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Department of Matter Sciences

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Chemical composition and combined in vitro-in silico approach of natural antioxydant agents applied for skin care - Formulation an anti-hyperpigmentation cream -

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بسم الله الرحمن الرحيم

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Dedication

To the source of tenderness, to the heart that does not age, to whose prayers was the secret of my success and happiness... To my beloved mother **KHADRA**, who instilled in me the seeds of love and hope, watered them with patience and sacrifices...Every word of thanks falls short of what you deserve, and every achievement that people deserve is you, you have my soul as redemption and my heart as gratitude.

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To someone who has a special place in my heart،To my dear cousin **said** ،Who has never spared me a word of support, a moment of attention, or a sincere presence at all stations of this road،I was more than close... you were a brother, a comrade, a real support. I dedicate this work to you as a thank you, and to every beautiful mart, you left on my journey.

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Elyamna Kherfi

Summary

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Chapter I

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List of abbreviations

A

- **ADMETOX: Absorption, Distribution, Metabolism, Excretion and Toxicity**
- **AFNOR: French Association for Standardization**

B

- **BHT: Butylated Hydroxy Toluene**

C

- **CAT: Catalase**

D

- **DMSO: Dimethyl Sulfoxide**
- **DNA: Deoxyribo Nucleic Acid**
- **DPPH: 2,2-Diphenyl-1-picrylhydrazyl**

E

- **EROS: Essential for Reactive Oxygen Species**

F

- **FAD: Flavin Adenine Dinucleotide**

G

- **GC-MS: Gas Chromatography-Mass Spectrometry**
- **GPX: Glutathione Peroxidase**
- **GR: Glutathione Reductase**
- **GSM: Glutathione Synthetase A**

I

- **ICM: Internal Coordinate Mechanics**

N

- **NADPH: Nicotinamide Adenine Dinucleotide Phosphate**
- **NBT: Nitro Blue Tetrazolium**
- **NLM: National Library of Medicine**
- **NO: Nitric Oxide**
- **NOS: Nitric Oxide Synthase**

P

- **PDB: Protein Data Bank**
- **PKCSM: Pharmacokinetic Properties using Graph-based Signatures Modeling**

R

- **RMSD: Root Mean Square Deviation**
- **RNS: Reactive Nitrogen Species**
- **ROS: Reactive Oxygen Species**

S

- **SDF: Structure-Data File**
- **SOD: Superoxide Dismutase**

T

- **TRX: Thioredoxin**
- **TYR: Tyrosinase / Tyrosine**
- **TYRP: Tyrosinase Related Protein**

U

- **USP: United States Pharmacopeia**
- **UV: Ultraviolet**

General Introduction

Introduction

The skin is the coating that covers the entire body, considered as the largest organ, which performs a protective function against external aggressions that include environmental pollutants, chemical materials, heat, cold, ultraviolet radiation, and pathogens. **[1]**

Since it defended the body from exterior agents, it would be the most affect organ from oxidative stress as a result of exogenous ROS sources, additionally to the endogenous sources. The oxidative stress is defined as an imbalance between the production of free radicals and the anti-oxidative systems. The body's cells produce free radicals during normal metabolic processes. However, cells also produce antioxidants that neutralize them. **[2]**

Oxidative stress is a crucial factor that affects skin cellular. Many research shows that oxidation is also a significant contributor to the extrinsic, or photo-aging of the skin. The older we get, the more our skin cells are damaged by external oxidising agents caused by UV irradiation. **[3]** It is known to be associated with skin aging and hyperpigmentation. **[4]**

The epidermis contains a pigment called melanin, which is responsible for giving color to the skin and providing protection from ultraviolet radiation to the underlying tissues**.[5]** Tyrosinase is one of the key enzymes in melanin synthesis. **[6]**

In efforts to resolve these problems, many have focused on the screening of skin whitening agents. In terms of melanin syntheses, tyrosinase plays a key role because it catalyzes the rate-limiting reactions of melanogenesis, Thus, many researchers have explored for potent tyrosinase inhibitors. **[7]**

In this context, our study came to search for natural products - derived antioxidants agents, contained within a study combining laboratory experimentation (in vitro) and simulation analysis (in silico), with the aim of evaluating the effectiveness of plant extracts as potential antioxidants and inhibitors of the enzyme tyrosinase. This research is structured into three comprehensive chapters:

The first chapter explores various concepts, we talked about oxidative stress, skin diseases associated with it, namely hyperpigmentation and dark spots, explained the role of the enzyme tyrosinase. The latter part of this chapter presents the importance of naturel antioxidants such as the essential oil and vegetable oil.

The second chapter outlines the experimental procedures, including two parts (phytochemical and biological studies), starting with the selected plants utilized in this work and methods

applied to extract oil (Soxhlet and Clevenger). The natural compounds obtained were also identified using gas chromatography (GC-MS); their antioxidant capacity was evaluated by means of biochemical tests: DPPH, DMSO, and phenanthroline, with analysis and interpretation of the results to determine the most effective extracts.

In the third chapter, we switched to a simulation study using the MOE and discovery Studio programs to perform molecular docking, in order to find out how compounds interact with the tyrosinase enzyme. Additionally, the physicochemical properties of these compounds were characterized, and their pharmacokinetic profiles including absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, were evaluated to predict their potential efficacy and safety for therapeutic use.

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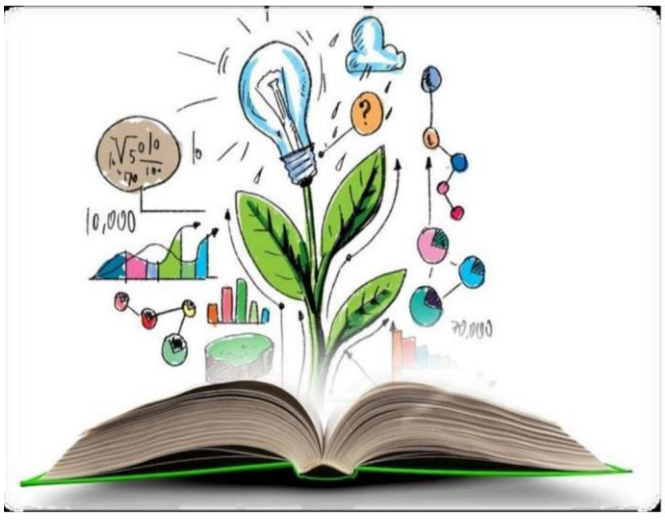
Chapter I

Overview of the literature



❖ Introduction:

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. **[1]**

The skin is a biological barrier that defends against multiple environmental insults. Free radicals, one of the forms of insult, stimulate or contribute to the occurrence of adverse effects on the skin, including erythema, edema, wrinkles. **[2]**

Melanin, the major pigment that gives color to skin, may be overproduced with sun exposure or in conditions such as melasma or hyperpigmentary diseases. Tyrosinase is a key enzyme that catalyzes melanin synthesis in melanocytes; therefore, inhibitors of the tyrosinase enzyme could be used for cosmetic skin whitening. **[3]**

Figure I.1: Dark Spots caused by Oxidative Stress

❖ General oxidative stress and antioxidant agent skin care

I. Oxidative stress

Oxidative stress is defined by an imbalance between increased levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms. An increased oxidative stress can induce damage to the cellular structure and potentially destroy tissues. However, ROS are needed for adequate cell function, including the production of energy by the mitochondria. Increased oxidative stress has been incriminated in physiological conditions, such as aging and exercise, and in several pathological conditions, including cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, inflammatory diseases, and intoxications. **[1]**

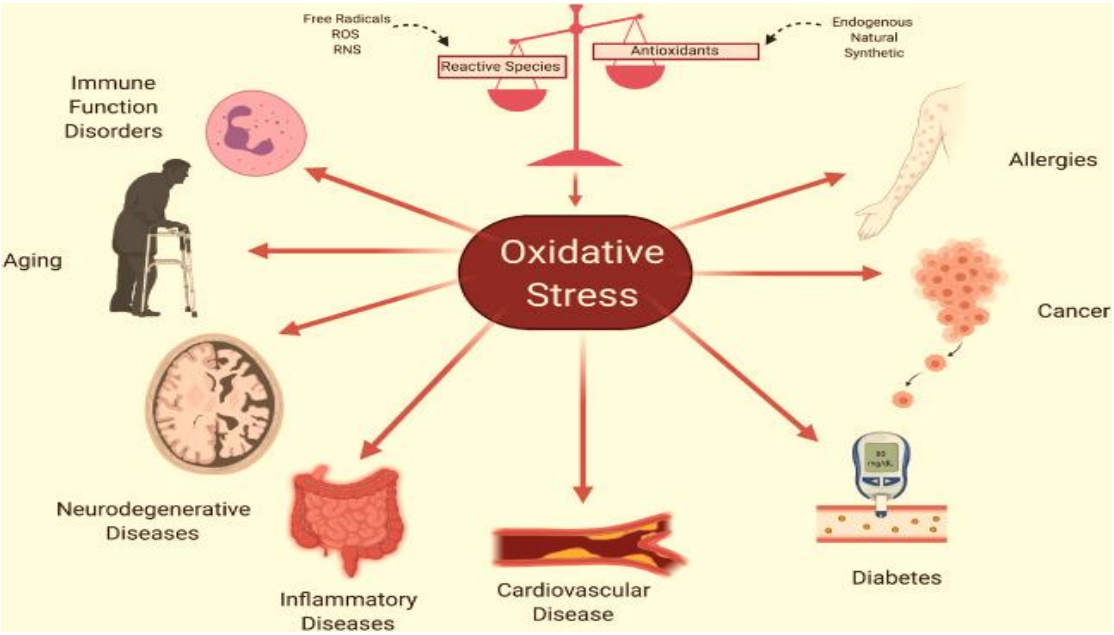


Figure I.2: Oxidative Stress

I.1. Free radicals

The activated oxygen species (free radicals). They are chemical substances, molecules, or simple atoms containing one or more unpaired electrons, making them highly reactive. Indeed The reactive oxygen species (ROS) and the reactive nitrogen species (RNS) represent all, a free radical will always tend to fill its orbital by capturing an electron to achieve stability: it will therefore reduce by oxidizing another compound. **[4]** Free radicals can cause large chain chemical reactions in your body because they react so easily with other molecules. These reactions are called oxidation. They can be beneficial or harmful.**[5]**

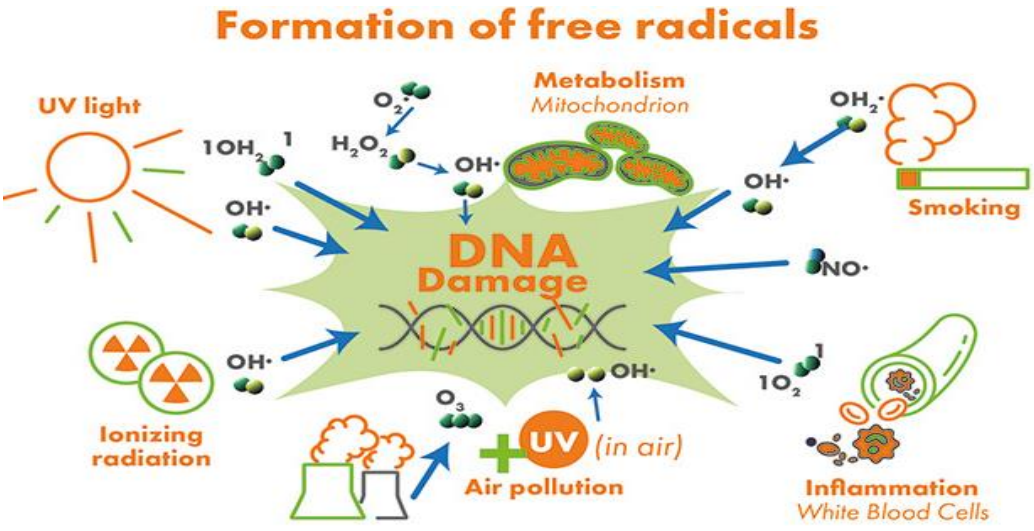


Figure I.3: Sources and Effects of Free Radical Formation on DNA Damage

I.1.1. Types of free radicals

In biological systems, the most important free radicals are Reactive oxygen gas species (ROS for "reactive oxygen species") and reactive nitrogen species (RNS for "reactive nitrogen species"). **[4] [6] [7]**

Table I.1: Types of Free radicale Generated by Oxidative Stress

Reactive oxygen species (ROS)	**Reactive Nitrogen Species (RNS)**
Reactive oxygen species (ROS) are chemically reactive radicals or non-radical molecules derived from molecular oxygen (O2), including singlet oxygen (O2), peroxide (02), superoxide (O), and hydroxyl radical (HO).	**-Nitrogen monoxide (NO)** Nitrogen monoxide is an ubiquitous free radical of a gaseous nature and highly dispersible. It is synthesized from L-arginine by NO synthases (NOS), in the presence of supporting agent such as NADPH, FAD, calmodulin and tetrahydrobiopterine (BH4).

I.1.2. Sources of free radicals

EROS can be generated by physical factors such as radiant energy, chemical processes and especially enzymatic. Indeed, any reaction involving O_2 and a reducing electron transport system is likely to release ROS. This is how the energy production chain causes a significant release of ROS. Other enzymatic activities also provide ROS, in particular NADPH oxidases during irritation and cytochromes P450 during purification of xenobiotics. Thus, the mitochondrion, the plasma membrane and the intracellular membrane system are the main sites of ROS release. There are many endogenous and exogenous sources producing ROS. **[4]**

Table I.2: Endogenous and Exogenous Sources of free Radicals

Endogenous source	**Exogenous source**
-Mitochondrial respiratory chaine. -NAD(P)H oxidase. -Xanthine oxidase. -Enzymes of the endoplasmic reticulum.	-Cigarette -Rayonnement UV -Various pollution -Physical exercise

II. Consequences of Oxidative Stress:

The main risk of free radicals comes from the harm they can cause when they interact with crucial cellular elements, such as DNA, lipids (peroxidation), proteins.
This oxidation causes damage to the entire organism, accelerating aging (disorders cardiovascular and neuro-degenerative diseases, cancer, diabetes.) and the degradation of body cells and tissues.**[6]**

III. Oxidative stress and skin diseases

Most diseases caused by oxidative stress develop with age, because aging reduces antioxidant defenses and raises the mitochondrial formation of radicals. Oxidative stress will be the primary cause of various diseases: cancer, cataracts, amyotrophic lateral sclerosis, acute respiratory distress syndrome, pulmonary edema, accelerated aging**. [8]** Which leads to accelerated skin aging and the appearance of a number of skin diseases. The skin plays a vital role in the body's defense, so it's constant covering its entire external surface and serving as a first-order physical barrier against the environment. Its functions include temperature regulation and protection against ultraviolet (UV) light, trauma, pathogens, microorganisms, and toxins. The skin is also highly adaptive with different thicknesses and specialized functions in different body sites.**[9] [10]**

The skin has a typical layered structure consisting of three main layers. From the outside to the inside, these are called: epidermis, which is the most superficial layer, dermis and hypodermis, the subcutaneous tissue**.**

- The **epidermis**, the outermost layer of skin, provides a waterproof barrier and creates our skin tone.
- The **dermis**, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands.
- The **hypodermis** is the deeper subcutaneous tissue and is made of fat and connective tissue

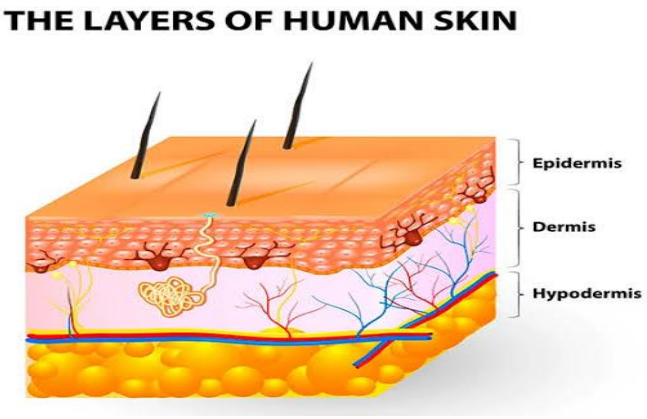


Figure I.4: The Layers of human skin

- **Melanin and hyperpigmentation**

Melanin is the main pigment responsible for the color of human skin, hair and eye. Its biosynthesis requires three melanogenic enzymes, tyrosinase (TYR), and the tyrosinase-related proteins TYRP1 and TYRP2. **[11]** Although melanin gives protection against DNA damage induced by the UV radiation of the sun, and from different chemical compounds, its overaccumulation can cause hyperpigmentation-related diseases, esthetic problems and even skin cancer. **[12]**

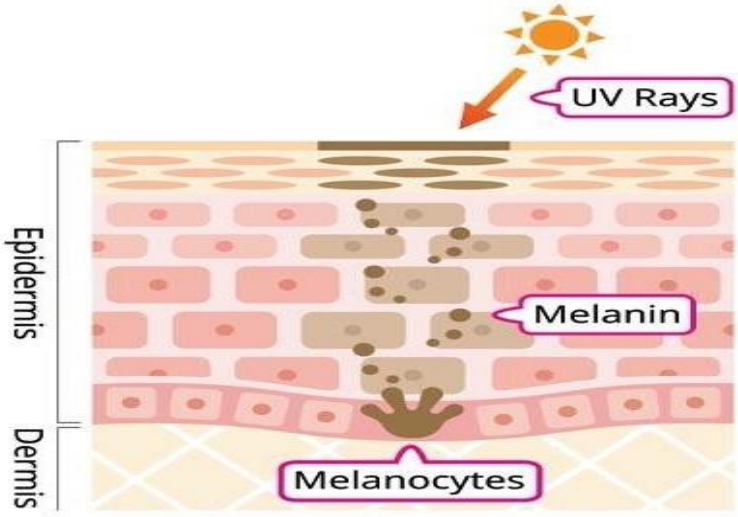


Figure I.5: Melanocytes and their Role in pigmentation and protection

Various dermatological disorders result in the accumulation of an excessive level of epidermal pigmentation. These hyperpigmented lentigines include melasma, age spots and sites of actinic damage.**[13]** Melanin also play a crucial role in the absorption of free radicals generated within the cytoplasm and in shielding the host from various types of ionizing

radiations, including UV light. Melanin can be of two basic types: eumelanin, which are is brown or black, and pheomelanin, which are is red or yellow.**[14]**

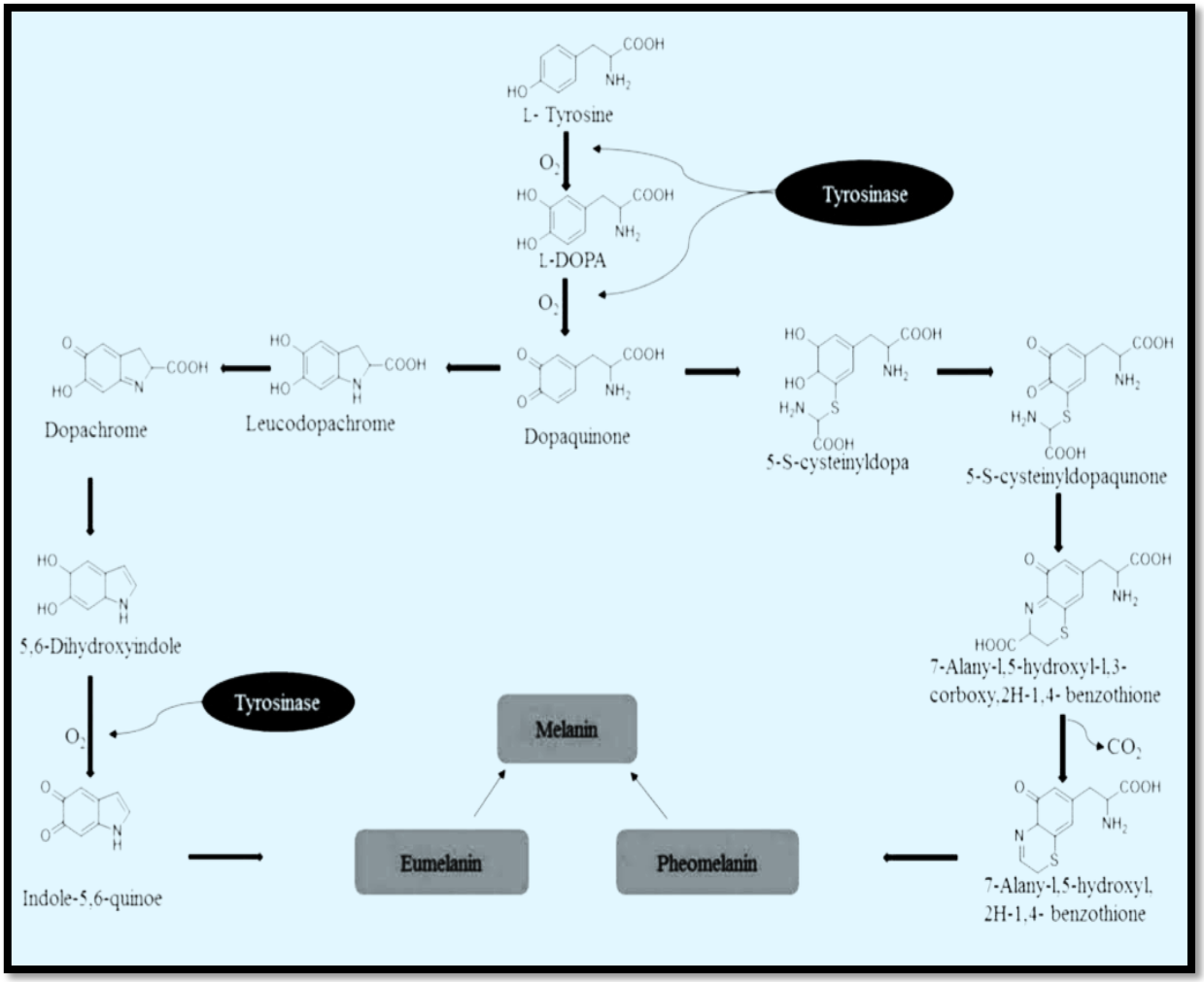


Figure I.6: Mechanism of Melanin Conversion into: eumelanin and pheomelanin

IV. Antioxidant agents

Antioxidant agents are chemical substances able to neutralizing or attenuating the harm resulting from free radicals in the body, while allowing maintaining non-cytotoxic ROS concentrations at the cell level. These substances are used to protect against getting older, due to the excessive production of free radicals. Antioxidants are very diverse substances comprising small molecules fat-soluble (such as vitamin E, β-carotene) or water-soluble (such as vitamin C, uric acid) as well as proteins with enzymatic activity (such as superoxide dismutase, glutathione peroxidase, catalase, etc.), no-enzymatic (such as the sequestering

metals). They can be of endogenous or exogenous origin, and it can be natural or synthetic compounds. **[6]**

IV.1. Types of antioxidants

A. Enzymatic antioxidants

They are endogenous antioxidants represent the first lane of defense of our organization against EROS.

