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FACULTY OF NATURAL SCIENCES AND LIFE

قسم البيولوجيا الخلوية والجزيئية

DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY

Master's Thesis

In order obtain a diploma of an Academic Master

In biological sciences

Specialty: Applied Biochemistry

Theme

Evaluation of the Physicochemical Properties and In Vitro Biological Activities of *Milk thistle (Silybum marianum) Seed Oil*: Exploring Its Potential Applications in Dermatological and Cosmetic Fields

Presented by: ADAIKA Amani and ADAIKA Djihad and BERGUIGA Keltoum

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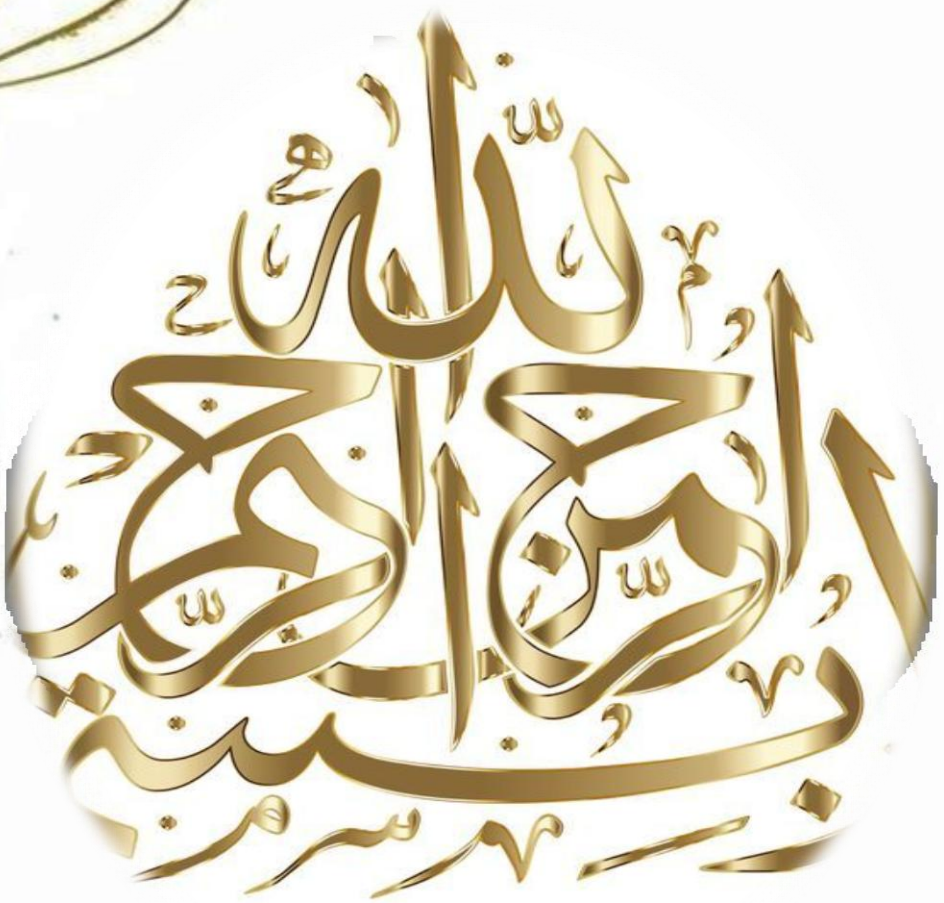

بسم الله الرحمن الرحيم

Dédicace:

In the name of Allah, the Most Gracious, the Most Merciful

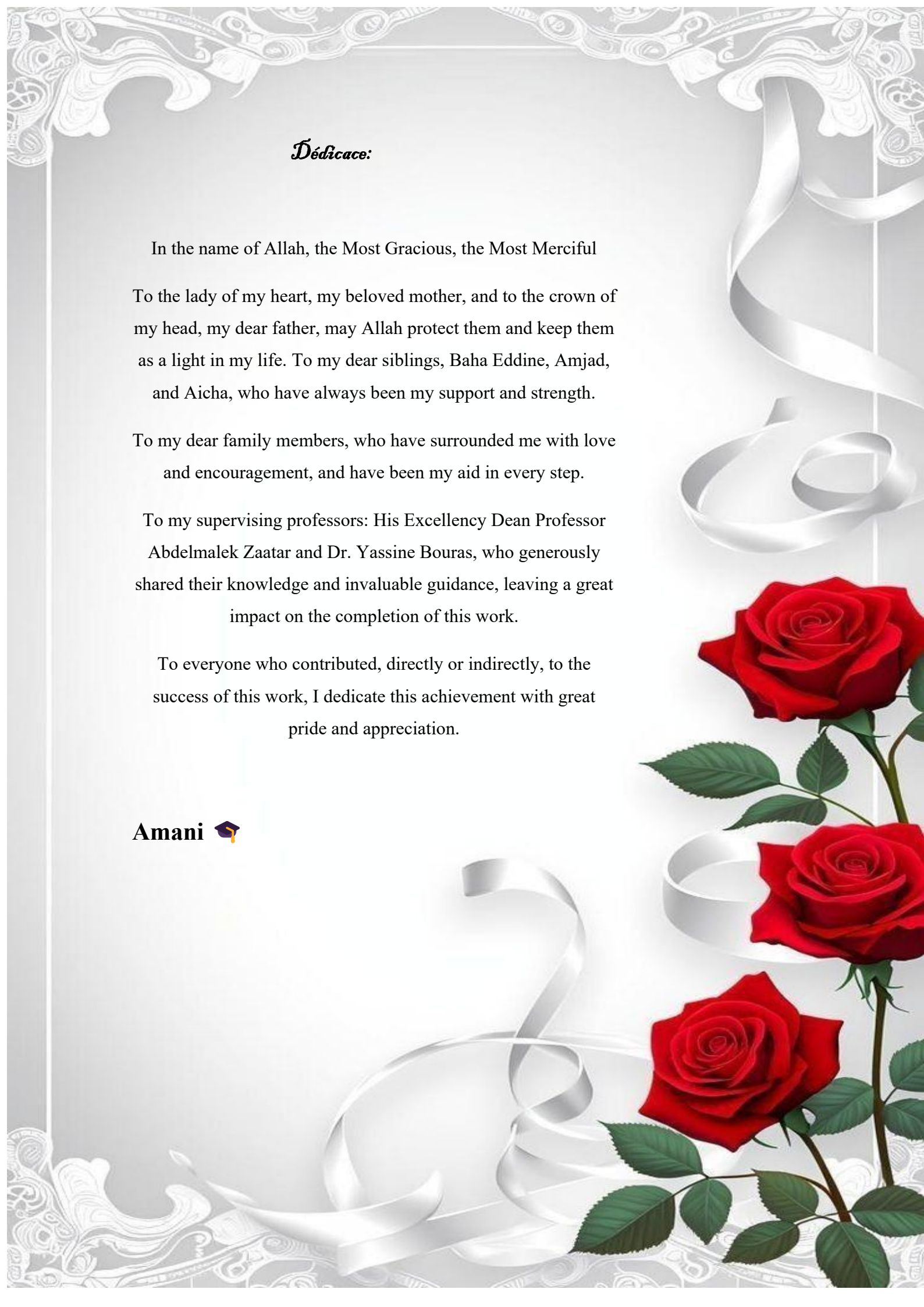
To the lady of my heart, my beloved mother, and to the crown of my head, my dear father, may Allah protect them and keep them as a light in my life. To my dear siblings, Baha Eddine, Amjad, and Aicha, who have always been my support and strength.

To my dear family members, who have surrounded me with love and encouragement, and have been my aid in every step.

To my supervising professors: His Excellency Dean Professor Abdelmalek Zaatar and Dr. Yassine Bouras, who generously shared their knowledge and invaluable guidance, leaving a great impact on the completion of this work.

To everyone who contributed, directly or indirectly, to the success of this work, I dedicate this achievement with great pride and appreciation.

Amani 🎓



Dédicace:

With the deepest feelings of gratitude and love, I dedicate this humble work to the people who have always been my greatest support.

To my beloved parents, my father Soltan and my mother Salima, whose endless love, sacrifices, prayers, and encouragement have been the light guiding my path. Your belief in me has been my greatest strength, and this achievement is as much yours as it is mine.

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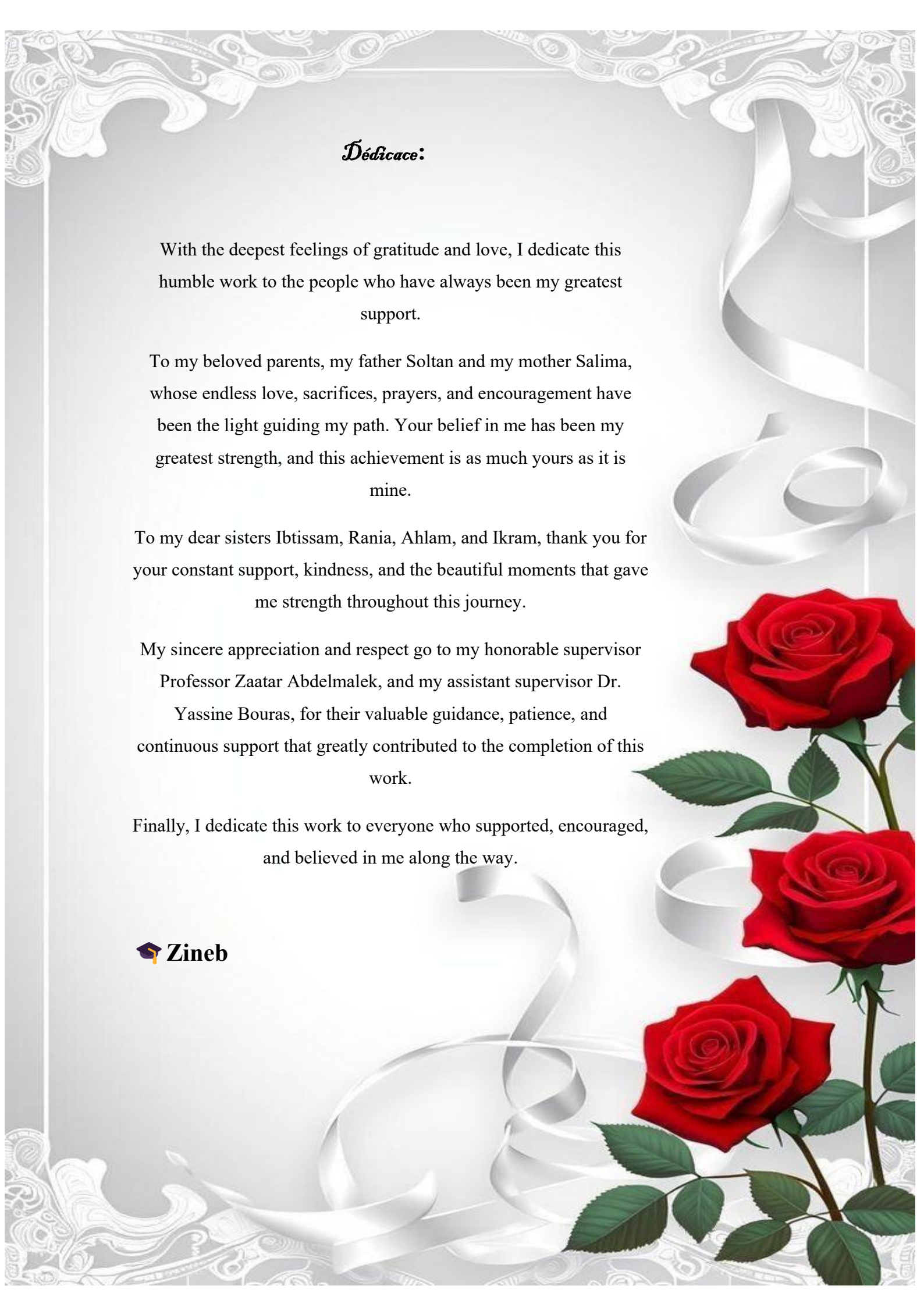
Finally, I dedicate this work to everyone who supported, encouraged, and believed in me along the way.

🎓 **Zineb**



Dédicace:

I dedicate this humble work to the most precious people in my life, my beloved parents, who have always been my support and constant source of encouragement throughout all stages of my life.

I also dedicate this success to my dear siblings, Abdelkarim, Youssef, and Ahmed, who have always been a pillar of support and a source of motivation, and to my beloved sisters, Aïcha, Safaa, Dziria, and Radia.

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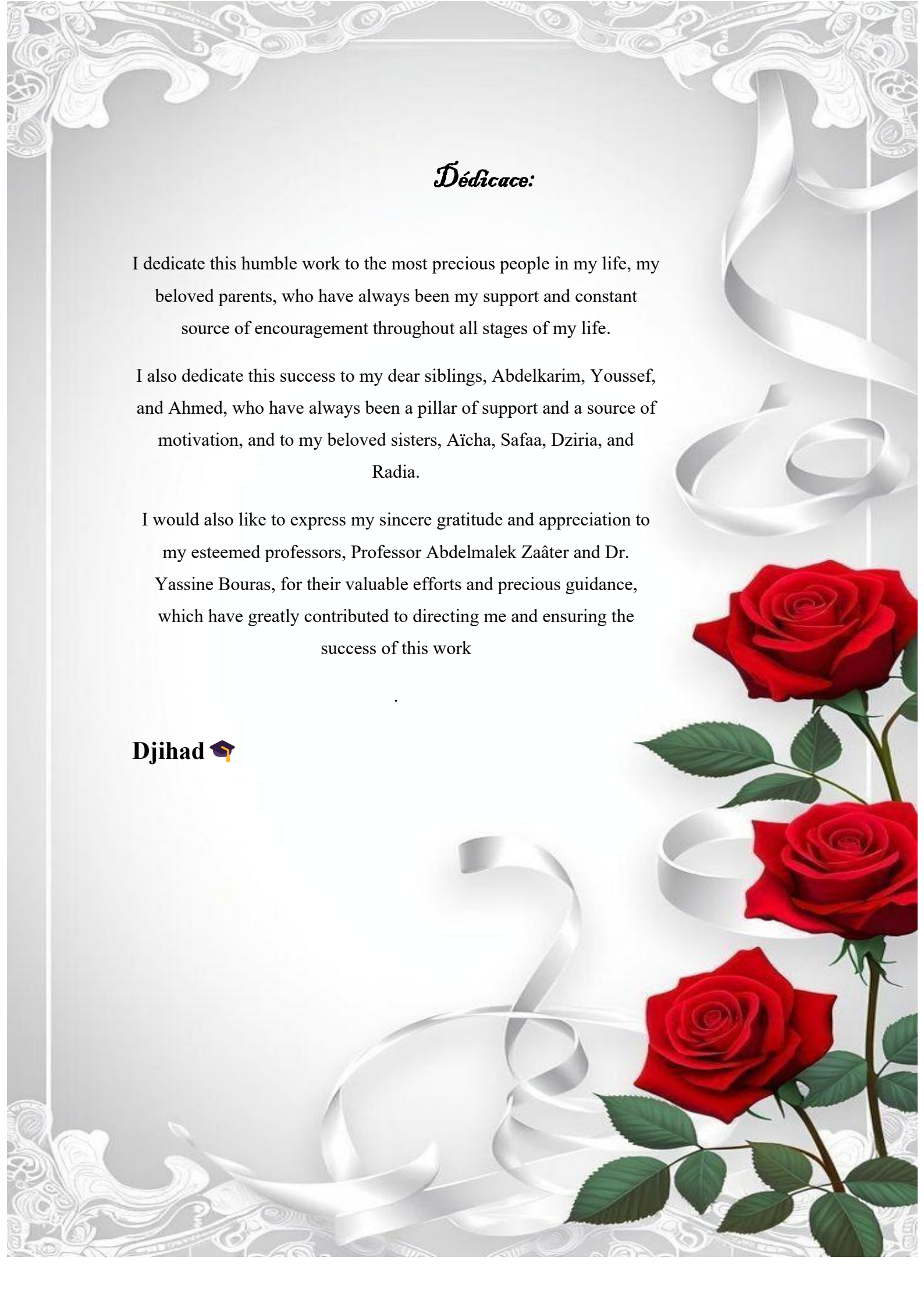
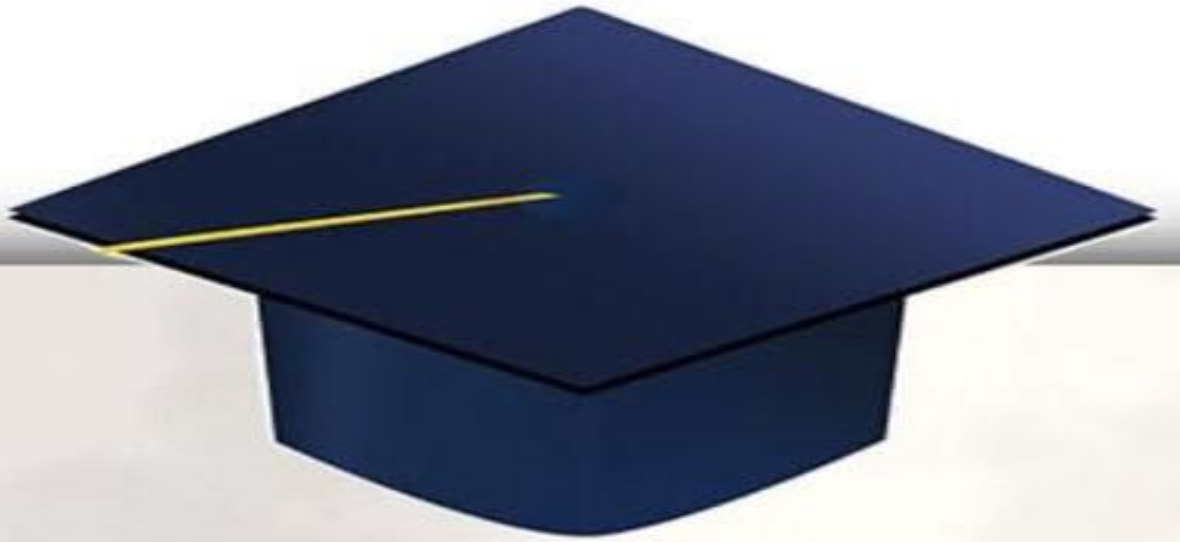
Djihad 🎓





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Finally, I would like to express my gratitude to everyone who contributed, directly or indirectly, to the completion of this work. May God reward them all abundantly and bless their efforts.

ملخص

تهدف هذه الدراسة إلى تقييم الخصائص الفيزيائية-الكيميائية والأنشطة البيولوجية لزيت بذور *Silybum marianum*، مع استكشاف إمكانياته في التطبيقات الجلدية والتجميلية. تم الحصول على الزيت باستخدام طريقة العصر البارد، حيث أظهرت النتائج مردود استخلاص قدره (11.52%)، مع درجة حموضة شبه متعادلة (pH = 6.24) وكثافة (0.92 غ/مل)، مما يدل على توافق جيد مع البشرة واستقرار في التركيب.

كما سجل الزيت قدرة عالية جدًا على الحماية من الأشعة فوق البنفسجية، حيث بلغ عامل الحماية من الشمس (SPF) قيمة (135.3). وفيما يتعلق بالنشاط المضاد للأكسدة وفق اختبار FRAP، تشير النتائج إلى وجود فرق إحصائي معنوي مرتفع بين زيت بذور شوك الجمل وحمض الأسكوربيك، حيث سُجلت قيمة EC_{50} للزيت (0.336 ± 0.006 µg/mL) مقارنة بـ (0.425 ± 0.004 µg/mL) لحمض الأسكوربيك. وقد أظهر التحليل الإحصائي باستخدام اختبار (Student's t-test) لعينتين مستقلتين أن هذا الفرق ذو دلالة إحصائية عالية جدًا (***, $P < 0.001$)، مما يؤكد تفوق الزيت في قدرته على اختزال الحديد ومعادلة الجذور الحرة.

كذلك أظهر الزيت نشاطًا تثبيطيًا قويًا لإنزيم التيروزيناز (IC_{50} = 16.8%) مقارنة بالمركب المرجعي (IC_{50} = 45%)، مما يعزز فعاليته المحتملة في تقليل تصنيع الميلانين. أما النشاط المضاد للبكتيريا، فقد بيّن فعالية معتبرة، حيث تراوحت قيم MIC وMBC بين (40 و80 ملغ/مل) حسب السلالات البكتيرية، مع اختلاف في الحساسية، في حين أكد معامل (MBC/MIC = 1) تأثيرًا قاتلًا للبكتيريا.

كما أظهر الزيت قدرة واضحة على تثبيط تكوين الأغشية الحيوية بشكل معتمد على التركيز، إضافة إلى تسجيل سمية خلوية منخفضة إلى متوسطة مرتبطة بالجرعة، مما يشير إلى هامش أمان مقبول.

وبناءً على هذه النتائج، يبرز زيت بذور *Silybum marianum* كمصدر طبيعي واعد متعدد الوظائف، يتميز بخصائص مضادة للأكسدة والبكتيريا ومفتحة للبشرة، مع إمكانية استخدامه في التطبيقات الصيدلانية والتجميلية.

الكلمات المفتاحية: شوك الجمل (*Silybum marianum*)، زيت بذور شوك الجمل، الاستخلاص، التركيب الكيميائي، النشاط المضاد للأكسدة، تثبيط إنزيم التيروزيناز، النشاط المضاد للبكتيريا، عامل الحماية من الشمس (SPF).

Résumé

Cette étude visait à évaluer les propriétés physico-chimiques et les activités biologiques de l'huile de graines de *Silybum marianum*, tout en explorant son potentiel dans les applications dermatologiques et cosmétiques. L'huile a été obtenue par pression à froid, avec un rendement d'extraction de 11,52 %, un pH quasi neutre de 6,24 et une densité de 0,92 g/mL, indiquant une bonne compatibilité cutanée et une stabilité structurale.

L'huile a également démontré une très forte capacité de protection contre les rayons ultraviolets, avec un facteur de protection solaire (SPF) de 135,3. Concernant l'activité antioxydante évaluée par le test FRAP, les résultats ont révélé une différence statistiquement très significative entre l'huile de graines de *Silybum marianum* et l'acide ascorbique. La valeur de EC_{50} de l'huile était de 0,336 ± 0,006 µg/mL, contre 0,425 ± 0,004 µg/mL pour l'acide ascorbique. L'analyse statistique réalisée à l'aide du test t de Student pour deux échantillons indépendants a confirmé que cette différence était hautement significative (***, $P < 0,001$), démontrant le pouvoir réducteur supérieur de l'huile dans la réduction du fer et la neutralisation des radicaux libres.

L'huile a également présenté une forte activité inhibitrice contre la tyrosinase, avec une valeur de IC_{50} de 16,8 %, contre 45 % pour le composé de référence, soulignant son efficacité potentielle dans la réduction de la synthèse de mélanine. En ce qui concerne l'activité antibactérienne, l'huile a montré une efficacité considérable, avec des valeurs de CMI et CMB comprises entre 40 et 80 mg/mL, selon les souches bactériennes testées. Le rapport CMB/CMI = 1 a confirmé son effet bactéricide.

En outre, l'huile a démontré une inhibition nette de la formation de biofilms de manière dépendante de la concentration, ainsi qu'une cytotoxicité faible à modérée liée à la dose, suggérant une marge de sécurité acceptable.

Sur la base de ces résultats, l'huile de graines de *Silybum marianum* apparaît comme une source naturelle multifonctionnelle prometteuse, dotée de propriétés antioxydantes, antibactériennes et éclaircissantes pour la peau, ce qui en fait une candidate potentielle pour des applications pharmaceutiques et cosmétiques.

Mots clés : Chardon-Marie (*Silybum marianum*)، Huile de graines de chardon-Marie، Extraction، Composition chimique، Activité antioxydante، Inhibition de l'enzyme tyrosinase، Activité antibactérienne، Facteur de protection solaire (SPF).

Abstract

This study aimed to evaluate the physicochemical properties and biological activities of *Silybum marianum seed oil*, while exploring its potential in dermatological and cosmetic applications. The oil was obtained by cold pressing, yielding an extraction rate of 11.52%, with a near-neutral pH of 6.24 and a density of 0.92 g/mL, indicating good skin compatibility and structural stability.

The oil also demonstrated a very high ultraviolet protection capacity, with a sun protection factor (SPF) of 135.3. Regarding antioxidant activity assessed by the FRAP assay, the results revealed a highly significant statistical difference between Silybum marianum seed oil and ascorbic acid. The EC_{50} value of the oil was 0.336 ± 0.006 µg/mL, compared to 0.425 ± 0.004 µg/mL for ascorbic acid. Statistical analysis using the Student's t-test for two independent samples confirmed that this difference was highly significant (***, $P < 0.001$), demonstrating the superior reducing power of the oil in iron reduction and free radical scavenging.

The oil also exhibited strong inhibitory activity against tyrosinase, with an IC_{50} value of 16.8%, compared to 45% for the reference compound, highlighting its potential effectiveness in reducing melanin synthesis. In terms of antibacterial activity, the oil showed considerable efficacy, with MIC and MBC values ranging from 40 to 80 mg/mL, depending on the bacterial strains tested. The MBC/MIC ratio of 1 confirmed its bactericidal effect.

Furthermore, the oil demonstrated a clear concentration-dependent inhibition of biofilm formation, along with low to moderate dose-dependent cytotoxicity, suggesting an acceptable safety margin.

Based on these findings, Silybum marianum seed oil appears to be a promising multifunctional natural source with antioxidant, antibacterial, and skin-lightening properties, making it a potential candidate for pharmaceutical and cosmetic applications.

Key words: *Silybum marianum*، *Milk thistle seed oil*، Extraction، Chemical composition، Antioxidant activity، Tyrosinase inhibition، Antibacterial activity، SPF.

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Liste of Abbreviation

EC_{50}: Half Maximal Effective Concentration

IC_{50}: Half Maximal Inhibitory Concentration

FRAP: Ferric Reducing Antioxidant Power

SPF: Sun Protection Factor

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

pH: Potential of Hydrogen

UV: Ultraviolet

ATP: Adenosine Triphosphate

SFE: Supercritical Fluid Extraction

EOs: Essential Oils

TPTZ: 2,4,6-Tripyridyl-s-triazine

MT: Milk Thistle

CO_2: Carbon Dioxide

SOD: Superoxide Dismutase

CAT: Catalase

UV Radiation :Ultraviolet Radiation

g/mL: gram per milliliter

mg/mL: milligram per milliliter

µg/mL: microgram per milliliter

%: Percentage

R^2: Coefficient of Determination

p: p-value

SD: Standard Deviation

UVB: Ultraviolet B

General Introduction

Medicinal plants are among the most important natural sources of bioactive compounds that have been used for centuries in traditional medicine to treat various diseases. With the significant advancements in biological and chemical sciences, scientific interest in studying plants and extracting their active compounds has increased due to their diverse pharmacological properties and important therapeutic effects. Furthermore, the global trend toward seeking natural alternatives to synthetic compounds, due to their potential side effects, has reinforced studies focusing on plant sources rich in phenolic compounds, antioxidants, and antimicrobial agents (**Cowan, 1999; Balunas & Kinghorn, 2005**).

