People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of El Oued

Faculty of Natural and Life Sciences

Laboratory of Valorization and Technology of Desert Resources (VTRS)

In order of obtaining the academic master's degree in biological sciences

Field: Biological Sciences

Specialization: Toxicology

Extract and immuno-toxico-biological study of Echinops spinosissimus

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Academic Year: 2024-2025 / 1446-1447 AH

Acknowledgements

In the Name of Allah, the Most Gracious, the Most Merciful.

Praise be to Allah, by whose grace all good deeds are accomplished, and may peace and blessings be upon the most honorable of prophets and messengers, our Master Muhammad, and upon his family and companions.

We extend our thanks and gratitude to all who contributed to the realization of this memorandum.

First and foremost, we offer our sincere thanks and deep appreciation to our supervising professor "**Adaika Aicha**", for her invaluable guidance, continuous support, and great patience. Her scientific guidance was instrumental in overcoming difficulties and reaching this stage.

We would like to express our deep gratitude to the co-supervisor **Guediri Imane** for her valuable guidance, constant support and insightful suggestions, which played a decisive role in shaping the quality and depth of this thesis.

We would like to express our sincere thanks and gratitude to the co-supervisor **Djarallah Maroua** for her valuable guidance and advice.

I extend my sincere thanks and gratitude to **Dr. Aouimer meriem**, Head of my thesis defense committee, **Dr. Zeghib khawla**, discussant, **Dr. Djedidi samiha**, incubator representative, and **Dr.Bahri abdelmoumen**, economic partner, for their constructive feedback that contributed to enriching this scientific work and guiding it towards improvement.we're proud you chaired our committee. It clearly showcases your academic generosity and dedication to scientific research.

We wish to thank all the professors and colleagues at the Chahid Hamma Lakhdar University of Oued Souf, Faculty of Nature and Life Sciences, Department of Biology, for their support and encouragement throughout our period of study.

May the peace, mercy, and blessings of Allah be upon you.

الحمد لله الذي بنعمته تتم الصالحات، وبتوفيقه تتيسر الصعوبات، وبعونه تتحقق الأمنيات

I dedicate the fruit of this academic endeavor, by the grace of Allah Almighty, to my **dear parents, Boutalbi Mohamed Tahar** and **Ben Djaloul Wahiba**, who gave me life and supported my journey with their sincere prayers and boundless love. To you both belongs the greatest credit for all I have achieved.

And to my **cherished family**: my brothers **Abdelhalim, Moussa, Aymen,** and **Nasreddine**, and my sisters **Wafa** and **Awatef** , you have been my constant support and aid at every turn. To my brothers' wives, **Touati Samia** and **Mohammedi Aicha**, and to my sisters' husbands, **Barakat Ibrahim** and **Said Choaib**. And to my beloved nieces and nephews: **Mohamed El-Bachir**, **Adam**, **Ghufran**, **Tawba**, **Buchra El-Rahman**, and **Hidaya**. To every single member of my family who has showered me with love and support, this achievement is for each one of you.

I also extend my sincere thanks and profound gratitude to my distinguished **professors**, the wellsprings of knowledge and wisdom, who spared no effort in guidance and instruction, serving as beacons that illuminated my path towards excellence.

And to my esteemed **colleagues**: **Yousra**, **Wadjdan**, **Djawahir**, **Haifa**, and **Angham**, my companions on this journey, and partners in moments of struggle and diligence.

Finally, to everyone who believed in my abilities, supported me even with a word of encouragement, and contributed to the realization of this achievement, I offer my deepest gratitude and appreciation. This work is for every hand that reached out, every heart that believed, and every dream that came true.

BOUTALBI ACHWAK

الحمد لله الذي بنعمته تتم الصالحات

And them

To the one who lives on In my heart and memory, to the soul of my beloved father "**Mohammed Said Benzina**", who taught me how to give without expecting, how to be content with little, and whose light still guides me even after his departure… You have all my prayers and endless gratitude. May your soul rest in eternal peace.

To my dearest mother "**Beskri Mania**", my sanctuary, my strength, and my endless source of love. Without you, none of this would have been possible. Thank you for your patience, your support, and your unconditional love.

To my dear brother "**Abderrahim BenZina**" and his kind wife "**Khemis Sara**", who stood by me with unwavering support and care. May God bless you both and reward you for your generosity and kindness.

To my loyal brother "**Laid BenZina**", companion through all seasons of life your presence has been a blessing I am forever grateful for

To my little stars "**Nour El islam** and **Ahmed**", you are the light that brightens my path and the hope that lifts my soul.

To my precious little ones: "**Mohammed Said**"," **Sraj Eddine**", "**siline**", and" **Ranim** you are the joy in my days and the innocence that keeps my heart warm.

To my cousin and brother In spirit "**Azmi BenZina**", your loyalty and unwavering support have been my rock. Thank you for being a constant light in my life

To my soul sister and life companion "**haifa Hammouda**", who walked with me through every step of the journey, shared my smiles and tears this achievement would not have felt the same without you.

To my cherished friends: "**Angham**, **Daouia**, **Ahlam**, **Karima**, **Achwak**, and **Djawaher**" thank you for the genuine friendship, the memories, and the unwavering support.

To the pure soul of my late grandmother "**Hawas Saida**", your memory lives in my prayers and heart. May your soul dwell in the highest heavens.

To all of you… I dedicate this graduation, the fruit of years of effort, tears, and dreams. You are the foundation of this success and the true meaning behind its joy

BEN ZINA WADJDAN

"Whoever follows the path will arrive"

This was the very first sentence I heard when I began my academic journey, and praise be to God who granted me success in following the path and arriving.

I toiled, stayed awake late, struggled, and strived. Indeed, the dark circles under my eyes were not insignificant, nor was the pallor of my face natural. I paid this price simply to reach that moment when I would be crowned with the wreath of pride and success.

I will carry a certificate that people have always seen as merely a piece of paper, but it means a great deal to me. Thanks to my persistence, I achieved what I wished for, and now the smile of joy adorns my lips.

I dedicate this graduation to those who made me strong, successful, ambitious, and always striving for the highest ranks:

To the one who stayed up late, toiled, raised me, and contributed to every step of my life—to my dearest mother **"Aribi Nawal"**

To the one who taught me that nothing is impossible with determination, to the one who instilled confidence in me since childhood—to my support and my pride, my dear father **"Zehani Massoud"**

To my beloved aunt(my second mother)**"Sabah"**

To my dear uncle (my second father)**"zohir"**

To my dear brothers**"Abdelmutalib" "Adel"** .

To my sisters, the source of my smiles and my support, this graduation is thanks to your constant backing: **"DJoumana"**, **"Houaria"**, **"Noujoud"**, **"Azza"**, **"Hibat El Rahman"**,**"Riham"**.

To my companions throughout my academic journey, to those who were my help and support, my dear friends.

"Sara",**"Hana"**,**"Wala"**,**"Achwak"**,**"Roufaida"**,**"Roumaisa"**,**"Wadjdan"**

To all of the **"Al Zehani"** and **"Al Aribi "families**, I present this success to you with all pride and gratitude.

To my revered teachers from elementary school to university, thanks to whom I achieved the best distinctions.

And to everyone who supported me with a word, a prayer, a smile...

To all of you, I dedicate this graduation, for you are my partners in it.

ZEHANI DJAWAHIR

Abstract

Résumé

ملخص

Abstract

This study investigates the biological properties of *E.spinosissimus* seeds extract through a series of in vitro assays. The antioxidant potential was evaluated using ascorbic acid as a reference. The skin whitening effect was assessed by measuring tyrosinase inhibition and compared to ascorbic acid. Anti-inflammatory activity was tested using the albumin denaturation method, employing diclofenac as a reference. Finally, the immunomodulatory effect was examined via a phagocytosis assay and its activity , using zinc as the standard compound.Additionally, in silico studies were conducted to further explore the anti-inflammatory and tyrosinase inhibitory mechanisms at the molecular level.

The in vitro experimental results were compared with in silico predictions using Molecular Docking, such as AutoDock, where the results showed good agreement supporting the effectiveness of the hydroethanolic extract as a natural source of bioactive compounds. The tyrosinase inhibition test confirmed its potential in skin-whitening applications by targeting melanin production, and when compared with medical-grade tyrosinase inhibitors, it showed equivalent efficacy. The study indicated that the extract is beneficial for the skin, making it suitable for the development of natural cosmetic products that promote skin regeneration and reduce redness and signs of aging.

Keywords: *Echinops spinosissimus*, Silymarin, Tyrosinase ,
Antioxidant ,Antibacterial, Anti-inflammatory, Molecular Docking, whitening

Résumé

Cette étude explore les propriétés biologiques de l'extrait de graines *d'Echinops spinosissimus* à travers une série de tests in vitro. Le potentiel antioxydant a été évalué en utilisant l'acide ascorbique comme référence. L'effet dépigmentant a été analysé par la mesure de l'inhibition de la tyrosinase et comparé à celui de l'acide ascorbique. L'activité anti-inflammatoire a été testée par la méthode de dénaturation de l'albumine, en utilisant le diclofénac comme substance de référence. Enfin, l'effet immunomodulateur a été examiné à l'aide d'un test de phagocytose et de son activité, le zinc servant de composé standard. Par ailleurs, des études in silico ont été réalisées afin d'approfondir les mécanismes anti-inflammatoires et d'inhibition de la tyrosinase au niveau moléculaire.

Les résultats des expériences in vitro ont été comparés aux prédictions in silico utilisant des techniques de docking moléculaire telles qu'AutoDock et Discovery Studio, où les résultats ont montré une bonne concordance, soutenant l'efficacité de l'extrait hydro-éthanolique en tant que source naturelle de composés bioactifs. Le test d'inhibition de la tyrosinase a confirmé son potentiel dans les applications de blanchiment de la peau en ciblant la production de mélanine, et lorsqu'il a été comparé aux inhibiteurs médicaux de la tyrosinase, il a montré une efficacité équivalente. L'étude a indiqué que l'extrait est bénéfique pour la peau, le rendant adapté au développement de produits cosmétiques naturels qui favorisent la régénération de la peau et réduisent les rougeurs et les signes du vieillissement.

Mots-clés : *Echinops spinosissimus*,Silymarine,Flavonoïdes,Antioxydant, Antibactérien, Anti-inflammatoire,docking moléculaire.

ملخص

تهدف هذه الدراسة إلى الخصائص البيولوجية لمستخلص بذور نبات *Echinops spinosissimus* من خلال سلسلة من الاختبارات المخبرية. in vitro تم تقييم النشاط المضاد للأكسدة باستخدام حمض الأسكوربيك كمرجع . وقد تم تحليل تأثير تفتيح البشرة من خلال قياس تثبيط إنزيم التيروزيناز، ومقارنته بحمض الأسكوربيك .أما النشاط المضاد للالتهاب فقد تم اختباره باستخدام طريقة تغير بنية الألبومين، مع استخدام الديكلوفيناك كمرجع . وأخيراً، تم فحص التأثير المعدل للمناعة عبر اختبار البلعمة ونشاطها، مع اعتماد الزنك كمركب معياري . بالإضافة إلى ذلك، أجريت دراسات in silico لاستكشاف آليات التثبيط للالتهاب وإنزيم التيروزيناز على المستوى الجزيئي .

تمت مقارنة نتائج التجارب المختبرية (in vitro) مع التنبؤات الحاسوبية باستخدام تقنية الالتحام الجزيئي مثل AutoDock و Discovery Studio، حيث أظهرت النتائج توافقًا جيدًا يدعم فعالية المستخلص الهيدروإيثانولي كمصدر طبيعي للمركبات النشطة بيولوجيًا. وأكد اختبار تثبيط التيروزيناز إمكانيته في تطبيقات تفتيح البشرة من خلال استهداف إنتاج الميلانين، وعند مقارنته بمثبطات التيروزيناز الطبية أظهر نفس الفعالية. وأشارت الدراسة إلى أن المستخلص مفيد للبشرة، مما يجعله مناسبًا لتطوير منتجات تجميل طبيعية تعزز تجديد البشرة وتقلل الاحمرار وعلامات الشيخوخة.

الكلمات المفتاحية : *Echinops spinosissimus*,سيليمارين,فلافينويد,مضاد للاكسدة,مضاد للبكتيريا,مضاد للالتهابات,النمذجة الجزيئية, تيروزين.

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Abbreviations list

Cm :centimeter

Hplc : High-performance liquid chromatography

GC-MS : gas chromatography-mass spectrometry

Gpp: geranyl pyrophosphate

NPP: neryl pyrophosphate

$C_{15}H_{24}$: Sesquiterpenes

GGPP : geranylgeranylpyrophosphate

C25H22O10 :silymarin

SMEDDS :self-microemulsifying drug delivery system

IL-12:interleukin-12

TNF-α : Tumor necrosis factor alpha

MCP-1:monocyte chemottractant protein-1

NF-κB: Nucler factor kappa-light-chain-enhancer of activated B cells

PGE2 :prostaglandin E2

COX :cyclooxygenase (cyclooxygenase -1; cyclooxygenase-2; cyclooxygenase-3)

NOS : Nitric Oxide Synthase

LTs : Leukotrienes

NO : nitric oxide

5-LOX :5-lipoxygenase

LTB4 :leukotriene B4

NSAIDs : Non steroidal anti-inflammatory drugs

SAIDs : Steroidal anti-inflammatory drugs

DM : Diabetes mellitus

IDDM : insulin-dependent diabetes mellitus

NIDDM : insulin-dependent diabetes mellitus

DNA : Deoxyribomucleic acid

L-DOPA :L-3,4-dihydroxyhpenylalanine

TRP : Tyrosine Related Peptide

DHI : 5,6-dihydroxyindole

DHIC: 5,6-dihydroxyindole-2-carboxylic acid

ER : endoplasmic reticulum

PDB : Protein Data Bank

ADT : AutoDockTools

QSAR :quantitative structure-activity relationship

ADMET : absorption, distribution, metabolism, excretion, and toxicity.