- Superoxide dismutase (SOD).
- Catalase (CAT) **[6]**
- Glutathione peroxidases (GPx).
- Glutathione reductase (GR). **[15]**
- Thioridoxine peroxidases (Trx).

B. Non-enzymatic antioxidants

Certain chemical compounds of low molecular weight, act as antioxidants, their role is not catalysis. There are two categories of them: antioxidants endogenous non-enzymatic (if the eukaryotic cell is capable of synthesizing them) and the exogenous non-enzymatic antioxidants (through food). **[6]**

Table I.3: Endogenous and Exogenous Non Enzymatic Antioxidants

a) Endogenous non-enzymatic antioxidants	b) Exogenous non-enzymatic antioxidants
Glutathione (GSH). Uric acid. **[16]** Bilirubin Lipoic acid	Vitamin E Vitamin C ß-carotene. **[17]** Selenium Polyphenols.

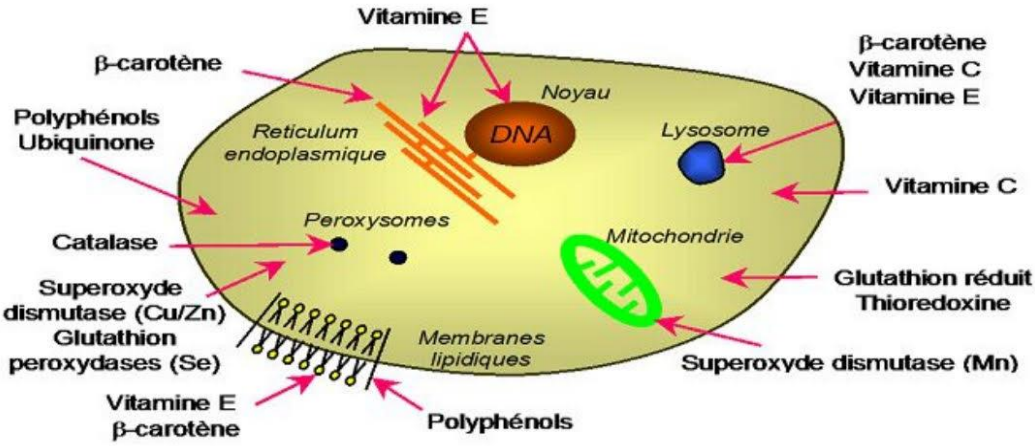


Figure I.7: Schematization of the molecules involved in cell protection

IV.2. Role of antioxidants in skin treatment:

Tyrosinase is responsible for the production of melanin. Excessive melanin production causes many skin disorders and aesthetic problems. Researchers have investigated the use of several strategies to reduce melanin hyperpigmentation. **[18]**

In turn, inhibition of the activity of the tyrosinase enzyme is a more attractive way to approach hyperpigmentation due to the key role that Tyrosinase plays in the production of melanin. Countless tyrosinase inhibitors Natural, synthetic, and semi-synthetic have been identified, some of them have been developed to reduce skin hyperpigmentation, but there is still a demand capable of considering new tyrosinase inhibitors that are more effective and safer than the ones identified so far. **[18]**

❖ Essential oil

I. Definition

There are several definitions available of an essential oil converge on the fact that oils essential oils. An essential oil is defined as a product obtained from a material first of vegetable origin, after extracted using physical methods separating it from water. The European pharmacopoeia defines essential oils as: "Odorous product, generally of complex composition, obtained from a vegetable raw material botanically defined, either by entrainment by water vapor, or by dry distillation, or by an appropriate mechanical process without heating. The essential oil is most often separated from the aqueous phase by a physical process that does not lead to a change significant of its composition" **[19]**

According to AFNOR (the French Association for Standardization), these are products generally odorous, obtained either by steam entrainment of water, plants or parts of plants, either by expression of the fresh pericarp of certain citrus fruits. This definition excluding products derived through alternative extraction methods. Essential oils are mixtures of many compounds that are molecules little complexes such as terpenes, phenols, methyl ethers, oxides, esters, and ketones... They have important medical applications either by their odorous quality or for relieve pain or for their physiological effectiveness. **[19]**

II. Role of essential oils

Plants use them to defend against viruses and all think that they are hormones vegetable. Others consider that oils are communicators between kind of parasites and microbes; work has shown that monoterpenes and sesquiterpenes can play important roles in the relationship of plants with their environment. For example, the 1.8-cineole and camphor inhibit the initiation of infected organs or the growth of pathogens infectious agents from these organs. Essential oils are used in a wide variety fields, particularly in the cosmetic and health industries, because, in addition to their odor, they serve as preservatives, active agents and additives that are beneficial for the skin. **[19]**

III. Properties of essential oils

The exposure of essential oils to light, heat and moisture to the occurrence of reactions with oxidation and analysis and polymerization, which leads to a change in its chemical and physical qualities. **[20]**

Table I.4:physical and chemical properties of Essential Oils

Oxidation	Due to its chemical composition as the presence of double bonds and functional groups such as hydroxyl, aldehyde and Ester Essential oils are vulnerable to oxidation by light, heat and air.
The color	Most volatile oils are colorless, a few are pale yellow, the rare ones are either blue or blue Green.
The smell	Most volatile oils are characterized by a fragrant aroma, rarely their smell is not desirable.
To fly	The vast majority of volatile and extracted oils vaporize or volatilize under natural and normal conditions, Except for a few of them.

the specific density	The specific density of volatile oils varies according to their different plant sources, and ranges from 1.17-0.8 And most essential oils have a density less than the correct one, that is, less than the density of water.

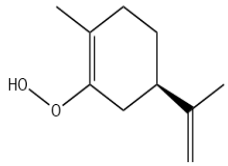
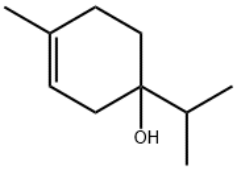
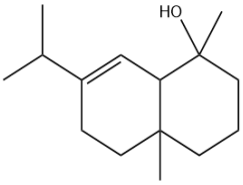
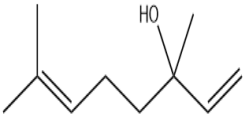
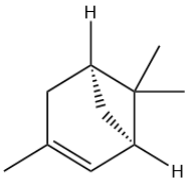
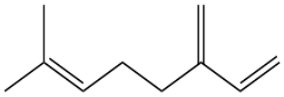
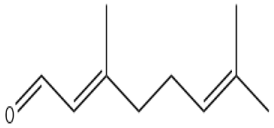
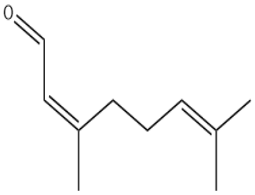
IV. Structure of essential oils :

Volatile oils are very complex mixtures; the constituents are mainly monoterpenes and sesquiterpenes of general formula (C_5H_8) n. The oxygen compounds derived from these hydrocarbons include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. It is estimated that there are more than 1000 monoterpenes and 3000 of sesquiterpene structures. Other compounds include phenylpropanes and compounds containing sulfur or nitrogen, Fig. 14. presents the structure of some components found in essential oils. Essential oils are a combination of several compounds: terpenoid, aromatic (phenolic), phenyl derivatives Propane and propane are the two most commonly found, and sometimes hydrocarbon derivatives that differ from each other chemically and with sources Different. **[20]**

Table I.5: Composition of Essential Oil

A. terpenes turbochargers	B. aromatic compounds	C. vehicles from different sources
They are also called Isoprene derivative and form the vast collection of natural products in the kingdom Plants, whose structures include series of 5 carbon atoms called isoprene, are seen as terpenes Monoterpenes the most important groups of volatile oils in addition to sesquiterpenes Phenylpropanoid.	They are derivatives of phenylpropane (C6-C3) where element C6 represents the benzene ring which the latter obtains its aromatic properties. These compounds are generally less present in essential oils relative to the aforementioned compounds.	Essential oil can also contain other substances.

Table I.6: Structure encountered in essential oils

Limonène	Terpinène-4-ol	Selina-6-en-4-ol	Linanool
			
α-Pinène	**β-Myrcène**	**Geranial**	**Néral**
			

❖ Vegetable oil

I. Definition

Vegetable oils are obtained from oil containing seeds, fruits, or nuts by different pressing methods, solvent ex-traction or a combination of these. Crude oils obtained are subjected to a number of refining processes, both physical and chemical. **[21]** Vegetable oils are biodegradable, non-toxic, less harmful to environment and locally available **[22]**, Most of the vegetable oil currently used as biodiesel feedstock could also be used as edible oil. **[23]**

II. Role of vegetable oils

Vegetable oils satisfy four main roles: nutritional support: provide of energy and nutrients (fatty acids, vitamins fat-soluble, minor constituents of interest such as phytosterols or phenolic compounds for olive oil); Organoleptic analysis (taste and smell addition); theological properties (texture); technological innovation (heat transfer fluid, for example in applications in frying). **[24]**

III. properties of vegetable oils:

The quality of vegetable oil has been reported to be evaluated by several physical and chemical parameters that are dependent on the source of oil processing and storage conditions. Some physical parameters (moisture content, refractive index, viscosity, specific gravity, color, etc) and chemical parameters (smoke point, saponification value, acid value, iodine value, ash content and peroxide value) can be used to evaluate the purity and quality of oils. **[25]**

Saponification value	lower the saponification value, the larger the molecular weight of fatty acids in the glycerides or the number of ester bonds is less and vice versa.
Specific gravity	Density or specific gravity of a vegetable oil depends on the type of oil and temperature. Different values of density may attribute to the different in fatty-acid composition, total solid content and degree of unsaturation.
Refractive index (RI)	The refractive index (RI) is the ratio of the speed of light in a vacuum to the speed of light through a given material.
Color	Refined oils have usually soft tastes, clear and transparent appearance.
Peroxide value	Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage and it is used as a good criterion for the prediction of the quality and stability of oils.

Table I.7: Physical and chemical properties of vegetable oils

IV. structure of vegetable oils

Vegetable oils are triglycerides of fatty acids and may be classified as: **[26]**

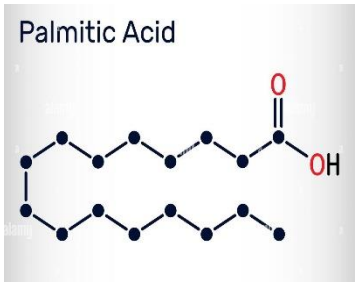
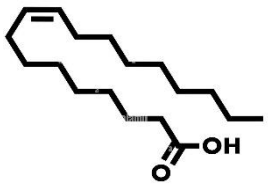
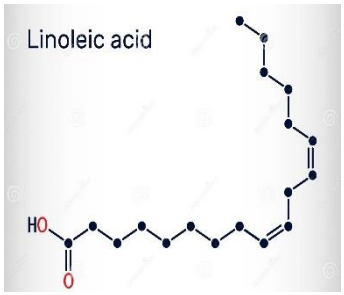
Saturated	Monounsaturated	Polyunsaturated
Examples of saturated fatty acids include palmitic and stearic acids. All carbon-hydrogen bonds are saturated, that is, they are methyl (CH) or methylene (CH2).	An example of a monounsaturated fatty acid is oleic acid (a common ester component in olive oil), which possesses one double bond (-CH=CH).	Polyunsaturated fatty acids possess two or more double bonds. (-CH=CH-) in conjugation with each other. An example of a fatty acid with two double bonds is linoleic acid (an omega-6 fatty acid) and a fatty acid with three double bonds is linolenic acid (an omega-3 fatty acid).
	 oleic acid	

Table I.8: Composition of vegetable Oil

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Chapter II

Phytochemical and Biological studies

❖ Introduction:

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health. **[1] [2]**

The focus of this investigation is to identify natural products with antioxidant properties for potential future drug development. Our choice intensive on the selection of six plants such as **Bay laurel**, **Lavender**, **Tigernut**, **Chia**, **Apricot kernel** and **Seed of Washingtonia robusta**. This research was done at the University of Biskra's Laboratory of Chemistry in the Faculty of Exact Sciences and Sciences of Matter department, in collaboration with the Scientific and Technical Research Center on Arid Region's (CRSTRA), Laboratory of Bioactive Compounds.

The principal objective of this research is to provide a comprehensive review of phytochemical investigations focused on the extraction, quantification, and characterization of bioactive compounds derived from natural plant sources. This review also evaluates antioxidant activities, specifically examining fatty acids from vegetable oils and volatile constituents of essential oils, with the aim of supporting their use in pharmaceutical formulations for skin care applications.This work is organized in three steps:

- **First step:** Extraction, separation, purification, and quantification of bioactive compounds in plant biochemical analyses.
- **Second step:** Characterization and identification of naturel products.
- **Third step:** Evaluation of antioxidant activity via three methods.

Part 1: Materials and methods

I. Identification of plants used:

- **Laurus nobilis (**Bay laurel)**:**

Bay laurel or bay leaf (Laurus nobilis) belonging to the Lauraceae family is a culinary herb. cultivated in the Mediterranean region and in the warm climates of the southern United States, Central America, Europe, the Middle East, and Asia. The oil obtained from the bay leaf is extracted from different parts of the plant and possesses potent biological and pharmacological properties and is used as an antibacterial agent, antifungal agent, antioxidant agent, and many more.**[3]**

Bay leaves is full of antioxidants, minerals and fibres. **[4]** Several studies have shown that flavonoids and phenolic acids, two classes of polyphenolic compounds, have antioxidant properties such as anti-inflammatory actions, inhibition of oxidative enzymes, and free radical scavenging. **[3]**

- **Lavandula angustifolia (**Lavender)**:**

Lavender, scientifically named Lavandula angustifolia, is part of the Lamiaceae family, related to mint, being very easy to identify due to the purple flowers and its sweet, floral scent. This plant is thought to have originated in the Mediterranean area, North Africa, the Middle East and India. Although the list of benefits of lavender essential oil is impressive, it has several valuable pharmacological properties that make it increasingly useful in alternative and complementary medicine, Due to its high content of antioxidants, it is a good skin moisturizer. Lavender oil also improves blood circulation, which will facilitate nutrient transfer to the face, stimulating the healing process, thus reducing the inflammation caused by episodes of acne, insect bites, allergic reactions and skin diseases.**[5]**

- **Cyperus esculentus** (Tigernut)**:**

tigernut (Cyperus esculentus) is a member of the division Magnoliophyta, class Liliopsida, order Cyperales, and family Cyperaceae. Many tropical and subtropical nations in the African sub region grow.

Due to the presence of flavonoids, tigernut has excellent antioxidant properties and can be used as a natural source of antioxidants. **[6]**

- **Salvia hispanica (**Chia)**:**

Chia (Salvia hispanica) is a summer annual herbaceous flowering plant belonging to the Lamiaceae family observed to flower with purple and/or white petals found in southern Mexico. It is native to central and southern Mexico and Guatemala. The USP defines chia seed oil as the oil extracted from the seeds by cold pressing and excludes the use of solvents or external heat in the extraction pro cess. For purposes of preserving the oil, tocopherols may be added as antioxidants.

Rahman et al. conducted a study that highlights & hispanica's antioxidative potential. Hydrolysis of chia produces bioactive peptides with low molecular weight. These molecules dem enzyme inhibitory and antioxidant activity. **[7]**

- **Apricot (*Prunus armeniaca* L.):**

Apricot (*Prunus armeniaca* L.) originated in China, and later it was introduced to various parts of Central Asia. The kernel is an organic product that positively affects human health and is often considered an unwanted part of the fruit. Apricot kernel is a rich source of proteins, vitamins, and carbohydrates. Moreover, it can be used for medicinal purposes and the formation of food ingredients. **[8]**,**[9]** It is also enormous properties in several industries inducing cosmetic, pharmaceutical, and food industries.**[10]**

- **Seed of Washingtonia robusta :**

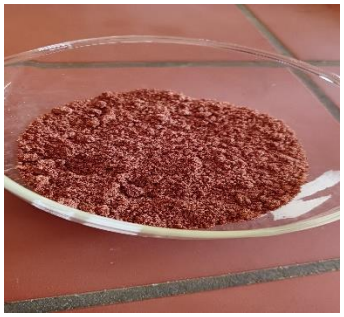
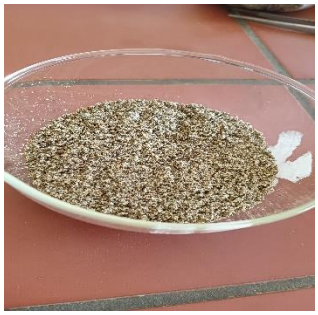
A large evergreen palm, a small, fast-growing mimosoid tree native to northwestern parts of Mexico, is now naturalized in Florida, California, Spain, and Italy. It is a popular landscape palm in areas where it is hardy throughout the world.**[11]** Washingtonia seeds are usually not used much in traditional nutrition, but like many plant seeds they contain phenolic compounds, flavonoids, and tannins, and these compounds are known for their properties as powerful antioxidants. Antioxidants help neutralize free radicals in the body, protecting cells from damage and reducing the risk of diseases.**[12]**

II. Methods of oil extraction:

II.1. Preparation of plant material:

Prepare plant material by collecting the desired plant parts, then carefully cleaned to remove impurities and dust, dried in a shaded and ventilated place to preserve effective compounds. After drying, the material is ground into a fine powder or cut into small pieces depending on the type of extraction method.

Table II.1: Plant Prepared in the laboratory

		
Washingtonia	Chia	Tigernut
		
Bay laurel	Apricot kernel	Lavender

II.2. Soxhlet extraction of vegetable oil:

Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction field, which has been used for a long time, is a standard technique and the main reference for evaluating the performance of other solid-liquid extraction methods. Extraction by Soxhlet is a general and well-established technique, and which exceeds in performance the other conventional extraction techniques, except in the case of the extraction of thermo-labile compounds. **[13]**

- **Principle:**

In conventional Soxhlet, the sample is placed in a thimble-holder and during operation is gradually filled with condensed fresh solvent from a distillation Sask. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This operation is repeated until complete extraction is achieved. **[14]**

- **Protocol:**

1) Accurately weigh 50 g of dried and ground sample in a clean cartridge

2) Put the cartridge with its contents in the extractor (the upper edge must be above the level of the siphon) Introduce 450 ml of n-hexane into the pre-calibrated lapped neck flask

3) Install the extractor on the flask and put everything below the refrigerant

4) Circulate the water in the refrigerants and turn on the plates. It should be noted that it is necessary that the Soxhlet appliance is well protected under a hood they must be away from any source of fire.

Figure II.1: Soxhlet Apparatus for Extracting plants oils

5) Stop boiling when the level of the condensed n-hexane in the extractor is a little below the siphon level, carefully remove the cartridge without losing the particles of the sample and empty the n-hexane contained in the extractor. The extraction time is 4 h

6) After that turn off the device and let it cool.

7) Remove the solvent by evaporation in a steam rotavapor

Dry the flask containing the extract and the little n-hexane in the oven at 105 ° C overnight (leave the oven door slightly open for the first 15 minutes)

8) Weigh the flask after cooling (cooling can be done at room temperature except that the flask must be equipped with its cap).

II.3. Extraction of essential oil by Clevenger hydro-distillation:

Hydro-distillation is the simplest and cheapest distillation method. It is employed in the manufacture of volatile compounds of various aromatic herbs and flowers. This method can result in two products: an essential oil as well as a watery herbal distillate. The essential oils are often used in perfumery and aromatherapy, while the watery distillates have many applications in aromatherapy, food processing, and skin care.

- **Principle**

A mixture of water and samples is brought to a boil in a round-bottomed flask. The water vaporizes. Under the effect of heat and water, the plant cells break and release the essential oil, which is entrained in a gaseous state with the water vapor towards the refrigerant. The latter therefore makes it possible to condense (make liquid) the vapors.
At the outlet of the refrigerant, the distillate consisting of:

➤ Above, a very fragrant organic phase containing the odorous compounds.

➤ Below, an aqueous phase containing a very small amount of the oil. This phase is called the hydrolat.

- **Protocol:**

The essential oils were extracted by hydro-distillation method using a Clevenger equipment, of two aromatic plant species of high therapeutic value: ***Laurus nobilis*** **(**Bay laurel) and ***Lavandula angustifolia*** **(**Lavender) followed the next steps:

1. **Solid-liquid extraction**

1) The operation consists in immersing a quantity of the vegetal mass in a large glass flask.

2) Containing a sufficient quantity of distilled water without completely filling the flask (the contents of the flask must not exceed two thirds) to avoid overflows during boiling. The mixture is brought to a boil using a balloon heater.

3) The vapors charged with the essential oil pass through the vertical tube.

Figure II.2: clevenger Apparatus for Essential oil Extraction

4) Then through the refrigerant, where the condensation will take place. The droplets thus produced accumulate in the tube filled beforehand with distilled water.