Among the medicinal plants that have received wide scientific attention is *Milk Thistle* (*Silybum marianum*), which belongs to the Asteraceae family. This plant is widely distributed across many regions of the world, particularly in the Mediterranean basin and North Africa, and has been used for thousands of years in traditional medicine due to its multiple therapeutic properties, especially in the treatment of liver disorders. The biological activity of this plant is mainly attributed to its bioactive compounds, such as silymarin, a group of flavonolignans known for their antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial properties (**Abenavoli et al., 2018**; **Federico et al., 2017**).

In addition to its active compounds, the seeds of *Milk Thistle* (*Silybum marianum*) are an important source of vegetable oil rich in various bioactive molecules, including unsaturated fatty acids, phenolic compounds, tocopherols, and phytosterols. These compounds contribute to the oil's biological properties, such as antioxidant and antimicrobial activities, making it increasingly interesting for applications in the food, pharmaceutical, and cosmetic industries (**El-Mallah et al., 2003; Meddeb et al., 2017**).

Oxidative stress is one of the main factors associated with the onset of many chronic diseases, including cardiovascular diseases, cancer, diabetes, and neurological disorders. Therefore, the search for natural antioxidants capable of reducing the effects of free radicals has become a highly significant topic in scientific research. Plant oils rich in phenolic compounds and tocopherols are considered promising natural antioxidants due to their ability to neutralize reactive oxygen species and protect cellular components from oxidative damage (**Lobo et al., 2010; Nile & Park, 2014**).

Additionally, the increasing resistance of bacteria to conventional antibiotics represents a global health challenge, driving researchers to explore new natural compounds with antibacterial activity capable of inhibiting the growth of pathogenic microorganisms. The severity of bacterial infections is further increased when bacteria form biofilms, which are complex microbial communities surrounded by an extracellular polymeric matrix secreted by

bacterial cells, enhancing their resistance to antimicrobial agents and the host immune response. Therefore, searching for natural substances capable of inhibiting biofilm formation has become a key focus in modern microbiological studies (**Costerton et al., 1999; Flemming & Wingender, 2010**).

Plant-derived compounds are also gaining growing interest in the cosmetic industry due to their ability to provide protection against ultraviolet (UV) radiation and contribute to the inhibition of certain enzymes associated with skin pigmentation processes, such as tyrosinase. Natural antioxidants and phenolic compounds have shown significant potential in cosmetic formulations because of their photoprotective, anti-aging, and skin-whitening properties (**Saewan & Jimtaisong, 2015; Solano, 2014**).

This study aims to evaluate the biological properties of *Milk Thistle seed oil*. To achieve this, its antioxidant activity was assessed using the FRAP assay, the sun protection factor (SPF) was determined, and its ability to inhibit tyrosinase activity was evaluated.

Furthermore, its antibacterial activity was investigated through the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as well as its effect on bacterial biofilm formation.

Thesis Problematic:

This study seeks to answer the following main research questions:

- ✓ To what extent does the *seed oil of Milk thistle (Silybum marianum)* exhibit physicochemical properties that reflect its quality and biological potential?
- ✓ Does the seed oil show antioxidant activity, particularly when evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay?
- ✓ Does *Milk thistle seed oil* possess antibacterial activity against certain pathogenic bacterial strains?
- ✓ Can the seed oil inhibit the formation of bacterial biofilms?
- ✓ Does the oil have the ability to inhibit the enzyme tyrosinase associated with pigmentation processes?
- ✓ Can *Milk thistle seed oil* exhibit protective properties against ultraviolet radiation through the evaluation of the Sun Protection Factor (SPF)?

Thesis Structure:

1. Theoretical Part – Includes two chapters:

➢ Chapter 1: General background and literature review on the seeds of the *plant Silybum marianum*.

➢ Chapter 2: This chapter deals with the biologically active compounds present in *Milk thistle seeds* and their oil, with a focus on phenolic compounds, flavonoids, and fatty acids. It also

discusses the main methods used for the extraction of vegetable oils, particularly those applied for seed oil extraction, such as mechanical pressing and solvent extraction.

2. Experimental Part – Includes two chapters:

- Chapter 1: Description of the materials, experimental methods, and protocols used in the study.
- Chapter 2: Presentation and discussion of the results, including antioxidant assays, evaluation of the sun protection factor (SPF), and antibacterial activity tests.

First part

Bibliographic synthiesis

Chapter 1:

General overview of *Silybum marianum*

1.1. Introduction:

This section introduces *Silybum marianum* (commonly known as *Milk Thistle*) as a medicinal and ecological plant of great importance. The chapter highlights its rising global interest due to its rich pharmacological profile, strong adaptability to harsh environments, and its significant value in traditional and modern medicine. Special emphasis is placed on its bioactive compounds, particularly silymarin, which has gained scientific attention for its therapeutic and protective effects.

1.2. *Silybum marianum*:

Milk thistle (MT; Silybum marianum), a member of the Asteraceae family, is a therapeutic herb with a 2,000-year history of use. MT fruits contain a mixture of flavonolignans collectively known as silymarin, being silybin (also named silibinin) the main component. This article reviews the chemistry of MT, the pharmacokinetics and bioavailability, the pharmacologically relevant actions for liver diseases (e.g., anti-inflammatory, immunomodulating, antifibrotic, antioxidant, and liver-regenerating properties) as well as the clinical potential in patients with alcoholic liver disease, nonalcoholic fatty liver disease, viral hepatitis, drug-induced liver injury, and mushroom poisoning. Overall, literature data suggest that, despite encouraging preclinical data, further well-designed randomized clinical trials are needed to fully substantiate the real value of MT preparations in liver diseases. **(Abenavoli et al., 2018).**

Figure 1. 1: Silybum marianum plant.

1.3. Taxonomic Classification of *Silybum marianum*:

The following table shows the taxonomic classification and scientific naming of the *Milk Thistle (Silybum marianum) plant*. **(Karimi et al., 2011).**

Table 1. 1: Classification and scientific naming of Silybum marianum.

Kingdom	**plantae**
Sub-Kingdom	Tracheobionta (Vascular plants)
Phylum	Magnoliophyta (Flowering plants)
Sub-Phylum	Spermatophyta (Seed plants)
Class	Magnoliopsida (Dicotyledons)
Sub-Class	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Silybum
Species	*Silybum Marianum (L.) Gaertn*

In fact, the genus Silybum (*Milk thistle*) includes several species, but *Silybum marianum (Milk thistle)* is the most widespread and extensively studied. This is due to its global availability, its well-established effectiveness in liver protection, and its rich content of active phenolic compounds such as silymarin.

1.4. Common Names of *Silybum marianum*:

Common name: *Silybum marianum*, Camel Horn, Wild Artichoke, *Milk Thistle*, Blessed Thistle, Wild Globe Artichoke. **(Ben R. 2012).**

1/The name in Arabic: شوك الجمل, بوزروال, عكوب,الخرشف الجبلي , الارضي الشوكي البري.

2/The name in French: Silybe de marie, Chardon marbré, Chardon-Marie.

3/The name in Tuareg: ثاورة.

4/The name in Berber: تاسكرت, امسكليل. **(Halimi A. 1997).**

5/**The name in English:** Milk thistle, Blessed Milk thistle.

6/The name in German: Mariendiste.

7/The name in Spanish: Cardo lechero, Cardo mariano.

8/The name in Chinese: Hui Fei Ji.

1.5. Morphological Description:

Milk thistle (Silybum marianum) is an annual or biennial herbaceous plant whose growth depends mainly on the climate and ecological conditions. It grows in rocky terrains and in dry and arid areas. Its height reaches 3–6 meters, and it belongs to the family Asteraceae. One of the main identifying characteristics of this plant is its leaves, which have a marbled appearance with white veins. This species is commonly found along roadsides and pathways, either in large or moderate populations. **(Julian Charbonnaud. 2007).**

Figure 1. 2: Drawing of the different parts of the Milk thistle plant.

1.5.1. Seeds:

The seeds are brown to gray in color, smooth, and shiny, with a hard texture and sometimes bearing small spots near the tip. Their length ranges between 2–6 mm.

Color: purplish to light brown.

Shape: rounded base with a truncated apex.

Size: 3.2–5.4 × 1.4–8 mm. **(Julian Charbonnaud. 2007).**

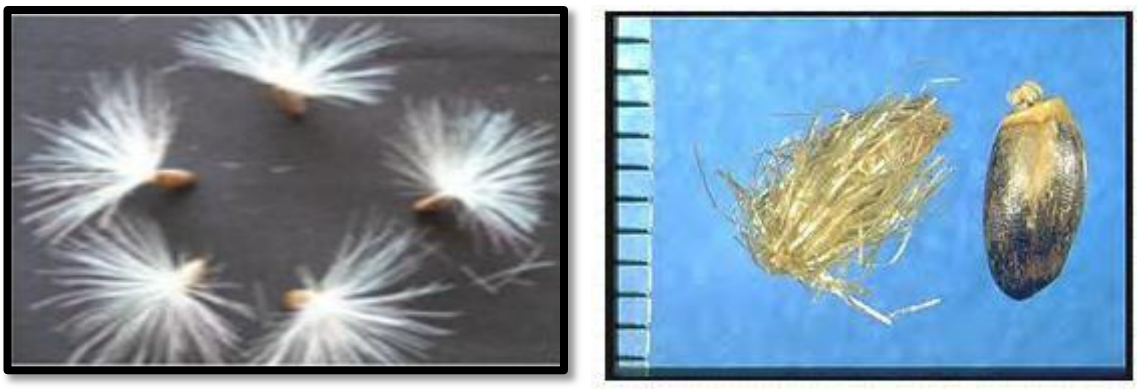


Figure 1. 3: Seeds of the Milk thistle plant.

1.5.2. Leaves:

Cotyledon leaf: elongated with a spoon-like shape.

Leaves: simple and serrated, with deeper teeth during the early stages of growth.

Leaves during development: elongated and lanceolate, bearing sharp spines. They are green in color, marked with white marbling along the veins. The upper leaves are amplexicaul (clasping the stem). **(Julian Charbonnaud. 2007).**

Figure 1. 4: Embryonic leaf of a plant.

1.5.3. Roots:

According to botanical descriptions, *Milk thistle* is characterized by a strong, long, thick,

1.5.4. Stems:

The stems are generally erect, reaching a height of 20 to 60 cm, and they are slightly branched near the upper part of the plant. **(Amarani O. 2008).**

1.5.5. Flowers:

The flowers are purplish and are grouped in globular flower heads, each measuring (4–5 cm) in diameter. They are surrounded by spiny bracts arranged along the central receptacle.

1.5.6. Fruits:

The fruits are shiny achenes, with a yellowish to brownish color similar to that of honey. Each fruit is topped by a long, silky pappus attached at its base .

The plant does not emit any particular odor and resembles an artichoke in structure.

The plant does not emit a particular smell and is characterized by an artichoke flavor. **(Dr. Jean clande laproz; Dr Alain Crillon. 201) .**

Figure 1. 5: Different parts of the Milk thistle plant. (Benchaachoua, A. 2019).

Figure 1. 6: Milk thistle flowers.

1.6. Habitat and Origin:

Milk thistle is a wild plant native to the Mediterranean basin **(Bezanger et al., 1980).** According to **Bayer and Buller (1990)**, it ranges from sea level to 700-1100 m altitude on dry, rocky, uncultivated land throughout Western and Southern Europe, as well as in North Africa. *Milk thistle* is cultivated in ornamental gardens **(Roche, 1991)**. According to Quezel and Santa (1963), this plant is cosmopolitan, preferring dry soils and warm, sunny locations. It is found in fields, wastelands, rubble, and roadsides. In Algeria, according to Belouahem (2009), *Milk thistle* is particularly widespread in the high plateaus, the steppe, the southern Saharan Atlas Mountains, sandy pastures, and slightly damp areas. According to **Sindel (1991)** and (**Gabay et al., 1994)**, *Milk thistle* is now widespread in North America, so much so that it is found in Canada, Mexico, New Zealand, Australia, South Africa, Chile, and Argentina.

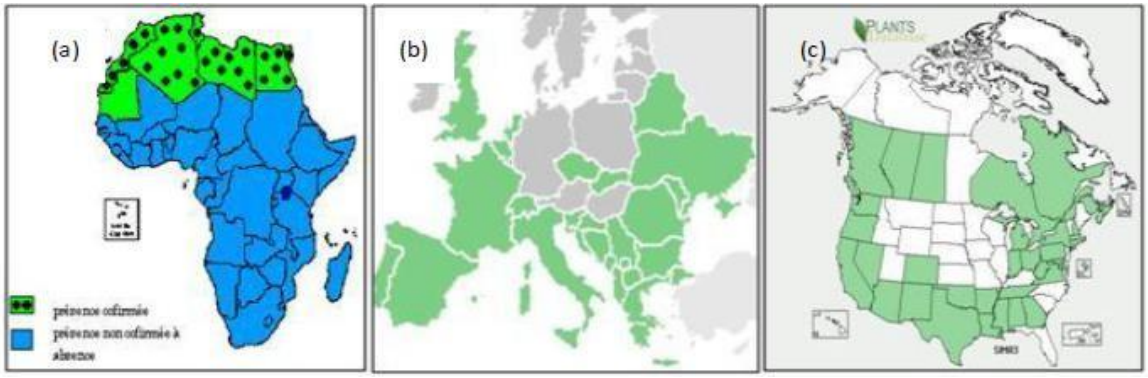


Figure 1. 7: Distribution of Milk thistle (Silybum marianum) in Africa (A), Europe (B), and America (C). (Ben R. 2012).

1.7. Types of *Silybum marianum (L)*:

A. *Silybum marianum (L.)* Gaertn. var.marianum

- Flowers are purple in color.
- Seeds are black, larger in size.

B. *Silybum marianum (L.)* Gaertn. var.albiflorum

- Flowers are white.
- Seeds are brown speckled, smaller in size.

Figure 1. 8: A photograph showing inflorescences terminal heads (capitula) of two varieties of Silybum mariaum L. heads large, solitary, discoid (tubular flower).

Figure 1. 9: A photograph showing seeds of two varieties of Silybum mariaum L.

The main difference between the two types:

Based on the results of the study conducted by the researchers. **(Azoz, S et al., 2019).**

Table 1. 2: Comparative Morphological and Biochemical Characteristics of Two Varieties of Silybum marianum L.

The attribute	**Var. marianum**	**Var. albiflorum**
Flower color	purple	white
Seed color	black	Spotted brown
Plant height	Top (59.3cm)	Less (43.6 cm)
leg thickness	Top (7.3 mm)	Less (3.7 mm)
Number of seeds	Top (145 seeds)	Less (82.66 seeds)
Weight of 1000 seeds	Highest (24.53g)	Less (15.73g)
Silymarin content	Higher (4.3 micrograms/ml)	Less than (2.0 micrograms/ml)
Amino acids	Higher in phenylalanine	relatively lower
fatty acids	Higher in linoleic acid	relatively lower

1.8. Chemical Composition:

The fruit contains 15 to 30% lipids, predominantly linoleic acid (60%), oleic acid (30%), and palmitic acid (9%), approximately 25 to 30% protein, as well as tocopherol (approximately 0.038%), phenolic derivatives, and sterols (approximately 0.063%), including ducampesterol, dutigmasterol, and sitosterol. Some sugars (arabinose, rhamnose, xylose, glucose) and mucilage are also present **(Abenavoli et al., 2010)**. It also contains flavonoids: quercetin, taxifolin, eryodictyol, chrysoeriol, and several others, including dihydrokaempferol, kaempferol, apigenol, and naringetol, which also possess interesting properties. According to **Bruneton (1999)**, they play only a minor role in the properties attributed to *Milk thistle*. The achene also contains 1.5 to 3% flavanolignans, the main fraction of which is called silymarin. Silymarin constitutes the most active molecules in milk thistle, and its major components are silybin (the largest constituent of the mixture, 50-60%), silydianin (approximately 10%), and silychristin (20%), along with their numerous stereoisomeric derivatives. isosilybin approximately 5%, dihydrosilybin, silimonine, and other compounds: silandrin, silhermin, neosilihermins A and B,

2,3-dehydrosilibinin, and tripentamer desilibinin (silybinomers). Some of these minor flavanolignans are present only in white-flowered varieties.

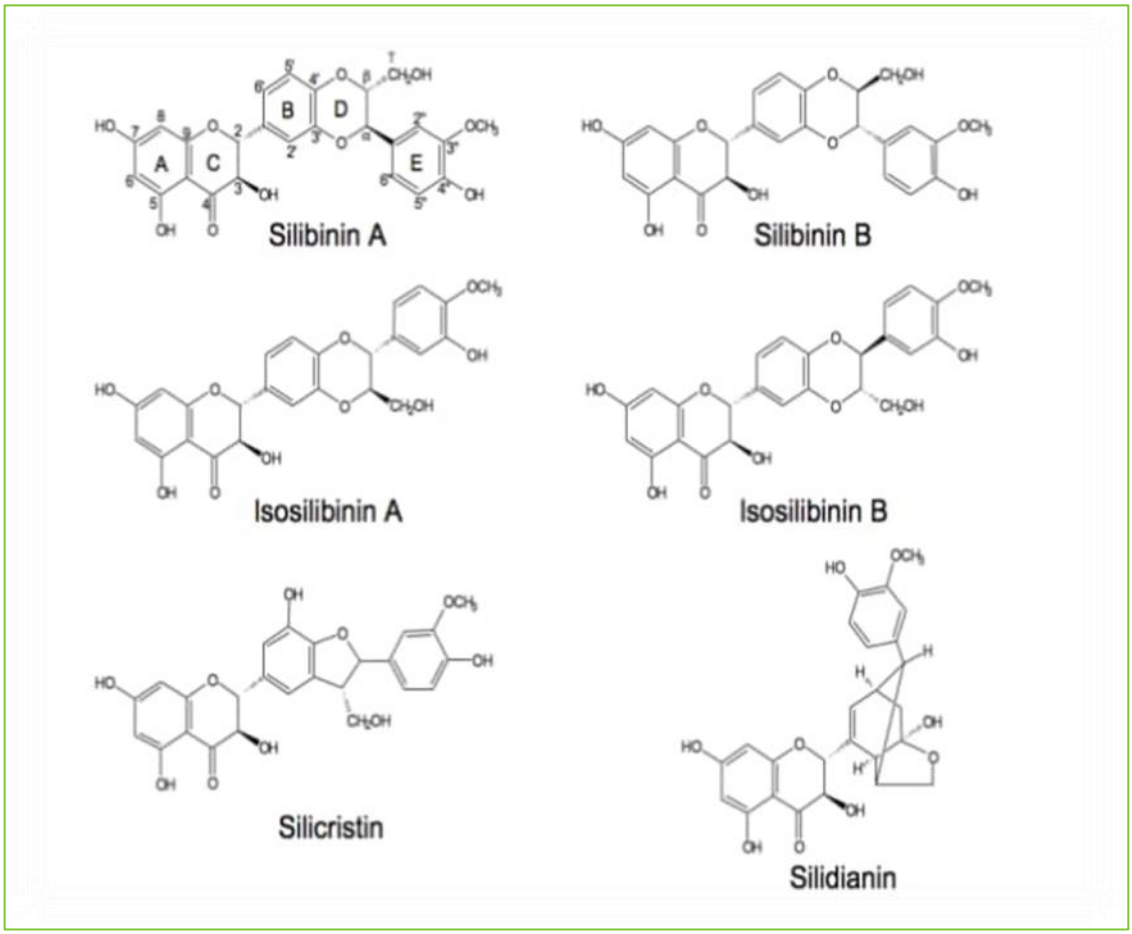


Figure 1. 10: Major flavanolignans from Silybimum (karimi, G et al., 2011).

1.9. Nutritional value of *Silybum marianum (Milk thistle)*:

Thistle seeds (Silybum marianum) are distinguished by their high nutritional value, making them of increasing interest in functional nutrition and the food industry. They are a significant source of energy, with a caloric content ranging from 430 to 480 kcal per 100 g of dry seeds. This is primarily attributed to their high vegetable fat content, which ranges from 20 to 30%. These fats consist mostly of unsaturated fatty acids, particularly linoleic acid (Omega-6) and oleic acid (Omega-9), which play an important role in maintaining heart health and regulating blood cholesterol levels **(Křen, V and Walterová, D. 2005)**. Furthermore, thistle seeds contain a considerable amount of plant protein, ranging from approximately 18–25%, making them a valuable dietary supplement, especially in vegetarian diets **(Abenavoli et al., 2018)**. These seeds are also a good source of dietary fiber, which contributes to improved digestion and the regulation of sugar and fat absorption. On a mineral level, they are rich in essential elements such as potassium, calcium, magnesium, phosphorus, and iron, which play vital roles in muscle

function, the nervous system, and bone formation **(Corchete, p. 2008).** In addition, *Milk thistle seeds* contain active phenolic compounds, most notably silymarin, known for its antioxidant properties, which enhances the health and nutritional value of these seeds and makes them a promising ingredient in the development of functional foods and dietary supplements **(Abenavoli et al., 2018)**.

1.10. Applications of *Silybum marianum (Milk Thistle):*

Milk thistle (Silybum marianum) is a medicinal plant of great importance in both traditional and modern medicine. It has been used for centuries to treat various health disorders, particularly those related to the liver and digestive system. These uses are attributed to its richness in phenolic compounds, especially silymarin, which is primarily responsible for most of its biological properties **(Abenavoli et al., 2018)**.

1.10.1. Medical and Pharmaceutical Uses:

Milk thistle is widely used in medicine, particularly in the treatment of liver diseases. Studies have shown that silymarin possesses hepatoprotective properties by inhibiting oxidative stress, promoting hepatocyte regeneration, and preventing the penetration of toxins into cells **(Polyak et al., 2010)**. It is also used in cases of hepatitis, cirrhosis, and liver toxicity caused by drugs or heavy metals **(Flora et al., 1998)**.

In addition, *Milk thistle* exhibits antioxidant activity due to its ability to scavenge free radicals and enhance the activity of antioxidant enzymes such as SOD and CAT, thus contributing to the prevention of chronic diseases associated with oxidative stress **(Surai, 2015)**. Recent research has also demonstrated its effectiveness as an anti-inflammatory and anticancer agent, particularly in inhibiting cancer cell proliferation and inducing apoptosis **(Deep & Agarwal, 2010)**.

1.10.2. Nutritional and Dietary Uses:

Milk thistle seeds are considered to have considerable nutritional value, containing high levels of vegetable oils (20–30%), proteins, dietary fiber, and unsaturated fatty acids, particularly linoleic and oleic acids **(Hadolin et al., 2001)**. Therefore, in some countries, the seeds are used as dietary supplements to promote liver health and improve digestion.

Milk thistle seed oil is also extracted and used as a potential edible oil due to its rich composition of essential fatty acids and phytosterols, which contribute to lowering LDL cholesterol and improving cardiovascular health **(Elwekeel et al., 2013)**.

1.10.3. Cosmetic Uses:

Milk thistle oil has recently entered the cosmetics and skincare industry due to its moisturizing, antioxidant, and anti-inflammatory properties. It is used in creams and ointments to help treat dry skin, eczema, and acne, as well as to delay skin aging **(Kroll et al., 2007)**.

The oil is also used in hair care products because of its ability to nourish the scalp and strengthen hair follicles, thanks to its vitamin E and fatty acid content **(Dabbour et al., 2014)**.

1.10.4. Therapeutic Uses for Wounds and Cell Protection:

Multiple studies have shown that *Milk thistle* extracts have the ability to promote wound healing by reducing inflammation and stimulating cell regeneration, in addition to their antimicrobial activity, which reduces the risk of infection **(Biedermann et al., 2014)**. Its phenolic compounds also contribute to protecting cells from oxidative damage, supporting its use in topical therapeutic applications.

1.11. Effects of *Milk Thistle (Silybum marianum):*

Milk thistle is known for its multiple therapeutic properties, mainly due to its content of silymarin. The most important effects include:

- **Hepatoprotective effect:** *Milk thistle* is used to protect liver cells from toxins and to stimulate their regeneration. Studies have shown that silymarin reduces liver damage caused by alcohol and drugs **(Flora et al., 1998)**.
- **Antioxidant effect:** Silymarin acts by inhibiting free radicals and reducing oxidative stress, thereby contributing to the protection of cells from damage **(Křen & Walterová, 2005)**.
- **Anticancer effect:** Laboratory studies have demonstrated that silymarin has the ability to inhibit the growth of certain cancer cells and to induce programmed cell death (apoptosis) **(Deep & Agarwal, 2007)**.
- **Antidiabetic effect:** *Milk thistle* contributes to improving insulin sensitivity and reducing blood glucose levels in patients with type 2 diabetes **(Huseini et al., 2006)**.