PDB : Protein Data Bank

EC : Escherichia coli

Kp: Klebsiella pseudomonas

Pa : Pseudomonas aeruginosa

EF : Enterococcus feacalis

BS : Bacillus subtiles

Sa : staphylococcus aureus

FeCl3 : ferric chloride solution

H2SO4 : sulfuric acid

HCl : hydrochloric acid

CHCl3 : chloroform

H2SO4 : methanol

NaOH : sodium hydroxide

NH_4OH :ammonium hydroxide

Alcl3 :aluminum chloride

NCCLS : National Committee for Clinical Laboratory Standards

mL : milliliter

DPPH : 1,1-diphenyl-2-picrylhydrazyl

I(%) : percentage of inhibition

Abs : absorption

BSA : Bovine Serum Albumin

K : Binding constant

ΔG : Gibbs free energy

EDTA :ethylenediamineteraacetic acid

HBSS:hanks balanced salt solution

µl :microliter

°C :degree celsius

Min :minute

AP: Phagocytic activity

cp : phagocytosis capacity

Ic50: Concentration causing 50% inhibition of an activity

LD 50: Lethal dose 50

Kj: Kiloujoule

M: Molar

Mg: Milligram

R: Gas constant

T°: Temperature

mRNA:messenger ribonucleic acid

GOLD: genetic optimizati on for ligand docking

UV-Vis:

R%: yield in %

Me: mass of the extract after evaporation of the solvent

Mv: mass of the plant material used for the extraction

KI:potassium iodide

A0 : absorbance of control

GAE : gallic acid equivalents

g :gram

RE :rutin equivalents

DM : dry matter

DAMPs: damage-associated molecular patterns

ROS :reactive oxygen species

TLRs : Toll-like receptors

µg :microgram

mm: millimeter

ERK: extracellular signal-regulated kinas

AKT3:AKT serine/theronine kinase3

GSK-3β :glycogen synthase kinase 3β

LPS:lipolysacch

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General Introduction

General Introduction

Medicinal plants have been employed since ancient times for the mitigation and treatment of human diseases. Indeed, their therapeutic efficacy is due to the presence of a diverse array of bioactive natural compounds, numbering in the hundreds or even thousands, collectively termed secondary metabolites. These compounds are subsequently accumulated within various plant tissues and occasionally within specialized cell types. The contemporary challenges of increasing microbial resistance to antibiotics and the inherent toxicity of synthetic antioxidants have motivated researchers to investigate the plant kingdom, with a particular focus on medicinal and culinary species, as a source of efficacious natural molecules with minimal or no adverse effects. A substantial body of research has demonstrated the presence of a wide range of secondary metabolites possessing significant biological activities, including polyphenols, tannins, alkaloids, flavonoids, and terpenes, among others . These molecules exhibit a restricted distribution within the plant organism and play a crucial role in its defense mechanisms against external stressors. These compounds confer appreciable curative properties that cannot be entirely duplicated by synthetic and combinatorial chemistry approaches**(Gheffour,K.2019).**

Phytochemical investigations of *Echinops spinosissimus* have revealed the presence of biologically active compounds such as thiophenes, terpenoids, sterols, fatty acids, and alkanes. This diverse composition of secondary metabolites has earned the plant its reputation as a "complete pharmacy," owing to its efficacy in treating a wide range of ailments, including infections, intestinal parasitic infestations, hemorrhoids, migraines, diarrhea, and heart pain. Additionally, its antimicrobial potential against multidrug-resistant pathogens has made it a promising candidate in the search for novel therapeutic agents. Researchers have focused on identifying new natural compounds from plants and microbes or developing synthetic drugs to combat microbial resistance.(**Al Masoudi, L. M *et al.*, 2023).**

Traditionally, plants of the *E.* genus have been employed to treat various infectious diseases, such as sepsis, trachoma, gonorrhea, typhoid, and ulcerative lymphangitis. They are also used for conditions potentially caused by bacterial or fungal infections, including respiratory ailments, fever, leucorrhea, earache, and

toothache. Studies have demonstrated the antimicrobial efficacy of *Echinops* extracts and isolated compounds against both Gram-negative and Gram-positive bacteria. Beyond its medicinal applications, *E. spinosissimus* has found a role in cosmetics, leveraging its bioactive components for skin health and antibacterial properties in skincare formulations(**Al Masoudi, L. M *et al.*, 2023)**.

The plant *E. spinosissimus* is considered a common medicinal plant in the Mediterranean region and North Africa. It has been used since ancient times in traditional medicine to treat many health issues, due to its content of biologically active compounds such as flavonoids, phenolic compounds, and sterols. These properties have contributed to introducing this plant into the field of cosmetics, where its active components are utilized to promote skin health, thanks to its ability to resist bacteria and oxidation, making it a promising ingredient in skincare products.

Our skin faces daily challenges, especially from continuous exposure to UV radiation, which leads to cell damage, dehydration, loss of elasticity, wrinkles, and dark spots it has become necessary to search for effective natural solutions. This highlights the importance of exploring medicinal plants like *E. spinosissimus* and relying on their active components to address these issues.

Based on this, the aim of this study is to evaluate the biological properties of *E. spinosissimus* seeds, focusing on their role as antioxidants and combatants against signs of aging, in addition to developing a natural cosmetic product based on the extract of these seeds. Can *E. spinosissimus* seeds provide a natural and effective solution for treating skin problems?

In this research , the content of the dissertation was divided into two main parts:

Part one: Theoretical part

- ✓ Chapter I: General information on plants *Echinops spinosissimus*
- ✓ ChapterII :The Biological Activities of *Echinops spinosissimus*
- ✓ Chapter III: Theoretical Context of Experimental Techniques

Part two: Experimental part

- ✓ Chapter I:Materials and methods
- ✓ Chapter II: Results and discussion

Part I: Theoretical Part

Chapter I

Echinops spinosissimus

I.1. Definition of Echinops spinosissimus

The genus *Echinops*, belonging to the Asteraceae family (formerly Compositae), comprises approximately 120 species distributed across the Mediterranean region, Central Asia, and tropical Africa. In Algeria, this genus is represented by the widespread species *Echinops spinosus* L.**(BOUDERSA ,2022)**

I.2. Botanical classification

Classification	**Name**
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Asterales
Family	Asteraceae
Genus	Echinops
Species	*Echinops spinosissimus*

Table I. 1: *the taxonomic classification of the plant* Echinops spinosissimus

Figure I. 1: *Echinops spinosissimus* (real image)

I.3. Morphological description

This herbaceous perennial grows over a meter tall, featuring upright, brownish-red stems and sparse, elongated (10-15 cm) hairy leaves with a cobweb-like texture. Its most notable characteristic is the abundance of very long spines. The plant produces a single, rounded flower head, up to 5 cm across, which is also surrounded by numerous long spines. This dense flower head contains small, bisexual flowers with a tubular shape that change color from green to white and finally to yellowish as

they mature. The small fruits are achenes, equipped with membranous scales for wind dispersal. **(Abdul Majid *et al.*, 2024).**

I.4. Vernacular names

In Algeria, Echinops spinosus L. has several vernacular names. In Berber, it's known as "Taskra," "Teskera," "Taskra Ameskelit T," and "Sarsor." Arabic names include "fouga el djemel," "chouk el djemel," "suk ej-jmal," Kachir, Ikchir, "Chouk el Hamir," Chicaou, and Sorr. The Tamahaq name is Téfariast.

The Arabic name "qounfoudzia" (meaning "hedgehog") is derived from the Greek "Ekhinos." Another Arabic name, "ri ayi el-ibil," translates to "Camel Pasture." In Morocco, the same species is called "tasekra," "asekra," "teskra," "chouk el hamir," "suk al-himar," and "tîmat."**(Bouzabata *et al.*, 2018).**

I.5. Geographic distribution

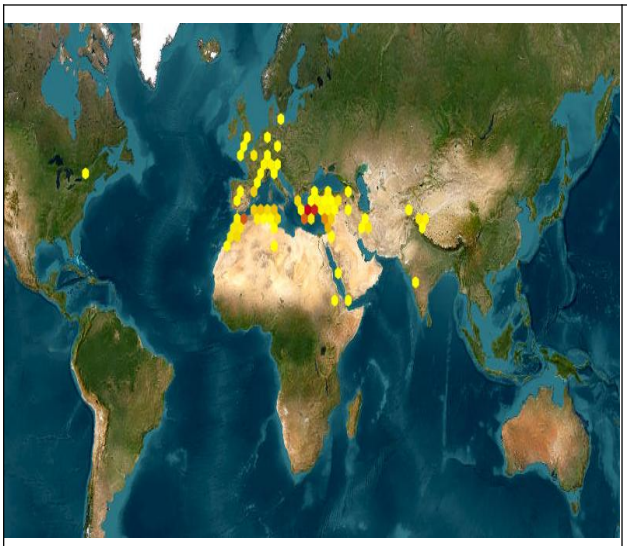


Figure I. 2: A geographical image showing the distribution of a plant *Echinops spinosissimus* in the world

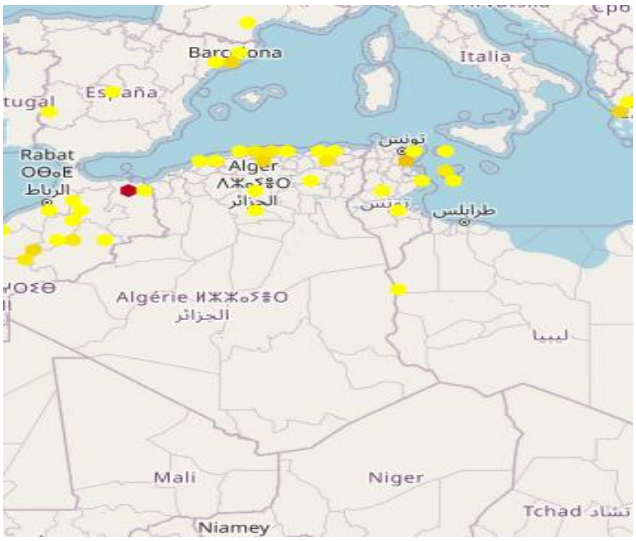


Figure I. 3: A geographical image showing the distribution of a plant Echinops spinosissimus in the world

I.6. Previous studies on treatments for different parts of Echinops spinosissimus

Table I. 3: Treatments for different parts of Echinops spinosissimus

Parts uses	Therapeutic uses	Pays	Reference
Root	hypoglycemic conditions, diuresis, post-partum care, hepatic disorders, appetite stimulation, depuration, obstetric applications.	**Morocco**	**(Bouzabata *et al.*, 2018)**
Root	Genital infections (post-abortion), urinary tract infections, Inflammation .	**Algeria** (Mascara)	**(Bouzabata *et al.*, 2018)**
Branches and Flowers	● Decoction: Treatment of kidney stones, Disinfectant, diuretic, hypoglycemic, Abortifacient, Inducing uterine contractions during childbirth. ● Infusion: Relieving neuralgia and fatigue.	**Morocco**	**(Zitouni Ep. Nourine, 2021)**
Seeds	Antidiabetic remedy	**Morocco**	**(Bouzabata *et al.*, 2018)**
Stem and Leaf	Hepatoprotective purposes ,abortifacient purposes	**Morocco**	**(Zitouni Ep. Nourine, 2021)**
Whole Plant	renal disorders, splenic disorders, hepatic disorders, gallbladder disorders.	**Morocco**	**(Marceddu *et al.*, 2022)**

I.7. Metabolites of *Echinops spinosissimus* responsible for biological activities

Phytochemical analysis of *Echinops* species has demonstrated a predominance of thiophenes and terpenes, with roots serving as the primary source of thiophenes and aerial sections containing higher concentrations of terpenes and flavonoids. Additional compounds, including flavonoids, phenolic compounds, alkaloids, lipids, and phenylpropanoids, have been identified. Essential oils are ubiquitously distributed

within the plant, and approximately 53 isolated compounds demonstrate various biological activities.**(Bitew & Hymete, 2019)** .

I.7.1. Thiophenes

The Echinops genus contains a variety of biologically active thiophenes, These compounds are biosynthesized through a process involving fatty acids and reduced sulfur. Characteristically, these thiophenes possess acetylenic functional groups and typically feature two thiophene rings as shown in (**Figure I. 4** and **Figure I. 5)** . Their presence has been documented in nine Echinops species, with prominent examples including 5-(4-hydroxybut-1-ynyl)-2-(pent-1,3-diynyl)-thiophene **(Zitouni Ep. Nourine, 2021)**. Certain thiophenes from this genus have demonstrated multiple pharmacological activities, such as antimalarial, insecticidal, and fungicidal properties.**(Abdul Majid *et al.,* 2024)** .

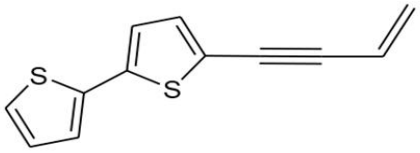


Figure I. 4: 5-(but-3-en-1- ynyl)-2,2'- bithiophene

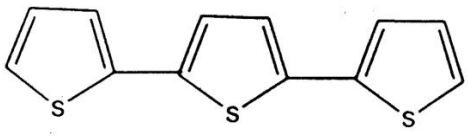


Figure I. 5: α-terthienyle

I.7.2. Terpenoids

Sesqui-and triterpenoids are chemical compounds found mainly in the whole plant and aerial parts of the echinops genus. The majority of the sesquiterpenoids contain lactones, the most common secondary metabolites in the Asteraceae family. Conversely, Triterpenoids exist in various forms, such as lactones, esters, sterols, and glycosides. The most reported sesquiterpenoids are costunolide, lupeol, and lupeol a.cetate.**(Abdul Majid *et al.,* 2024)** .

I.7.2.1. Monoterpenes

Are a diverse group of over 900 chemical compounds, classified by their structure as linear (acyclic), single-ring (monocyclic), or double-ring. They are formed through the head-to-tail connection of isoprene units.

- **Acyclic monoterpenes :**

Are linear-structured compounds that originate from geranyl pyrophosphate (GPP), which is the first product formed from mevalonate in biochemical pathways. Among these acyclic monoterpenes, geraniol is the most prevalent in plants. **(Malecky,2008).**

- **Monocyclic monoterpenes:**

Characterized by a single ring structure, are derived from neryl pyrophosphate (NPP) or geranyl pyrophosphate (GPP). Aromatic compounds like p-cymene and its hydroxyl derivatives are prominent in this group, often found with γ-terpinene.

This category includes four subgroups:

- ✓ C10H16 hydrocarbons with two double bonds (e.g., D-limonene, phellandrenes).
- ✓ C10H18 hydrocarbons with one double bond.
- ✓ C10H20 saturated hydrocarbons (menthanes) whose alcohol (menthol) and ketone (menthone) derivatives exist naturally.
- ✓ C10H20 hydrocarbons containing an oxide (e.g., cineole/eucalyptol), which are abundant.**(Malecky,2008).**

- **Bicyclic monoterpenes:**

Characterized by a two-ring structure, are abundant in many essential oils, particularly those from conifers. They are primarily classified into the pinane, bornane, and thujane families, with fenchane and carane being less common.**(Malecky ,2008).**

I.7.2.2. Sesquiterpenes

Sesquiterpenes, composed of three isoprene units ($C_{15}H_{24}$), are the most diverse class of terpenes and can be acyclic, monocyclic, bicyclic, tricyclic, or polycyclic. They occur as hydrocarbons or oxygenated derivatives like alcohols, ketones, aldehydes, acids, and lactones.**(EL HAIB, 2011).**The plant *Echinops spinosus* is rich in sesquiterpenes, mainly due to echinopsine A and B. A new sesquiterpenoid, 11-hydroxyisocom-2-en-5-one (S41), was discovered in the dichloromethane root extract of *Echinops spinosissimus* subspecies. **(Bouzabata *et al*., 2018).**

I.7.2.3. Diterpenes

Are C20 compounds made from four isoprene units, derived from geranylgeranyl pyrophosphate (GGPP). They are mainly found in plant resins, gibberellins, and fungi. Most diterpenes are cyclic, while linear ones like phytol (part of chlorophyll and vitamins K and E) belong to the phytane family. Cyclic diterpenes are based on the cyclophytane structure. **(Malecky,2008).**

I.7.3. Alkaloids

Quinoline alkaloids from *Echinops* species are derived from anthranilic acid, a tryptophan metabolite. These compounds have a bicyclic structure, with the furoquinoline subgroup, like furacridone, containing an extra furan ring. Known for their medicinal properties, they have inspired synthetic quinoline drugs.**(Enas J *et al.*,2014)** In 2011, echinopsine a natural quinoline alkaloid was extracted from the flower heads of *Echinops spinosus* L. in an Egyptian study. **(Zitouni Ep. Nourine, 2021).**

- ✓ **Echinopsine** as shown in (**Figure I. 6**) This alkaline chemical,does not fluoresce under light. Its dissolved salt forms react with common alkaloid detection reagents to produce a solid precipitate. It has a very bitter taste, and its physiological effects are similar to, but weaker than, a combination of brucine and strychnine.

