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THEME

Herbal extract-based phytocosmetic as potential tyrosinase inhibitors Antioxidants, anti-inflammatory and skin-whitening cosmetic intgredients.

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Thanks

First and foremost, praise be to Allah, the Almighty, who said in His noble book

" لئن شكرتم لازيدنكم" All praise is due to Allah, out of love, gratitude, and appreciation, at the beginning(وآخر دعواهم ان الحمد لله رب العالمين) and the end

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Dedication

All praise is due to Allah, by whose grace all good deeds are completed. May the peace and blessings of Allah be upon our Master Muhammad, and upon his family and companions.

We first thank Allah, then express our deepest gratitude to our beloved parents ,

whose sacrifices paved the way for this achievement.

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And the last of our call is: "الحمد لله الذي بنعمته تتم الصالحات"

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ

﴿39﴾ وَأَنَّ سَعْيَهُ سَوْفَ يُرَىٰ))

صدق الله العظيم

SUMMARY

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- **Å²: Unit for Topological Polar Surface Area**
- **ADT: AutoDockTools**
- **BBB: Blood-Brain Barrier**
- **Bcl-2: B-cell lymphoma 2**
- **BSA: Bovine Serum Albumin**
- **CNS: Central Nervous System**
- **COX-2: cyclooxygenase-2**
- **CYP: Cytochrome P450**
- **DPPH：2,2-diphenyl 1-1picrylhydrazyl**
- **ECG: epicatechin gallate**
- **GAE: Gallic Acid Equivalents**
- **GI: Gastrointestinal**
- **HAs: Anthocyanins**
- **HLP: Hibiscus Leaf Polyphenols**
- **Hs: Hibiscus sabdariffa**
- **I %: Inhibition percentage**
- **IC50: half maximal inhibitory concentration**
- **IC50: The effective concentration of the substrate that causes the 50% reduction of DPPH in solution**
- **IL: interleukins**
- **JNK: c-Jun N-terminal kinase**
- **LD50: Lethal Dose, 50%**
- **L-DOPA: dihydroxyphenylalanine**
- **LXRα: liver-X receptor alpha**
- **M : Molar**
- **MCF-7: Michigan Cancer Foundation-7**
- **mg :Milligram**
- **mg GAE/g: Milligrams of gallic acid equivalents per gram of extract**
- **mg RE/g: Milligrams of rutin equivalent per gram of plant extract**

- **min : Minutes**
- **mn : Millimeter**
- **MR: Molar Refractivity**
- **nm : Nanometer**
- **NMU: N-Nitroso-N-methylurea**
- **OxLDL: oxidized low-density lipoprotein**
- **p38 MAPK: p38 mitogen-activated protein kinase**
- **PBR: Publisher**
- **PCA: protocatechuic acid**
- **PDBQT: Protein Data Bank, Partial Charge (q), & Atom Type**
- **P-gp: P-glycoprotein**
- **Ph: Potential hydrogen**
- **PTC: Chronic Toxicity Class**
- **RE: Rutin Equivalent**
- **ROS: Reactive Oxygen Species**
- **SMILES: Simplified Molecular Input Line Entry System**
- **TNF-α: tumor necrosis factor-alpha**
- **TPSA: Topological Polar Surface Area**
- **UV: Ultraviolet**
- **V : Volume**
- **µL : Micro liter**

Abstract

This study aims to evaluate the therapeutic potential of plant extracts from three species: Cyperus rotundus Linn, Glycyrrhiza glabra, and Hibiscus sabdariffa, with a focus on their chemical composition and biological activities, particularly their tyrosinase inhibitory activity, which represents an important pharmacological target in the development of natural skin-lightening cosmetic products.

The study begins with a preliminary phytochemical screening of the plant extracts to detect the presence of secondary metabolites such as flavonoids and polyphenols.

Subsequently, the biological activities of the extracts were evaluated using in vitro assays, including antioxidant, anti-inflammatory, antimicrobial, and tyrosinase inhibition activities. on the other hand, The results showed good biological activity compared with the chosen standards.

In addition, in silico tools by Molecular docking were employed to predict binding affinity between active compounds and tyrosinase enzyme and cyclooxygenase 2, alongside assessment of physicochemical properties, drug-likeness (Lipinski's Rule), bioavailability, and potential toxicity of selected compounds by swiss ADME and ProTox. The results reveal drug-like properties of different compounds, moreover the values of k and ΔG show a spontaneous electrostatic type interaction.

Overall, this study highlights the significant potential of these plant species as natural sources of bioactive compounds with promising applications in the cosmetic industry, particularly for skin-whitening formulations. It also underscores the importance of integrating both experimental and computational approaches for a comprehensive and accurate evaluation of such compounds.

Key words: plants extract, tyrosinase inhibition, molecular Docking,swiss ADME, whitening formulation, cyclooxygenase

Résumé

Cette étude vise à évaluer le potentiel thérapeutique d'extraits de trois espèces végétales : Cyperus rotundus Linn, Glycyrrhiza glabra et Hibiscus sabdariffa, en mettant l'accent sur leur composition chimique et leurs activités biologiques, notamment leur activité inhibitrice de la tyrosinase, qui représente une cible pharmacologique importante dans le développement de produits cosmétiques dépigmentants naturels.

L'étude a débuté par un criblage phytochimique préliminaire des extraits végétaux afin de détecter la présence de métabolites secondaires tels que les flavonoïdes et les polyphénols. Ensuite, les activités biologiques des extraits ont été évaluées au moyen de tests in vitro, notamment les activités antioxydante, anti-inflammatoire, antimicrobienne et inhibitrice de la tyrosinase. Par ailleurs , Les résultats obtenus ont révélé une bonne activité biologique par rapport aux standards utilisés.

En outre, des outils in silico ont été utilisés pour analyser les interactions entre les composés actifs présents dans ces plantes et les enzymes tyrosinase et cyclooxygénase. Des simulations de docking moléculaire ont été réalisées pour prédire l'affinité de liaison, ainsi que pour évaluer les propriétés physicochimiques, le caractère « drug-like » (selon la règle de Lipinski), la biodisponibilité et la toxicité potentielle des composés sélectionnés à l'aide des outils SwissADME et ProTox. Les résultats montrent que plusieurs composés possèdent des caractéristiques proches de celles des médicaments, et les valeurs de K et ΔG indiquent des interactions électrostatiques spontanées.

Dans l'ensemble, cette étude met en lumière le potentiel important de ces espèces végétales comme sources naturelles de composés bioactifs ayant des applications prometteuses dans l'industrie cosmétique, en particulier pour les formulations dépigmentantes. Elle souligne également l'importance d'intégrer à la fois des approches expérimentales et computationnelles pour une évaluation complète et précise de ces composés.

Mots-clés : extrait de plantes, inhibition de la tyrosinase, docking moléculaire, SwissADME, formulation dépigmentante, cyclooxygénase

الملخص

تهدف هذه الدراسة إلى تقييم الجوانب العلاجية لمستخلصات نباتية من ثلاث أنواع : نبات السعد وعرق سوس وزهور الكركدية ، مع التركيز على تركيبها الكيميائي وفعالياتها البيولوجية، وخاصة فعاليتها المثبطة لإنزيم التيروسيناز، الذي يُعد هدفًا دوائيًا مهمًا في تطوير مستحضرات تجميل طبيعية مبيضة للجلد.

بدأ البحث بفحص كيميائي أولي للمستخلصات النباتية لكشف وجود مركبات ثانوية مثل الفلافونويدات والبولي فنولات. بعد ذلك، تم تقييم الأنشطة البيولوجية للمستخلصات باستخدام اختبارات in vitro ، تضمنت الأنشطة المضادة للأكسدة، والمضادة للالتهابات، والمضادة للميكروبات، بالإضافة إلى قياس التأثير المثبط لإنزيم التيروسيناز. أظهرت النتائج فعالية بيولوجية جيدة مقارنة بالمعايير المستخدمة.

بالإضافة إلى ذلك، تم استخدام أدوات حاسوبية (in silico) لتحليل التفاعلات بين المركبات الفعالة الموجودة في النباتات وإنزيمي التيروسيناز والسيكلوكسجيناز. وأجريت محاكاة ارتباط جزيئي (molecular docking) لتوقع مدى قوة الارتباط، إلى جانب تقييم الخصائص الفيزيوكيميائية، و"الملاءمة الدوائية" (وفقًا لقاعدة ليبينسكي)، والتوافر الحيوي، والسمية المحتملة للمركبات المختارة باستخدام أدوات SwissADME و .ProTox أظهرت النتائج أن العديد من هذه المركبات تمتلك خصائص تشبه تلك الموجودة في الأدوية، كما أن قيم كل من ثابت الارتباط (K) وطاقة غيبس الحرة (ΔG) تدل على وجود تفاعلات إلكتروستاتيكية طفيفة وطبيعية.

بشكل عام، تسلط هذه الدراسة الضوء على الأهمية الكبيرة لهذه الأنواع النباتية كمصادر طبيعية لمكونات فعالة بيولوجيًا لها تطبيقات واعدة في الصناعة التجميلية، خاصةً في صياغة المنتجات المبيضة للجلد. كما تؤكد الدراسة أهمية الجمع بين الأساليب التجريبية والحاسوبية لتقييم شامل ودقيق لهذه المركبات.

الكلمات المفتاحية : مستخلصات نباتية، تثبيط التيروسيناز، الارتباط الجزيئي، SwissADME، صيغة تبييض، السيكلوكسجيناز

Introduction

Introduction

Cosmetics are an essential part of daily life, widely used to enhance aesthetic appearance, care for skin and hair, and improve overall health. With significant advancements in pharmaceutical and cosmetic industries, there has been increased reliance on synthetic chemical compounds as primary ingredients in the formulation of these products, due to their high efficacy in achieving desired results within a short period. However, the use of these compounds has raised growing concerns regarding their potential side effects, especially after long-term exposure, including skin irritations, allergic reactions, and even certain toxic effects on internal organs (Chéret et al., 2017).

In response to these challenges, recent years have witnessed a noticeable shift toward the use of natural sources in cosmetic formulations, particularly those derived from medicinal plants. This interest stems from the diverse biological properties exhibited by plant secondary metabolites such as flavonoids, polyphenols, and alkaloids, which have demonstrated effectiveness in influencing oxidative processes, inflammation, skin pigmentation, and antimicrobial activities (Khan et al., 2020). Moreover, these substances are generally considered safer and less toxic compared to their synthetic counterparts, making them a promising option for development in the field of functional cosmetics.

Based on these trends, three plants with high medicinal and cosmetic importance were selected for **use in this study:**Cyperus rotundus(licorice),Glycyrrhiza glabra (licorice) and Hibiscus sabdariffa (roselle), Glycyrrhiza glabra (licorice). These plants have been traditionally used for centuries across various cultures to treat skin disorders, strengthen hair, and lighten skin tone. Modern studies have supported these traditional uses by confirming that they contain active compounds such as lawsone, anthocyanins, glabridin, and hesperidin, which have shown strong biological activity in both laboratory and clinical settings (Ahmad et al., 2019; Zarepour et al., 2021).

This study aims to explore and reassess the efficacy of active compounds extracted from these plants using in vitro study(antibacterien, wigthening, anti-oxydant and anti-inflammatory assay) and computational tools (in silico) to determine their physicochemical properties, compliance with "Lipinski's Rule of Five", bioavailability, and potential toxicity, with the ultimate goal of developing effective and safe natural-based cosmetic products.

Furthermore, in silico tools were employed in this section to analyze the interaction between the active compounds in each plant and tyrosinase—an important enzyme target in the development of skin-lightening cosmetics—to understand the inhibition mechanism and identify the types of bonds involved in ligand-enzyme binding.

Thus, the diversity of approaches followed in this study, combining theoretical background, experimental testing, and computational modeling, enhances the reliability of the findings and directs the development of natural and sustainable cosmetic formulations.

The study is divided into two main parts:

Part One (Theoretical): Includes comprehensive information about the three studied plants, covering botanical characteristics, traditional uses, and the active compounds isolated from each.

Part Two (Applied): Involves conducting a series of in vitro laboratory experiments to evaluate the biological activity of the plant extracts, including antioxidant, anti-inflammatory, antibacterial assays, and tyrosinase inhibition efficiency, in addition to measuring the total flavonoid and polyphenol content of each extract.

Finally, here is a conclusion along with some perspectives and recommendations for further information.