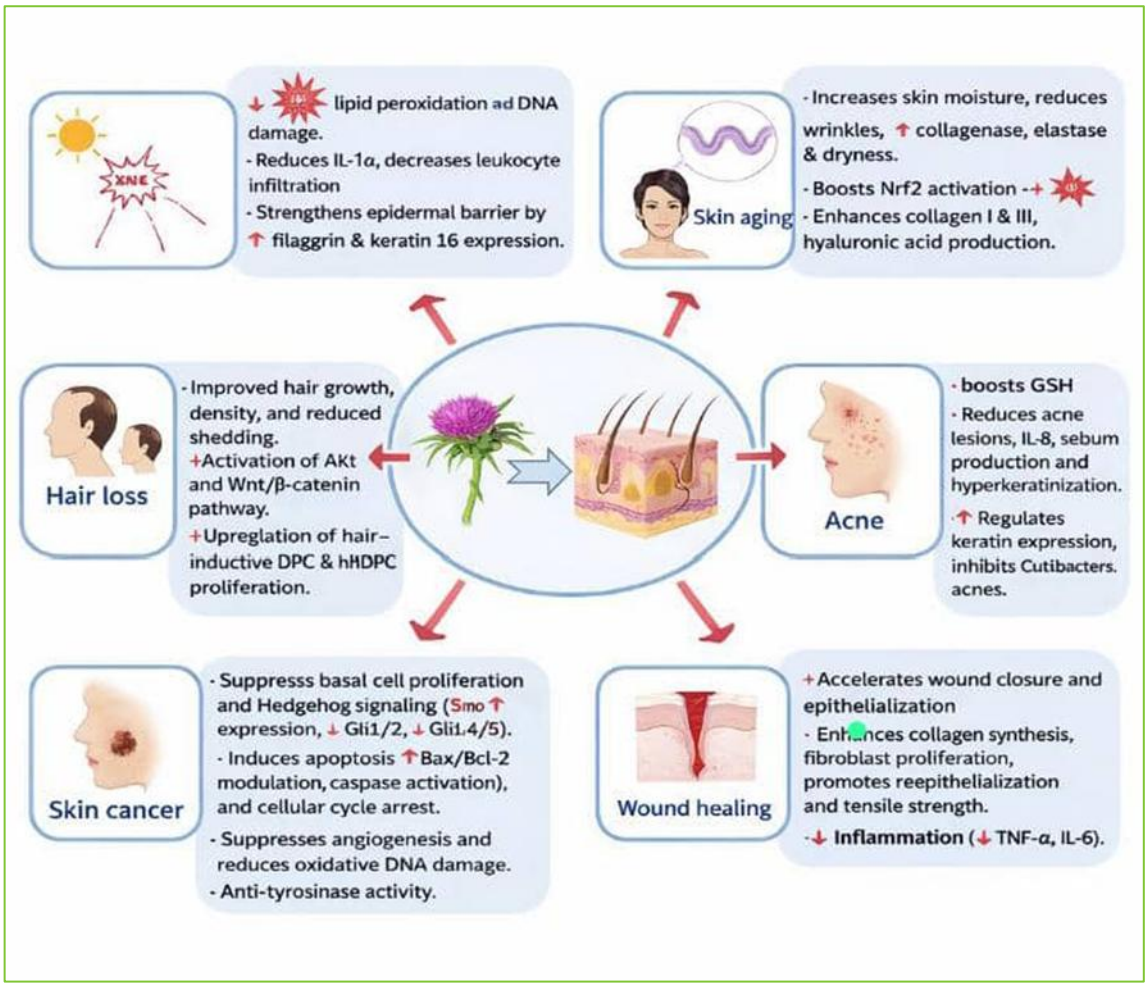


Figure 1. 11: Effets biologiques et dermatologiques du Silybum marianum.

Chapter 2:

Essential oil: *Silybum seed extract oil*

2.1. Introduction to Essential Oils:

Essential oils are considered one of the organic food-derived products of plants and represent some of the most important secondary products, as they originate from primary secretions that are naturally produced or excreted by certain specialized plants known as aromatic plants. Essential oils constitute the main substances responsible for the characteristic aroma of plants and their various organs. They are distinguished by the ease with which they can be separated from the plant organs that contain them using different distillation and extraction methods. Among these methods, solvent extraction is one of the most commonly used techniques in the fragrance industry **(Boulanouae, G et al., 2013)**.

Notably, significant variations are often observed among extracted essential oils, and these differences can be attributed to factors such as the degree of plant maturity, harvesting and storage methods, as well as environmental and growing conditions **(Maes, J et al., 2021)**.

2.2. Definition of Essential Oils:

Essential oils are oily substances with distinctive aromatic odors that readily volatilize and evaporate at normal temperatures without undergoing decomposition, in contrast to fixed oils, which do not volatilize but decompose when exposed to evaporation or heating. Essential oils are considered products of secondary metabolism and are soluble in ethanol, chloroform, and ether, but insoluble in water. Their color may change, their odor may deteriorate, and their viscosity may increase upon exposure to air. In addition, they are highly flammable**.**

Volatile oils mainly consist of monoterpenes and sesquiterpenes. Essential oils are also known by several names, including **(Al-Hajj, N et al., 2014); (Schmidt, E. 2020)**:

- Aromatic oils
- Ethereal oils
- Essential oils (Essence)

Figure 2. 1: Various essential oil bottles with herbs.

2.3. Essential oil extraction sites:

Essential oils are stored in plant tissues within storage sites known as secretory structures. Volatile plant oils may be present in all parts of the plant or localized in specific organs. For example, they are found in the leaves of mint plants, in the flower petals of rose and jasmine, or in the fruits or fruit peels of orange. In some plants, essential oils may occur in more than one part, with their concentration varying from one organ to another **(paolini, J et al., 2010)**. Examples:

- **Family Rutaceae:** Essential oils are concentrated in the leaves, flowers, and seeds; for example, Citrus aurantium L.
- **Family Myrtaceae:** Essential oils are mainly localized in the leaves, as in the case of Eucalyptus.
- **Family Apiaceae (Umbelliferae):** Essential oils are concentrated in vascular or secretory canals, as observed in Angelica archangelica.
- **Family Lamiaceae (Labiatae):** Essential oils are concentrated in the aerial parts of plants, such as mint and lemon balm (Melissa officinalis).

2.4. The Role of Essential Oils in Plants:

y a vital role in plants, including **(Z, Arshad et al., 2014)**:

- Repelling or attracting certain insects to flowers for pollination.
- Serving as an energy source that facilitates some chemical reactions.
- Maintaining sufficient moisture for plants exposed to desert climates.
- Acting as natural repellents or killers of fungal and bacterial pests that cause plant diseases.
- Promoting faster wound healing and preventing the external leakage of sap.

2.5. Chemical Composition of Essential Oils:

The chemical composition of essential oils is highly complex and diverse, with each oil containing anywhere from 20 to 200 different chemical compounds. These compounds can be broadly classified into several groups:

2.5.1 Terpenes:

Terpenes are a large and diverse class of organic compounds produced by a variety of plants ,
particularly conifers. They are the primary constituents of essential oils and are derived from isoprene units (C_5H_8). Terpenes are classified based on the number of isoprene units they contain:

- **Monoterpenes ($C_{10}H_{16}$):** Contain two isoprene units and are the most abundant molecules in essential oils, constituting up to 90% of some oils. Examples include limonene (citrus oils), pinene (pine oil), and myrcene (hops, bay leaves). Monoterpenes are known for their antiseptic, bactericidal, antiviral, and anti-inflammatory properties.
- **Sesquiterpenes ($C_{15}H_{24}$):** Contain three isoprene units and are less volatile than monoterpenes. Examples include β-caryophyllene (clove oil), humulene (hops), and farnesene (apple skin). Sesquiterpenes often have anti-inflammatory, analgesic, and sedative properties.
- **Diterpenes ($C_{20}H_{32}$):** Contain four isoprene units and are less common in essential oils due to their low volatility. Examples include cafestol and kahweol (coffee oil) and taxol (Pacific yew). Diterpenes often have biological activities, including anticancer, antifungal, and anti-inflammatory properties.

2.5.2 Terpenoids:

Terpenoids (or isoprenoids) are modified terpenes that have been chemically altered through oxidation or rearrangement of the carbon skeleton. Important subgroups include:

- **Alcohols:** Such as linalool (lavender oil), geraniol (rose oil), and menthol (peppermintoil). These compounds often have antimicrobial, antiseptic, tonifying, and balancing properties.
- **Aldehydes:** Such as citral (lemongrass oil), citronellal (citronella oil), and cinnamaldehyde (cinnamon oil). Aldehydes often have sedative, anti-inflammatory, and antimicrobial properties.
- **Ketones:** Such as carvone (caraway oil), menthone (peppermint oil), and thujone (wormwood oil). Ketones can have mucolytic, cell-regenerating, and neurotoxic properties.
- **Esters:** Such as linalyl acetate (lavender oil), geranyl acetate (sweet marjoram oil), and bornyl acetate (pine oil). Esters are known for their antispasmodic, sedative, and anti-fungal properties.
- **Ethers:** Such as 1,8-cineole (eucalyptus oil), anethole (anise oil), and methyl chavicol (basil oil). Ethers often have expectorant and stimulant properties.
- **Phenols:** Such as thymol (thyme oil), carvacrol (oregano oil), and eugenol (clove oil). Phenols are powerful antimicrobials but can be skin and mucous membrane irritants.

2.5.3. Phenylpropanoids:

Phenylpropanoids are a diverse family of organic compounds synthesized by plants from the amino acid phenylalanine. They contain a six-carbon aromatic phenyl group and a three-carbon propene tail. Examples include:

- **Eugenol:** Found in clove oil and cinnamon oil, known for its analgesic and antiseptic properties.
- **Cinnamaldehyde:** The main component of cinnamon oil, with antimicrobial and anti-inflammatory properties.
- **Anethole:** Found in anise and fennel oils, with antimicrobial and antispasmodic properties.

2.5.4. Other Compounds:

Essential oils may also contain various other compounds, including:

- **Sulfur compounds:** Found in oils from the Allium family (garlic, onion) and some

Brassicaceae plants.

- **Nitrogen compounds:** Such as indole in jasmine oil and methyl anthranilate in neroli oil.
- **Coumarins:** Such as bergapten in bergamot oil and umbelliferone in many plant oils.
- **Acids:** Such as benzoic acid and cinnamic acid in various plant oils.

The specific combination and proportion of these compounds determine the unique characteristics and therapeutic properties of each essential oil. The chemical composition can vary significantly even within the same plant species due to factors such as geographical location, climate, soil conditions, and extraction methods.

2.6. Methods for Extracting Essential Oils:

There are several methods for extracting essential oils. The choice of the most appropriate method depends on the nature of the plant material to be processed, as well as on the physical and chemical properties of the extract.

The main extraction methods are:

water distillation, steam distillation, extraction using organic solvents, and expression (cold pressing).

The steps for extracting essential oils of plant origin remain essentially the same regardless of the extraction method used. First, it is necessary to extract the aromatic molecules from the plant material that constitute the essential oil, and then, in a second step, to separate these molecules from the medium by distillation, as illustrated in the figure **(M. -E. Lucchesi. 2005)**.

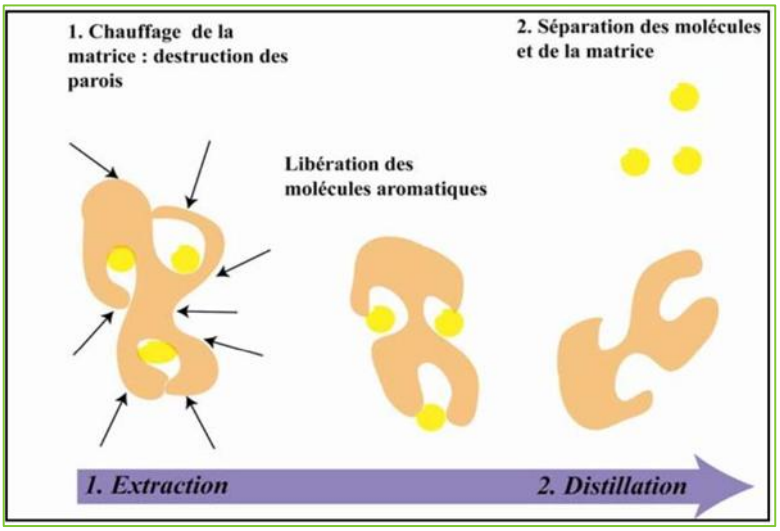


Figure 2. 2: Steps for obtaining an essential oil.

Most essential oils are obtained by steam distillation, which is generally applicable to all plant species that are not significantly altered by water at 100 °C.

2.6.1. Distillation:

The plant material from which the essential oil is to be extracted is mixed with water and heated together until boiling. The resulting steam carries with it the essential oil molecules, which are then condensed using a special condenser **(B. Benjilali. 2004)**. The oil and water subsequently separate under the effect of density differences and are collected. Distillation can be carried out using two methods:

- **Water Distillation (Hydrodistillation):**

Hydrodistillation is a method standardized by AFNOR for the extraction of essential oils as well as for quality control. In this method, the plant material is immersed in water and the whole mixture is heated until boiling **(C. Boutekedjiret et al., 2003)**, as shown in the figure.
High temperatures allow plant cells to rupture and release aromatic molecules. These aromatic molecules form, together with water vapor, an azeotropic mixture (mélange azéotropique). Hydrodistillation can be performed with or without water recycling; the recycling principle is known as cohobation. In laboratories, the system equipped with a cohobation device commonly used for essential oil extraction and compliant with the European Pharmacopoeia is the Clevenger apparatus.

The duration of hydrodistillation can vary greatly and may last several hours, depending on the equipment used and the plant material being processed. Distillation time affects not only the yield but also the composition of the extract.

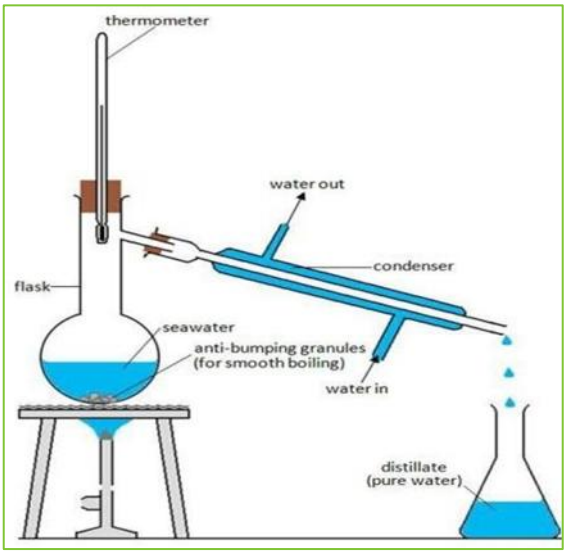


Figure 2. 3: The Clevenger apparatus used in the hydrodistillation process.

- **Steam Distillation:**

Plants are placed in perforated or mesh containers in a way that allows steam to pass through them. The steam extracts the volatile oils and carries them to condensation tubes, where they are converted into the liquid state and easily separated from water. It is preferable to break the plant material into small pieces to allow steam penetration and to collect a greater amount of volatile oil. This method can be used for all types of plants containing volatile oils that can withstand high temperatures **(B. Meyer- Warnod. 1984)**, as shown in the figure.

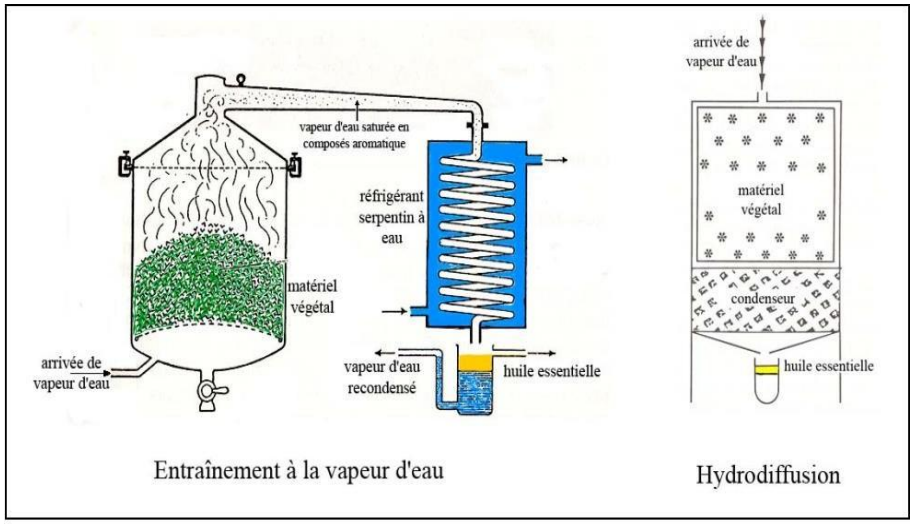


Figure 2. 4: The Clevenger apparatus used in the hydrodistillation process (M. -E, Lucchesi. 2005).

2.6.2 Solvent Extraction:

Due to the importance of essential oils in the perfume industry, expensive aromatic oils known as “natural oils” have been developed. These oils are not extracted by distillation methods but rather by extraction using organic solvents **(F. Peng et al., 2004)**. In this case, the oil obtained is identical to its natural state in the plant, that is, a true natural oil, as shown in the figure.

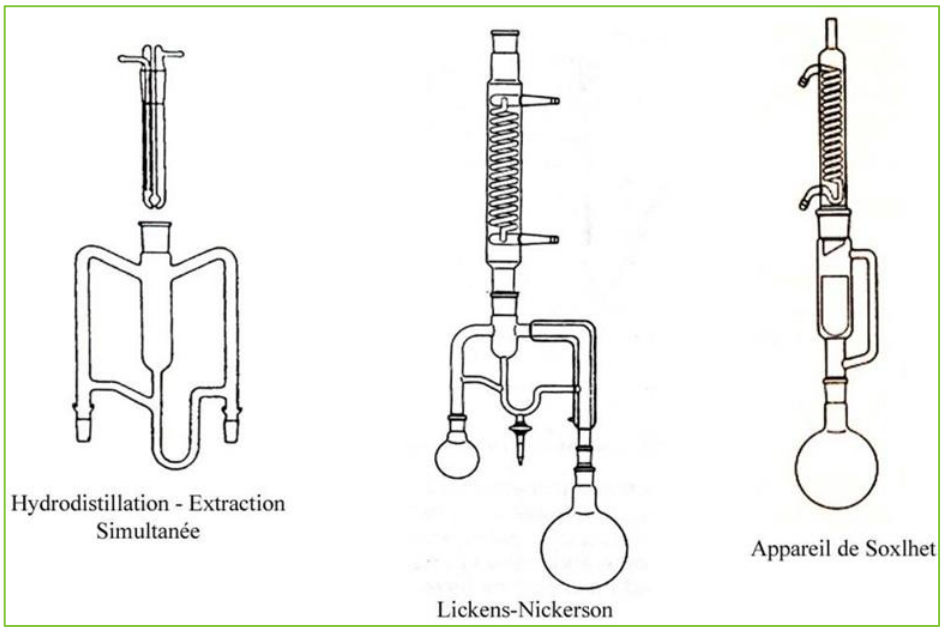


Figure 2. 5: Upward and downward steam distillation (M. -E, Lucchesi. 2005).

2.6.3 Expression (Cold Pressing):

This method is used to extract volatile oils located in the main glands of the outer layer of fruit peels. The nature and chemical composition of these oils do not allow extraction by distillation due to their sensitivity to heat **(L. Maignial et al., 1992)** Therefore, the expression method is used, as in the case of lemon oil, orange oil, and others.

Cold pressing is a mechanical extraction method that preserves thermolabile bioactive compounds and produces high-quality oil without the use of organic solvents. **(Harborne, 1998; Rahman, 2007)**.

2.6.4. Supercritical Fluid Extraction (SFE):

Supercritical Fluid Extraction is similar to Soxhlet extraction method, but the solvent used is supercritical fluid. SFE is one of the emerging extraction techniques, which is usually faster and more selective in extraction of the compounds from plant materials. As well as it is 'green' technologies which means the resultant waste materials can be easily recycled and reused. Thus, SFE may be defined as separation and extraction of chemical compound of interest from samples or products such as tea, herbs, and spices etc. using supercritical fluid as an extracting solvent. When the extraction mixtures are subjected to temperature and pressure above their critical points the state obtain is SF state. Supercritical fluid exhibits physico-chemical properties which are intermediate between liquid and gas. These fluids can diffuse through solid like a gas and dissolve material like a liquid **(Mendiola et al., 2013)**. Additionally, small changes close to the critical point in temperature or pressures results in greater changes in the

densities allowing many properties of SCF to be fined tuned. Thus, supercritical fluids (SCF) are preferably a substitute for organic solvent in the many industrial and laboratory processes. The supercritical solvent of choice for the extraction of plants compounds is carbon-di-oxide, since it is odourless, nontoxic, inflammable and inexpensive. Carbon dioxide facilitates supercritical activity at relatively low pressure and close to ambient temperatures **(Knez et al., 2010; Zizovic et al., 2007)**. The critical temperature for CO2 is 31.1°C and 72 bar. The SFE method consists of extraction of the soluble components in supercritical solvent and isolation of the solutes from solvent. The segregation of soluble solutes from the solvent may be executed by simply changing the temperature and pressure of the system thus changing the thermodynamics of supercritical solvent. The desired effect in these cases is a reduction of a solvent power **(Pereda et al., 2007)**. A standard SFE unit includes a high-pressure pump that supplies the SCF and an extraction tank with sample which is held at the specified temperature and pressure. The selectivity of a SCF can be modified by adjusting temperature and pressure, allowing access for selective extraction from a mixture of different compounds. Examples of procedures for selectively extracting or separating different compounds with SFE are the fractional extraction process (FEP), single stage extraction (SSE), and sequential depressurisation (SD) **(Shi et al., 2006; Zizovic et al., 2007)**.

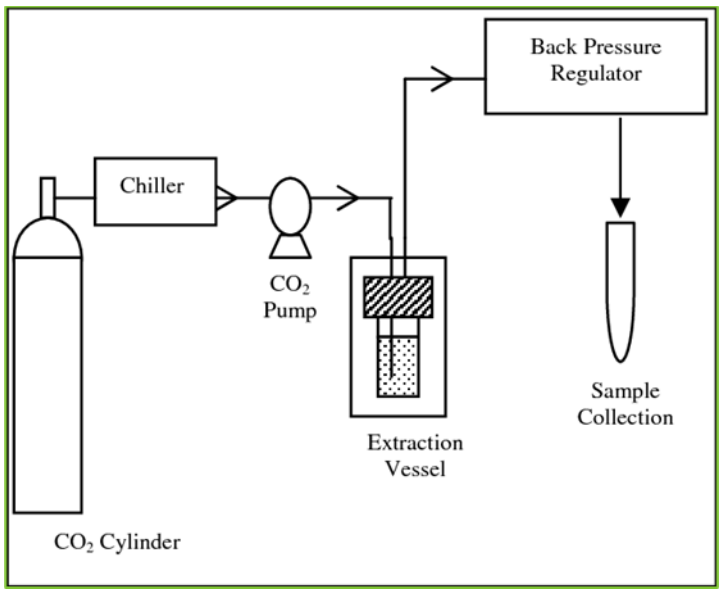


Figure 2. 6: Flow diagram of SCF (Puah et al., 2005).

2.6.5. Microwave-Assisted Extraction:

In this method, the heat produced by the microwave radiation is used in heating the mixture of plant materials and solvent. Heating with microwave causes dipole rotation of the molecules which disrupts weak hydrogen bonds and changes the cell structure helping in very fast extraction of the essential oil. The heat caused by the microwave is instantaneous and

occurs in the interior of the plant materials helping in fast extractions. Utilizing microwave technology helps in reducing the extraction time by more than half as well as the solvent requirement when compared with the conventional methods like steam distillation or solvent extraction methods without compromising the quality of the essential oil **(Flamini et al., 2007; Bendahou et al., 2008)**. With the development of the green extraction concept and to reduce energy inputs in the extraction of essential oils, more emphasis has been given to the exploitation of microwave technology which has led to the development of several methods such as solvent-free microwave extraction **(Lucchesi et al., 2004 a,b)**, vacuum microwave hydro-distillation **(Huma et al., 2011)**, microwave assisted solvent extraction **(Tomaniova et al., 1998)**, microwave hydro- diffusion and gravity **(Vian et al., 2008)** and microwave assisted simultaneous distillation- solvent extraction **(Ferhat et al., 2007)**.

2.7. Physical and Chemical Properties of Essential Oils (EOs):

2.7.1. Physicochemical Properties:

In general, the properties of essential oils include various indices, optical rotation, viscosity, density, solubility in alcohol, boiling and freezing points. Many researchers have focused on the physicochemical properties of essential oils, which can be summarized as follows:

They are generally homogeneous liquids at ambient temperature, except for some that exist in the solid state (such as anise, fennel, and Japanese mint). They contain volatile compounds present in plants, along with traces of fixed oils.

They are rarely colored (with some exceptions). However, all volatile oils are highly flammable and strongly aromatic. Some oils gradually take on a light blue color (such as chamomile and patchouli), which is due to the presence of chamazulene.

2.8. Benefits and Uses of Essential Oils:

For thousands of years, essential (aromatic) oils have been used in China and in ancient Egyptian civilization. They were also widely used in Arab and Islamic civilization. Over time, their use has developed until they became widely applied in beauty institutes and cosmetic centers. They were used in the treatment of diseases as a medical system aimed at maintaining human health.

As for the benefits of essential oils for plants, they help attract insects that carry out the pollination process, which increases fruit production. Moreover, some oils are insect-repellent

or toxic to animals, thus playing a protective role for plants against insects that cause damage to them **(A. Kasrati et al., 2015)**.

In addition, essential oils help eliminate the by-products of metabolic processes and expel them outside the plant. There are several other benefits and uses of essential oils for all living organisms, as they contribute to strengthening most body systems, including the immune system. They also help stimulate blood circulation and energize the body.

Some essential oils have the ability to promote hair growth, a property found in lavender. They also increase oxygen absorption and the production of ATP (adenosine triphosphate). Table (2.1) summarizes the main benefits and uses of essential oils **(W. Brud. 2020); (P. Tongnuanchan and S. Benjakul, 2014)**.

Table 2. 1: Uses of Essential Oils.

Medical Uses	
Gives some medicines a pleasant taste and smell.	Sedative, Calming (Sedative and Antiseptic).
Used as a flavoring or spice to foods.	Hypotensive (Fever Reducer).
Used in perfumes and cosmetics.	Antiviral.
Used the manufacture of pharmaceutical preparations and soaps.	Anti-inflammatory.
Worm and parasite repellent.	Restores balance to the internal nervous system.
Insect repellent, such as mosquitoes.	Antispasmodic (Muscle spasm).

2.9. Economic importance of essential oils:

The quantities of essential oils produced worldwide vary greatly, as the annual production of some essential oils exceeds 35,000 tons, while others may reach only a few kilograms. Table shows the production figures, estimated in tons. Similarly, wide differences are also observed in the monetary value of different essential oils, with prices ranging from $1.8 per kilogram for orange oil to $120,000 per kilogram for orris (iris) oil. The total annual value of the global

market has reached billions of US dollars, and the production of essential oils represents an important source of annual income for some African countries **(B. M. Lawrence, 2009)**. Indonesia, Sri Lanka, and India occupy leading positions in the production and export of these oils.

Table 2. 2: National production of essential oils in some countries of the world.

Essential Oil:	Production in tons:	Main Producing Countries:
Orange Oil	51000	United States – Brazil.
Peppermint Oil	32000	India - China – Argentina.
Lemon Oil	9200	Spain - Italy – Argentina.
Eucalyptus Oil	4000	China - India – Australia.
Clove Oil	1800	Indonesia – Madagascar.
Cedarwood Oil	1650	United States – China.