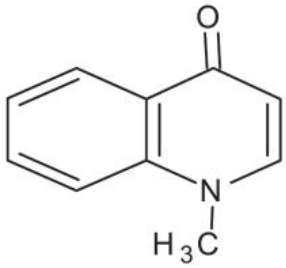


Figure I. 6: Echinopsine(1-methyl-4-quinolone).

- ✓ **Echinorine** as shown in (**Figure I. 7**) is a chemical substance whose structure has been identified as 1-methyl-4-methoxyquinolinium. When echinorine is dissolved in a basic (alkaline) liquid, it breaks down into another compound called echinopsine, which has the chemical name 1-methyl-quinolinone-(4).

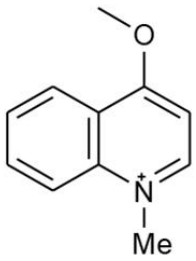


Figure I. 7: Echinorine

I.7.4. Flavonoids and other phenolics

I.7.4.1. Phenolic Acids

Are a non-flavonoid polyphenols.They are divided into two main groups: benzoic acid derivatives (one carbon attached to a six-carbon ring) and cinnamic acid derivatives (a three-carbon chain attached to a six-carbon ring, as shown in (**Figure I. 8**).They are found in free form in many fruits and vegetables.In grains and seeds (especially outer layers), they are often bound to other molecules and require acidic/basic hydrolysis or enzymes for release.**(Rong.2010).**

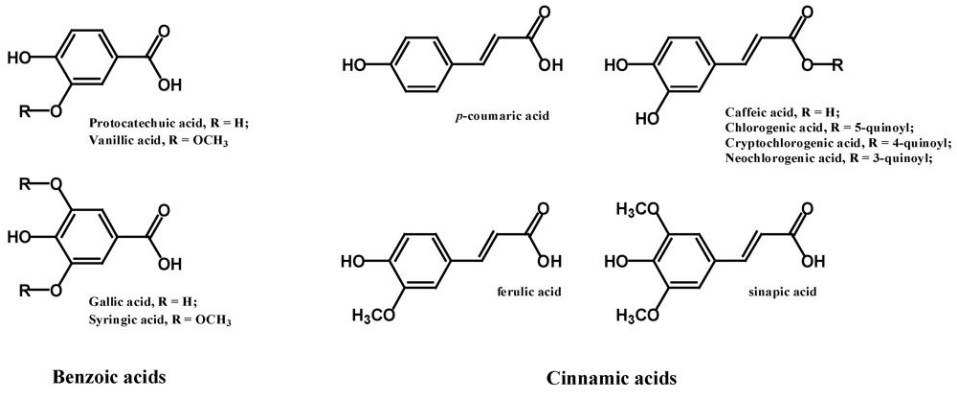


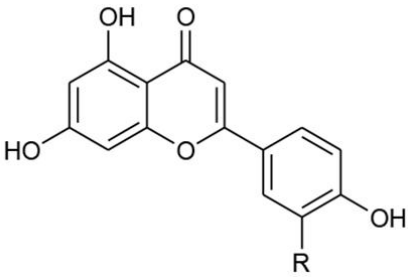
Figure I. 8: Typical phenolic acids in food: Left, Benzoic acids; right, Cinnamic acids.**(Rong,2010)**

I.7.4.2. Flavonoids

Flavonoids in Echinops plants, especially flavones like apigenin as shown in **(Figure I. 9)** , offer significant medicinal benefits. Found mainly in the aerial parts and flowers, they act as strong antioxidants and also have anti-inflammatory, antibacterial, and antifungal properties. Additionally, they help protect the stomach

lining and may aid in cancer prevention by affecting various cellular processes. **(Abdul Majid *et al.*, 2024)** .

Echinops spinosus contains three identified flavonoid compounds: apigenin, apigenin-7-O-β-glucopyranoside as shown in **(Figure I. 10)** ,and apigenin-7-β-D-O-(6''-O-E-p-coumaroyl)-glucopyranoside as chown in **(Figure I. 11)**. Notably, this study is the first to discover the presence of these specific compounds within this particular plant species.**(Boumaraf ,2016)** .



R=H

Figure I. 9: Apigenine

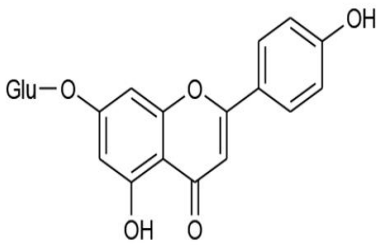


Figure I. 10: Apigenine 7-*O*glucoside

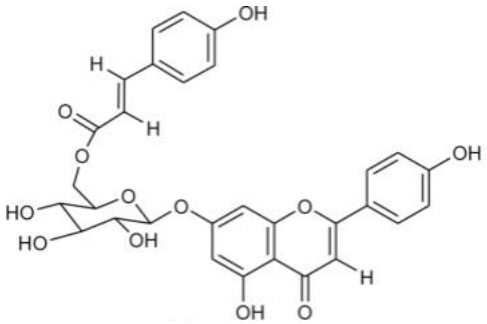


Figure I. 11: Apigenine-7-*O*- (6''-transpcoumaroyl- β -Dglucopyranoside **(Zitouni Ep. Nourine, 2021)**

I.7.4.2.1. Isoflavones

Are a class of flavonoids characterized by a phenyl group attached to the third carbon atom of the chromen-4-one structure. This C-3 phenyl group distinguishes them from flavones, where the phenyl group is attached to the second carbon atom. Despite this difference in the phenyl group's position, isoflavones as shown in **(Figure I. 12)** and flavones possess very similar chemical structures and are considered isomers.**(Rong,2010).**

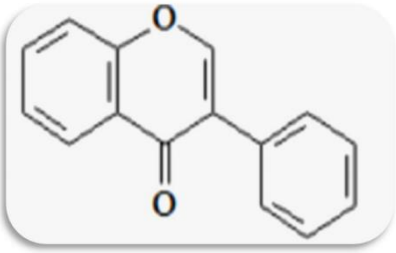


Figure I. 12: Basic chemical structure of isoflavones**(Dias *et al.*, 2021).**

I.7.4.2.2. Flavanols

Flavanols as shown in **(Figure I. 13)** , or flavan-3-ols/catechins, are common compounds in edible plants, with catechin and epicatechin being the most prevalent forms. 1 These flavanols can polymerize into proanthocyanidins (condensed tannins), a name derived from their ability to be broken down into anthocyanidins through acid treatment.**(SAIDI,2019)** .

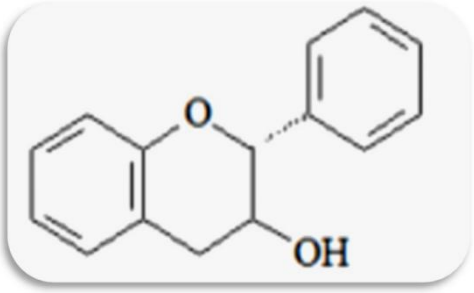


Figure I. 13: Basic chemical structure of flavanols**(Dias *et al.*, 2021).**

I.7.4.2.3. Flavones

When there is a double bond between carbon atoms C2 and C3 in the flavanone structure, the molecule is classified as a flavone as shown in **(Figure I. 14)**, which is a derivative of 2-phenylchromen-4-one.**(Bessam,2015)** .

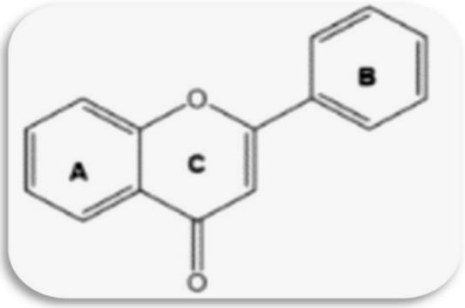


Figure I. 14: Basic chemical structure of flavones**(Dias *et al.*, 2021).**

I.7.4.2.4. Flavanones and Hydroflavonols

Flavanones and hydroflavonols are related compounds that differ from flavones and flavonols by lacking a double bond between carbons 2 and 3 and having asymmetry. Dihydroflavonols are a subtype of flavanones distinguished by an additional hydroxyl group at the C3 position. **(AKROUM , 2011).**

I.7.4.2.5. Anthocyanidins

Are charged flavonoids that produce red, violet, and blue colors in nature, crucial for attracting pollinators.**(Rong ,2010)** Are anthocyanidins as shown in **(Figure I. 15)** with a sugar molecule attached to the hydroxyl group at their C3 position through a heterosidic bond. Examples include palargonidol-3,4-O-glucoside and cyamidol-3-O-rutinose (keracyanin).**(AKROUM , 2011).**

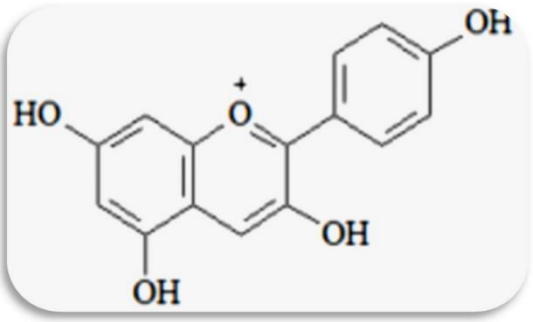


Figure I. 15: Basic chemical structure of anthocyanidins**(Dias *et al.*, 2021)**

I.7.4.2.6. Chalcones and Aurones

Chalcones as shown in **(Figure I. 16)** have an open central ring with two aromatic rings connected by a three-carbon unsaturated ketone chain. 1 Their B ring is often plain, while the A ring has substitutions like other flavonoids. Aurones have a 2-benzylidene coumarone structure. Some classify anthocyanins as flavonoids due to their structural similarities, particularly to anthocyanidols.**(AKROUM , 2011).**

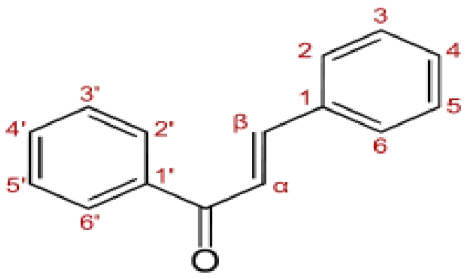


Figure I. 16: Structure of a chalcone**(BESSAM,2015)**

I.7.4.3. Tannins

Tannins are common phenolic compounds in Angiosperms, Gymnosperms, and Dicotyledons that can bind to and precipitate proteins, with the strength of the binding varying depending on the protein.**(AKROUM ,2011).**

I.7.5. Essential Oils and Lipids

The genus Echinops is rich in bioactive essential oil constituents, which were mainly found in the roots. **(Bitew & Hymete, 2019)** .The most prevalent compound was isoshyobunone (152), which was followed by modephene (157) , isocomene (150), β-phellandrene (164) , α- pinene (165) , dehydrocostuslactone (140), β-pinene (166) , and β-isocomene (151) .**(Elseragy *et al.*, 2024)** .

I.8. Seeds of Echinops spinosissimus

I.8.1. Definition of Seeds

The fruits as shown in **(Figure I. 17)**, specifically the achenes, are characterized by a long, white pappus and display a color range from black to glossy brown, with gray and mottled variants also frequently observed. The 1000-seed weight ranges between 20 and 30 grams. Seed yield per flower head varies from approximately 65–100 up to around 190 seeds, depending on genotype and growth conditions, resulting in a total of over 6,000 seeds per plant.**(Roberto ,2022).**

Figure I. 17: Seeds of echinops spinosissimus (real image)

I.8.2. Chemical composition of seeds

Milk thistle (Silybum marianum) seeds are a valuable source of medicinally active compounds, primarily due to their high silymarin content. This part of the plant is of particular interest, as silymarin the principal bioactive compound can be extracted from the seeds either alone or in combination with other oil constituents summarized in (**Table I. 4)**.

Table I. 4: Chemical composition of seeds**(BEN RAHAL,2012)**

Type	Percentage
Silymarin	%6-4
Lipids	%30-20
Proteins	%30-25
Minerals	-
Calcium	-
Phosphorus	0.6 g/kg
Vitamin E	60-50 mg/100g
Flavonoids	%0.25
Unsaturated fatty acids	56% polyunsaturated, 21% monounsaturated

I.8.2.1. Silymarin

Silymarin, an extract derived from milk thistle, is a complex phytochemical mixture primarily composed of flavonolignans, flavonoids (such as taxifolin and quercetin), and various polyphenolic compounds. These constituents exhibit potent antioxidant activity and a broad range of biological effects. The principal flavonolignan isomers as shown in **(Figure I. 18)** in silymarin include silibinin, isosilibinin, silicristin, and silidianin, with silibinin (also known as silybin) being the most abundant and biologically active. Silibinin accounts for approximately 50–60% of the silymarin complex, while the remaining flavonolignans contribute roughly 35%, distributed as follows: silicristin (~20%), silidianin (~10%), and isosilibinin (~5%). Silibinin, a polyphenolic flavonoid with antioxidant properties, has the molecular formula $C_{25}H_{22}O_{10}$ and a molecular weight of 482.44 g/mol. It exists as an

approximately equimolar mixture of two diastereomers, silibinin A and silibinin B.**(Gillessen & Schmidt, 2020)** .

