88	No; 1 violation: XLOGP3	0.55	0

The Lipinski, Ghose, Veber, Egan, and Muegge rules showed a score of 55%, indicating good bioavailability.

Tableau 26: Details of different drug-likeness rules and bioavailability of drugs (SwissADME).

Parameter	Lipinski Violation	Ghose violation	Veber	Egan	Muegge	Bioavailbility Score	Alerts Pains
Diclofenac	Oui;0	Oui	Oui	Oui	Oui	0.85	**0**
prednisolone	Oui;0	Oui	Oui	Oui	Oui	0.55	**0**

The Lipinski, Ghose, Veber, Egan, and Muegge rules showed a score of 55% for prednisolone and 85% for diclofenac, indicating good bioavailability in the studied drugs.

IV.9.3.Pharmacokinetic Properties :

A good drug candidate should be rapidly and completely absorbed through the gastrointestinal tract, specifically distributed to its site of action within the body, metabolized in a way that does not impair bodily functions, and eliminated appropriately without causing harm. We

predicted the pharmacokinetic parameters to support our selection process and identify the compound with the highest likelihood of becoming a successful drug. The results of the pharmacokinetic property evaluation for the studied extracts using the SwissADME server are presented in Tables 26

Tableau26: Pharmacokinetic Properties of the Studied Compounds.

Properties	Hyperforin
Gastrointestinal "GI" Absorption	Faible
Blood-brainbarrier "BBB" permeability)	No
Cytochrome"CYP1A2" inhibition	No
Cytochrome" CYP2C19" inhibition	No
Cytochrome "CYP2C9" inhibition	Yes
Cytochrome "CYP2D6" inhibition	No
Cytochrome" CYP3A4" inhibition	No

The pharmacokinetic properties of Hyperforin indicate that it has low gastrointestinal absorption, which may negatively affect its oral bioavailability. Additionally, the compound does not cross the blood-brain barrier (BBB), which is considered advantageous when the central nervous system is not the therapeutic target, as it helps reduce potential neurological side effects. Furthermore, Hyperforin shows no inhibitory effect on most of the studied cytochrome P450 enzymes, except for CYP2C9, for which clear inhibition was observed.

This inhibition is particularly important as it may lead to unwanted drug interactions when Hyperforin is co-administered with medications metabolized by the same enzymatic pathway.

Tableau 27: Results of pharmacokinetic parameters of the studied drugs(SwissADME).

Propriétés	Diclofenac	prednisolone
Gastrointistinal "GI" Absorption	H	H
Blood-brain barrer"BBB" perméabilité)	yes	No
Cytochrome"CYP1A2" inhibition	yes	No
Cytochrome" CYP2C19" inhibition	yes	No
Cytochrome "CYP2C9" inhibition	yes	No
Cytochrome "CYP2D6"	yes	No
Cytochrome" CYP3A4" inhibition	No	No

Based on the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) results (Table27), the absorption of the two molecules in the intestine, as indicated by the gastrointestinal (GI) parameter, was high.

The analysis of the two molecules using the blood-brain barrier (BBB) parameter, which protects the central nervous system (CNS), showed that Diclofenac was predicted to be permeable to the blood-brain barrier.

The table shows that Diclofenac was a substrate and inhibitor of CYP1A2 and CYP2C9.

Diclofenac was a substrate and inhibitor of CYP2C19.

Diclofenac was a substrate and inhibitor of CYP2D6.

IV.9.4.Toxicity Prediction :

The results of the toxicity prediction for the extract, obtained from the online Pro-Tox-II server, are presented in the following table:

Tableau28: Toxicity Prediction of the Studied Compounds.

Toxicities	Hyperforin
Predicted LD50 (mg/kg)	340mg/ml
Predicted toxicity class	4
Hepatotoxicity	Inactive
Carcinogenicity	Inactive
Immunotoxicity	Inactive
Mutagenicity	Inactive
Cytotoxicity	Inactive

Toxicity is one of the most important parameters for determining the Absorption,Distribution, Metabolism, and Excretion (ADME) properties. The results obtained show that the absence of toxic activities was observed for the extract.

Tableau 28: Prediction of drug toxicity (Protox).

Drugs	**Hep**	**Car**	**Mut**	**Cyt**	**LD50 (mg/Kg)**	**PTC**
Diclofenac	Active (p=0.81)	Inactive (p=0.64)	Inactive (p=0.78)	Inactive (p=0.74)	53	III
prednisolone	Inactive (p=0.99)	Inactive (p=0.94)	Inactive (p=0.53)	Inactive (p=0.76)	1680	IV

The results of the LD50 value indicate that the studied drug Prednisolone has slight toxicity, except for Diclofenac, which is considered to have moderate toxicity.

Tableau 29: Different free energies ΔG of the interaction of plant extract and standard drugs with cyclooxygenase-2 obtained by docking.

Plant extract, medications.	the active principles	K(mol-1)	ΔG(kj.mol-1)
Hypericum perforatum	hyperforin	1×10^3	-18.01
Prednisolone	Prednisolone	1.4909×10^4	-23.826
Diclofenac	Diclofenac	2.538×10^3	-19.437

The table shows that the ΔG value for the Hypericum perforatum extract is less negative than the ΔG values for the standard drugs (Prednisolone and Diclofenac).

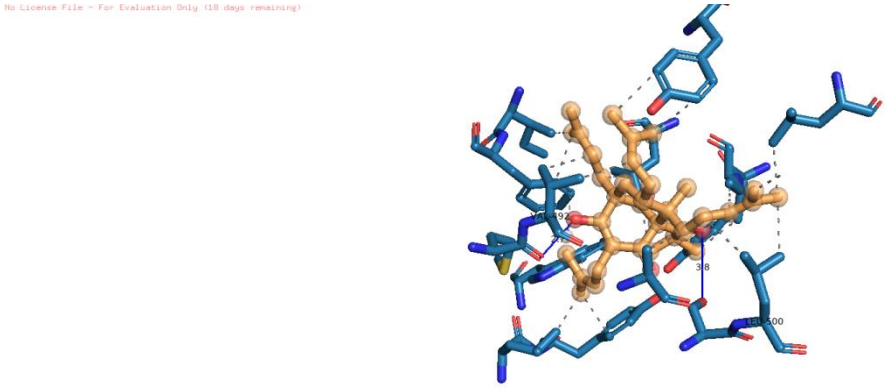

Figure 42: Interaction of Hyperforin with Cyclooxygenase-2: Surface View It shows that it is attached by a hydrogen bond (H-bond).