2.10. *Milk thistle seed oil*:

2.10.1. Extraction Methods:

Several methods are used to extract oil from *Milk thistle seeds,* each with its advantages and limitations:

2.10.1.1. Cold Pressing:

Cold pressing is a mechanical extraction method that involves applying pressure to the seeds to release the oil, without the application of external heat. This method preserves the natural .

properties of the oil, including its nutritional value, aroma, and color. Cold-pressed *Milk thistle seed oil* is considered premium quality and is often used in high-end cosmetic and culinary applications.

The process typically involves cleaning and sorting the seeds, removing the seed coats (dehulling), grinding the seeds into a paste, and then pressing the paste in a mechanical press. The extracted oil is then filtered to remove any solid particles. Cold pressing typically yields less oil compared to solvent extraction (approximately 25-30% of the seed weight), but the quality of the oil is generally higher.

Figure 2. 7: Clod pressing machine.

2.10.1.2. Solvent Extraction:

Solvent extraction involves the use of organic solvents, such as hexane, to dissolve the oil from the seed material. The process typically includes:

1 .Grinding the seeds to increase the surface area

2 .Mixing the ground seeds with the solvent

3 .Separating the solvent-oil mixture from the seed residue

4 .Evaporating the solvent to recover the oil

5 .Refining the oil to remove any remaining solvent and impurities

Solvent extraction typically yields more oil than cold pressing (up to 35-40% of the seed weight), but there are concerns about potential solvent residues in the final product, even after refining. This method is more commonly used for industrial-scale production and for oils intended for non-food applications.

2.10.1.3. Supercritical Fluid Extraction (SFE):

Supercritical fluid extraction, particularly using carbon dioxide (CO_2), is a modern extraction technique that offers several advantages for *Milk thistle seed oil* extraction. In this method, CO_2 is pressurized and heated to a supercritical state, where it has properties of both a liquid and a gas. The supercritical CO_2 acts as a solvent, extracting the oil from the seed material. When the pressure is released, the CO_2 returns to a gaseous state, leaving behind the pure oil.SFE with CO_2 offers several advantages: - It operates at relatively low temperatures , preserving heat-sensitive compounds - CO_2 is non-toxic and leaves no residues - The extraction parameters can be adjusted to target specific compounds - The method produces a high-quality oil free from solvent residues However, the equipment is expensive, and the method may not be as economically viable for large-scale production as solvent extraction.

2.10.2. Composition and Characteristics:

The fixed oil of *Milk thistle seed oil* is rich in unsaturated fatty acids, mainly linoleic acid (ω-6) and oleic acid (ω-9), in addition to saturated fatty acids such as palmitic and stearic acids, which contribute to its nutritional and functional value. **(Duke, 2002; Rahman, 2007)**.

Besides fatty acids, *Milk thistle seed oil* contains several bioactive compounds, including phenolic compounds, flavonoids, phytosterols and tocopherols (vitamin E), which are responsible for its antioxidant and protective properties. **(Harborne, 1998; Rahman, 2007)**.

Physicochemical properties of *Milk thistle seed oil* include a yellow to light brown color, non-volatility, lipophilic nature and relative oxidative stability due to its natural antioxidant content. **(Duke, 2002; Said & Fulder, 2011)**.

2.10.3. Biological Activities:

Milk thistle seed oil exhibits significant antioxidant activity due to its content of phenolic compounds and tocopherols, which help neutralize free radicals and reduce oxidative stress. **(Harborne, 1998; Rahman, 2007)**.

Moderate antimicrobial activity of *Milk thistle seed oil* has been reported against several pathogenic microorganisms, supporting its traditional medicinal uses. **(Said & Fulder, 2011; Duke, 2002)**.

The anti-inflammatory activity of *Milk thistle seed oil* is attributed mainly to its unsaturated fatty acids and flavonoid content. **(Said & Fulder, 2011; Rahman, 2007)**.
From a nutritional and therapeutic perspective, *Milk thistle seed oil* may contribute to improved lipid metabolism and protection against chronic diseases related to oxidative damage. **(Duke, 2002; Said & Fulder, 2011)**.

2.10.4. Uses of *Milk thistle seed oil*:

2.10.4.1. Medicinal and Therapeutic Uses:

Milk thistle seed oil has been traditionally used in folk medicine due to its bioactive constituents. The oil exhibits antioxidant, anti-inflammatory, and antimicrobial properties, which make it potentially useful in the prevention and management of various diseases. These biological effects are mainly attributed to its phenolic compounds, flavonoids, and unsaturated fatty acids. **(Harborne, 1998; Said & Fulder, 2011)**.

2.10.4.2. Nutritional Applications:

From a nutritional perspective, *Milk thistle seed oil* is considered a valuable source of essential fatty acids, particularly unsaturated fatty acids, which contribute to improved lipid metabolism and cardiovascular health. Its antioxidant components may also help protect against oxidative stress-related chronic diseases. **(Duke, 2002; Rahman, 2007)**.

2.10.4.3. Cosmetic and Dermatological Uses:

Due to its emollient and antioxidant properties, *Milk thistle seed oil* may be used in cosmetic formulations such as skin creams, massage oils, and hair care products. The oil helps maintain skin hydration, improve skin elasticity, and protect the skin from oxidative damage and premature aging. **(Said & Fulder, 2011; Duke, 2002)**.

2.10.4.4. Pharmaceutical Potential:

Milk thistle seed oil represents a promising natural source for pharmaceutical applications. Its antimicrobial and anti-inflammatory activities support its potential use as a natural ingredient in the development of topical formulations and herbal remedies. Further studies are required to standardize its composition and evaluate its clinical efficacy. **(Harborne, 1998; Rahman, 2007)**.

2.10.4.5. Traditional and Ethnobotanical Uses:

In traditional medicine systems, *Milk thistle* and its derivatives have been used to treat inflammatory conditions, digestive disorders, and infections. The seed oil complements these traditional uses by providing concentrated bioactive compounds with therapeutic relevance. **(Said & Fulder, 2011)**.

2.10.5. Challenges and Future Perspectives of *Milk thistle seed oil*:

2.10.5.1. Challenges:

- **Limited Scientific Studies:**

One of the main challenges associated with *Milk thistle seed oil* is the limited number of comprehensive scientific studies focusing specifically on its chemical composition, biological activities, and safety profile. Most available information is derived from ethnobotanical knowledge or generalized studies on medicinal plants, which limits its wider scientific and industrial acceptance. **(Harborne, 1998; Duke, 2002)**.

- **Variability in Chemical Composition:**

The chemical composition of *Milk thistle seed oil* may vary significantly depending on environmental factors such as climate, soil type, geographical origin, and harvesting conditions. This variability affects oil quality, biological activity, and reproducibility, making standardization difficult for pharmaceutical and industrial applications. **(Rahman, 2007)**.

- **Extraction and Processing Constraints:**

Efficient extraction of *Milk thistle seed oil* remains a challenge due to the lack of optimized extraction protocols. Traditional extraction methods may result in low oil yields or degradation of thermolabile bioactive compounds, while advanced techniques require higher costs and technical expertise. **(Harborne, 1998)**.

- **Lack of Toxicological and Clinical Data:**

Another major limitation is the scarcity of toxicological and clinical studies evaluating the safety, dosage, and long-term effects of *Milk thistle seed oil* in humans. This gap restricts its use in pharmaceutical formulations and functional foods. **(Duke, 2002; Said & Fulder, 2011)**.

2.10.5.2. Future Perspectives:

- **Advanced Extraction Technologies:**

Future research should focus on the development and optimization of modern extraction techniques, such as supercritical fluid extraction and green solvent technologies, to improve oil yield while preserving its bioactive compounds and ensuring environmental sustainability. **(Rahman, 2007)**.

- **Chemical Characterization and Standardization:**

Detailed chemical profiling using advanced analytical techniques (GC–MS, HPLC) is necessary to identify and quantify the major and minor constituents of *Milk thistle seed oil*. This will facilitate standardization and quality control for pharmaceutical, cosmetic, and nutritional applications. **(Harborne, 1998)**.

- **Expanded Biological and Pharmacological Studies:**

Further in vitro and in vivo studies are required to confirm the antioxidant, anti-inflammatory, antimicrobial, and other pharmacological activities of *Milk thistle seed oil*. These studies may support its development as a natural therapeutic agent. **(Said & Fulder, 2011)**.

- **Industrial and Commercial Potential:**

With increasing demand for natural and plant-based products, *Milk thistle seed oil* has promising potential for use in the food, cosmetic, and pharmaceutical industries. Sustainable cultivation and valorization of *Milk thistle seeds* could also contribute to economic development in arid and semi-arid regions. **(Duke, 2002)**.

- **Integration into Functional Foods and Natural Products:**

In the future, *Milk thistle seed oil* may be incorporated into functional foods, dietary supplements, and natural health products aimed at preventing oxidative stress-related diseases and improving overall health. **(Rahman, 2007; Said & Fulder, 2011)**.

Second part

Experimental Analysis

Chapter 1:

Materials and Methods

1.1. Introduction:

This chapter presents the comprehensive methodological framework adopted in the experimental study of the biological properties of *Milk thistle seed oil* and its potential applications in the pharmaceutical and cosmetic fields. The study design integrates several analytical approaches aimed at evaluating the physicochemical properties of the oil, as well as identifying its various biological activities.

Starting with the careful selection and preparation of the plant material, this chapter outlines the methodological steps taken to ensure scientific accuracy and the reproducibility of experiments under similar conditions. The extraction method was prioritized to preserve the bioactive compounds within the oil, ensuring that its active components were not degraded during processing.

Subsequent analyses focused on determining the physicochemical constants of the oil, along with studying its biological activities, including its antioxidant capacity, UV-protective factor, and antibacterial properties. The potential for inhibiting certain enzymes associated with skin disorders was also assessed. Rigorous experimental protocols and clear measurement criteria were employed, supported by appropriate statistical analysis methods to ensure the reliability of the results. This methodological framework provides the scientific basis for presenting and subsequently discussing the results, and contributes to expanding the understanding of the applied potential of *Milk thistle seed oil* as a promising natural ingredient in the development of products with therapeutic and cosmetic value.

1.2. Materials:

1.2.1. Plant Materials:

The plant material used in this study consisted of dried *Milk thistle (Silybum marianum) seeds*, which were obtained from the local market. After collection, the seeds underwent a thorough cleaning process to remove impurities and adhering materials, followed by manual separation of unwanted parts to ensure the acquisition of sound and pure seeds. The seeds were then dried—where necessary—under suitable conditions to preserve their active components before being stored in airtight containers away from moisture and light until use in the oil extraction process.

Figure 1. 1: Milk thistle seeds used in the study.

1.2.2. Bacterial Strains:

The bacterial strains used in this study are standard reference strains used to evaluate the biological activity of the vegetable oil under investigation. These strains were obtained from a certified laboratory source and are commonly used as test specimens in microbiological studies. The strains were kept under appropriate refrigerated conditions on nutrient agar to ensure their viability and stability, and were activated prior to the experiments according to standard laboratory procedures.

Table1. 1: The microbial species used during the experiment.

Gram Type	Strains	Reference	Origin
Gram +	*Staphylococcus aureus*	*ATCC 25932*	Human skin and nose (normal flora that may become pathogenic).
Gram+	*Bacillus subtilis*	*ATCC 25973*	Soil and natural environment.
Gram-	*Escherichia coli*	*ATCC 25922*	The intestines of humans and animals (normal flora).
Gram-	*Pseudomonas aeruginosa*	*ATCC 27853*	The environment (water, soil) and it can exist as an opportunistic pathogen

1.2.3. The enzyme used:

Table 1. 2: Properties of the tyrosinase enzyme used

Element	Description
Enzyme name:	*Tyrosinase*
Classification:	*Oxidoreductase (EC 1.14.18.1)*
Origin:	Tyrosinase extracted from mushroom (mushroom tyrosinase)
Supplier (if available):	Sigma-Aldrich
Form:	Powder (lyophilized)
Storage conditions:	Stored at −20 °C until use

1.3. Methods:

1.3.1. Extraction Method:

The oil was extracted from *Milk thistle seeds* using a cold-pressing method with a mechanical oil press specifically designed for vegetable oils. This non-thermal, solvent-free technique is considered effective in preserving biologically active compounds, particularly silymarin and phenolic compounds, allowing the production of pure oil without degradation of its sensitive constituents while maintaining its natural properties and chemical stability **(Anwar et al., 2005)**.

After extraction, the oil was collected and stored in tightly sealed dark bottles and kept in a cool, dry place away from light and heat sources to minimize oxidation and preserve its physicochemical properties for as long as possible **(Manzoor et al., 2007)**.

Figure 1. 2: Steps for extracting Milk thistle seed oil.

The extracted oil was filtered to remove suspended solids and seed residues. The filtration process was carried out using sterile filter paper under gravity at room temperature

Two values were recorded:

Volume of crude oil before filtration: 200 ml

Volume of oil after filtration: 72 ml

Figure 1. 3: Milk thistle seed oil extract.

1.3.2 .Determination of the physical constants of the oil sample:

The color and viscosity of the oil extracted from *Milk thistle seeds* were described, the pH was measured in the laboratory, and both the density of the oil and its extraction rate were calculated.

1.3.2.1. Determination of Extraction Yield:

To calculate the percentage of oil extracted, we divide the weight of the oil by the initial weight of the sample. The oil yield was calculated using the following equation. **(Ventura et al., 2021) (Lani et al., 2017)**.

$$R\% = \frac{M_{HE(g)}}{M_{E(g)}} \times 100\%$$

R% = Oil yield percentage

MHE = Mass of extracted oil (g)

ME = Mass of sample (g)

1.3.2.2. PH Measuremen:

Figure 1. 4: pH measuring device.

Measuring the pH of *Milk thistle (Silybum marianum) oil* is essential, as it can directly affect the physical stability of the oil and the efficacy of its active compounds. An inappropriate pH may lead to the degradation of some active components or alter the oil's properties, reducing its therapeutic and cosmetic benefits. For skincare applications, it is preferable that the pH of topical preparations derived from *Milk thistle oil* ranges between 4 and 7 to ensure compatibility with the natural skin barrier. The pH was measured in the laboratory using a digital pH meter, with the electrode directly immersed in the extracted oil to obtain an accurate reading **(Maaloul et al., 2024)**.

1.3.3. Biological Activities:

1.3.3.1. Antioxidant Activity: Ferric Reducing Antioxidant Power Assay (FRAP Assay):

The FRAP (Ferric Reducing Antioxidant Power) test is one of the most widely used methods for evaluating the antioxidant capacity of biological samples or plant extracts. This test is based on the principle of reducing the ferric ion (Fe^{3+}) bound to the TPTZ (2,4,6-tripyridyl-s-triazine) compound to its ferric form (Fe^{2+}) in an acidic medium, in the presence of antioxidant compounds. This reduction results in the formation of a blue Fe^{2+}-TPTZ complex, the intensity of which is assessed spectroscopically. The increase in absorbance is directly proportional to the reducing power of the sample, reflecting its antioxidant activity. Results are often expressed in units of Trolox equivalents or $FeSO_4$. The FRAP test is characterized by its simplicity, speed, and high reproducibility.

$$Fe^{3+} + TPTZ + antioxidants \rightarrow Fe^{2+} - TPTZ\ (blue)$$

In this study, the antioxidant activity of *Milk thistle (Silybum marianum) seed oil* was evaluated using the FRAP assay according to the method of **(Benzie et al. 1996)** with some modifications.

Experimental Protocol:

Table1. 3: Materials and Equipment.

Materials:	Equipment:
• **Ascorbic acid (standard antioxidant)** • ***Milk thistle seed oil (Silybum marianum)*** • **Distilled water** • **Phosphate buffer solution (0.2 M, pH = 6.6)** • **Potassium ferricyanide ($K_3Fe(CN)_6$)** • **Trichloroacetic acid (TCA, 10%)** • **Ferric chloride ($FeCl_3$, 0.1%)**	• Water bath (set at 50°C) • Centrifuge • Spectrophotometer • Test tubes and micropipettes

a) Sample preparation:

A series of diluted standard solutions of ascorbic acid were prepared in distilled water to establish a calibration curve. Diluted solutions of *Milk thistle (Silybum marianum) seed oil* were also prepared in dimethyl sulfoxide (DMSO), all at different and known concentrations.

b) Protocol:

1. 1000 µl of sample of each test solution was taken.
2. 1.25 ml of phosphate buffer solution (0.2 M, pH = 6.6) and 1.25 ml of potassium ferricyanide ($K_3Fe(CN)_6$) was added.
3. The reaction mixture was incubated at 50°C for 20 minutes in a water bath.
4. After cooling to room temperature, 1.25 ml of trichloroacetic acid solution TCA (10%) was added to terminate the reaction.
5. The mixture was then centrifuged at 3000 rpm for 10 minutes.
6. A volume of 650 µl of the resulting supernatant of each test tube was taken.
7. 650 µl of DMSO (distilled water for ascorbic acid) was added and 250 µl of ferric chloride $FeCl_3$ (0.1%).
8. The absorbance of the final mixture was measured at 700 nm, against a blank.

c) Quantification and expression of antioxidant capacity:

The antioxidant capacity of the sample (oil) was then expressed as a percentage relative to the reducing power of the standard antioxidant using the following formula:

$$\textit{FRAP inhibition percentage}\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where:

- ***Inhibition* (%):** The percentage of free radicals scavenged or neutralized by the sample.
- $A_{control}$**:** The absorbance of the control solution (without antioxidant).
- A_{sample}**:** The absorbance of the solution containing the sample or antioxidant extract

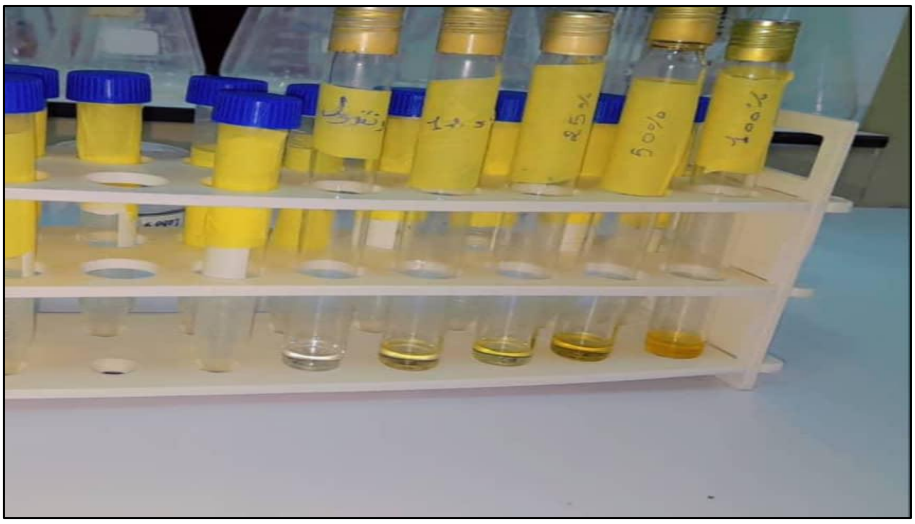


Figure 1. 5: Samples of solutions prepared for the FRAP experiment.

1.3.3.2 .Sun Protection Factor (SPF) Determination:

The sun protection factor (SPF) is a parameter used to assess the efficacy of sunscreen products in protecting against harmful ultraviolet (UV) radiation, particularly UVB rays responsible for erythema (sunburn). In this study, the SPF value of *Milk thistle seed oil* was determined in vitro using UV-Visible spectrophotometry, following the method originally developed by **(Mansur et al., 1986)**.

Experimental Protocol:

Table1. 4: Materials and Equipment.

Materials:	Equipment:
• ***Milk thistle seed oil*** • **Dimethyl sulfoxide (DMSO) used as a solvent** • **Prepared sample solution (oil dissolved in DMSO)** • **Distilled water**	• UV–Visible spectrophotometer • Vortex mixer • Micropipette • Test tubes • Cuvettes • Test tube rack • Magnetic stirrer

a) Sample Preparation:

- A mixture was prepared by dissolving 1 ml of *Milk thistle seed oil* in 3 ml of dimethyl sulfoxide (DMSO). DMSO was chosen as the solvent due to its ability to dissolve lipophilic compounds and its transparency in the ultraviolet range.

- The solution was gently vortexed by vortex device for 5 minutes until fully homogeneous.

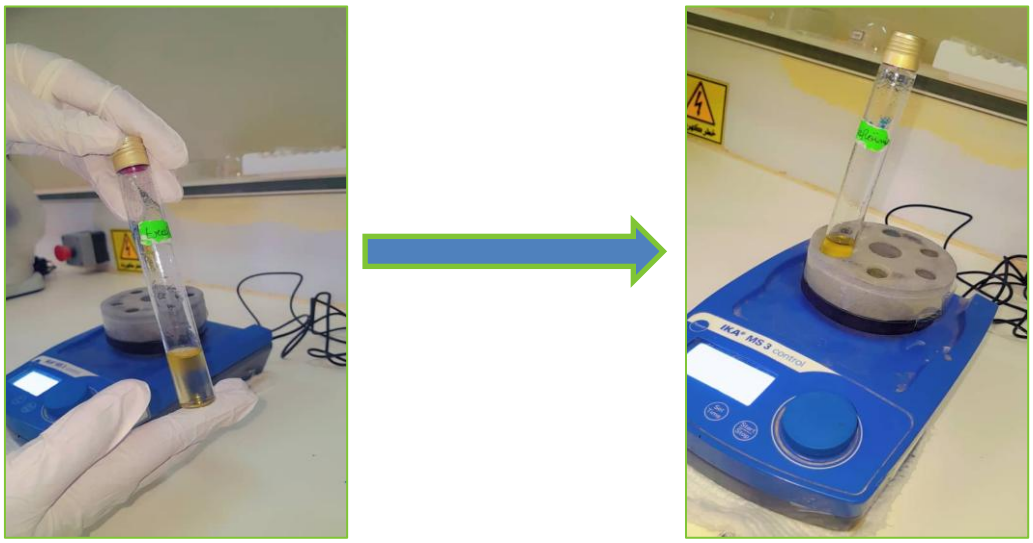


Figure 1. 6: SPF sample preparation.

b) **Spectrophotometric measurement:**

Immediately after preparation, the absorbance of the solution was measured using a UV-Visible spectrophotometer in the 290-320 nm range at 5 nm intervals, with DMSO used as the blank to calibrate the spectrophotometer.

Figure 1. 7: Measurement of the solution absorbance.

c) **SPF calculation:** The Sun protection factor calculation was based on absorbance values measured in vitro by the application of the mathematical equation by **(Mansur. 1986)** as follows:

$$SPF\ spectrophotometric = CF \times \sum EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where:

- $EE(\lambda)$: Erythemal effect spectrum
- $I(\lambda)$: Solar intensity spectrum
- $Abs(\lambda)$: Absorbance of sunscreen product
- CF: Correction factor (= 10)

Table1. 5: Normalized product function used in the calculation of SPF **(Sayre et al., 1979)**

Wavelength λ (nm)	**290**	**295**	**300**	**305**	**310**	**315**	**320**	**Total**
EEM× I(λ) (Norms)	0.0150	0.0817	0.2874	0.3278	0.1864	0.0839	0.0180	1

Where EEM is the Erythemal effect spectrum, and I is the solar intensity spectrum SPF classification standards: Following European recommendations in 2006 **(Verheugen, 2006)**, sun protection products were categorized based on their SPF values as shown in Table 1.3.

Table1. 6: Sunscreen protection categories per EU regulation (Verheugen, 2006).

Protection Level	**SPF Value**
Low protection	6-10
Medium protection	15-20-25
High protection	30-40
Very high protection	50+

1.3.3.3. Tyrosinase Inhibition:

The tyrosinase inhibition assay is used to evaluate the ability of various extracts or compounds to inhibit the activity of tyrosinase, an essential enzyme in melanin formation. This assay utilizes mushroom tyrosinase and an L-DOPA substrate. The enzyme oxidizes L-DOPA to dopaquinone, which is subsequently converted to dopachrome, a colorant that can be

measured spectroscopically. The reaction is carried out in a buffer at a pH of approximately 6.8. After incubating the enzyme with the sample or control, L-DOPA is added, and the change in absorbance is measured at a wavelength of 475 nm using a spectrometer. A decrease in absorbance compared to the control indicates tyrosinase inhibitory activity, and the inhibition percentage is calculated to determine the efficacy of the tested sample. This assay is commonly used in research for cosmetics, skin lightening, and natural anti-pigmentation products. **(Chang, 2009)**; **(Masuda et al., 2005)**; **(Orhan et al., 2016)**.

Experimental Protocol:

Table 1. 7: Materials and Equipment.

Materials:	Equipment:
• **Mushroom tyrosinase** • **L-DOPA (L-3,4-dihydroxyphenylalanine)** • **Phosphate buffer solution (pH ≈ 6.8)** • **Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$)** • **Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$)** • **Distilled water** • **Control solution** • ***Milk thistle seed oil***	• Spectrophotometer • pH meter • Incubator • Micropipettes • Cuvettes • Analytical balance • Volumetric flask • Beaker

a) Preparation of the Buffer Solution:

- Two buffer solutions must be prepared: one with pH = 8 and the other with pH = 7, as follows:
- Tampon pH 6.8:
- Weigh 8.890 g of Na_2HPO_4, $2H_2O$ and dissolve it in 500 ml of distilled water (MA = 177.99 g/mol).
- Weigh 1.56 g of NaH_2PO_4, $2H_2O$ and dissolve it in 100 ml of distilled water (MA = 156.01 g/mol).
- Take 49 ml of the Na_2HPO_4, $2H_2O$ solution and add 51 ml of the NaH_2PO_4, $2H_2O$ solution.

Then measure the pH using a pH meter. If the pH is 6.8, add more distilled water until the total volume reaches 200 ml and allow it to stand for several minutes (approximately 10

minutes) while shaking. The solution obtained has a concentration of 100 mM, but the solution we should use in this study has a concentration of 50 mM, meaning we must double the volume by adding distilled water. For example, in this case, we add 200 ml of distilled water.

Figure 1. 8: Buffer solution (tampon ph = 6.8).

Figure 1. 9: A solution with a concentration of 50 Mm.

b) Enzyme Preparation:

Add the enzyme to 250 µl of tampon (pH = 6.8).

Divide the solution into Eppendorf tubes (approximately 50 µl per tube). Store in the freezer until ready to use.

Figure 1. 10: Tyrosinase from mushroom (T3824-250KU).