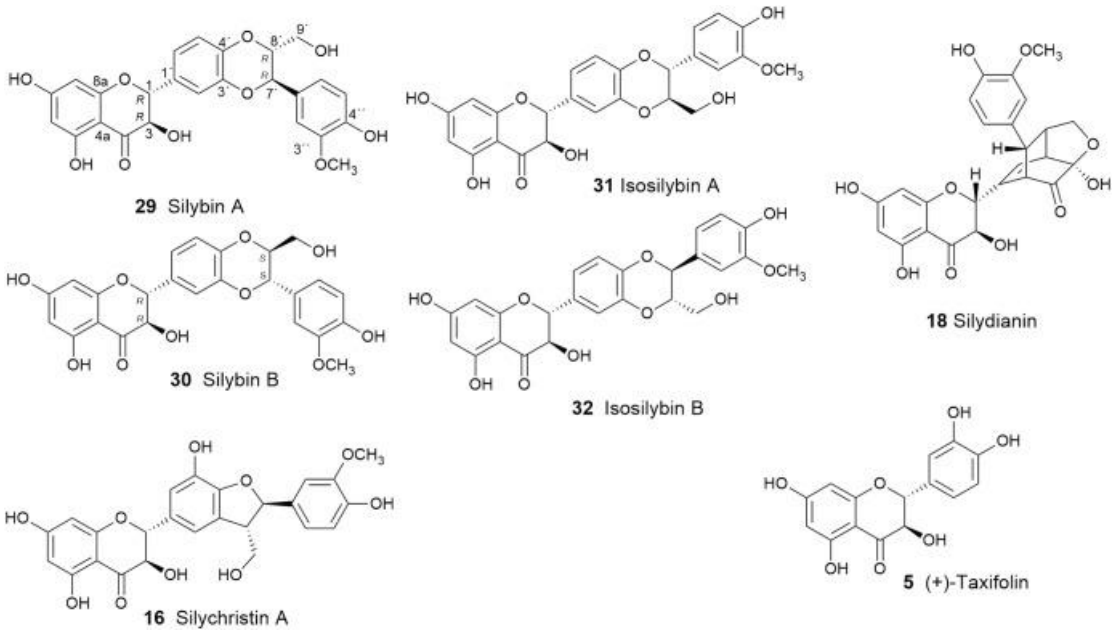


Figure I. 18: Chemical structure of silymarin.**(Pferschy-Wenzig *et al.*, 2023)**

I.8.2.1.1. Mode of action of silymarin.

Milk thistle supports liver health and function primarily by inhibiting harmful substances such as leukotrienes and free radicals that can cause hepatic damage. Silymarin, the active constituent of milk thistle, protects the liver through multiple mechanisms:

1. It stops fats from being damaged by free radicals.
2. It helps the liver cells repair themselves by boosting protein production.
3. It improves the liver's ability to remove toxins by affecting the first stage of detoxification.
4. It helps the liver process substances and prevents the loss of an important protective compound called glutathione.
5. It reduces inflammation by blocking the production of certain inflammatory molecules and by affecting specific immune cells in the liver.
6. It can slow down or even reverse liver scarring by preventing certain liver cells from turning into scar tissue.
7. It might help fight cancer by interfering with cancer cell growth.

8. It can also the immune response in a diseased liver.**(Bouhalit,2018).**

I.8.3. Pharmacokinetics

The oral bioavailability of silybin, a key pharmacokinetic molecule, is generally low and is influenced by the composition and concentration of the silymarin extract. This limited absorption can be significantly enhanced by forming a complex with phosphatidylcholine or by incorporating silybin into advanced drug delivery systems such as SMEDDS® (Self-Microemulsifying Drug Delivery Systems) or liposomes, which can increase absorption by two- to threefold. The plasma concentration of silymarin correlates with its intestinal absorption. In healthy individuals, only 23–47% of orally administered silymarin is absorbed, reaching peak plasma levels within 2 to 9 hours, with an elimination half-life of approximately 6 hours. The primary route of elimination is biliary excretion, accounting for 20–40% of the dose as conjugates over a period exceeding 24 hours, with minor urinary excretion (3–8%) and the remainder eliminated via feces.**(Bouhalit.2018) .**

I.8.4. Toxicity, side effects, interactions

Milk thistle is generally regarded as a safe herbal remedy with a low risk of toxicity, even at high doses. Although the methodologies used to assess its effects are not always thoroughly detailed, some individuals may experience mild gastrointestinal side effects, such as a laxative effect, and rare allergic reactions have been documented. Additionally, transient elevations in liver enzymes and bile acids have been observed during the initial phase of milk thistle administration.

Toxicological studies evaluating the acute effects of intravenously administered silymarin (a principal constituent of milk thistle) have been conducted in various animal models. The median lethal dose (LD_{50}) varied among species: approximately 400 mg/kg in mice, 385 mg/kg in rats, and 140 mg/kg in both dogs and rabbits. Notably, the LD_{50} increased when silymarin infusion was administered slowly over 2–3 hours in rats (up to 2 g/kg) and was significantly higher when administered orally (up to 10 g/kg). Other investigations using specific silymarin formulations administered intravenously also reported variable LD_{50} values across species.

Overall, these findings suggest that milk thistle and its active components exhibit very low acute, subacute, and chronic toxicity profiles.**(Bouhalit.2018).**

I.9. Cosmetic use

Milk thistle is a valuable cosmetic ingredient due to its antioxidant properties, which help reduce redness and prevent oxidative damage, making it especially suitable for anti-aging formulations. Additionally, it promotes skin renewal. The oil extracted from milk thistle improves skin condition and is commonly incorporated into the oily phase of cosmetic products, providing moisturizing and regenerative effects.

Consequently, milk thistle extracts and oil are particularly beneficial in formulations designed for:

- sensitive or stressed skin with redness.
- anti-aging night creams.
- eye wrinkle treatments.
- hydrating body lotions.
- massage creams for tired legs and feet.
- hair care for damaged and weak hair.**(BESSAM ,2015)**

I.10. Pharmacological activities

Echinops spinosissimus contains various bioactive compounds, including thiophenes and terpenoids, and has been traditionally used as a broad-spectrum remedy for infections, parasitic worms, pain, and digestive disorders. Current research primarily focuses on its antimicrobial potential against drug-resistant microorganisms, investigating both natural compounds and synthetic analogs. The *Echinops* genus has a longstanding history of use in the treatment of various infectious diseases, including bacterial and fungal infections. Several studies have confirmed the antimicrobial activity of *Echinops* species against a range of bacterial strains. Additionally, *E. spinosissimus* is rich in antioxidants such as phenolic compounds and flavonoids, which contribute to its capacity to mitigate oxidative stress a major factor implicated in numerous health conditions.**(Al Masoudi & Hashim, 2023).**

Chapter II
The Biological Activities

II.1. Antioxydant activity

II.1.1. Oxidative stress

II.1.1.1. Definition of oxidative stress

Oxidative stress refers to a condition in which the normal equilibrium between the production of unstable molecules, known as free radicals, and the body's antioxidant defense system is disrupted, resulting in an excess of free radicals. This imbalance may occur due to diminished antioxidant levels or excessive generation of free radicals.**(Gupta, 2015).**

II.1.2. Free radicals

Free radicals are chemical species such as atoms, molecules, or ions that contain at least one unpaired electron. This unstable electron configuration renders them highly reactive, enabling them to either donate or accept electrons from other molecules. Consequently, free radicals can act as both oxidizing and reducing agents in chemical reactions.**(FERDJIOUI,2020)** .

II.1.2.1. Nature of free radicals

- **Reactive Oxygen Species (ROS):**

Reactive Oxygen Species (ROS) are a group of highly reactive molecules that contain oxygen. This category includes radicals formed from oxygen, such as the extremely reactive hydroxyl radical (OH•), the superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the peroxyl radical (RCOO•), among others.**(FERDJIOUI,2020)** .

II.1.3. Antioxidants

II.1.3.1. Definition

Antioxidants are substances that protect susceptible molecules from oxidative damage. Their mechanisms of action vary depending on the source of oxidation. For instance, antioxidants can neutralize harmful free radicals, inhibit enzymes that catalyze oxidative processes, or chelate active metal ions that may initiate oxidation.

Essentially, antioxidants function as a defense system that slows down or prevents the detrimental effects of oxidative stress.**(HOUAS,2022).**

II.1.3.2. Mechanisms of action of antioxidants

Antioxidants protect the body through various mechanisms. They can scavenge and neutralize reactive oxygen species, stabilize damaging molecules known as free radicals by binding to them, reduce the reactivity of radicals and peroxides, and chelate metal ions that contribute to cellular damage.**(WEYEPE LAH ,2021)** .

II.2. Antibacterial activity

II.2.1. Bacteria

II.2.2.1. Definition

Bacteria are single-celled, simple organisms classified as prokaryotes and are ubiquitously distributed in diverse environments. They typically possess a cell wall composed of peptidoglycan, which is a carbohydrate-based polymer. Bacterial size generally ranges from 0.5 to 2 micrometers in width and 2 to 6 micrometers in length, although some species may be considerably larger. Bacteria exhibit various morphological shapes, including cocci (spherical), which may occur singly, in pairs, clusters, or chains, and bacilli (rod-shaped), which can be straight, curved, or spiral. These morphological differences are primarily determined by the composition of their peptidoglycan cell wall and their mode of cell division.**(Touhami, 2017)** .

II.2.2.2. Bacterial resistance

Bacterial antimicrobial resistance (AMR) refers to the ability of bacteria to withstand the effects of antibiotic drugs, rendering infections increasingly difficult or, in some cases, impossible to treat. This phenomenon poses a significant threat to public health in the 21st century **(Xuan *et al*., 2023)**. A bacterial strain is classified as resistant if it survives exposure to an antibiotic treatment. However, in vivo success of antibiotic therapy depends not only on the intrinsic resistance of the bacterial strain but also on additional factors such as the site of infection, the antibiotic dosage and administration route, and the patient's immune system competence.**(Muylaert et Mainil, 2012).**

II.2.2.3. Bacterial resistance mechanism

Bacteria develop resistance to antibiotics through multiple mechanisms as shown in **(Figure II. 1)**. The most common strategies include enzymatic inactivation of the drug, modification or replacement of the antibiotic's target site, active efflux of the antibiotic from the cell, and decreased permeability to limit drug entry. Less common mechanisms involve target protection or overproduction, which tend to be specific to certain classes of antibiotics.**(Muylaert et Mainil, 2012)** .

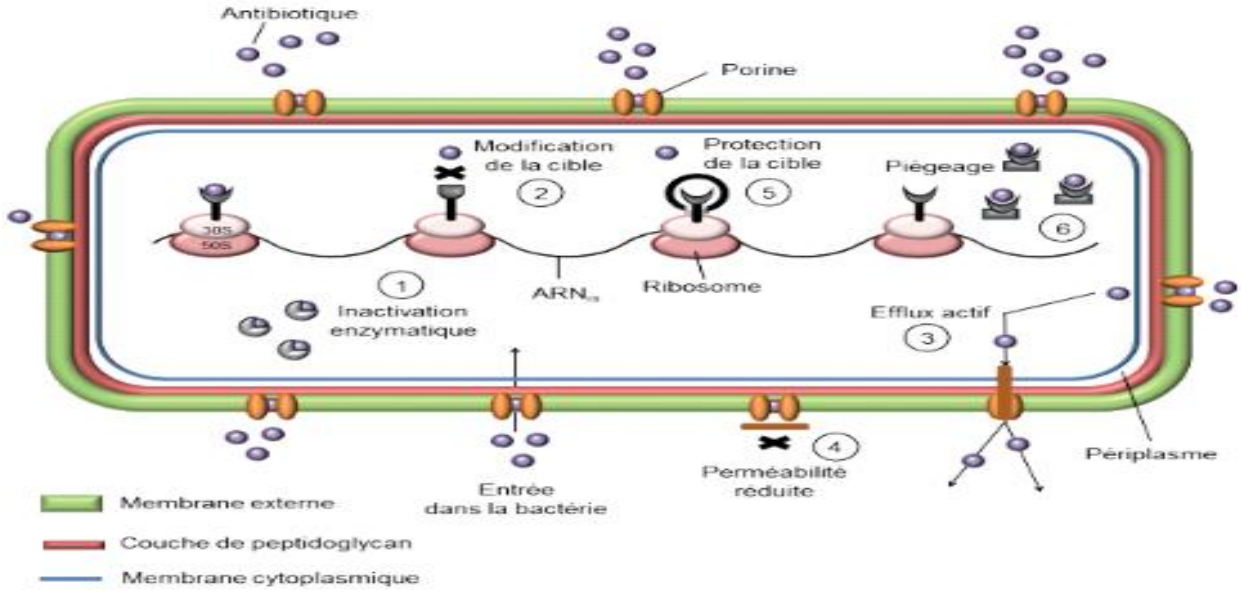


Figure II. 1: Different antibiotic resistance mechanisms in a Gram-negative bacterium

II.3 Antidiabetic activity

A plethora of plants serve as abundant reservoirs of bioactive compounds exhibiting specific pharmacological properties, often with minimal undesirable side effects. For generations, communities in developing countries have relied extensively on plant-based therapies, utilizing cost-effective medicinal plants as a common approach for the management of diabetes.(**Przeor, 2022)** .

II.3.1. Diabetes

Diabetes mellitus comprises a group of metabolic disorders with diverse underlying causes. Its defining feature is persistent hyperglycemia (chronic elevated blood glucose levels). This sustained high blood sugar is accompanied by disturbances in the metabolism of carbohydrates, fats, and proteins. These metabolic abnormalities result from either insufficient insulin production by the pancreas or the body's ineffective use of the insulin produced (insulin resistance) **(DeFronzo *et al.*, 2015)** .

II.3.2. Types of diabetes

- **Type 1 diabetes**: is caused by an autoimmune response in which the body's immune system destroys the insulin-producing beta cells in the pancreas. This destruction results in the body's inability to produce insulin, leading to a complete deficiency of this essential
- **Type 2 diabetes**: occurs when the body's cells do not respond effectively to insulin, a condition known as insulin resistance.
- **Gestational diabetes (GDM)**: is a form of diabetes first diagnosed during pregnancy, in women who did not have a prior diagnosis of diabetes before becoming pregnant. **(Kebir, 2018)** .

II.3.3. The enzymatic regulation of glucose

II.3.3.1. Alpha-amylases

Are enzymes that break down large carbohydrates (like starch and glycogen) by randomly cutting the links between their sugar units. They belong to the glycoside hydrolase family 13 (GH-13) and generate glucose, maltose, and branched oligosaccharides known as alpha-limit dextrins. These enzymes retain the α-configuration of the sugar molecules during catalysis and are capable of bypassing branch points in the carbohydrate chains.**(Kherouf,2021)** .

II.3.3.1. 1. Mechanism of Action

The α-amylase enzyme catalyzes the hydrolysis of polysaccharides using three key amino acids in its active site. Glu219 functions as a proton donor, Asp193 acts as the nucleophile that attacks the glycosidic bond, and Asp294 helps bind the substrate. The process involves:

- Glu219 donates a proton to the oxygen atom in the glycosidic bond between two sugar units. Then, Asp193 attacks one of the carbon atoms (C1) of the first sugar unit, leading to the release of the second sugar molecule. Asp294 ensures the large sugar molecule is held securely in place.
- A water molecule is made reactive, probably with the help of Glu 219 after it has donated its proton.
- The enzyme returns to its original state by breaking the temporary bond that formed between Asp193 and the first sugar molecule using the activated water. This releases the second sugar fragment.**(Kherouf,2021).**

II.3.4. Diabetes Treatment

Diabetes treatments are of two types: non-pharmacological (lifestyle and dietary management) and pharmacological (oral antidiabetic drugs (OADs) and insulin therapy).

➢ **Non-drug treatments:**

✓ weight loss and regular, tailored exercise improve blood sugar control and insulin resistance.

✓ Balanced diet low in saturated fats, sugars, and alcohol is key.

✓ Lifestyle changes should begin when HbA1c levels rise above 6.

➢ **Drug Treatments**

Oral Antidiabetic Drugs (OADs)

✓ Les sulfamides hypoglycémiants (Carbutamide, Glipizide, Glibenclamide, Gliclazide, glibornuride, glimépiride).