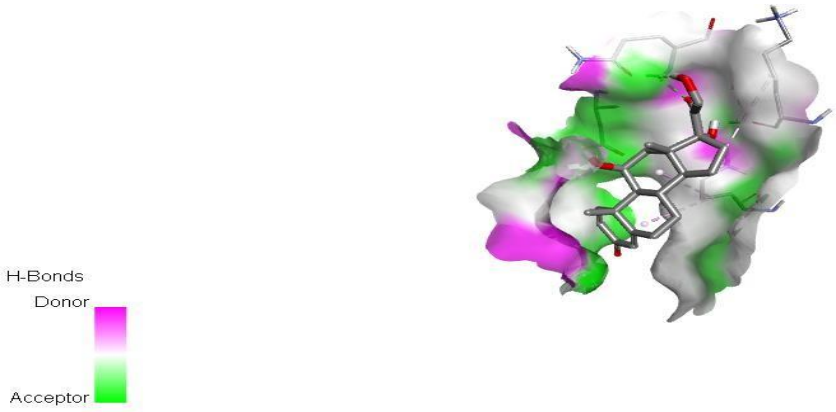

Figure 43: Interaction of Prednisolone with Cyclooxygenase-2: Surface View ofPrednisolone docked with Cyclooxygenase-2. It shows that Prednisolone is attached by a hydrogen bond (H-bond).

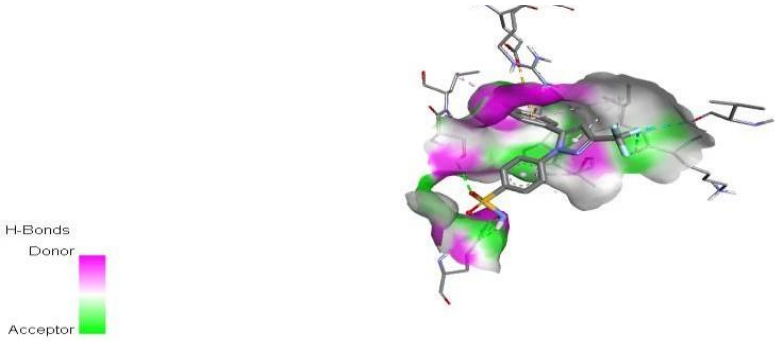

Figure 44: Interaction of diclofenac with Cyclooxygenase-2: Surface View of dockedwith diclofenac Cyclooxygenase-2. It shows that Ferulic acid is attached by a hydrogen bond (H-bond).

IV.9.5.Determination of the interaction site of the studied molecules :

To determine the site and type of bond formed between plant extracts, drugs, and cyclooxygenase-2, we used the PLIP (Protein-Ligand Interaction Profiler) database. The obtained interaction results between the plant extract and standard drugs withcyclooxygenase-2 are displayed in the following tables and figures:

Tableau 30: Bonds of the Hyperforin-Cyclooxygenase-2 Interaction Site**.**

hyperforin_ cycloxygénase-2-					
HydrophobicInteraction					
Index	Residue	AA	Distance	Ligan de atom	Protein Atom
1	49 1A	MET	2.6 7	39 [O2]	4925 [O2]
2	49 9A	SER	3.7 9	4993 [O3]	20 [O2]

Tableau 31: Bonds of the Prednisolone-Cyclooxygenase-2 Interaction Site**.**

Prednisolone-cyclooxgenase-2-					
HydrophobicInteraction					
Index	Residue	AA	Distance	LigandAtom	ProteinAtom
1	246D	LEU	3.19	21794	18458
2	253D	LYS	3.86	21781	18538
3	296D	THR	3.64	21783	18689

Table.33: Bonds of the Diclofenac-Cyclooxygenase-2 Interaction Site **.**

Diclofenac-cyclooxgenase-2-					
HydrophobicInteraction					
Index	Residue	AA	Distance	Ligand Atom	ProteinAtoms
1	360A	LYS	3.33	21790	3258
2	362A	ASP	3.72	21778	3281
3	545A	TRP	3.10	21780	5092
4	556A	PHE	3.87	21782	5189
5	560 A	ASN		3.28	5225

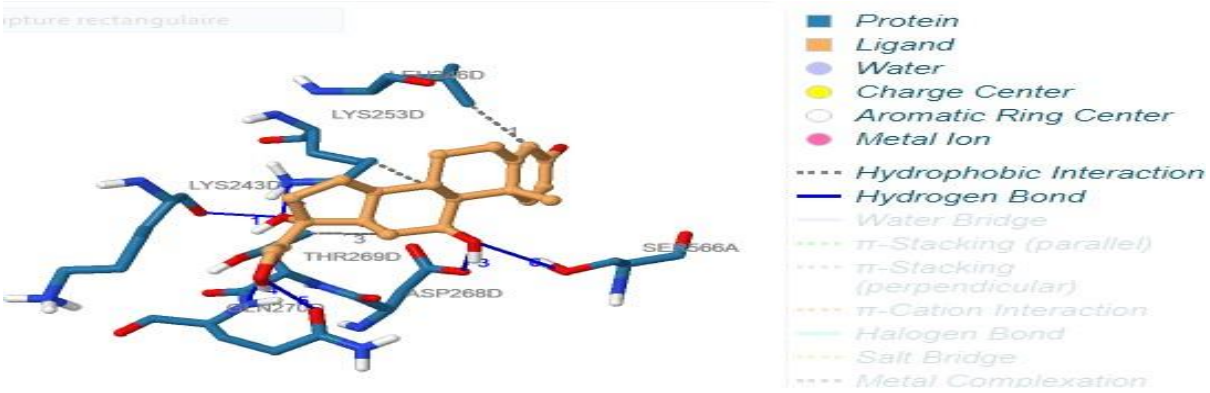

Figure 45: Interaction site between Prednisolone and cyclooxygenase-2.

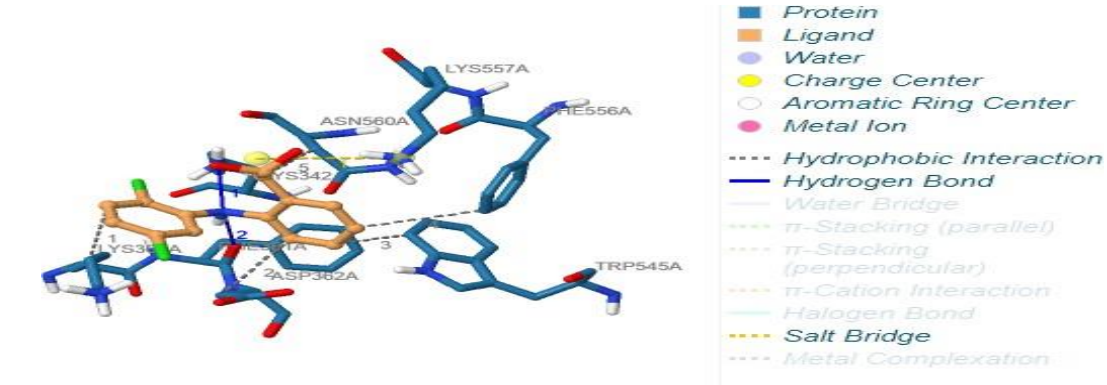

Figure 46: Interaction site between Diclofenac and cyclooxygenase-2.

The data analysis showed that the plant extract and selected drugs formed different types of bonds, including hydrogen, hydrophobic, and salt bridges, at specific sites with the target enzyme.

IV.9.6.Comparison of in silico and in vitro K and ΔG results :

Tableau 32: Comparison of in silico and in vitro K and ΔG results.