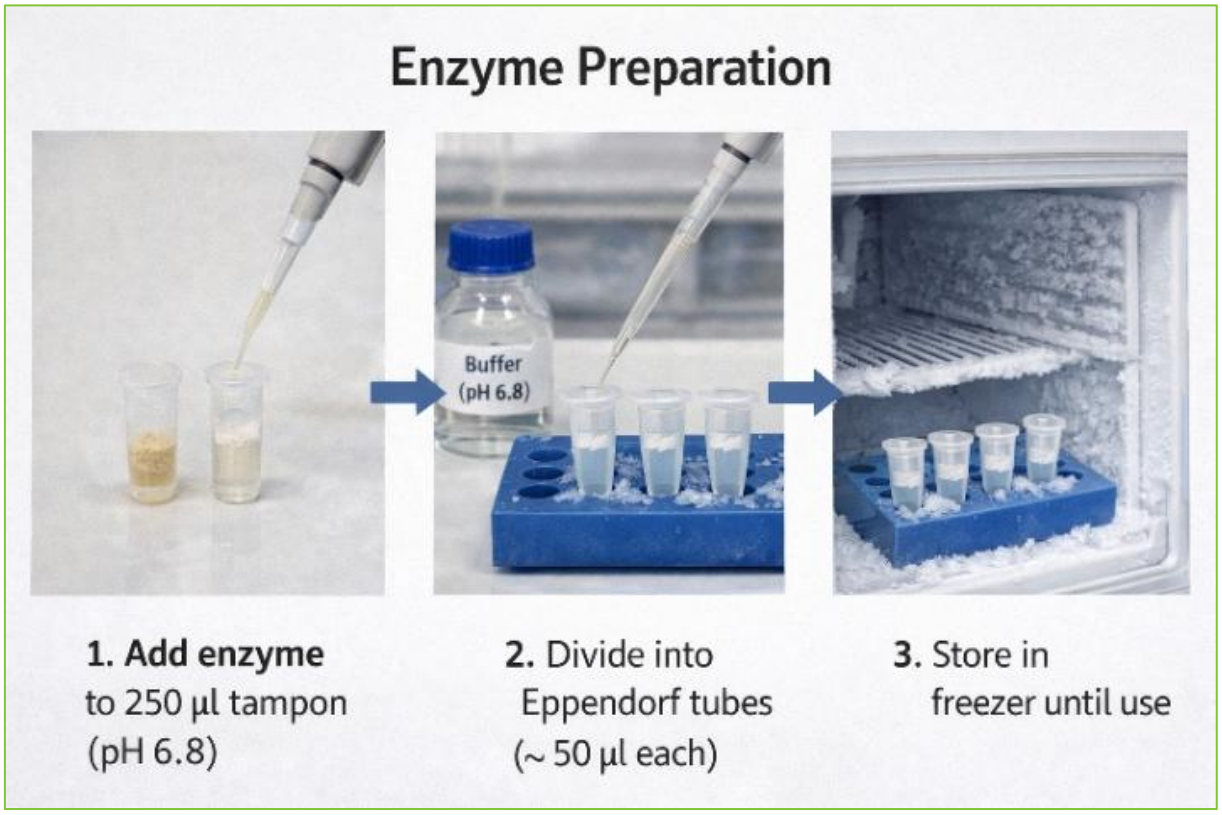


Figure 1. 11: Enzyme Preparation.

During use, 2000 µl of buffer (pH = 6.8) is added to a 50 µl volume if the solvent used is alcoholic (control). During use, 3000 µl of buffer (pH = 6.8) is added to a 50 µl volume if the solvent used is water (control).

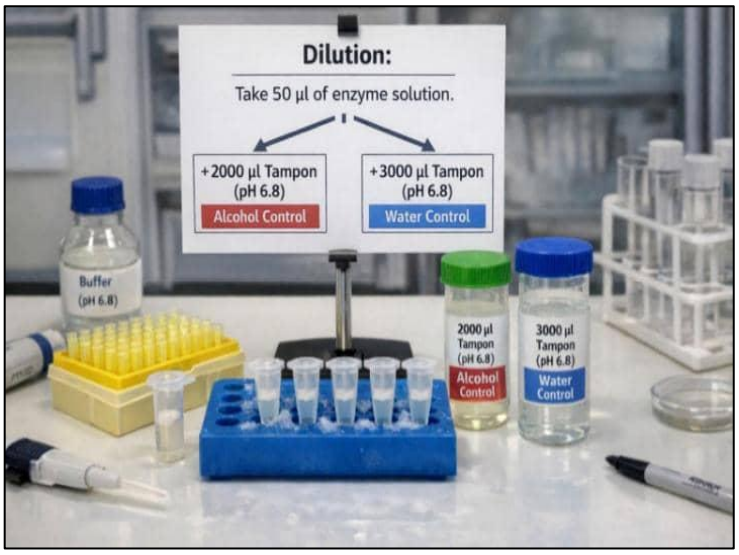


Figure 1. 12: Dilution of the enzyme solution.

c) Preparation of L-DOPA:

5 mg of L-DOPA are dissolved in 5 mL of distilled water and mixed well.

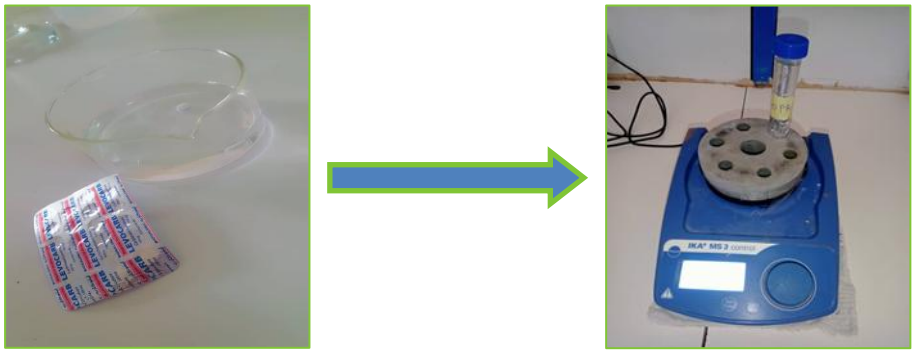


Figure 1. 13: Preparation of L-DOPA.

d) Preparation of the dilution series:

A series of dilutions was prepared starting from *Milk thistle seed oil* using the appropriate solvent (DMSO), with the aim of obtaining different concentrations for testing. The preparation was carried out in tubes while maintaining a constant final volume of 1000 µL in each tube. The first tube contained 100 µL of *Milk thistle seed oil* and 900 µL of solvent, while the second tube contained 50 µL of the oil and 950 µL of solvent. The third tube contained 25 µL of the oil and 975 µL of solvent, whereas the fourth tube contained 12.5 µL of the oil and 987.5 µL of solvent. A fifth tube was used as a control sample, containing only the solvent without the addition of the oil. After preparation, all tubes were thoroughly homogenized before being used in subsequent experiments.

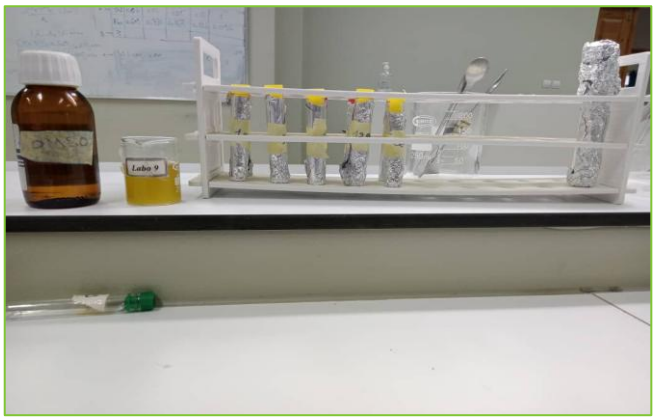


Figure 1. 14: Prepared solution samples for the tyrosinase inhibition assay.

- **Practical steps:**

150 µl (tampon pH=6.8)

+

10 µl extract or control

+

20 µl enzyme

(minutes of incubation with shaking in the spectrophotometer 3)

10 minutes of incubation at 37 °C

20 µl L-DOPA

(Kinetic reading using the spectrophotometer) 475 nm

Figure 1. 15: Incubation of samples at 37 °C.

After incubating the samples for ten (10) minutes under the specified experimental conditions, absorbance was measured using a spectrophotometer at a wavelength of 475 nm at successive time intervals in order to monitor the progression of the reaction over time.

1.3.3.4. Evaluation of the cytotoxicity of *Milk thistle seed oil*:

A cytotoxicity test is used to assess the toxic effects of natural compounds or extracts on cells or simple organisms and is an important tool in preliminary biosafety studies. This test aims to determine the ability of the substance under investigation to induce cell death or reduce cell viability, allowing for an assessment of its safety before proceeding to more complex tests. The Artemia salina lethality assay is widely used as a rapid, simple, and low-cost model for preliminary toxicity detection, demonstrating a good correlation with the overall cytotoxicity and biological activity of the compounds under investigation. **(Meyer et al., 1982)**.

Experimental Protocol :

Table 1. 8: Materials and Equipment.

Materials:	Equipment:
• ***Milk thistle seed oil*** • **Dimethyl sulfoxide (DMSO)** • **Commercial sea salt** • **Distilled water** • **Artemia salina sachets/eggs** • **Potassium dichromate ($K_2Cr_2O_7$)** • **Artificial seawater.**	• 96-well microplate • Micropipettes • Room temperature incubator/incubation area • Continuous light source • Gentle ventilation • Stereomicroscope • Solution preparation vessels

➢ **Protocol:**

The cytotoxic potential of the tested plant essential oil was evaluated using the *Artemia salina* lethality bioassay, which is widely used for preliminary toxicity screening. The procedure was adapted from published methods with minor adjustments to suit a 96-well microplate format and to ensure good reproducibility. *Artemia salina* cysts were hatched in artificial seawater prepared by dissolving 35 g/L of commercial sea salt in distilled water, and then incubated under continuous light with gentle aeration at room temperature (approximately 25 °C) for 24–48 h **(Rajabi et al., 2015)**. Actively swimming nauplii were collected and used for the assay. Stock solutions of the essential oil were prepared in dimethyl sulfoxide (DMSO) and then diluted with artificial seawater to obtain final test concentrations of 40, 20, 10, and 5% (v/v), while keeping the final DMSO concentration at a non-toxic level. In each well of a sterile 96-well microplate, 100 µL of seawater containing about 20 viable nauplii was added, followed

by the appropriate volume of the essential oil solution and artificial seawater to reach the desired concentration. The plates were incubated at room temperature under constant light for 24 h. After incubation, the number of surviving larvae was counted under a stereomicroscope, and nauplii were considered dead when no movement was observed. The survival percentage was calculated as:

Survival (%) = (number of live larvae / total number of larvae) × 100.

All treatments were carried out in triplicate. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control to verify assay sensitivity, while seawater containing the same concentration of DMSO without essential oil served as the negative control **(Nguta et al., 2012)**.

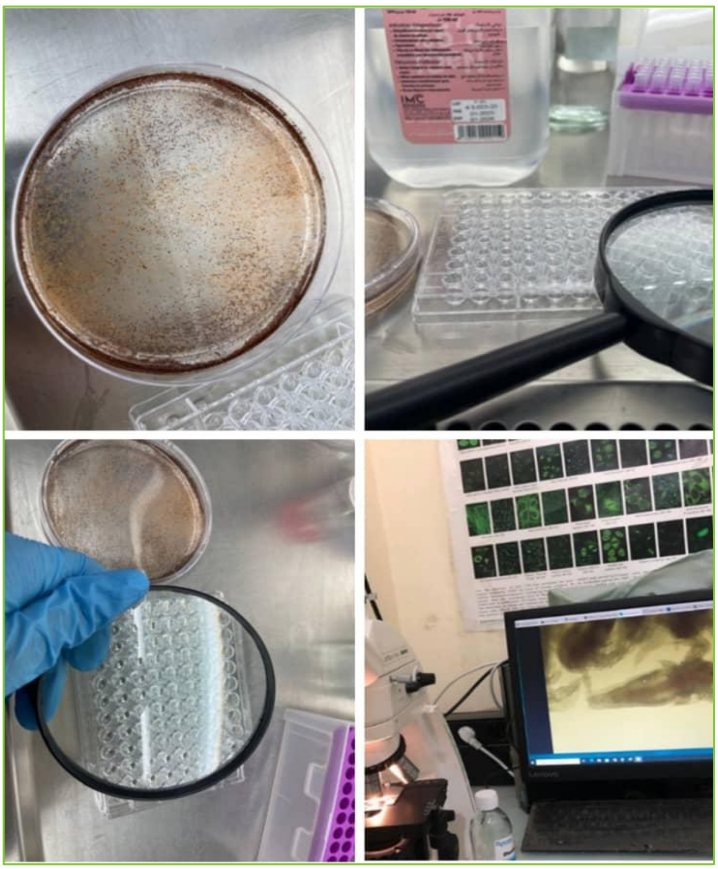


Figure 1. 16: Devices and methods for studying cytotoxicity of Milk thistle seed oil.

1.3.4. Study of Antibacterial Activity:

This study aimed to evaluate the antibacterial potential of *Milk thistle seed oil* as a natural antimicrobial agent using a series of biological tests that provide insight into its mechanism of action against bacterial strains.

1.3.4.1. MIC and MBC:

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay is a standard methodology used to evaluate the antibacterial activity of natural compounds such as vegetable oils. In this assay, a series of graded dilutions of the oil are prepared in a suitable medium, to which samples of the bacteria to be tested are added. The MIC is calculated as the lowest concentration that prevents visible bacterial growth after incubation. Solutions in which no growth is observed are then transferred to solid media to determine if bacterial cells survive. The MBC is considered the lowest concentration that completely kills the bacteria. The difference between the MIC and MBC helps determine whether a compound acts solely as an inhibitor or possesses a bactericidal effect, which is important when evaluating antimicrobial efficacy. This methodology is based on antimicrobial susceptibility methods used in scientific studies, such as those described in the study by **Cristina Rodríguez-Melcón et al., (2021)**, which determined the MIC and MBC values of multiple antimicrobials using conventional liquid dilution methods.

Experimental Protocol :

Table 1. 9: Materials and Equipment.

Materials:	Equipment:
• ***Milk thistle seed oil*** • **Dimethyl sulfoxide (DMSO)** • **Cation-amended Mueller-Hinton broth** • **Agar-Mueller-Hinton** • **Sabouraud dextrose agar** • **Bacterial/Yeast Isolates** • **McFarland Standard 0.5** • **Mueller-Hinton broth with DMSO (see growth)** • **Sterilized broth only (see sterilization)**	• Sterile microplates, 96 wells • Micropipeline and sterile tips • DensiCHEK Turbidity Meter • Bacterial incubator (37°C) • sterile Petri dishes • Sterilization tools (flame/alcohol) • Biosafety cabinet (optional)

- **protocol:**

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested plant essential oil were determined using the broth microdilution method, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Bacterial strains were first subcultured on Mueller–Hinton agar (MHA) and then transferred into cation-adjusted Mueller–Hinton broth (MHB). The cultures were incubated until visible turbidity was obtained, and the inoculum density was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using a DensiCHEK densitometer. The essential oil was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 2.5% (v/v) to ensure solubility without affecting microbial growth. Serial dilutions of the essential oil were prepared in MHB to obtain final test concentrations of 80, 40, 20, 10, 5 and 2.5% (v/v). In a sterile 96-well microtiter plate, 100 µL of each essential oil dilution was added to the wells, followed by 50 µL of the standardized microbial suspension. Wells containing MHB with the same concentration of DMSO but without essential oil served as the growth control, while wells containing MHB only were used as the sterile control. The microplates were incubated at 37 °C for 18–24 h. After incubation, the MIC was defined as the lowest concentration of the essential oil that showed no visible microbial growth when compared with the growth control. For MBC determination, a spot test method was applied by aseptically transferring 3 µL from wells showing no visible growth onto agar plates free of essential oil (Mueller–Hinton agar for bacteria and Sabouraud dextrose agar for yeast). The plates were further incubated at 37 °C for 24 h, and the MBC was defined as the lowest essential oil concentration at which no colony growth was observed, indicating complete microbial killing **(Qaiyumi, 2007; Weinstein, 2018) (PA, 2002) .**

Figure 1. 17: Photo of the broth microdilution method used to assess the antimicrobial activity of the oil against bacterial strains.

1.3.4.2. Effect of essential oil on biofilm formation:

Biofilm assays are used to assess a bacteria's ability to form a biofilm, an organized microbial community that adheres to surfaces and is surrounded by a protective polymer matrix. This increases bacterial resistance to antibiotics and environmental factors. One of the most common methods is the crystal violet microplate assay, where bacteria are cultured in microplate wells and incubated to allow biofilm formation. After incubation, non-adhering cells are removed by washing, and the biofilm is stained with crystal violet. The stain is then dissolved, and the spectral absorbance is measured, which is directly proportional to the amount of biofilm formed. This method is widely used because it is simple, reproducible, and allows for comparison of the effects of compounds, oils, or inhibitors on biofilm formation. It is of great importance in medical, nutritional, and pharmaceutical studies due to the role of biofilms in chronic infections and treatment resistance, as demonstrated by guidelines and scientific reviews from organizations such as the Centers for Disease Control and Prevention. **(Stepanović et al., 2007)**.

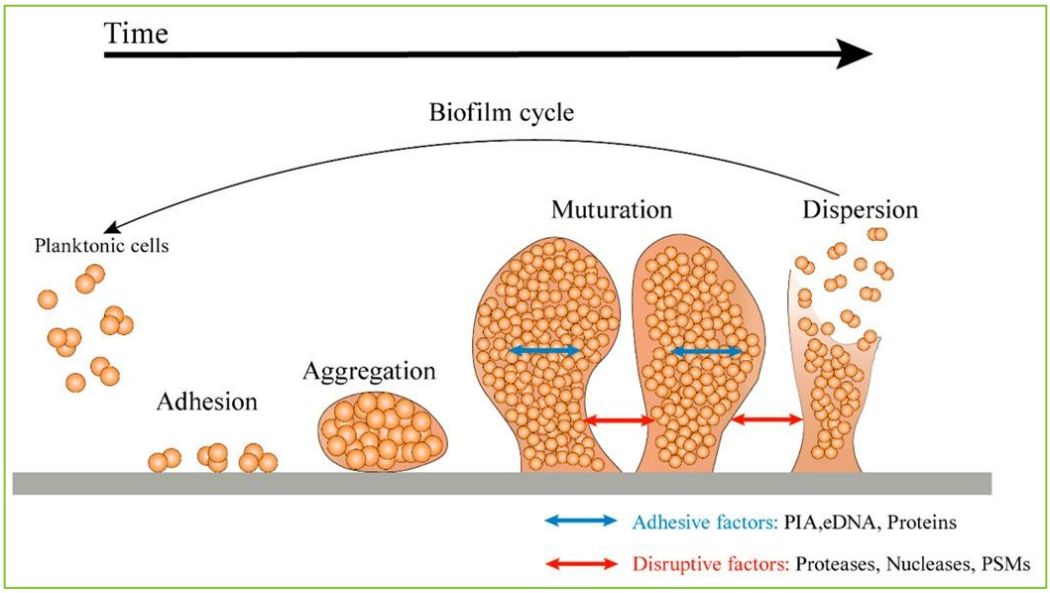


Figure 1. 17: Biofilm cycle.

Experimental Protocol :

Table 1. 10: Materials and Equipment.

Materials:	Equipment:
• ***Milk thistle seed oil*** • **Dimethyl sulfoxide (DMSO)** • **Reference bacterial strains** • **amid bacterial growth** • **Sterile PBS (Phosphate Buffered Saline) solution** • **Crystal Violet 0.1% Dye** • **distilled water** • **Pre-prepared bacterial suspensions**	• 96-well sterile microplate • Micropipettes • Incubator at 37°C • Microplate reader • Sterile laboratory tubes • Sheet drying rack • Essential sterilization tools

➢ **Protocol:**

The effect of the tested plant essential oil on bacterial biofilm formation was evaluated using a quantitative microtiter plate assay. Four reference bacterial strains were used, including *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as Gram-negative bacteria, and *Staphylococcus aureus* ATCC 25932 and *Bacillus subtilis* ATCC 25973 as Gram-positive bacteria. Overnight cultures of each strain were prepared in fresh growth medium and then diluted to obtain standardized inocula. Aliquots of the bacterial suspensions were dispensed into sterile 96-well flat-bottom microplates, and the essential oil, previously dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 2.5% (v/v), was added at sub-inhibitory concentrations of 80, 40, 20, 10, and 5% (v/v). Wells containing the same concentration of DMSO without essential oil served as the untreated control. The plates were incubated statically at 37 °C for 48 h to allow biofilm formation. After incubation, the culture medium was gently removed, and the wells were washed twice with sterile phosphate-buffered saline (PBS) to eliminate non-adherent cells. The remaining attached biofilms were air-dried and stained with 200 µL of 0.1% (w/v) crystal violet for 30 min at room temperature. Excess stain was removed by thorough washing with distilled water, and the plates were left to dry. Bound crystal violet was then solubilized by adding DMSO, and the absorbance was measured at 570 nm using a microplate reader to quantify biofilm biomass. All experiments were performed in triplicate, and the results were expressed relative to the untreated control to determine the inhibitory effect of the essential oil on biofilm formation **(Fan et al., 2018)**.

The percentage of biofilm inhibition was calculated using the following equation:

Inhibitory rate (%) = [1 − OD_{570nm} (Sample) /OD_{570nm} (positive control)] × 100. The whole experiment was replicated three times independently.

The entire experiment was performed independently in triplicate to ensure reproducibility and reliability of the results.

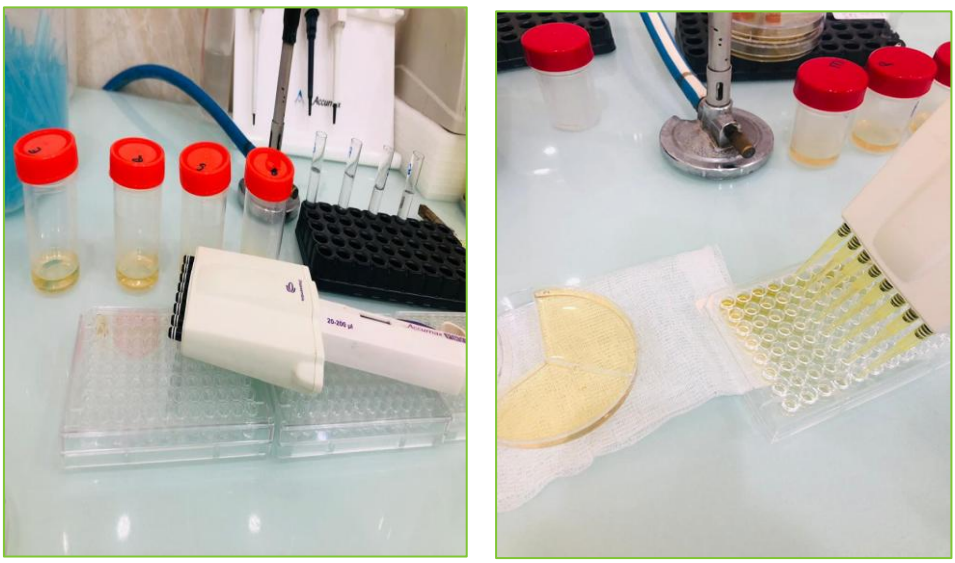


Figure 1. 18: Materials and Equipment used for biofilm testing.

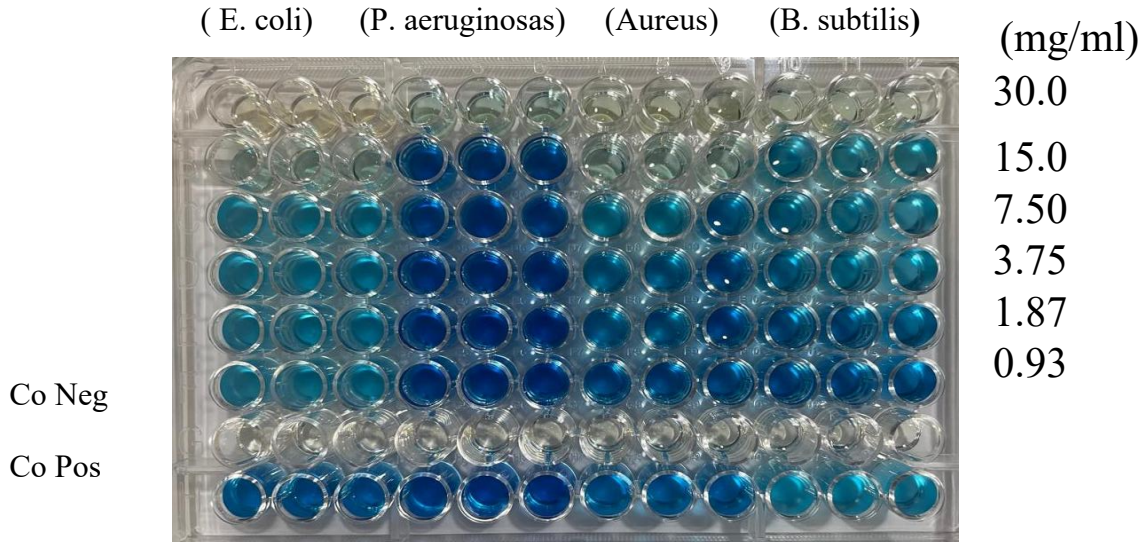


Figure 1. 20: The effect of laboratory-containing *Milk thistle seed oil* on biofilm formation in four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis*) at different concentrations.

Chapter 2:

Results and discussion

2.1. Introduction:

This chapter will present and analyze the results obtained from various biological and chemical tests conducted to evaluate the cosmetic potential of *Milk thistle seed oil*. The study included testing antioxidant activity using the FRAP method, determining the sun protection factor (SPF), testing tyrosinase inhibition, and assessing antimicrobial activity by determining the minimum effective concentration (MIC) and maximum normal concentration (MBC). The oil's effect on biofilm formation was also investigated. Furthermore, a cytotoxicity test was performed to determine the oil's compatibility with cells at the studied concentrations.

This chapter will present the results systematically, supported by tables and illustrative figures. These results will be discussed and compared with relevant scientific studies, without drawing conclusions before a detailed presentation of the data.

2.2. Results:

2.2.1. Determination of Physical Constants of the Oil Sample:

The physicochemical properties of *Milk thistle seed oil* were evaluated to determine its fundamental constants and establish reference parameters reflecting its quality and compositional characteristics. The assessment included the determination of color, viscosity, density, and extraction yield. The obtained results are summarized in the table below, providing a clear characterization of the studied sample and supporting its potential research and practical applications.

Table 2. 1: Physical and chemical properties of Milk thistle seed oil.