✓ Les biguanides (metformine).

✓ Les inhibiteurs des alpha-glucosidases (acarbose, miglitol).

II.4. Immunomodulatory activity:

II.4.1. immune system

The human immune system is a two-part defense network, featuring the rapid-acting innate system and the more specific adaptive system. Within the innate arm, myeloid cells like neutrophils, macrophages, and dendritic cells are crucial. Their role is to identify and eliminate bacteria, providing an immediate response to infection.**(Hirayama,2017)** .

II.4.1.1. Types the immunity

II.4.1.1.1. Adaptive immunity

Adaptive immunity, also referred to as acquired immunity, corresponds to the development of specific resistance to an antigen. This resistance may result from an active immune response, in which the body endogenously produces antibodies or effector cells specific to the antigen. Alternatively, it may arise through passive immunity, characterized by the exogenous transfer of pre-formed antibodies or immune cells.**(Song,2021)** .

II.4.1.1.1. Innate immunity

In the initial stages of infection, the innate immune system mounts a rapid response aimed at eliminating and blocking invading pathogens. This intrinsic defense mechanism, which is an essential aspect of human physiology, plays a vital role in maintaining survival by providing immediate protection. It does so by recognizing harmful microorganisms and internal physiological stress signals, subsequently initiating an inflammatory response to neutralize these threats. This inflammation is triggered when pattern recognition receptors on innate immune cells detect specific molecular signals associated with infection or tissue damage, thereby activating downstream immune pathways to contain and resolve the threat **(Hirayama,2017).**

II.4.2. phagocytic activity

II.4.2.1. Definition

Phagocytosis is a fundamental mechanism of innate immunity, employed by specialized cells to internalize and eliminate pathogens. It is recognized as one of the primary strategies through which vertebrates combat microbial infections. The

process involves several distinct stages and is carried out by phagocytic cells that play a multifaceted role in immune defense. The phagocytic activity of these cells can be evaluated in vitro by microscopically quantifying their uptake of particles, such as latex beads,and zymosan.**(Benaoun, 2016)** .

II.4.2.2. Macrophage

Macrophages, as key players in our initial immune defense, engulf bacteria and release molecules that trigger inflammation and fight microbes. Furthermore, they are crucial for clearing out unhealthy or injured cells by inducing their self-destruction.**(Hirayama,2017)** .

II.4.2.3. Mechanism activity phagocytic

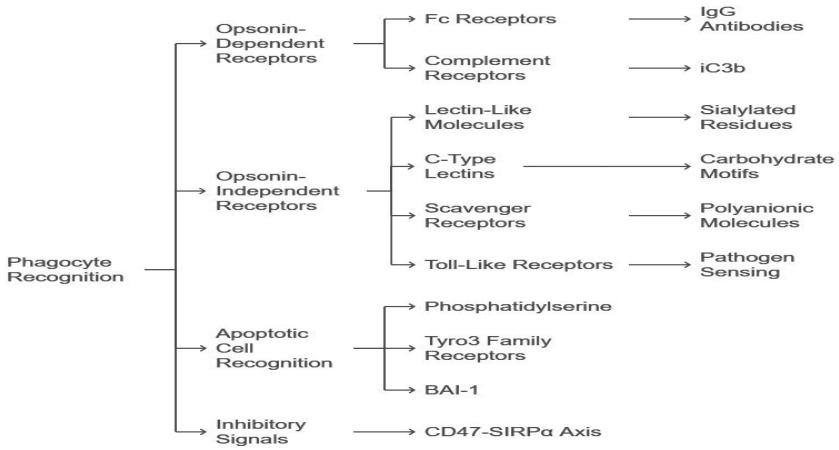


Figure II. 2: Mechanism the phagocytosis(**Gordon,2016).**

II.5. Anti-inflammatory activity

Milk thistle (Silybum marianum) appears to be a strong anti-inflammatory. 1 This effect seems to come from compounds produced as silymarin breaks down, which then act to reduce inflammation in body tissues. It's important to note that these findings come from early-stage research**(M. C. Sidhu1 , Punima Saini,2012)** .

II.5.1. Definition of inflammation

Inflammation is how your body defends itself. Whether it's a physical injury, exposure to chemicals, or an infection from tiny organisms, inflammation kicks in.

This vital immune process works to clear out the problem and heal the damaged area **(FERDJIOUI,2020)** .

II.5.2. Types of inflammation

- **The acute of inflammation:** this process is often vigorous, exhibiting prominent vasculoexudative changes.(**Kouachi, M. 2017).**
- **Chronic inflammation:** acute inflammatory responses transition into prolonged subacute and chronic inflammation when the initial pathogenic agent persists within tissues(**Kouachi, M. 2017).**

II.5.3. Mediators of inflammation

Inflammatory responses trigger the production and release of numerous inflammatory mediators as shown in **(Figure II. 3)**, which are typically categorized as either pro- or anti-inflammatory. Interestingly, some mediators, like IL-12, exhibit both properties. Cytokines (including interferons, interleukins, and TNF-α), chemokines (such as MCP-1), eicosanoids (like prostaglandins and leukotrienes), and the important inflammatory regulator NF-κB are among the extensively studied mediators and cellular pathways linked to human diseases.**(Azab,2016)** .

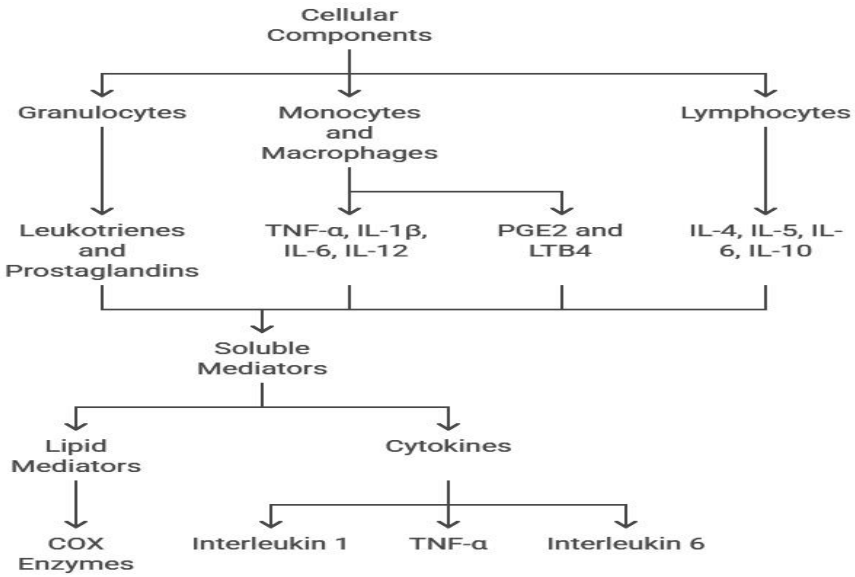


Figure II. 3: Different from Mediators of inflammation (**Bahi, 2015).**

II.5.3.1. Cyclooxygenase

Cyclooxygenase, also known as prostaglandin G/H synthase, is a crucial enzyme in the series of reactions involving arachidonic acid. This enzyme is responsible for creating various signaling molecules called prostanoids, which are key players in causing inflammation.**(Copeland ;1994)** .

PGE2 stands out as one of the most extensively studied prostaglandins due to its involvement in numerous aspects of human physiology and the development of various diseases. In a healthy body, PGE2 contributes to essential functions like maintaining a normal body temperature, safeguarding the stomach's inner layer, regulating blood flow in the kidneys, and supporting the female reproductive system.However, when PGE2 activity is altered, it can contribute to the development of pathological conditions. These include inflammatory diseases, disruptions in body temperature control, and even colorectal cancer.The synthesis of prostaglandins begins with the action of the PLA2 enzyme, which releases arachidonic acid from the phospholipids in cell membranes. Subsequently, the COX enzyme family converts this arachidonic acid into prostaglandins. Among the different COX enzymes (COX-1, COX-2, and COX-3), COX-2 is particularly noteworthy for its role in inflammation, as its activity increases significantly during inflammatory processes.Leukotrienes (LTs) and Nitric Oxide Synthase (NOS): Other Key Inflammatory MediatorsBesides prostaglandins, leukotrienes (LTs), with LTB4 being a prominent example, are also implicated in various human illnesses, such as inflammation, asthma, and even depression. The 5-LOX enzyme is responsible for the production of leukotrienes.Another important player in inflammatory conditions is the enzyme nitric oxide synthase (NOS), which generates nitric oxide (NO). Similar to COX-2, the inducible form of NOS, known as iNOS, is considered the most pro-inflammatory variant of this enzyme.**(Azab,2016).**

II.5.4. anti-inflammatory

II.5.4.1. Non steroidal anti-inflammatory drugs

Non steroidal anti-inflammatory drugs (NSAIDs) are the main drugs used to fight inflammation by blocking COX enzymes, which are involved in producing inflammatory molecules called prostaglandins. There are three types of COX enzymes: COX-1 (always present and handles vital functions), COX-2 (increases

during inflammation and is the main target of NSAIDs for pain relief), and COX-3 (in the brain, its exact role is still being studied). Blocking COX-1 can cause side effects like kidney issues and stomach ulcers by shifting the body's chemical pathways to produce more inflammatory leukotrienes, potentially leading to allergic reactions. NSAIDs are classified based on how strongly they inhibit COX-1 and COX-2, ranging from traditional non-selective drugs to those that prefer COX-2 and highly selective COX-2 inhibitors (Coxibs) **(FERDJIOUI,2020)** .

II.5.4.2. Steroidal anti-inflammatory drugs

Steroidal anti-inflammatory drugs (SAIDs) are synthetic versions of cortisol, a natural stress hormone. They have broad effects, including regulating stress response and energy release (hence the name glucocorticoids). As anti-inflammatories, SAIDs reduce inflammation by decreasing the activity of pro-inflammatory genes (like cytokines), increasing anti-inflammatory genes, and blocking the production of prostaglandins and leukotrienes by inhibiting phospholipase A2. They also reduce swelling, dampen immune cell activity, and hinder the release of other inflammatory substances. Their immunosuppressive effects stem from blocking cytokine production, which is vital for both cell-mediated and humoral immunity. Because SAIDs mimic a powerful hormone and affect many bodily functions, their side effects are a direct result of these widespread actions.**(Ferdjioui,2020).**

II.6. Antityrosinase activity

II.6.1. Tyrosinase

II.6.1.1. Definition

Tyrosinase is a protein containing a metal that speeds up the initial two shared steps in the production of melanin. Because of this role, it acts as the key enzyme that controls the overall process. If tyrosinase is missing or if there are changes in its gene, this can lead to less pigmentation or even a complete stop in color production. It has been shown that a mutation in the tyrosinase gene is linked to type I oculocutaneous albinism.**(Okombi,2005)** .

II.6.1.1. Structure of tyrosinase

The tyrosinase found in humans and other mammals is a type I transmembrane protein. This means it's a protein that spans a cell membrane. Crucially, it's a metalloprotein, containing two copper atoms at its active site, which are essential for its enzyme activity. The area where these copper atoms bind is located inside the melanosome, the cellular compartment where melanin is produced.

Despite its importance, the complete sequence of amino acids (primary structure) and the 3D arrangement (crystallization) of human tyrosinase haven't been fully determined yet. This difficulty in studying its structure is mainly due to a part of the protein that crosses the membrane and the presence of sugar molecules attached to one end (N-glycosylation).

However, spectroscopic studies have revealed that the copper-containing active site, which is involved in melanin production, has a structural similarity to the active sites of catechol oxidases (another type of enzyme) and hemocyanins (copper-containing proteins that transport oxygen in some animals) **(Okombi,2005)** .

II.6.1.3. Source and localization

First found in mushrooms, the enzyme tyrosinase is common throughout the natural world. Later research revealed its presence in a diverse range of living things, from single-celled organisms like prokaryotes to more complex life forms such as plants, arthropods, insects, amphibians, mammals, and fungi. Tyrosinase is a water-soluble enzyme that can be found in various parts of cells, including chloroplasts, mitochondria, microsomes, peroxisomes, or the cell's cytoplasm **(Leboukh,2018)** .

II.6.1.4. Biosynthesis

Tyrosinase, a protein that crosses cell membranes and can have up to seven sugar attachments, is created when its gene's instructions are copied into mRNA, which then guides the protein's assembly. This newly formed protein enters the endoplasmic reticulum (ER), where it first gets sugar molecules attached and begins to mature. A helper protein called calnexin is needed in the ER to ensure tyrosinase folds correctly and binds to copper, a necessary component. Calnexin keeps tyrosinase in the ER for the time it needs for these steps. After this initial processing, tyrosinase moves to the Golgi apparatus for further maturation. Finally, it reaches its

working location, an organelle called the melanosome, by traveling in small transport sacs **(Okombi,2005)** .

II.6.1.5. Roles of Tyrosinase

In humans, the tyrosinase enzyme, whose instructions are found on a specific part of chromosome 11, controls the very first step in the production of melanin (the pigment that gives us our skin, hair, and eye color). In mammals, this enzyme carries out a few key chemical reactions:

- It adds a hydroxyl group (oxygen and hydrogen) to the molecule L-tyrosine, turning it into L-DOPA. This specific action is called monophenol hydroxylase activity.
- It then converts L-DOPA into another molecule called dopaquinone. This second action is known as catechol oxidase activity.
- It also plays a role in changing 5,6-dihydroxyindole (DHI) into indolequinone.

Beyond mammals, tyrosinase has other jobs. In insects, it's important for hardening their exoskeletons (a process called sclerotization, where chitin becomes tough) and for their defense systems.

In the world of food, tyrosinase is the enzyme that causes fruits and vegetables to turn brown when they are cut or bruised. This browning happens because tyrosinase helps to oxidize certain compounds (diphenols) into quinones **(Okombi,2005)** .

II.6.2. Melanogenesis

- **Melanocytes**

Melanocytes, the pigment-producing cells, originate from the neural crest, the same embryonic tissue that forms nerve cells. During fetal development (weeks 8-14), their precursors, melanoblasts, migrate to the skin's base (epidermis) and hair follicles, where they mature into functional melanocytes **(Okombi,2005)** .

- **Melanosomes**

Melanosomes are oval vesicles from the Golgi apparatus where melanin production occurs through a series of chemical reactions involving tyrosine and

enzymes like tyrosinase. Produced in melanocytes, tyrosinase becomes active in melanosomes but its activity decreases as they migrate to the upper skin layers. Melanosomes are transferred to keratinocytes via phagocytosis, organizing around the nucleus ("capping") to protect DNA from UV radiation. Eventually, they are broken down in keratinocytes, releasing melanin. A melanocyte and its associated keratinocytes form the "epidermal melanin unit," responsible for melanin production, a multi-stage process **(Okombi,2005)** .

II.6.2.1. Biosynthesis of Melanins

Melanin biosynthesis as shown in (**Figure II. 4**) is a genetically and enzymatically controlled process within melanocytes' melanosomes, converting tyrosine from the blood into melanin pigments through several steps. The initial oxidation of L-tyrosine to L-DOPA, and then to dopaquinone, is primarily catalyzed by tyrosinase, sometimes in competition with tyrosine hydroxylase. The subsequent pathway determines whether dark brown eumelanin or yellow-orange pheomelanin is produced, a decision influenced by the levels of sulfur-containing compounds like cysteine and glutathione in the melanocytes. Sufficient sulfur compounds lead to pheomelanin production, while insufficient levels result in eumelanin production **(Okombi,2005)** .