6) Due to the difference in density, the oil supernatants on the surface of the distilled water.

2. **liquid liquid extraction**

Transfer the distillate to a decanting funnel. Carry out three extractions each time with 20 ml of chloroform (or dichloromethane). Review the organic phases.

3. **Drying and filtration:**

The oil obtained is recovered and then dried with a desiccant, sodium sulfate, to eliminate the little water likely to have been retained in the oil and filter.

4. **Evaporation:**

Remove the solvent by distillation in a rotary evaporator. In practice, a rotary evaporator is used which makes it possible to remove the solvent under vacuum.
All extracts were stored at 4 °C until analysis.

III. Characterization of the extracted oils:

III.1. Organoleptic study:

Organoleptic is defined as being perceivable by the senses such as smell, appearance, taste, touch, odor etc. **[15] [16]**

- **Color:** is the first organoleptic attribute that seen by consumers in consuming a product.
- **Aroma:** is a smell that caused by chemical stimuli that were smelled by olfactory nerves that are in the nasal cavity.
- **Aspect**: Aspect refers to a set of physical and visual characteristics that can be observed with the naked eye, such as the physical state (solid, liquid, semi-solid, or gel-like).

III.2. Extraction yield: The percentage extraction yield (%) is defined as being the ratio between the weight of the see extract in grams and the weight of the dry plant in powder. It is calculated using the following equation:

$$\text{Yield } \% = M_0/M_1 \times 100$$

M_0: mass in grams of the dry extract.

M_1: mass in grams of the initial dry plant material**.**

IV. Evaluation of the antioxidant activity:

The antioxidant activity of the various plant extracts were determined by the following methods: free radical DPPH test, alkaline DMSO superoxide and phenanthroline activity.

IV.1. Free radical DPPH test:

- **Principle:**

The reduction of the free radical DPPH by an antioxidant can be followed by UV-visible spectrophotometry, by measuring the decrease in absorbance at 517 nm caused by the presence of the synthesized products. The DPPH is initially violated, decolorizes when the single electron pairs. This discoloration is representative of the ability of the products to trap these free radicals independently of any enzymatic activities. **[17]**

- **Protocol:**

1) Preparation of the DPPH Solution:

-Dissolve 1 mg of DPPH (powder) in 10 ml of methanol and mix until completely dissolved (Use a magnetic stirrer or stir manually).

-Measure the absorbance at 517 nm using a Spectrophotometer up to A = 0.6.

2) Sample preparation:

-Dilute the extracts in the same solvent as that used for the DPPH.

3) Mixing:

-Mix 40 µl of the sample with 160 µl of DPPH Solution and shake well.

-Incubation for 30 minutes at room temperature.

-Measure the absorbance at 517 nm using a Spectrophotometer.

-The % inhibition of the anti-radical activity was calculated using the following equation:

$$\text{IC50 (\%)} = (A_{control} \times A_{sample}/A_{control}) \times 100$$

A control: is the absorbance of the control reaction containing all the reagents with the exception of the extract.

A Sample: is the absorbance of the compound to be tested.

IV.2. Alkaline DMSO superoxide test:

- **Principle:**

Some studies have demonstrated antioxidant activity for DMSO substance as reported by Engelmann and Velasco et al. These authors reported on the antioxidant capacity related DMSO deriving promoting activity with calcium ions and its ease to interact at the molecular level with various elements such as proteins, lipids, carbohydrates and, consequently, radical stabilizing and reducing the levels of free electrons. For Sturion et al. **[18]**

- **Protocol:**

1) Preparation of the DMSO Solution:

In a 100ml vial dissolve 20mg of NaOH in 1ml H_2O and complete with DMSO up to 100ml

2) Preparation of the NBT Solution:

In a beaker mix 10 mg NBT and 10 ml H_2O

3) Mixing:

40 l extract + 130 µl DMSO alkaline solution + 30 µl NBT solution

IV.3. Phenanthroline activity:

- **Principle:**

Ortho substituted phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro-oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is measured al 510 nm. **[19]**

- **Protocol:**

1) Preparation Phenanthroline (0.5%):

0.05g of 1, 10-Phenanthroline in 10ml of MeOH

2) Preparation Ferric chloride FeCl3 (0.2%):

0.02g of FeCl3 in 10 ml of H_2O

3) Mixing:

10 µl extract + 50 µlFeCl3 (0.2%) +30 µl Phenanthroline (0.5%) + 110 µl MeOH+ incubation in the dark for 20 min at 30°C + reading at 510 nm. The BHT is used as a standard.

V. Identification by Gas Chromatography-Mass Spectrometry GC-MS:

- **Definition:**

Gas chromatography coupled with mass spectrometry is today one of the most widely used techniques in analytical chemistry. Compound identification in gas chromatography mass spectrometry (GC-MS) is currently achieved by comparing a query mass spectrum with reference mass spectra in a library via spectrum matching. **[20]**

Figure II.3: Gas chromatography Mass Spectrometry (GC-MS) for chemical analysis

- **Principle:**

The CG-SM is an analysis technique that combines the performance of gas chromatography, it is suitable for volatile samples that can evaporate upon heating.

Gas chromatography can separate analyte components by partitioning between the gaseous mobile phase and the stationary phase at different retention times; finally, all components are eluted, and the detector identifies them. The detector is a mass spectrometer that can decompose molecules into ionized fragments and detect these fragments at their characteristic mass-to-charge ratio (m/z). **[21]**

- **Protocol**

The chemical profile of the extract oils was screened using Gas Chromatography-Mass Spectrometry-GCMS. The analysis was performed using a Saturn 2000 gas chromatograph, which was outfitted with a fused silica capillary DB-5MS column (30 × 0.25 mm, with a film thickness of 0.25 µm). He_2 gas served as the carrier in this analytical procedure. The chromatographic conditions were programmed as follows: an initial hold at 60 ◦C for 1 min, a subsequent increase to 150 ◦C at a rate of 10 ◦C/min, followed by a 1 min hold. A second gradient was then set to reach 260 ◦C at a rate of 12 ◦C/min, where it was held for 10 min. The temperature of the trap was maintained at 220 ◦C, while the transfer line was heated to 240 ◦C. Mass spectra were recorded within the range of 70 to 650 m/z. 5 µL of each prepared

extract was injected. Identification of the compound was obtained by comparing the retention time with the original compound with spectral data obtained from the appropriate compound data library. The number of compounds is represented as a percentage of the relative area originating from the integrator. The chemical structure profile screening was based on analysis of the mass spectrum fragmentation pattern compared with the mass spectrum in the National Institute of Standards and Technology (NIST) and Wiley compound profile databases.

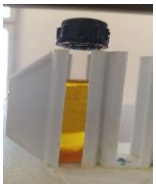
Part 2: Discussion of results

I. Characterization Organoleptic study of the oils:

Conducting an organoleptic Descriptive Study of oils extracted from various plant sources, by evaluating their aspect, color and aroma.

Table II.2: A Descriptive Sensory Evaluation of Oils Extracted from Various plant Sources

Plants	Picture	Aspect	Color	Aroma
Lavender		liquid	Yellowish green	Aromatic and sharp
Bay laurel		liquid	Transparent to very pale yellow	Floral and fresh
Tigernut		liquid	yellow	Nutty and sweet

Chia		liquid	yellow to golden	Light natural and mildly nutty
Apricot kernel		liquid	yellow	Nutty and mild
Seed of Washingtonia robusta		liquid	Light yellow to golden	Light, oily and slightly earthy

- ✓ The organoleptic study showed a pronounced unevenness of color and smell, reflecting the chemical diversity of their components. The colors of the oils ranged from pale yellow to gold, with bay laurel oil recording a characteristic greenish tint, which may indicate the presence of chlorophyll or phenolic compounds.
- ✓ In terms of Aroma, Essential oils such as Laurel and lavender have been characterized by strong, sharp or fresh floral aromas, which supports their use in the cosmetic and therapeutic fields. As for vegetal oils such as apricot kernel oil, shea oil, and Washingtonia love oil, they were characterized by a light or nutty smell.
- ✓ It is noted that Washingtonia seed oil possesses mild aromatic properties with a slight greasy character, which makes it a potential candidate in the food or cosmetic industries.

II. Extraction yield:

The oil recovery yield ratio is calculated according to the relationship:

$$\text{Yield \%} = M0/M1 \times 100$$

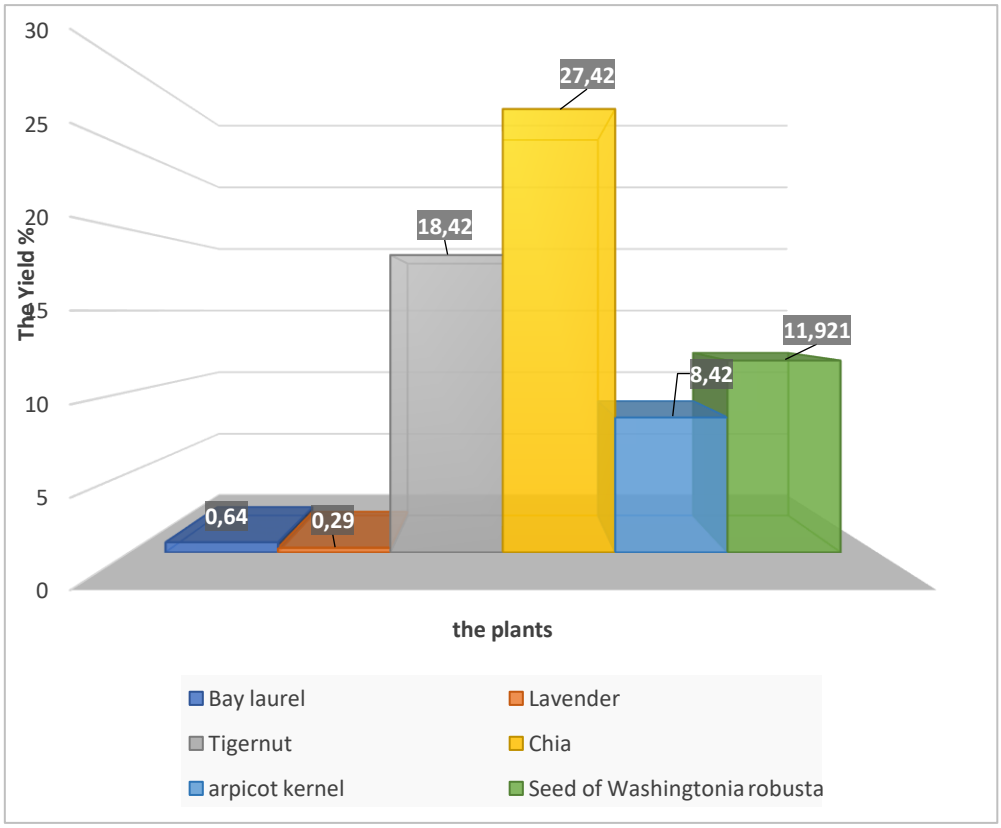


Figure II.4: Bar chart comparison of extracted oils based on yield

The above **Figure II.4** shows the yield ratios (%) for extracting oils from six different types of plants. The graph shows that the Chia plant recorded the highest yield of 27.42%, followed by Tigernut with 18.42%, and then Washingtonia robusta seeds with 11.921%, Apricot kernel are also scored by 8.42%, Other plants, such as Lavender and bay laurel recorded relatively low yields of 0.29% and 0.64%, respectively. Compared to vegetable oils, however, these ratios are good for the basic ones.

The difference between the nature of fixed oils and volatile oils is clear, as the former is characterized by its high concentration, which makes it effective in therapeutic.

III. Evaluation of the antioxidant activity:

The following table shows the IC50 or A0.5 results of three antioxidant tests DPPH, DMSO, phenanthroline where the smaller the value the more the extract is able to neutralize free radicals or iron reductive.

Table II.3: The antioxidant assays results

Source oil	DPPH	DMSO	phenanthroline
Lavender	381.01±3.2	712.49±2.8	66.006±1.4
Bay laurel	<50	270.78±7.2	>1.25
Apricot kernel	<80	92.91±2.7	15.48±1.34
Chia	71.10±3.42	62.05±7.7	21.37±2.6
Tigernut	>800	112.21±0.7	157.62±11,4
Washingtonia	>800	120.93±8.8	128.77±2.04
BHT	38.87±0.1	40.21±0.3	6.52±0.07
	IC50	**IC50**	**A0.5**

- The results showed that Bay laurel, Chia and apricot kernel showed the best free radical inhibitory ability in the DPPH test compared to other vegetable oils, indicating that it contains effective various bioactive compounds, while the Chia plant and apricot kernel has a remarkable activity in the phenanthroline test, which may reflect a wealth of compounds with a reducing ability.
- The rest of the plant extracts showed a marked variation in their antioxidant activity depending on the type of test. Apricot kernel oil extract was acceptable effective in the DPPH test, but showed good activity in the polar medium (DMSO), which may indicate the presence of compounds with suitable polarity. As for the chia extract, despite its limited effectiveness in neutralizing free radicals, it was characterized by a high reductive ability in the phenanthroline test, reflecting its content of electron-donor compounds. On the other hand, both Tigernut and Washingtonia showed poor efficacy in all tests, indicating that their chemical composition may lack effective antioxidant compounds or that their concentration is insufficient to achieve a pronounced effect.

Figure II.5: Spectrophotometer for measuring antioxidants activity (DPPH assay)

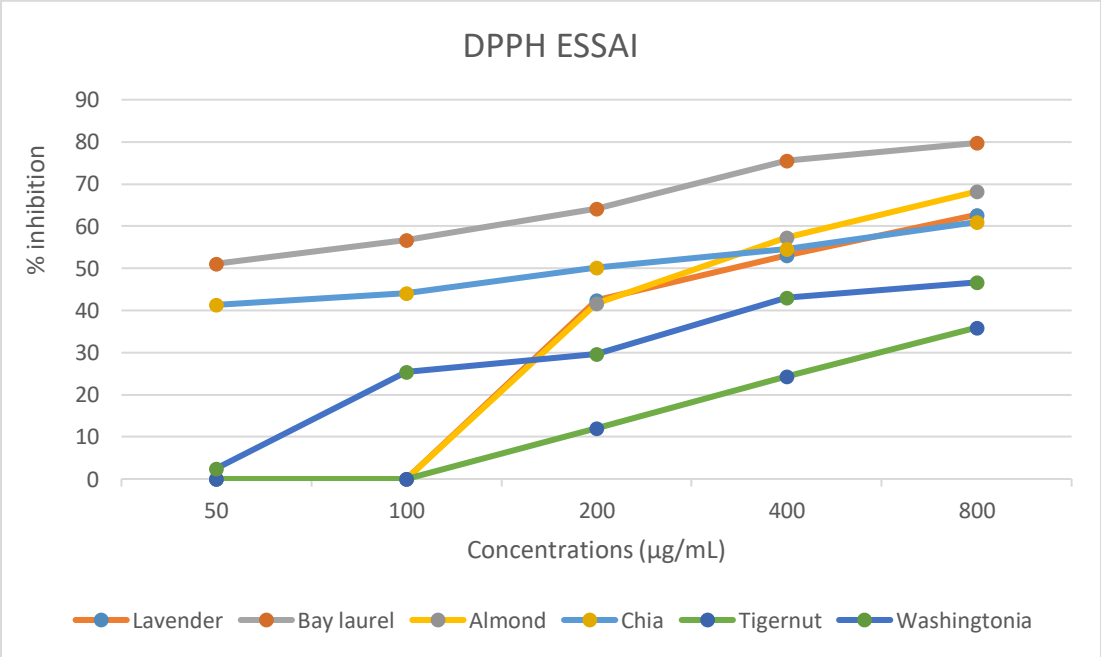


Figure II.6: Effect of plant extract concentration on DPPH activity

- The antioxidant activity of several plant extracts was analyzed using DPPH Testing, the concentrations ranged from 50 to 800 (µg/mL), and the percentage inhibition was measured.

- All extracts showed a direct relationship between concentration and inhibition ratio, which shows that their effectiveness increases with increasing concentration, where between bay leaf extract and lavender, good effectiveness with a rapid rise in the

inhibition ratio of bay leaf, as for apricot kernel extract and Shea showed good activity and the rest showed relatively less activity.

Figure II.7: Spectrophotometer for measuring antioxidants activity (DMSO assay)

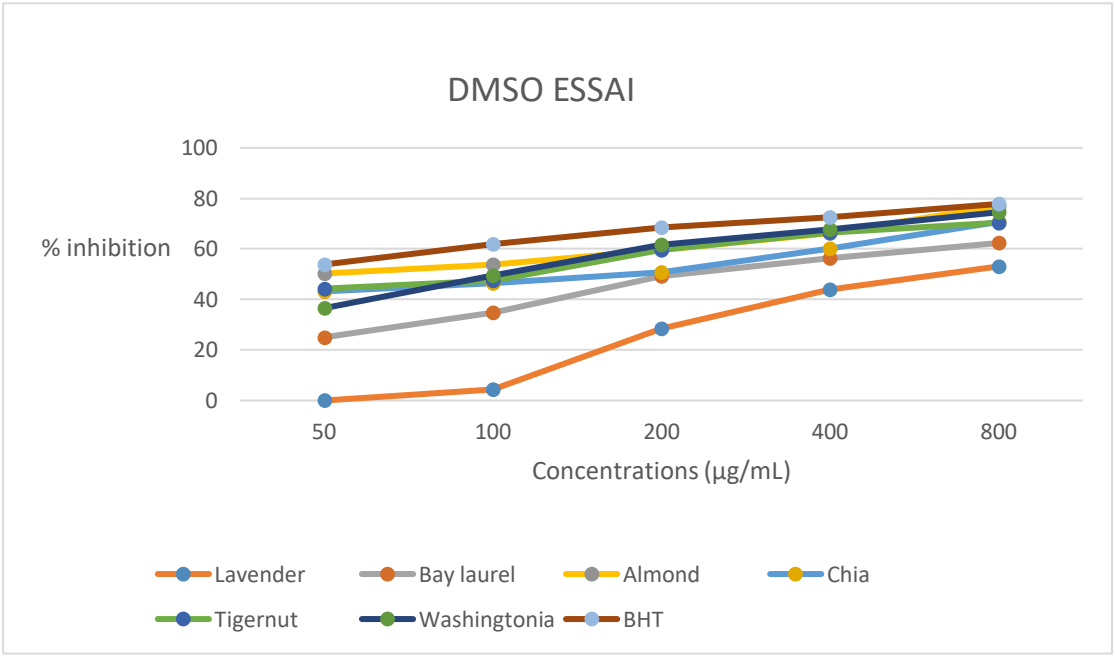


Figure II.8: Inhibition (%) of DMSO at different concentration

- The graph shows the percentage of inhibition of free radicals by various plant extracts at concentrations ranging from 50 to 800 µg / mL. Use BHT as a reference material for comparison. Most extracts showed a gradual increase in efficacy with higher

concentration, while BHT achieved the highest inhibition as expected from a strong reference, allowing a relative assessment of the antioxidant efficacy of the extracts.

Figure II.9: Spectrophotometer for measuring antioxidants activity (phenanthroline assay)

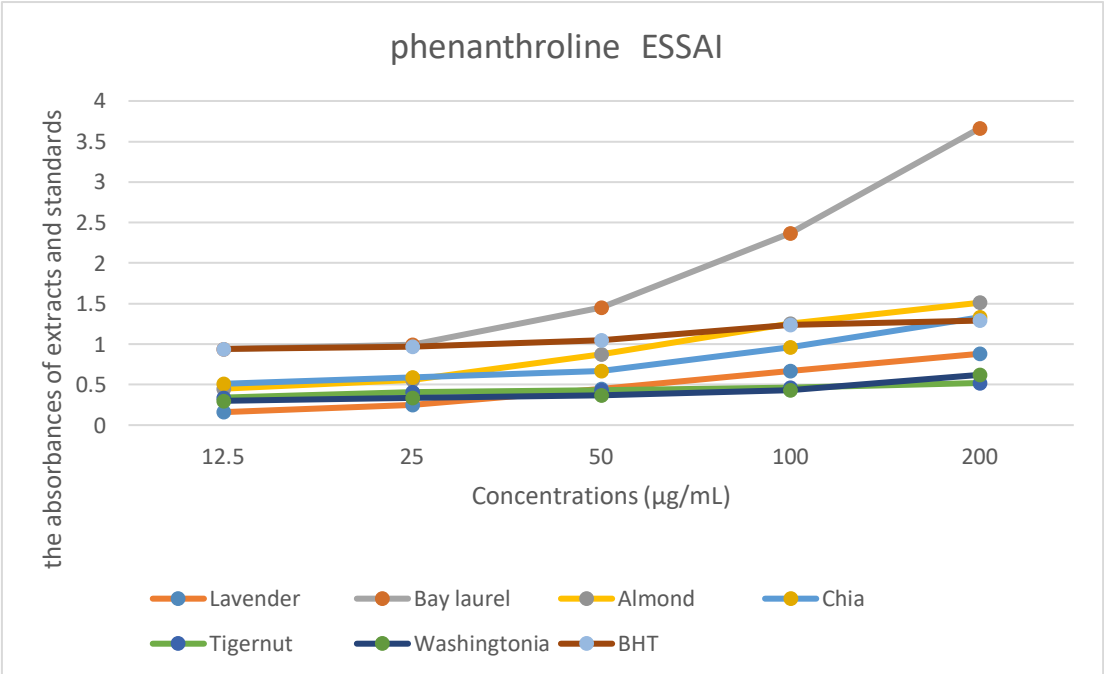


Figure II.10: Absorbance of extracts and Standards at different concentration using the Phenanthroline assay

- The graph shows the absorbability of various plant extracts at increased concentrations in the phenanthroline test. The BHT compound was used as a reference material to compare the reductive efficacy. All extracts showed a slight increase in absorbability

with high concentration, with efficacy remaining significantly lower than the reference BHT, reflecting a limited reductive ability compared to the standard substance.

