People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Echahid Hamma Lakhdar University of El-Oued

Faculty of Natural and Life Sciences
Department of Cellular and Molecular Biology

Master's Thesis
In order to obtain a diploma of an Academic Master
In biological sciences
Specialty: Applied Biochemistry

Study and Application of *Moringa oleifera*: Development of a Seed Oil Extract and Evaluation of Its Effectiveness in Skin Health

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2024/2025

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DEDICACES

To the greatest man in the world, for me, Lakhder, my dear father

To the most beautiful mother, my heart, Fatma

To my dear grandmother, Fatima

To my dear brothers — Aissa, Yasser, and Aymen

To Zahra, my sister and my greatest example in life

To my supervisor, Prof. A. ZAATER, and my co-supervisor, Dr. Y. BOURAS

I dedicate this humble work.

KAWTHER

Acknowledgments

First and foremost, I would like to thank God for giving me the strength, perseverance, and guidance throughout this journey. This thesis represents the culmination of several years of hard work, challenges, and growth. During this time, I have gained valuable knowledge and experience in my field, which have shaped both my academic and personal development.

I would like to express my deepest gratitude to my supervisors, ***Dr. Zaater Abdelmalek*** *and* ***Dr. Bouras Yassine****, for their continuous support, insightful guidance, and encouragement throughout the course of this research. Their expertise and constructive feedback were instrumental in shaping this work.*

*I would also like to sincerely thank the members of the jury****, Dr. Lanez Elhafnaoui*** *and* ***Dr. Laiche Touhami****, for accepting to evaluate my work and for their valuable comments and suggestions.*

Throughout this thesis, I have had the privilege of collaborating with and receiving support from many people. I would like to extend my heartfelt thanks to my sister, ***Dr. Hadjaidji Fatma Zahra****, whose unconditional help, support, and presence have been a great source of motivation and comfort during the most demanding moments.*

Finally, I dedicate special thanks to my family -my parents and siblings- for their unwavering support, love, and encouragement. Their sacrifices, prayers, and belief in me have made this achievement possible.

To all who contributed in any way to this journey, please accept my sincere appreciation.

ملـــَـــخـص

تهـدف هـذه الدراسـة إلـى تقيـيم الفعاليـة البيولوجيـة و العلاجيـة لزيـت مسـتخلص مـن بـذور المورينغـا، مـع التركيـز علـى دوره فـي تسـريع التئـام الجـروح الجلديـة عنـد التطبيـق الموضـعي. وقـد جـاء اختيـار هـذه النبتـة نظـرا لغناهـا بالمركبـات النشـطة بيولوجيـا مثـل الفلافونويـدات، الفينـولات، و الأحمـاض الدهنيـة، إلـى جانـب استخدامها الواسع في الطب التقليدي كمضاد للميكروبات،الالتهابات و معزز لتجديد الأنسجة.

و لتحقيـق هـذا الهـدف تـم تنفيـذ سلسـلة مـن الاختبـارات البيوكيميائيـة و البيولوجيـة شـملت تجـارب داخل المختبر(in vitro) و على نموذج حيواني حي (in vivo).

فـي البدايـة، تـم اسـتخراج الزيـت مـن بـذور المورينغـا بطريقـة العصـر علـى البـارد تمـت بآلـة عصـر مخصصـة و ذلـك للحفـاظ علـى المركبـات النشـطة فـي الزيـت، و مـن ثـم تـم تحديـد الخـواص الفيزيائيـة التـي أعطت مردودا وفير قدر ب 38.97% و قيمة pH مناسبة (4.11).

قيمـت القـدرة المضـادة للاكسـدة باسـتخدام اختبـار FRAP(القـدرة المختزلـة للحديـديك) ، و قـد أظهـرت النتـائج القـدرة و الفعاليـة العاليـة للزيـت فـي اختـزال ايونـات الحديـد بالمقارنـة مـع مضـاد اكسـدة قياسـي (حمـض الاسـكوربيك)، و اثبـت ذلـك التحليـل الاحصـائي اعتمـادا علـى قيمـة EC50 حيـث : EC50EXT:0.873+_0.030 و EC50AS:0.5243+_1.10, ممايشـر إلـى القـدرة الجيـدة للزيـت على مكافحة الجذور الحرة.

كمـا تـم تقيـيم الفعاليـة الواقيـة مـن الأشـعة فـوق البنفسـجية مـن خـلال تحديـد معامـل الحمايـة مـن الشـمس SPF حيـث سـجل الزيـت قيمـة 73.25 و هـي نتيجـة معتبـرة تظهـر إمكانيـة اسـتخدامه كعامـل واقـي طبيعي.

امـا علـى المسـتوى الميكروبـي، فقـد أجـري تقيـيم للنشـاط المضـاد للبكتيريـا مـن خـلال تحديـد التركيـز المثـبط الأدنـى MIC و التركيـز القاتـل الأدنـى MBC ضـد سـلالات بكتيريـة مختـارة (Gram+ and Gram-) تـراوح بـين 25% بالنسـبة P.aeruginosa , E.coli، B.subtilis، و 50% بالنسـبة ل P.aureus. بينـت هـذه النتـائج قـدرة الزيـت الفعالـة فـي التثبيـت ممـا يشـير إلـى خصائصـه المضـادة للميكروبات.

كمـا تـم إجـراء اختبـار تثبـيط تكـوين الأغشـية الحيويـة biofilm inhibition و تثبـيط انـزيم البروتيـاز الـذي يعـد مؤشـرا علـى تقليـل الالتهابـات و منـع تـدمير مكونـات الأنسـجة كـالكولاجين و الايلاسـتين، و خاصة في سياق التئام الجروح.

امـا الجانـب التطبيقـي للدراسـة، فقـد اسـتخدم زيـت بـذور المورينغـا موضـعيا علـى نمـوذج جـروح جلديـة مسـتحدثة عنـد الجـرذان، إذ قسـمت الحيوانـات إلـى مجموعـات: مجموعـة ضـابطة، مجموعـة عولجـت بمـرهم تجـاري لالتئـام الجـروح (mebo) و مجموعـة عولجـت بزيـت بـذور المورينغـا، حيـث طبـق العـلاج كـل يـومين لمـدة 20 يـوم. كشـفت النتـائج ان المجموعـة المعالجـة بالزيـت حققـت وتيـرة اسـرع بـإغلاق الجـرح بحلـول اليـوم 12 مقارنـة بالمعالجـة بـالمرهم الـذي تـم بحلـول اليـوم 15 و الشـاهدة بعـد اليـوم 20. كـم اثبـت ذلـك بدراسـة احصـائية تمـت بتحليـل تبـاين ثنـائي الاتجـاه ANOVA، و أظهـر عـن وجـود فـروق معنويـة دالـة($p<0.05$)لصـالح مجموعـة الزيـت مـن حيـث تسـارع انكمـاش الجـرح، اعـادة التظهيـر و تحسـن عمليـة اعادة التغطية بمرور الوقت.

تؤكـد النتـائج تمتـع زيـت بـذور المورينغـا بخصـائص بيولوجيـة متعـددة تبـرز امكانيتـه الواعـدة كمكـون طبيعـي فعـال فـي مجـال التئـام الجـروح و الـدمج فـي تطـوير مستحضـرات علاجيـة و تجميليـة بصـفة طبيعية.

كلمـات مفتاحيـة : زيـت مسـتخلص بـذور المورينجـا أوليفيـرا، النشـاط المضـاد للأكسـدة، اختبـار FRAP، النشـاط المضـاد للبكتيريـا، تثبـيط تكـوّن الأغشـية الحيويـة، التئـام الجـروح، عامـل الوقايـة مـن الشـمس (SPF).

Résumé

Cette étude vise à évaluer l'efficacité biologique et thérapeutique de l'huile extraite des graines de Moringa oleifera, en mettant particulièrement l'accent sur son rôle dans l'accélération de la cicatrisation des plaies lorsqu'elle est appliquée localement. Le choix de cette plante a été motivé par sa richesse en composés biologiquement actifs tels que les flavonoïdes, les composés phénoliques et les acides gras, ainsi que par son usage répandu en médecine traditionnelle comme agent antimicrobien, anti-inflammatoire et régénérant des tissus.

Pour atteindre cet objectif, une série de tests biochimiques et biologiques ont été réalisés, incluant des expériences in vitro et in vivo.

L'huile a été initialement extraite des graines de moringa par pression à froid, à l'aide d'une presse mécanique spécialisée permettant de préserver les constituants actifs. Le rendement en huile a été significatif, atteignant 38,97 %, avec une valeur de pH appropriée de 4,11.

La capacité antioxydante a été évaluée à l'aide du test FRAP (Ferric Reducing Antioxidant Power). Les résultats ont montré une efficacité élevée dans la réduction des ions ferriques, comparable à celle d'un antioxydant standard (acide ascorbique), comme le confirme l'analyse statistique basée sur les valeurs de CE_{50} : CE_{50} (extrait) : 0,873 ± 0,030 contre CE_{50} (acide ascorbique) : 0,5243 ± 1,10, indiquant un fort potentiel de piégeage des radicaux libres.

L'effet photoprotecteur de l'huile a également été évalué par la détermination du Facteur de Protection Solaire (FPS), qui a donné une valeur de 73,25, soulignant son potentiel comme agent photoprotecteur naturel.

Au niveau microbiologique, l'activité antibactérienne a été étudiée par la détermination de la Concentration Minimale Inhibitrice (CMI) et de la Concentration Bactéricide Minimale (CBM) contre des souches bactériennes sélectionnées (Gram+ et Gram-). Les valeurs de CMI variaient de 25 % pour E. coli, P. aeruginosa et B. subtilis à 50 % pour P. aureus, confirmant les propriétés antimicrobiennes de l'huile.