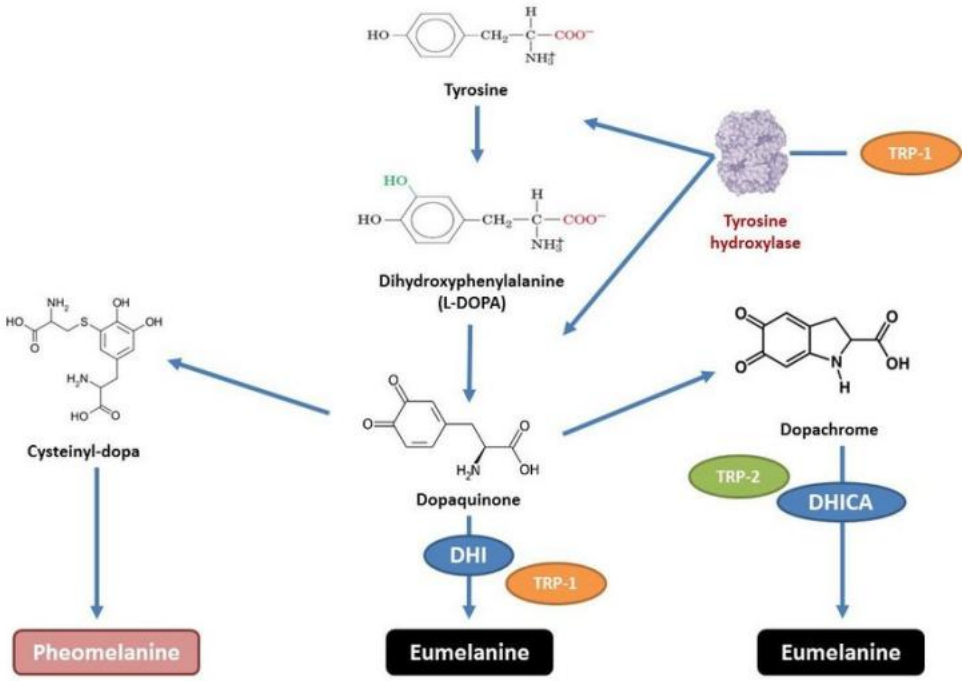


Figure II. 4: Pathways for Melanin Production

The enzyme tyrosine hydroxylase plays a key role in converting tyrosine into melanin. Following a series of metabolic steps, the produced melanin is stored within small sacs called melanosomes. These melanosomes are later engulfed by skin cells known as keratinocytes, located in the outer layer of the skin (epidermis). Dopamine, a substance formed during melanin production, is further processed through oxidation into dopaquinone and then dopachrome. Finally, dopachrome molecules link together (polymerize) to form melanin. Increased exposure to sunlight stimulates this entire production process (DHI: 5,6-dihydroxyindole, DHIC: 5,6-dihydroxyindole-2-carboxylic acid, TRP: Tyrosine Related Peptide) **(Segaoula ,2017)** .

II.6.3. Tyrosinase inhibitors

Substances that block tyrosinase, an enzyme involved in pigment production, can work in different ways. They might stop the gene that makes tyrosinase from being read, alter the sugar attachments on the enzyme, affect how the enzyme is processed after being made, or directly prevent the enzyme from doing its job.

- **Controlling the production of tyrosinase:** The production of tyrosinase is regulated by a protein called MITF. Compounds that can reduce the amount of MITF or its activity can lighten skin. For example, tretinoin (all-trans retinoic acid) encourages immature pigment cells to develop and then die off.
- **Blocking the proper development of tyrosinase:** If the sugar molecules attached to tyrosinase are changed, the enzyme becomes less active and is not transported correctly within the pigment-producing cells. Certain sugars like glucosamine and galactosamine may cause this disruption.
- **Directly stopping tyrosinase from working:** Most skin-lightening agents work by directly interfering with the activity of tyrosinase. They can mimic the molecules that tyrosinase normally acts on, block the active site of the enzyme, or bind to copper, a necessary component for tyrosinase to function.**(Okombi,2005)**

ChapterIII

Theoretical Context of Experimental Techniques

III.1. Experimental Techniques

III.1.1. UV-Vis spectroscopy

UV-Vis spectroscopy is a common and powerful technique in molecular analysis that relies on how substances absorb light. By measuring this absorption in the ultraviolet and visible regions of the electromagnetic spectrum (specifically wavelengths from 100 to 800 nanometers), we can learn about the structure of some molecules and determine the amounts of various substances present. This method, known as spectrophotometry, is also very useful in physical chemistry for investigating how chemical reactions reach equilibrium and how fast they occur in solutions. Light itself acts as an electromagnetic wave, and its energy is carried in tiny packets called photons. As the wave propagates, it exhibits oscillations characterized by peaks and troughs. The wavelength, a crucial property of light, is defined as the distance between two successive peaks or troughs of this wave.

- ✓ UV rays (from 100 to 400 nm).
- ✓ Visible light (from 400 to 800 nm).**(Benbahmed et Outaleb, 2018).**
- ✓ the Beer-Lambert Law is a fundamental principle that tells us how much light a substance absorbs.
- ✓ This relationship is written: **Log10 (I0/I)= *f* εCl.**
- ✓ Or, as it is currently written:**A : *f* εCl.**

With:

A: absorbance.

ε: the extinction coefficient (mol-1.cm-1.L).

C: concentration (mol/L).

l:Thickness of the cuvette.

I_0: ntensity of the irradiation energy arriving at the sample (Incident light).

I:Intensity of the radiation that has passed through the sample (Transmitted light).**(Ibrahim et Ouazine, 2014).**

III.1.1.1. Principle of UV-Visible Spectroscopy

The electronic transitions observed in molecules using UV-Vis spectroscopy entail significant energy changes , ranging from roughly 160 to 665 kJ per mole. These energy magnitudes are comparable to the strengths of chemical bonds, indicating that absorbed UV or visible light can, in some instances, possess sufficient energy to induce bond dissociation. However, more commonly, the absorption of such light results in the excitation of electrons to higher energy levels within the molecular orbitals (**Baudouin, A.,2012)** .

III.1.2. Molecular docking

Molecular docking is a crucial step in identifying how small molecules, called ligands, can bind to a specific site on a larger molecule. This computational technique involves the virtual exploration of various conformations, positions, and orientations of the ligand within the designated binding site. Only the most energetically favorable arrangements, indicative of robust interactions, are retained for subsequent analysis. This computational approach is highly valuable in biology, pharmacy, and medicine, given that a significant number of therapeutic agents exert their effects through specific interactions with biological targets **(EL Hadj Said, 2016 ; Lanez, 2016)** .

III.1.2.1. Principles of Molecular Docking

Molecular docking comprises a two-phase process. Initially, the ligand is positioned within the protein's active site, and a comprehensive exploration of its conformational landscape, spatial localization, and orientation is undertaken. Only configurations indicative of the most robust binding interactions are retained. Subsequently, these promising arrangements undergo evaluation and ranking based on their predicted binding affinity to the protein. This scoring methodology facilitates the identification of the most probable binding mode **(EL Hadj Said, 2016)** .

The best molecular docking algorithms would accurately predict how a molecule actually binds in experiments. Furthermore, their scoring systems should identify this correct binding arrangement as the most likely one compared to all other possibilities they generate.**(Bouchagra, 2018).**

The docking score roughly estimates the free energy change when a protein and a ligand bind to form a complex, based on thermodynamics.**(Arrault, 2007):**

ΔG = ΔG complexe – ΔG ligand - ΔG Protéine

In theory, for a protein and a ligand to bind spontaneously and form a stable complex, the overall change in Gibbs free energy during this binding process must be negative (ΔG complexation < 0).)**(Lebbad, 2016).**

III.1.2.2. Steps of Molecular Docking

Molecular docking typically involves these main steps: selecting the 3D structures of the target and ligand, preparing these structures for the specific docking method, and then analyzing the results **(Morris, 2008).**

III.1.2.3. Tools of Molecular Docking

The practical implementation of molecular docking requires the following parameters:

a) Protein: Macromolecules

To actually perform molecular docking, the most important initial step is having the 3D shape of the molecule you're studying (in this case, an enzyme) **(Dar et Mir, 2017)**,Fortunately, this information is readily available in protein databases like the Protein Data Bank (PDB).

b) Ligand

Obtaining the ligand's 3D structure is essential for molecular docking. These structures can be found in databases (like ZINC or PubChem) or created using modeling software (like Chemsketch or Gaussian) through geometry optimization based on quantum chemistry **(Jade et Gupta, 2016; El Hadji Said, 2016)** .

- **Molecular Modeling**

For molecular docking, the ligand structures need to be optimized using molecular modeling.

III.1.2.4. Docking Programs

Molecular docking programs are essential tools in biological, pharmaceutical, and medical research.**(Amina & Khaoula, 2017).**With more than 30 options available commercially and non-commercially, programs like Surflex, GOLD, FlexX,

DOCK, and AutoDock are frequently used. A key advantage of these programs is their ability to rapidly screen large collections of molecules. They achieve this using various specialized computational approaches, such as Genetic Algorithms, Monte Carlo methods, and Incremental Construction algorithms **(Dias et *al*., 2008 ; Forli et *al*., 2016)** .

III.1.2.4.1. AutoDock molecular docking software

AutoDock is a molecular docking software that employs trajectory simulation for precise binding prediction. It starts with the ligand outside the receptor's active site and explores the binding region through iterative translations, rotations, and conformational changes. An energy function evaluates the ligand-receptor interaction after each movement, and these energy changes guide subsequent movements. The simulation stops when the optimal binding pose is identified. This method effectively accounts for ligand flexibility and allows for extensive exploration of the binding site **(LANEZ,2019)** .

Servers Used

III.1.3. SwissADME

SwissADME **(Figure III. 1)** is a free online tool that uses computer models to quickly and reliably predict important properties of drug-like molecules. These properties include their physical and chemical behavior, how they might be processed by the body (ADME), their similarity to existing drugs, and their suitability for medicinal use. The website is user-friendly, allowing both experts and non-experts to easily input and understand the predictions for multiple molecules, which can aid in the drug discovery process.(**Dainaet *al*., 2017).**

Figure III. 1: Home page of the SwissADME server

III.1.4. The Pubchem database

The PubChem database**(Figure III. 2)** was introduced in 2004 and has since grown to be a source important chemical facts for the public, students, and scientists. Every month, our programming and internet services offer information to millions of users worldwide. PubChem mostly includes little molecules as well as bigger ones like nucleotides, peptides, lipids, carbohydrates, and macromolecules with chemical modifications. We Let's collect data on chemical IDs, structures, and characteristics. biological, chemical, and physical activities, patents, safety, health, and data poisoning, among other things.**(Trad, 2020).**

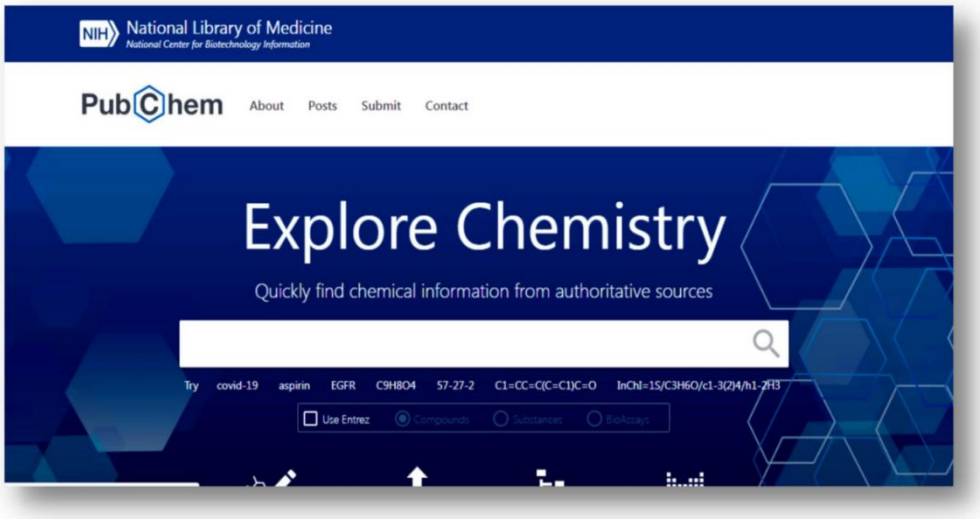


Figure III. 2: Home page of the Pubchem database server.

III.1.5. ProTox

ProTox-II **(Figure III. 3)** is a free online tool (accessible at http://tox.charite.de/protox_II without needing to log in) that helps predict how toxic a chemical might be. It's designed for toxicologists, regulators, computer and medicinal chemists, and anyone else interested. You just enter the 2D structure of a chemical, and ProTox-II will give you a possible toxicity profile based on 33 different models, along with how confident it is in each prediction. It also shows a radar chart summarizing the overall toxicity and compares your chemical to the three most similar compounds with known immediate (acute) toxicity.**(Banerjee et *al.*, 2018).**

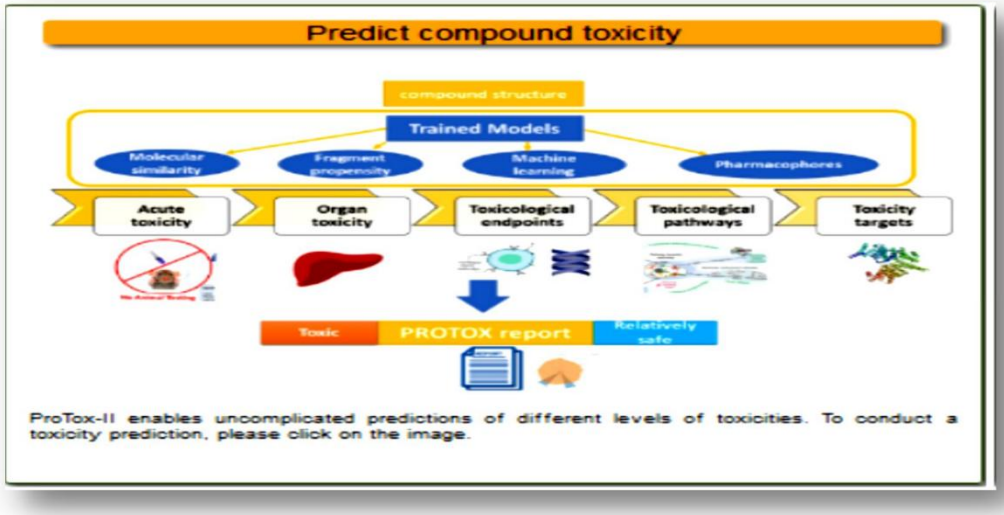


Figure III. 3: Home page of the proTox server

III.1.6. AutoDockTools

ADT, or AutoDockTools **(Figure III. 4)**, is a free graphical interface created by the AutoDock developers to facilitate the setup, execution, and analysis of AutoDock docking and AutoGrid calculations. This software also offers features for molecular surface computation, secondary structure visualization, hydrogen bond analysis, and other molecular modeling tasks.**(Ahmedou, 2021).**

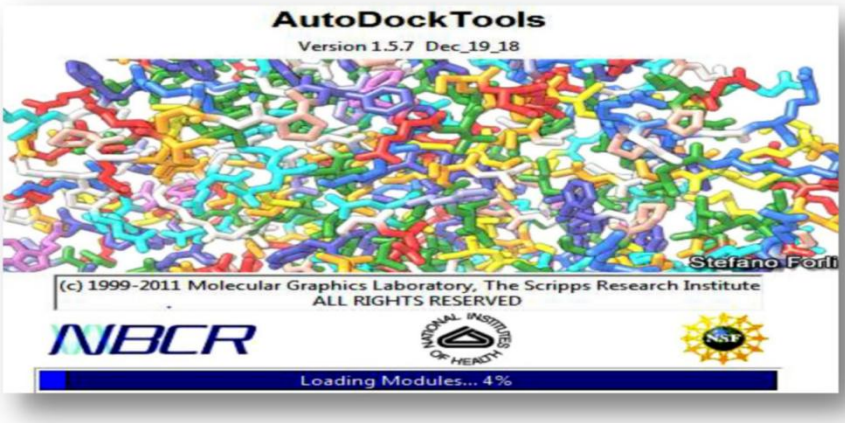


Figure III. 4: AutoDockTools server home page.