Parameter	Value
Color	Golden yellow
Viscosity	Medium
Density	0.92 g/ml
pH	6،24
Percentage yield	11.52%

The analysis of the oil extracted from *Milk thistle (Silybum marianum) seeds* revealed a golden-yellow color and moderate viscosity, indicating balanced physical properties that

facilitate its use in biological applications. The oil density was 0.92 g/mL, a value within the reference range for most vegetable oils, reflecting the stability of its chemical composition.

The recorded pH value was 6.24, which is close to neutrality and suggests good biocompatibility, particularly for dermatological applications. The extraction yield reached 11.52% of the dried seeds, which can be considered acceptable and indicative of the effectiveness of the extraction method as well as the quality of the plant material used, consistent with previous findings reported for *Milk thistle seed oil* **(Chikhoune et al., 2024)**.

2.2.2. Biological Activities:

2.2.2.1. Antioxidant Activity: Ferric Reducing Antioxidant Power Assay (FRAP Assay):

The antioxidant capacity of *Milk thistle seed oil* was evaluated and compared with that of ascorbic acid using the Ferric Reducing Antioxidant Power (FRAP) assay. Based on the experimental data obtained from a series of previously prepared concentrations expressed in µg/mL, the relative reducing activity (Activity %) was calculated for each concentration using the highest recorded response value, according to the following equation:

$$\textbf{Activity}\ (\%)\ = (\frac{\textbf{Asample}}{\textbf{Amax}}) \times\ \textbf{100}$$

Subsequently, calibration curves were constructed for both *Milk thistle seed oil* and ascorbic acid (used as a reference antioxidant) by plotting the relative reducing activity (%) against concentration (µg/mL). This graphical representation enabled the evaluation of the dose–response relationship and the graphical determination of the EC_{50} value, defined as the concentration (µg/mL) required to achieve 50% of the maximum reducing activity.

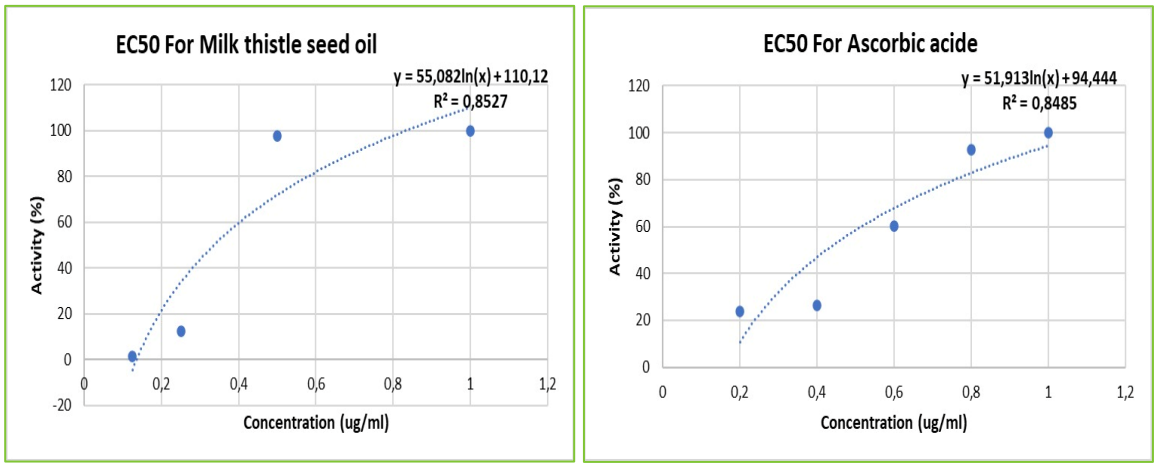


Figure 2. 1: Comparative evaluation of the antioxidant activity (EC_{50}) of ascorbic acid and Milk thistle seed oil using the FRAP assay.

The results obtained from the FRAP assay indicate a logarithmic relationship between concentration and antioxidant activity for both *Milk thistle seed oil* and ascorbic acid, with the data fitted using logarithmic regression equations. For *Milk thistle seed oil*, the regression equation obtained was $y = 55.082 \ln(x) + 110.12$ with a coefficient of determination $R^2 = 0.8527$, indicating a good agreement between the mathematical model and the experimental data, with an $EC_{50} = 0.336$ µg/mL. For ascorbic acid, the regression equation was $y = 51.913 \ln(x) + 94.444$ with a coefficient of determination $R^2 = 0.8485$, also reflecting an acceptable fit of the model, with an $EC_{50} = 0.425$ µg/mL. These values show that antioxidant activity increases with increasing concentration in a non-linear manner, which is commonly observed in biochemical assays related to reducing power. Moreover, the close R^2 values (≈ 0.85) indicate good reliability of the results despite slight variability attributable to experimental factors. Based on the EC_{50} values, the lower value reflects a higher reducing capacity.

Table 2. 2: The EC_{50} values of Milk thistle (Silybum marianum) seed oil and ascorbic acid in the FRAP assay.

Reducing Power	A 0.5 (µg/ml)
Seed oil of the plant Silybum marianum	0.336 ± 0.006
Ascorbic acid	0.425 ± 0.004

The results showed that the EC_{50} value of *Milk thistle seed oil* was 0.336 ± 0.006 µg/mL, while a value of 0.425 ± 0.004 µg/mL was recorded for ascorbic acid. These values represent the mean ± standard deviation (Mean ± SD) and were calculated from three replicates (n = 3) for each sample.

Statistical Analysis:

To assess the significance of the difference between the two EC_{50} values, a statistical analysis was performed as shown in the following Figure 2.2:

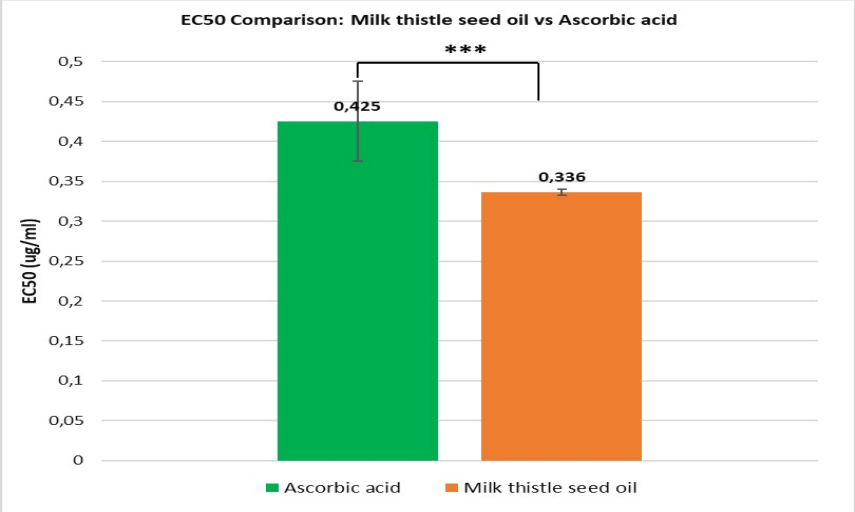


Figure 2. 2: Bar graphs showing EC50 comparison between Milk thistle seed oil and Ascorbic Acid.

The results shown in the figure indicate a statistically significant difference between the two values. The EC50 value of *Milk thistle seed oil* (0.336 ± 0.006 µg/mL) was lower compared to that of ascorbic acid (0.425 ± 0.004 µg/mL). Statistical analysis using Student's t-test for two independent samples revealed a very highly significant difference (***, $P < 0.001$; very highly significant difference), confirming that the observed variation between the two samples is not due to chance but reflects a real difference in antioxidant activity.

2.2.2.2. SPF Test:

In our study, we evaluated the sun protection factor (SPF) of *Milk Thistle (Silybum marianum) seed oil* using an in vitro spectrophotometric method described by **(Mansur et al., 1996)**. This method is based on measuring the absorbance (Abs) of the sample within the harmful ultraviolet (UV) radiation spectrum, particularly in the ultraviolet B (UVB) range (290–320 nm), wh300ich is mainly responsible for skin erythema and photodamage. The absorbance values obtained at the selected wavelengths were recorded and used for the subsequent calculation of the SPF.

Table 2. 3: Absorption values of Milk thistle seed oil at different wavelengths.

Wavelength λ (nm)	Absorbance (Abs)
290	1.42
295	1.836
300	2.343
305	2.656
310	2.075
315	1.658
320	1.542

Upon analyzing the spectrophotometric absorption results of *Milk thistle seed oil* within the 290–320 nm range, a gradual increase in absorbance values was observed, reaching a maximum at 305 nm (Abs = 2.656). This was followed by a progressive decrease at 310, 315, and 320 nm, without any further statistically significant increase. This spectral behavior indicates that a stabilization point was reached at 305 nm, which is consistent with findings reported in the scientific literature. It is considered acceptable to rely on absorbance values within the 290–320 nm range for the calculation of the Sun Protection Factor (SPF) according to the spectrophotometric method described by **(Mansur et al., 1986)**, which is based on the contribution of this range to protection against ultraviolet B (UVB) radiation. Furthermore, **Kaur and Saraf (2010)** emphasized that effective absorption within this range is the main determinant of SPF value, whereas values that do not show a significant increase beyond the maximum do not meaningfully enhance photoprotective efficacy.

Based on the data recorded in the table, the Sun Protection Factor of *Milk thistle seed oil* was calculated using the Mansur equation, yielding a value of SPF = 135.3. This very high value reflects a strong capacity to absorb ultraviolet radiation within the UVB range. According to the classification adopted by the European Commission **(European Commission, 2006)** for sunscreen products, SPF values exceeding 50 are categorized as "Very High Protection," highlighting the promising potential of *Milk thistle seed oil* as an effective natural ingredient in sunscreen formulations.

2.2.2.2.1. Comparison of SPF:

To provide a practical look at the sun protection performance of *Milk thistle seed oil*, we compared it to a well-known commercial product with high protection, namely Avène High Protection Cream with SPF 50+, a leading formula in the European skincare market:

Table 2. 4: Comparison of SPF values between Milk Thistle seed oil and commercial sunscreen

Product	SPF
Milk thistle seed oil	135.3 ± 0.283
Avène	41 ± 0.141

Based on the data presented in the table, *Milk thistle (Silybum marianum) seed oil* exhibited a markedly higher SPF value (135.3 ± 0.283) compared to the tested commercial sunscreen (41 ± 0.141). These results indicate that *Milk thistle seed oil* provides superior photoprotective efficacy. Moreover, the lower variability observed in its measurements (± 0.283 versus ± 0.141) suggests greater consistency and spectral stability, highlighting its potential as an effective natural alternative for UV protection.

2.2.2.3. Tyrosinase Inhibition:

The evolution of absorbance was tracked at different time points to evaluate the effect of *Milk thistle seed oil* on enzyme activity and to study its inhibitory role over time. Spectroscopic measurements were performed under the same experimental conditions to ensure the accuracy of the results and the comparability of different time periods. This temporal analysis allows for determining the extent of the decrease in enzyme activity in the presence of the oil, and thus estimating the intensity and effectiveness of its inhibitory effect over time. The values obtained are presented in the following tables :

- **the time :**
  1. **0 Second (s) :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	1.483	1.121	0.920	0.589	0.997

  2. **5 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	1.396	0.965	0.749	0.525	1.055

3. **10 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	1.093	0.894	0.931	0.526	1.087

4. **15 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	0.952	0.867	0.904	0.519	1.121

5. **20 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	0.936	0.862	0.820	0.516	1.127

6. **25 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	0.934	0.858	0.802	0.512	1.161

7. **30 Minutes :**

Concentration	100%	50%	25%	12.5%	Control
Absorbance	0.931	0.852	0.774	0.509	1.200

After presenting the experimentally recorded absorbance values at different time points, the results will be analyzed based on a kinetic study of tyrosinase inhibition by calculating the percentage of inhibition (%) for each concentration and at each incubation time. This analysis aims to assess the time-dependent nature of the inhibitory activity and to determine the nature of the enzyme reaction's evolution in the presence of the inhibitor.

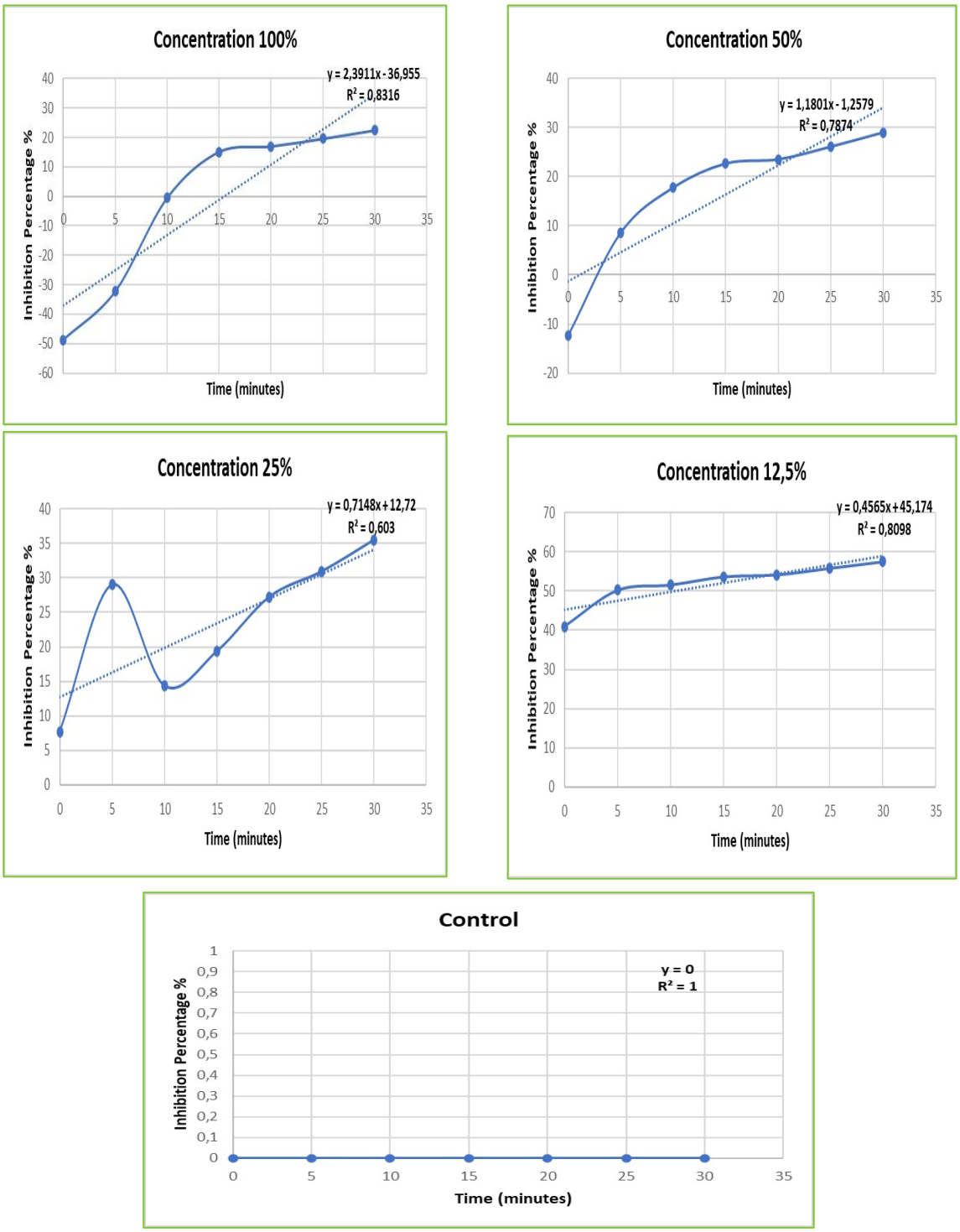


Figure 2. 3: Kinetic analysis of the inhibition percentage (%) as a function of time at progressively increasing concentrations of the studied sample.

The results of the tyrosinase inhibition assay demonstrated that *Milk thistle seed oil (Silybum marianum)* exhibited a clear and time-dependent inhibitory activity compared to the control, which maintained 0% inhibition throughout the incubation period ($R^2 = 1$), confirming that the observed variation in enzymatic activity is exclusively attributable to the tested oil. The percentage of inhibition was calculated using the following equation:

$$inhibition\ percentage\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

At a concentration of 12.5%, the highest inhibitory activity was recorded, with inhibition progressively increasing from approximately 40% at the beginning of the reaction to nearly 58% after 30 minutes (R^2 = 0.8098), indicating significant inhibitory efficacy and relative temporal stability. The 25% concentration exhibited moderate inhibition ranging from 7% to 35% (R^2 = 0.603), with a gradual increase over the incubation period. In contrast, the higher concentrations (50% and 100%) showed initial negative inhibition values, which may be attributed to possible spectral interference or transient effects on enzyme kinetics, before stabilizing at the end of the incubation period at approximately 29% and 22%, respectively (R^2 = 0.7874 and 0.8316). This behavior reflects a non-linear response pattern that may be associated with complex interactions between the oil constituents and the enzyme, or with physicochemical properties of the extract at higher concentrations.

To determine the half-maximal inhibitory concentration (IC_{50}), the value was estimated using the inhibition data at 30 minutes, as this time point represents a quasi-steady state of the reaction. Since the inhibition profile did not follow a classical sigmoidal dose–response model, linear interpolation between the two concentrations surrounding 50% inhibition (25% and 12.5%) was applied. The IC_{50} value was estimated to be approximately 16.8%, confirming a considerable inhibitory potential of *Milk thistle seed oil (Silybum marianum)* within the tested range, with maximal activity observed at intermediate to lower concentrations, consistent with the non-linear behavior recorded.

2.2.2.4. Evaluation of the cytotoxicity of *Milk thistle seed oil*:

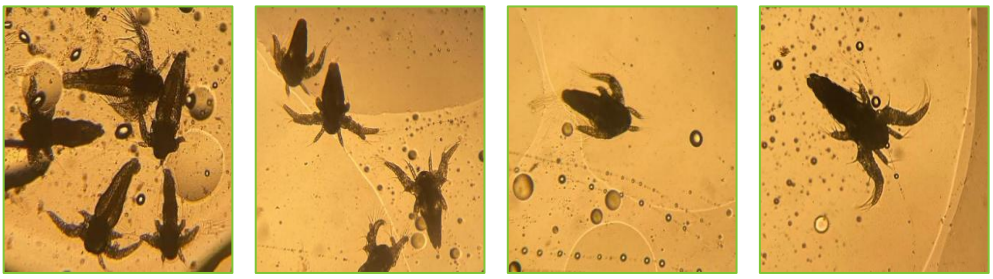
Table 2. 5: Cytotoxic Effects of Milk thistle seed oil (Silybum marianum) on Artemia salina Larvae Over Time.

Concentration	T_0	T_{30}	T_{60}	T_{180}	T_{360}	T_{24H}
Control Neg	100.00 ± 0.00	100.00 ± 0.00	99.67 ± 0.58	99.33 ± 0.58	98.00 ± 1.00	96.67 ± 1.53
Control Pos ($K_2Cr_2O_7$)	100.00 ± 0.00	46.67 ± 2.08	26.67 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
40%	100.00 ± 0.00	98.67 ± 1.15	96.67 ± 1.53	90.00 ± 2.00	80.00 ± 2.65	70.00 ± 3.00
20%	100.00 ± 0.00	99.33 ± 0.58	97.33 ± 1.15	93.33 ± 1.53	85.33 ± 2.31	76.67 ± 2.52
10%	100.00 ± 0.00	99.67 ± 0.58	98.67 ± 0.58	96.00 ± 1.00	90.67 ± 1.53	83.33 ± 2.08
5%	100.00 ± 0.00	100.00 ± 0.00	99.33 ± 0.58	97.33 ± 0.58	94.00 ± 1.00	88.67 ± 1.53

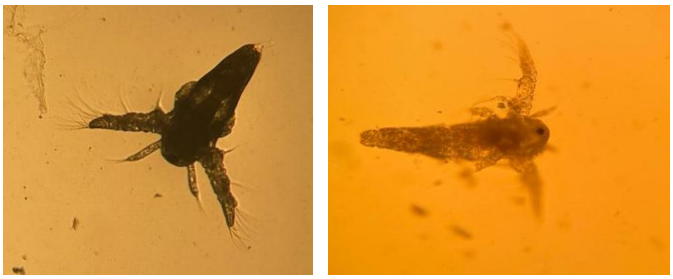
This table presents the survival percentages of *Artemia salina* larvae after exposure to the OIL (5 to 40 %), along with control groups, at various time intervals (T_0, T_{30}, T_{60}, T_{180}, T_{360}, T_{24H}).

The results presented in Table 2.4 show a clear cytotoxic effect of oil that depends *Milk thistle seed oil* on both concentration and exposure time on *Artemia salina* larvae. At the initial time (T_0), the survival rate reached 100% in all groups, confirming the uniform viability of the larvae prior to exposure. The negative control maintained high survival rates throughout the experimental period, with only a slight decrease observed at $T_{72}H$ (96.67 ± 1.53%), indicating stable and appropriate experimental conditions. In contrast, the positive control ($K_2Cr_2O_7$) exhibited a rapid and complete loss of viability, with survival reaching 0% starting from T_{180}, confirming the sensitivity of the bioassay.

Regarding the tested concentrations of *Milk thistle seed oil* (5–40%), a progressive decrease in survival percentage was observed over time. The highest concentration (40%) showed the strongest cytotoxic effect, reducing survival to 70.00 ± 3.00% at $T_{72}H$. Moderate toxicity was observed at concentrations of 20% and 10%, where survival rates reached 76.67 ± 2.52% and 83.33 ± 2.08%, respectively, at the end of the exposure period. The lowest concentration (5%) showed the least effect, maintaining a survival rate of 88.67 ± 1.53%. These findings indicate that exerts a dose-depende *Milk thistle seed oil* nt cytotoxic effect that becomes more pronounced with increasing exposure time.



40% **20%** **10%** **5%**



Control Neg **Control Pos ($K_2Cr_2O_7$)**

Figure 2. 4: Microscopic images illustrating the effect of different concentrations of Milk thistle seed oil on the viability of Artemia salina larvae compared to controls.

2.2.3. Study of Antibacterial Activity:

2.2.3.1. MIC and MBC:

Table 2. 6: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), MBC/MIC Ratio, and Antibacterial Effect of Milk thistle seed oil (Silybum marianum) Against Reference Bacterial Strains.

Bacteria strains (n = 3)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Antibacterial Effect
***Escherichia coli* ATCC 25922**	40.0±0.00	40.0±0.00	1	Bactericidal
***Pseudomonas aeruginosa* ATCC 27853**	80.0±0.00	80.0±0.00	1	Bactericidal
***Staphylococcus aureus* ATCC 25932**	40.0±0.00	40.0±0.00	1	Bactericidal
***Bacillus subtilis* ATCC 25973**	80.0±0.00	80.0±0.00	1	Bactericidal

NB. An MBC/MIC ratio of ≥4 is generally considered to indicate a bactericidal compound, whereas an MBC/MIC ratio of >4 is generally considered to indicate a bacteriostatic compound **(Lushniak, 2014; Suzuki et al., 1953)**.

The antibacterial activity of *Milk thistle seed oil* was evaluated by determining the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC), and calculating the corresponding MBC/MIC ratios against four reference bacterial strains.

The results showed that Escherichia coli and Staphylococcus aureus were the most sensitive to *Milk thistle seed oil*, with both MIC and MBC values recorded at 40 mg/mL. In contrast, Pseudomonas aeruginosa and Bacillus subtilis required a higher concentration of 80 mg/mL for both MIC and MBC, indicating relatively lower sensitivity to the oil.

All tested bacterial strains exhibited an MBC/MIC ratio equal to 1, demonstrating that *Milk thistle seed oil* exerts a bactericidal effect rather than merely a bacteriostatic one.

Overall, these findings suggest that *Milk thistle seed oil* possesses broad-spectrum antibacterial activity, although the degree of bacterial susceptibility varies depending on the strain type.

2.2.3.2. Effect of essential oil on biofilm formation:

Table 2. 7: Inhibitory rate (%) of biofilm formation (Mean ± SD, n = 3).

Concentration (mg/mL)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
80	92.5 ± 0.9	96.1 ± 0.7	92.0 ± 1.1	92.7 ± 0.8
40	90.6 ± 1.4	13.9 ± 1.2	91.4 ± 1.3	89.3 ± 1.0
20	1.9 ± 0.8	14.0 ± 1.5	89.6 ± 1.6	84.1 ± 1.9
10	4.9 ± 2.1	16.8 ± 1.7	29.2 ± 3.4	43.8 ± 3.1
5	8.1 ± 2.6	15.3 ± 1.4	11.0 ± 2.8	29.4 ± 2.7
2.5	2.7 ± 1.2	9.2 ± 1.6	1.3 ± 0.9	25.9 ± 2.9

Table 2. 8: Inhibitory rate (%) of biofilm formation by Milk thistle seed oil (Silybum marianum) at different concentrations.

Concentration (mg/mL)	*E. coli* (%)	*P. aeruginosa* (%)	*S. aureus* (%)	*B. subtilis* (%)
80	92.5 ± 0.9^{a}	96.1 ± 0.7^{a}	92.0 ± 1.1^{a}	92.7 ± 0.8^{a}
40	90.6 ± 1.4^{a}	13.9 ± 1.2^{c}	91.4 ± 1.3^{a}	89.3 ± 1.0^{a}
20	1.9 ± 0.8^{c}	14.0 ± 1.5^{c}	89.6 ± 1.6^{a}	84.1 ± 1.9^{b}
10	4.9 ± 2.1^{c}	16.8 ± 1.7^{b}	29.2 ± 3.4^{c}	43.8 ± 3.1^{c}
5	8.1 ± 2.6^{bc}	15.3 ± 1.4^{bc}	11.0 ± 2.8^{d}	29.4 ± 2.7^{d}
2.5	2.7 ± 1.2^{c}	9.2 ± 1.6^{d}	1.3 ± 0.9^{d}	25.9 ± 2.9^{d}

This table presents the inhibitory rate (%) of biofilm formation for each bacterial strain at different extract concentrations. Data are expressed as mean values (n = 3). Different superscript letters (a, b, c, d) within the same column indicate statistically significant differences

between concentrations according to one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

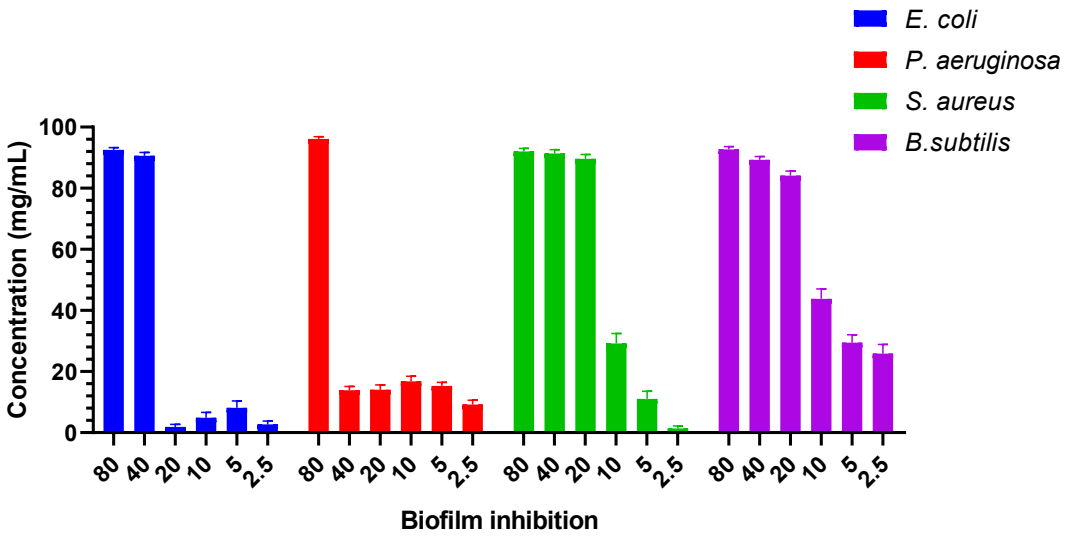


Figure 2. 5: The inhibitory effect of Milk thistle seed oil on biofilm formation at different concentrations.