IV. Result of chromatography GC-MS Analysis:

Gas chromatography-mass spectrometry (GC/MS), which has become a standard technique using different phases and dimensions for their separation, carried out the identification of the essential oil and vegetal oil components in order to achieve their correct characterization and even their isolation through preparative applications. It is the main and the most universally employed analytical tool for quantitative and qualitative analysis of these complex chemicals. **Figure II.11** shows chromatograms of the vegetal oil of chia and apricot kernel, in which efficient separation of different components, including geometric isomers such as cis and trans.

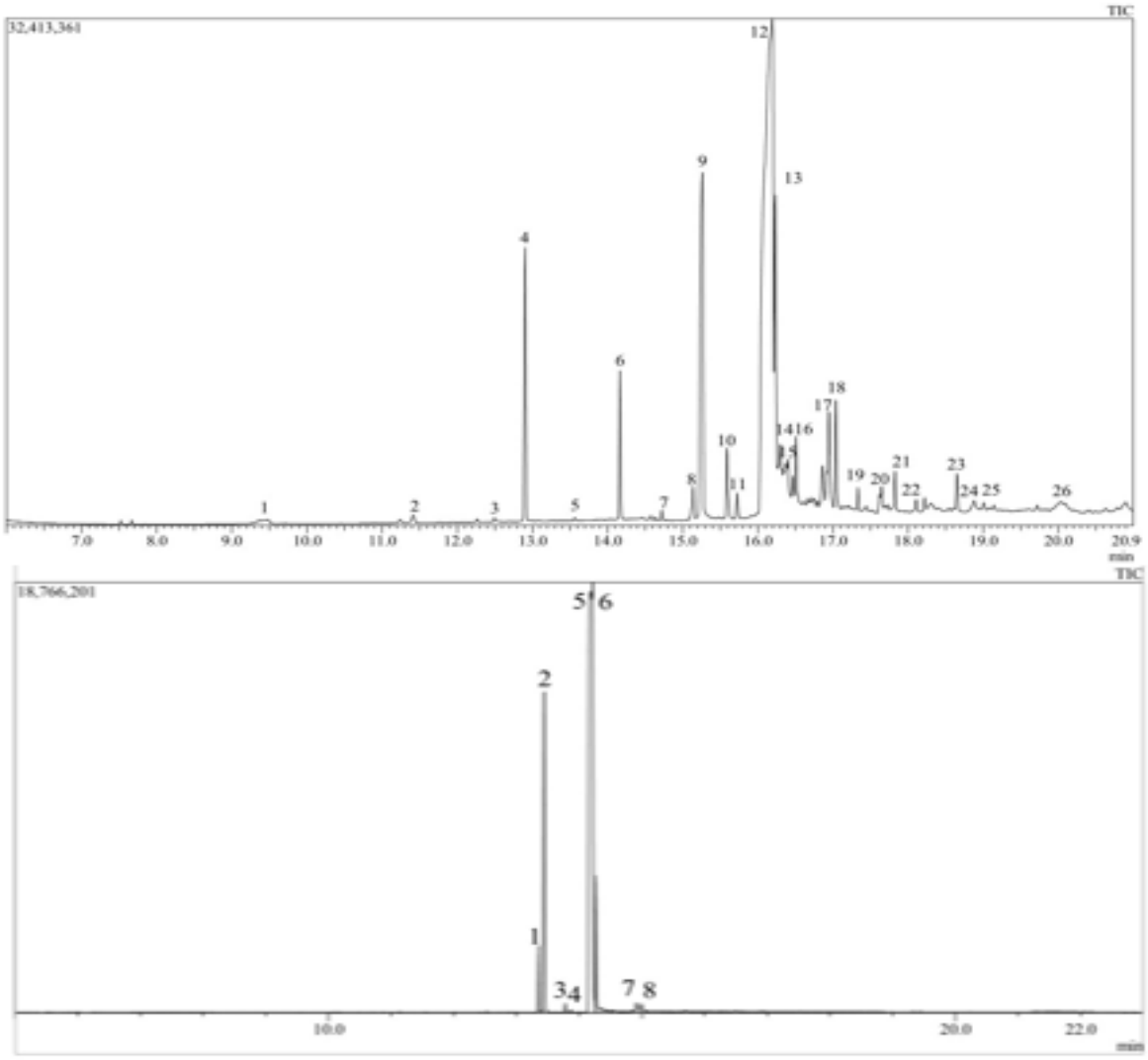


Figure II.11: Chromatograms showing the components of chia and apricot kernel oils

According to the previous study, these two fractions have excellent antioxidant activity with good yields. The analysis by GC-MS method has led to the identification of 26 compounds in vegetal oil of **Chia** and 08 compounds in **Apricot kernel** oil, all the result are rassembled in the following tables.

Table II.4: Chemical profile of extract oils, A Chia oil, B Apricat oil

Peak Number	Ret Time	Area	Area %	Name of compounds	A
1	9.365	3267548	0,51	OCTANOIC ACID, METHYL ESTER	
2	11.412	1578160	0,25	DECANOIC ACID, METHYL ESTER	
3	12.263	375653	0.06	Nonanoic acid, 9-oxo-, methyl ester	
4	12.904	25096703	4.00	DODECANOIC ACID, METHYL ESTER	
5	13.500	140236	0.02	8,11,14-Eicosatrienoic acid, methyl ester	
6	14.164	13147060	2.10	TETRADECANOIC ACID, METHYL ESTER	
7	14.723	788385	0.13	PENTADECANOIC ACID, METHYL ESTER	
8	15.140	3806785	0.61	9-Hexadecenoic acid, methyl ester, (Z)-	
9	15.263	64237524	10.24	HEXADECANOIC ACID, METHYL ESTER	
10	15.591	7240224	1.15	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	
11	15.725	2644480	0.42	Hexadecanoic acid, 15-methyl-, methyl ester	
12	16.193	271094213	43.38	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	
13	16.233	34028459	5.43	OCTADECANOIC ACID, METHYL ESTER	
14	16.298	15642024	2.49	1,E-11,Z-13-Hexadecatriene	
15	16.403	7336041	1.17	10-NONADECENOIC ACID, METHYL ESTER	
16	16.509	13937901	2,22	NONADECANOIC ACID, METHYL ESTER	
17	16.946	18595957	2.96	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	
18	17.040	11756305	1.87	EICOSANOIC ACID, METHYL ESTER	
19	17.332	3285567	0.52	HENEICOSANOIC ACID, METHYL ESTER	
20	17.648	3214441	0.51	ETHYL (9Z,12Z)- OCTADECADIENOATE	
21	17.826	6146441	0.98	DOCOSANOIC ACID, METHYL ESTER	
22	18.109	5 995 499	0.96	TRICOSANOIC ACID, METHYL ESTER	
23	18.651	5250124	0.84	TETRACOSANOIC ACID, METHYL ESTER	
24	18.878	5407686	0.86	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-	
25	19.007	2164343	0.35	Pentacosanoic acid, methyl ester	
26	20.039	5320786	0.85	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	

Peak Number	Ret Time	Area	Area %	Name	B
1	13.354	3184125	2.48	HEXADECANOIC ACID, METHYL ESTER	
2	13.442	17492587	13.61	8,11,14-Eicosatrienoic acid, methyl ester	
3	13.779	438967	0.34	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	
4	13.868	131735	0.10	Hexadecanoic acid, 15-methyl-, methyl ester	
5	14.182	87184149	67.93	8,11-Octadecadienoic acid, methyl ester	
6	14.270	6658501	5.18	Octadecanoic acid, methyl ester	
7	14.915	1098382	0.85	11-EICOSENOIC ACID, METHYL ESTER	
8	15.140	3806785	0.61	9-Hexadecenoic acid, methyl ester, (Z)-	

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Chapter III

In-silico study

I. Introduction:

Hyperpigmentation occurs when an area of skin becomes darker than the surrounding skin. Hyperpigmentation may worsen after sun exposure, as melanin absorbs UV rays. Application of cream may help protect against further darkening of hyperpigmented spots. It is a darkening of the skin that is most often caused by an abnormally high level of melanin.

Free radicals play a major role in causing oxidative damage to cells, which contributes to accelerated aging and pigmentation disorders, tyrosinase is one of the main enzymes in the process of melanin synthesis, which contributes to oxidative stress, and therefore, inhibition of tyrosinase is an important goal to reduce free radicals.

In this study, molecular docking techniques were used to evaluate the effectiveness of a group of compounds obtained as potential inhibitors of the tyrosinase enzyme. A simulation of the interaction between these compounds and the active site of the enzyme was performed. This calculation method enables experimental studies to be directed towards the most effective compounds as natural antioxidants, which is an asset for research in this field.

II. molecular docking:

Molecular docking is the in-silico method and a kind of computational modeling that anticipates the favoured orientation of ligand against receptor to make a stable complex and uses electrostatic, Van der waals, coulombic interactions and hydrogen bonds to quantify it. The sum of all these interactions is approximated by a docking score, which represents the potentiality of binding. **[1]**

The receptor is most of the time a protein, while the ligand can be another protein, a nucleic acid or a small molecule (a potential drug, substrate, inhibitor, etc.). **[2]**, **[3]**

II.1 The principle of docking:

Molecular docking is a method that makes it possible to predict the structure of a molecular complex by studying the modes of interaction between two isolated molecules. This method is often used in biology, pharmacy and medicine, because it makes it possible to predict the interaction between a small compound (ligand) and a biological target of therapeutic interest, usually a protein. Docking software are therefore valuable tools for the discovery of new drugs, because they make it possible to reduce the number of tests necessary to find active compounds by simulating their interaction with the biological target. **[4]**

The simulation is carried out in two main stages:

a. Docking: This stage involves predicting the optimal spatial conformation of the ligand when it binds to the active site of the protein. The docking programmes calculate the possible positions of the ligand relative to the protein, taking into account the physicochemical interactions between the two molecules, such as hydrogen bonds and van der Waals interactions. **[5]**

b. Scoring: This step evaluates the quality of the poses. It is based on different criteria, such as binding energy, shape complementarity, and other factors that determine the strength of the interaction between the ligand and the protein. Poses with the highest scores are considered the most probable. **[5]**

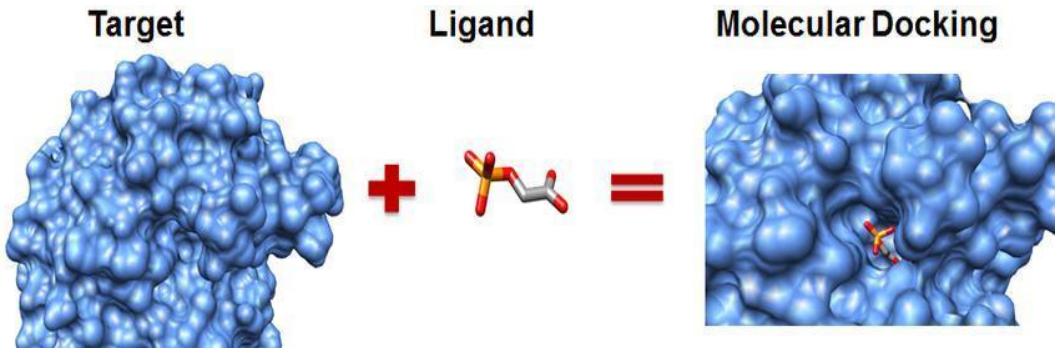
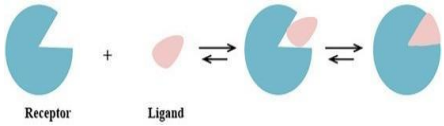
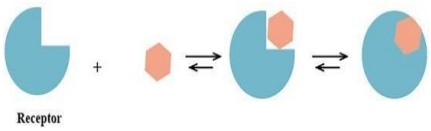


Figure III.1: Illustration of the Molecular Docking Process Between a Target Protein and a Ligand

II.2 Types of molecular docking:

The molecular docking simulation is mainly based on the ligand -protein association which can be considered rigid, flexible or semi-flexible (**Table III.1**).

Table III.1: Types of Molecular Docking: rigid, Semi Flexible and Flexible

Rigid docking	Semi-flexible docking	Flexible docking
The ligand and the receptor (protein) are considered as two rigid entities, the search for the optimal (stable) conformation is limited to positioning, listing all the rotations and translations possible for the ligand inside the interaction site, and assigning the appropriate energy values. **[6]** 	The molecular system is separated into two parts, a flexible part containing the ligand and the flexible residues of the active site and a rigid part containing the rest of the protein without the ligand. Semi-flexible Docking programs are the most effective. **[6]** 	In flexible docking, the ligand and the protein are flexible (the flexibility of the receptor concerns the lateral chains of the residues of the active site). The conformational degrees of freedom of the receptor can be limited to certain side chains or even consider wider movements involving, for example, the different possible arrangements between domains of a protein. **[7]** 

II.3 The molecular docking programs:

Initiated in the early 1980, this field has developed to become, nowadays, an essential tool in the search for biologically active products. **[7]** Currently, several molecular docking programs (commercial or not) are available. The most frequently cited are respectively: Doc, MOE, Auto-Dock Vina, GOLD, Flex, DOCK and ICM. **[8]**

III. Prediction ADMET in silico:

Pharmacokinetics (PK) is the study of how the body interacts with administered substances for the entire duration of exposure. The ADME-tox profile of a molecule is the set of parameters characterizing its bioavailability in the body, that is to say, its absorption, its distribution, its metabolism, its excretion and its toxicity. The democratization of in silico screening has led to the need for ADME-tox models to eliminate quickly compounds with the least similar physicochemical properties with the drugs available on the market (which are not "drug like").

Possessing an understanding of these processes allows practitioners the flexibility to prescribe and administer medications that will provide the greatest benefit at the lowest risk and allow them to make adjustments as necessary. **[9]**

III.1. Absorption

Absorption is the process that brings a drug from the administration, into the systemic circulation. Absorption affects the speed and concentration at which a drug may arrive at its desired location of effect, eg, plasma. There are many possible methods of drug administration, including but not limited to oral, intravenous, intramuscular, intrathecal, subcutaneous, buccal, rectal, vaginal, ocular, otic, inhaled, nebulized, and transdermal. **[10]**

III.2. Distribution

Distribution describes how a substance is spread throughout the body. This varies based on the biochemical properties of the drug as well as the physiology of the individual taking that medication. In the simplest sense, the distribution may be influenced by two main factors: diffusion and convection. **[11]**

These factors may be influenced by the polarity, size, or binding abilities of the drug, the fluid status of the patient (hydration and protein concentrations), or the body habitus of the individual. **[12]**

III.3. Metabolism

Metabolism is the processing of the drug by the body into subsequent compounds. This is often used to convert the drug into more water-soluble substances that will progress to renal clearance or, in the case of prodrug administration, such as codeine, metabolism may be required to convert the drug into active metabolites. **[13]**

III.4. Excretion

Excretion is the process by which the drug is eliminated from the body. The kidneys most commonly conduct excretion, but for certain drugs, it may be via the lungs, skin, or gastrointestinal tract. Medications may be cleared in the kidneys by passive filtration in the glomerulus or secretion in the tubules, complicated by reabsorption in some compounds.**[14]**

Part1: Materials and methods

I. Materials:

I.1. Microcomputer:

In our work, we used the MOE 2014 (0901) program. This software was installed on our computer named DESKTOP-P5EEVPP, which is equipped with 8.00 GB of RAM, an Intel Core i5-7200U processor running at 2.50 GHz (up to 2.71 GHz), and operates under the Windows 10 Professional 64-bit operating system.

I.2. Programs used:

- **ChemDraw 3D 17.1:**

ChemDraw is a molecular design software used to draw the structures of molecules used in molecular docking studies.

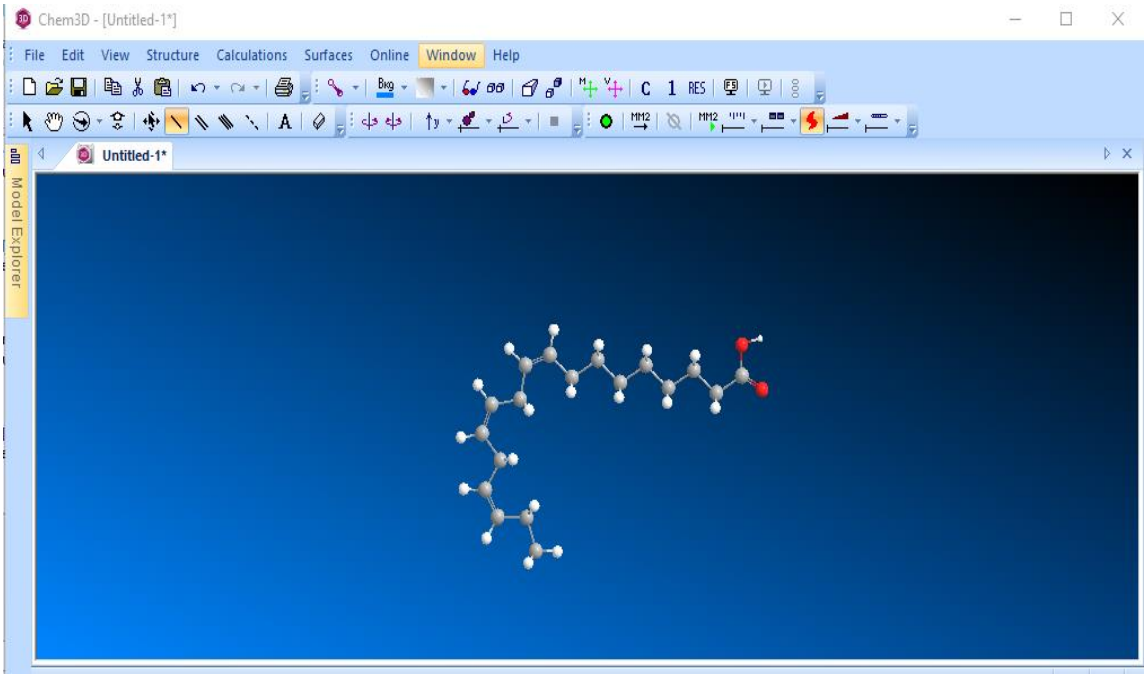


Figure III.2: chem Draw User interface

- **MOE software:**

The MOE software is based on the semi-flexible method and is commonly used for Target-ligand docking, the ligand was considered flexible and the main chain of the enzyme was kept fixed, while the side chains remained flexible. It uses the MMFF94x force field to optimize the conformations during the calculations. **[15]**

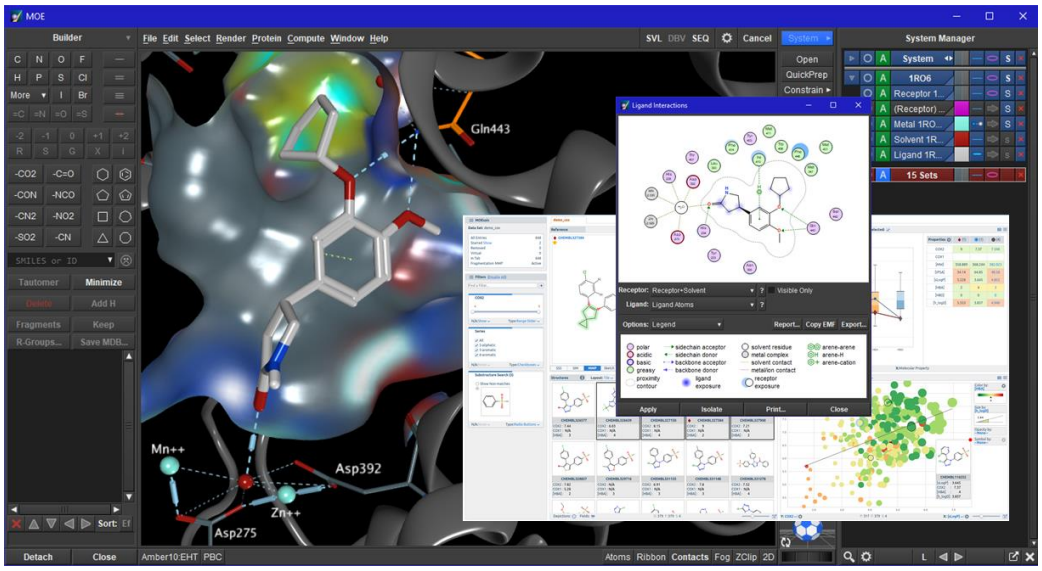


Figure III.3: the interface of the Moe Software

- **Discovery Studio Visualizer**

Discovery Studio is a complete of programs modelling and simulations environment for Life Science Researchers, providing functionality for visualizing and analyzing biological and chemical data. It has been used for visualizing results, generating 2D and 3D ligand-target interaction diagrams, and constructing ligands.