De plus, l'huile a démontré une capacité à inhiber la formation de biofilms et l'activité enzymatique des protéases, deux facteurs cruciaux pour réduire l'inflammation et protéger les protéines structurelles telles que le collagène et l'élastine, ce qui est particulièrement pertinent dans les processus de cicatrisation.

Lors de la phase d'application in vivo, l'huile a été appliquée localement sur des plaies cutanées induites expérimentalement chez des rats. Les animaux ont été répartis en trois groupes : un groupe témoin, un groupe traité avec une pommade cicatrisante commerciale (Mebo) et un groupe traité avec l'huile de graines de moringa. Les traitements ont été administrés un jour sur deux pendant une période totale de 20 jours.

Les résultats ont révélé que le groupe traité avec l'huile de moringa a obtenu une fermeture plus rapide des plaies dès le 12e jour, contre le 15e jour pour le groupe traité avec Mebo et après le 20e jour pour le groupe témoin. Ces résultats ont été validés statistiquement

par une ANOVA à deux facteurs, qui a montré des différences significatives ($p < 0,05$) en faveur du groupe traité à l'huile de moringa en ce qui concerne la contraction des plaies, la réépithélialisation et l'amélioration du remodelage tissulaire au fil du temps.

En conclusion, les résultats confirment que l'huile de graines de moringa possède de multiples propriétés biologiques, faisant d'elle un agent naturel prometteur pour les applications de cicatrisation des plaies et un candidat potentiel pour le développement de formulations thérapeutiques et cosmétiques.

Mots clés : Huile extraite des graines de *Moringa oleifera*, Activité antioxydante, Test FRAP, Activité antibactérienne, Inhibition des biofilms, Cicatrisation des plaies, Facteur de protection solaire (SPF).

Abstract

This study aims to evaluate the biological and therapeutic efficacy of oil extracted from Moringa oleifera seeds, with a specific focus on its role in accelerating wound healing when applied topically. The selection of this plant was motivated by its richness in biologically active compounds, such as flavonoids, phenolics, and fatty acids, in addition to its widespread use in traditional medicine as an antimicrobial, anti-inflammatory, and tissue-regenerating agent.

To achieve this objective, a series of biochemical and biological tests were conducted, including both in vitro and in vivo experiments.

Initially, the oil was extracted from Moringa seeds by cold pressing, using a specialized mechanical press to preserve the active constituents. The oil yield was considerable, reaching 38.97%, with a suitable pH value of 4.11.

The antioxidant capacity was assessed using the FRAP assay (Ferric Reducing Antioxidant Power). The results demonstrated high efficacy in reducing ferric ions, comparable to the standard antioxidant (ascorbic acid), as confirmed by statistical analysis based on EC_{50} values: EC_{50} (extract): 0.873 ± 0.030 vs. EC_{50} (ascorbic acid): 0.5243 ± 1.10, indicating a strong free radical scavenging potential.

The UV-protective effect of the oil was also evaluated by determining the Sun Protection Factor (SPF), which yielded a value of 73.25, highlighting its potential use as a natural photoprotective agent.

At the microbial level, the antibacterial activity was assessed through the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against selected (Gram+ and Gram-) bacterial strains. The MIC values ranged from 25% for E. coli, P. aeruginosa, and B. subtilis to 50% for P. aureus, confirming the oil's antimicrobial properties.

In addition, the oil demonstrated the ability to inhibit biofilm formation and protease enzyme activity, both of which are crucial factors in reducing inflammation and protecting

structural proteins such as collagen and elastin, especially relevant in wound healing processes.

In the in vivo application phase, the oil was applied topically to experimentally induced skin wounds in rats. The animals were divided into three groups: a control group, a group treated with a commercial wound-healing ointment (Mebo), and a group treated with Moringa seed oil. Treatments were administered every other day for a total of 20 days.

The results revealed that the Moringa oil-treated group achieved faster wound closure by day 12, compared to day 15 for the Mebo-treated group and after day 20 for the control group. These findings were statistically validated through two-way ANOVA, which showed significant differences ($p < 0.05$) in favor of the Moringa oil group regarding wound contraction, re-epithelialization, and improved tissue remodeling over time.

In conclusion, the findings confirm that Moringa seed oil possesses multiple biological properties, making it a promising natural agent for wound healing applications and a potential candidate for use in the development of therapeutic and cosmetic formulations.

Key words: *Moringa oleifera* seed extract oil, Antioxidant activity, FRAP assay, Antibacterial activity, Biofilm inhibition, Wound healing, Sun Protection Factor (SPF).

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First part: Bibliographic synthesis

Second part: Experimental Analysis

Abbreviation list

AO	Antioxidant
SPF	Sun Protection Factor
FRAP	Ferric Reducing Antioxidant Power
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
UV	Ultraviolet
UVB	Ultraviolet B
UVA	Ultraviolet A
ROS	Reactive Oxygen Species
DPPH	2,2-Diphenyl-1-picrylhydrazyl
MMP	Matrix Metalloproteinase
HPLC	High-Performance Liquid Chromatography
FTIR	Fourier-Transform Infrared Spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
TLC	Thin Layer Chromatography
ANOVA	Analysis of Variance
SD	Standard Deviation
SEM	Standard Error of the Mean
CFU	Colony-Forming Units
WHO	World Health Organization
PBS	Phosphate Buffered Saline
rpm	Revolutions per Minute
IC_{50}	Half Maximal Inhibitory Concentration
v/v	Volume per Volume
°C	Degrees Celsius
cm	Centimeter
cm^2	Square Centimeters
mg/mL	Milligrams per Milliliter
g/mL	Grams per Milliliter
OD	Optical Density
min	Minute(s)
h	Hour(s)
g/mol	Gram-molecule
pH	Potential of Hydrogen
QC	Quality Control

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General Introduction

FOR centuries, Medicinal plants have long been recognized for their therapeutic value and continue to be a rich source of bioactive compounds for the development of natural remedies. In recent decades, the global demand for plant-based products has grown significantly, driven by increased awareness of the limitations of synthetic drugs and the need for safer, environmentally friendly alternatives. According to the World Health Organization (WHO), over 80% of the global population still relies on traditional plant-based medicine for primary healthcare. In Algeria, a country rich in biodiversity and ethnobotanical heritage, medicinal plants remain widely used both in traditional and modern therapeutic practices, particularly in rural regions where access to pharmaceutical products may be limited.

Among the most promising medicinal plants that have captured the attention of researchers is Moringa oleifera, commonly referred to as the "miracle tree," due to its diverse bioactive components and its broad spectrum of traditional and modern medicinal, cosmetic, and nutritional applications (Leone et al., 2015; Anwar et al., 2007).

Moringa oleifera belongs to the Moringaceae family and is native to the Indian subcontinent. It is now cultivated widely across Africa, including southern regions of Algeria such as El Oued and Ouargla, owing to its drought-resistant nature and numerous health benefits.

Various parts of the moringa plant contain a wide range of biologically active compounds, including phenolics, flavonoids, tocopherols, carotenoids, and essential vitamins such as A, C, and E. These are accompanied by vital minerals such as zinc and selenium, which collectively confer antioxidant, antimicrobial, and tissue-regenerating properties (Vergara-Jimenez et al., 2017; Mbikay, 2012; Rockwood et al., 2013). Within this context, skin health and wound healing have emerged as areas of growing scientific interest, given their reliance on complex biological processes such as inflammation control, collagen production, and the regeneration of damaged tissue (Choudhary et al., 2013; Aderinola et al., 2020).

Among the most therapeutically significant parts of the moringa plant are its seeds, which contain a high yield of oil (ranging between 30–40%), typically extracted via cold

pressing to preserve its rich profile of compounds, including oleic acid (approximately 76%), phytosterols, phenolics, and tocopherols (Anwar & Bhanger, 2003; Lalas & Tsaknis, 2002). Several studies have demonstrated that moringa seed oil promotes collagen synthesis, inhibits oxidative stress, reduces inflammation, and counters the proliferation of pathogenic organisms (Kooltheat et al., 2014).

Based on this background, the present study aims to evaluate the biological and therapeutic efficacy of moringa seed oil when applied topically, particularly its potential in accelerating the healing process of cutaneous wounds. The plant was selected based on its scientifically supported properties, and the study seeks to explore its potential use in bioactive formulations for skin therapy and care.

To achieve this goal, a structured experimental approach was adopted, consisting of two main phases:

The first phase (in vitro) involved a series of biological assays, including evaluation of antioxidant activity using the FRAP method, antimicrobial potential (MIC and MBC determination), inhibition of biofilm formation, and protease enzyme inhibition—an indicator of tissue degradation suppression, particularly of components like collagen.

The second phase (in vivo) focused on the topical application of moringa seed oil on experimentally induced skin wounds in rats, aiming to assess its effectiveness in enhancing wound contraction, re-epithelialization, and tissue regeneration.

This study was conducted with scientific rigor and supported by reliable academic literature, with the aspiration of obtaining promising results that would validate the efficacy of moringa seed oil as a natural agent in skin wound treatment and its integration into future pharmaceutical and cosmetic formulations.

Thesis Problematic

This study seeks to answer the following key questions:

- ✓ To what extent does Moringa oleifera seed extract contain phenolic and flavonoid compounds?
- ✓ Does the seed extract exhibit antioxidant activity, particularly as measured by the FRAP assay?
- ✓ Does it have antibacterial activity against common pathogens?
- ✓ Does Moringa oleifera seed oil have wound-healing potential?