III.1.7. Discovery Studio.

Discovery Studio **(Figure III. 5)** is a versatile software platform used for simulating and modeling systems involving small and large molecules. Its applications include simulations, ligand design, pharmacophore development, structure-based design strategies, QSAR analysis, and ADMET profiling.**(Ahmedou, 2021).**

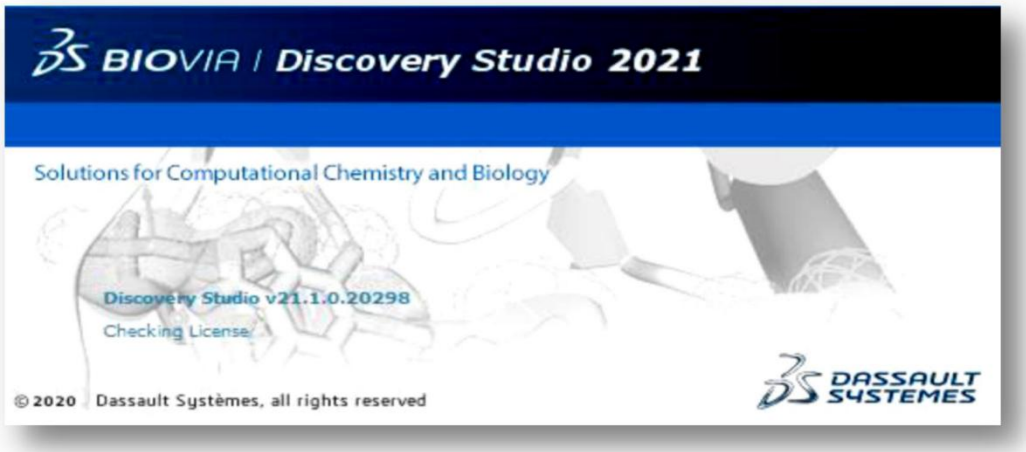


Figure III. 5: Discovery Studio server home page.

III.1.8. The Protein Data Bank PDB

The PDB, or Protein Data Bank **(Figure III. 6)**, serves as the primary global repository for freely accessible three-dimensional structures of biological macromolecules. Researchers worldwide contribute their structural data to this

database, a practice often mandated by leading scientific publications. The PDB boasts a diverse international user base, encompassing biologists in various disciplines (like structural biology and pharmacology), bioinformaticians, software developers, educators and students at all levels, science communicators, and the general public. Consequently, the PDB attracts around 140,000 unique visitors monthly from approximately 140 nations.**(Dorey, 2012).**

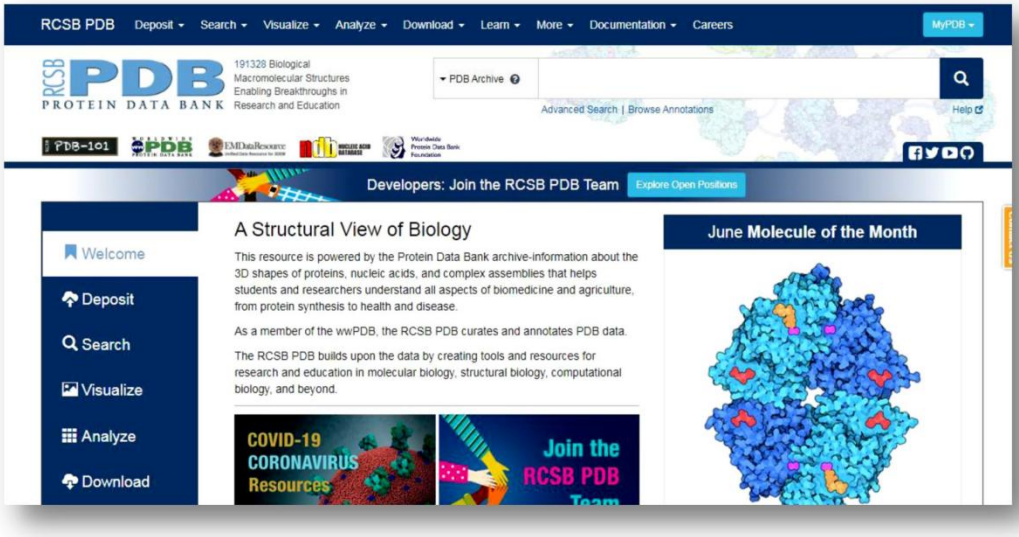


Figure III. 6: Protein Data Bank (PDB) server home page.

III.1.9. PyMOL

PyMOL(Pleomorphic Analysis Methodology) **(Figure III. 7)** is a free, open-source software developed by Warren Lyford DeLano for 3D molecular visualization. It produces high-quality representations of both small and large molecules, making it a valuable tool for scientific research and teaching, particularly in structural biology. Its open-source availability enhances its use in both educational and scientific communities.

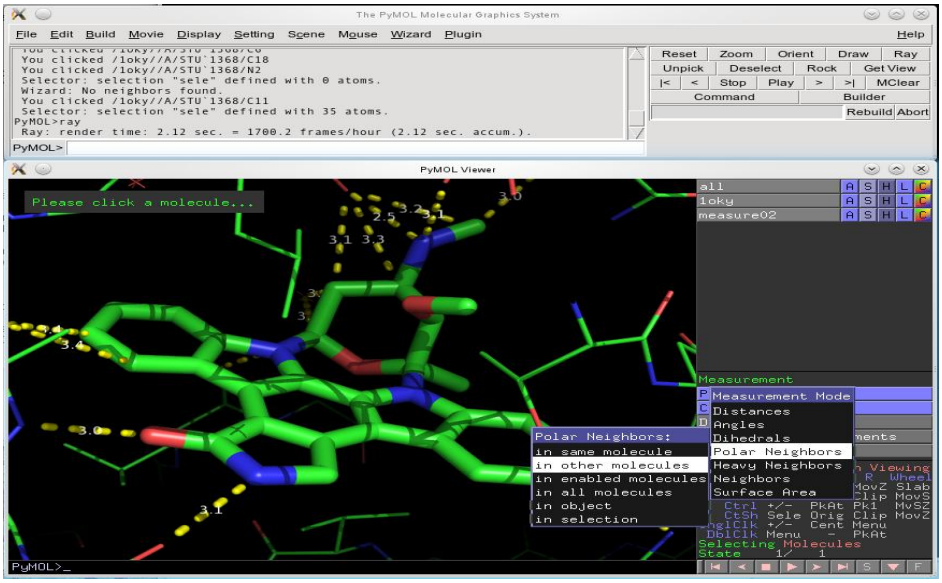


Figure III. 7: PyMOL software interface

III.1.10. PLIP

PLIP(Protein-Ligand Interaction Profiler)**(Figure III. 8)**, is an online tool for examining and displaying how proteins and small molecules interact in three dimensions. This server can also identify where molecules bind to proteins, predict potential interactions, and thoroughly analyze the specific atoms and molecular forces involved in these interactions.**(Salentin,2015) .**

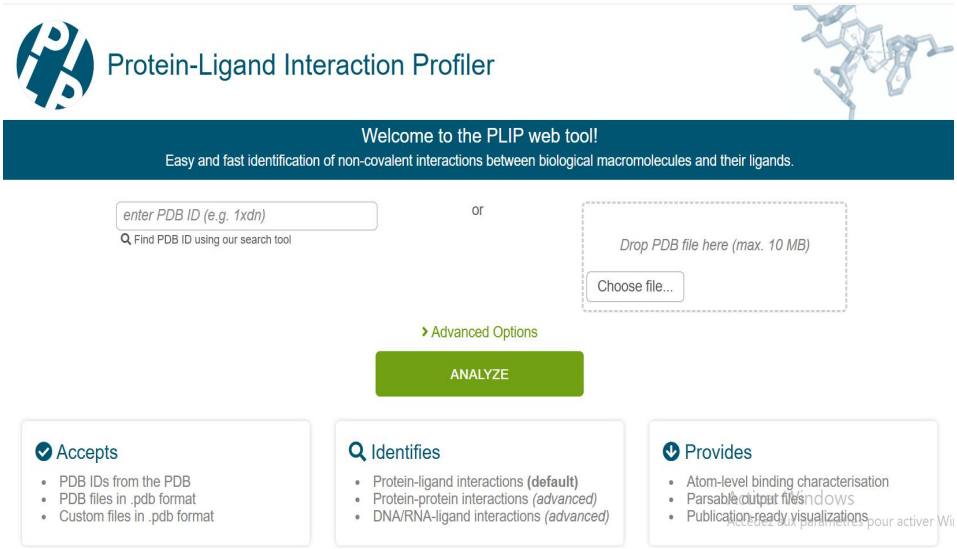


Figure III. 8: PLIP home page

General conclusion

General Conclusion

This investigation explored the bioactive potential of *Echinops spinosissimus* seeds through aqueous and methanolic extraction, followed by a comprehensive suite of in vitro and in silico analyses.

The extracts demonstrated significant antioxidant capabilities, effectively scavenging free radicals. Notably, they exhibited considerable tyrosinase inhibitory activity, suggesting their potential application in dermatological formulations for skin lightening or cosmetic purposes. Furthermore, the extracts displayed anti-inflammatory properties and notable antibacterial effects against several pathogenic strains, highlighting their potential as a source of natural bioactive compounds. Immunomodulatory potential was also indicated by a phagocytosis assay, revealing promising effects on immune responses. Complementary in silico simulations predicted interactions between putative bioactive constituents and relevant molecular targets.

Collectively, these findings underscore the promising biological activities of *E.spinosissimus* seeds, warranting further research to isolate and identify the specific active compounds, evaluate their safety profiles, and assess their efficacy in vivo through animal models and clinical trials.

Future research on Echinops spinosissimus seeds should prioritize the isolation and structural elucidation of bioactive compounds via advanced analytical techniques. Subsequent investigations must focus on delineating their precise molecular and cellular mechanisms of action. Toxicology is pivotal in these investigations, assessing the safety profile of Echinops spinosissimus seed extract and comparator drugs via predictive tools like the ProTox server. This evaluation is crucial for establishing safe dosages and identifying potential adverse effects, thereby validating the extract's suitability for pharmaceutical and cosmetic development. Furthermore, comprehensive toxicity and efficacy assessments in advanced in vitro and in vivo models are crucial, encompassing acute and chronic studies and therapeutic dose determination. Lastly, exploring the development of stable and effective pharmaceutical and cosmetic formulations is recommended, along with ensuring the sustainable utilization and conservation of this promising natural resource .

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Annex

Annex 1:Experimental part

The instrumentation utilized in this study

Figure 1: Spectrophotometer Visible

Figure 2: Oven

Figure 3: Hotplate and stirrer

Figure 4: Balance

Figure 5: Conductivity meter

Figure 7: Water bath

Figure 6: Centrifuges Clinic

Figure 8: Photomic microscope

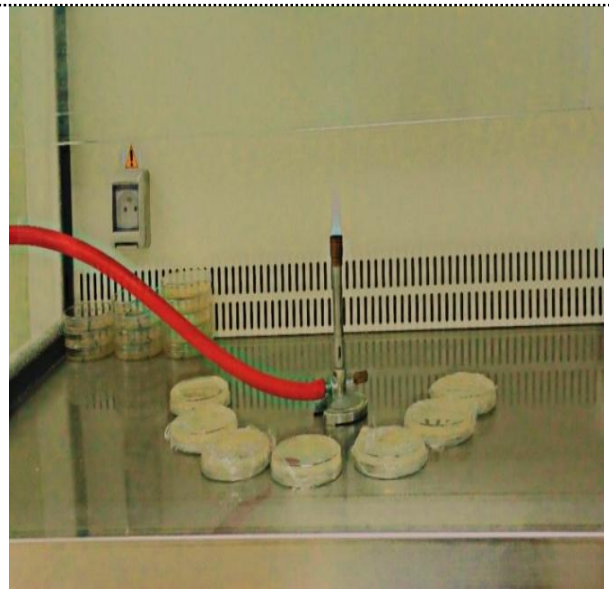

Figure 9: Bec Bunsen

Figure 10: Morter and pestle

- **The linear inhibition curves representing the extract's efficacy against various bacterial species are presented**

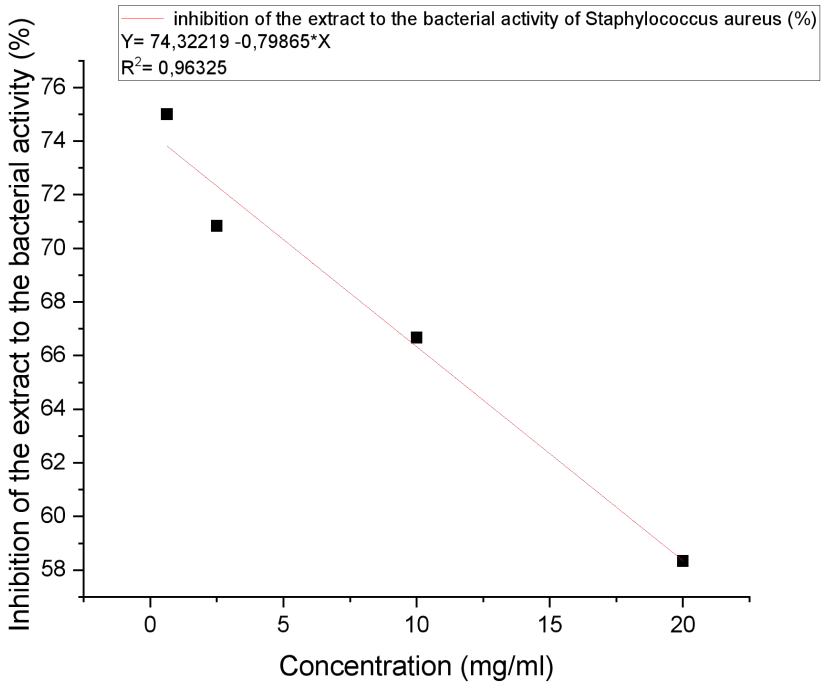

Figure 11: Inhibition of the extract to the bacterial activity of *Staphylococcus aureus* (%)