Literature Review

CHAPTER I:

Medicinal Plants And Biological Activities

I.1.1. Cyperus rotundus linn

Cyperus rotundus L, commonly known as nutgrass, is a widely distributed weed found in temperate, tropical, and subtropical regions across the world. Due to its rich composition of bioactive compounds and significant pharmacological properties, extensive research has been conducted to identify its active constituents. Since 1965, approximately 192 compounds have been identified, including terpenoids, flavonoids, stilbenes, aromatic compounds, and aliphatic fatty acids**.(Babiaka *et al.*,2021).**

This perennial plant belongs to the Cyperaceae family and is characterized by an umbel inflorescence and fibrous roots. It propagates extensively through rhizomes and tubers. Despite being one of the most troublesome weeds in agriculture due to its strong competition for soil nutrients, high adaptability, and resistance to herbicides—resulting in significant crop losses—it also possesses valuable medicinal properties **(Kandikattu *et al*,2022).**

Figure 1: Cyperus rotundus linn (Kandikattum, Amrutam Khanum,Narayana, & Srinivasulu,2022).

I.1.2. Scientific classification of Cyperus rotundus linn

Table 01:Classification of Cyperus rotundus linn (Pasam *et al*, 2023)

Kingdom	**Plantae**
Subkingdm	Tracheophyta
Infrakingdom	Streptophyta
Genus	Cyperus
Family	Cyperaceae
Order	Cyperales
Subclass	Commelinidae
Class	Liliopsida
Species	C.rotundus
Division	Magnolinidae
Subclass	Commelinidae
Binomial name	**Cyperus rotundus linn**

I.1.3. Geographical spread of the Cyperus rotundus linn in the world

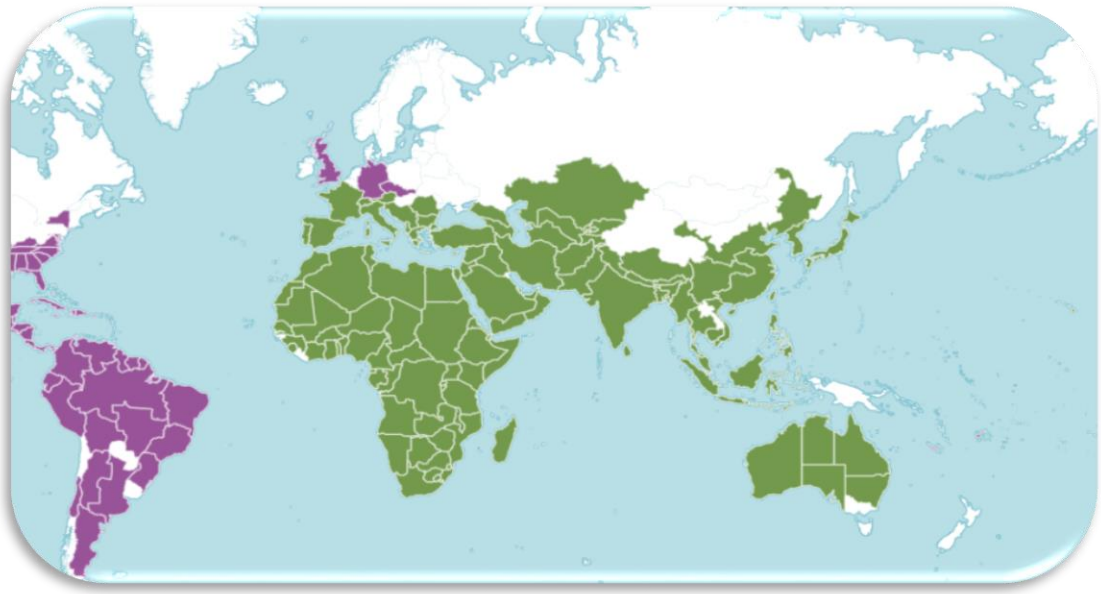

Figure 02: geographical spread of the Cyperus rotundus linn in the world (Brazil Flora ,2014)

I.1.4. Chemical composition of Cyperus rotundus Linn

Cyperus rotundus L. (Nutgrass, family Cyperaceae) is a highly invasive weed that is widely distributed across temperate, tropical, and subtropical regions of the globe. Due to its chemical richness and significant pharmacological potential, extensive research has been conducted to identify its bioactive components. Since 1965, approximately 192 compounds, including terpenoids, flavonoids, stilbenes, aromatic compounds, and aliphatic fatty acids, have been isolated and characterized. **This table** provides an overview of the biological activities and mechanisms of action of some key compounds derived from Cyperus rotundus L, highlighting their therapeutic potential**.(Babiaka , Moumbock , Günther,& Ntie-Kang,2021)**

Table 02: Chemical composition of Cyperus rotundus Linn. (Babiaka , Moumbock , Günther,& Ntie-Kang,2021)

Plant	Chemical compounds	Classification by chemical family	bioactive compounds derived
Cyperus rotundus Linn	Terpenoids	(−)- (Sesquiterpene)	Cypera-2,4-diene
		(+)-(Sesquiterpene ketone)	Nootkatone
		Sesquiterpene oxide	Rotundone
		Sesquiterpene ketone	α-Cyperone
	Flavonoids	Flavone	Luteolin
		Flavonol	Quercetin
		Flavone	Tricin
		Isoflavone	Biochanin A
	stilbenes and derivatives	Stilbene	trans-Scirpusin A
		Stilbene	Scirpusin B
		Phenolic acid	Ellagic Acid
		Stilbene derivative	Cyperusphenol A
	Miscellaneous	Organic Phenolic Acid	(−)-(E)-Caffeoylmalic
		Furanocoumarin	Khellin
		Furanocoumarin	Visnagin
		Saturated Fatty Acid	Myristic Acid

I.1.5. Cyperus Rotundus Oil: A Safe and Effective Alternative to Laser for Underarm Hair Reduction

A pilot study compared the efficacy of topical Cyperus rotundus oil, Alexandrite laser, and saline solution for reducing unwanted underarm hair. Sixty participants were randomly assigned to three groups: Cyperus rotundus oil, saline, or laser. Results showed no significant difference in overall hair reduction between the oil and laser ($P > .05$). However, the oil was significantly more effective in reducing white hair growth ($P < .05$). No side effects were reported. Researchers concluded that Cyperus rotundus oil is as effective as laser for reducing underarm hair, including both dark and white hair**.(Mohammed, 2014)**

I.1.6. Pharmacological activity of Cyperus rotundus Linn

Table 03: Pharmacological activity of Cyperus rotundus Linn **(Kamala,Middha & Karigar 2018).**

Plant	Plant part used	Solvent used for extraction	Pharmacological activity	References
Cyperus rotundus Linn	Rhizome	Ethanol	Anti-oxidant property	Kamala, Middha , Gopinath , Sindhura, & Karigar, 2018)
	Tuber	Ethanol/ether/distilled water	Anti-inflammatory activity	(Chithran , Ramesh Babu, & Himaja , 2012).
	Essential oil	Methanol	Anti-helmintic activity	(Kasala , Ramanjaneyulu , Himabindhu , Alluri , & Babu , 2016)
	Rhizome-essential oil	Ethanol	Anti-candida activity	(Duarte , Figueira , Sartoratto , Rehder , & Delarmelina , 2005).

I.1.7. Activity Biology of Cyperus rotundus

I.1.7.1. Anti-cancer Activity of Cyperus rotundus

In recent years, the anti-tumor activities of C. rotundus have attracted the attention of researchers and become a hot topic of study, including its potential effects against cervical cancer, breast cancer, ovarian cancer, esophageal cancer, hepatocellular carcinoma, human rectal cancer, prostate cancer, and colorectal cancer.

It has been demonstrated that the ethanolic extract of C. rotundus possesses potential effects against cervical cancer and breast cancer in human cervical carcinoma cells (HeLa) and breast cancer cells (MCF-7). Additionally, the ethanolic extract of C. rotundus was found to exhibit cytotoxic activity against cervical cancer cells (HeLa**).(Xue *et al*.,2023).**

I.1.7.2. Anti-Inflammatory Activity of Cyperus rotundus

Cyperus rotundus has demonstrated significant anti-inflammatory effects, particularly through its active compounds found in the rhizomes. The key compound α-cyperone reduces inflammation by activating protective pathways such as Akt/Nrf2/HO-1 and suppressing the NF-κB pathway, thereby decreasing the production of harmful cytokines. Studies have also shown that other compounds, such as cyperalin A and sugetriol triacetate, inhibit enzymes associated with inflammation, including COX-2 and LOX-5. In animal models, α-cyperone effectively treated acute lung injury by reducing inflammatory cell infiltration. Additionally, topical application of the extract reduced ear swelling and dermatitis-related inflammation in mice. These findings highlight the potential of Cyperus rotundus as a natural therapeutic agent for inflammatory disorders**. (Taheri *et al.*, 2021).**

I.1.7.3. Antimicrobial Activity of Cyperus rotundus

The plant Cyperus rotundus has demonstrated significant antimicrobial activity against bacteria and fungi, including drug-resistant strains such as multidrug-resistant Mycobacterium tuberculosis and antibiotic-resistant bacteria like Staphylococcus aureus. Alcoholic extracts and essential oils exhibited high efficacy, particularly against Gram-positive bacteria and fungi such as Candida albicans. The compounds β-Sitosterol and α-amyrin were identified as key contributors to this antimicrobial effect. Combining the extract with ampicillin enhanced its efficacy against ampicillin-resistant S. aureus, showing synergistic activity by damaging the bacterial cell wall and membrane, increasing permeability, and inhibiting β-lactamase. Fermented extracts also displayed inhibitory effects on bacteria such as Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli. Additionally, copper oxide nanostructures synthesized using C. rotundus extracts showed excellent antibacterial activity against

Klebsiella pneumoniae, likely due to mechanisms involving mechanical damage, oxidative stress, and genotoxicity. These findings highlight C. rotundus as a promising natural source for combating microbial infections. **(Taheri *et al*., 2021).**

I.1.7.4. Anti-oxidant activity

The plant Cyperus rotundus is a rich source of active compounds with potent antioxidant properties, such as phenolic acids, alkaloids, quinones, and essential oils. Among these compounds, "nootkatone" stands out as the most effective in scavenging free radicals (DPPH), with an IC50 value of 4.81 µg/mL, followed by compounds like "aristolone" and "solavetivone." Additionally, the aqueous extract of the plant has shown remarkable efficacy in combating oxidative stress compared to other extracts.

This plant also demonstrates the ability to neutralize various types of free radicals, including hydroxyl radicals and superoxide radicals, making it a promising candidate for preventing and treating diseases associated with oxidative stress, such as epilepsy, neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), liver damage, and diabetes.

Studies indicate that C. rotundus helps boost levels of antioxidant enzymes like SOD and GSH-Px while reducing harmful factors such as MDA, contributing to improved brain health and alleviating epileptic seizures in mice. Thus, this plant represents a promising natural option for enhancing health and combating oxidative stress-related diseases**.(Xue *et al,* 2023).**

I.1.7.5. Anti-ulcer activity

The anti-ulcer activity of the rhizome powder of Cyperus rotundus was investigated in a study conducted on two different animal models. Gastric ulcers were induced in guinea pigs by intraperitoneal administration of histamine (50 mg/kg) and in albino rats by oral administration of aspirin (500 mg/kg). The rhizome powder was administered orally 45 minutes prior to histamine injection and 1 hour before aspirin administration. In both cases, the treatment with Cyperus rotundus significantly reduced the ulcer index, showing effects comparable to those of the standard drug ranitidine. The observed anti-ulcer activity of Cyperus rotundus can be attributed to its potent antioxidant properties, which likely contribute to mitigating oxidative stress associated with ulcer formation**.(Mohammad , Nagarajaiah & Kudagi , 2012).**

I.1.8. Traditional Uses of Cyperus Rotundus linn in Folk Medicine

Cyperus rotundus, or nutgrass, has been traditionally utilized across various continents, with significant applications documented in Asian countries like India and Pakistan, as well as in Brazil, Morocco, and Papua New Guinea. The plant's roots, rhizomes, and tubers are the most frequently used components, comprising 33%, 22%, and 16% of its applications, respectively, while leaves and the whole plant are less commonly employed (13% and 9%, respectively). These parts are incorporated into remedies aimed at addressing a wide array of health issues. Notably, Cyperus rotundus is used to treat stomach disorders, diarrhea, and dysentery (each accounting for 11% of reported uses), alongside fever (10%), menstrual issues (9%), diuresis promotion (9%), and worm infections (8%). Additional medicinal applications have also been identified, emphasizing the plant's diverse role in traditional medicine systems globally. This broad spectrum of uses highlights the cultural significance and therapeutic versatility of Cyperus rotundus in addressing human health concerns across different regions.**(Bezerra & Pinheiro, 2022)**

I.2.1. Glycyrrhiza glabra (licorice)

lycyrrhiza glabra Linn, commonly known as licorice, is a hardy perennial shrub that can reach a height of approximately 2.5 meters. The plant exhibits compound leaves of the imparipinnate type, arranged alternately along the stem, comprising 4 to 7 pairs of oblong to lanceolate leaflets.

The flowers are typically papilionaceous, borne in axillary spikes, and display a color range from lavender to violet. The calyx is short and campanulate, with lanceolate tips and glandular hairs onits surface.