To address these questions, the thesis is divided into two main parts:

Thesis Structure

1. Theoretical Part – Contains three chapters:

Chapter 1: General background and literature review on Moringa oleifera.

Chapter 2: General study of essential oils, followed by a specific focus on the extraction and characterization of Moringa oleifera seed essential oil.

Chapter 3: Phytochemical profiling and biological evaluation of Moringa oleifera seed extract oil, focusing on its antioxidant and antibacterial activities, focusing on mechanisms and biofilm inhibition.

2. Experimental Part – Contains two chapters:

Chapter 1: Description of the materials, experimental methods, and protocols used.

Chapter 2: Presentation and discussion of results, including antioxidant assays, SPF evaluation, antibacterial tests, and wound-healing experiments.

First part

Bibliographic synthesis

Chapter 1

General overview of *Moringa Oleifera*

1.1. Introduction

THIS section introduces Moringa oleifera as a multipurpose tree known for its medicinal, nutritional, and agricultural value. It will highlight its increasing global importance, especially in regions with limited resources, due to its adaptability and health-promoting properties.

Figure 1.1. Moringa oleifera tree

1.2. Moringa Oleifera

Moringa Oleifera is a perennial tree known for its exceptional medicinal and nutritional properties. It belongs to the Moringaceae family and is scientifically classified as Moringa oleifera. The plant is commonly known by various names including the Drumstick tree, Horseradish tree, and Ben oil tree. It is often referred to as the "Miracle Tree" or "Tree of Life" due to its significant importance and diverse applications (Islam et al. 2021). Moringa is considered one of the most beneficial trees in the world, as virtually all parts of the tree can be utilized for food, medicinal applications, or other beneficial purposes.

In recent years, Moringa oleifera has gained increasing attention due to its high nutritional value and content of bioactive compounds, making it the subject of numerous scientific studies worldwide. The tree is characterized by its ability to grow in diverse climatic conditions and its drought resistance, making it an ideal choice for cultivation in regions with limited resources (Pareek et al. 2023).

1.3. Taxonomic Classification of *Moringa Oleifera*

The following table shows the classification and scientific naming of the Moringa plant.

Table 1.1 Classification and scientific naming of *Moringa oleifera* (Oslon M.E., 1999).

Kingdom	**Plantae**
Sub-kingdom	Vascular plants (Tracheobionta)
Phylum	Spermatophyta (Seed plants)
Sub-phylum	Magnoliophyta (Flowering plants)
Class	Eudicots
Subclass	Rosids
Order	Brassicales
Family	Moringaceae
Genus	*Moringa*
Species	*Moringa oleifera* Lam.

In fact, the genus Moringa comprises 13 known species, but M. oleifera is by far the most widely cultivated and researched due to its ease of propagation, rapid growth, and rich phytochemical content.

1.4. Moringa Species

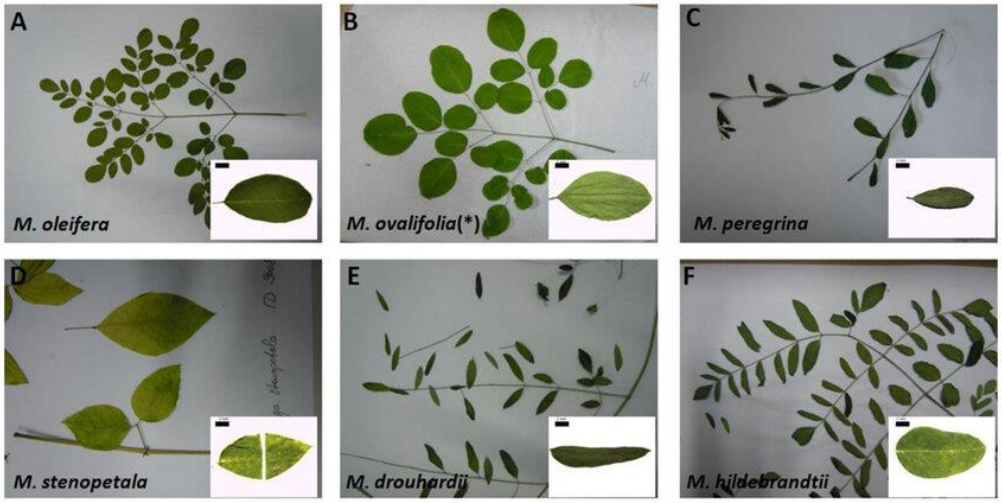


Figure 1.2. Comparison of leaf appearance of Moringa species (Wetters et al., 2024)

There are approximately 13 recognized species in the genus Moringa, most of which are native to Africa and the Indian subcontinent. Some of the notable species include:

- Moringa stenopetala: Common in East Africa; similar in use and composition to M. oleifera.
- Moringa peregrina: Found in the Arabian Peninsula and parts of North Africa.
- Moringa drouhardii: Native to Madagascar.

Despite the diversity of the genus, M. oleifera remains the most economically significant and globally cultivated species.

1.5. Common Names of *Moringa Oleifera*

Moringa oleifera is known by many names worldwide, reflecting its wide distribution and diverse uses. The table below shows the common names of Moringa oleifera. These names often emphasize its healing properties or physical characteristics.

Table 1.2 Common names of Moringa oleifera

Arabic	**English**	**French**	**Hindi**	**Portuguese**
الحبة الغالية	Precious seed	Graine précieuse	Anmol beej	Semente preciosa
شجرة اليسر	Tree of ease	Arbre de facilité	Sukh ki jhaad	Árvore da facilidade
البان الزيتي	Oil tree	Arbre à huile	Tel ka ped	Árvore do óleo
الشجرة المعجزة	Miracle tree	Arbre miracle	Chamatkari ped	Árvore milagrosa
صديقة الفقراء	Poor's friend	Amie des pauvres	Garibon ki saheli	Amiga dos pobres
شجرة عصا الطبل	Drumstick tree	Arbre tambour	Sainjna	Moringa
شجرة فجل الحصان	Horseradish tree	Raifort arbre	Mooly jhaad	Árvore de rabanete
شجرة الروّاق	Purifier tree	Arbre purificateur	Shuddh karne wala ped	Árvore purificadora
شجرة الثوم البري	Wild garlic tree	Arbre à ail sauvage	Jangli lehsun jhaad	Árvore de alho selvagem
شجرة الحياة	Tree of Life	Arbre de vie	Jeevan ka ped	Árvore da vida

1.6. Morphological Description of *Moringa Oleifera*

Moringa oleifera is a small to medium-sized deciduous tree that can grow up to 10–12 meters tall. It has a straight or crooked trunk and a spreading canopy. The leaves are compound, tripinnate, and can grow up to 45 cm long, providing dense foliage. The flowers are fragrant, white or cream-colored, and borne in loose panicles. The fruit is a

hanging pod, typically 30–50 cm long, containing dark brown seeds with three papery wings (Pareek et al. 2023). The bark is gray and corky, while the roots are thick and have a pungent smell reminiscent of horseradish.

1.7. Morphological Description of *Moringa Oleifera*

Moringa oleifera is a fast-growing perennial tree that can reach a height of 7 to 12 meters. It is characterized by an open, umbrella-shaped crown with fragile branches and is evergreen in suitable climatic conditions (Pareek et al., 2023). The tree consists of several distinctive parts:

- Trunk

The trunk is generally straight but can be quite variable in some cases. It reaches a height of 1.5 to 2 meters before branching, and in some cases can reach up to 3 meters. The bark is smooth, with large lenticels, and has a dark gray-purplish color.

Figure 1.3. Moringa Tree Trunk

- Branches

The branches grow irregularly and form an umbrella-like shape. They are characterized by their fragility and ease of breaking, and they bear leaves, flowers, and fruits.

Figure 1.4. Moringa Oleifera Branches

- Leaves

Moringa leaves are compound bipinnate, reaching 30-60 cm in length. They consist of small, oval-shaped leaflets that are green in color and have a smooth texture. The leaflets are arranged oppositely on the main axis of the leaf. The leaves contain a high percentage of proteins, vitamins, and minerals, making them of high nutritional value.

Figure 1.5. Moringa oleifera compound leaves

- Flowers

Moringa flowers are white or cream-colored, fragrant, and appear in clusters. They consist of five unequal petals, five fertile stamens, and five sterile stamens. The flowers bloom throughout the year in tropical regions, while they bloom seasonally in other regions.

Figure 1.6. Moringa oleifera flower

- Fruits (Pods)

Moringa fruits are long, triangular pods, ranging from 20-45 cm in length. They are green when immature and turn brown when ripe. The pods contain seeds arranged in rows inside the pod.

Figure 1.7. Moringa oleifera pods

- Seeds

Moringa seeds are round or triangular in shape, surrounded by thin white wings. The seed diameter is about 1 cm. The seeds contain a high percentage of oils (up to 40%), which is a non-volatile oil known as Ben oil, used in the manufacture of cosmetics and soaps.

Figure 1.8. Moringa seeds

- Roots

Moringa roots are strong and taproot, extending deep into the soil, which helps the tree withstand drought.

Figure 1.9. Moringa roots

The roots contain bioactive compounds and are used in some cultures as a substitute for horseradish due to their pungent taste.

1.8. Origin and Geographical Distribution of *Moringa Oleifera*

Moringa oleifera is native to northeastern India, specifically the Himalayan region. Over time, the cultivation of this tree has spread to various parts of the world, especially in tropical and subtropical regions. In the early 20th century, its cultivation began in Africa through trade and commercial exchange.