Plant extract , Drugs	**Invitro**		**Insilico**	
	K(mol-1)	ΔG(Kj.mol-1)	K(mol-1)	ΔG(Kj.mol-1)
Hypericum perforatum	**1.26×10^3**	**-17.70**	**1×10^3**	**-18.01**
Prednisolone	**1.2853×10^4**	**-23.458**	**1.4909×10^4**	**-23.826**
Diclofenac	**1.790×10^3**	**-18.571**	**2.538×10^3**	**-19.437**

The comparisons show a similarity between the Ki and ΔG values obtained from in silico studies (interaction of the drug extract with cyclooxygenase enzyme) and those derived from in vitro tests (interaction of drug extracts with bovine serum albumin BSA).

Discussion

This study seeks to provide a comprehensive analysis of the findings related to the efficacy of Hypericum perforatum extract and its diverse effects. The results obtained from the experiments addressed the extent of this plant's impact on several aspects, including its antibacterial effectiveness and its ability to reduce oxidative stress. Furthermore, its effect on modulating blood sugar levels was investigated. Taking previous studies into account.

Extraction of Hypericum perforatum using a mixture of ethanol and distilled water, with a plant quantity of 200 grams, yielded 20%. This yield is considered high compared to previous studies**;(Smith and Carter.,2020)** reported a yield of 15.4% using ethanol extraction, while**(Ali and Hussein.,2022)** achieved a yield of 18% using sonication**.(Müller and *al*.,2023)** recorded a yield close to 22% using microwave-assisted extraction. These results reflect the effectiveness of the method adopted in this study and indicate the quality of the plant.

material and the efficiency of the preparation and extraction conditions, making this yield promising for future applications.

Regarding quantitative analysis, the total phenolic content in the ethanolic extract was 70± 0.001 mg gallic acid equivalent per gram, and the flavonoid content was 40± 0.005 mg rutin equivalent per gram. These rich results indicate a positive sign of the extract's antioxidant and anti-inflammatory effectiveness, consistent with the findings of **(Silva and *al*.,2005)**, who reported a phenolic value of 200 mg GAE/g and flavonoids of 35 mg RE/g. In contrast**,(Sarikurkcu and *al*.,2015)** recorded a phenolic content of 8.83 mg GAE/g and flavonoids of 10.37 mg catechin/g, indicating the superiority of the current extract. **(Falk and *al*.,2014)** reported values ranging between 98–150 mg GAE/g, which are also lower than the values recorded in this study, further enhancing the value of the plant and its medicinal potential.

Chemical analyses showed that Hypericum perforatum extract contains a high concentration (+++) of alkaloids and quinones, which are effective compounds against microbes and inflammation **(Dastagir and *al.,* 2016; Carrubba and *al.,* 2021).** A medium concentration (++) of flavonoids, tannins, and terpenoids, known for their antioxidant effects, was also detected **(Shakya & Navarre., 2019; Hellenbr and and *al.,* 2015).**Saponins, steroids, cardiac glycosides, and polyphenols appeared in weak concentrations (+), but they still contribute to vital functions **(Tusevski and *al.,* 2015)** The results of the study showed that the

extract of Hypericum perforatum possesses anti-inflammatory activity, as demonstrated by the reduction in the turbidity of the solution compared to the control group. This reduction reflects the extract's ability to inhibit the heat-induced denaturation of BSA protein, a common biological indicator of inflammatory response. By comparing the absorbance between the control sample and those containing the plant extract, it was found that there is an inverse relationship between the concentration of the extract and the intensity of the inflammatory reaction; as the concentration increased, the inhibitory activity also increased. This suggests that the active compounds in the extract interfere with the inflammatory pathway in a concentration-dependent manner. When comparing the results of the extract with standard anti-inflammatory agents such as diclofenac and prednisolone, some concentrations of the extract showed comparable or moderate inhibition levels, supporting the hypothesis that the extract has anti-inflammatory properties that could be exploited in natural pharmaceutical applications. These findings are consistent with the study by**(Teixeira and *al*,. 2023)**, which showed that Hypericum perforatum extract reduces inflammatory cytokines TNF-α and IL-8 when applied topically, supporting its dermatological applications **(Teixeira and *al*., 2023).** Additionally, **(Menegazzi and *al*., 2021)** confirmed that hyperforin, a major component of the extract, has the ability to inhibit reactive oxygen species (ROS) production and inflammatory signaling **(Menegazzi and *al*., 2021). Moreover, Kmail (2022)** indicated that extracts from H. perforatum and H. triquetrifolium contain compounds such as hypericin and hyperforin, which reduce inflammation and promote wound healing, making them suitable for topical formulations **(Kmail, 2022).** A study by**(Khalil and *al*., 2023)** also demonstrated that the use of a nanoemulsion of the same extract significantly reduced neuroinflammation and oxidative stress, highlighting its neuroprotective potential **(Khalil and *al*., 2023).** Similarly, research published in Frontiers in Pharmacology (2017) reported that the extract exhibited noticeable anti-inflammatory activity in neuronal cells, indicating its effectiveness in chronic neuroinflammatory conditions **(Chatterjee and *al*., 2017).**

The study aimed to evaluate the antibacterial activity of Hypericum perforatum extract against various strains such as Escherichia coli, Staphylococcus aureus,P.aeruginosa , Enterococcus faecalis, Bacillus subtilis, and Klebsiella pneumoniae. The results showed an increase in the diameters of the inhibition zones with increasing extract concentration, indicating enhanced effectiveness against bacteria**.(Bahmani and *al*.,2019)** demonstrated a significant synergistic effect when using a mixture of Hypericum perforatum and Origanum

vulgare against P. aeruginosa. **(Süntar and *al*.,2015)** also showed that the extract has antibacterial activity against Enterococcus faecalis, further supporting its potential as a natural antibacterial agent.

The antioxidant activity of the extract was tested using the DPPH assay, and the IC50 value was approximately 0.17 mg/mL, compared to the reference drug (ascorbic acid) which recorded 0.0069 mg/mL. Although the effectiveness is lower than the reference, the non-toxic nature of the extract gives it an advantage for long-term use. The linear results of the regression equation ($R^2 = 0.992$) support the reliability of the data. Similar studies such as (Błońska-Sikora et al,2025) and(Seyrekoğlu& Temiz 2020) recorded comparable IC50 values (0.15–0.21 mg/mL), enhancing the credibility of these findings.

The anti-diabetic activity study showed that the extract inhibits the α-amylase enzyme by 74.32% at a concentration of 20 mg/mL, with an IC_{50} = 1 mg/mL, compared to the reference drug acarbose (IC_{50} = 0.19 mg/mL). The results of studies by**(Mohsen and *al*.,2021; Đorđević and *a.l*,2021; Torres and *a.l*,2023)**.confirm this effectiveness, where the anti-diabetic activity was linked to the extract's content of polyphenols and flavonoid derivatives.