This bar chart illustrates the inhibitory rate (%) of biofilm formation for each bacterial strain at different extract concentrations. Data are expressed as mean ± SD (n = 3). Bars represent mean values, and error bars indicate standard deviation. Different superscript letters (a, b, c, d) above the bars denote statistically significant differences between concentrations within the same bacterial strain, as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

The inhibitory effect of *Milk thistle seed oil* on biofilm formation was clearly concentration-dependent across all studied bacterial strains. As shown in Figure 2.4, the highest inhibitory activity was recorded at 80 mg/mL, where the inhibition rate exceeded 90% in all strains, reaching 92.5% against *Escherichia coli*, 96.1% against *Pseudomonas aeruginosa*, 92.0% against *Staphylococcus aureus*, and 92.7% against *Bacillus subtilis*.

At 40 mg/mL, both *S. aureus* and *B. subtilis* exhibited high sensitivity, with inhibition percentages exceeding 89%, whereas the effect decreased noticeably in *P. aeruginosa*. With further reduction of the concentration to 20 and 10 mg/mL, a gradual decline in inhibition percentages was observed. However, Gram-positive strains, particularly *B. subtilis*, maintained considerable inhibitory levels compared to the other strains.

At lower concentrations (5 and 2.5 mg/mL), the inhibitory activity markedly decreased in most strains; nevertheless, *B. subtilis* demonstrated relatively better responsiveness compared

to the other bacteria. Overall, *P. aeruginosa* appeared to be the most sensitive strain at the highest concentration, while *B. subtilis* showed relative consistency in its response across the different concentrations. These findings reflect the effectiveness of *Milk thistle seed oil* in inhibiting biofilm formation, especially in Gram-positive bacteria.

2.3. Discussion:

2.3.1. Physicochemical Properties:

The oil extracted from *Milk thistle seeds (Silybum marianum)* exhibited a golden-yellow color and a medium viscosity, which are physical characteristics reflecting its purity and significant content of unsaturated fatty acids, particularly linoleic acid. The oil density was 0.92 g/mL, a value within the natural range for vegetable oils (0.90–0.95 g/mL), indicating the stability of its physical and chemical composition. The recorded pH was 6.24, which is close to neutral, suggesting good biocompatibility and suitability for dermatological and pharmaceutical applications. The extraction yield, on the other hand, was 11.52% of the dry weight of the seeds, a moderate value that can be explained by variations in extraction factors such as solvent type, temperature, extraction time, as well as the genetic and environmental differences of the plant. Studies indicate that *Milk thistle seed oil* yield can range between 10–25% depending on extraction conditions and seed quality, making the observed result scientifically acceptable **(Abenavoli et al., 2010)**; **(Ramadan et al., 2007)**.

2.3.2. Biological Activities:

2.3.2.1. Antioxidant Activity: Ferric Reducing Antioxidant Power Assay (FRAP Assay):

The obtained EC50 values indicate that *Milk thistle seed oil* exhibits a higher antioxidant capacity compared to ascorbic acid, as the lower EC50 value reflects greater efficiency in iron reduction in the FRAP assay. This result can be explained by the oil's composition, which is rich in phenolic compounds and tocopherols, known for their strong ability to donate electrons and neutralize free radicals. Previous studies have shown that silymarin compounds found in milk thistle, particularly silybin, significantly contribute to antioxidant activity by inhibiting oxidative stress and enhancing cellular defense mechanisms **(Soto et al., 2010).**

Moreover, the presence of vitamin E (tocopherol) in vegetable oils enhances their reducing power, which explains the good performance of *Milk thistle seed oil* in the FRAP test (Kamal-Eldin & Appelqvist, 1996). On the other hand, although ascorbic acid is considered a standard reference antioxidant, its effectiveness may vary depending on the type of assay used. The FRAP test primarily depends on the reducing ability of compounds in an acidic medium, which

may highlight the superiority of certain lipidic or phenolic compounds **(Benzie & Strain, 1996).**

Therefore, the highly significant statistical difference ($P < 0.001$) between the two values confirms that the antioxidant activity of *Milk thistle seed oil* is not only noticeable but also scientifically significant, supporting its potential use as a promising natural source of antioxidants in food or pharmaceutical applications. Laboratory studies have shown that flavonolignans such as silybin, silychristin, and taxifolin possess strong radical-scavenging abilities, explaining the oil's potent antioxidant activity compared to conventional antioxidants like ascorbic acid **(Křen & Walterová, 2005)**. Furthermore, recent reviews indicate that *Silybum marianum seed* extracts can protect proteins, lipids, and DNA from oxidative damage, highlighting their potential as a natural source of antioxidants for pharmaceutical and food applications **(Polyak et al., 2010)**.

The present results, supported by low variability between experimental replicates ($n = 2$), confirm the reliability of the measurements and the stable antioxidant capacity of *Milk thistle seed oil*. These observations underscore the importance of future studies to optimize extraction methods and enhance the concentration of bioactive compounds to maximize the oil's biological efficacy **(Kaur et al., 2021)**.

2.3.2.2. SPF Test:

The results obtained demonstrated that *Milk thistle (Silybum marianum) seed oil* possesses a remarkably high photoprotective capacity, with a Sun Protection Factor (SPF) of 135.3 ± 0.283 calculated using the spectrophotometric method described by **(Mansur et al., 1986)**. This value falls within the "Very High Protection" **category according to the European Commission (2006)**, which classifies sunscreen products with SPF values above 50 as providing very high protection against ultraviolet radiation. Such a result reflects the strong ability of the oil to absorb radiation within the UVB range (290–320 nm), which is primarily responsible for erythema (skin redness), oxidative stress, and UV-induced mutagenic changes associated with skin carcinogenesis **(Young, 2006)**.

The high SPF value can be attributed to the distinctive chemical composition of *Milk thistle seed oil*, particularly its richness in phenolic compounds, notably the silymarin complex. Silymarin is a mixture of flavonolignans, including silibinin, silychristin, and silydianin, which are known for their dual action: partial absorption of ultraviolet radiation and potent antioxidant activity. These compounds have been shown to inhibit the formation of reactive oxygen species, reduce lipid peroxidation, and protect cellular DNA from UV-induced damage **(Katiyar, 2005)**;

(Agarwal et al., 2006). Furthermore, silymarin has been reported to modulate inflammatory pathways associated with photoaging by inhibiting NF-κB activation and decreasing pro-inflammatory cytokine expression, thereby enhancing overall photoprotection **(Nichols & Katiyar, 2010)**.

Moreover, the relatively low standard deviation (±0.283) indicates consistent spectrophotometric measurements and stable absorption behavior within the investigated wavelength range. Collectively, these findings suggest that *Milk thistle seed oil* represents a promising natural candidate for incorporation into sunscreen formulations, either as a primary active ingredient or as an adjunct component enhancing antioxidant and photoprotective efficacy.

2.3.2.3. Tyrosinase Inhibition:

The present results demonstrated that *Milk thistle seed oil (Silybum marianum)* exhibits significant inhibitory activity against tyrosinase, with the highest effect observed at a 12.5% concentration, achieving approximately 58% inhibition after 30 minutes and an estimated IC_{50} of around 16.8%. This behavior reflects a non-linear response pattern, as a relative decrease in efficacy was observed at higher concentrations (50–100%), which may be attributed to physicochemical factors such as spectral interference, changes in component solubility, or aggregation phenomena that can affect enzyme kinetics and the interaction of the inhibitor with its active site. Similar non-conventional responses have been reported in several plant extracts rich in phenolic compounds, which may display concentration- and medium-dependent effects **(Chang, 2009)**.

The inhibitory effect of this seed oil is primarily attributed to its richness in silymarin, a flavonolignan complex comprising silibinin, silydianin, and silychristin, which are well-documented for their antioxidant properties and ability to modulate the activity of various oxidizing enzymes **(Křen & Walterová, 2005)**; **(Abenavoli et al., 2010)**. It is well known that phenolic compounds and flavonoids can inhibit tyrosinase through multiple mechanisms, including copper chelation at the enzyme's catalytic site or competitive inhibition of the substrate, in addition to involvement in redox reactions related to quinone formation **(Kim & Uyama, 2005)**; **(Chang, 2009)**. Moreover, the antioxidant activity of silymarin may reduce the formation of intermediate oxidative products, thereby enhancing the observed inhibitory effect **(Singh et al., 2008)**.

The gradual increase in inhibition over time suggests the possibility of slow-binding inhibition or the formation of stable complexes between the oil constituents and the enzyme, justifying the selection of the 30-minute time point for IC_{50} estimation as it represents a quasi-steady-state phase of the reaction. Compared to reports on crude plant extracts, the estimated IC_{50} (16.8%) indicates a considerable inhibitory potential, although pure standard inhibitors such as kojic acid often exhibit higher efficacy due to their specific mode of action **(Chang, 2009)**.

Overall, these findings support the scientific evidence that the phenolic and flavonolignan constituents of *Milk thistle seeds* may represent a promising natural source of tyrosinase inhibitors, highlighting the need for further studies including detailed chemical profiling and kinetic modeling to elucidate the precise mechanism of inhibition and identify the active compounds responsible.

2.3.2.4. Evaluation of the cytotoxicity of *Milk thistle seed oil*:

The results of the cytotoxicity test using *Artemia salina* larvae showed that *Milk thistle seed oil* exhibits low to moderate cytotoxicity, clearly depending on both concentration and exposure time. All treatments showed a survival rate of 100% at the beginning of the experiment (T_0), indicating uniformity of the Artemia larvae and appropriate experimental conditions. The negative control maintained high survival rates throughout the experimental period, with only a slight decrease after 24 hours, confirming that the observed mortality in the different treatments was due to the effect of the oil rather than environmental or experimental factors. In contrast, the positive control ($K_2Cr_2O_7$) showed a rapid decrease in survival rates until reaching 0%, demonstrating the sensitivity of the *Artemia salina* assay and its reliability for evaluating the cytotoxicity of natural compounds **(Meyer et al., 1982)**.

The results showed a gradual decrease in larval survival percentages with increasing oil concentration and exposure time. The highest concentration (40%) exhibited the strongest cytotoxic effect, reducing the survival rate to approximately 70% after 24 hours. In contrast, survival rates ranged between approximately 76–83% at concentrations of 20% and 10%, respectively, while the lowest concentration (5%) maintained a relatively high survival rate of about 89%. This progressive reduction in survival indicates that *Milk thistle seed oil* exerts a dose-dependent cytotoxic effect, which is a common characteristic of plant oils containing biologically active compounds **(Carballo et al., 2002)**.

The observed cytotoxic effect of *Milk thistle seed oil* may be attributed to the presence of bioactive compounds such as flavonoids, phenolic compounds, and unsaturated fatty acids, which can affect cell membrane permeability and cellular metabolic processes, leading to a gradual decrease in larval viability with increasing concentration and exposure time. It is well known that *Milk thistle seed oil (Silybum marianum)* contain silymarin and its derivatives, which are biologically active compounds capable of affecting cellular activity at high concentrations despite their protective effects at lower concentrations **(Karkanis et al., 2011)**.

Despite the observed cytotoxic effect at higher concentrations, survival rates remained relatively high even at the highest concentration, exceeding 70% after 24 hours. This suggests that *Milk thistle seed oil* has a good biological safety profile and low acute toxicity compared to the positive control. These findings indicate the potential use of the oil in pharmaceutical and biological applications at moderate concentrations without causing significant toxic effects, which is consistent with studies reporting the relative safety of *Milk thistle seed oil* extracts and their wide use in medicinal and nutritional applications.

Overall, these results demonstrate that *Milk thistle seed oil* exhibits limited cytotoxicity dependent on concentration and exposure time, with a good safety margin at low and moderate concentrations, making it a promising candidate for biological and medical applications, particularly due to its content of biologically active compounds with antioxidant and antimicrobial properties.

2.3.3. Study of Antibacterial Activity:

2.3.3.1. MIC and MBC:

The antibacterial activity of *Milk thistle seed oil (Silybum marianum*) was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), revealing a clear inhibitory and bactericidal effect against all tested bacterial strains. MIC values ranged between 40 and 80 mg/mL, indicating moderate antibacterial efficacy with notable biological significance. The lowest MIC and MBC values (40 mg/mL) were recorded against *Escherichia coli* and *Staphylococcus aureus,* indicating higher sensitivity of these strains, whereas *Pseudomonas aeruginosa* and *Bacillus subtilis* required higher concentrations (80 mg/mL), reflecting relatively lower susceptibility.

This variation in sensitivity among the tested strains can be explained by structural and compositional differences in bacterial cell envelopes. Gram-negative bacteria, such as *Pseudomonas aeruginosa*, possess an outer membrane rich in lipopolysaccharides (LPS), which

acts as a permeability barrier limiting the penetration of lipid-soluble antimicrobial compounds **(Nikaido, 2003)**. This structure generally confers intrinsic resistance to many plant oils and lipophilic compounds. In contrast, Gram-positive bacteria, such as *Staphylococcus aureus*, lack this outer membrane, making their cytoplasmic membrane more susceptible to penetration by active lipid-soluble compounds, which explains the lower MIC values observed.

Notably, MBC values were equal to MIC values for all strains (MBC/MIC ratio = 1), clearly indicating a bactericidal rather than a merely bacteriostatic mode of action. According to established criteria, an antimicrobial agent is considered bactericidal when the MBC/MIC ratio ≤ 4 **(Pankey & Sabath, 2004)**. Thus, the recorded ratio (1) strongly supports the ability of *Milk thistle seed oil* to induce irreversible cellular damage leading to bacterial cell death.

The bactericidal effect is associated with the chemical composition of the oil, particularly its richness in unsaturated fatty acids (such as oleic and linoleic acids), as well as tocopherols and phenolic compounds. Studies have shown that unsaturated fatty acids can integrate into the phospholipid layer of the bacterial cell membrane, disrupting its structure, increasing fluidity, and causing leakage of intracellular contents **(Desbois & Smith, 2010)**. Phenolic compounds are also known to disrupt essential enzymatic systems, increase membrane permeability, and induce oxidative stress within microbial cells **(Burt, 2004)**.

Furthermore, natural antioxidants present in *Milk thistle seeds*, such as flavonoids and tocopherols, may synergistically contribute to antibacterial activity by destabilizing microbial membranes and affecting critical metabolic pathways **(Abenavoli et al., 2018)**. The concurrent presence of lipophilic fatty acids and phenolic compounds is likely to enhance antibacterial efficacy through multi-target mechanisms, reducing the likelihood of bacterial resistance development.

Although the MIC values recorded in this study are higher compared to some conventional antibiotics, it should be noted that crude plant oils represent a complex natural mixture of compounds rather than a purified single substance. Their biological significance lies in their multiple activities, including antioxidant, anti-inflammatory, and antimicrobial effects, which may provide an advantage in complementary therapeutic applications or alternative medicine.

2.3.3.2. Effect of essential oil on biofilm formation:

The results of the present study demonstrate that *Milk thistle seed oil (Silybum marianum)* exhibits significant antibiofilm activity against the tested bacterial strains, with inhibition percentages generally increasing as the oil concentration increased. This concentration-

dependent pattern of inhibition is consistent with previous reports describing the antibiofilm activity of plant extracts, which have been shown to reduce biofilm formation in a dose-dependent manner across various multidrug-resistant Gram-positive and Gram-negative bacteria. Such effects are often mediated through interference with quorum sensing systems and disruption of extracellular polymeric substances (EPS) **(Akhtar & Saadia, 2026)**; **(Al-Sheddi et al., 2019)**.

Recent studies on *Milk thistle seed* extracts and their active constituents further indicate that these extracts can reduce biofilm formation and inhibit bacterial cell adhesion. This activity reflects the ability of phenolic and flavonoid compounds present in the seeds to interfere with biofilm development primarily through indirect regulatory mechanisms rather than direct bactericidal action **(Akhtar & Saadia, 2026)**. Notably, bioactive compounds identified in *Milk thistle seeds*, such as silibinin and silymarin, are well recognized for their antioxidant and antimicrobial properties, which may contribute to the observed partial inhibition of biofilm formation **(Rasool et al., 2021)**.

When comparing the susceptibility of different bacterial strains, variations in inhibition levels were observed, suggesting that certain strains are more sensitive to the oil than others. This variability may be explained by structural differences in the cell wall architecture of Gram-positive and Gram-negative bacteria, which can influence the penetration and activity of oil constituents as well as their interaction with adhesion mechanisms **(Rasool et al., 2021)**; **(Akhtar & Saadia, 2026)**.

Based on the present findings and supporting literature, *Milk thistle seed oil* can be considered a promising natural agent with antibiofilm potential. These results reinforce growing scientific evidence that plants rich in bioactive phytochemicals represent valuable sources of antibiofilm compounds with potential medical applications in preventing biofilm-associated infections **(Akhtar & Saadia, 2026)**; **(Al-Sheddi et al., 2019)**.

General Conclusion

General Conclusion:

At the end of this study, the scientific and biological importance of *Milk thistle* (*Silybum marianum*) was highlighted. This plant is considered one of the medicinal plants with high therapeutic and nutritional value due to its richness in several biologically active compounds, such as phenolic compounds, flavonoids, unsaturated fatty acids, and silymarin, which is known for its protective and therapeutic properties.

The objective of this study was to extract the oil from the seeds of *Milk thistle* (*Silybum marianum*) and to evaluate some of its physicochemical properties, in addition to studying several of its biological activities that may open new perspectives for its use in various fields.

The obtained results showed that *Milk thistle seed oil* possesses suitable physicochemical properties that reflect the quality of the extracted oil and its potential for use in different applications. The extraction yield obtained also indicated that the seeds of this plant represent a promising source for oil production, which further enhances its economic and scientific importance.

Regarding the biological activities, the results demonstrated that *Milk thistle seed oil* exhibits a considerable reducing capacity in the Ferric Reducing Antioxidant Power (FRAP) assay, indicating its antioxidant activity, which may be attributed to its content of phenolic compounds and active fatty acids. This activity is of great importance due to the role of antioxidants in reducing oxidative stress associated with many chronic diseases.

The study also showed that the oil has the ability to inhibit the enzyme tyrosinase, a key enzyme in the process of melanin synthesis in the skin, suggesting its potential use in cosmetic industries, particularly in products intended for skin care and the regulation of skin pigmentation.

In addition, microbiological tests revealed antibacterial activity against certain bacterial strains, as well as an inhibitory effect on biofilm formation, highlighting the promising potential of *Milk thistle seed oil* as a natural source of antimicrobial compounds.

Moreover, the evaluation of the Sun Protection Factor (SPF) showed that *Milk thistle seed oil* has a certain capacity to absorb ultraviolet radiation, suggesting the possibility of incorporating it as a natural ingredient in sunscreen formulations. This opens new perspectives for its use in the cosmetic and pharmaceutical industries.

Based on the obtained results, *Milk thistle seed oil* can be considered a natural source rich in bioactive compounds with multiple biological properties, making it promising for applications in nutrition, pharmaceuticals, and cosmetics.

However, further in-depth studies are still required, particularly to determine the precise chemical composition of the oil using advanced analytical techniques, to investigate its biological mechanisms of action, and to evaluate its effectiveness and safety in industrial and clinical applications.

References

References:

Abenavoli, L., Capasso, R., Milic, N., & Capasso, F. (2010). Milk thistle in liver diseases: Past, present, future. Phytotherapy Research, 24(10), 1423–1432.

Abenavoli, L., Capasso, R., Milic, N., & Capasso, F. (2018). Milk thistle in liver diseases: Past, present, future. Phytotherapy Research, 32(5), 903–915.

Abenavoli, L., Capasso, R., Milic, N., & Capasso, F. (2018). Milk thistle in liver diseases: Past, present, future. Phytotherapy Research, 32(1), 14–23.

Abenavoli, L., Izzo, A. A., Milic, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Phytotherapy Research, 32(11), 2202–2213.

Abenavoli, L., Izzo, A. A., Milic, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Hepatic Medicine: Evidence and Research, 10, 1–12.

Agarwal, R., Agarwal, C., Ichikawa, H., Singh, R. P., & Aggarwal, B. B. (2006). Anticancer potential of silymarin: From bench to bedside. Anticancer Research, 26, 4457–4498.

Akhtar, M. T., & Saadia, M. (2026). Silybum marianum extract as a next-generation multifunctional therapeutic: Potent antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anti-biofilm activities validated by phytochemical profiling and molecular docking. 3 Biotech, 16(1), 26.

Alhajj, N. Q. M., Ma, C., Thabit, R., Gasmalla, M. A., Musa, A., Aboshora, W., et al. (2014). Chemical composition of essential oil and mineral contents of Pulicaria inuloides.

Al-Sheddi, E. S., Farshori, N. N., Al-Oqail, M. M., Musarrat, J., Al-Khedhairy, A. A., & Siddiqui, M. A. (2019). Antimicrobial and antibiofilm potential of plant-derived compounds against multidrug-resistant bacteria. Saudi Journal of Biological Sciences, 26(7), 1615–1622.

Amrani, O. (2008). Nutritional value of Milk thistle (Doctoral dissertation, Faculty of Sciences, Department of Agronomy, El-Hadj Lakhdar University of Batna, Algeria).

Anwar, F., Ashraf, M., & Bhanger, M. I. (2005). Properties and extraction of vegetable oils and the effect of extraction methods on quality.

Arshad, Z., Hanif, M. A., Qadri, R. W. K., Khan, M., Babarinde, A., Omisore, G., et al. (2014). Role of essential oils in plant diseases protection: A review. International Journal of Chemical and Biochemical Sciences, 6, 11–17.

Azoz, S. N., Farag, H. M., & Salama, A. M. (2019). Comparative botanical studies two varieties of Silybum marianum (L.) Gaertn. (Asteraceae) in Egypt. International Journal of Advanced Research in Biological Sciences, 6(6), 154–168. DOI: 10.22192/ijarbs.2019.06.06.019.

B. Boulanouae, G. Abdelaziz, S. Aazza, C. Gago, and M. G. Miguel,(2013). "Antioxidant activities of eight Algerian plant extracts and two essential oils," Industrial Crops and Products, vol. 46, pp. 85-96.

Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. Life Sciences, 78(5), 431–441.

Bayer, E., Finkenzeller, J.G.R., Buller, K. (1990). Guide to Mediterranean Flora: Characteristics, Habitats, Distribution, and Particularities of 536 Species. Paris: Edition De la Chaux and Nestlé, 222 pp.

Belouahem, AD. (2009) Floristic Biodiversity and Vulnerability of Black Alder Groves in Numidia, Algeria (NE Algeria). European Journal of Scientific Research; 32 (3) p 345.

Ben Rahal, N. (2012). Extraction, identification, and characterization of bioactive molecules from the seed and oil of Silybum marianum: Study of their antioxidant and antitumor activities (Doctoral dissertation, University of Lorraine & University of Carthage).

Ben Youcef, F. Z., & Feritas, H. (2018). Phytochemical approach and biological activities of the medicinal plant Silybum marianum (Master's thesis, University of Frères Mentouri Constantine).

Benchaachoua, A. (2019). Characterization and Valorization of a plant from the Asteraceae family in the Sidi Bel Abbès region: Evaluation of the bioactive substances of Silybum marianum (Doctoral dissertation).

Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J.and Bernardini, A. (2008). Antimicrobial activity and chemical composition of Origanum glandulosum Desf. Essential oil and extract obtained by microwave extraction: comparison with hydrodistillation. Food Chemistry, 106(1):132-139.

Benjilali, B. (2004). Extraction of aromatic and medicinal plants: Particular case of steam distillation and its equipment. In Practical manual. Essential oils: From the plant to commercialization (pp. 17–59).

Benzie, I. F. F., & Strain, J. J., (1996). The ferric reduction ability of Plazma (FRAP) as a measure of « antioxidant power »: the FRAP assay. Analytical Biochemistry, 239(1), 70-76.

Bezanger, BJ., Pinkas, M0., Torck, M., Trot, F. (1980). Medicinal plants of temperate regions. Edition. Vlaloine S.A. Paris: 416 p.

Biedermann, D., Vavříková, E., Cvak, L., & Křen, V. (2014). Chemistry of silybin. Natural Product Reports, 31(9), 1138–1157.

Boulanouar, B., Abdelaziz, G., Aazza, S., Gago, C., & Miguel, M. G. (2013). Antioxidant activities of eight Algerian plant extracts and two essential oils. Industrial Crops and Products, 46, 85–96.

Boutekedjiret, C., Bentahar, F., Belabbes, R., & Bessière, J. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour and Fragrance Journal, 18, 481–484.

Brud, W. (2020). Industrial uses of essential oils. In Handbook of essential oils (pp. 1029–1040). CRC Press.

Bruneton, J. (1999). Pharmacognosie : Phytochimie, Plantes médicinales. 3ème édition.Tec & Doc Lavoisier. 1120.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology, 94(3), 223–253.

Carballo, J. L., Hernández-Inda, Z. L., Pérez, P., & García-Grávalos, M. D. (2002). A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnology, 2, Article 17.

Chang, T. S. (2009). An updated review of tyrosinase inhibitors. International Journal of Molecular Sciences, 10(6), 2440–2475.