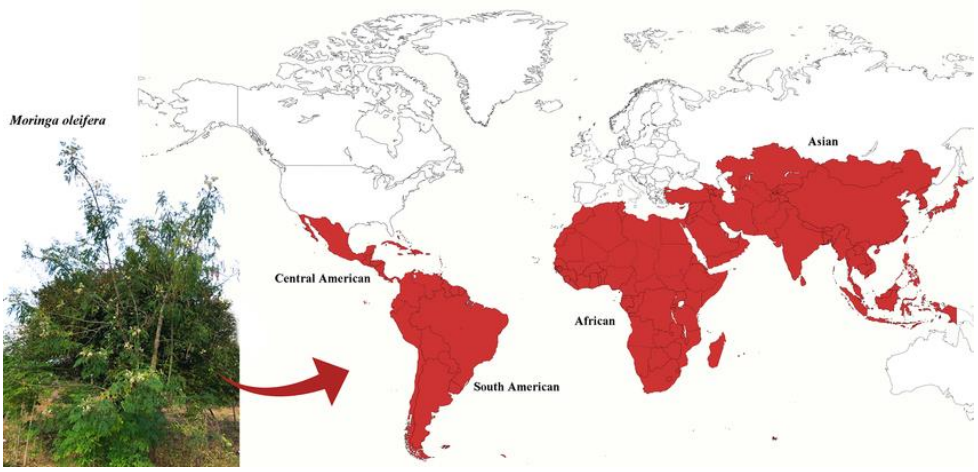


Figure 1.10. Geographic distribution of Moringa oleifera (Bezerra et al., 2023)

Currently, Moringa oleifera can be found in more than fifty countries in tropical and subtropical regions, including: - South and Southeast Asia: India, Pakistan, Bangladesh, Afghanistan, Sri Lanka - Pacific Islands - South and Central America: Brazil, Mexico, Paraguay - Caribbean region - Africa: Sudan, Ethiopia, Kenya, Madagascar, Nigeria, Tanzania - Middle East: Egypt, Saudi Arabia, UAE, Oman, Yemen, Algeria.

Moringa grows well in areas with high temperatures and low humidity and tolerates a wide range of soil conditions, from acidic to alkaline, but prefers well-drained soil. Its ability to withstand drought has made it an ideal choice for cultivation in arid and semi-arid regions.

1.9. Chemical Constituents

Different parts of the Moringa oleifera plant contain a variety of biologically active chemical compounds, which explains its multiple uses in traditional medicine and nutrition (Islam et al. 2021). These constituents include:

- Leaves

Moringa leaves contain a high percentage of proteins (up to 27% of dry weight), fiber,

minerals (calcium, potassium, magnesium, iron, zinc), vitamins (A, B, C, E), antioxidants such as flavonoids and phenols, and essential amino acids.

- Seeds

Moringa seeds contain 35-40% oils, which is a non-volatile oil rich in oleic acid (constituting about 70% of the fatty acid content). They also contain proteins and peptides that have antimicrobial and water-coagulating properties.

- Roots

Moringa roots contain glucosinolates and isothiocyanates that give them a pungent taste similar to horseradish. They also contain alkaloid compounds such as moringine and moringinine.

- Bark

Moringa bark contains alkaloid compounds, tannins, and gum.

- Flowers

Moringa flowers contain flavonoids, carotenoids, and amino acids.

- Pods

Moringa pods contain vitamin C, minerals, and dietary fiber.

These diverse chemical constituents give Moringa oleifera its antioxidant, anti-inflammatory, antimicrobial, blood sugar-lowering, cholesterol-lowering, and other therapeutic properties.

1.10. Nutritional Value of *Moringa Oleifera*

Moringa oleifera is distinguished by its high nutritional value, especially its leaves, which are considered one of the richest plant sources of nutrients (Islam et al., 2021). Moringa leaves contain:

- Proteins: They contain all eight essential amino acids, at a higher percentage than most other vegetables.
- Vitamins: Rich in vitamin A (10 times more than in carrots), vitamin C (7 times more than in oranges), vitamin B, vitamin E, and vitamin K.
- Minerals: Rich in calcium (17 times more than in milk), potassium (15 times more than in bananas), iron (25 times more than in spinach), magnesium, phosphorus, and zinc.
- Antioxidants: They contain high levels of antioxidants such as quercetin, kaempferol, beta-sitosterol, and zeatin.
- Omega-3 and omega-6 fatty acids.
- Dietary fiber: Helps improve digestion and regulate blood sugar levels.

Moringa seeds are rich in oils (35-40%) that contain a high percentage of oleic acid, a monounsaturated fatty acid beneficial for heart health. Moringa oil is also resistant to rancidity, making it suitable for cooking and long-term storage.

Green (immature) Moringa pods are rich in vitamin C and fiber and are used as vegetables in many cultures.

Due to its high nutritional value, Moringa is used in malnutrition programs in many developing countries and is added to foods to enhance their nutritional value.

1.11. Uses of *Moringa Oleifera*

Moringa oleifera is characterized by its multiple uses in various fields, which has earned it the title of "Miracle Tree." Here are its most important uses:

1.11.1. Medical and Pharmaceutical Applications

Moringa has been used in traditional medicine to treat many diseases and health conditions, and with the advancement of scientific research, many of these uses have been confirmed:

- Anti-inflammatory: Helps reduce various inflammations in the body.
- Antioxidant: Contains high levels of antioxidants that protect cells from oxidative damage.
- Blood sugar-lowering: Helps regulate blood sugar levels, making it beneficial for diabetic patients.
- Cholesterol-lowering: Helps lower harmful cholesterol levels (LDL) and improve beneficial cholesterol levels (HDL).
- Antimicrobial: Has antibacterial, antifungal, and antiviral properties.
- Immune-boosting: Enhances the immune system due to its high content of vitamins and minerals.
- Diuretic: Helps increase urine production, which helps cleanse the kidneys and reduce fluid retention.
- Anti-cancer: Some studies have shown that Moringa extracts may have anti-cancer properties.
- Treatment of skin diseases: Used in the treatment of wounds, burns, rashes, and acne.
- Improving bone health: Due to its high content of calcium and magnesium.
- Improving eye health: Due to its content of vitamin A and beta-carotene.

1.11.2. Nutritional Applications

Different parts of the Moringa tree are used as food in many cultures:

- Leaves: Eaten fresh as a salad, cooked like spinach, or dried and ground as a nutritional supplement added to soups, sauces, juices, and bread.
- Green (immature) pods: Cooked as vegetables and used in soups and curries.
- Seeds: Eaten roasted like nuts or used to extract oil.
- Roots: Used as a substitute for horseradish in some cultures.
- Flowers: Eaten fried or boiled, or added to salads.

Moringa is also used in malnutrition programs, especially for children and pregnant women, due to its high content of proteins, vitamins, and minerals.

1.11.3. Cosmetic Applications

Moringa seed oil is used in the manufacture of cosmetics and skin and hair care products:

- Hair oils: Help nourish the scalp, strengthen hair, and prevent hair loss.
- Skin creams: Used in the manufacture of moisturizing and nourishing creams for the skin.
- Soap: Used in the manufacture of natural soap.
- Perfumes: Used as a fragrance fixative in the perfume industry.
- Sunscreen products: Help protect the skin from ultraviolet rays.

1.11.4. Water Treatment Applications

Moringa seeds are used in water purification:

- The seeds contain natural proteins that act as coagulants for impurities in water.
- Seed powder can be used to precipitate impurities and bacteria in contaminated water.
- It is considered a natural and safe alternative to chemicals used in water treatment.
- It is used in rural and remote areas where traditional water treatment technologies are unavailable or expensive.

In addition to these uses, Moringa is also used as animal feed, organic fertilizer, a source of biofuel, and in fiber production. It is also planted as windbreaks and to reduce soil erosion.

This diversity of uses makes Moringa a tree of great economic, environmental, and social value, especially in developing regions.

1.11.5. Burns and Wound Healing

Burns represent a significant global health challenge, requiring intensive treatment due to their extensive tissue damage and high risk of infection. Burns are classified into different degrees based on the depth of tissue damage (Żwierełło et al., 2023).

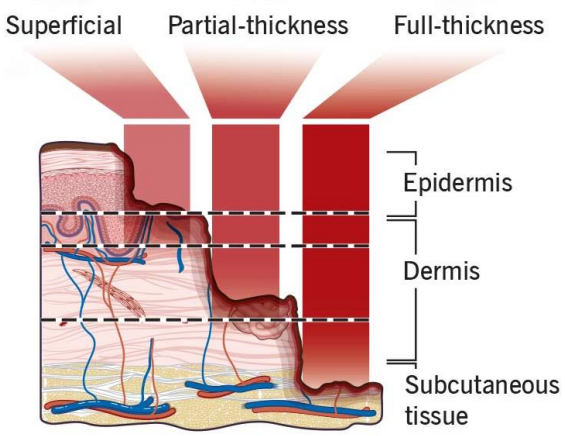


Figure 1.11. Classification of burns by degree, showing first-degree (superficial), second-degree (partial thickness), and third-degree (full thickness) burns (Cleveland Clinic., 2025)

1.11.5.1. Classification of Burns

Burns are classified into four degrees based on the depth of tissue damage:

✓ First-degree burns: These affect only the epidermis, causing erythematous (red) lesions that are painful but without blisters. They typically heal without scarring in 4-5 days.

✓ Second-degree superficial burns: These involve destruction of nearly all the epidermis. Blisters appear, and the wound is red, vascular, and extremely painful. Healing occurs without scarring in 10-14 days.

Second-degree deep burns: These involve destruction of the epidermis, the basal layer, and a significant thickness of the dermis. The floor of the blister is whitish-pink, poorly vascularized, and painful. In the absence of infection, healing is slow (21-35 days) and results in significant scarring.