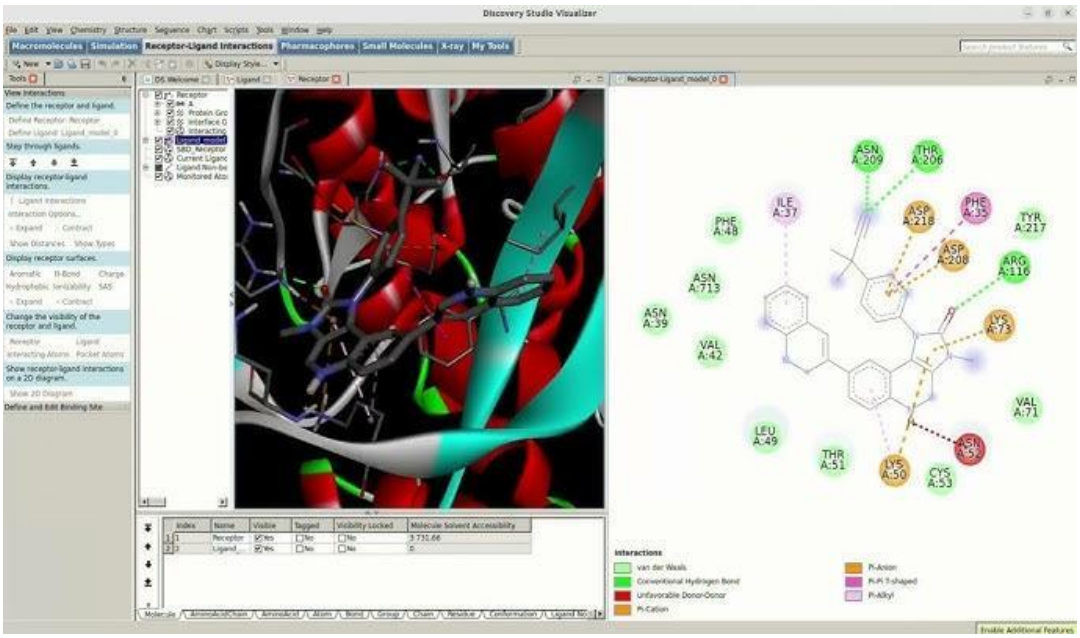


Figure III.4: the interface of the Discovery Studio

I.3. Data banks:

- **Protein Data Bank (PDB)**

The Protein Data Bank (PDB) the single global repository of experimentally determined 3D structures of biological macromolecules and their complexes was established in 1971, becoming the first open-access digital resource in the biological sciences. The PDB archive currently houses -130,000 entries. **[16]**

Figure III.5: Protein Data Bank (PDB) website

- **Pubchem**

The PubChem is a chemical database. Public from the National Library of Medicine (NLM), an institute of the (NLM), an institute of the National Institutes of Health (NIH) of the United States. It collects chemical information from more than 750 data sources and disseminates it to the public free of charge. **[17]**

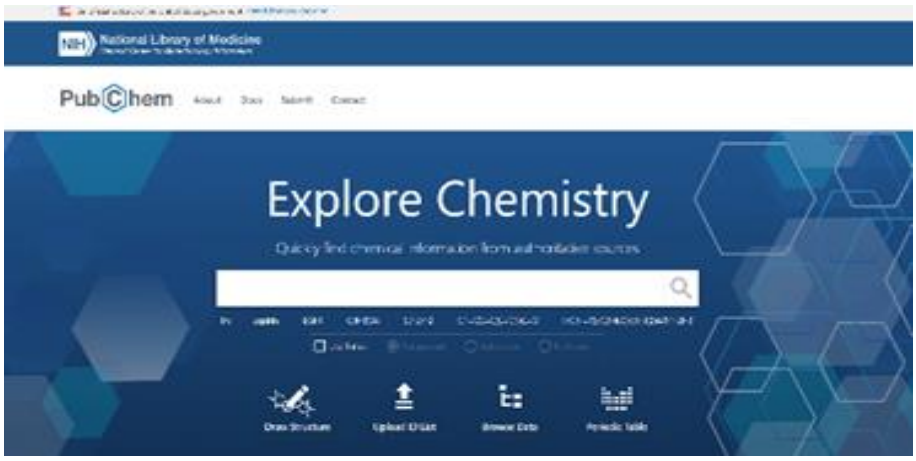


Figure III.6: Homepage of the Pubchem database

- **ADMETlab 3.0:**

ADMETlab 3.0 was used to assess the physico-chemical and therapeutic qualities of the chemicals found. **[18]**

Figure III.7: Homepage of the ADMETlab3.0 Web site

- **PkCSM:**

physicochemical and pharmacokinetic analyses were performed using the pkCSM web tool. This method provides valuable insights into the absorption, distribution, metabolism, excretion, and toxicity (ADMET). **[19]**

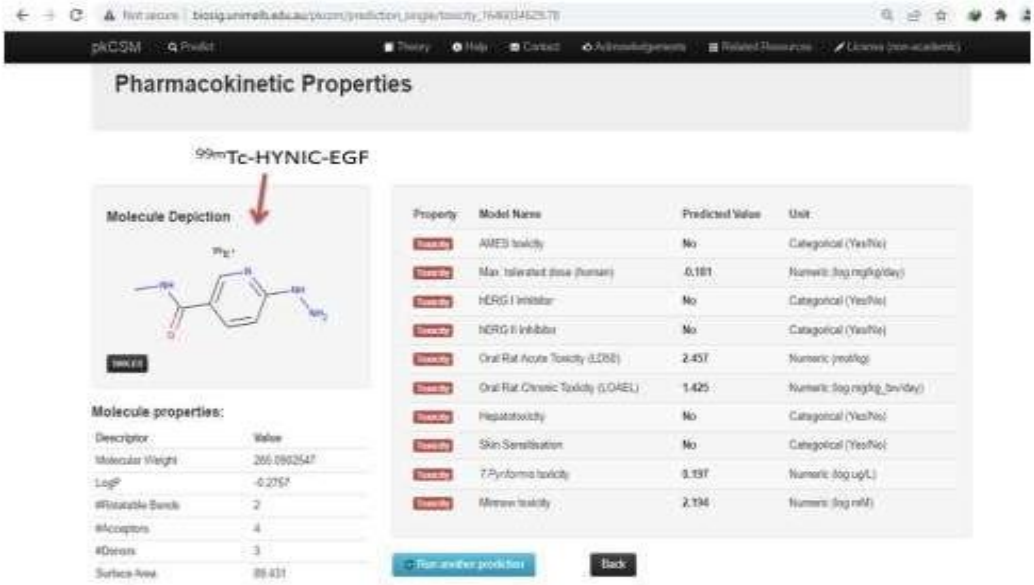


Figure III.8: Homepage of the PKCSM Website

II. Methods:

II.1. Calculation steps:

1) Preparation of Ligand:

The previous phytochemical and biological experimental study (chapter II) found the presence of biomolecules in the two plants ***Salvia hispanica*** (Chia) and ***Prunus armeniaca* L** (Apricot kernel), which exhibit an excellent antioxidant activity. The structure of these biomolecules was previously identified by a coupling technique, gas chromatography coupled with GC-MS mass spectrometry, in vegetable oil of selected plants. The results of the chemical profile of the prepared oils were presented in **Table II. 4 (chapter II).**

These myths are prepared for docking studies using various tools built into the Moe software, according to the following steps:

1. Obtaining molecular structures:

The structures of the compounds were obtained using GC-MS technology, and were hand-painted using ChemDraw. In the final step, the Lewis structures were corrected and any errors were removed.

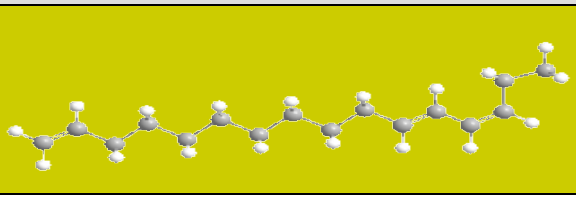
2. Molecular registratio

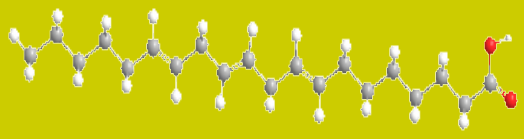
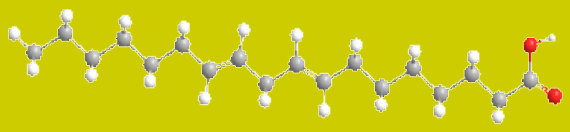
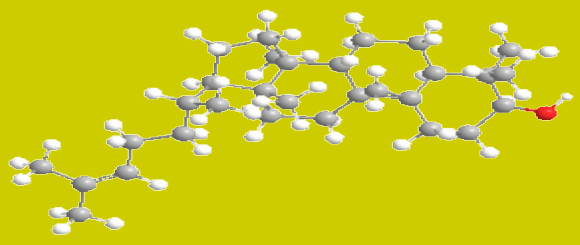
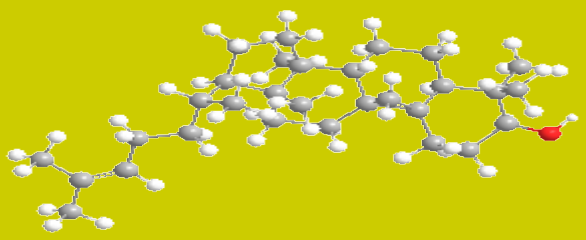
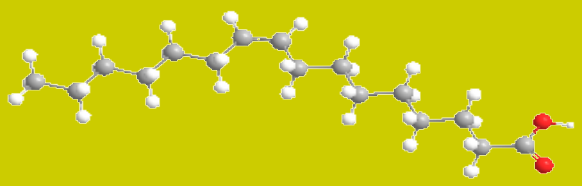
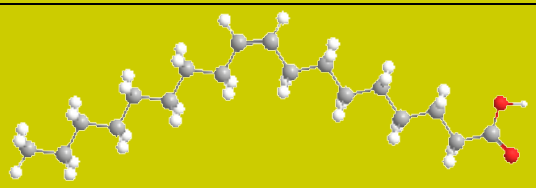
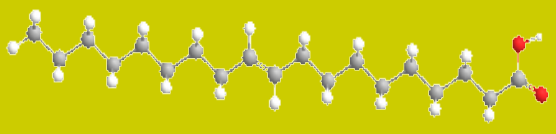
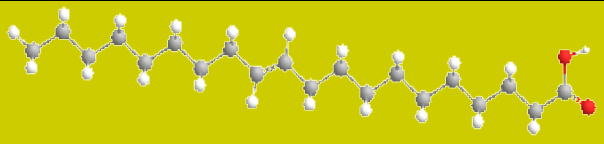
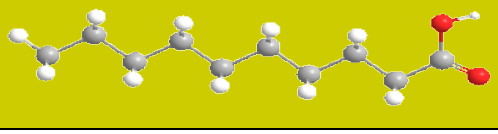
Twenty-eight molecules (28) were selected. The ChemDraw 3D software was used to convert the structures into 3D format. The files were then saved to SDF format, a standard format for molecular structures. **Table III.2** displays all the chemical structures of the ligand compounds utilized in this study.

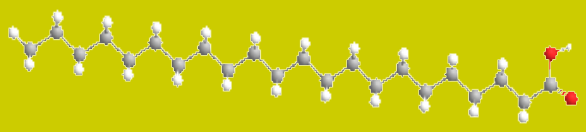
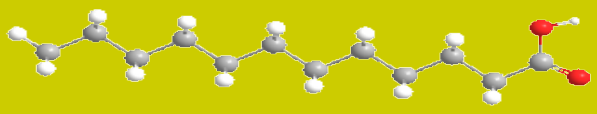
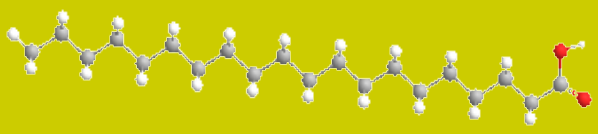
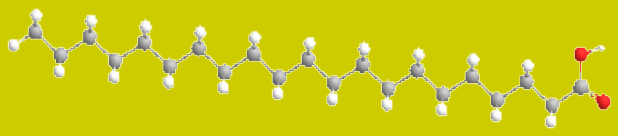
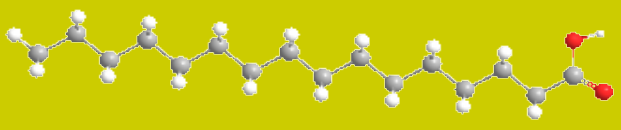
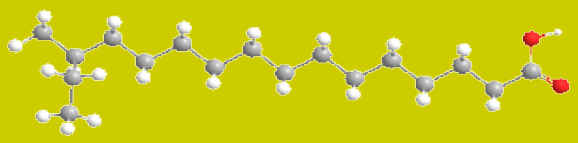
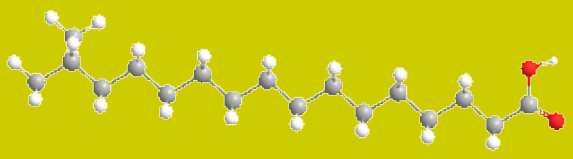
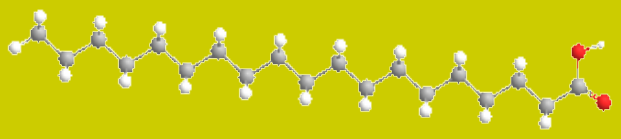
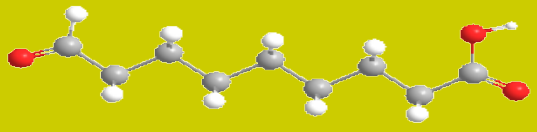
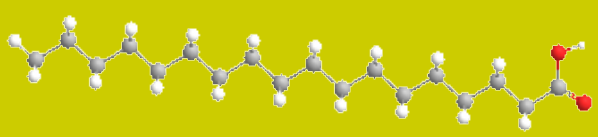
3. Improvement of structures:

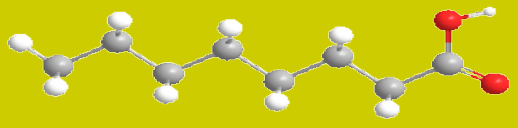
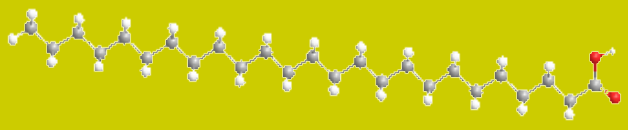
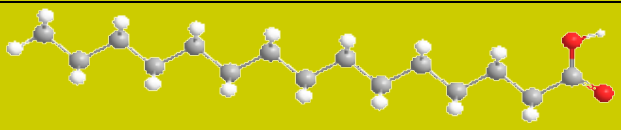
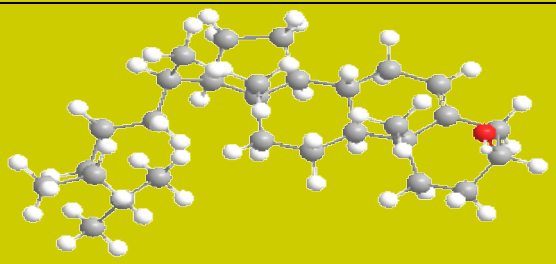
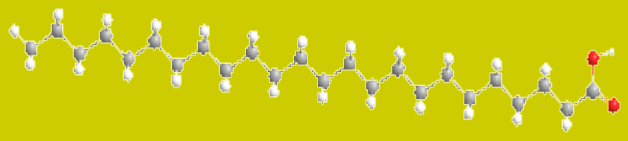
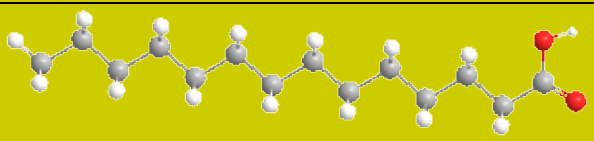
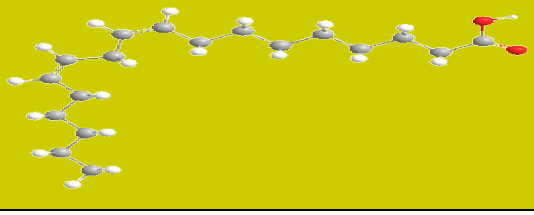
The files were then optimized using the Moe program, in order to obtain a stable matching with a docking-ready power.

Table III.2: Molecular Structures of compounds in chia and Apricot kernel Oil

ligands	Structures 3D	Formulas
L1 **1,11E,13Z-HEXADECATRIENE**		$C_{16}H_{28}$

L2 **8,11,14-EICOSATRIENOIC ACID**		$C_{20}H_{34}O_2$
L3 **8,11-OCTADECADIENOIC ACID**		$C_{18}H_{32}O_2$
L4 **9,12,15-OCTADECATRIENOIC ACID (Z,Z,Z)**		$C_{36}H_{60}O_4$
L5 **9,19-CYCLOLANOST-24-EN-3-OL, (3.BETA.)**		$C_{32}H_{53}O$
L6 **9-HEXADECENOIC ACID (Z)**		$C_{19}H_{36}O_4$
L7 **9-OCTADECENOIC ACID (Z)**		$C_{21}H_{45}O_8$
L8 **10-NONADECENOIC ACID**		$C_{19}H_{36}O_2$
L9 **11-EICOSENOIC ACID**		$C_{20}H_{38}O_2$
L10 **DECANOIC ACID**		$C_{10}H_{20}O_2$

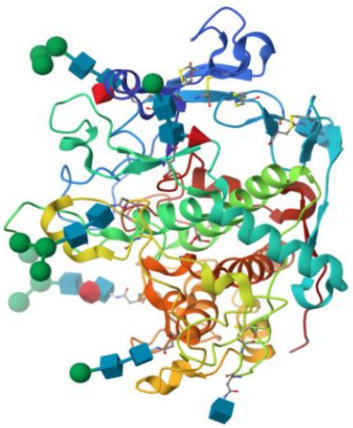
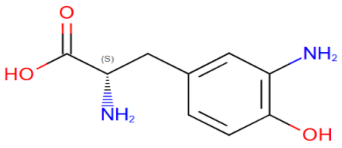
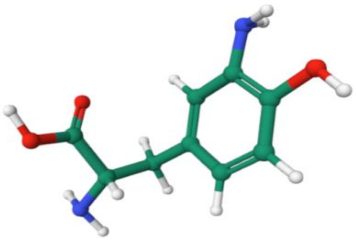
L11 **DOCOSANOIC ACID**		$C_{22}H_{44}O_2$
L12 **DODECANOIC ACID**		$C_{12}H_{24}O_2$
L13 **EICOSANOIC ACID**		$C_{20}H_{40}O_2$
L14 **HENEICOSANOIC ACID**		$C_{21}H_{42}O_2$
L15 **HEXADECANOIC ACID**		$C_{16}H_{32}O_2$
L16 **HEXADECANOIC ACID, 14-METHYL**		$C_{17}H_{34}O_2$
L17 **HEXADECANOIC ACID, 15-METHYL**		$C_{17}H_{34}O_2$
L18 **NONADECANOIC ACID**		$C_{19}H_{38}O_2$
L19 **NONANOIC ACID, 9-OXO-**		$C_9H_{16}O_3$
L20 **OCTADECANOIC ACID**		$C_{18}H_{36}O_2$

L21 **OCTANOIC ACID**		$C_8H_{16}O_2$
L22 **PENTACOSANOIC ACID**		$C_{25}H_{50}O_2$
L23 **PENTADECANOIC ACID**		$C_{15}H_{30}O_2$
L24 **STIGMAST-5-EN-3-OL, (3.BETA.,24S)**		$C_{29}H_{50}O$
L25 **TETRACOSANOIC ACID**		$C_{24}H_{48}O_2$
L26 **TETRADECANOIC ACID**		$C_{14}H_{28}O_2$
L27 **TRICOSANOIC ACID**		$C_{23}H_{46}O_2$
L28 **9Z,12Z-OCTADECADIENOIQUE ACID**		$C_{18}H_{32}O_2$

2) Preparation of proteins:

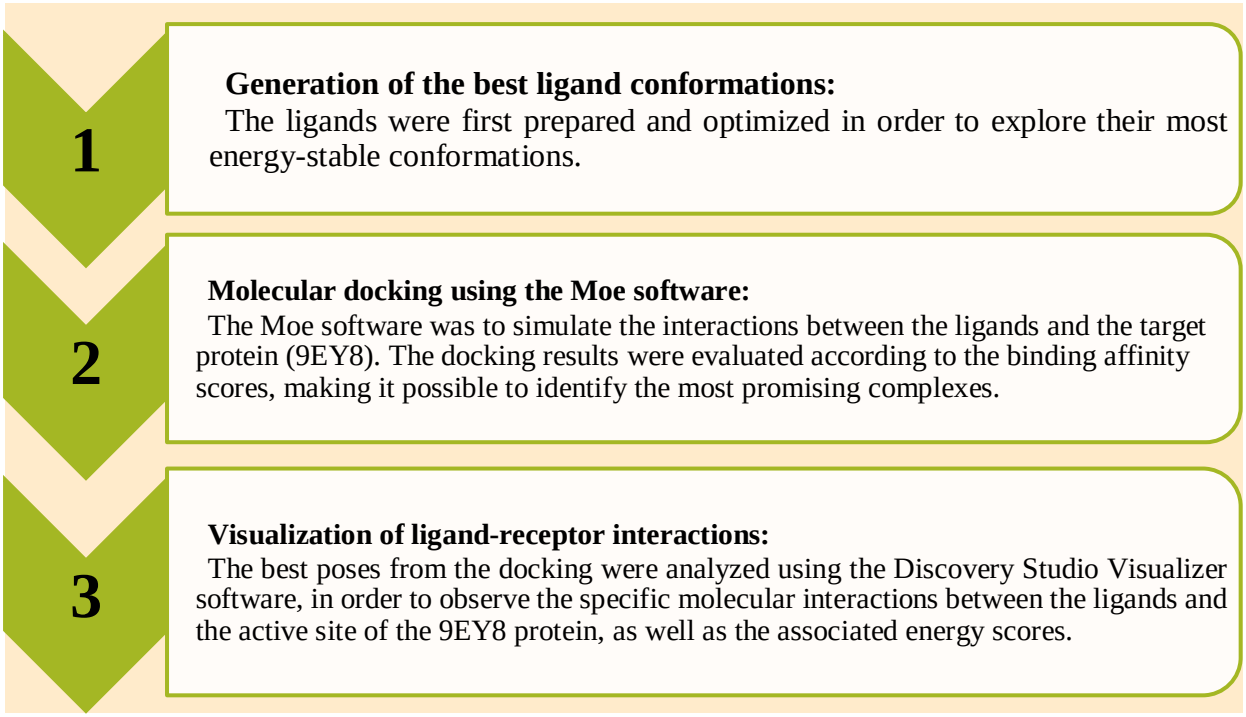
The 3D structure of the enzyme tyrosinase (pdb: 9EY8) uploaded to the database is in the form of a complex where it is linked to the Co-crystallized ligands (Ty2), the preparation of the receptor was done by elimination of the cofactors then the other protein chains.