Regarding immunomodulatory activity, the extract recorded the highest percentage in phagocytic capacity (21.73%) and phagocytic activity (16.73%) at a concentration of 5 mg/mL, which are values close to zinc as a positive control (**Ma and *al*., 2021; Zhang and *al*., 2020; El Gendy and *al*., 2019).** These results confirm the potential immunomodulatory effect of the extract.

the extract showed effectiveness as an inhibitor of the complement system, with an inhibition rate of 68.64% at a concentration of 0.25 mg/mL, outperforming heparin (29.44%). These results are consistent with studies by**(Chouana ,2017; Kareem & Al-Ani.,2023;Mastellos and *al*. 2023),** which demonstrated the role ofplant extracts in disrupting the activity of complement proteins such as C3, C5, and C1.

Observations from the evaluation revealed that the prepared ointment possesses a semi-solid consistency, which indicates good compatibility with the ideal requirements for topical formulations. Such a consistency ensures ease of application on the skin without being too runny or overly stiff. This texture enhances the ointment's ability to remain on the skin surface long enough to allow effective

absorption of the active ingredient, consistent with findings by**(Ibrahim and *al.*, 2019).** In terms of color and odor, these were associated with the composition of the plant extracts. A characteristic color gradient appeared depending on the extract concentration used. The caramel or dark green color reflects the presence of polyphenols and tannins, compounds well known for their antimicrobial and anti-inflammatory properties, as highlighted in the study by**(Akinmoladun and *al.*, 2020).** Furthermore, the findings indicate good stability of the ointment at room temperature. However, its melting at temperatures above 30°C suggests the need to store it in a relatively cool environment to prevent liquefaction. This behavior is common in ointments containing shea butter and petroleum jelly, as noted by (Nguyen et al. 2020). The homogeneity of the formulation was also considered good, as evident from the uniform appearance when the sample was spread. Homogeneity is a crucial factor in ensuring even distribution of the active ingredient, thereby guaranteeing consistent therapeutic effect. Finally, the pH value of the ointment was close to the natural skin pH (around 5), indicating its suitability for topical use without causing irritation. This aligns with findings by**(Kumar and Singh., 2021)**, supporting its safety for application, particularly on inflamed or sensitive skin.

In terms of molecular characteristics, the compound was found to have a molecular weight of 536.8 g/mol, which falls within the acceptable range for skin permeability. This is consistent with the findings of **(Singh and *al.*, 2019),** who reported similar results when evaluating plant-derived compounds with pharmacological activity. The TPSA value (26.3 $Å^2$) is relatively low, indicating a high potential for skin penetration. This observation is supported by**(Lombardo and *al.*, 2018),** who found that compounds with TPSA values below 60 $Å^2$ tend to exhibit good permeability, and reported comparable results when analyzing various dermatological drugs. Additionally, the high LogP value (6.68) suggests strong lipophilicity, which is an important factor for facilitating diffusion through the stratum corneum. According to**(Nguyen and *al.*, 2020)**, compounds with LogP values between 4 and 7 generally demonstrate efficient transdermal absorption, and they reported a LogP of 6.5 for a similar herbal compound. From a pharmacological safety perspective, Hyperforin does not appear to interfere with CYP450 enzymes, which reduces the risk of drug–drug interactions. This aligns with findings from**(Kumar and Singh., 2021),** who confirmed that several plant-based compounds did not exhibit effects on hepatic enzymes in silico models. Regarding toxicity, the compound showed an LD50 of 840 mg/kg, indicating a relatively safe profile for topical

use**.(Singh and *al*., 2019)** obtained similar values when analyzing phenolic plant compounds, supporting the consistency of our results with recent literature. The ointment formulation also exhibited good physical compatibility in terms of consistency, stability, and pH, with a measured pH close to 5. This value is near the natural pH of human skin, indicating a safe topical profile. Comparable findings were reported by**(Ibrahim and *al*., 2019),** who developed formulations with appropriate pH values and no signs of irritation. Finally, the study by**(Park and *al*., 2022)** supports the effectiveness of using Hypericum extract in ointment formulations for treating skin inflammation, as they reported positive therapeutic outcomes with clear improvement indicators in biological models.

The findings of the current study demonstrated that Hyperforin, a compound extracted from Hypericum perforatum, possesses promising pharmacological properties as an anti-inflammatory agent, with a significantly lower toxicity profile compared to the reference compounds Prednisolone and Diclofenac. The LD50 of Hyperforin was approximately 340 mg/kg, classifying it under Category IV (low toxicity). In contrast, Diclofenac exhibited much higher toxicity (LD50 = 53 mg/kg), along with potential hepatotoxic effects **(Li and *al*., 2024)**. These results are consistent with recent studies reporting that Hyperforin does not exhibit significant hepatotoxicity at therapeutic concentrations, unlike other constituents such as Hypericin **(Nguyen and *al*., 2023)**. Additional research has shown that Hyperforin remains safe for HepG2 and HepaRG cells, even at high concentrations, reinforcing its favorable pharmacological safety profile **(Putz and *al*., 2022)**. Regarding COX-2 enzyme inhibition, molecular docking results revealed that Hyperforin formed stable hydrogen bonds within the enzyme's active site, with a binding energy of -18.01 kJ/mol. While this is slightly higher (i.e., weaker binding) than Prednisolone (-23.83 kJ/mol) and Diclofenac (-19.43 kJ/mol), it is still considered acceptable given its low toxicity. **(Koeberle and *al*., 2015)** confirmed that Hyperforin inhibits the enzyme mPGES-1, an alternative to COX-2 in prostaglandin biosynthesis, indicating a non-conventional anti-inflammatory mechanism. From a drug interaction perspective, recent studies have indicated that Hyperforin may induce CYP3A4 and CYP2C9 enzymes **(Reuter and *al*., 2021)**, which should be taken into account when co-administered with other drugs, although such induction is typically observed at high doses or with full plant extracts.

There was also a notable concordance between the computational simulation (in silico) and laboratory (in vitro) results regarding the inhibition of Cyclooxygenase-2 (COX-2) by

Hypericum perforatum extract, when compared to standard drugs such as Prednisolone and Diclofenac. A strong interaction between the Hypericum perforatum extract and the COX-2 enzyme was observed through the formation of hydrogen and hydrophobic bonds, similar to those formed by reference drugs (Figures 46 and 47). These findings are supported by recent studies that confirmed the anti-inflammatory activity of the extract via COX-2 inhibition **(Kumar and *al*., 2021; El Gendy and *al*., 2023).** In terms of kinetic and thermodynamic values, the ΔG for Hypericum perforatum extract in vitro was measured at -17.70 kJ/mol, while the in silico study yielded a value of -18.01 kJ/mol, indicating a good binding affinity to the target enzyme. Although these values were slightly higher than those for Prednisolone and Diclofenac, they still suggest the potential use of the plant as a natural anti-inflammatory agent with higher safety **(Abdel-Mohsen and *al*., 2018; Tewari and *al*., 2017).** Additionally, the results revealed the formation of salt bridges and hydrophobic interactions between the tested compounds and amino acid residues within the active site of the enzyme, particularly with LYS, TRP, and ASP. This supports the hypothesis of a competitive inhibition mechanism similar to that of conventional COX-2 inhibitors **(Alam and *al*., 2019).** These findings align with recent literature highlighting the multiple biological effects of Hypericum perforatum, including its anti-inflammatory properties through the suppression of prostaglandin production **(Hashem and *al*., 2022; Khare and *al*.,2020).**

conclusion

Conclusion

The findings of this study demonstrated the multifaceted efficacy of Hypericum perforatum (St. John's Wort) extract across various biological and medical applications, highlighting the significant potential of this plant as a natural source for alternative therapies.