✓ Third-degree burns: These involve destruction of the entire epidermis, basal membrane, deep dermis, and sometimes the hypodermis. There are no blisters; the lesion is black and white, dry, and cartilaginous. Treatment requires excision of the necrotic tissue followed by grafting.

1.11.5.2. Moringa oleifera in Burns Treatment

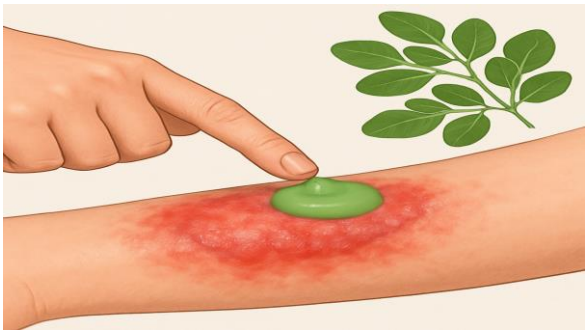


Figure 1.12. Application of Moringa oleifera extract ointment on a second-degree burn.

Recent scientific research has demonstrated the effectiveness of Moringa oleifera in the treatment of burns through several mechanisms:

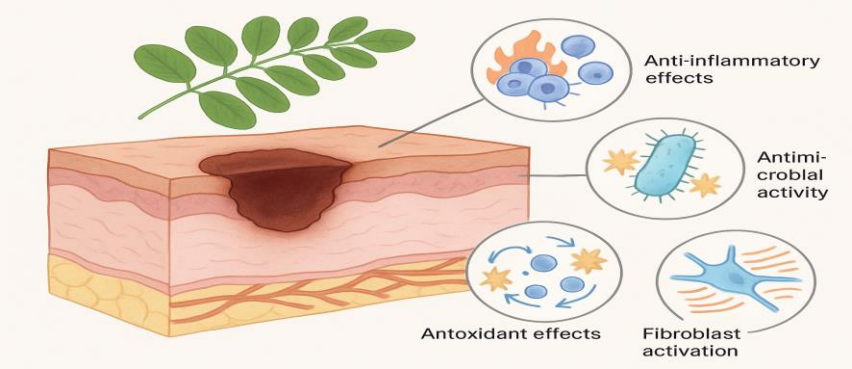


Figure 1.13. Mechanisms of action of Moringa oleifera in burn wound healing, showing anti-inflammatory effects, antimicrobial activity, antioxidant effects, and fibroblast activation.

- **Anti-inflammatory Properties**

In vivo studies have demonstrated that Moringa oleifera extract significantly reduces inflammation in burn wounds. A 2022 study published in Bioscientia Medicina found that Moringa extract decreased the number of polymorphonuclear (PMN) cells in burn tissues of experimental rats. This anti-inflammatory action is crucial during the initial phases of burn healing, as excessive inflammation can delay the healing process and increase scarring.

The anti-inflammatory effect is primarily attributed to bioactive compounds such as flavonoids, phenolic acids, and isothiocyanates present in Moringa leaves. These compounds inhibit pro-inflammatory cytokines and reduce oxidative stress in the wound environment.

- **Fibroblast Activation and Proliferation**

Fibroblasts play a critical role in wound healing by synthesizing collagen and other extracellular matrix components essential for tissue repair. Research has shown that Moringa oleifera extract promotes fibroblast activation and proliferation in burn wounds.

A combination of Moringa extract with standard treatments like silver sulfadiazine has been shown to significantly increase the number of fibroblasts in burn tissues compared to control groups. This synergistic effect accelerates the formation of granulation tissue and subsequent re-epithelialization of the burn wound.

- **Antimicrobial Activity**

Burns are highly susceptible to infection, which can significantly impede the healing process. Moringa oleifera possesses potent antimicrobial properties that help prevent and combat wound infections.

Studies have demonstrated that Moringa leaf extracts exhibit antibacterial activity against common burn wound pathogens, including methicillin-resistant Staphylococcus

aureus (MRSA). A 2022 study published in Pharmaceuticals showed that Moringa oleifera leaf extract effectively promoted healing of MRSA-infected wounds in diabetic rats, highlighting its potential for treating complicated burn infections.

The antimicrobial activity is attributed to various compounds, including alkaloids, tannins, and saponins, which disrupt bacterial cell membranes and inhibit essential bacterial enzymes.

- **Antioxidant Effects**

Burns generate significant oxidative stress, which can damage healthy tissues and delay healing. Moringa oleifera is rich in antioxidants that neutralize free radicals and reduce oxidative damage.

Research has shown that Moringa extract increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in wound tissues. This antioxidant action protects healthy cells from damage and creates an optimal environment for tissue regeneration.

- **Growth Factor Stimulation**

Moringa oleifera extract has been shown to stimulate the expression of key growth factors involved in wound healing. In vitro studies using human keratinocyte cell lines (HaCaT) have demonstrated that Moringa extract increases the expression of vascular endothelial growth factor (VEGF) and transforming growth factor-beta 1 (TGF-β1).

VEGF promotes angiogenesis (formation of new blood vessels), which is essential for delivering oxygen and nutrients to the healing burn wound. TGF-β1 stimulates collagen synthesis and regulates the remodeling phase of wound healing, contributing to improved wound strength and reduced scarring.

1.11.5.3. Clinical Applications and Formulations

Various formulations of Moringa oleifera have been developed and tested for burn treatment:

- **Topical Ointments and Creams**

Moringa leaf extract has been formulated into ointments and creams for direct application to burn wounds. These formulations typically contain 5-10% Moringa extract and have shown promising results in experimental studies.

A study using 10% Moringa ointment demonstrated a wound closure and tissue repair rate of 83.04% ± 0.89% in experimental burn models, significantly higher than control groups. These formulations are particularly effective for first and second-degree burns.

- **Hydrogels**

Novel hydrogel formulations incorporating Moringa oleifera extract have been developed to enhance wound healing. These hydrogels provide a moist environment conducive to

healing while delivering the bioactive compounds from Moringa directly to the wound site.

Recent research reported that hydrogels formulated with Moringa gum with a polyacrylamide base showed significant wound healing activity through antioxidant action, fluid absorption, and mucoadhesion. These hydrogels demonstrated superior fluid absorption, mucoadhesion, and sustained release of bioactive compounds.

- **Combination Therapies**

Research has shown that combining Moringa oleifera extract with conventional burn treatments can enhance healing outcomes. The combination of silver sulfadiazine (the standard topical antimicrobial for burns) with Moringa extract has demonstrated synergistic effects, accelerating burn healing by simultaneously combating infection, reducing inflammation, and promoting tissue regeneration.

1.11.5.4. Advantages Over Conventional Treatments

Compared to conventional burn treatments, Moringa oleifera offers several advantages:

1. Reduced Side Effects: Unlike some synthetic antimicrobials that can cause allergic reactions or delay wound healing, Moringa extract has shown minimal side effects in experimental studies.

2. Multi-target Action: While conventional treatments often target a single aspect of wound healing (e.g., antimicrobial activity), Moringa provides a multi-faceted approach by simultaneously addressing inflammation, infection, oxidative stress, and tissue regeneration.

3. Accessibility and Cost-effectiveness: As a plant-based remedy that grows abundantly in tropical and subtropical regions, Moringa offers a potentially accessible and affordable treatment option, particularly in resource-limited settings where burn incidence is high.

4. Potential for Reducing Scarring: Preliminary evidence suggests that the anti-inflammatory and antioxidant properties of Moringa may contribute to reduced scarring in healed burn wounds, though more research is needed in this area.

1.11.5.5. Future Directions in Burns Treatment

Despite the promising results, several challenges and research gaps remain:

1. Standardization: There is a need for standardized extraction methods and quality control parameters to ensure consistent efficacy of Moringa-based burn treatments.

2. Clinical Trials: Most evidence comes from in vitro and animal studies. Well-designed clinical trials are needed to confirm the efficacy and safety of Moringa for human burn treatment.

3. Optimal Formulations: Further research is needed to determine the optimal formulations, concentrations, and delivery systems for Moringa-based burn treatments.

4. Mechanism Elucidation: While several mechanisms have been identified, a more comprehensive understanding of how Moringa compounds interact with the complex burn healing process is needed.

The growing body of scientific evidence supports the traditional use of Moringa oleifera for burn treatment and highlights its potential as a valuable addition to the burn care arsenal. As research continues to advance, Moringa-based treatments may offer new hope for improved burn healing outcomes, particularly in settings where conventional treatments are limited or inaccessible.

Chapter 2

Essential oils: *Moringa Oleifera* seed extract oil

2.1. Introduction to Essential Oil

ESSENTIAL oils are concentrated hydrophobic liquids containing volatile chemical compounds extracted from plants. These oils are called “essential” because they contain the essence of the plant’s fragrance, the characteristic scent of the plant from which they are derived. Essential oils are obtained through several extraction methods, with steam distillation being the most common. They have been used for thousands of years in various cultures for medicinal and health purposes, including aromatherapy, personal care, and household cleaning products.

The chemical composition of essential oils is complex and varies depending on the plant species, geographical location, climate, soil conditions, cultivation methods, harvesting time, and extraction technique. Essential oils typically contain a mixture of terpenes, terpenoids, phenylpropanoids, and other aromatic and aliphatic compounds. This complex mixture gives each essential oil its unique chemical profile, aroma, and therapeutic properties.

Figure 2.1. Various essential oil bottles with herbs

Essential oils play important ecological roles in plants, including protection against herbivores and pathogens, attraction of pollinators, and communication between plants. For humans, essential oils have become valuable in various industries, including pharmaceuticals, cosmetics, food, and agriculture, due to their diverse biological activities and aromatic properties.