The fruit is a compressed, erect legume, about 1.5 cm in length, glabrous, and sometimes faintly reticulated. Each pod generally contains 3 to 5 brown, kidney-shaped (reniform) seeds. The primary root is taprooted, measuring approximately 1.5 cm in length, and branches into several secondary roots. From these arise horizontal, woody stolons that may extend up to 8 meters.

When harvested, dried, and cut, both the roots and stolons constitute the source of commercial licorice, which is available in peeled or unpeeled forms. The roots exhibit a fibrous fracture upon breaking, revealing a yellowish interior with a distinctive aroma and a characteristically sweet taste. **(Zadeh , Kor, & Goftar, 2013)**

Figure 03: Licorice plant (left) and its root (right). (Abraham, & Florentine,2021)

I.2.2.Scientific classification of Glycyrrhiza glabra

Table 04: Scientific classification of G. glabra (Sharma & Agrawal ,2013)

Kingdom	**Plantae**
Division	Angiospermae
Class	Dicotyledoneae
Order	Rosales
Family	Leguminosae
Genus	Glycyrrhiza
Species	glabra Linn
Binomial Name	Glycyrrhiza glabra L

I.2.3. Vernacular Nanes (Lohar, Wankhade, Faisal, & Jagtap, 2020).

Sanskrit :Yashtimadhu, Madhuka

Telugu : Atimadhuramu, Madhuka

Tamil : Atimadhuram

Kannada : Yashtimadhuka, atimaddhura

Malayalam : Irattimadhuram

Hindi : Mulethi

English :Liquorice root

Gujarati :Jethimadh

Bengali : Yashtimadhu

I.2.4. Biodiversity of Licorice

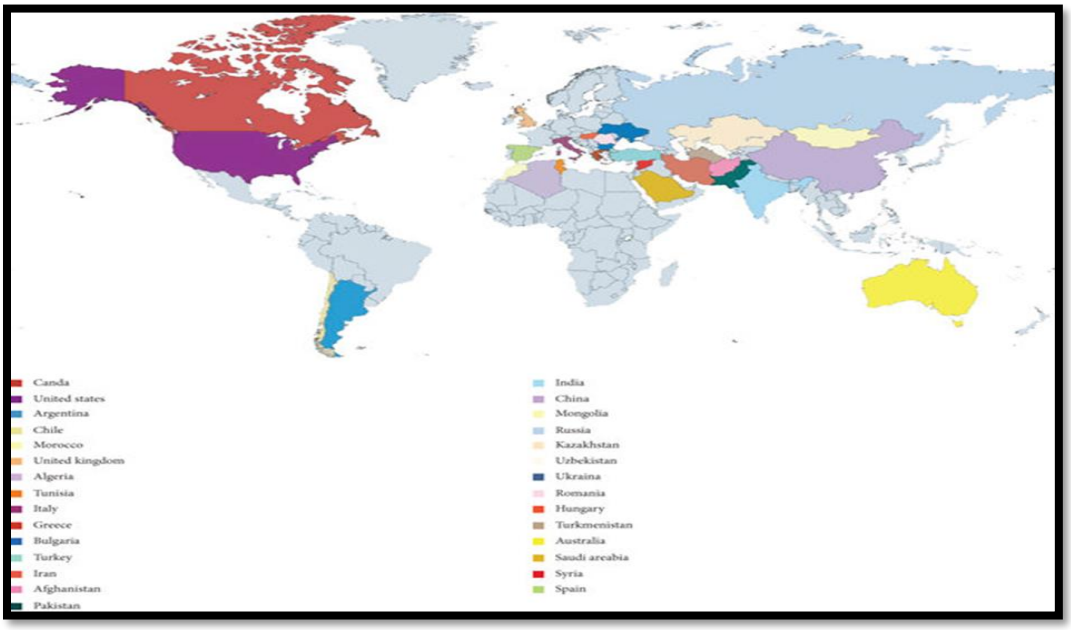

Figure 04: World map showing the major countries producing Glycyrrhiza spp.(Sharifi-Rad *et al*.,2021)

I.2.5. Chemical Constituents of Glycyrrhiza glabra Roots

The roots of Glycyrrhiza glabra are rich in a variety of active compounds, including flavonoids such as liquirtin, rhamnoliquirilin, liquiritigenin, prenyllicoflavone A, glucoliquiritin apioside, and 1-methoxyphaseolin, alongside other constituents like shinpterocarpin, shinflavanone, licopyranocoumarin, glisoflavone, licoarylcoumarin, and coumarin-GU-12. Saponins, particularly glycyrrhizin—a compound 60 times sweeter than sugarcane—are also prominent. Four isoprenoid-substituted phenolic compounds, namely isoangustone A, semilicoisoflavone B, licoriphenone, and 1-methoxyficifolinol, have been identified, along with kanzonol R, a prenylated isoflavan derivative. Volatile components such as pentanol, tetramethyl pyrazine, hexanol, terpinen-4-ol, linalool oxide A and B, geraniol, and α-terpineol have also been reported. Additionally, propionic acid, 1-methyl-2-formylpyrrole, 2,3-butanediol, benzoic

acid, ethyl linoleate, furfuryl formate, trimethylpyrazine, furfuraldehyde, methyl ethyl ketone, and maltol were isolated from the essential oil. Glycyrrhizin, a saponin, and its aglycone glycyrrhetinic acid are key bioactive components in *G. glabra* . Glycyrrhizin consists of glycyrrhetic acid, a triterpenoid aglycone, and a glucuronic acid disaccharide, naturally occurring as calcium and potassium salts in licorice root. In humans, glycyrrhizin is metabolized into glycyrrhetinic acid, resulting in similar pharmacological activities between the two compounds. **(Sharma, Namdeo, & Singh, 2021).**

I.2.6. Pharmacological activity

The pharmacological value of Glycyrrhiza glabra is primarily attributed to extracts or powders derived from its roots and rhizomes. These parts of the plant are rich in bioactive compounds that are therapeutically significant. A schematic overview highlights the key ailments treated and their corresponding active constituents. **(Hasan, Ara, Mondal, & Kabir, 2021)**

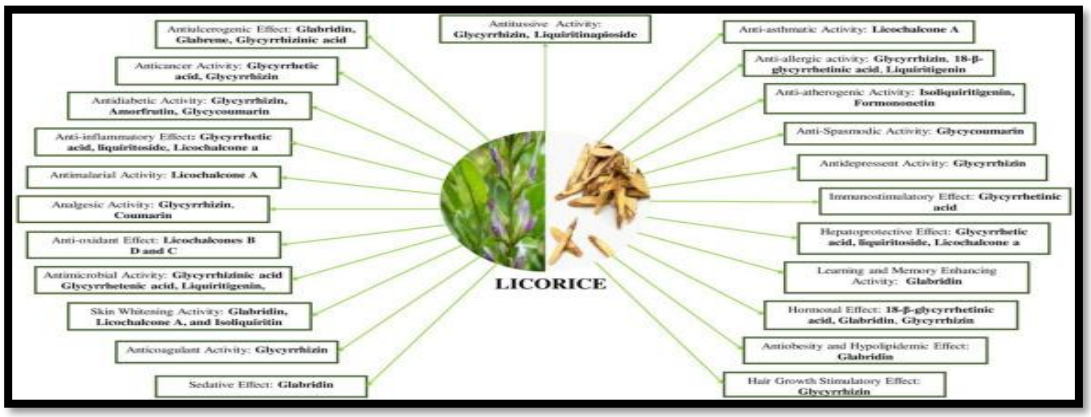

Figure 05: List of Bioactive compounds available in Licorice and their role in alleviating different physiological ailments. (Hasan, Ara, Mondal, & Kabir, 2021)

I.2.7. Activity Biology of Glycyrrhiza glabra

I.2.7.1. Anti-tussive and Expectorant

Licorice, in both powdered and extract forms, has demonstrated effectiveness in alleviating symptoms of sore throat, cough, and bronchial congestion. It possesses antitussive, demulcent, and expectorant properties, which contribute to loosening mucus and facilitating its expulsion from the upper respiratory tract. These effects are largely attributed to the presence of glycyrrhizin, a compound known to stimulate tracheal mucus secretion. Additionally, recent findings have identified liquiritin apioside as an active constituent in licorice extract, capable

of suppressing cough reflexes induced by respiratory tract irritation**. (Sharma, Katiyar, & Agrawal, 2017)**

I.2.7.2. Antioxidant activity

Glycyrrhiza glabra (licorice) possesses significant antioxidant potential, largely attributed to its high content of bioactive flavonoids. These compounds exhibit a robust free-radical scavenging capacity, reportedly surpassing the antioxidant efficiency of vitamin E by more than one hundredfold. Such potency underscores the extract's value in mitigating oxidative stress, making it a promising candidate for incorporation into dermatological and hair care formulations. Its application in cosmetic science is particularly relevant for the prevention of cellular damage induced by environmental factors and reactive oxygen species**. (Lohar, Wankhade, Faisal, & Jagtap, 2020)**

I.2.8. Effect of Licorice on Cancer

Licorice, a natural product rich in flavonoids and triterpenoid saponins, exhibits significant anti-cancer properties. Key bioactive compounds, such as glycyrrhizic acid, modulate critical signaling pathways like AKT/mTOR in breast and endometrial cancer cells, while also inhibiting the proliferation of leukemia cells. Studies demonstrate that licorice extracts and purified compounds suppress tumor growth through multiple mechanisms, including cell cycle arrest, apoptosis, autophagy, and differentiation. These compounds also inhibit angiogenesis (formation of new blood vessels) and metastasis (cancer spread). Notably, combining licorice-derived agents with conventional chemotherapy enhances therapeutic efficacy while reducing side effects. Specific compounds, such as Lic E , glycyrrhizin , and isoliquiritigenin , show potent cytotoxic effects against various cancers, including breast, oral, and prostate cancers. Additionally, isoliquiritigenin acts as a chemopreventive agent by inducing detoxifying enzymes like quinone reductase-1. These findings highlight the potential of licorice-derived compounds as promising anti-cancer agents, warranting further clinical investigation into their therapeutic applications**.(Noreen *et al*., 2021)**

I.3.1. Hibiscus sabdariffa

Hibiscus sabdariffa, a member of the Malvaceae family and the Hibiscus genus, is an annual erect shrub with a reddish or green stem that is typically unbranched or has basal branches. The stem is glabrous or slightly hairy, with small tubercles on its surface. Its serrated leaves vary

in shape, with lower leaves being ovate and undivided, while upper leaves are palmately 3-5 lobed. The plant produces large yellow flowers with a dark crimson center, featuring an epicalyx fused at the base and a dark red to purple calyx. The fruit is a fleshy, ovoid capsule containing numerous seeds, completing its distinctive botanical characteristics. **(Onyeukwu, Dibie, & Njideaka, 2023).**

I.3.1.1 Plant description and ecology

he plant demonstrates remarkable adaptability, thriving in diverse soil conditions, though it prefers nutrient-rich soils for optimal economic yield. It tolerates high temperatures and requires 45-50 cm of rainfall distributed over 90-120 days. Reaching up to 3.5 meters in height, it features deep taproots, smooth green-to-red stems, and alternate leaves that are green with reddish veins, varying from simple to deeply lobed with toothed margins. Solitary flowers appear in leaf axils, displaying yellow or buff colors with a maroon center, turning pink as they wither. The red calyx, composed of 5 sepals surrounded by 8-12 bracts, encloses the velvety capsule fruit, which contains kidney-shaped, light-brown seeds covered in fine stellate hairs. Taking 3-4 months to mature, the plant is suited to tropical climates with 1500-2000 mm of annual rainfall, warm temperatures above 21°C, and 13 hours of sunlight during early growth to avoid premature flowering, while remaining sensitive to frost and fog**.(Shruthi *et al*,.2016).**

Figure 06:Roselle plant . (Sapian *et al.*, 2023).