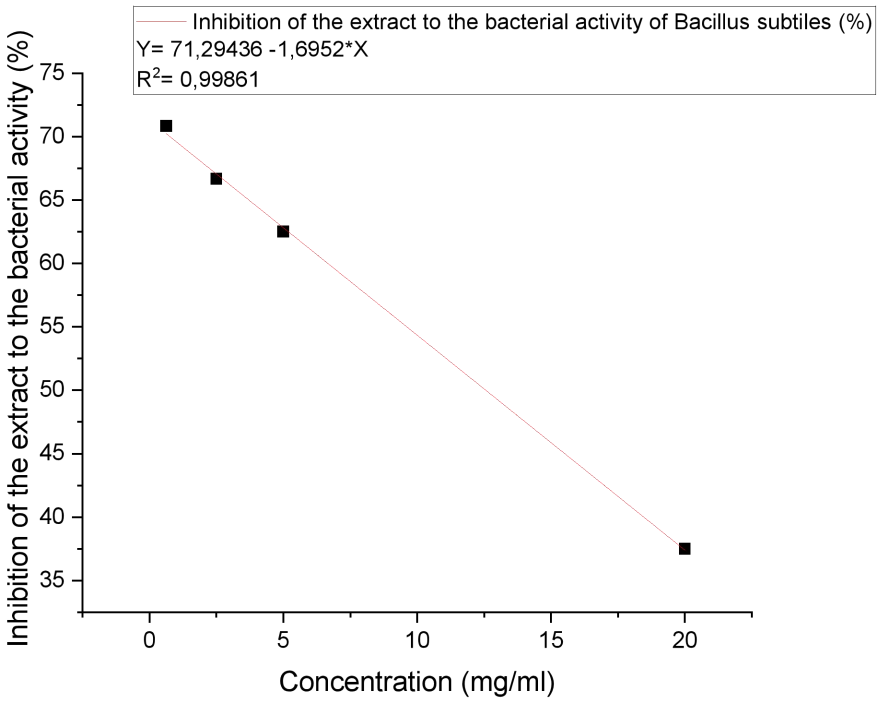

Figure 12: Inhibition of the extract to the bacterial activity of *Bacillus subtiles* (%)

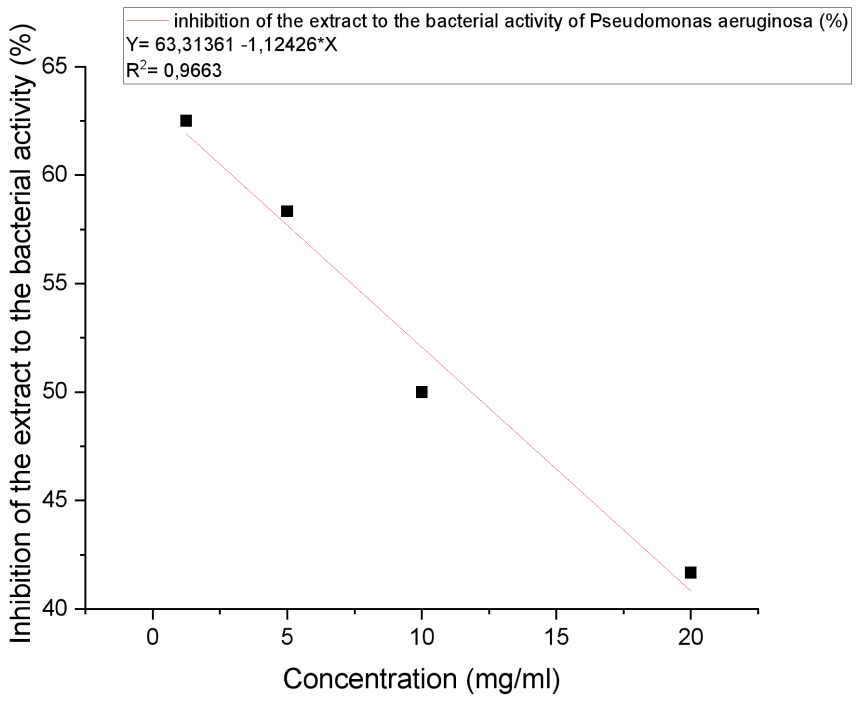

Figure 13: Inhibition of the extract to the bacterial activity of *Pseudomonas aeruginosa* (%)

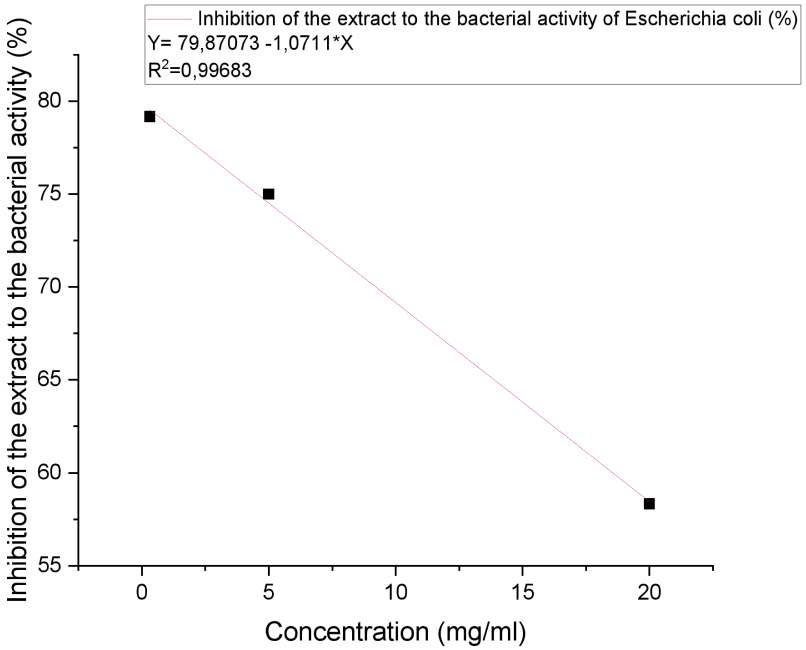

Figure 14: Inhibition of the extract to the bacterial activity of *Escherichia coli* (%)

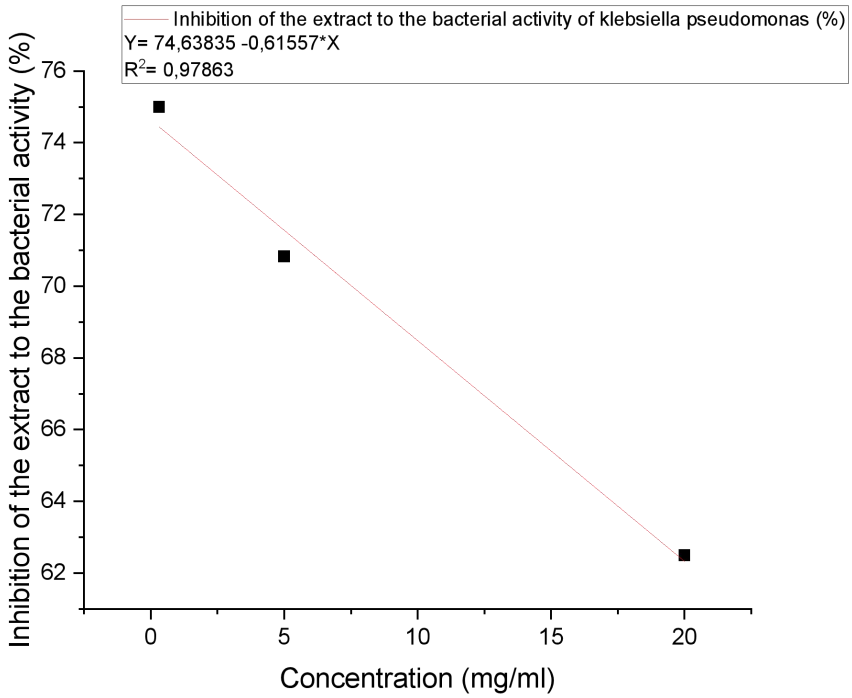

Figure 15: Inhibition of the extract to the bacterial activity of Klebsiella pseudomonnas (%)

الملاحق الخاصة بمؤسسة مصغرة *Annex 2*

نموذج العمل التجاري

الجدوى الاقتصادية

اولا: الدراسة الفنية

1. وصف المشروع

- اسم المشروع : مؤسسة jawa beauty لانتاج مستحضرات التجميل الطبيعية .
- نوع المشروع : صناعة وانتاج مستحضرات العناية بالبشرة .
- الفئة المستهدفة : الرجال والنساء .
- الموقع: محل صغير مرخص داخل المدينة .

2. الطاقة الاستيعابية

الطاقة الاستيعابية لمؤسسة مصغّرة تستوعب الات و عمال و مواد خامكما يلي:

◆**المواد الخام:**

- زيوت نباتية، شمع، عطور طبيعية، مستخلصات نباتية.
- عبوات (بلاستيكية أو زجاجية)
- ملصقات وأغلفة .

◆**التجهيزات:**

- خلاطات ومحمصات صغيرة.
- أواني ستانلس ستيل.
- ميزان دقيق.
- أدوات تعبئة يدوية أو شبه آلية.
- أدوات تعقيم.
- طاولات ومعدات نظافة.

◆**الموارد البشرية:**

- صاحب المشروع.
- مساعد/ة في التحضير والتعبئة.
- (اختياري) مصمم لتغليف العلامة التجارية.

- العمل يكون مدة 5 ايام في الاسبوع. الطاقة الشهرية حوالي 60000 علبة شهريا.

3. الخدمات المقدمة

- منتج طبيعي خالي من المواد الكيميائية .
- توفير ميزة التجربة المجانية لعينة من المنتج
- تقديم نصائح حول العناية بالبشرة وكيفية اتباع روتين يومي

4. المساحة المطلوبة

- تكون حوالي من 30-50م^2 مقسمة كما يلي :

➢ **منطقة التحضير(fabrication)**

- **10إلى 15 م^2**
- لتحضير الكريمات
- تحتاج إلى مساحة لآلات الخلط، الطهي، الطاولات.

- **منطقة التعبئة والتغليف(conditionnement)**

- **6 إلى 10 م²**
- وضع المنتجات في العلب والقوارير، وضع الملصقات

- **منطقة التخزين**

- **8 إلى 12 م²**
- لتخزين المواد الأولية (زيوت، عبوات، عطور...) والمنتجات الجاهزة.

- **مكتب إداري صغير أو استقبال**

- **5 م² تقريبًا**
- إذا كان هناك تسيير أو استقبال موردين.

- **ممرات وتهوية + شروط النظافة**

- يجب ترك فراغات كافية للحركة والتنظيف.

5. المعدات المطلوبة

- معدات للخلط والمزج(خلاطات صناعية.)
- معدات المختبر(انابيب اختبار, موازين دقيقه ,اجهزة تحليل جودة المنتج
- مواد التغليف(عبوات زجاجية او بلاستيكية و علب كرتونية و ملصقات)
- معدات التعبئة والتغليف

ثانيا :الدراسة المالية (تقديرية لمدة سنة)

1. تكاليف تأسيس(رأس المال الاولي)

البند	التكلفة التقديرية بالدينار الجزائري
ايجار المصنع	حسب الموقع والمساحة 30000,00 – 50000,00
معدات التصنيع والتعبئة	500000,00 -2500000 ,00
شراء المواد الخام الاولية	300000,00-150000,00
التراخيص والتسجيل القانوني	100000,00 -50000,00

تصميم وتطوير العلامة التجارية	40000,00-10000,00
تصميم العبوات والتغليف	80000,00-20000,00
بناء متجر الكتروني وتطبيقات البيع	60000,00-30000,00
التسويق والاعلانات الاولية	40000,00-10000,00
رواتب اول 3 اشهر	300000,00-200000,00
احتياطي طوارئ	100000,00-50000,00
اجمالي تكاليف تاسيسية	1930000,00-1070000,00

2. التكاليف التشغيلية الشهرية

البند	التكلفة التقديرية بالدينار الجزائري
ايجار المصنع او ورشة الانتاج	حسب الموقع والمساحة 30000,00 – 50000,00
شراء المواد الخام الاولية	100000,00-50000,00
الكهرباء والمياه واللوجستيات	80000,00 -30000,00
الصيانة والاصلاحات	100000,00-10000,00
نقل وتوزيع المنتجات	50000,00-20000,00
التسويق والاعلانات	40000,00-10000,00
رواتب الموظفين	300000,00-20000,00
اجمالي التكاليف الشهرية	720000,00-350000,00

3. الإيرادات المتوقعة (شهرية)

عدد العملاء اليومي 10 - 30

المنتج	الكمية الشهرية	متوسط السعر	الايراد الكلي
كريمات وزيوت	500	400000 دج	4800000 دج
صابون طبيعي	1000	300000 دج	3600000 دج
مقشر وماسك	300	150000 دج	1800000 دج
سيرومات وبلسم	300	1500000 دج	1800000 دج

4. **الربح الصافي المتوقع سنويا :**

الإجمالي السنوي = 12000000 دج

- الايرادات السنوية لدخل المالي الذي تحققه المؤسسة المصغّرة من بيع المنتجات أو تقديم الخدمات خلال سنة كاملة، قبل خصم المصاريف والتكاليف
- المواد الخام: 3,000,000 دج / السنة
- الأجور: 1,200,000 دج
- الإيجار والكهرباء والماء: 600,000 دج
- التغليف والتسويق: 500,000 دج
- مصاريف أخرى: 300,000 دج

الإجمالي = 5,600,000 دج

- المصروفات التشغيلية +التأسيسية(سنة أولى 6400000=5600000-12000000)**دج**
- الخسارة في السنة الأولى

من الطبيعي ان تكون هناك خسارة خلال السنة الاولي و هدا راجع طبعا الي كمية المنتوج و طبيعة المنتج سعر المنتج و ايضا المصاريف الخاصة بالمشروع كهرباء و اجور وووووالخ

- بدء الربحية من السنة الثانية بنسبة نمو

ابتداء من السنة احيانا يبدا المشروح بارباح طائلة نتيجة للخبرة المكتسبة في انتاج مواد تكون اكثر طلبا في السوق مع تحديد اسعار مقننة على حسب الفئة المستهدفة و ايضا استعمال طرق تسويق اكثر جلب للانتباه سواء عبر المحلات المستهدفة او عن طريق مواقع التواصل الالكتروني من المنتج الى المستهلك مباشرة مما يقلص نسبة الخسارة من منتج الى الاقتصاديين

العلامة التجارية وصورة المنتج

JAWA
BEAUTY

صورة توضيحية : العلامة التجارية للمنتج

صورة توضيحية :المنتج التجاري