2.2. Essential Oil of *Moringa Oleifera* Seed

Moringa oleifera seed oil is a fixed oil rather than a true essential oil, as it is primarily composed of fatty acids rather than volatile compounds. However, the seed oil does contain a small fraction of volatile compounds that contribute to its aroma and some of its biological properties (Leone et al., 2016). The oil is extracted from the seeds of the Moringa oleifera tree, which are known for their high oil content (approximately 35-40% by weight).

The fatty acid composition of Moringa oleifera seed oil is characterized by a high percentage of oleic acid (C18:1), a monounsaturated fatty acid, which typically constitutes 65-75% of the total fatty acids. Other fatty acids present in significant amounts include:

- Palmitic acid (C16:0): 5-10%
- Stearic acid (C18:0): 4-8%
- Behenic acid (C22:0): 5-8%
- Arachidic acid (C20:0): 2-4%
- Linoleic acid (C18:2): 1-3%

This unique fatty acid profile, particularly the high oleic acid content, contributes to the oil's stability against oxidative rancidity and its suitability for cooking and cosmetic applications.

In addition to fatty acids, Moringa oleifera seed oil contains various bioactive compounds, including:

- **Tocopherols (Vitamin E)**: These natural antioxidants help protect the oil from oxidation and contribute to its stability and shelf life. They also provide antioxidant benefits when the oil is used in cosmetic or pharmaceutical formulations.
- **Sterols**: Plant sterols such as β-sitosterol, stigmasterol, and campesterol are present in Moringa seed oil and contribute to its cholesterol-lowering effects when consumed.
- **Phenolic Compounds**: Various phenolic compounds with antioxidant properties have been identified in Moringa seed oil, although in lower concentrations than in the leaf extracts.
- **Carotenoids**: These pigments contribute to the oil's color and have antioxidant properties.

The volatile fraction of Moringa oleifera seed oil, though small, contains various compounds that contribute to its characteristic aroma. These include aldehydes, ketones, alcohols, and terpenes. Some of the identified volatile compounds include hexanal, 2-hexenal, 2-heptanone, octanal, nonanal, and various sesquiterpenes.

Physically, Moringa oleifera seed oil is a pale yellow to golden liquid at room temperature, with a mild, nutty aroma. It has a relatively high smoke point (approximately 200°C), making it suitable for cooking. The oil is also characterized by its low viscosity and good spreadability, properties that make it valuable in cosmetic formulations.

2.3. Extraction Methods

Several methods are used to extract oil from Moringa oleifera seeds, each with its advantages and limitations:

2.3.1. 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 Moringa 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.

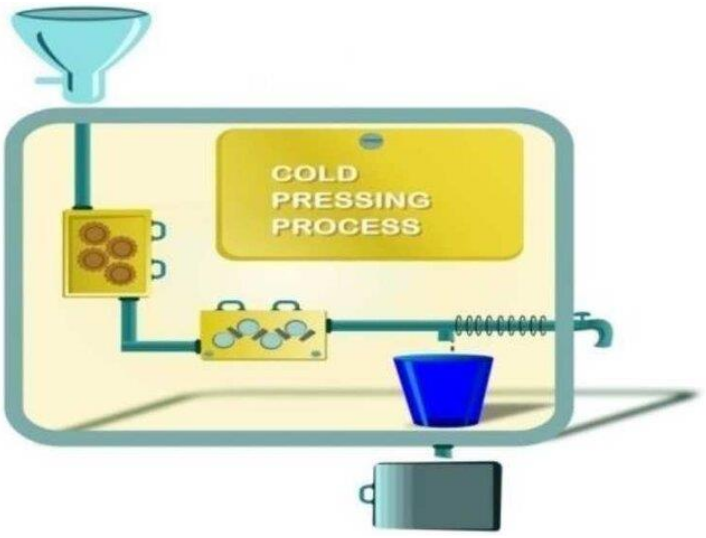


Figure 2.2. Cold pressing extraction method (Geramitcioski et al., 2018)

2.3.2. 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.

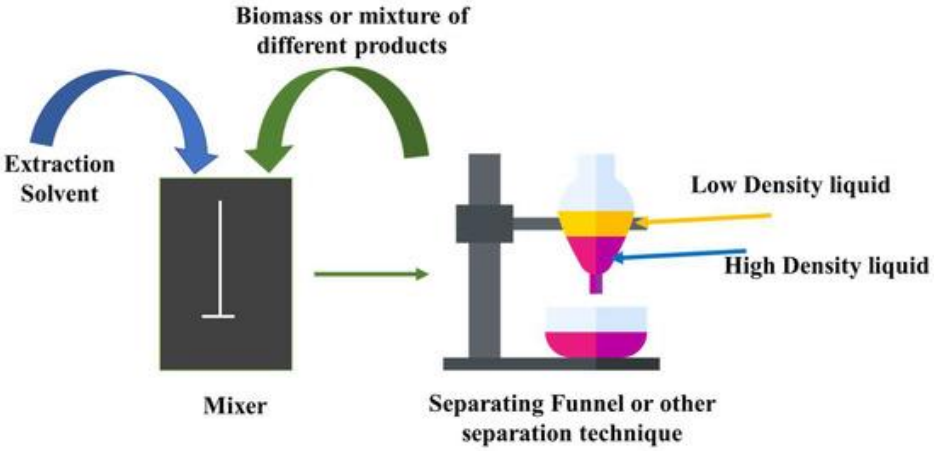


Figure 2.3. Schematic diagram of the solvent extraction method (Usman et al., 2023)

2.3.3. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction, particularly using carbon dioxide (CO_2), is a modern extraction technique that offers several advantages for Moringa 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.

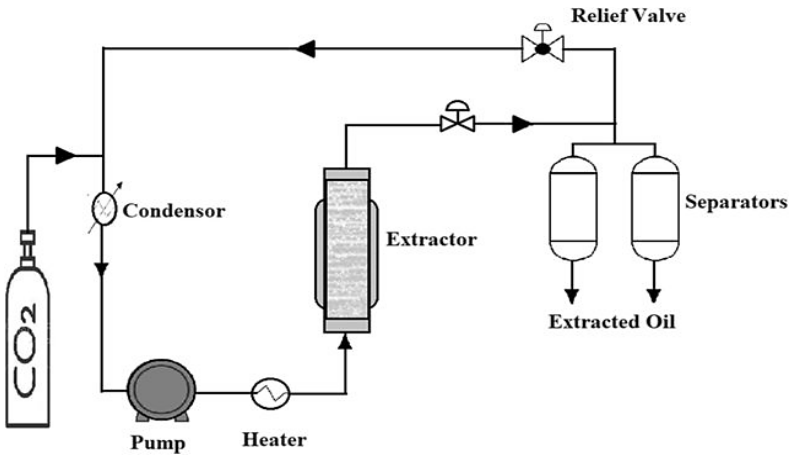


Figure 2.4. Supercritical CO2 extraction method (Gaber et al., 2018)

2.3.4. Aqueous Enzymatic Extraction

Aqueous enzymatic extraction is an eco-friendly alternative to solvent extraction. This method uses water and enzymes to break down the cell walls of the seed material, releasing the oil. The process typically involves:

1. Grinding the seeds and mixing them with water
2. Adding enzymes (such as cellulases, hemicellulases, and proteases) to break down the cell walls
3. Incubating the mixture at the optimal temperature and pH for enzyme activity
4. Separating the oil from the aqueous phase and solid residue
5. Purifying the oil

This method avoids the use of organic solvents, making it environmentally friendly and suitable for food-grade oil production. However, it typically yields less oil than solvent extraction and requires careful control of the extraction conditions.

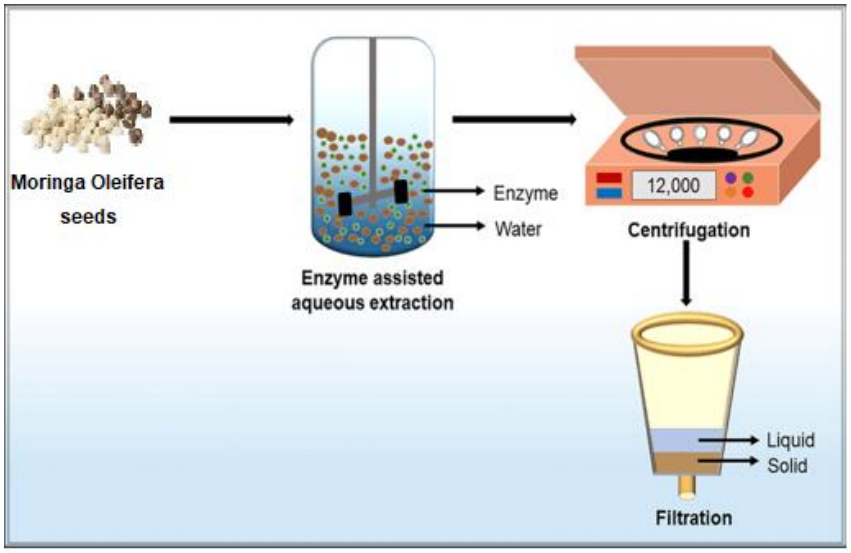


Figure 2.5. Enzymatic Extraction Method

2.4. Biological Activities

Moringa oleifera seed oil exhibits various biological activities that make it valuable for medicinal, cosmetic, and other applications:

2.4.1. Antioxidant Activity

Moringa seed oil contains natural antioxidants, including tocopherols, phenolic compounds, and carotenoids, which can neutralize free radicals and prevent oxidative damage. The antioxidant activity of the oil helps protect it from rancidity and contributes to its potential health benefits when consumed or applied topically (Wang et al., 2017).