I.3.2. Classification of hibiscus sabdariffa

Table 05 :**Classification of hibiscus sabdariffa (Edo *et al*,.2023 ; Kéllou *et al*,. 2024)**

Kingdom	**Plantae**

Division	Tracheophyta
Subdivision	Angiospermae
Class	Magnoliopsida
Branch	Spermaphytes
Clade	Dicotyledons
Sub-clade	Dialypetals
Family	Malvaceae
Genus	Hibiscus
Specie	Sabdariffa

I.3.3. Vernacular Names of Hibiscus sabdariffa

Hibiscus sabdariffa, commonly known as Roselle, is recognized by various vernacular names across different regions and cultures. In general English usage, it is referred to as Roselle, Hemp, or Kenaf. In India, names like Mesta, Pusa, Pusa hemp, and Kasi gogu (especially in Andhra Pradesh) are commonly used. The plant is also known as Roselle in Indonesia, while in Thailand, it is referred to as Siam jute or Paw keo. In Madagascar, it is called Voam bombazaha, whereas in Brazil, the names Azedinha, Curura-de-guiné, and Quiabo-roséo are used. In West Africa, particularly among French-speaking regions, it is known as Oiselle-de-Guinée. These diverse names reflect the plant's wide distribution and its cultural and economic significance in different parts of the world. **(Islam, 2019).**

I.3.4. Bioactive Compounds of Roselle Bioactive Compounds of Roselle

The roselle plant (Roselle) is a rich source of bioactive compounds, primarily concentrated in its calyx. Studies indicate that the main chemical components of this plant include phenolic acids, flavonoids, anthocyanins, and organic acids, which contribute to numerous biological properties associated with it. Anthocyanins are the key compounds responsible for the vibrant color of the calyx, with major components including delphinidin-3-sambubioside and cyanidin-3-sambubioside, along with smaller amounts of delphinidin-3-glucoside and cyanidin-3-glucoside. A variety of flavonoids have also been identified in the calyx and leaves, such as quercetin-3-glucoside, rutin, kaempferol, and methyl epigallocatechin, in addition to other compounds like sabdaritrin, hibiscitrin, and luteolin. Furthermore, important phenolic acids

such as protocatechuic acid and chlorogenic acid are present in higher concentrations compared to other phenolic compounds, while organic acids like caffeic acid and gallic acid are also found in the plant. The distribution of phenolic compounds varies among different parts of the plant, with the calyx being the richest in these compounds, followed by the leaves and then the stems. These compounds collectively provide multiple biological effects, including antioxidant, anti-inflammatory, and other health-promoting properties, highlighting the significant nutritional and therapeutic value of roselle**.(Sapianetal.,2023).**

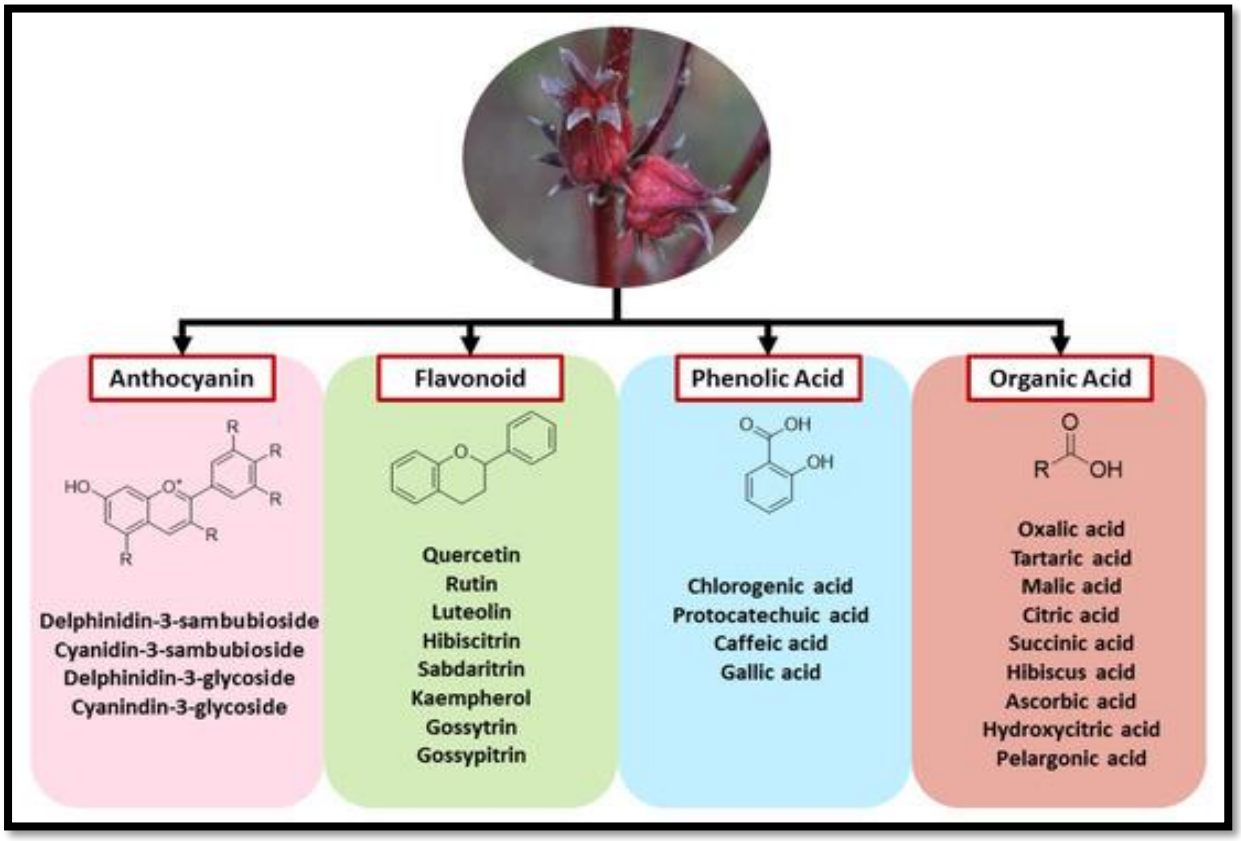

Figure 07: The bioactive compounds found in roselle.(Sapian *et al.*, 2023).

I.3.5. Traditional therapeutic usage

Hibiscus sabdariffa is a medicinal plant with diverse applications in traditional medicine. Various parts of the plant, including the calyx, leaves, and dried flowers, are utilized to address numerous health conditions. The calyx is employed to treat urinary system disorders, such as kidney and bladder stones, and to alleviate pain during urination. It is also used to improve digestion, reduce bloating, and manage diarrhea; its extract mixed with salt is effective in

treating diarrhea and dysentery in both humans and animals. In Mexico, infusions of the calyx and leaves are used to control high blood pressure and reduce cholesterol levels. Postpartum, it is utilized to relieve back pain and gynecological disorders. Additionally, "Sudan Tea," prepared from the calyx, is consumed to reduce fever and treat coughs and biliousness. The plant also exhibits antispasmodic and antimicrobial properties, making it effective in managing various health conditions. **(Riaz,& Chopra, 2018)**

I.3.6. Phytochemical compounds of H. sabdariffa Linn

I.3.6.1. Anti-hyperlipidemia properties

The main compounds of hibiscus, such as anthocyanins, flavonoids, and polyphenolic acids, exhibit anti-hyperlipidemic properties by acting as resistance agents that restrict the onset of oxidized low-density lipoprotein (OxLDL). Fernández et al. demonstrated that hibiscus extract significantly reduced serum triglyceride levels by over 50% in hyperlipidemic animal models under a hypercaloric diet. Similarly, Hirunpanich et al. reported that hibiscus lowered not only serum cholesterol but also triglycerides and LDL levels in hypercholesterolemic animal models. Chen et al. further highlighted the inhibitory effects of hibiscus on OxLDL through an in vitro study, showing that hibiscus flavonoids reduced foam cell formation and intracellular lipid accumulation in macrophages exposed to OxLDL at non-cytotoxic concentrations. Molecular data suggest that hibiscus prevents atherosclerosis by upregulating the liver-X receptor alpha (LXRα)/ATP-binding cassette transporter A1 (ABCA1) pathway, which enhances cholesterol removal from macrophages. By promoting cholesterol efflux, hibiscus prevents the transformation of macrophages into foam cells, a critical step in atherosclerotic lesion formation, thereby delaying the progression of atherosclerosis. The presence of polyphenols, particularly flavonoids, makes hibiscus a promising candidate for combating hyperlipidemia and preventing atherosclerosis. **(Putra, Paramita, & Santoso,2025).**

I.3.6.2. Anti microbial activity

Many naturally occurring compounds derived from medicinal plants, herbs, and spices have demonstrated significant antimicrobial activity against a wide range of pathogens. These bioactive compounds offer a promising alternative to the extensive use of synthetic antibiotics, which are often associated with adverse effects and microbial resistance. The increasing global demand for plant-based therapeutics, particularly in both developing and developed countries is driven by the recognition of their natural origin, non-narcotic properties, and

biodegradability , which collectively minimize environmental hazards. Furthermore, these natural products are associated with fewer adverse side effects compared to synthetic pharmaceuticals, making them an attractive option for combating microbial infections while promoting environmental and human health. **(Bedi, Bekele, & Gure, 2020).**

I.3.6.3. Anticancer activity

Anthocyanins (HAs) and protocatechuic acid (PCA) from Hibiscus sabdariffa exhibit significant anticancer properties. HAs induce apoptosis in gastric cancer and leukemia cells, suppressing NMU-induced leukemia progression by 33.3% in rats and activating pathways like p38-FasL and ROS-mediated mitochondrial dysfunction. PCA protects against cytotoxicity, inhibits skin tumor formation in mice, and suppresses anti-apoptotic proteins like Bcl-2. Hibiscus leaf extracts, rich in polyphenols, show potent anticancer effects on prostate cancer cells via intrinsic and extrinsic apoptotic pathways and inhibit tumor growth in mice. Ethanolic extracts also demonstrated moderate antiproliferative activity in breast cancer cells (MCF-7) due to high levels of phytoestrogens like quercetin. Additionally, HLP extracts rich in epicatechin gallate (ECG) exhibited anticancer effects against melanoma cells by regulating caspase cleavage and Fas/FasL activation. These findings highlight H. sabdariffa as a promising natural source for anticancer therapies. **(Hassan, Berchová, & Šudomová, 2016).**

I.3.6.4. Antipyretic, antinociceptic and anti-inflammatory activities

Despite its traditional use in relieving fever, limited studies have investigated the antipyretic effects of Hibiscus sabdariffa (Hs). In vivo studies on the crude extract of Hs revealed significant antipyretic and anti-inflammatory potential. Both ethanol (more potent) and aqueous extracts demonstrated antipyretic effects by reversing yeast-induced fever in rats, with a mechanism distinct from aspirin, as it involves the inhibition of cytokines such as interleukins (IL), interferons, and tumor necrosis factor-alpha (TNF-α), contributing to its anti-inflammatory properties. Earlier studies suggest that flavonoids, polysaccharides, and organic acids may be responsible for these pharmacological activities, while a more recent study confirmed the antinociceptive effect of the ethanolic extract from the calyces in a rat model. Additionally, two fractions of the crude aqueous-ethanolic extract of dried Hs exhibited significant immunostimulatory activity by increasing interleukin-10 (IL-10) production and reducing TNF-α levels. The polyphenol-rich extract also exerts its anti-inflammatory effects by impairing cyclooxygenase-2 (COX-2) induction through the down-regulation of JNK and

p38 MAPK pathways. These findings highlight the diverse therapeutic potential of Hs in managing fever, pain, and inflammation**. (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich,2014).**

CHAPTERII:

Tyrosinase and Whitening Activity

In this chapter, we will study the enzyme tyrosinase in terms of its structure and function, as well as its central role in melanin synthesis. Furthermore, the enzyme's direct involvement in skin whitening will be discussed, highlighting its status as a key target for cosmetic agents aimed at reducing pigmentation and lightening skin tone.

II.1. La tyrosinase

Tyrosinase is an enzyme belonging to the oxidase family, playing a vital role in catalyzing a series of biochemical reactions that lead to the production of melanin, the pigment responsible for skin, hair, and eye color. This enzyme facilitates the oxidation of the amino acid tyrosine into dihydroxyphenylalanine (DOPA), which is subsequently converted into DOPA-quinone and ultimately into melanin. Structurally, tyrosinase contains copper as an essential cofactor, which is critical for its unique ability to perform oxidative processes. The absence of tyrosinase in humans results in a genetic condition known as albinism, characterized by the inability to produce melanin, leading to the loss of natural pigmentation in the skin, hair, and eyes. Tyrosinase thus serves as a central enzyme in biological processes associated with pigmentation. **(Collins, 2021).**

II.2. Melanin

is a natural pigment produced through the oxidation and polymerization of the amino acid tyrosine in animals or phenolic compounds in lower organisms. Melanin is characterized by its diverse structure and sources, and it exists primarily in three forms: eumelanin (a black-brown pigment), pheomelanin (a yellow to reddish-brown pigment), and neuromelanin (a dark pigment found in neurons). Eumelanin is the most common form and is generally associated with the general meaning of the term "melanin." It is produced through the oxidation and polymerization of the amino acid L-dopa chemical intermediate called 5,6-dihydroxyindole **.(Mostert, 2021).**

II.3. Mechanism of Melanin Synthesis

Melanin synthesis occurs in specialized organelles called melanosomes, which are formed near the nucleus of melanocytes. The process begins with the structural organization of melanosomes by the protein Pmel17, which creates a fibrillar matrix for melanin deposition. The enzyme tyrosinase is then recruited to the melanosomes, where it catalyzes the conversion of L-tyrosine to L-DOPA, the key step in melanin production. This enzyme's activity is regulated by a transporter protein known as MATP.