Charbonnaud, J. (2007, August). Existing control methods against milk thistle. Oléotech, (3), [page numbers if available], Paris.

Chikhoune, A., Benyelles, N. G., Madani, K., & Dairi, S. (2024). Bioactive potential of milk thistle (Silybum marianum) seeds and applicability of its edible oil in food processing. Processes, 12(1), 2.

Clinical and Laboratory Standards Institute (CLSI). (2021). Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100.

Corchete, P. (2008). Silybum marianum (L.) Gaertn.: The source of silymarin. Fitoterapia, 79(5), 327–347.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. Science, 284(5418), 1318–1322.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.

Dabbour, I. R., Heurtel, B., & Lecomte, J. (2014). Milk thistle oil: Cosmetic potential and antioxidant activity. Industrial Crops and Products, 52, 33–41.

Deep, G., & Agarwal, R. (2007). Chemopreventive efficacy of silymarin in skin and prostate cancer. Integrative Cancer Therapies, 6(2), 130–145.

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology, 85(6), 1629–1642.

Dr.Jean clande laproz; Dr Alain Crillon, Plantes médicinales phytothérapie clinique intégrative et médecine endobiogénique , édition Brigitte Peyrot, page 256-257,(2012).

Duke, J. A. (2002). Handbook of Medicinal Herbs (2nd ed.). CRC Press, Boca Raton, Florida.

El-Mallah, M. H., El-Shami, S. M., & Hassanein, M. M. (2003). Detailed studies on some lipids of Silybum marianum (L.) seed oil. Grasas y Aceites, 54(4), 397–402.

Elwekeel, A., Elfishawy, A., & AbouZid, S. (2013). Chemical composition and biological evaluation of Milk thistle seed oil. Journal of Food Science, 78(11), C1695–C1701.

European Commission. (2006). Commission Recommendation on the efficacy of sunscreen products and the claims made relating thereto (2006/647/EC). Official Journal of the European Union.

Fan, Q., Zhang, Y., Yang, H., Wu, Q., Shi, C., Zhang, C., Xia, X., & Wang, X. (2018). Effect of Coenzyme Q0 on biofilm formation and attachment-invasion efficiency of Listeria monocytogenes. *Food Control*, *90*, 274-281.

Federico, A., Dallio, M., & Loguercio, C. (2017). Silymarin/silybin and chronic liver disease: A marriage of many years. Molecules, 22(2), 191.

Ferhat, M., Tigrine-Kordjani, N., Chemat, S., Meklati, B. and Chemat, F. (2007). Rapid extraction of volatile compounds using a new simultaneous microwave distillation: solvent extraction device. Chromatographia, 65(3-4):217-222.

Flamini, G., Tebano, M., Cioni, P., Ceccarini, L., Ricci, A. and Longo, I. (2007). Comparison between the conventional method of extraction of essential oil of Laurus nobilis L. and a novel method which uses microwaves applied in situ, without resorting to an oven. Journal of Chromatography A, 1143:36-40.

Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. Nature Reviews Microbiology, 8(9), 623–633.

Flora, K., Hahn, M., Rosen, H., & Benner, K. (1998) .Milk thistle (Silybum marianum) for the therapy of liver disease. The American Journal of Gastroenterology, 93(2), 139–143.

Gabay, R., Plitmann, U., Danin, A. (1994). Factors affecting the dominance of Silybum marianum L.(Asteraceae) in its specific habitats. Flora, 189(3), 201-206.

Hadolin, M., Škerget, M., Knez, Ž., & Bauman, D. (2001). High pressure extraction of oil from milk thistle seeds. Food Chemistry, 74(3), 355–364.

Halimi, A. (1997). Directory of Medicinal Plants in Algeria. Algiers, Algeria: IUCN / ANA.

Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall.

Huma, Z., Vian, M., Elmaataoui, M., Chemat, F. (2011). A novel idea in food extraction field: study of vacuum microwave hydrodiffusion technique for by-products extraction. Journal of Food Engineering, 105(2):351-360.

Huseini, H. F., Larijani, B., Heshmat, R., et al. (2006). The efficacy of Silybum marianum in type II diabetes. Phytotherapy Research, 20(12), 1036–1039.

Julian Charbonnaud, Des moyens de lutte existent contre le chardon marie ,Oléotech, N° 3 , Paris, Aout(2007).

Kamal-Eldin, A., & Appelqvist, L. Å. (1996). The chemistry and antioxidant properties of tocopherols. Lipids, 31(7), 671–701.

Karimi, A., Vahabzadeh, M., Lari, J., & Heidari, R. (2011). Milk Thistle (Silybum marianum): A review on its phytochemistry and pharmacological properties. Journal of Medicinal Plants Research, 5(10), 1891–1899.

Karimi, G., Vahabzadeh, M., Lari, P., Rashedinia, M., Moshiri, M. (2011). "Silymarin", a

Karkanis, A., Bilalis, D., & Efthimiadou, A. (2011). Cultivation of milk thistle (Silybum marianum L. Gaertn.), a medicinal weed. Industrial Crops and Products, 34(1), 825–830.

Kasrati, A., Alaoui Jamali, C., Bekkouche, K., Spooner-Hart, R., Leach, D., & Abbad, A. (2015). Chemical characterization and insecticidal properties of essential oils from different wild populations of Mentha suaveolens subsp. timija (Briq.) Harley from Morocco. Chemistry & Biodiversity, 12, 823–831.

Katiyar, S. K. (2005). Silymarin and skin cancer prevention: Anti-inflammatory, antioxidant and immunomodulatory effects. International Journal of Oncology, 26(1), 169–176.

Kaur, C. D., & Saraf, S. (2010). In vitro sun protection factor determination of herbal oils used in cosmetics. Pharmacognosy Research, 2(1), 22–25.

Kim, Y. J., & Uyama, H. (2005). Tyrosinase inhibitors from natural and synthetic sources: Structure, inhibition mechanism and perspective for the future. Cellular and Molecular Life Sciences, 62(15), 1707–1723.

Křen, V., & Walterová, D. (2005). Silybin and silymarin – new effects and applications. Biomed Pap Med Fac Univ Palacky Olomouc, 149(1), 29–41.

Křen, V., & Walterová, D. (2005). Silybin and silymarin – New effects and applications. Biomedicine & Pharmacotherapy, 59(3), 137–145.

Kroll, D. J., Shaw, H. S., & Oberlies, N. H. (2007). Milk thistle nomenclature: Why it matters in cancer research and pharmacokinetic studies. Integrative Cancer Therapies, 6(2), 110–119.

Lani, N. S., et al. (2017). Synthesis, characterization and performance of silica impregnated

calcium oxide as heterogeneous catalyst in biodiesel production. Journal of Cleaner Production, 146, 116–124.

Laproz, J.-C., & Crillon, A. (2012). Medicinal plants: Integrative clinical phytotherapy and endobiogenic medicine (pp. 256–257). Brigitte Peyrot Edition.

Lawrence, B. M. (2009). A preliminary report on the world production of some selected essential oils and countries. Perfumer & Flavorist, 34, 38–44.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118–126.

Lucchesi, M.-E. (2005). Solvent-free microwave-assisted extraction: Design and application to the extraction of essential oils (Doctoral dissertation, University of Réunion).

Lucchesi, M.E., Chemat, F. and Smadja, J., (2004a). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydrodistillation. Journal of Chromatography A, 1043: 323-327.

Lucchesi, M.E., Chemat, F. and Smadja, J., (2004b). An original solvent free microwave extraction of essential oils from spices. Flavour Fragrance Journal, 19: 134-138.

Lushniak, B. D. (2014). Antibiotic resistance: a public health crisis. *Public Health Reports*, *129*(4), 314-316.

Maaloul, S., Ghzaiel, I., Mahmoudi, M., Mighri, H., Pires, V., Vejux, A., et al. (2024). Characterization of Silybum marianum and Silybum eburneum seed oils: Phytochemical profiles and antioxidant properties supporting important nutritional interests. PLOS ONE, 19(6), e0304021.

Maes, C., Meersmans, J., Lins, L., Bouquillon, S., & Fauconnier, M.-L. (2021). Essential oil-based bioherbicides: Human health risks analysis. International Journal of Molecular Sciences, 22, 9396.

Maignial, L., Pibarot, P., Bonetti, G., Chaintreau, A., & Marion, J. (1992). Simultaneous distillation—extraction under static vacuum: Isolation of volatile compounds at room temperature. Journal of Chromatography A, 606, 87–94.

Mansur, J. S., Breder, M. N. R., Mansur, M. C. A., & Azulay, R. D. (1986). Determination of sun protection factor by spectrophotometry. Anais Brasileiros de Dermatologia, 61(3), 121–124.

Manzoor, M., Anwar, F., Iqbal, T., & Bhanger, M. I. (2007). Physico-chemical characterization of Moringa concanensis seeds and seed oil. Journal of the American Oil Chemists' Society, 84(5), 413–419.

Masuda, T., Yamashita, D., Takeda, Y., & Yonemori, S. (2005). Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica. Food Chemistry, 91(4), 745–750.

Meddeb, W., Rezig, L., Abderrabba, M., Lizard, G., & Mejri, M. (2017). Tunisian milk thistle: An investigation of the chemical composition and the characterization of its cold-pressed seed oils. International Journal of Molecular Sciences, 18(12), 2582.

Mendiola, J. A., Herrero, M., Cifuentes, A., & Ibáñez, E. (2013). Use of compressed fluids for sample preparation: Food applications. Journal of Chromatography A, 1152(1–2), 234–246.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica, 45(5), 31–34.

Meyer-Warnod, B. (1984). Natural essential oils: Extraction processes and application to some major oils. Perfumer & Flavorist, 9, 93–104.

Nguta, J., Mbaria, J., Gakuya, D., Gathumbi, P., Kabasa, J., & Kiama, S. (2012). Evaluation of acute toxicity of crude plant extracts from kenyan biodi-versity using brine shrimp, artemia salina l.(artemiidae).

Nichols, J. A., & Katiyar, S. K. (2010). Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. Archives of Dermatological Research, 302, 71–83.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability. Microbiology and Molecular Biology Reviews, 67(4), 593–656.

Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. Nutrition, 30(2), 134–144.

Orhan, I. E., Tosun, F., & Skalicka-Woźniak, K. (2016). Cholinesterase and tyrosinase inhibitory, and antioxidant potential of randomly selected umbelliferous plant species and the chromatographic profile of Heracleum platytaenium Boiss. and Angelica sylvestris L. var. sylvestris. Journal of the Serbian Chemical Society, 81(4), 357–368.

PA, W. (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved standard. *CLSI document M27-A2*.

Pankey, G. A., & Sabath, L. D. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clinical Infectious Diseases, 38(6), 864–870.

Paolini, J., Barboni, T., Desjobert, J.-M., Djabou, N., Muselli, A., & Costa, J. (2010). Chemical composition, intraspecies variation and seasonal variation in essential oils of Calendula arvensis L. Biochemical Systematics and Ecology, 38, 865–874.

Peng, F., Sheng, L., Liu, B., Tong, H., & Liu, S. (2004). Comparison of different extraction methods: Steam distillation, simultaneous distillation and extraction, and headspace co-distillation, used for the analysis of the volatile components in aged flue-cured tobacco leaves. Journal of Chromatography A, 1040, 1–17.

Pereda, S., Bottini, S., and Brignole, E. (2007). Fundamentals of supercritical fluid technology. In: Martı´nez J (ed) *Supercritical fluid extraction of nutraceuticals and bioactive compounds.* CRC Press, Boca Raton, pp. 2–18.

Polyak, S. J., Morishima, C., Shuhart, M. C., Wang, C. C., Liu, Y., & Lee, D. Y. (2010). Identification of hepatoprotective flavonolignans from Silybum marianum. PLoS ONE, 5(7), e11506.

Professor Dr. Halimi Abdelkader, Medicinal Plants, National Agency for Nature Conservation, International Union for Conservation of Nature, p. 1222 (102-122).

promising pharmacological agent for treatment of diseases. Iran J Basic Med Sci. 14(4): 308-17.

Puah, C.W., Choo, Y.M., Ma Ah, N., and Chuah, C.H. (2005).Supercitical Fluid Extraction of Palm Carotenoids. American Journal of Environmental Sciences, 1:264-269.

Qaiyumi, S. (2007). Macro-and microdilution methods of antimicrobial susceptibility testing. In *Antimicrobial susceptibility testing protocols* (pp. 75-79). Taylor & Francis Boca Raton.

Qavami, N., Naghdi Badi, H., Labbafi, M. R., & Mehrafarin, A. (2013). A review on pharmacological, cultivation and biotechnology aspects of milk thistle (Silybum marianum (L.) Gaertn.). Journal of Medicinal Plants, 12(47), 19–37.

Quezel, P., Santa, S. (1963). New flora of Algeria and desert regions. CNRS Éd 1011 p.

Rahman, A. U. (2007). Studies in Natural Products Chemistry. Elsevier Science Publishers, Amsterdam.

Rajabi, S., Ramazani, A., Hamidi, M., & Naji, T. (2015). Artemia salina as a model organism in toxicity assessment of nanoparticles. *DARU Journal of Pharmaceutical Sciences*, *23*(1), 20.

Ramadan, M. F., Kroh, L. W., & Mörsel, J. T. (2007). Radical scavenging activity of black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.), and niger (Guizotia abyssinica Cass.) crude seed oils and oil fractions. Journal of Agricultural and Food Chemistry, 55(12), 4245–4252.

Rasool, M., Malik, A., Basit Ashraf, M. A., et al. (2021). Therapeutic potential of Silybum marianum (milk thistle): A review of its phytochemistry and pharmacological activities. Phytotherapy Research, 35(7), 3755–3773.

Rizvi S. (ed) *Separation, extraction and concentration processes in the food, beverage and nutraceutical industries.* Woodhead, USA, pp 3–21.

Roche, C. (1991). Milk thistle (Silybum marianum (L.) Gaertn.). PNW-Pacific Northwest Extension Publication, Washington, Oregon, and Idaho State Universities, Cooperative Extension Service (USA).382p.

Saewan, N., & Jimtaisong, A. (2015). Natural products as photoprotection. Journal of Cosmetic Dermatology, 14(1), 47–63.

Said, H. M., & Fulder, S. J. (2011). Herbs and Health: New Perspectives. McGraw-Hill, New York.

Said, O., & Fulder, S. (2011). Herbal Medicine in the Middle East. CRC Press.

Sayre, R. M., Agin, P. P., LeVee, G. J., & Marlowe, E. (1979). A comparison of in vivo and in vitro testing of sunscreening formulas. Photochemistry and Photobiology, 29(3), 559-566.

Schmidt, E. (2020). Production of essential oils. In Handbook of essential oils (pp. 125–160). CRC Press.

Shi, J., Kassama, L., and Kakuda, Y. (2006). Supercritical fluids technology for extraction of bioactive components. In: Shi P (ed) *Functional food ingredients and nutraceuticals: processing technologies.* CRC Press, Boca Raton, pp 5–30.

Sindel, B. M. (1991). A review of the ecology and control of thistles in Australia. Weed Research; (31): 189-201.

Singh, R. P., Tyagi, S. C., & Sharma, D. (2008). Silymarin protects against oxidative stress and lipid peroxidation. Food and Chemical Toxicology, 46(1), 365–370.

Solano, F. (2014). Melanins: Skin pigments and tyrosinase inhibitors. International Journal of Molecular Sciences, 15(10), 17980–18018.

Soto, C., Recoba, R., Barrón, H., Alvarez, C., & Favari, L. (2010). Antioxidant properties of silymarin and its role in oxidative stress protection. Food and Chemical Toxicology, 48(3), 803–806.

Stepanović, S., Vuković, D., Hola, V., et al. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations. APMIS, 115(8), 891–899.

Surai, P. F. (2015). Silymarin as a natural antioxidant: Chemistry and biological effects. Antioxidants, 4(1), 1–24.

Suzuki, S., Suzuki, H., Nishizawa, T., Kaneko, F., Ootani, S., Muraoka, H., Saito, Y., Kobayashi, I., & Hibi, T. (1953). Past rifampicin dosing determines rifabutin resistance of Helicobacter pylori. *Digestion*, *79*(1), 1-4.

Tomaniova, M., Hajslova, J., PavelkaJr, J., Kocourek, V., Holadova, K. and Kl1'mova ,I.(1998). Microwave-assisted solvent extraction: a new method for isolation of polynuclear aromatic hydrocarbons from plants. Journal of Chromatography A,827:21-29.

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science, 79, R1231–R1249.

Ventura, M. G., et al. (2021). Composite catalytic materials based on κ-carrageenan and CaO used on the transesterification of soybean oil for the process of biodiesel obtention. Catalysis Today, 379, 96–104.

Verheugen, G. (2006). Commission recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto. Official Journal of the European Union, 265, 39-43.

Vian, M., Fernandez, X., Visinoni, F., Chemat, F. (2008). Microwave hydrodiffusion and gravity, a new technique for extraction of essential oils. Journal of Chromatography A, 1190(1-2):14-17.

Wayne, P. (2010). Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. *CLSI document M100-S20*.

Weinstein, M. P. (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *(No Title)*.

Young, A. R. (2006). Acute effects of UVR on human eyes and skin. Progress in Biophysics and Molecular Biology, 92(1), 80–85.

Z. Arshad, M. A. Hanif, R. W. K. Qadri, M. Khan, A. Babarinde, G. Omisore, et al., "Role of essential oils in plant diseases protection: a review," International Journal of Chemical and Biochemical Sciences, vol. 6, pp. 11-17, 2014.

Zizovic, I., Stamenic, M., Orlovic, A., and Skala, D. (2007). Supercritical carbon-dioxide extraction of essential oils and mathematical modelling on the micro-scale. In: Berton L (ed) Chemical engineering research trends. Nova Science Publishers, New York.

Annexes

Annexes:

1) Results statistical analysis of biofilm formation:

mg/ml	E. coli			P. aeruginosa			S. aureus			B.subtilis		
80	0,071	0,056	0,061	0,078	0,091	0,088	0,066	0,081	0,075	0,071	0,074	0,079
40	0,076	0,082	0,079	1,862	1,905	1,928	0,086	0,080	0,072	0,110	0,105	0,112
20	0,826	0,835	0,821	1,834	1,912	1,941	0,101	0,095	0,093	0,192	0,106	0,188
10	0,828	0,858	0,719	1,892	1,897	1,715	0,706	0,608	0,648	0,548	0,659	0,516
5	0,839	0,787	0,699	1,842	1,869	1,892	0,908	0,828	0,731	0,702	0,767	0,693
2,5	0,841	0,802	0,818	2,012	1,991	2,006	0,879	0,968	0,888	0,785	0,839	0,647
co Neg	0,074	0,071	0,059	0,081	0,084	0,089	0,074	0,095	0,071	0,063	0,065	0,070
co Pos	0,941	0,801	0,786	2,104	2,192	2,318	0,989	0,914	0,868	1,002	1,061	1,004

Concentration (mg/ml)	E. coli Mean (%)	E. coli SD	P. aeruginosa Mean (%)	P. aeruginosa SD	S. aureus Mean (%)	S. aureus SD	B. subtilis Mean (%)	B. subtilis SD
80	92.5	0.9	96.1	0.7	92	1.1	92.7	0.8
40	90.6	1.4	13.9	1.2	91.4	1.3	89.3	1
20	1.9	0.8	14	1.5	89.6	1.6	84.1	1.9
10	4.9	2.1	16.8	1.7	29.2	3.4	43.8	3.1
5	8.1	2.6	15.3	1.4	11	2.8	29.4	2.7
2.5	2.7	1.2	9.2	1.6	1.3	0.9	25.9	2.9

2) Results statistical analysis of Tyrosinase inhibition:

Inhibitory capacity	IC_{50} (%)
Seed oil of the plant Silybum marianum	16.8 ± 0.14
Sunscreen	45 ± 0.42

Biofilm: Photos of experiments and relevant protocols

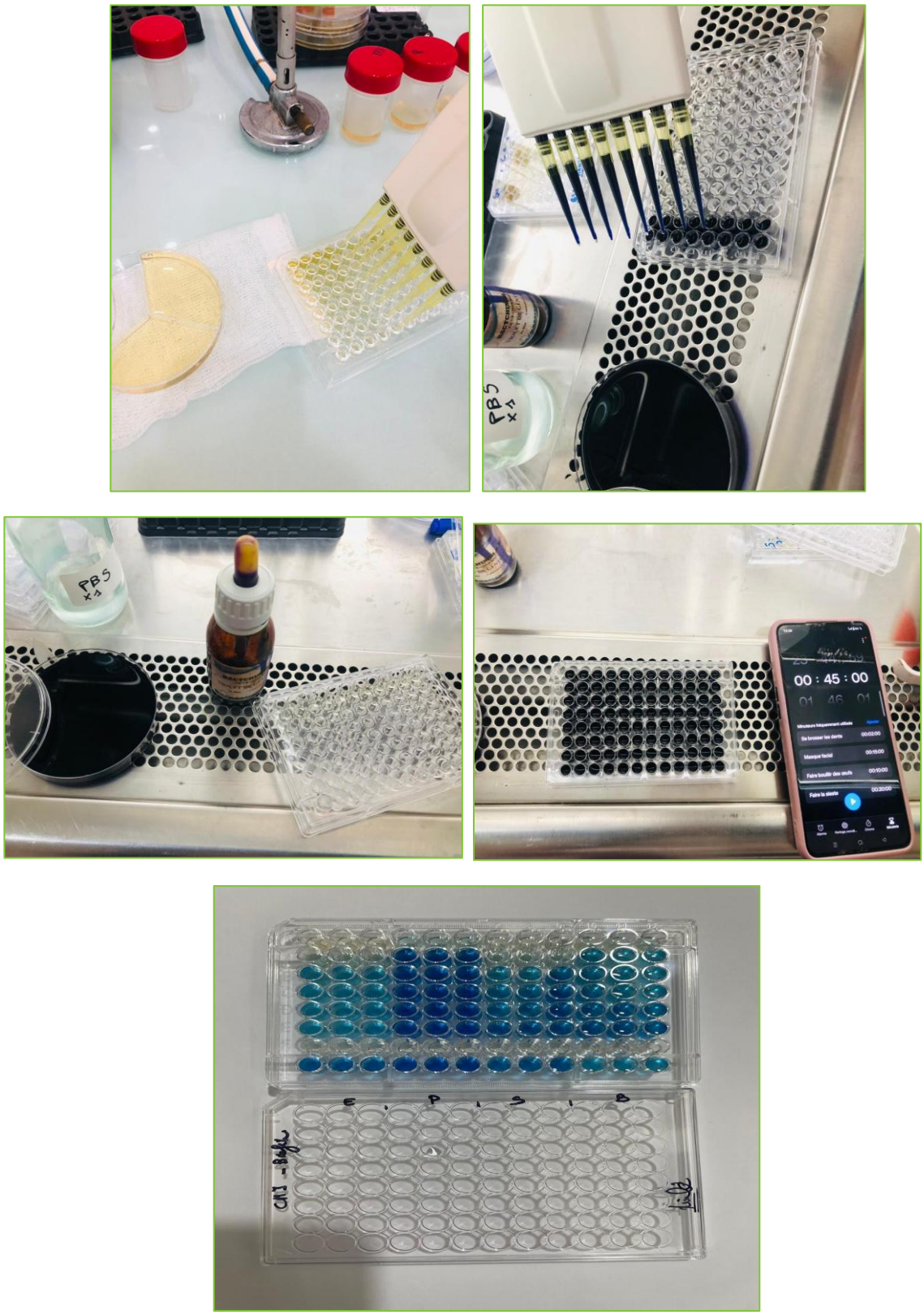


Figure A. 1: Experimental steps used to evaluate the effect of *Milk thistle seed oil* on biofilm formation.

MBC and MIC: Photos of experiments and relevant protocols

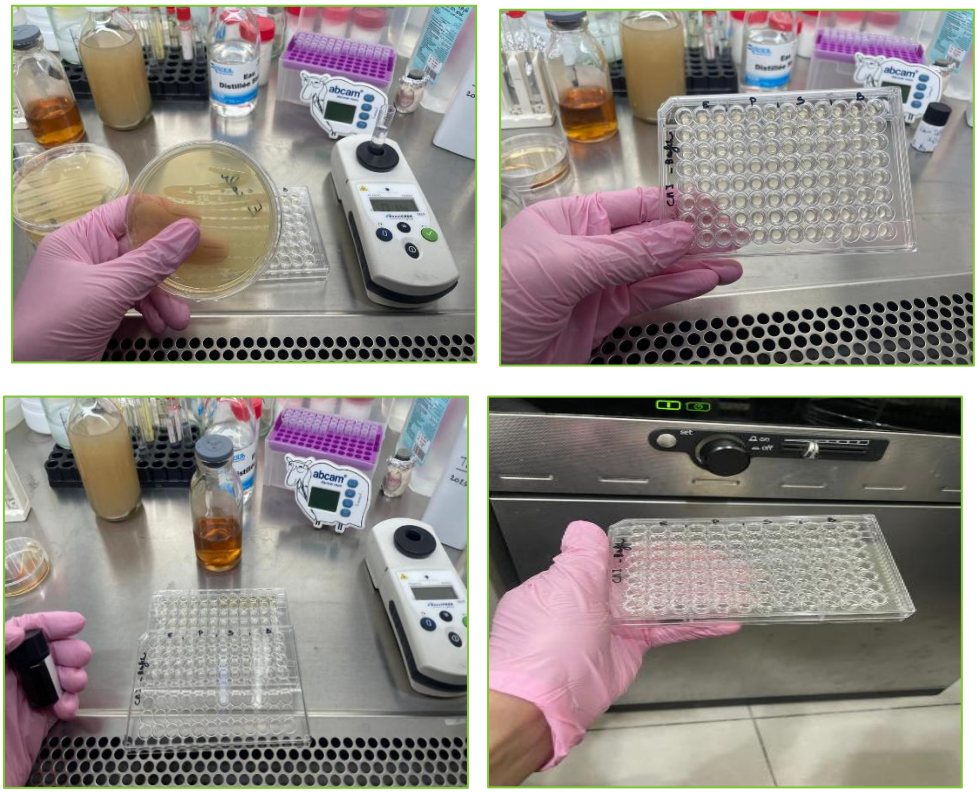


Figure A. 2: Broth microdilution method to determine the antimicrobial activity (MIC/MBC) of *Milk thistle seed oil*.

Figure A. 3: The *Milk thistle seed oil* (*Silybum marianum*) extracted in this experiment.

Figure A. 4: Image of the product studied in this research.