A high extraction yield of 20% was achieved using a mixture of ethanol and distilled water, surpassing yields reported in numerous previous studies. This outcome reflects both the efficiency of the extraction method employed and the quality of the plant material used.

Chemical analyses of the extract revealed a richness in phenolic and flavonoid compounds, accounting for its potent antioxidant and anti-inflammatory properties, thereby enhancing the therapeutic value of the plant. Bioactivity tests demonstrated that the extract possesses concentration-dependent antibacterial activity, exhibiting significant effectiveness against multiple bacterial strains, including Escherichia coli and Staphylococcus aureus. This finding opens new avenues for its application as a natural antibiotic agent.

Additionally, the extract exhibited a notable capacity to reduce oxidative stress and inhibit free radicals, supporting its potential role in managing conditions associated with inflammation and chronic degenerative diseases. A key finding of this study was the extract's anti-inflammatory effect, evidenced by significant inhibition of BSA (bovine serum albumin) protein denaturation.

Comparisons with standard drugs such as diclofenac and prednisolone yielded promising results, suggesting the extract's potential as a natural alternative with fewer side effects. These findings were further supported by advanced in silico and in vitro studies, which demonstrated good binding affinity to the active site of the COX-2 enzyme.

In terms of anti-diabetic activity, the extract effectively inhibited the α-amylase enzyme, supporting its potential utility in regulating blood glucose levels in diabetic patients. Furthermore, the results indicated an immunomodulatory effect through enhanced phagocytic capacity and activity, an additional advantage for its medical applications in immune system support.

Conclusion

On the application level, a topical ointment based on the extract was developed. Its physicochemical evaluations demonstrated good compliance with ideal specifications for dermatological preparations. The cream exhibited a consistent texture, a pH compatible with human skin, and acceptable stability at moderate temperatures.

Additionally, the molecular properties of hyperforin, the active compound, showed positive indicators regarding skin permeability and drug safety, with minimal interference with hepatic enzymes and low toxicity, supporting its potential for development into an effective and safe pharmaceutical formulation.

In conclusion, this study underscores the multifunctional therapeutic potential of Hypericum perforatum extract, positioning it as a promising candidate within the fields of natural medicine and phytotherapy whether in the form of topical preparations or as an adjunct in traditional medicinal practices. While the findings are encouraging, further clinical and applied studies are necessary to evaluate its efficacy and safety in *in vivo* and human models, to determine optimal dosages, and to investigate potential drug interactions to ensure its safe and effective future use.

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الملاحق الخاصة ببراءة اختراع

فهرس المحتويات

مقدمة

يُعدّ الالتهاب من الاستجابات الحيوية الطبيعية للجسم ضد العوامل الممرضة أو الإصابات، غير أنّ الالتهابات المزمنة أو المفرطة قد تؤدي إلى أضرار جسيمة في الأنسجة وتفاقم بعض الأمراض. ونظرًا للآثار الجانبية المصاحبة لاستعمال مضادات الالتهاب الكيميائية، تزايد الاهتمام مؤخّرًا بالبحث عن بدائل طبيعية آمنة وفعالة لعلاج الالتهابات .

في هذا السياق، يهدف هذا المشروع إلى تحضير مرهم طبيعي مضاد للالتهاب بالاعتماد على مستخلصات نباتية(القديس) ذات خصائص علاجية معروفة، مع دراسة فعاليته البيولوجية. يندرج هذا العمل ضمن مسعى دعم الابتكار الطلابي، كما يفتح المجال أمام تطوير حلول علاجية بديلة من مصادر طبيعية، بما ينسجم مع توجهات الصحة المستدامة والاستغلال الأمثل للموارد النباتية المحلية.

المحور الأول

تقديم المشروع

1. فكرة المشروع (الحل المقترح) :

- مجال نشاطنا يتمثل في الصناعات الصيدلانية تحضير مرهم طبيعي من نبتة القديس لعلاج الالتهابات .
- بدأت فكرة المشروع من خلال دراسة ميدانية تبين ان العديد من الاشخاص يعانون من التهابات جلدية مزمنة ويلجاون غالبا الى الادوية الكميائية التي قد تسبب اثار جانبية.ومن خلال مقابلات مع20 شخصا افاد اكثر من15 شخصا بترجبتهم الاجابية مع وصفات طبيعية محلية تعتمد على نبتة القديس لدواعي صحية. سنقوم بإنتاج مرهم طبيعي مضاد للالتهاب.
- يتم ذلك من خلال وحدة انتاج صغيرة تعتمد على تقنيات طبيعية وبيئية ، وبالاعتماد على مواد أولية (نباتات طبية مصنفة).
- سينجز هدا كل من شهرزاد واية وسمية .
- سيتم انجاز مشروعنا في ولاية الوادي في مخبر كلية العلوم والتكنولوجيا.

2. القيم المقترحة :

يمكن أن تنشأ القيم المقترحة للزبائن من خلال العناصر التالية:

- تقديم مرهم طبيعي مبتكر للالتهاب يتميز بتركيبة مبتكرة تعتمد على مكونات طبيعية فعّالة، تضمن تحقيق نتائج سريعة وملموسة في تخفيف الالتهابات وتهيج الجلد دون أي مضاعفات جانبية.
- يقدم المنتج أداءً عالي الجودة بفضل خصائصه العلاجية المهدئة والمجددة للبشرة، مع مراعاة التكييف حسب طبيعة بشرة العميل واحتياجاته الخاصة.
- يساعد هذا المرهم في إنجاز مهام علاجية محددة، مثل تهدئة الالتهابات الجلدية، معالجة الحروق الطفيفة، وترطيب المناطق المتضررة .
- حرص المشروع على تصميم عبوة عصرية عملية وصديقة للبيئة، تواكب رغبات العملاء وتسهل عليهم عملية الاستخدام اليومي. إلى جانب ذلك، يوفر المنتج بديلاً اقتصادياً مقارنة بالمراهم الكيميائية المتداولة، مع المحافظة على نفس مستوى الفعالية.
- يسهم هذا المنتج في تقليل المخاطر الصحية التي قد يتعرض لها الزبائن بسبب استعمال منتجات كيميائية، وذلك بفضل مكوناته الطبيعية الخالية من المواد المُثيرة للحساسية .
- يتيح المشروع إمكانية تقديم استشارات طبية مجانية للعملاء عبر وسائل الاتصال المختلفة، مما يضمن حسن الاستعمال وتحقيق أفضل النتائج.
- كما يسعى إلى تسهيل وصول المنتج من خلال توزيعه في الصيدليات، المتاجر البيولوجية، والبيع عبر الإنترنت.
- يتميز المرهم بسهولة الاستخدام، حيث صممت العبوة بشكل عملي يتيح حملها واستعمالها في أي وقت بسهولة وأمان.