Once melanin is synthesized, melanosomes are transported toward the dendrites of melanocytes along microtubules, facilitated by proteins such as kinesin and dynein. At the tips of the dendrites, melanosomes bind to actin filaments through a complex involving Myosin Va, Rab27a, and Melanophilin.Melanosome maturation also requires an increase in internal pH from 5 to 6.8, controlled by a proton pump located in the melanosome membrane.

Mature melanosomes are transferred to keratinocytes, where melanin is positioned above the nucleus to shield DNA from UV damage. Transfer mechanisms include exocytosis, cytophagocytosis, or vesicle-mediated processes. This coordinated process ensures proper pigment distribution in the skin. **(Zhou, *et al.*, 2021).**

II.4. The overall structure of tyrosinase

consists of three main domains: the central domain, the N-terminal domain, and the C-terminal domain. The central domain is the most conserved among different tyrosinase types, containing six conserved histidine residues that play a critical role in binding the copper ions CuA and CuB. The CuB site exhibits a higher degree of conservation compared to CuA. The N-terminal domain acts as a transit peptide that determines the final localization of the enzyme within the cell; it directs the enzyme to chloroplasts in plants or is involved in melanosome transport in humans and mice, whereas it is absent in fungi, whose enzymes are cytoplasmic. Finally, the C-terminal domain regulates enzyme activity by acting as a barrier that blocks substrate access to the active site in the latent pro-tyrosinase form, and the enzyme is activated through the removal or modification of this domain. **(Kanteev, Goldfeder , & Fishman, 2015).**

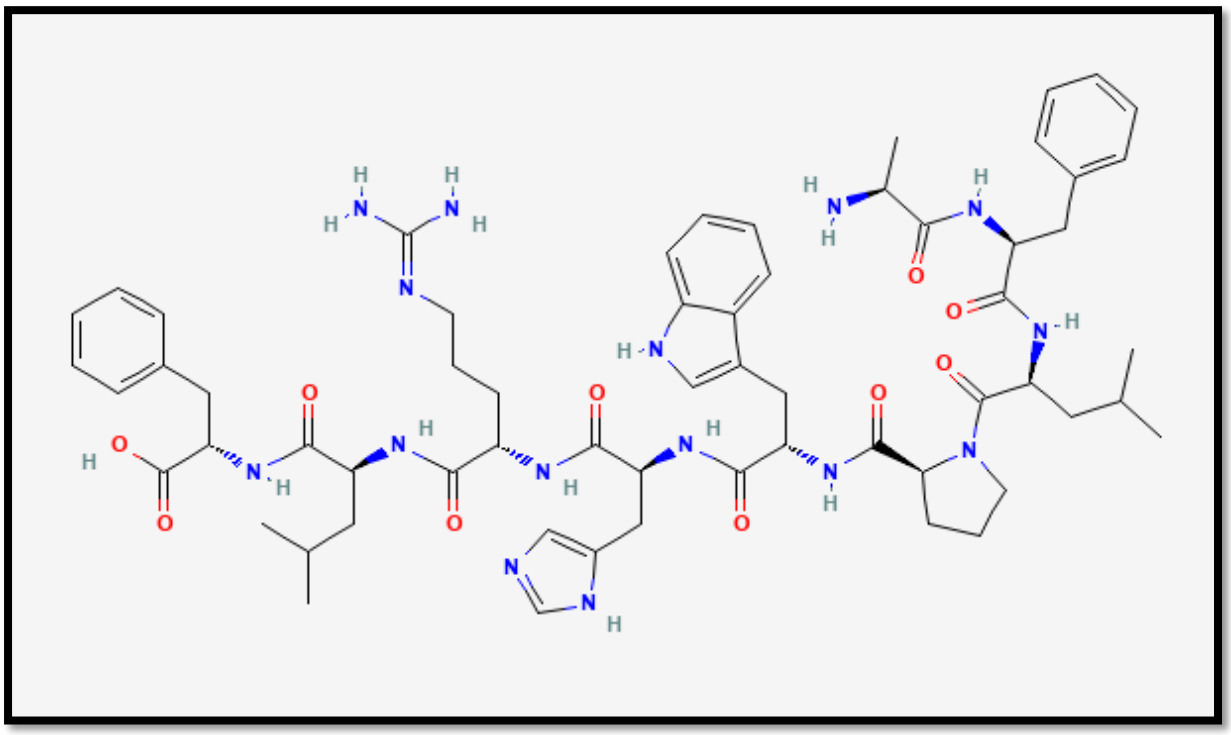

Figure 08: The chemical structure depiction of tyrosinase (2D).(Kanteev, Goldfeder , & Fishman, 2015).

II.5. The mechanism of melanin Melanogenesis

Melanin synthesis is a complex process that primarily relies on the enzyme tyrosinase, which plays a pivotal role in initiating the biochemical cascade for melanin production. Tyrosinase is synthesized within melanocytes in the Golgi apparatus and endoplasmic reticulum and is subsequently transported to melanosomes, where melanin synthesis occurs. Tyrosinase exhibits two major activities: monophenolase activity, which converts L-tyrosine to L-DOPA, and diphenolase activity, which oxidizes L-DOPA to dopaquinone. The conversion of L-tyrosine to dopaquinone represents the rate-limiting step in this process, as subsequent steps proceed spontaneously following this stage. Thereafter, the pathway branches into the production of two types of melanin: eumelanin (brown-black), which is synthesized via the involvement of enzymes such as TRP-2 and TRP-1, and pheomelanin (red-yellow), which forms in the presence of sulfur-containing compounds like cysteine or glutathione. Tyrosinase is characterized by three active states (Oxy, Met, and Deoxy), which regulate its activity. The lag time associated with its monophenolase activity can be reduced using reducing agents such as L-DOPA. In addition to its role in melanin synthesis, tyrosinase also contributes to the production of neuromelanin in brain cells, making it highly significant in the study of neurodegenerative diseases such as Huntington's and Parkinson's diseases. Finally, melanin

synthesis can be regulated by inhibiting tyrosinase activity using compounds such as hydroquinone or kojic acid, offering promising strategies for addressing hyperpigmentation disorders.**(El-Sayed *et al* .,2021).**

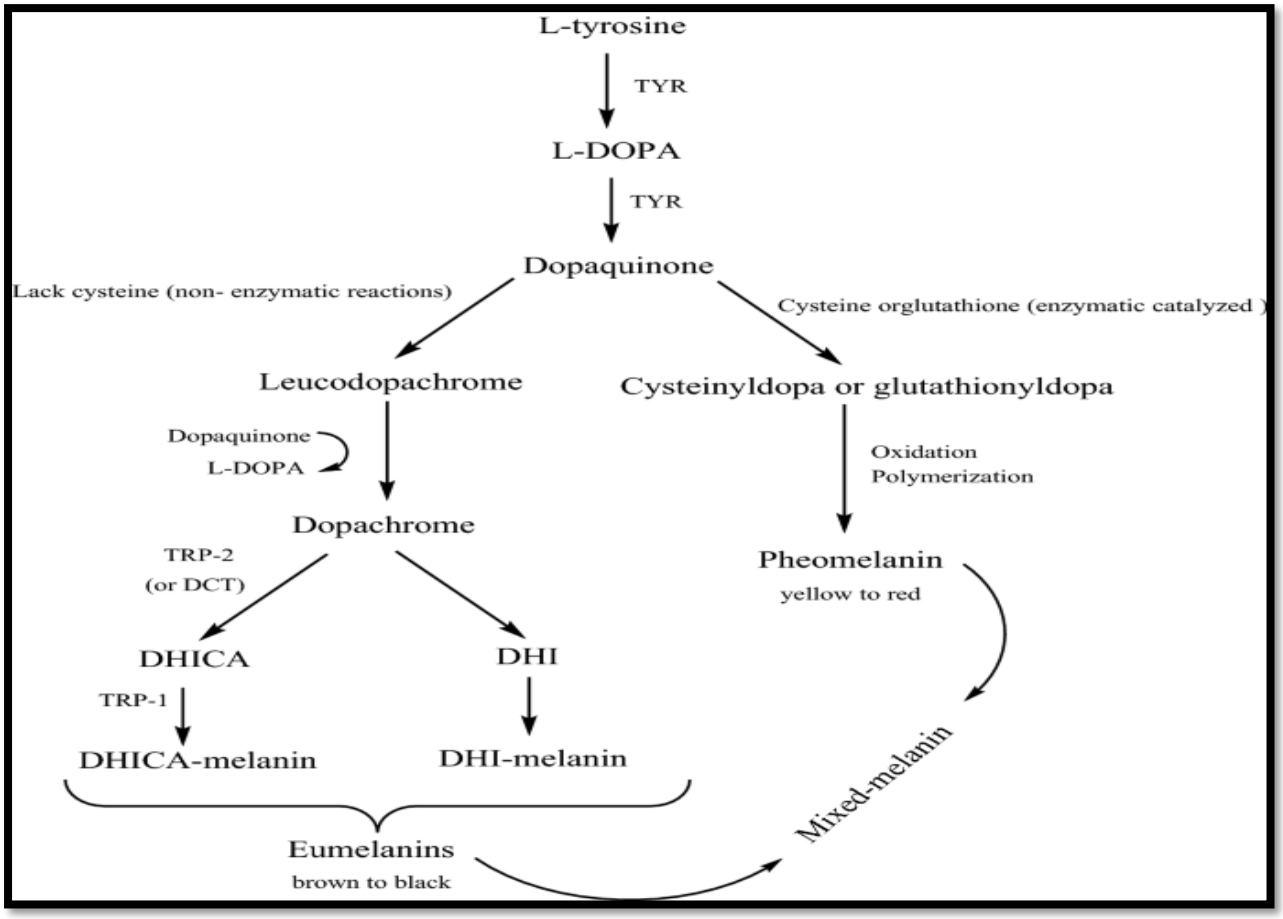

Figure 09: Biosynthes is of melaninin human (Vaezi, 2022).

II.6. Tyrosinase Inhibitor Types Based on Action Mechanism

II.6.1. Classical Tyrosinase Inhibitor Types

Tyrosinase inhibitors are conceptually classified into two types—inactivators and reversible inhibitors—based on the molecular recognition properties of the enzyme, particularly in the context of anti-melanoma agents and cosmetics. Tyrosinase inactivators, also known as suicide inactivators, work by causing tertiary structural and conformational changes in the enzyme. These changes result in shifts in the enzyme's confirmation, ultimately leading to its inactivation.**(Garcia-Jimenez *et al.*,2017).**

II.6.2. Tyrosinase Inhibitor Types Based on Action Mechanism

Tyrosinase enzyme inhibitors are typically categorized into four types based on their mechanisms of inhibitory action. These include competitive inhibitors, uncompetitive inhibitors, mixed-type inhibitors (which exhibit both competitive and uncompetitive characteristics), and noncompetitive inhibitors**. (Kim*, et al*.,2023).**

II.6.3. Competitive Inhibitory Type

Competitive inhibitors function by binding to the active site of tyrosinase, thereby preventing natural substrates from accessing it. Common examples of such inhibitors include hydroquinones like α-/β-arbutins, phenolic compounds such as polyphenols, flavonoids (e.g., flavones, isoflavones), and tannins. These compounds are characterized by aromatic rings bearing hydroxyl (-OH) groups. Additionally, glycoside derivatives, such as glucosides, demonstrate strong inhibitory activity against tyrosinase. For instance, glucose, xylose, and maltose derivatives are among the most potent competitive inhibitors due to their unique structures, which often combine sugars with active components like resorcinol**. (Avonto, Wang, Avula, Wang, Rua, & Khan, 2016).**

II.7. The Role of Melanin and Tyrosinase in Skin Protection Against Skin Cancer

Dark skin is highly resistant to skin cancer compared to light skin, primarily due to the presence of melanin, which reduces the risk of cancer by up to 500-1000 times. Skin cancer typically occurs in sun-exposed areas among individuals with light skin, while it tends to appear in sun-protected areas in those with dark skin. However, genetic factors such as mutations in the MC1R gene can increase the risk of skin cancer regardless of skin color. Additionally, the protein p53 plays a crucial role in enhancing pigmentation as a defense mechanism against ultraviolet radiation, whereas its absence may inhibit the tanning response. Moreover, UVA radiation is believed to elevate the risk of melanoma due to its oxidative effects on DNA. Thus, while melanin provides significant protection, genetic and environmental factors remain critical determinants of skin cancer susceptibility**.(Brenner, & Hearing, 2008).**

II.8. Antioxidant Mechanisms of Tyrosinase Inhibitory Peptides (TIPs)

Tyrosinase Inhibitory Peptides (TIPs) function through antioxidant mechanisms that help reduce oxidative stress and regulate melanin production. This mechanism relies on the ability of TIPs to inhibit the activity of the tyrosinase enzyme, which plays a key role in catalyzing melanin production, by interacting with free radicals and copper ions. Some peptides possess

copper-chelating properties, preventing copper from participating in oxidative processes and reducing its harmful effects. Although copper is an essential element in the body, it can contribute to oxidative reactions under certain conditions, making its chelation by TIPs crucial for enhancing their antioxidant effectiveness.