Table III.3: crystal Structures of Human Tyrosinase (PDB-9E48) with co-crystallized Ligand

receiver	**tyrosinase**	**3D structure of 9EY8**
code	9EY8	
resolution	2.20 Å	
expression system	Homo sapiens	
the chains	A, B, C, D	
classification	METAL BINDING PROTEIN	
crystallized co ligand (Ty2)		

3) Molecular Docking:

The docking calculations were carried out in three main steps:



II Pharmacokinetics and toxicity properties prediction:

Evaluating pharmacokinetic properties of molecules is considered a key feature in most drug development and high-throughput screening processes. Generally, pharmacokinetics, which represent the fate of drugs in the human body, This field generally examines these four main parameters: absorption, distribution, metabolism, and excretion, all of which are closely related to a fifth perspective, toxicity (ADMET).

The pharmacokinetic and toxic properties of the best covalent/competitive binding molecule inhibitor obtained in this study were predicted using ADMETLab3.0 (https://admetlab3.scbdd.com/) We also have to use the PkCSM program.**[20]**

Part2: Discussion of results

I Results of molecular docking:

The **Table III.4**presents the docking scores (in kcal/mol) of ligands L1 to L28 with the target protein 9EY8.pdb. The docking scores were used as an indicator of the interaction strength between each ligand and the protein's active site, compare to the reference ligand.

- ☞ The lowest energy corresponds to the highest affinity of the ligand and its target.

Table III.4: the docking scores of ligands, Compared with the reference ligand

ligands	S score (kcal/mol)	ligands	S score (kcal/mol)
L1	-4.852	**L15**	-4.840
L2	-6.237	**L16**	-5.146
L3	-5.462	**L17**	-5.161
L4	-5.633	**L18**	-7.197
L5	-5.063	**L19**	-4.884
L6	-5.255	**L20**	-5.209
L7	-5.231	**L21**	-4.541
L8	-5.458	**L22**	-5.566
L9	-5.489	**L23**	-4.947
L10	-4.774	**L24**	-3.139
L11	-6.230	**L25**	-6.295
L12	-4.564	**L26**	-5.109
L13	-5.568	**L27**	-5.822
L14	-6.063	**L28**	-5.585
Lref(TY2)	**-5.842**		

According to the results obtained, we found that the majority of the compounds tested have an interaction energy around to that of the initial ligand (inhibitor). Whereas L2, L11, L14, L8 and L25 present the best inhibitors with low score energy: **L2 (-6.23729706 kcal/mol)**; **L11(-6.23008299 kcal/mol)**; **L14 (-6.06354666 kcal/mol)**; **L18 (-7.19737482 kcal/mol)**; **L25 (-6.29524803 kcal/mol)**, compared to the reference molecule, which is **Ty2** with an interaction energy = -5.843kcal/mol. So we can predict that these are the molecules that may be responsible for the antioxydante vegetable oil activity of Chia and Apricot kernel, among all the molecules present. These ligands demonstrate promising characteristics as potential candidates for the development of new inhibitors.

- **RMSD value:** The quality of the adjustment was also evaluated using the legend's RMSD value at the following interval:

- RMSD 1.0 Å, correct pose;
- 1.0A < RMSD ≤2.0A, near pose;
- 2.0 <RMS =3.0 A, pose with errors,
- RMSD > 3.0 A, incorrect pose. **[21]**

In our study, the value of **RMSD** is equal to **0.792** this value is best posed.

II Interaction of ligands-9EY8:

In this step, we only selected the best results, that is, those with the best docking score. The interaction of this compound within the active site of the **9EY8** protein was visualized using Discovery Studio Visualizer. The binding energy scores of the top-ranked ligands are presented in the following table.

Table III.5: Scores and interaction binding between ligand atoms and active site residue

ligands	score (kcal/ mol)	Binding between ligand atoms and active site residues					
		Ligand atom	Atom involved in AA	Residue	Categories	Type of connection	Distance (Å)
L2	-6.237	O	H1	HOH646	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,214
		O	HE1	HIS377	Hydrogen Bond	Carbon Hydrogen Bond	3,058
L11	-6.230	O	H1	HOH646	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,890
		O	H1	HOH646	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,819
		O	H1	HOH699	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	3,066
		H	O	HOH704	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,239
		C	6 ring	TYR348	Hydrophobic	Pi-Alkyl	5,237
L14	-6.063	O	H1	HOH646	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,154
		O	H1	HOH699	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	3,039
		H	O	HOH704	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	3,026
L18	-7.197	O	H1	HOH646	Hydrogen Bond	Water Hydrogen Bond; Conventional Hydrogen Bond	2,304
		O	HE1	HIS377	Hydrogen Bond	Carbon Hydrogen Bond	3,016
		C	C	LYS198	Hydrophobic	Alkyl	4,327
L25	-6.295	C	5 ring	PRO371	Hydrophobic	Alkyl	5,087

- **8,11,14-Eicosatetraenoic acid L2:**

This compound showed a negative binding energy of -6.237 kcal / mol, which indicates a good interaction with protein 9EY8. The compound reacts via hydrogen bonds between an atom O in the ligand and an atom H1 in the residue HOH646, where the distance between the two atoms is 2.21 **Å**, which is an ideal distance for the hydrogen bond. Another interaction with the amino acid HIS377 is shown at the HE1 atom, at a distance of 3.05 **Å**, which enhances its stability within the active site of the protein.

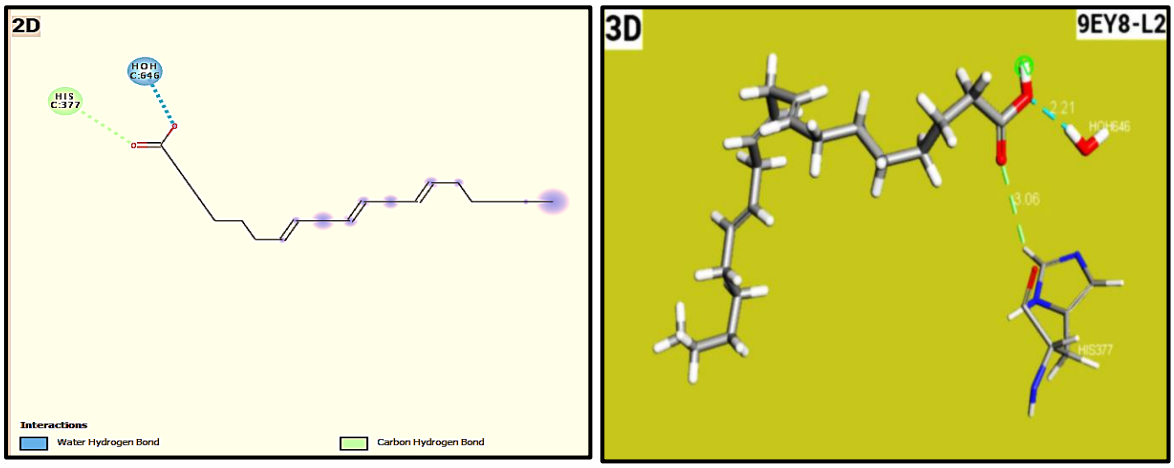


Figure III.9:2D and 3D projection of the interactions of the natural inhibitor 8,11,14-Eicosatetraenoic acid with the Sude cham residues of the active Site of 9Ey8

- **Docosanoic acid L11:**

This acid has a binding energy of -6.230 kcal/mol, which is also an indicator of stable binding to the protein. It interacts with several water molecules such as HOH646, HOH699 and HOH704 via hydrogen bonds, the distances being between 2.23 and 3.06 **Å**. This multiplicity of ligands enhances the stability of the compound within the ligand pocket. All of these bonds are made via hydrogen or oxygen atoms indicating an efficient hydrogen reaction network, as it also forms a bond between an atom C in the ligand and an 6 ring in the residue TYR348.

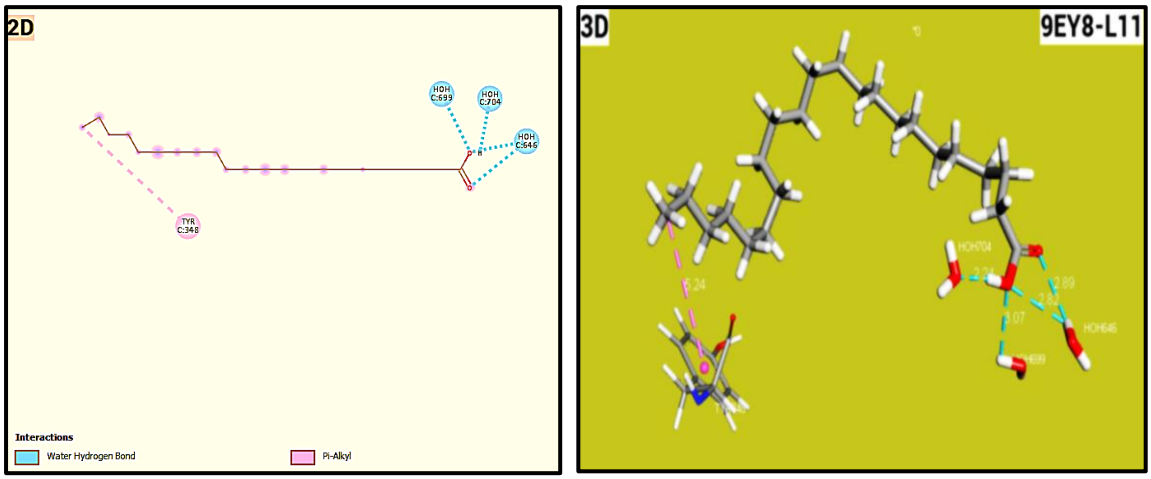


Figure III.10:2D and 3D projection of the interactions of the natural inhibitor Docosanoic acid with the Sude cham residues of the active Site of 9Ey8

- **Henicosanoic acid:**

This compound showed a relatively lower binding energy -5.063 kcal/mol that is also an indicator of stable binding to the protein, It interacts with several water molecules such as HOH646, HOH699 and HOH704 via hydrogen bonds, with distances between 2.15 and 3.03 **Å**, providing additional stability.

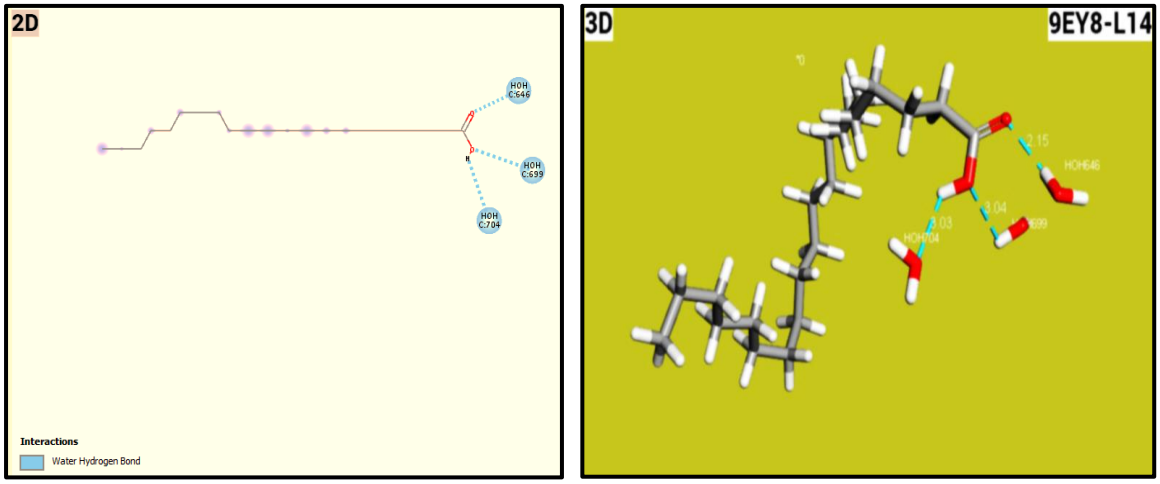


Figure III.11:2D and 3D projection of the interactions of the natural inhibitor Henicosanoic acid with the Sude cham residues of the active Site of 9Ey8

- **Nonadecanoic acid:**

This compound achieved the highest negative binding energy in the table -7.197 kcal/mol, which indicates the highest stability among the mentioned compounds. Its main interaction is through a strong hydrogen bond with the water molecule HOH646 at the hydrogen atom

H1, with a distance of 2.30 **Å**, an ideal distance that enhances its stability within the active binding site, and reacts with the amino acid HIS 377 via a hydrogen bond at the HE1 atom with a distance of 3.01 **Å**. It also has hydrophobic interactions with the amino acid LYS198 at a distance of 4.32 **Å**.

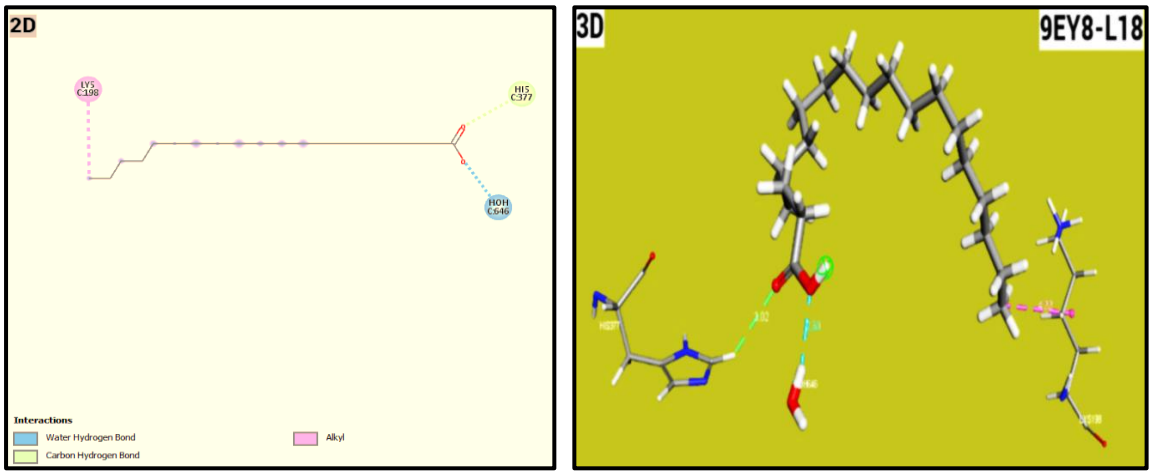


Figure III.12:2D and 3D projection of the interactions of the natural inhibitor Nonadecanoic acid with the Sude cham residues of the active Site of 9Ey8

- **Tetracosanoic acid:**

This compound has a binding energy of -6.295 kcal/mol, and interacts with protein 9EY8 via a single hydrophobic reaction with amino acid PRO371 of the Alkyl type, with a distance of approximately 5.09 **Å**.

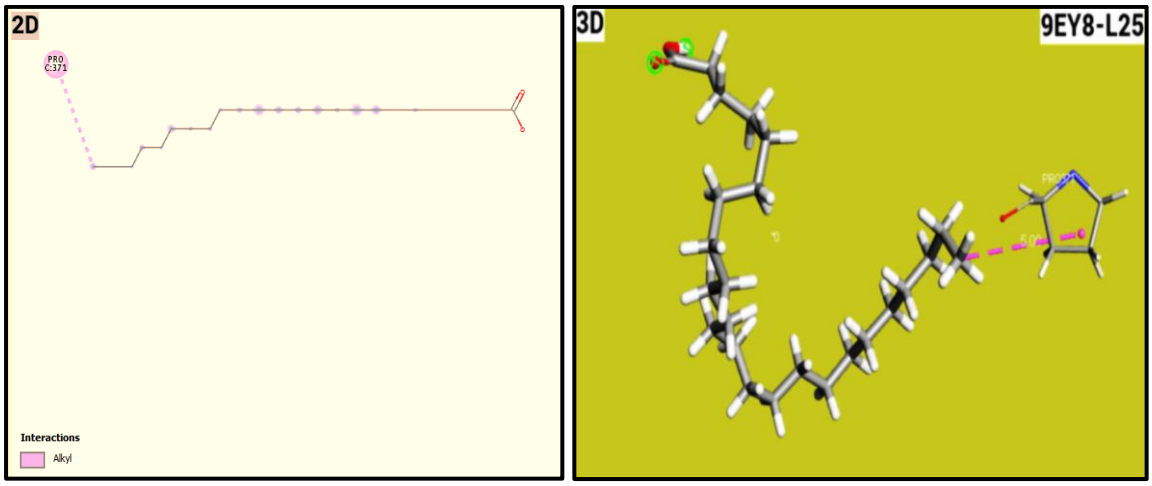


Figure III.13:2D and 3D projection of the interactions of the natural inhibitor Tetracosanoic acid with the Sude cham residues of the active Site of 9Ey8

III Evaluation of ADME properties:

- **physico-chemical propertie**

From previous studies, we have identified five compounds as being the best inhibitors compared to the reference ligands. For more detailed studies, evaluate the molecular properties of these compounds according to different rules.

Table III.6: Different physic-chemical parameters for the best ligands and the residues of the active Site of tyrosinase

ligands	physico-chemical properties					
	TPSA (Å²) (0~140)	n-Rot (0~11)	MW(g/mol) (100~500)	Log P (0~5)	n-HA (0~12)	n-HD (0~7)
L2	37.3	15	306.26	7.44	2	1
L11	37.3	20	340.33	9.069	2	1
L14	37.3	19	326.32	8.873	2	1
L18	37.3	17	298.29	8.056	2	1
L25	37.3	22	368.37	9.46	2	1
Lref(TY2)	109.57	3	196.08	-2.246	5	6

The parameters evaluated include the lipid/water partition coefficient (**logP**), the number of donors (**nHD**) and acceptors (**nHA**) of hydrogen bonds, the number of rotary bonds (**nRot**), the molecular weight (**Mw**), and the topological polar surface (**TPSA**).

Evaluation of the physicochemical properties of five of the best ligands compared to the reference Lref(TY2), where all the selected ligands (L2, L11, L14, L18, L25) showed low TPSA values (37.3 $Å^2$) < 140 $Å^2$, reflecting their good cellular permeability, unlike the reference which has a TPSA (109.57 $Å^2$) proximate a 140 $Å^2$. The molecular weights of all compounds also came within the ideal range (100-500 g/mol). However, the log P values of all ligands exceeded the ideal upper limit (5), which indicates their hydrophobic nature and may affect their bio-solubility. On the other hand, the number of cyclic bonds and the number of donors and acceptors of hydrogen bonds came within the permissible limits. In general, these ligands show good properties in terms of absorption and distribution compared to the reference,

From the above results, we can conclude that our compounds have strong dermal absorption.

- **Pharmacokinetic properties:**

The study of the profile ADMET of the five compounds is of great importance to understand the ability of these compounds to produce the desired therapeutic effects and to avoid the appearance of side effects that can lead to negative results. On the other hand, the predictive objective of ADMET also makes it easier to determine the best potential compound among all the compounds obtained.

Table III.7: ADMET/Pharmacokinetic properties of the selected compounds

ADME	parameters	L2	L11	L14	L18	L25	L ref
Absorption	skin permeability(logKp)	**-2.729**	**-2.734**	**-2.724**	**-2.729**	**-2.743**	**-2.931**
Distribution	VDss	**0.395**	**16.054**	**12.453**	**7.55**	**25.925**	**0.81**
	PPB	**97.6%**	**99.7%**	**99.4%**	**98.8%**	**100.3%**	**33.0%**
	BBB (logBB)	**-0.199**	**-0.363**	**-0.049**	**-0.237**	**-0.094**	**-0.626**
Metabolism	CYP2D6 substrate	**NO**	**NO**	**NO**	**NO**	**YES**	**NO**
	CYP3A4 substrate	**YES**	**YES**	**NO**	**YES**	**NO**	**NO**
	CYP1A2 inhibitior	**YES**	**YRS**	**YES**	**YES**	**NO**	**NO**
	CYP2C19 inhibitior	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
	CYP2C9 inhibitior	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
	CYP2D6 inhibitior	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
	CYP3A4 inhibitior	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
Excretion	total clearance	**1.778**	**1.967**	**1.749**	**1.866**	**1.858**	**0.516**
	T1/2	**0.457**	**1.956**	**1.719**	**1.314**	**2.494**	**1.956**
Toxicity	AMES Toxicity	**NO**	**NO**	**NO**	**NO**	**NO**	**YES**
	Rat Oral Acute Toxicity	**0.111**	**0.1**	**0.103**	**0.1**	**0.092**	**0.272**
	skin sensitization	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
	Eye corrosion	**YES**	**YES**	**YES**	**YES**	**YES**	**NO**
	Ototoxicity	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**
	Hematotoxicity	**NO**	**NO**	**NO**	**NO**	**NO**	**NO**

The distribution properties include the volume of distribution (**VD**), the permeability of the blood-brain barrier (**BBB**) and the binding to plasma proteins (**PPB**). Elimination was

assessed by total clearance and half-life (**T1/2**). Finally, the toxicity properties include skin sensitization, eye corrosion.

This table shows that:

1. The data show that all five compounds have good skin absorption ability compared to the reference compound (L_ref), which confirms that these compounds had a high permeability, enhances their potential for transdermal use.

2. All compounds show a weak ability in terms of crossing the blood-brain barrier (logBB)<3, which is positive if the therapeutic targets are outside the central nervous system.

   All values of distribution volume VDss are optimal; between (0.04 –20 L/Kg), except VDss of L25 is poor. In addition, it appears that all compounds have PPB (plasma protein binding) values > 90%; hence the fixation of these molecules to plasma proteins ,On the other hand, As shown in the table, all compounds show a weak ability in terms of crossing the blood-brain barrier (logBB) <3 , which is positive if the therapeutic targets are outside the central nervous system.