Studies have shown that Moringa seed oil can scavenge free radicals, inhibit lipid peroxidation, and protect against oxidative stress-induced damage. The antioxidant activity is often measured using assays such as DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP).

The antioxidant properties of Moringa seed oil make it valuable in anti-aging skincare formulations, as oxidative stress is a major contributor to skin aging. The oil can help protect the skin from environmental stressors such as UV radiation and pollution.

2.4.2. Antimicrobial Activity

Moringa seed oil has demonstrated antimicrobial activity against various bacteria and fungi. This activity is attributed to certain fatty acids and other bioactive compounds present in the oil. Studies have shown that the oil can inhibit the growth of both Gram-positive bacteria (such as Staphylococcus aureus) and Gram-negative bacteria (such as Escherichia coli), as well as some fungi (such as Candida albicans).

The antimicrobial properties of Moringa seed oil make it useful in formulations for treating skin infections, acne, and other conditions associated with microbial overgrowth. The oil is also used in natural preservative systems for cosmetics and personal care products (Ruttarattanamongkol & Petrasch, 2015).

2.4.3. Anti-Inflammatory Activity

Moringa seed oil has shown anti-inflammatory properties in various studies. The oil can inhibit the production of pro-inflammatory mediators and reduce inflammation in different experimental models. This activity is attributed to various bioactive compounds, including oleic acid, which is known for its anti-inflammatory effects.

The anti-inflammatory properties of Moringa seed oil make it valuable in formulations for treating inflammatory skin conditions such as eczema, psoriasis, and dermatitis. The oil can help soothe irritated skin, reduce redness, and promote healing (Cretella et al., 2020).

2.4.4. Moisturizing and Emollient Properties

Moringa seed oil is known for its excellent moisturizing and emollient properties. The oil can penetrate the skin easily, providing deep hydration without leaving a greasy residue. It helps strengthen the skin barrier, prevent water loss, and improve skin elasticity and smoothness (Athikomkulchai et al., 2020).

The moisturizing properties of Moringa seed oil are attributed to its fatty acid composition, particularly the high oleic acid content. Oleic acid is compatible with the skin's natural oils and can help restore the skin's lipid barrier.

2.4.5. Wound Healing Properties

Moringa seed oil has shown wound healing properties in various studies. The oil can promote cell proliferation, collagen synthesis, and tissue regeneration, accelerating the healing process. It also helps prevent infection due to its antimicrobial properties and reduces inflammation, creating an optimal environment for wound healing.

The wound-healing properties of Moringa seed oil make it valuable in formulations for treating minor cuts, burns, and other skin injuries. The oil is also used in post-surgical care to promote healing and reduce scarring (Shady et al., 2022).

2.5. Applications of *Moringa Oleifera* Seed Oil

Moringa oleifera seed oil has a wide range of applications due to its unique properties and biological activities (Cao et al., 2023; Gharsallah et al., 2023):

2.5.1. Cosmetic and Personal Care Applications

Moringa seed oil is widely used in the cosmetic and personal care industry:

- **Skincare**: The oil is used in various skincare products, including moisturizers, serums, facial oils, and anti-aging formulations. It helps hydrate the skin, reduce inflammation, protect against environmental stressors, and promote a healthy, radiant complexion.
- **Haircare**: Moringa seed oil is used in haircare products for its moisturizing, conditioning, and strengthening properties. It can help nourish the scalp, reduce dandruff, prevent hair breakage, and add shine to the hair.
- **Massage Oils**: The oil's good spreadability, mild aroma, and skin-nourishing properties make it an excellent base for massage oils. It can be used alone or blended with essential oils for added therapeutic benefits.
- **Soap Making**: Moringa seed oil is used in natural soap formulations for its moisturizing properties and skin benefits. It produces a mild, creamy lather and helps create a soap that is gentle on the skin.

- **Lip Care**: The oil is used in lip balms and other lip care products for its moisturizing and protective properties. It helps keep the lips soft, smooth, and protected from environmental stressors.

2.5.2. Culinary Applications

Moringa seed oil is used in culinary applications, particularly in regions where the Moringa tree is native:

- **Cooking Oil**: The oil's high smoke point and stability make it suitable for various cooking methods, including frying, sautéing, and baking. It imparts a mild, nutty flavor to dishes.
- **Salad Dressing**: The oil can be used in salad dressings and marinades, providing a nutritious alternative to other vegetable oils. Its mild flavor complements a wide range of ingredients.
- **Nutritional Supplement**: Due to its high oleic acid content and presence of various bioactive compounds, Moringa seed oil is sometimes used as a nutritional supplement to support heart health, improve cholesterol levels, and provide antioxidant benefits.

2.5.3. Medicinal and Pharmaceutical Applications

Moringa seed oil has various medicinal and pharmaceutical applications:

- **Topical Formulations**: The oil is used in topical formulations for treating various skin conditions, including eczema, psoriasis, acne, and minor wounds. Its anti-inflammatory, antimicrobial, and wound-healing properties make it valuable in dermatological preparations.
- **Carrier Oil for Essential Oils**: Moringa seed oil is used as a carrier oil for essential oils in aromatherapy and other therapeutic applications. It helps dilute the essential oils to safe levels for topical application and enhances their absorption into the skin.
- **Pharmaceutical Excipient**: The oil is used as an excipient in various pharmaceutical formulations, including creams, ointments, and suppositories. It helps improve the stability, spreadability, and bioavailability of active pharmaceutical ingredients.

2.5.4. Industrial Applications

Moringa seed oil has various industrial applications:

- **Biodiesel Production**: The oil can be converted into biodiesel through transesterification, providing a renewable alternative to fossil fuels. Moringa biodiesel has properties comparable to or better than conventional diesel fuel.

- **Lubricants**: The oil's good lubricity and stability make it suitable for use in certain lubricant formulations, particularly those requiring biodegradability and low toxicity.
- **Biopolymer Production**: The oil can be used as a raw material for producing biopolymers, which are biodegradable alternatives to conventional plastics.

2.6. Challenges and Future Prospects

Despite its numerous benefits and applications, the commercial exploitation of Moringa oleifera seed oil faces several challenges:

- **Limited Supply**: The global production of Moringa seeds is relatively small compared to major oilseeds like soybean, rapeseed, and sunflower. This limits the availability of the oil for large-scale applications.
- **Variability in Quality**: The quality of Moringa seed oil can vary significantly depending on factors such as the geographical origin of the seeds, cultivation practices, harvesting time, and extraction method. This variability can affect the oil's properties and performance in different applications.
- **Limited Scientific Research**: While there is growing scientific interest in Moringa seed oil, the research is still limited compared to more established oils. More comprehensive studies are needed to fully understand the oil's properties, biological activities, and potential applications.
- **Regulatory Challenges**: In some regions, there may be regulatory challenges related to the use of Moringa seed oil in food, cosmetic, or pharmaceutical products, particularly if it is not traditionally used in those regions.

Despite these challenges, the future prospects for Moringa oleifera seed oil are promising:

- **Growing Demand for Natural Products**: The increasing consumer preference for natural, plant-based products in the food, cosmetic, and pharmaceutical industries creates opportunities for Moringa seed oil.
- **Sustainable Agriculture**: Moringa trees are drought-resistant and can grow in poor soil conditions, making them suitable for sustainable agriculture in regions facing water scarcity and soil degradation. This could help increase the supply of Moringa seeds and oil.
- **Technological Advancements**: Advances in extraction and processing technologies could improve the yield, quality, and cost-effectiveness of Moringa seed oil production, making it more competitive with established oils.
- **Expanding Applications**: Ongoing research is likely to uncover new properties and applications for Moringa seed oil, expanding its market potential.

- **Value-Added Products**: The development of value-added products incorporating Moringa seed oil, such as specialized cosmetic formulations, functional foods, and pharmaceutical preparations, could increase the economic value of the oil.

In summary, essential oils, including the oil extracted from Moringa oleifera seeds, represent a fascinating intersection of traditional knowledge and modern science. These complex mixtures of volatile compounds offer a wide range of biological activities and applications, from healthcare and cosmetics to food and agriculture.

The unique properties of Moringa oleifera seed oil, particularly its high oleic acid content, stability, and biological activities, make it a valuable resource with diverse applications. As research continues to uncover the full potential of this oil, and as sustainable production methods are developed, Moringa seed oil is likely to play an increasingly important role in various industries.

The growing interest in natural, plant-based products and sustainable resources creates opportunities for essential oils in general, and Moringa seed oil in particular, to contribute to addressing various challenges in healthcare, personal care, food security, and environmental sustainability. However, realizing this potential will require continued research, technological innovation, and collaborative efforts across disciplines and sectors.

In conclusion, essential oils, with their complex chemistry and diverse properties, offer a rich field for scientific exploration and practical application. The ongoing study of these oils, including Moringa oleifera seed oil, promises to yield new insights and innovations that can benefit both human health and the environment.

Chapter 3

Phytochemical Characterization and Biological Evaluation

3.1. Introduction

MORINGA oleifera plant is distinguished by its exceptional medicinal and nutritional value, containing a diverse range of bioactive compounds responsible for its health benefits. These include flavonoids, phenolic acids, glucosinolates, alkaloids, and isothiocyanates, which have been shown to exhibit significant pharmacological properties. This chapter examines the relationship between these compounds and their biological activities, particularly their antioxidant and antibacterial properties, paving the way for their applications in pharmaceutical, food, and cosmetic industries. Due to the growing interest in natural alternatives, these bioactive compounds have become a focus of modern research for disease treatment and the development of effective, safe products (Anwar et al., 2007; Saini et al., 2016; Leone et al., 2015; Vergara-Jimenez et al., 2017).