Additionally, many TIPs exhibit multifunctional effects, such as moisturizing, improving skin elasticity, and combating aging, which are often linked to their antioxidant properties. For instance, crude extracts contain a mixture of compounds that may have synergistic effects, making them more effective in reducing oxidative damage compared to pure compounds. This synergistic effect enhances both the antioxidant and tyrosinase-inhibiting properties of TIPs, contributing to overall skin health improvement.

Furthermore, most tyrosinase inhibitors work through powerful antioxidant mechanisms, helping to mitigate the harmful effects of free radicals that cause premature aging and hyperpigmentation. However, continuous exposure of the skin to light triggers a complex set of biochemical reactions that go beyond simple sun tanning, increasing the importance of using TIPs to counteract these effects. **(Song, Chen, Li, Zeng, & Hu, 2022).**

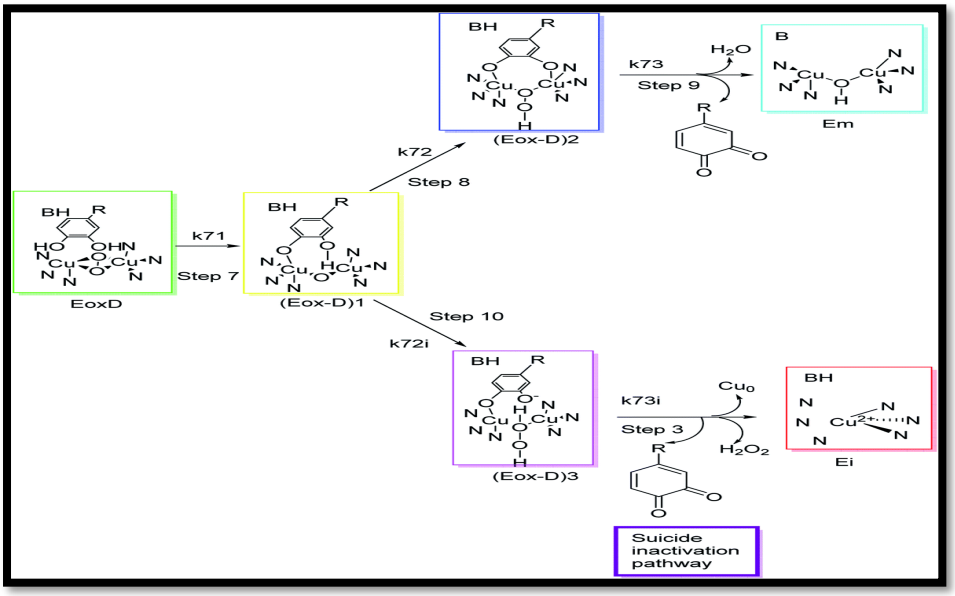

Figure10: Molecular mechanism of tyrosinase suicide deactivation by oxidation of an o-diphenol substrate. The curly arrows showed that deprotonation results in the reduction of copper from bivalent to zero-valent form, and the elimination of an o-quinone and tyrosinase inactivationNaturally occurring tyrosinase inhibitors (Obaid, *et al.*, 2021).

II.9. Natural and synthetic sources of inhibitors

Below, we present a general classification of the main inhibitors targeting tyrosinase, which include various types such as flavonoids, azolides, kojic acid, phenols, and others :

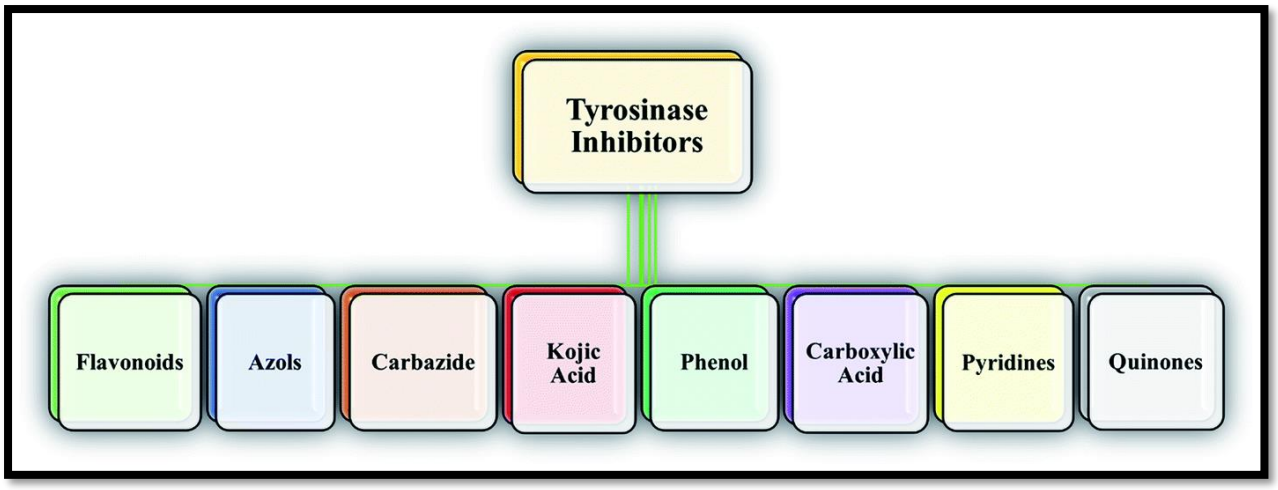

Figure11: Representative anti-tyrosinase scaffolds.(Obaid *et at.*,2021).

II.9.1. Flavonoids

Flavonoids are polyphenolic secondary metabolites widely distributed in plants, with more than 4000 identified compounds found in various plant parts such as leaves, seeds, bark, and flowers. These compounds play a protective role against ultraviolet radiation, pathogens, and herbivores, and are recognized as important natural inhibitors of tyrosinase. They are classified into six major groups: flavanols, flavones, flavonols, flavanones, isoflavones, and anthocyanidins, each exhibiting structural variations that influence their biochemical functions. Certain flavonoids, including kaempferol, quercetin, and morin, demonstrate strong tyrosinase inhibitory activity, whereas others such as catechin and rhamnetin may act as substrates or cofactors. Notably, steppogenin, isolated from Cudrania tricuspidata , exhibited potent tyrosinase inhibition, showing an activity level ten times higher than that of kojic acid**.(Lee , Baek , & Nam , 2016).**

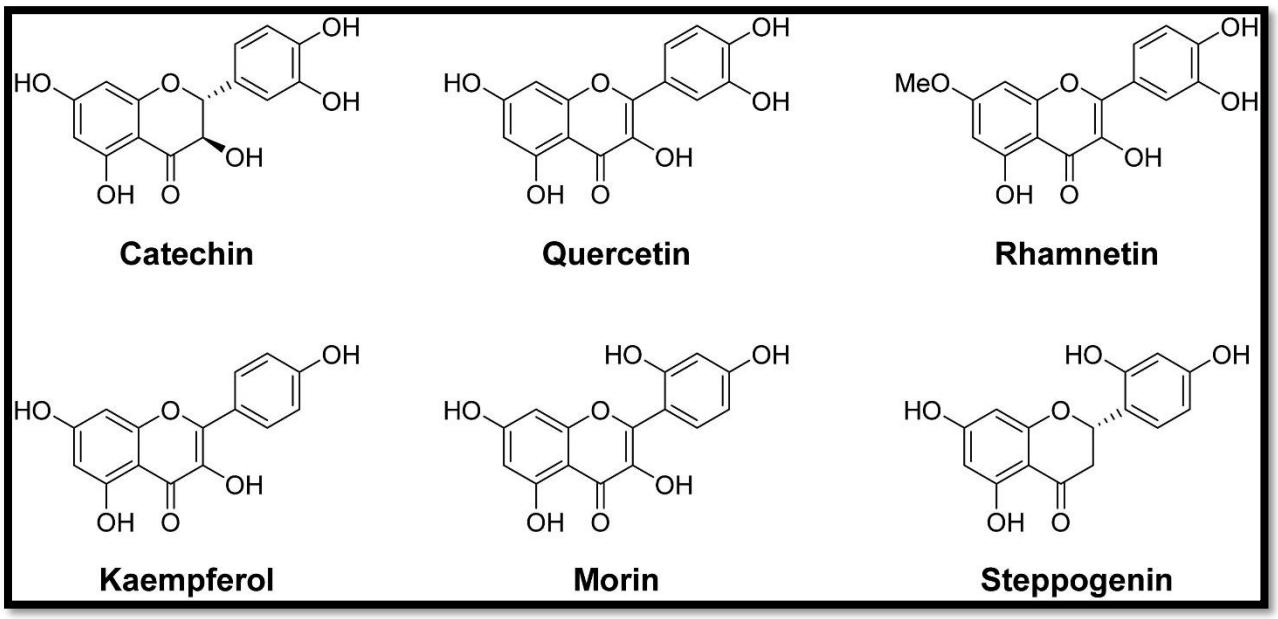

Figure12:**Representative anti-tyrosinase flavonoids** (**Lee , Baek , & Nam , 2016).**

II.9.2. Kojic acid

Tyrosinase inhibition is considered one of the primary targets in the development of skin-whitening agents or substances used to reduce skin pigmentation. Tyrosinase inhibition assays were performed in 19 out of 25 studies, utilizing different sources of the enzyme, with mushroom-derived tyrosinase being the most commonly employed, alongside human tyrosinase. Derivatives of kojic acid demonstrated greater efficacy in inhibiting tyrosinase compared to the parent molecule; however, results varied depending on the enzyme source. For instance, the IC50 values of kojic acid ranged from less than 20 μM to over 100 μM, depending on the enzyme type, while it reached 500 μM for human tyrosinase, suggesting that kojic acid may be less effective against human tyrosinase compared to other sources. The primary mechanism of tyrosinase inhibition involves the chelation of copper ions at the enzyme's active site, as well as direct binding to this site. Additionally, chemical modifications, such as the addition of nitro (NO2) or hydroxymethyl groups, can enhance inhibition by improving binding affinity or altering the microenvironment around the enzyme**. (Zilles, Dos Santos, Kulkamp-Guerreiro, & Contri, 2022).**

Conclusion

In this study, the following plants were selected: Cyperus rotundus Linn, Hibiscus sabdariffa, and Glycyrrhiza glabra, with the aim of evaluating their biological properties particularly antimicrobial, antioxidant, anti-inflammatory, and tyrosinase inhibitory activities to assess their potential use in cosmetic and pharmaceutical formulations.
The results indicated promising biological efficacy , showing that ***Glycyrrhiza glabra*** was the most effective plant in the anti-tyrosinase, anti-inflammatory, and antibacterial tests, while ***Cyperus rotundus*** Linn exhibited the strongest antioxidant activity. Moreover, ***Glycyrrhiza glabra*** was the only plant that demonstrated effectiveness across all tested categories, making it a strong candidate for further development. According to the study findings, all the tested plants showed notable efficacy, suggesting their potential application in treating hyperpigmentation and promoting skin whitening.

However, certain complementary biological tests, such as *in vivo* and *in silico* studies, could not be conducted at this stage, although they are essential for further validation and support of the obtained results.

Therefore, it is recommended that future research expand the investigation by evaluating the efficacy of these plant extracts in treating specific skin conditions such as acne or dandruff-like symptoms, as well as conducting antifungal activity assays. These additional studies would enrich the findings and confirm the practical applicability of these natural resources in the cosmeceutical and pharmaceutical industries.