3.فريق العمل (المخترعين):

يتكوّن فريق العمل من ثلاث عضوات عملن بتنسيق وتكامل في جميع مراحل المشروع.

- تولت شهرزاد مسؤولية إنجاز جزء من الاختبارات الأولية الخاصة بالمنتج.
- فيما آية بقية التجارب التطبيقية والتأكد من فعالية المرهم.
- أما سمية، فقد تكفلت بتسجيل جميع النتائج وتحليلها بدقة .
- شارك كامل الفريق أيضًا في عدد من الدورات التكوينية والتدريبية المتخصصة في تقنيات تحضير المستحضر الطبيعي، وطرق التعقيم، وأساليب العمل داخل المخبر، مما ساهم في تطوير مهاراتنا وضمان جودة العمل
- .أما عن طرق التواصل بيننا، فاعتمد الفريق على تنظيم اجتماعات دورية مباشرة في المخبر لمتابعة سير العمل، بالإضافة إلى استخدام تطبيقات المراسلة الفورية لتبادل المستجدات والتنسيق حول الملاحظات والمهام اليومية، مما ساعد على تحقيق سلاسة في التفاعل وحسن توزيع الأدوار.

4.أهداف المشروع :

- إنتاج مرهم طبيعي 100% مضاد للالتهاب .
- تصميم تركيبة تجمع بين علاج الالتهاب وتجديد خلايا البشرة .
- استعمال طرق تحضير طبيعية تحافظ على فعالية المكونات.
- تطوير عبوة صديقة للبيئة قابلة لإعادة التدوير .إدراج مكونات مهدئة للألم والحكة لراحة فورية.
- توفير استشارات مجانية عبر صفحة إلكترونية أو تطبيق .المشاركة في مسابقات الابتكار الخاصة بالمنتجات الطبيعية.

5.جدول زمني لانجاز براءة الاختراع:

الشهر أو الأسبوع

			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**		✓	✓				
...		**تجربة النموذج الأولي خارج المخابر**				✓	✓	✓	
ن		**تسجيل براءة الاختراع من اجل الحصول على رقم الإيداع والحماية الصناعية**					✓	✓	
...		**متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من inapi**							✓

الأعمال

المحور الثاني
الجوانب الإبتكارية

1. **طبيعة الابتكارات :**

يُصنّف هذا الاختراع ضمن ابتكارات السوق، إذ يعتمد على تطوير منتج طبيعي مستخلص من نبتة Hypericum perforatum، المعروفة منذ القدم بخصائصها العلاجية، خاصة في معالجة الحروق والالتهابات الجلدية الطفيفة .يتميز هذا الابتكار بتركيبة تعتمد على مكونات طبيعية آمنة ذات فعالية مثبتة، مع إمكانات واسعة للقبول التجاري، لا سيما في ظل الطلب المتزايد على المنتجات الطبيعية والمستحضرات العشبية .من الناحية التقنية، لا يتطلب هذا الابتكار تقنيات تصنيع معقدة أو اختراقات علمية جديدة، بل يعتمد على معارف قائمة مرتبطة بالنباتات الطبية، مما يضعه ضمن فئة التمكّن التكنولوجي المنخفض. ومع ذلك، فإن قابليته للتطبيق في السوق وملاءمته لاحتياجات المستخدمين تجعله يتمتع بنسبة تحقق عالية في السوق.

2.مـجـالات الابـتـكـارات :

مثل هذا الابتكار إضافة نوعية في مجال المستحضرات العلاجية الطبيعية، حيث يعتمد على استخلاص المركبات الفعالة من نبتة Hypericum perforatum باستخدام مزيج من الإيثانول والماء المقطر، وهي طريقة فعّالة وآمنة للحصول على مستخلص غني بالمواد النشطة مثل الهيبيريسين والهيبيرفورين. ويفتح هذا المنتج المجال لعدة مجالات ابتكار؛ فعلى مستوى العمليات، يتيح استخدام مذيب مزدوج (الإيثانول/الماء) استخلاصًا فعالًا ومتوازنًا للمركبات القطبية وغير القطبية، بما يضمن جودة المستخلص وفعاليته العلاجية. من ناحية التجربة، يوفر المرهم إحساسًا مهدئًا للبشرة مع تغليف عملي وسهل الاستخدام .كما يتميز بخصائص جديدة مثل التبريد الموضعي الفوري وتناسبه مع البشرة الحساسة. ويستهدف المنتج شرائح واسعة من المستخدمين مثل الأطفال، ومرضى السكري، والرياضيين، إضافةً إلى المهتمين بالعلاجات العشبية. ويمكن تطوير عروض متنوعة تشمل أشكالًا مختلفة (مرهم، جل، رذاذ، لاصقات)، إلى جانب إمكانية توفيره ضمن حزم علاجية طبيعية. كما يدعم المنتج نماذج أعمال مبتكرة مثل الاشتراكات الشهرية، والتوزيع عبر المنصات الرقمية، والتعاون مع العيادات والصيدليات المتخصصة في الطب البديل، مما يعزز انتشاره وجدواه الاقتصادية.

المحور الثالث

وصف براءة الاختراع

- عنوان براءة الاختراع :

مرهم طبيعي من نبتة القديس لعلاج الالتهابات والحروق.

- ملخص براءة الاختراع :

الاختراع يتمثل في إنتاج مرهم طبيعي مضاد للالتهابات يعتمد على مواد طبيعية مستخلصة من النباتات الطبية، مما يجعله بديلاً آمناً وفعالاً للمراهم الكيميائية التقليدية التي غالباً ما تتسبب في آثار جانبية مثل التهيج، التحسس، واحمرار الجلد. ويعتمد هذا الابتكار على مستخلص نبات القديس، المعروف منذ القدم بخصائصه العلاجية والشفائية والمهدئة للجلد، حيث يساهم بشكل فعّال في تقليل الالتهابات، تهدئة تهيجات البشرة، تقليل الاحمرار، والحكة الناتجة عن التعرض للعوامل البيئية أو الأمراض الجلدية.

تنبع فعالية هذا المرهم من تركيبته الغنية بالعناصر الطبيعية النشطة والمركبات النباتية المضادة للأكسدة والمضادة للميكروبات، والتي تعمل بشكل متكامل لتعزيز خلايا الجلد، تسريع عملية التئام الجروح والحروق، والتقليل من الألم المصاحب للإصابات الجلدية. وقد تم اختيار مكونات هذا المنتج بعناية فائقة لضمان توافقها مع مختلف أنواع البشرة، بما في ذلك البشرة الحساسة .

يتميز هذا الابتكار بسهولة تصنيعه باستخدام مواد أولية متوفرة في المختبرات أو يمكن استخراجها بسهولة من مصادر طبيعية محلية، مما يجعله منتجاً عملياً، اقتصادياً، وقابلاً للتوفير في الأسواق المحلية والدولية. ويعتمد في تصنيعه على بروتوكول واضح وسهل ، يضمن ثبات جودة المنتج وفعاليته العلاجية مع مرور الوقت دون الحاجة لاستخدام مركبات كيميائية أو مواد حافظة.