3. The table analysis can announce that no compounds are substrates of CYP2D6 with the exception of L25. On the contrary, they are substrates of CYP3A4 with the exception of L14, L25 and Lréf. On the other hand, it's easy to see that not all ligands are CYP2C19, CYP2C9, CYP2D6 or CYP3A4 inhibitors, while the ligands (L2, L11, L14, L18) are CYP1A2 inhibitors.

4. Furthermore, it is clear that these compounds have a low excretion clearance (<5 mL/min/kg); given that our dermal study excretes via sweat, we can also note the short half-life (T1/2) of these compounds, Unlike the ligand L25 has (T1/2) (2.494 hours), Which may indicate that its effect lasts longer compared to other ligands.

5. In the final analysis, all the compounds do not show AMES toxicity to Lréf reverse, nor do all the compounds show Rat Oral Acute Toxicity > (500 mg/Kg). In addition, all the compounds do not show derma-toxicity, and this is the result we want, as our objective is skin application Again, all compounds and reference ligands show no ototoxicity, hematotoxicity, however, they all show eye irritant effects, so we recommend apply away from the eye contour.

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Conclusion

Conclusion

The environment, have shown increasing interest in the study of medicinal plants and their traditional use in different regions of the world. Plants used to cure various diseases and relieve pain and suffering are called medicinal plants. Natural resources and associated biological diversity provide the basis of livelihood for many human populations.

The in-depth study of the healing properties of medicinal plants, evaluating their antioxidant activity, represents a promising approach to the discovery of new natural remedies.

In this study, this note aims to explore the effectiveness of some plant extracts as natural sources of antioxidants agents, and to study their ability to inhibit the tyrosinase enzyme associated with the appearance of dark spots and skin pigmentation. Starting from a deep understanding of the oxidation mechanism and the role of tyrosinase in the production of melanin, we propose a scientific approach based on the inhibition of this enzyme using antioxidants of natural origin.

Various tests in-vitro were conducted to evaluate the antioxidant potential of the extracts oils, including DPPH free radical scavenging, DMSO and phenanthroline tests. The results showed that chia oil extract showed a higher effect in all tests, while Apricot keep oil extract showed slightly lower than chia oil extract in most tests. These results allowed evaluating the possible effectiveness of chia and Apricot keep oil extracts as excellent antioxidants, and having good yields.

The chemical profile was determined with analysis by GC-MS method, which has led to the identification of 26 compounds in chia oil, and eight compounds in Apricot keep, rich in fatty acids.

Early in this work, we selected a series of naturally derived compounds previously obtained, to evaluate their potential effectiveness as tyrosinase inhibitors, through a molecular docking simulation study. This analysis was carried out using specialized software (MOE and discovery Studio), with the aim of studying the mechanism of interaction of these molecules with the active site of the enzyme responsible for the production of melanin, and therefore involved in the phenomenon of skin hyperpigmentation.

The discussion of the results obtained was based on three main criteria: The Binding energy value, the RMSD value, and the nature of the reactions (hydrogenic, hydrophobic...) Between the tested molecules and the residues of the active site of the tyrosinase enzyme, as well as the distances between the active atoms. The results of molecular docking revealed that that the five

ligands L2, L11, L14, L18, L25 are the best tyrosinase inhibitors (9EY8) this justified by the presence of different types of interactions (mainly hydrogen and hydrophobic bonds) between these molecules and the active site residues of the protein. Additionally these ligands have the lowest score energies compared to the others.

The physicochemical properties of the five best-selected candidates demonstrate a hydrophobic nature of our compounds, which makes their solubility in aqueous environments difficult and easy in lipids, indicating that these compounds have high permeability. This confirms that these candidates made sure that they correspond to the ideal properties for dermatological applications, and can be administered topically without any issues, as they possess no toxicity. However, as we mentioned earlier, they all present eye irritant effects, so we recommend applying them away from the eye area.

To conclude, in light of the results obtained in this research, we can say that the five compounds L2, L11, L14, L18, L25 would probably be the best tyrosinase inhibitors and good drug candidates without adverse effects for topical application in skin hyperpigmentation, paving the way for the development of safe and effective cosmetics based on natural compounds.

Preparation of prototype

Numerous dermatological conditions can lead to the development of hyperpigmented lesions, including brown spots. These hyperpigmented areas often result from cumulative sun exposure but may also be influenced by dietary factors. Aesthetically unappealing, such spots can cause significant distress, particularly when they occur on highly visible areas such as the hands, face, or décolleté. Consequently, many individuals seek effective treatments to diminish or eliminate these lesions. One medical approach involves the use of depigmenting creams, which act directly on melanin—the natural dark pigment in the skin responsible for absorbing UV radiation and imparting color to these pigmented spots.

This part of my research, take account to evaluate the results obtained from the combined study of phytochemistry, biological and molecular simulation, which encouraged us to move on to the formulation of a cream to combat hyperpigmentation using natural products applied as active ingredients. Its function is to slow down the synthesis of melanin by inhibiting the enzyme responsible for this synthesis and to promote cell renewal, which helps to reduce skin pigmentation.

A cream is a mixture of an aqueous phase and an oil phase, in the form of an emulsion. Consequently, a cream will always be composed of these three main ingredients.

Aqueous phase: Rose hydrolat, Sodium carbonate, Isocide and Urea.

Oily phase: Chia Seed Oil, Apricot kernel oil, and Vitamin E.

Emulsifier: Cutina

- **Method of preparation**

The aqueous phase was introduced into a glass beaker, and the oily phase into another identical beaker. Both beakers, with their respective phases, were then placed in a 60°C water bath.

When both phases had reached 50°C, we poured the oil phase drop by drop into the water phase under stirring, and kept the mixture in the water bath for 15 min, stirring until a homogeneous mass was obtained. The mixture was then removed from the bain-marie and left to cool in the open air to obtain a homogeneous cream.

❖ Principal Ingrédients

Chia Seed Oil 	Chia Seed Oil in the skin care, hair care, and cosmetic industries. This rich emollient is best blended into formulas targeting dryness found in matured skin. While Chia Seed Oil may work wonders in repairing dry patches and restoring moisture, it may not be best for those with oily skin due to its potential to be comedogenic, or pore clogging. Very rich in vitamins and antioxidants, it naturally contributes to cell regeneration. It's an ideal food for fighting dark spots, wrinkles, and expression lines on the face. It also helps keep skin firm and youthful.
Apricot kernel oil 	Apricot kernel oil offers numerous benefits for skin due to its rich composition of vitamins, fatty acids, and antioxidants. It deeply moisturizes, softens, and soothes the skin, making it a great choice for dry and sensitive skin types. It also helps to improve skin elasticity, reduce the appearance of fine lines and wrinkles, and protect against environmental damage. It can have a great depigmenting power on scientifically proven skin.
AHA 	A powerful cell regenerator thanks to its peeling effect, fruit acids reduce pigment spots, regenerate tissues and stimulate skin microcirculation. They brighten the complexion and improve the appearance of the skin by erasing imperfections

- **Other ingredients**

Rose hydrolat: Also known as, rose water, this hydrosol has toning and refreshing properties. Regenerating and anti-wrinkle, this hydrosol is one of the most active for mature skin.

Sodium carbonate: Sodium carbonate is used in a variety of cosmetic and personal care products as a buffering agent. It helps in reducing premature aging and irritation of the skin. PH adjusters maintain the PH balance of the product and avoid the skin's pH from shifting too far from the normal one.

Isocide: Isocide is a very effective preservative for aqueous phases. Its powerful antibacterial and antifungal properties prevent the development of microorganisms (bacteria, yeasts, germs, molds, etc.). Incorporated into cosmetic preparations, Isocide will ensure their good preservation for several months at room temperature. It is used in cosmetics-Bio. Isocide can also be replaced with grapefruit seed extract.

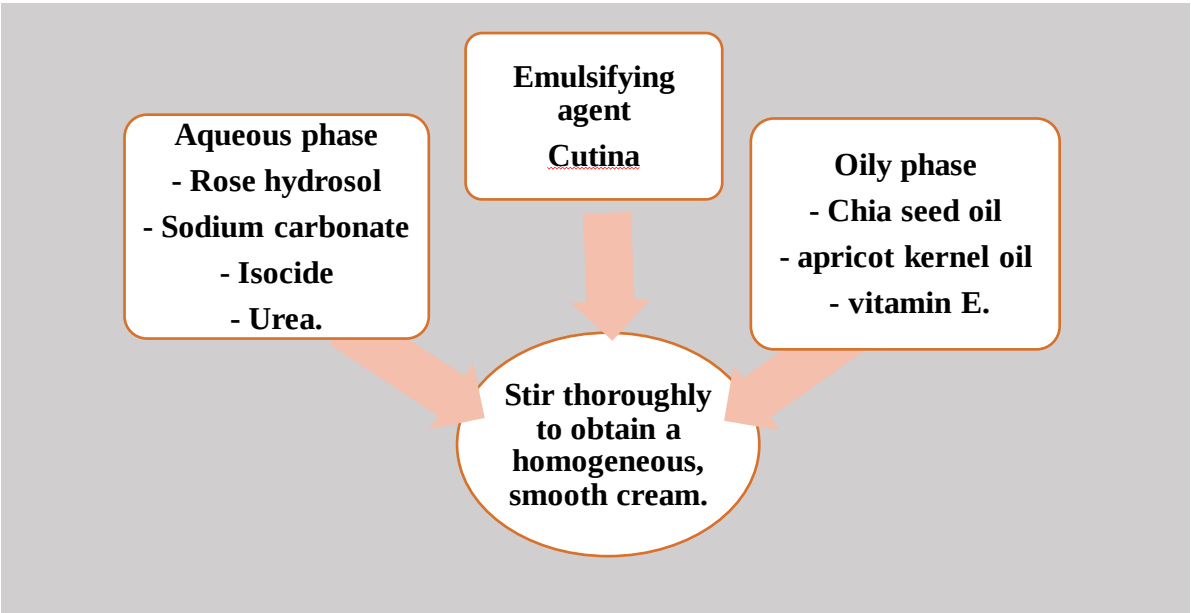
Cutina : is commonly used as an emulsifier in the cosmetics industry. Emulsifiers are ingredients that help to blend two immiscible substances, such as oil and water, to form a stable, homogeneous mixture. It can also help improve product consistency and enhance its moisturizing properties. Cutina can also act as a thickener and stabilizer, helping to prevent separation of the various components of a cosmetic product. Cutina is a natural emulsifier often used in organic cosmetics to make all kinds of smooth, creamy creams.

Urea: Well known for its moisturizing and emollient properties, it gives the skin a supple and smooth feel. At higher concentrations, urea also serves as a gentle exfoliate, removing scales and dead cells.

Vitamin E: preservative of oily phases, it is also an excellent antioxidant. The perfect ally of all anti-aging creams, it protects the skin and helps prevent skin aging.

❖ **Ingredients quantity**

Hydrolat de rose	59.09 %	30 gr	30 ml
Huile végétale de chia	27.07 %	13.8 gr	15 ml
Huile végétale des noix d'abricot	0.15 %	/	3 gouttes
Bicarbonate de soude	0.60 %	0.3 gr	/
Isocide	0.78 %	/	12 gouttes
Cutina	5.95 %	3 gr	/
Vitamine E	0.37 %	/	6 gouttes
Urée	3.97 %	2 gr	/
AHA	2.03 %	1.2 gr	1 ml



Cream prepared ((Phyt-Meladerm))

Line: skin care

Product Type: face peeling

Product Properties: anti dark spots, moisturizing, repair

Volume: 50 ml

When To Use: universal

Gender: for women and man

Age: 18+

Skin Type: all types

BENEFITS

- **Powerful antioxidant**
- **Suitable for natural sources**
- **Improves and evens skin tint.**
- **Corrects and reduces dark spots.**

☞ It is necessary to complete the work with quality control to ensure and confirm their application, such as PH measurement, consistency, homogeneity, stability...etc.

Abstract

Due to the high prevalence of hyperpigmentation phenomena and skin spots associated with oxidative stress, numerous researchers have focused on identifying new antioxidant compounds capable of preventing or treating these skin diseases.

Firstly, this study aimed to evaluate the antioxidant activity of oils extracted from plant sources, applying various methods such as DPPH, DMSO and phenanthroline. The results showed that chia and apricot kernel oil obtained with good yield, possessing remarkable ability to inhibit free radicals. Subsequently, the presence of fatty acids was then demonstrated using the GC-MS chromatography method, which identified 26 chemicals in chia vegetable oil and 8 compounds in apricot kernel oil.

Secondly, this work aims to discover, through in silico new structures acting as tyrosinase inhibitors that play a role in inhibiting oxidative stress and improving melanin production.To conduct this study, we combined two methods: molecular modeling (molecular docking) and ADME-T calculations on 28 compounds selected from the previous study. This approach enabled the validation of their therapeutic potential and elucidation of their inhibitory mechanism.

The findings highlighted five ligands (L2, L11, L14, L18, and L25) exhibiting strong inhibitory affinity toward tyrosinase (9EY8) along with favorable pharmacokinetic profiles, making them suitable for topical use without major adverse effects for the treatment of skin hyperpigmentation.

All these results thus pave the way for the development of a cosmetic cream formulation designed to prevent and reduce pigmented spots.

Key words: Hyperpigmentation - Natural product - Antioxidant activity- GC-MS - Molecular docking.

ملخص

نظرا لكثرة حدوث فرط في التصبغات والبقع الداكنة الناجمة عن الاجهاد التأكسدي كرس عدد من الباحثين جهودهم في البحث عن مواد جديدة مضادة للأكسدة من اجل محاربة الاجهاد التأكسدي والامراض الجلدية المتعلقة به.

هدفت هذه الدراسة أولاً إلى تقييم النشاط المضاد للأكسدة في الزيوت المستخرجة من مصادر نباتية، باستخدام طرق مختلفة مثل DPPH وDMSO والفينانثرولين. أظهرت النتائج أن زيت بذور الشيا والمشمش المُستخلص بمردود جيد، يمتلك قدرة ملحوظة على تثبيط الجذور الحرة. بعد ذلك، تم إثبات وجود الأحماض الدهنية باستخدام طريقة كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS)، والتي حددت 26 مركب كيميائي في زيت الشيا النباتي و8 مركبات في زيت بذور المشمش.

ثانيًا، يهدف هذا العمل إلى اكتشاف مركبات جديدة، من خلال محاكاة حاسوبية، تعمل كمثبطات للتيروزيناز، وتلعب دورًا في تثبيط الإجهاد التأكسدي وتحسين إنتاج الميلانين. لإجراء هذه الدراسة، جمعنا بين طريقتين: النمذجة الجزيئية (الالتحام الجزيئي) وحسابات ADME-T على 28 مركبًا مختارة من الدراسة السابقة. وقد أكد هذا على صحة هذه الطرق وساعدنا على فهم آلية التثبيط في هذا المرض بشكل أفضل.

أظهرت النتائج أن الربائط الخمسة L2 وL11 وL14 وL18 وL25 هي أفضل مثبطات التيروزيناز (EY89) ومرشحات دوائية جيدة بدون آثار جانبية للاستخدام الجلدي لعلاج فرط تصبغ الجلد.

شجعتنا كل هذه النتائج إلى تركيب كريم لمكافحة فرط التصبغ والبقع الداكنة.

الكلمات المفتاحية: فرط التصبغ - مركب طبيعي - نشاط مضاد للأكسدة - كروماتوغرافيا الغاز/مطياف الكتلة - الالتحام الجزيئي.

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي و البحث العلمي

جامعة محمد خيضر بسكرة

عنوان المشروع:

انتاج كريم لعلاج التصبغات و البقع الداكنة بمستخلصات نباتية (زيت بذور الشيا و زيت نوى المشمش)

مشروع لنيل شهادة مؤسسة إقتصادية (مؤسسة مصغرة) ضمن القرار 1275

الإسم التجاري:

Phyt-Meladerm

العلامة التجارية:

Phyt-Meladerm

السنة الجامعية

2025-2024

بطاقة معلومات :

حول فريق الإشراف و فريق العمل

1- فريق الإشراف:

فريق الإشراف	
المشرف الرئيسي1 : فتاح اسماء	**التخصص: كيمياء عضوية وكيمياء النبات**
المشروف الرئيسي2 : قارون سميرة	**التخصص: علم بيئة والنبات**

2- فريق العمل:

فريق العمل		
فريق المشروع	**التخصص**	**الكلية**
خرفي اليامنة	**كيمياء الصيدلانية**	**علوم الدقيقة**

أولا . وصف فكرة المشروع:

يهدف مشروعي إلى إنتاج كريم طبيعي لتفتيح البشرة وعلاج التصبغات، قمنا بإجراء سلسلة من التحاليل والاختبارات لتقييم الفعالية البيولوجية لمستخلصات زيت الشيا وزيت نوى المشمش المتحصل عليها على مستوى مخبر الكيمياء. شملت هذه الاختبارات تحليل النشاط المضاد للأكسدة باستخدام اختبار DPPH الذي يقيس قدرة الزيوت على تثبيط الجذور الحرة، واختبار PHENANTHROLINE الذي يؤكد هذه الفعالية من خلال قياس قدرة المركبات على اختزال أيونات الحديد. كما استُخدم اختبار DMSO لتحديد الفعالية الكيميائية للمضادات الطبيعية. تم التعرف ايضا على التركيبة الكيميائية لهذه المستخلصات بواسطة التحليل الازدواجي الكروماتوغرافيا الطور الغازي – مطيافية الكتلة.

بالإضافة إلى ذلك، تم تعزيز الدراسة من خلال اختبارات المحاكاة الحاسوبية (Molecular Docking) باستخدام برامج متخصصة، وذلك لتحليل قدرة المركبات المستخلصة على تثبيط إنزيم التيروزيناز المسؤول عن تصبغات الجلد، وتحديد أفضل الروابط من حيث التفاعل مع الموقع النشط للإنزيم.

- أولًا: السلعة (المنتج) التي سيوفرها مشروعي :كريم تبييض طبيعي للبشرة موجه خصيصًا لعلاج التصبغات، البقع الداكنة، الكلف، وآثار الشمس يحتوي على مركبات طبيعية فعالة مضادة للأكسدة ومثبطة لإنزيم التيروزيناز (المسؤول عن إنتاج الميلانين).
- ثانيا: الفئة المستهدفة :نساء ورجال يعانون من مشاكل تصبغ البشرة.أصحاب البشرة الحساسة أو الباحثين عن منتجات خالية من المواد الكيميائية الضارة. المهتمون بالتجميل الطبيعي.
- ثالثا: موقع مشروعي وطرق التوصيل

الموقع: محل صغير/ورشة مهيأة بالقرب من الجامعة.

الهدف: تحضير كريم، إجراء التحاليل الأساسية، والتعبئة.

طرق توصيل المنتوجات للزبائن :

1. البيع المباشر

2. البيع عبر الإنترنت (التوصيل):

إنشاء صفحة على فيسبوك، إنستغرام أو موقع بسيط لعرض المنتوجات.

استقبال الطلبات والدفع عند الاستلام.استخدام خدمات توصيل معروفة.

- رابعا : كيفية تشغيل المشروع و من سيعمل معي

سيتم تسيير المشروع مرحلة البداية، حيث يتم تحضير الكريم، اختيار المكونات، ومتابعة جودة الإنتاج. بمساعدة شخص واحد في بعض المهام مثل الوزن، التعبئة، والتنظيف. أما توصيل المنتوجات، فسيتم إما بالتسليم المباشر داخل الحي أو عبر خدمات التوصيل المتوفرة.

- خامسا :أسباب اختيار فكرة المشروع:

جاء اختيار فكرة هذا المشروع استجابة لعدة عوامل مرتبطة بالسوق المحلي، أبرزها الطلب المتزايد على مستحضرات العناية بالبشرة الطبيعية، وخاصة المنتجات المخصصة لتفتيح البشرة وعلاج فرط التصبغ والبقع الداكنة. في السابق، كانت المنتجات المستوردة تهيمن على هذا النوع من السوق، رغم ارتفاع أسعارها واحتوائها في كثير من الأحيان على مكونات كيميائية قد تُسبب آثارًا جانبية على المدى الطويل.

إلا أن الوضع عرف تغيرًا ملحوظًا، خاصة بعد أن قامت السلطات المعنية، في شهر ماي 2025، بمصادرة كميات كبيرة من منتجات التجميل المستوردة غير المصرّح بها، مما كشف عن ثغرات في الرقابة وجودة هذه المنتجات. هذا الحدث فتح المجال أمام تشجيع المبادرات المحلية لتقديم بدائل طبيعية وآمنة، مصنعة وفقًا لمعايير الجودة والسلامة، وتتماشى مع خصوصيات واحتياجات المستهلك الجزائري.