References

References

- **Abdelgaleil, S. A., El-Awady, A. M., & Nakatani, M. (2021). Antibacterial activity of Hibiscus sabdariffa polyphenols against foodborne pathogens. Food Control, 130, 108299.**
- **Abraham, J., & Florentine, S. (2021). Licorice (Glycyrrhiza glabra) extracts-suitable pharmacological interventions for COVID-19? A review. Plants, 10(12), 2600. Chen, Y., Liu, X., Zhang, W., & Wang, Q. (2021). Purification and kinetic**
- **Al-Abdullah, S., Al-Othman, Z., & Yilmaz, E. (2023). Extraction and biochemical**
- **Ali, A., Ashraf, Z., Rafiq, M., Kumar, A., Jabeen, F., Lee, G. J., ... & Choi, E. H. (2019). Novel amide derivatives as potent tyrosinase inhibitors; in-vitro, in-vivo antimelanogenic activity and computational studies. Medicinal Chemistry, 15(7), 715-728.**
- **Ali, M., Khan, M. S., & Rahman, M. A. (2022). Synergistic effects of anthocyanins and polyphenols in tyrosinase inhibition. Phytomedicine, 99, 153971.**
- **Ali, M., Khan, M. S., & Rahman, M. A. (2023). Polyphenols and antioxidant activity in Hibiscus sabdariffa and Glycyrrhiza glabra. Journal of Functional Foods, 99, 105348.**
- **Al-Massri, A. H., Abu-Lafi, S., & Qabajah, M. (2021). In silico identification of natural products as potential COX-2 inhibitors for anti-inflammatory therapy. Journal of Biomolecular Structure and Dynamics, 39 (12), 4321–4332.**
- **Al-Massri, A. Y., Al-Dabbagh, B., & Ahmad, H. (2021). Comparative analysis of tyrosinase from different sources: Structural and spectral studies. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 258, 119851.**
- **Al-Massri, A., Qaqish, B., & Korfali, E. (2021). Molecular docking and ADMET-based screening of natural compounds as potential COX-2 inhibitors . Journal of Molecular Modeling, 27(6), 145.**
  application in inhibitor screening . International Journal of Biological
- **Aslani, M. R., Dadras, H., & Ranjbar, V. R. (2020). Anti-inflammatory effects of glabridin and hispaglabridin A from Glycyrrhiza glabra. Planta Medica, 86(3), 226–232.**

- **Avonto, C., Wang, Y. H., Avula, B., Wang, M., Rua, D., & Khan, I. A. (2016). Comparative studies on the chemical and enzymatic stability of alpha-and beta-arbutin. International Journal of Cosmetic Science, 38(2), 187-193.**
- **Babiaka, S. B., Moumbock, A. F., Günther, S., & Ntie-Kang, F. (2021). Natural products in Cyperus rotundus L.(Cyperaceae): an update of the chemistry and pharmacological activities. RSC Advances, 11(25), 15060-15077.**
- **Bedi, P. S., Bekele, M., & Gure, G. (2020). Phyto-chemistry and pharmacological activities of Hibiscus sabdariffa Linn.—A review. Int. J. Pure Appl. Chem, 21, 41-54.**
- **Bezerra, J. J. L., & Pinheiro, A. A. V. (2022). Traditional uses, phytochemistry, and anticancer potential of Cyperus rotundus L.(Cyperaceae): A systematic review. South African Journal of Botany, 144, 175-186.**
- **Bickford, L. A., & Salem, H. (2019). Screening Smokes: Applications, Toxicology, Clinical Considerations, and Medical Management. In *Chemical Warfare Agents* (pp. 277-300). CRC Press.**
- **Boligon, A. A., Machado, M. M., & Athayde, M. L. (2014). Technical evaluation of antioxidant activity. Med. Chem, 4(7), 517-522.**
- **Brazil Flora G (2014): Brazilian Flora 2020 project - Projeto Flora do Brasil 2020. v393.147. Instituto de Pesquisas Jardim Botanico do Rio de Janeiro. Dataset/Checklist.**
- **Brenner, M., & Hearing, V. J. (2008). The protective role of melanin against UV damage in human skin. Photochemistry and photobiology, 84(3), 539-549.**
- **characterization of tyrosinase from eggplant (Solanum melongena L.) and its characterization of tyrosinase from Prunus domestica and its application in natural**
- **Chen, J., Lin, C., & Yang, Y. (2015). Spectral characteristics of tyrosinase extracted from eggplant. Biochemistry Research International, 2015, 605935.**
- **Chen, L., Wang, Y., & Yu, J. (2022). Polyphenols in Glycyrrhiza glabra and their pharmacological effects. Molecules, 27(3), 745.**
- **Chen, X., Zhang, L., & Zhang, Q. (2020). Phytochemicals and biological activity of medicinal plants with anti-inflammatory and immunomodulatory properties. Frontiers in Pharmacology, 11, 123.**
- **Chen, Y., Li, H., Zhang, Y., & Wang, X. (2019). In silico and in vitro evaluation of flavonoids from Glycyrrhiza glabra as COX-2 inhibitors . International Journal of Molecular Sciences, 20(15), 3756.**

- **Chen, Y., Zhang, J., & Li, Z. (2019). Antimicrobial activities of flavonoids and glycyrrhizin from Glycyrrhiza glabra. Journal of Applied Microbiology, 126(1), 135–144.**
- **Chithran, A., Ramesh Babu, T., & Himaja, N. (2012). Comparative study on anti-inflammatory activity of Cyperus rotundus (L.) using different solvent system in carragenan induced paw edema in albino wistar rats. Int J Phytopharmacol, 3, 130-134.**
- **Collins, J. F. (2021). Copper nutrition and biochemistry and human (patho) physiology. In Advances in food and nutrition research (Vol. 96, pp. 311-364). Academic Press.**
- **Corradi, I. D. F., de Souza, E., Sande, D., & Takahashi, J. A. (2018). Correlation between phenolic compounds contents, anti-tyrosinase and antioxidant activities of plant extracts.**
- **Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L.–A phytochemical and pharmacological review. Food chemistry, 165, 424-443**.
- **Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7 (1), 42717.**
- **Dara, E. M., Zarei, A., & Saha, S. K. (2023). In silico approaches for predicting drug toxicity: Advances and challenges . Toxicology and Applied Pharmacology , 461, 116345.**
- **Djaber, M., Adaika, A., & Boulekbache-Makhlouf, L. (2021). Extraction, caractérisation et évaluation in silico et in vitro de l'activité anti-inflammatoire de certains extraits de plantes médicinales du Sahara algérien (Master's thesis, Université Echahid Hamma Lakhdar – El Oued, Algérie)**
- **Duarte, M. C. T., Figueira, G. M., Sartoratto, A., Rehder, V. L. G., & Delarmelina, C. (2005). Anti-Candida activity of Brazilian medicinal plants. Journal of ethnopharmacology, 97(2), 305-311.**
- **Echresh, S., Behbahani, B. A., Falah, F., Noshad, M., & Ibrahim, S. A. (2024). Assessment of the probiotic, anti-bacterial, and anti-biofilm characteristics of Lacticaseibacillus rhamnosus CWKu-12, along with its potential impact on the**

expression of virulence genes in Listeria monocytogenes ATCC 19115. Lwt, 203, 116391.

- **Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2015). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7), 685–688.**
- **Edo, G. I., Samuel, P. O., Jikah, A. N., Oloni, G. O., Ifejika, M. N., Oghenegueke, O. & Essaghah, A. E. A. (2023). Proximate composition and health benefit of Roselle leaf (Hibiscus sabdariffa). Insight on food and health benefits. Food Chemistry Advances, 3, 100437.**
- **Egan, W. J., Merz, K. M., & Baldwin, J. J. (2000). Prediction of drug absorption using multivariate statistics . Journal of Medicinal Chemistry, 43(24), 3867–3877.**
- **El-Gendy, A. E., Mohamed, H. M., & Saeed, S. A. (2022). Impact of anthocyanins on flavonoid assays in Hibiscus sabdariffa. Analytical Biochemistry, 645, 114638.**
- **El-Sayed, A. M., Abd-ElGawad, A. M., & Al-Rowaily, S. L. (2021). Phenolic content and antioxidant activity of Cyperus rotundus L. under abiotic stress. Environmental Science and Pollution Research, 28(24), 30497–30509.**
- **El-Sayed, A. S., Maamoun, H. S., Rabie, G. H., Shaker, I., Alaidaroos, B. A., & Ali, M. G. (2021). Microbial tyrosinase: Biochemical, molecular properties and pharmaceutical applications. Biomedical and Pharmacology Journal, 14(3), 1281-1295.**
- **Evans, W. C. (2016). Trease and Evans' pharmacognosy. Elsevier Health Sciences.**
- **Farag, M. A., El-Kersh, D. M., & El-Gendy, A. O. (2022). Plant-derived antioxidants and their free radical scavenging activity. Antioxidants, 11(1), 45.**
- **Farombi, E. O., & Fakoya, A. (2021). Anti-inflammatory and antioxidant properties of Hibiscus sabdariffa anthocyanins. Journal of Inflammation Research, 14, 453–465.**
- **Fetni, S., & Bertella, N. (2020). Etude in vitro des propriétés anti-inflammatoires de l'extrait méthanolique des fruits de Rosa canina L.(Rosacées). Nutrition & Santé, 9(2), 117-125.**
- **Garcia-Jimenez, A., Teruel-Puche, J. A., Garcia-Ruiz, P. A., Saura-Sanmartin, A., Berna, J., Garcia-Canovas, F., & Rodriguez-Lopez, J. N. (2017). Structural and kinetic considerations on the catalysis of deoxyarbutin by tyrosinase. Plos one, 12(11), e0187845.**

- **Gupta, R., Sharma, A., & Singh, S. (2021). Tyrosinase inhibitory activity of selected plant-derived flavonoids: In silico and in vitro analysis . Biochemistry and Biophysics Reports, 26, 101012.**
- **Haida, Z., & Hakiman, M. (2019). A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food science & nutrition, 7(5), 1555-1563.**
- **Harborne, J. B. (2015). Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall.**
- **Hasan, M. K., Ara, I., Mondal, M. S. A., & Kabir, Y. (2021). Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra. Heliyon, 7(6).**
- **Hassan, S. T., Berchová, K., & Šudomová, M. (2016). Antimicrobial, antiparasitic and anticancer properties of Hibiscus sabdariffa (L.) and its phytochemicals: In vitro and in vivo studies. Ceska Slov. Farm, 65(1), 10-14.**
- **Horowitz, M. (1992). Mechanisms of establishment and spreading of Cyperus rotundus—the worst weed of warm regions. Proc. First Int. Weed Cont. Congr, 1, 94-97**

  inhibitor screening . Journal of Enzyme Inhibition and Medicinal Chemistry , 38(1),
- **Iqbal, E., Salim, K. A., & Lim, L. B. (2021). Comparative study of total flavonoid content in medicinal plants. Journal of Medicinal Plants Research, 15(1), 34–41.**
- **Islam, M. (2019). Food and medicinal values of roselle (Hibiscus sabdariffa L. Linn e malvaceae) plant parts. Review, 1, 14-20.**
- **Jo, S., Kim, S., Shin, D. H., & Kim, M. S. (2020). Inhibition of SARS-CoV 3CL protease by flavonoids. Journal of enzyme inhibition and medicinal chemistry, 35(1), 145-151.**
- **Kamala, A., Middha, S. K., & Karigar, C. S. (2018). Plants in traditional medicine with special reference to Cyperus rotundus L.: a review. 3 Biotech, 8(7), 309.**
- **Kandikattu, H. K., Amruta, N., Khanum, F., Narayana, V. V. P. C., & Srinivasulu, D. (2022). A review on Cyperus rotundus: ancient weed to modern elixir of life phytochemistry and therapeutic uses of Cyperus rotundus (Mustaka). Pharmaceutical and Biomedical Research.**
- **Kanteev, M., Goldfeder, M., & Fishman, A. (2015). Structure–function correlations in tyrosinases. Protein Science, 24(9), 1360-1369**

- **Karimou, R., Salami, H. A., Agossou, E., Boya, B., Assouma, F. F., Bouko, B. O. M. B., ... & Sina, H. (2024). Assessment of antimicrobial and anti-biofilm activities of lemongrass and bay leaf extracts on microorganisms from fermented cereal-based porridges in northern Benin. Scientific African, 24, e02241.**
- **Kasala, S., Ramanjaneyulu, K., Himabindhu, J., Alluri, R., & Babu, R. R. (2016). Preliminary phytochemical screening and in vitro anthelmintic activity of Cyperus rotundus (L). Journal of Pharmacognosy and Phytochemistry, 5(5), 407.**
- **Kéllou, K. K. B., Abdourazak, M. A., Boukar, M. C. M., Diagra, M., Saley, A. S., & Yacoubou, B. (2024). Roselle (Hibiscus Sabdariffa L.): Overview of Its Biology, Ecology, Socio-Economic Importance and Cultivation Constraints In West Africa. World Journal of Pharmaceutical and Life Sciences, 10(3), 47-56.**
- **Kim, H. D., Choi, H., Abekura, F., Park, J. Y., Yang, W. S., Yang, S. H., & Kim, C. H. (2023). Naturally-occurring tyrosinase inhibitors classified by enzyme kinetics and copper chelation. International Journal of Molecular Sciences, 24(9), 8226.**
- **Kim, J. H., Cho, E. J., & Lee, S. Y. (2020). Antibacterial properties of phenolic compounds from Cyperus rotundus. Molecules, 25(12), 2789.**
- **Kim, J. H., Cho, E. J., & Lee, S. Y. (2021). Anti-tyrosinase effect of phenolic compounds from Cyperus rotundus. International Journal of Cosmetic Science, 43(5), 543–552.**
- **Kumar, A., Singh, P., & Pandey, R. (2021). Integrating computational and experimental approaches for the discovery of novel COX-2 inhibitors from natural sources . Frontiers in Pharmacology, 12, 667890.**
- **Kumar, A., Singh, S. K., & Prakash, A. (2023). Molecular docking and dynamics insights into NSAIDs–COX interaction: Implications for drug discovery. Journal of Enzyme Inhibition and Medicinal Chemistry , 38(1), 2198765.**
- **Kumar, A., Verma, S., & Agarwal, S. (2022). Structure-based virtual screening and molecular dynamics studies of glabridin and hispaglabridin A as tyrosinase inhibitors . Journal of Enzyme Inhibition and Medicinal Chemistry, 37(1), 1243–1254.**
- **Kumar, R., Kashyap, R., Sharma, D., & Chaudhary, A. (2022). Role of computational methods in drug discovery: An overview. International Journal of Pharmaceutical Sciences and Research, 13 (1), 45–56.**