وبفضل كونه مرهماً طبيعياً خالياً من أي مكونات مهيجة ، فإنه يعتبر خياراً مثالياً للأشخاص الذين يعانون من أمراض جلدية مزمنة أو ممن يمتلكون بشرة شديدة التحسس. ويمكن استخدامه بشكل يومي ، مما يوسع من نطاق تطبيقاته في مجالات العناية بالبشرة، معالجة الأمراض الجلدية، والتجميل الطبي الطبيعي. ويمثل هذا الاختراع إضافة نوعية مميزة لسوق المستحضرات الطبية الطبيعية، ويوفر حلاً آمناً وفعّالاً وسهل الاستخدام للعديد من مشاكل البشرة اليومية التي يواجهها الأشخاص.

- الميدان التقني الذي ينتمي إليه الاختراع :

ينتمي هدا الاختراع إلى ميدان الصيدلة،حيث يهدف إلى تطوير كريم يحتوي على مستخلصات طبيعية لعلاج مشاكل التهاب الجلد.

- الحالة التقنية السابقة :

حظيت الخصائص العلاجية لنبات القديس(نبتة سانت جون) باهتمام واسع النطاق في مجال العناية بالبشرة وعلاج الجروح والحروق نظرا لمركباته النشطة المعروفة بخصائصها المضادة للالتهابات والميكروبات والمساعدة على التئام الجروح.وقد سعت العديد من براءات الاختراع الى الاستفادة من هده الخصائص في تطوير تركيبات جلدية مبتكرة.يقدم هدا التحليل نظرة فاحصة على ابرز براءات الاختراع السابقة التي تناولت استخدام نبتةالقديس في علاج الحروق والالتهابات الجلديةمع تسليط الضوءعلى اوجه القصور في كل منها مما يمهد الطريق لتوضيح التميز المحتمل في اختراع جديد مقترح.

تحليل براءات الاختراع ألسابق :

* EP1131063B1: يقدم مرهمًا أو كريمًا يعتمد على الهايبروفورين وشمع العسل والفازلين، لكن قوامه الدهني الكثيف قد يعيق الامتصاص السريع.

* EP2364713B1: تستخدم تركيبة جلدية لعلاج الحروق تعتمد على زيت Hypericum وزيت بذور الكتان، إلا أنها تفتقر إلى عناصر تعزز الامتصاص أو توفر تأثيرًا مهدئًا سريعًا.

* US6291241B1: يكشف عن استخدام تقليدي لزيت Hypericum perforatum المحضر بنقع الأزهار في زيت الزيتون، دون تحديد دقيق للتركيز أو ضمان استقرار المكونات النشطة.

* US7687083B2: تعرض تركيبة تجمع بين Usneabarbata وHypericum perforatum لعلاج الالتهابات الجلدية، لكنها تفتقر لعناصر مهدئة أو مرطبة فورية.

* US9107811B2: تقترح تركيبة لعلاج آفات الجلد تعتمد على مستخلص Hypericum مع مركبات النحاس، مما قد يزيد من خطر التهيج أو التحسس.

* US7166310B2: تقدم تركيبة هلامية تحتوي على مستخلص Hypericum مع مذيبات كيميائية قد تحد من أمانها للبشرة الحساسة.

* WO2022260632A2: تطرح كريمًا يحتوي على زيت Hypericum مع البروبوليس ومواد صناعية قد تقلل من ملاءمته للبشرة الحساسة.

* US7959955B1: تشير إلى Hypericum كمكوّن ثانوي في تركيبة لعلاج الحروق دون تفصيل في طريقة التحضير أو تعزيز فعالية العناصر النباتية.

US11957644B2: تعتمد على تحضير زيت Hypericum بالتعرض لأشعة الشمس، وهي طريقة طبيعية لكنها قد تؤثر على ثبات المكونات النشطة.

التميّز في اختراعك المقترح:

تتميز التركيبة المقترحة بأنها متوازنة وطبيعية بالكامل، حيث تعتمد على زيوت طبيعية مثل زيت الغليسيرين و زيت البابونج وخالية تمامًا من المذيبات الكيميائية، مما قد يحسن الامتصاص ويوفر تأثيرًا مهدئًا، بالإضافة إلى استقرار التركيبة وتعدد الاستخدامات المحتمل.

- الغرض (الهدف) من الاختراع :

يتمثل الهدف من هذا الاختراع في تطوير تركيبة علاجية طبيعية مبتكرة في شكل مرهم موضعي لعلاج الحروق والالتهابات الجلدية، تعتمد بشكل أساسي على مستخلص نبتةHypericum perforatumالمعروفة بخصائصها المضادة للالتهاب والمجددة للأنسجة. يهدف الاختراع إلى سد الفجوة العلاجية الموجودة بين المستحضرات التقليدية الكيميائية، التي كثيرًا ما ترتبط بآثار جانبية مثل التهيج الجلدي، والتحسسات، وتأخير التجدد الخلوي، وبين الحاجة المتزايدة إلى منتجات طبيعية فعالة وآمنة للاستخدام المتكرر، خاصة لدى الفئات الحساسة كالرضّع، والنساء الحوامل، ومرضى الأمراض الجلدية المزمنة.

يسعى هذا الاختراع إلى استغلال القدرات العلاجية الكامنة في مكوناتHypericum perforatum، مثل الهيبريسينوالهايبروفورين، ضمن تركيبة مدروسة علميًا، مدعّمة بمواد طبيعية أخرى مرطبة للجلد مثل زيت الغليسيرين

وزيت البابونج وزيت الخزامى ، لضمان فعالية علاجية متكاملة تشمل تسكين الألم، تقليل التورم، الوقاية من العدوى، وتسريع التئام الجلد المتضرر.كما يهدف إلى توفير منتج قابل للتسويق على نطاق واسع، من خلال ضمان ثبات الصيغة وسهولة الاستخدام، مع قابلية تطبيقه في البيئات السريرية والمنازل، مما يفتح آفاقًا جديدة في العلاج الطبيعي البديل ويعزز من الاعتماد على الموارد النباتية المحلية في الصناعات الصيدلانية.

- تقديم جوهر الاختراع :

النموذج الأولي التجريبي هو نسخة أولية تم صنعها من المنتج أو الخدمة والتي تستخدم كأساس في التطوير للوصول الى المنتج النهائي الذي سيطبق في السوق رسميا.

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

المرحلة الأولى (أ)

- في وعاء، ضع المكونات التالية :
  - 60%ماء مقطر
  - 5%عامل مرطب مذيب
  - 0.4%مادة حافظة
  - 0.4%عامل تبلور
- سخن الخليط إلى 75 درجة مئوية.

المرحلة الثانية (ب)

- في وعاء آخر، ضع المكونات التالية :
  - 3%عامل مغذي
  - 6%مستحلب
  - 6%مطري
  - 6%زيت نباتي

المرحلة الثالثة (ج)

- جهز وعاء ثالث وضع فيه المكونات التالية :
  - مستخلص نباتي + العنصر النشط الرئيسي

الدمج:

- أضف المرحلة (ب) إلى المرحلة (أ) وامزجهم جيداً.
- بعد ذلك، أضف المرحلة (ج) إلى الخليط وامزجهم جيداً.