ثانيا - بيانات المشروع

تقديم مختصر للمشروع	هذا المشروع يهدف إلى صناعة كريم بمستحضرات طبيعية مخصص لتفتيح البشرة والتقليل من التصبغات والبقع الداكنة، باستخدام مواد نباتية فعالة وآمنة. جاءت الفكرة بعد ملاحظة الإقبال الكبير على المنتجات الطبيعية، خاصة مع منع بعض المنتجات المستوردة في سنة 2025 بسبب عدم مطابقتها للمعايير. يهدف المشروع إلى توفير منتج محلي بجودة جيدة وبمكونات صحية تناسب احتياجات الزبائن.
ما هي أسباب اختيارك لهذا المشروع؟	تم اختيار هذا المشروع نظرًا للطلب المتزايد على منتجات طبيعية وآمنة لتفتيح البشرة والتقليل من التصبغات. هذا ما فتح المجال لتقديم بديل محلّي صحي وفعّال، يعتمد على مكونات نباتية، ويستجيب لحاجة المستهلك بجودة مناسبة وسعر معقول.اضافة الى ذلك تلبية حاجات المنطقة حيث منطقة بسكرة بها درجة حرارة عالية و سكانها الأكثر عرضة للتصبغات و البقع الداكنة. ولا تقتصر أهمية هذا الابتكار على منطقة بسكرة التي تشهد درجات حرارة مرتفعة تزيد من مشاكل

	التصبغات الجلدية، بل تشمل مختلف ولايات الجزائر، حيث يؤدي التعرض الطويل لأشعة الشمس القوية وتنوع الظروف المناخية إلى زيادة انتشار البقع الداكنة وتغير لون البشرة. لذلك، يساهم المشروع في توفير حل عملي وآمن يستجيب لتطلعات المستهلكين في عموم البلاد.
نوع النشاط (صناعي، زراعي، خدمي، سياحي، غذائي،.....اخرى)	صناعي (علاجي - تجميلي)
موقع المشروع:	المشروع يقع في محل صغير بمنطقة هادئة وقريبة من الجامعة لتسهيل إدارة المشروع و تسييره من طرف المخابر الجامعية و دار المقاولاتية.
تكلفة الاستثمارية بـ دج	**3.339.900 دج**

الصفة القانونية	*سجل تجاري	* بطاقة	* نشاط حر
	شخص طبيعي ✓ او شخص معنوي ☐	حرفي ☐ او فلاح ☐	✓

ثالثا - بيانات الدراسة التسويقية

1-الزبائن:

من هم زبائنك؟:	أفراد: ✓ مؤسسات: ☐
ما هو عددهم:.	عدد الزبائن المتوقعون شهريا (معدل تقديري) :حوالي 80 إلى 100 شهريا في البداية, مع إمكانية الزيادة لاحقا.
مكان الشراء؟:	محلاتك: ✓ محلات شركائك: ☐ باعة الجملة والتجزئة: ☐ عن طريق الإنترنت: ✓ الاسواق الاسبوعية او اليومية ☐
كم مرة يقومون بالشراء في:اليوم/الشهر/ السنة؟	في اليوم: حوالي 3 الى 5 طلبيات في البداية. في الشهر: ما بين 90 الى 150 علبة. في السنة: ما بين 1080 الى 1800 علبة (حسب تطور المشروع وانتشاره).
أذكر خصائص أخرى لزبائنك إن وجدت	الفئة العمرية: من 18 سنة فما فوق. الجنس :كلا الجنسين. مستواهم الاجتماعي اوالمهني: غير محدد.

2- المنافسين :

م	اسم المنافس	المنتجات / الخدمات	السعر	أهم المميزات	أهم العيوب
1	**Biopharma**	1. كريم تفتيح البشرة	1,200دج	*مصنوع من مكونات طبيعية	*قد لا يكون فعالًا للبقع الداكنة العميقة

				*متوفر في الصيدليات ومحلات التجميل *موثوق من قبل المستهلكين	*التأثير يظهر بعد استخدام طويل
2	**Chifa Derm**	**1. كريم لتفتيح البشرة**	**1,000 دج**	***مناسب للبشرة الحساسة *يحتوي على مكونات مهدئة *متوفر في الصيدليات**	*قد يحتاج لفترة طويلة لرؤية النتائج *التغليف بسيط
3	**DermaCare**	1. كريم لتفتيح البشرة 2.	1,500دج	*يحتوي على مكونات طبيعية *يعمل على توحيد لون البشرة *متوفر عبر الإنترنت	*قلة التوزيع في المتاجر *قلة التقييمات من المستخدمين

3-المنتج: (سلعة/ خدمة)

/	المنتجات / الخدمات	خصائص ومميزات منتجاتك / خدماتك	المميزات	الاحتياجات التي تلبيها
1	**كريم تفتيح طبيعي** 	**1) الجودة :منتج عالي الجودة بمكونات طبيعية 100% مصنع وفق شروط النقاوة**	**فعّال وآمن على البشرة.**	**حاجة الزبائن لمنتج طبيعي يعالج البقع الداكنة دون أضرار جانبية**
		2) الشكل، اللون، الحجم: عبوة أنيقة، لون أبيض، 50 مل	**سهل الاستخدام والتخزين**	**منتج مناسب للاستخدام اليومي**
		3) الضمان وخدمات ما بعد البيع: متابعة الزبائن بعد الشراء، تقديم نصائح الاستعمال، استقبال الآراء والتقييمات.	**منتج يجمع بين العناية و الجمال**	**بناء ثقة الزبائن وتطوير المنتج حسب تجاربهم**
		4) القيمة المضافة التي تقدمها منتجاتك / خدماتك. خالٍ من المواد الكيميائية،	**علاج طبيعي آمن**	**رغبة الزبائن في تحسين البشرة بطريقة صحية وآمنة**

		غني بخلاصات نباتية مرطبة ومغذية.		
		5) مميزات أخرى: توصيل للمنازل، استشارات مجانية، عروض خاصة للطلبة .	سهولة الشراء والمتابعة	توفير منتج وخدمة مريحة ومناسبة للفئة المستهدفة

4-التسعير :

المنتجات / الخدمات	أسعار المنافسين		السعر المقبول من العملاء	أسعاري المبدئية
	أقل سعر	أعلى سعر		
1.	1000دج	1500دج	1400دج	1300دج

5- الموقع:

جاهز بالإيجار [✓] ملك خاص [ ] أرض سيتم بناؤ[ ]

البيان	التفصيل
وصف الموقع	يقع المشروع في محل صغير بالقرب من جامعة محلية، في منطقة نشطة تجاريا، ما يجعله في موقع استراتيجي من حيث الحركة اليومية للطلبة والموظفين. يتميز الموقع بسهولة الوصول إليه من مختلف الاتجاهات، سواء سيرًا على الأقدام أو عبر وسائل النقل.
مساحة الموقع	إجمالي المساحة: 40 م^2. الـــطول: 8م الـــعرض: 5م
أسباب اختيار الموقع	➕ قربه من الجامعة يتيح استهداف شريحة واسعة من الطالبات والموظفات المهتمات بمنتجات التجميل الطبيعية. ➕ الموقع حيوي ويوفر حركة مستمرة، مما يُسهّل التسويق المباشر. ➕ تكلفة إيجاره مقبولة مقارنة بمواقع أخرى. ➕ مناسب لبدء مشروع صغير دون تكاليف كبيرة.
ايجابيات الموقع	✓ موقع نشِط بالقرب من الجامعة ومراكز خدمات. ✓ سهولة الوصول للزبائن، خاصة من الفئة المستهدفة. ✓ إمكانية توزيع عينات مباشرة للطالبات واستقبال آراءهن. ✓ الموقع مناسب للتوصيل داخل المدينة بسرعة.
سلبيات الموقع	☒ المساحة محدودة وقد لا تسمح بتوسعة كبيرة مستقبلاً.
قنوات التوزيع	1. البيع المباشر من المحل: للزبائن القاطنين بالقرب من موقع المشروع أو الجامعة. 2. خدمات التوصيل المحلية: مثل "يالبريد"، "ليفري"، أو خدمة توصيل خاصة لتوصيل الطلبيات إلى المنازل داخل المدينة.
الإيجار السنوي	قيمة الإيجار الشهري: 90,000 دينار جزائري (قابلة للتفاوض حسب المنطقة) الإيجار السنوي = 90,000 × 12 = 1.080.000 دينار جزائري

6- الترويج

طريقة الترويج	الوصف	الكمية	التكلفة (سعر الوحدة)	عدد مرات التكرار	التكلفة السنوية
الاشهار	الإشهار الشفوي (مجاني)	غير محدود	0	اسبوعيا	0
	الإعلانات(جرائد؛ إذاعة، تلفزيون)	/	/	/	/
	المطويات	50	60 دج	شهريا	36.000 دج
	اللوحات الإشهارية	1	100.000دج	مرة واحدة	100.000دج
المبيعات الترويجية	لمعارض	2	60.000دج	مرتين سنويا	120.000دج
	التخفيضات والتنزيلات على المبيعات	/	/	/	/
	منتجات وخدمات تكميلية	/	/	/	/
المجموع					256.000 دج

رابعا ـ الدراسة التقنية:

موقف المشروع حالياً: ✓ **مشروع جديد(تأسيس)**	مشروعي هو مشروع جديد في طور التأسيس، يهدف إلى إنتاج كريمات تفتيح ومعالجة البقع الداكنة باستخدام مكونات طبيعية وآمنة. حاليًا، يتم العمل على تجهيز الموقع، اقتناء المعدات الأساسية، واختيار الموردين المناسبين للمواد الأولية. كما يجري إعداد دراسة مخبرية لاختبار جودة المنتج وبدء عملية الإنتاج الفعلي في أقرب الآجال.
مراحل عملية الإنتاج أو البيع. **قم بوصف عملية دورة الإنتاج (الاستغلال) لمنتجاتك أو خدماتك (ومرافقتها بمخطط إذا لزم الأمر):**	تبدأ مراحل تنفيذ المشروع بإعداد الكريم من خلال عدة خطوات منظمة. أولًا، يتم شراء المواد الأولية الطبيعية من مصادر موثوقة. ثم تُحضّر هذه المواد وتُوزن بدقة حسب التركيبة المعتمدة. بعد ذلك، تُخلط المكونات حتى يتم الحصول على كريم متجانس، ثم يُعبّأ في علب خاصة ويُغلف بطريقة صحية. يُخزَّن المنتج مؤقتًا في مكان مناسب إلى حين بيعه. أما مراحل البيع، فتبدأ بالترويج للمنتج عبر الإنترنت ومنصات التواصل الاجتماعي. بعدها، تُستقبل الطلبات من الزبائن، ويتم تجهيزها بدقة. تُرسل الطلبيات عن طريق خدمات توصيل محلية موثوقة، مع متابعة الزبائن لاحقًا لضمان رضاهم وتكرار عملية الشراء.
• **دورة الإنتاج (عدد أيام العمل في الأسبوع):**	دورة الإنتاج وتشغيل المشروع *عدد أيام العمل في الأسبوع: 5 أيام (من الأحد إلى الخميس)

• **الزمن اللازم لدورة تشغيل الشهور.....شهر**	*عدد أشهر العمل في السنة: 11 شهرًا (مع احتساب شهر للراحة أو الصيانة أو التحديث) *عدد دورات التشغيل في الشهر: 4 دورات (دورة واحدة كل أسبوع) *الزمن اللازم لدورة تشغيل واحدة: 2 إلى 3 أيام
القدرة الإنتاجية (عدد الوحدات المنتجة في اليوم/ الشهر/ السنة):	*في اليوم الواحد: عدد العبوات المنتجة: 20 إلى 30 عبوة كريم *في الشهر الواحد: عدد أيام العمل: 20 يوم (5 أيام × 4 أسابيع) الإنتاج الشهري = 20 يوم × 25 عبوة (متوسط) =حوالي 500 عبوة شهريًا *في السنة: عدد أشهر العمل: 11 شهرًا الإنتاج السنوي = 11 شهر × 500 عبوة =حوالي 5500 عبوة سنويًا

1-تحديد المبيعات

ما هو تقديرك للمبيعات التي ستحققها خلال السنة الأولى من النشاط؟

الرقم	الشهر	1	2	3	4	5	6	7	8	9	10	11	12
1	الكمية المباعة	300	320	400	440	470	470	480	480	500	500	530	550
	سعر الوحدة (دج)	1300	1300	1300	1300	1300	1300	1300	1300	1300	1300	1300	1300
	قيمة المبيعات (دج)	390.000	416.000	520.000	572.000	611.000	611.000	624.000	624.000	650.000	650.000	689.000	715.000

2-إجمالي مبيعاتك

ما هو إجمالي مبيعاتك (إيراداتك) السنوية:

الرقم	أنواع المنتجات	الوحدة	سعر بيع الوحدة	الكمية (سنويا)	اجمالي القيمة
1	كريم تفتيح طبيعي	عبوة	1300 دج	5500 عبوة	7.150.000 دج
إجمالي مبيعاتك السنوية					7.150.000 دج

يتم حساب رقم الاعمال باليوم ثم الشهر ثم السنة السنة

تطور قيمة المبيعات على مدى خمس سنوات (10%)

السنة	1	2	3	4	5
المبيعات السنوية	7.150.000 دج	7.865.000 دج	8.651.500 دج	9.516.650 دج	10.468.315 دج

3- المواد الأولية السنوية

. ما هي المواد الأولية اللازمة من أجل بداية نشاطك؟:

الرقم	البيان	الوحدة	تكلفة الوحدة	الكمية (على مدار السنة)	اجمالي القيمة (سنويا)
1	ماء الورد	عبوة سعة 100 مل	150 دج	11	1.650 دج
2	بذور الشيا	1 كلغ	700 دج	11	7.700 دج
3	نوى المشمش	1 كلغ	300 دج	11	3.300 دج
4	فيتامين E	عبوة سعة 30 مل	500 دج	11	5.500 دج
5	كوتينا	30 غ	560 دج	11	6.160 دج
6	بيكربونات الصوديوم	100 غ	50 دج	11	550 دج
7	ايزوكسيد	5 مل	300 دج	11	3.300 دج
8	أحماض الفا هيدروكسي	1 كغ	3700 دج	11	40.700 دج
9	اليوريا	1 كلغ	40 دج	11	440 دج
	نسبة ما نفقده من المواد الأولية 5 % (هدر من الإجمالي)				69.300 دج

4- الرواتب والأجور السنوية

الوظيفة	العدد	الراتب الشهري	الراتب السنوي	إجمالي الرواتب السنوية
المسير (صاحبة المشروع)	1	45.000 دج	540.000 دج	540.000 دج
فني تشـغيل	1	25.000دج	300.000دج	300.000دج
عمالة فنية	1	35.000دج	420.000دج	420.000دج
المساهمة في التأمينات الاجتماعية		%26		327.000دج
اجمالي الاجور السنوية				1.587.000 دج
إجمالي عدد العاملين (بمافيهم صاحب المشروع): 3، منهم 2عمالة.				

5- مصاريف / أعباء أخرى: (تحتسب بالشهر أو حسب مدة الاشتراك)

الأعباء والمصاريف	لمبلغ (دج)
1. مصاريف النقل 2. الصيانة والتصليح 3. الكهرباء والغاز والماء 4. الهاتف والأنترنت	1. 42.000 دج سنويا (400 دج شهريا) 2. 20.000 دج سنويا مرة او مرتين. 3. 120.000 دج سنويا. (10.000 دج شهريا). 4. 36.000 دج سنويا. (3000 دج شهريا).
المجموع (دج)	218.000 دج سنويا

6- نفقات الاستغلال

الشهر	1	2	3	4	5	6	7	8	9	10	11	12
المشتريات(السلع ،المواد الأ لولية والمواد المستهلكة)	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج	6300 دج
نفقات التسيير (الإيجار، النقل، كهرباء وغاز ، ماء، الصيانة، هاتف ، أنترنيت،)...	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج	107.000 دج
نفقات الموارد البشرية (أجور العمال)	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج	132.300 دج
مجموع نفقات الاستغلال	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج	245.600 دج

7-احتياجات المشروع من الآلات والمعدات

الرقم	البيان	العدد	الوحدة قيمة	الإجمالي القيمة
1	ميزان الكتروني دقيق (1كغ - 5كغ)	1	3500 دج	3500دج
2	مطحنة كهربائية (1كغ)	1	20.000 دج	20.000 دج
3	آلة عصر الزيت كهربائية	1	70.000 دج	70.000 دج
4	خلاط مخبري	1	50.000 دج	50.000 دج
5	آلة تعبئة يدوية	1	40.000 دج	40.000 دج
6	صفيحة تسخين كهربائية	2	30.000 دج	60.000 دج
7	بيشر صغير100 مل	1	500 دج	500 دج
8	بيشر متوسط250 مل- 500 مل	1	800 دج	800 دج
9	بيشر كبير 1000 مل	1	1200 دج	1200 دج
10	مخبار مدرج 10 مل	1	500 دج	500 دج
11	مخبار مدرج 50 مل	1	800 دج	800 دج
12	ملعة مخبرية صغيرة	1	300 دج	300 دج
13	ملعقة مخبرية كبيرة	1	400 دج	400 دج
14	بوتقه	1	500 دج	500 دج
15	حوض حمام مائي	1	4000 دج	4000 دج
المجموع				**252.500 دج**

8- احتياجات المشروع من الأثاث والتجهيزات المكتبية

البيان	التكلفة دج
الأثاث المكتبي (مكتب استقبال + كراسي)	50.000دج+40.000دج=90.000د ج
أجهزة الحاسب الآلي والبرامج (حاسوب مكتبي+ طابعة)	80.000دج+40.000دج=120.000 دج
أجهزة اتصالات (هاتف ثابت + انترنت)	30.000دج+6000دج=36.000دج
الإجمالي	**246.000دج**

9- احتياجات المشروع من وسائل النقل والآليات

في هذا المشروع نحتاج إلى وسيلة نقل واحدة لنقل المواد الأولية من عند الموردين إلى مقر التصنيع وكبداية سنعتمد على كراء سيارة بقيمة 4000 دج شهريا (نقل المواد مرة كل أسبوع حسب الدورة الإنتاجية).

النـوع	العدد	السعر(دج)	القيـمة(دج) سنويا
سيارة عادية	**1**	**4000 دج شهريا**	**48.000 دج**
المجمـوع			**48.000 دج**

د/ بيانات الدراسة المالية

1- إجمالي رأس المال الثابت

البيان	القيمـة (دج)
المباني (كراء محل)	**90.000 دج شهريا**
الات و المعـدات	**252.500 دج**

وسائل النقل	**48.000 دج سنويا**
ادوات و تجهيزات مكتبية	**156.000 دج**
الأثـــاث	**90.000 دج**
مصـــاريف التأسيس (مواد أولية)	**69.300 دج**
مصاريف التعبئة والتغليف	**50.000 دج**
المجمـــــوع	**755.800 دج**

2- تقدير رأس المال العامل

البيان	التكلفة لدورة انتاجية	التكلفة السنوية
إيجار	**90.000 دج**	**1.080.000 دج**
المواد الخام	**6300 دج**	**69.300 دج**
أجور ومرتبات	**132.300 دج**	**1.587.000 دج**
مصروفات تسويق	/	**256.000 دج**
مصروفات صيانة وإصلاح	**10.000 دج (مرتين سنويا)**	**20.000 دج**
مصروفات التأمين	**27.300 دج**	**327.600 دج**
الإجمالي	**0**	**3.339.900 دج**
إجمالي رأس المال العامل		

العملاء	العلاقة مع العملاء	عرض القيمة	الأنشطة الرئيسية	الشركاء الرئيسيون
*النساء والرجال الذين يعانون من تصبغات. * فئتهم العمرية: 18 سنة فما فوق.	*خدمة العملاء (سريعة) للرد على الأسئلة أو حل المشاكل. *نشر معلومات على عناية بالبشرة، طريقة الاستخدام. *تجارب مجانية أي توزيع عينات لجذب العملاء الجدد وتشجيعهم على تجربة المنتج. *متابعتهم عبر البريد الإلكتروني أو الرسائل.	*استخدام مستخلصات نباتية محلية متوفرة. *مفعول ظاهر خلال فترة ز منية قصيرة. *رائحة طبيعية لطيفه وغير مزعجة (جذب العملاء الذين يهتمون بالرائحة). *اختيار عبوة أنيقة وسهلة ا لاستخدام. *توفير منتج بجودة وفعالية عالية وسعر معقول.	*تطوير المنتج من حيث ال جودة. *تصنيع المنتج وفق معايير صحية. *توزيع ذاتي. *تسويق المنتج.	*الموردون (مكونات طبيعية، عبوات، مواد التغليف). *المختبرات (مخابر كيمياء جامعة محمد خيضر بسكرة، مخابر مركز البحث العلمي). *شركات التوصيل. *حاضنة أعمال جامعة بسكرة.
	قنوات توزيع *منصات التواصل الاجتماعي للترويج وبناء العلامة والموقع الالكتروني الرسمي للمؤسسة. *موقع المشروع (على مستوى المحل).		**الموارد الرئيسية** * بذور الشيا ونوى المشمش. *المواد الكيميائية. *مستخلصات طبيعية ومحلية.	

	*التسويق عبر عرض المنتج في المعارض الدولية.		*آلات التصنيع. * العمال.	
الأرباح *مبيعات المنتج.			**تكاليف** *تكاليف تأسيسية: معدات الإنتاج. *تكاليف ثابتة: كهرباء، ماء، الاتصالات، الكراء وأجور العمال. *تكاليف متغيرة: تكاليف المواد الأولية (نباتات ومواد كيميائية.)	

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Université Med Khider Biskra

Faculté des Sciences Exactes
Département des Sciences de la Matière

Filière de Chimie

الجمهورية الجزائرية الديموقراطية الشعبية
وزارة التعليم العالي و البحث العلمي
جامعة محمد خيضر بسكرة

كلية العلوم الدقيقة
قسم علوم المادة
شعبة الكيمياء

تصريح شرفي

خاص بالالتزام بقواعد النزاهة العلمية لإنجاز بحث

(ملحق القرار 1082 المؤرخ في 2021/12/27)

أنا الممضي أسفله،

السيد(ة): خريفي الياسمينة

الصفة: طالب سنة ثانية ماستر كيمياء تخصص: كيمياء صيدلانية

الحامل(ة) لبطاقة التعريف الوطنية رقم: .... الصادرة بتاريخ: 2019/11/03

المسجل بكلية: العلوم الدقيقة قسم: علوم المادة والمكلف

بانجاز أعمال بحث : مذكرة ماستر في الكيمياء

عنوانها: chemical composition and combined in vitro - in silico approach of natural antioxydant agents for Skin care

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات المهنية والنزاهة الاكاديمية المطلوبة في انجاز البحث المذكور أعلاه وفق ما ينص عليه القرار رقم 1082 المؤرخ في 2021/12/27 المحدد للقواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.

التاريخ: 2025/06/15

إمضاء المعني بالمر

Khri