- **Kumar, R., Singh, H., & Chaudhary, G. (2023). Mechanistic insights into diclofenac binding with cyclooxygenase-2: A molecular dynamics approach . Journal of Biomolecular Structure and Dynamics, 41(5), 1987–2001.**
- **Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview . The Scientific World Journal, 2013, 1-16.**
- **Kumar, V., Abbas, M., & Ali, M. (2020). Extraction of bioactive compounds from medicinal plants using ethanol–water mixtures: A review. Journal of Pharmacognosy and Phytochemistry, 9(2), 1234–1241.**
- **Kuriyan, R., Suresh, S., & Mathew, A. (2023). Anthocyanin composition and bioactivity of Hibiscus sabdariffa. Journal of Food Biochemistry, 47(2), e13589.**
- **Lee, J., & Park, K. (2018). Phenolic compounds as tyrosinase inhibitors: Structural requirements and molecular mechanisms . Archives of Pharmacal Research, 41(3), 259–273.**
- **Lee, S. Y., Baek, N., & Nam, T. G. (2016). Natural, semisynthetic and synthetic tyrosinase inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry, 31(1), 1-13.**
- **Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies , 1(4), 337–341.**
- **Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews , 64, 4–17.**
- **Lohar, A. V., Wankhade, A. M., Faisal, M., & Jagtap, A. (2020). A review on Glycyrrhiza glabra linn (liquorice)—An excellent medicinal plant. Eur. J. Biomed. Pharm. Sci, 7, 330-334.**

  Macromolecules , 182, 654–662.
- **Marston, A., & Hostettmann, K. (2015). Developments in the application of counter-current chromatography to plant analysis. Journal of Chromatography A, 1376, 1–18.**
- **Mohammad, A., Nagarajaiah, B. H., & Kudagi, B. L. (2012). Experimental evaluation of antiulcer activity of Cyperus rotundus. Asian J Biochem Pharm Res, 2(2), 261-268.**

- **Mohammed, G. F. A. E. K. (2014). Topical Cyperus rotundus oil: a new therapeutic modality with comparable efficacy to Alexandrite laser photo-epilation. Aesthetic Surgery Journal, 34(2), 298-305.**
- **Mostert, A. B. (2021). Melanin, the what, the why and the how: An introductory review for materials scientists interested in flexible and versatile polymers. Polymers, 13(10), 1670.**
- **Ngamsurach, P., & Praipipat, P. (2022). Antibacterial activities against Staphylococcus aureus and Escherichia coli of extracted Piper betle leaf materials by disc diffusion assay and batch experiments. RSC Advances, 12(40), 26435–26454.**
- **Noreen, S., Mubarik, F., Farooq, F., Khan, M., Khan, A. U., & Pane, Y. S. (2021). Medicinal uses of licorice (Glycyrrhiza glabra L.): A comprehensive review. Open Access Macedonian Journal of Medical Sciences, 9(F), 668-675.**
- **Obaid, R. J., Mughal, E. U., Naeem, N., Sadiq, A., Alsantali, R. I., Jassas, R. S., ... & Ahmed, S. A. (2021). Natural and synthetic flavonoid derivatives as new potential tyrosinase inhibitors: A systematic review. RSC advances, 11(36), 22159-22198.**
- **Okuda, T., Yoshida, T., & Hatano, T. (2016). Classification of plant polyphenols: Tannins and flavonoids. Natural Product Reports, 23(4), 524–544.**
- **Oloyede, F. M. (2014). Anti-nutritional factors and antioxidant properties of some lesser-known edible fruits in Nigeria. African Journal of Food Science, 8(1), 35–40.**
- **Onyeukwu, O. B., Dibie, D. C., & Njideaka, O. T. (2023). Hibiscus sabdariffa-uses, nutritional and therapeutic benefits-A review. Journal of Bioscience and Biotechnology Discovery, 8(2), 18-23.**
- **Pasam, S., Pragada, V. R., Bakshi, V., & Boggula, N. (2023). Cyperus Rotundus L. Plant in Traditional Medicine: A Review. Journal of Advancement in Pharmacognosy, 3(1).**
- **Prasad, S. K., & Hemalatha, S. (2018). In vitro antioxidant potentials of Cyperus rotundus L. rhizome extracts and their phytochemical analysis. Pharmacognosy Magazine, 14(54), 261–267.**
- **Putra, F. G., Paramita, N., & Santoso, D. I. S. Effect of Hibiscus sabdariffa Linn. on the development of atherosclerosis in diabetes mellitus: a rapid review. Indonesian Journal of Biomedicine and Clinical Sciences, 57(1).**
- **Riaz, G., & Chopra, R. (2018). A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L. Biomedicine & Pharmacotherapy, 102, 575-586. Riendeau,**

D., Percival, M. D., Boyce, S., & Brideau, C. (2020). BIOCHEMICAL AND PHARMACOLOGICAL PROFILE OF MELOXICAM: A SELECTIVE INHIBITOR OF CYCLOOXYGENASE-2 . British Journal of Pharmacology, 130(1), 110–116.

- **Riendeau, D., Percival, M. D., Boyce, S., & Mancini, J. (2020). Biochemical and pharmacological profile of a selective COX-2 inhibitor. British Journal of Pharmacology , 160(3), 541–551**
- **Sankeshwari, R. M., Ankola, A. V., Bhat, K., & Hullatti, K. (2018). Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against: Streptococcus mutans:. Journal of the Scientific Society, 45(2), 67-71.**
- **Sapian, S., Ibrahim Mze, A. A., Jubaidi, F. F., Mohd Nor, N. A., Taib, I. S., Abd Hamid, Z., ... & Budin, S. B. (2023). Therapeutic potential of Hibiscus sabdariffa Linn. in attenuating cardiovascular risk factors. Pharmaceuticals, 16(6), 807.**
- **Sarker, S. D., & Nahar, L. (2018). Chemistry for pharmacy students: General, organic and natural product chemistry. John Wiley & Sons.**
- **Shanmugam Ramaswamy, S. R., Dwarampudi, L. P., Madhuri Kadiyala, M. K., Gowthamarajan Kuppuswamy, G. K., Reddy, K. V. V. S., Kumar, C. K. A., & Murali Paranjothy, M. P. (2017). Formulation and characterization of chitosan encapsulated phytoconstituents of curcumin and rutin nanoparticles.**
- **Sharifi-Rad, J., Quispe, C., Herrera-Bravo, J., Belén, L. H., Kaur, R., Kregiel, D. & Suleria, H. A. R. (2021). Glycyrrhiza genus: Enlightening phytochemical components for pharmacological and health-promoting abilities. Oxidative medicine and cellular longevity, 2021(1), 7571132.**
- **Sharma, D., Namdeo, P., & Singh, P. (2021). Phytochemistry & pharmacological studies of glycyrrhiza glabra: A medicinal plant review. Int. J. Pharm. Sci. Rev. Res, 67(1), 187-94.**
- **Sharma, V. A. R. S. H. A., & Agrawal, R. C. (2013). Glycyrrhiza glabra-a plant for the future. Mintage J Pharm Med Sci, 2(3), 15-20.**
- **Sharma, V., Katiyar, A., & Agrawal, R. C. (2017). Glycyrrhiza glabra: chemistry and pharmacological activity. Sweeteners, 87.**
- **Shruthi, V. H., Ramachandra, C. T., Udaykumar Nidoni, U. N., Sharanagouda Hiregoudar, S. H., Nagaraj Naik, N. N., & Kurubar, A. R. (2016). Roselle (Hibiscus sabdariffa L.) as a source of natural colour: a review.**

- **Singleton, V. L., & Rossi, J. A. (2014). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16(3), 144–158.**
- **Smith, J., Johnson, K., & Williams, T. (2020). Binding affinity prediction of natural products against tyrosinase using molecular docking simulations . Computational Biology and Chemistry, 89, 107384.**
- **Smith, R., Taylor, L., & Brown, M. (2021). Structural insights into COX-2 inhibition by NSAIDs: A molecular interaction perspective . European Journal of Medicinal Chemistry, 215, 113210.**
- **Smith, W. L., DeWitt, D. L., & Garavito, R. M. (2021). Cyclooxygenases: Structural, functional, and pathophysiological implications. Annual Review of Pharmacology and Toxicology , 61, 213–234.**
- **Song, Y., Chen, S., Li, L., Zeng, Y., & Hu, X. (2022). The hypopigmentation mechanism of tyrosinase inhibitory peptides derived from food proteins: An overview. Molecules, 27(9), 2710.**
- **Sushko, I., Novotarskyi, S., Körner, R., & Tetko, I. V. (2010). Online chemical modeling environment (OCHEM) . Journal of Chemical Information and Modeling, 50(4), 599–620.**
- **Sushko, I., Novotarskyi, S., Körner, R., Pandey, A. K., Rupp, M., Teetz, W., ... & Tetko, I. V. (2010). ADMET predictor™: A web-and vlife-based tool for predicting human ADME/toxicity properties. QSAR & Combinatorial Science, 29 (5), 845–846.**
- **Taheri, Y., Herrera-Bravo, J., Huala, L., Salazar, L. A., Sharifi-Rad, J., Akram, M., ... & Cho, W. C. (2021). Cyperus spp.: A Review on Phytochemical Composition, Biological Activity, and Health-Promoting Effects. Oxidative Medicine and Cellular Longevity, 2021(1), 4014867.**
- **Tiwari, B. K., Brunton, N. P., & Brennan, C. S. (2013). Plant food phytochemicals. Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction, 1-4.**
- **Vaezi, M. (2022). In silico and in vitro studies of naturally occurring tyrosinase inhibitors: structure–activity relationship. Chemistry Africa, 5(6), 1873-1887**
- **Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Wardescamden, M., Kopelowicz, A., & Fowler, R. H. (2002). Molecular properties that influence the oral bioavailability of drug candidates . Journal of Medicinal Chemistry, 45(12).**

- **Wang, Q., Liu, Y., & Zhao, Z. (2020). Discrepancies between experimental and computational binding affinities of rutin with COX-2: Role of solvation and conformational flexibility . Molecules, 25(19), 4452.**
- **Williams, C. A., & Harborne, J. B. (2020). The flavonoids: Advances in research since 1986.**
- **Xue, B. X., He, R. S., Lai, J. X., Mireku-Gyimah, N. A., Zhang, L. H., & Wu, H. H. (2023). Phytochemistry, data mining, pharmacology, toxicology and the analytical methods of Cyperus rotundus L.(Cyperaceae): a comprehensive review. Phytochemistry Reviews, 22(5), 1353-1398.**
- **Zadeh, J. B., Kor, Z. M., & Goftar, M. K. (2013). Licorice (Glycyrrhiza glabra Linn) as a valuable medicinal plant. International journal of Advanced Biological and Biomedical Research, 1(10), 1281-1288.**
- **Zhang, Y., Chen, X., & Li, W. (2017). Experimental and computational comparison of quercetin derivatives as COX-2 inhibitors . Bioorganic & Medicinal Chemistry Letters, 27(14), 3112–3117.**
- **Zhang, Y., Liu, X., Wang, L., & Chen, Z. (2023). Thermodynamic insights into protein-ligand interactions: Implications for drug design. Frontiers in Chemistry, 11 , Article 1123456.**
- **Zhao, H., & Bourne, P. E. (2022). Salt bridges: Essential interactions for biomolecular structures and functions. Computational and Structural Biotechnology Journal , 20, 1234–1245**
- **Zhao, Y., & Bourne, P. E. (2022). Structure-based drug design targeting cyclooxygenase-2: Advances and challenges . Current Topics in Medicinal Chemistry, 22(1), 34–47.**
- **Zhou, S., Zeng, H., Huang, J., Lei, L., Tong, X., Li, S., ... & Zeng, Q. (2021). Epigenetic regulation of melanogenesis. Ageing research reviews, 69, 101349.**
- **Zhou, Y., Zhang, X., & Zhang, W. (2022). Bioactive compounds in traditional herbs and their pharmacological activities. Journal of Ethnopharmacology, 289, 115056.**
- **Zilles, J. C., Dos Santos, F. L., Kulkamp-Guerreiro, I. C., & Contri, R. V. (2022). Biological activities and safety data of kojic acid and its derivatives: A review. Experimental dermatology, 31(10), 1500-1521.**