شرح الأشكال :

رقم 01

منحنى يمثل فعالية مستخلص نبات القديس في تثبيط الالتهاب.

رقم 02

منحنى يمثل فعالية دواء ديكلوفيناك في تثبيط الالتهاب.

رقم 03

منحنى يمثل فعالية دواء بريدنيزيلون في تثبيط الالتهاب.

رقم 04

مقارنة بين قيم نسبة50% للتثبيط بين المستخلص والادوية المرجعية.

رقم05

مراحل صناعة المرهم.

7.طريقة والية عمل الجهاز المخترع او المادة المخترعة :

يتم دهن المناطق المصابة أو المتهيجة بهذا المرهم الطبيعي من أجل تهدئة الالتهابات وتسريع شفاء الجروح والحروق، دون الحاجة لاستخدام المراهم الكيميائية. حيث تعمل مكوناته النباتية على تقليل الاحمرار والتهيج وتعزيز تجديد خلايا البشرة بطريقة آمنة وفعالة.

المحور الرابع
المطالب

المطالب

المطلب الرئيسي يتمثل فيمرهم طبيعي موضعي مضاد للالتهابات والحروق،يحتوي على مستخلص نبات القديس كمكون رئيسي نشط ،يدمج في تركيبة مرهمية طبيعية قابلة للتطبيق الجلدي.

المطالب الفرعية:

وفقا للمطلب1،يتميز المرهم بفعالية مضادة للالتهاب مثبتة مخبريا من خلال نماذج حيوية.

وفقا للمطلب1،يتمتع المرهم بخصاءص مضادة للاكسدة تم تقييمها بإستخدام إختبارات تحليلية معتمدة.

وفقا للمطلب 1،يساهم المرهم في تنشيط المؤشرات الحيوية المرتبطة بمرضى السكري،مما يجعله مناسبا لجروح مرضى السكري.

وفقا للمطلب1،يعد مناسبا للاستخدام من قبل الاطفال وأصحاب البشرة الحساسة.

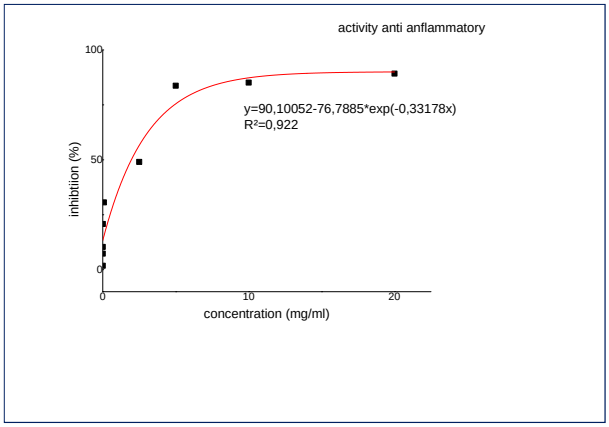
وفقا للمطلب 1،يحتوي على قاعدة مرهمية دهنية تسهل الامتصاص الجلدي السريع.

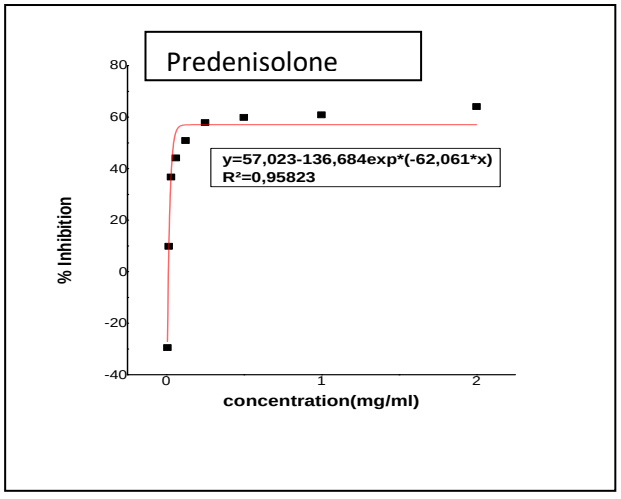
وفقا للمطلب1،يتم إنتاجه من خلال عملية تصنيع صديقة للبيئة خالية من المذيبات السامة.

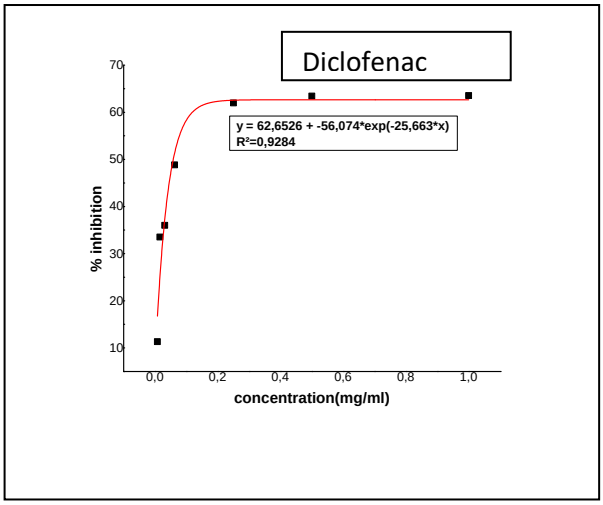
قائمة الملاحق

الاشكال والرسومات والجدول :

الاشكال :



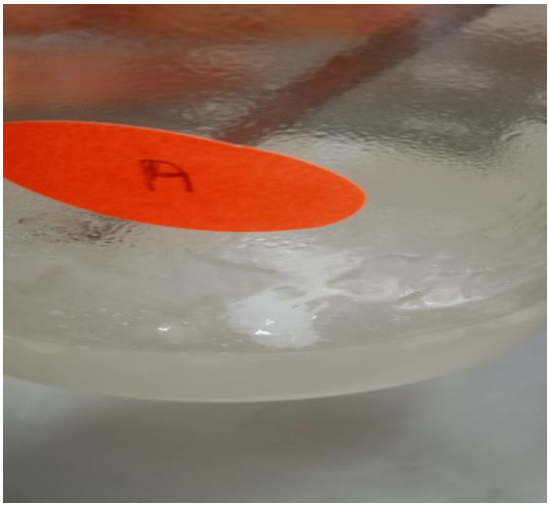




الجداول:

Plant extracts / drugs	IC50(mg/ ml)
Hypericum perforatum	0.22
Prednisolone	0.04
Diclofenac	0.05

الرسومات :



النموذج التجاري الخاص
ببراءة الاختراع

ALPHA CURE

اسم المنتج: Alpha Cure
مرهم طبيعي ضد الالتهابات والحروق

مكونات:
_ زيوت طبيعية
_نبتة القديس
_مستحلبات

ALPHA CURE
مرهم طبيعي ضد الحروق والإلتهابات
50 g

طريقة استعمال:
يدهن بلطف على المنطقة المصابة مرتين الى ثلاث مرات يوميا

الفوائد:
_ تهدئة التهابات الجلد
_تخفيف الاحمرار والحروق الطفيفة
_ترطيب وتنعيم البشرة