3.2. Bioactive Products in *Moringa Oleifera*

3.2.1. Definition of Secondary Metabolism

Secondary metabolism refers to the biochemical processes that occur in plants to produce compounds that are not directly essential for the basic growth and development of the plant but play an important role in the plant's adaptation to its environment (Anwar et al. 2007). Unlike primary metabolic pathways that produce essential compounds such as carbohydrates, proteins, lipids, and nucleic acids, secondary metabolic pathways produce specialized compounds that vary from one plant species to another.

Secondary metabolites are of great importance to the plant, helping it survive in different environmental conditions through several functions, including: - Defense against insects and herbivores - Protection against pathogens such as fungi and bacteria - Protection against environmental stress such as ultraviolet radiation and drought - Attraction of pollinators and beneficial organisms - Communication between plants and between plants and other organisms.

Secondary metabolites are characterized by their great diversity, with more than 200,000 different secondary compounds discovered in plants. These compounds are also of great importance to humans, as they are used in pharmaceutical, food, cosmetic, and other industries.

In Moringa oleifera, secondary metabolites play an important role in giving it its distinctive medicinal and nutritional properties. Different parts of the plant contain a variety of these compounds, which explains their multiple uses in traditional medicine and nutrition.

3.2.2. Classification of Secondary Metabolites

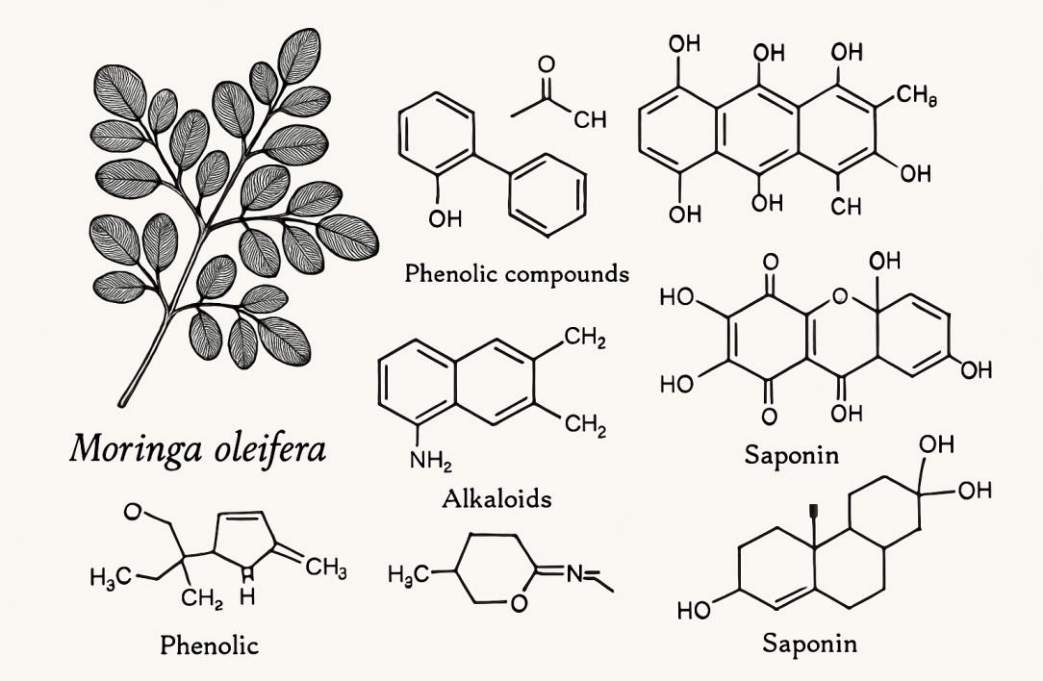


Figure 3.1. Major bioactive compounds found in Moringa oleifera, including phenolic compounds, alkaloids, and saponins, with their molecular structures

Secondary metabolites in plants are classified into several main groups based on their chemical structure and biosynthetic pathways. In Moringa oleifera, the following groups of secondary metabolites have been identified:

3.2.2.1. Phenolic Compounds

Phenolic compounds are a broad group of chemicals that contain at least one aromatic ring attached to one or more hydroxyl groups. They are among the most widespread secondary metabolites in plants and are characterized by their high ability to combat free radicals due to their antioxidant properties (Fahey, J. W. 2005).

In Moringa oleifera, many phenolic compounds have been discovered in various parts of the plant, especially in the leaves and seeds. The most important of these compounds include: gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and cinnamic acid. These compounds contribute to the antioxidant and anti-inflammatory properties that characterize Moringa oleifera.

3.2.2.2. Flavonoids

Flavonoids are a large group of phenolic compounds characterized by a basic structure consisting of 15 carbon atoms arranged in three rings (C6-C3-C6). Flavonoids are among the most widespread secondary metabolites in plants and are characterized by their diverse colors ranging from yellow to red and blue.

In Moringa oleifera, many flavonoids have been discovered, especially in the leaves and flowers. The most important of these flavonoids include: quercetin, kaempferol, myricetin, and rutin. These flavonoids contribute to the antioxidant, anti-inflammatory, and anti-cancer properties that characterize Moringa oleifera.

3.2.2.3. Alkaloids

Alkaloids are complex nitrogenous organic compounds with a bitter taste, characterized by their strong physiological effect on living organisms. Alkaloids are formed from nitrogen derived from amino acids and are often basic (alkaline) in nature, which gives them their name.

In Moringa oleifera, several alkaloids have been discovered, especially in the roots and bark. The most important of these alkaloids include: moringine and moringinine. These alkaloids contribute to the antimicrobial and anti-inflammatory properties that characterize Moringa oleifera.

3.2.2.4. Saponins

Saponins are glycosidic compounds consisting of a non-sugar part (aglycone) linked to a sugar part. Saponins are characterized by their ability to form foam when mixed with water, giving them soap-like properties (hence their name).

In Moringa oleifera, saponins have been discovered in various parts of the plant, especially in the seeds and roots. These saponins contribute to the antimicrobial and anti-inflammatory properties that characterize Moringa oleifera. The saponins in Moringa seeds also play an important role in their water-coagulating properties, making them useful in water purification.

3.2.2.5. Terpenes

Terpenes are a large and diverse group of organic compounds derived from isoprene units (C_5H_8). Terpenes are among the largest groups of natural products and are characterized by their great diversity in structure and function.

In Moringa oleifera, many terpenes have been discovered, especially in the leaves and seeds. The most important of these terpenes include: beta-sitosterol, stigmasterol, and campesterol. These terpenes contribute to the anti-inflammatory and cholesterol-lowering properties that characterize Moringa oleifera.

3.2.2.6. Glucosinolates

Glucosinolates are sulfur-containing compounds that, upon hydrolysis, produce

isothiocyanates, thiocyanates, and nitriles. These compounds are known for their pungent flavor and antimicrobial properties.

In Moringa oleifera, several glucosinolates have been identified, particularly in the seeds and roots. The most notable is 4-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate, which upon hydrolysis produces 4-(α-L-rhamnopyranosyloxy)-benzyl isothiocyanate. This compound has shown significant antimicrobial and anticancer properties.

3.2.3. Extraction and Analysis of Bioactive Products

Bioactive products are extracted from Moringa oleifera using several methods, depending on the nature of the compounds to be extracted and their physical and chemical properties (Pop et al., 2022). Here are the most important extraction methods used:

3.2.3.1. Extraction with Organic Solvents

Extraction with organic solvents is one of the most common extraction methods. Different solvents such as ethanol, methanol, acetone, hexane, chloroform, and others are used, depending on the polarity of the compounds to be extracted.

Extraction can be performed by several methods, such as: - Maceration: The plant material is soaked in the solvent for a specific period, then the extract is filtered. - Soxhlet Extraction: The Soxhlet apparatus is used to extract compounds repeatedly, which increases extraction efficiency. - Ultrasonic Extraction: Ultrasonic waves are used to accelerate the extraction process and increase its efficiency.

3.2.3.2. Extraction with Water

Water is used to extract polar compounds such as sugars, amino acids, glycosides, and some phenolic compounds. Water extraction can be performed by several methods, such as: - Decoction: The plant material is boiled in water for a specific period, then the extract is filtered. - Infusion: The plant material is soaked in hot water for a specific period, then the extract is filtered.

3.2.3.3. Fixed Oil Extraction

Fixed oils are extracted from Moringa seeds using several methods, such as: - Cold Pressing: The seeds are mechanically pressed without using heat, which preserves the quality of the oil. - Extraction with Organic Solvents: Solvents such as hexane are used to extract the oil, then the solvent is evaporated. - Supercritical CO2 Extraction: Supercritical carbon dioxide is used to extract the oil, which provides a clean and efficient extraction.

3.2.3.4. Analysis of Bioactive Products

After extracting the bioactive products, they are analyzed and their identity and quantity are determined using several analytical techniques (Leone et al. 2015), such as:

- High-Performance Liquid Chromatography (HPLC): Used to separate and quantify phenolic compounds, flavonoids, alkaloids, and others.

- Gas Chromatography (GC): Used to analyze volatile compounds and essential oils.

- Mass Spectrometry (MS): Used to determine the molecular weight and structure of compounds.

- Nuclear Magnetic Resonance (NMR): Used to determine the chemical structure of compounds.

- UV-Vis Spectroscopy: Used to determine the quantity of phenolic compounds and flavonoids.

- IR Spectroscopy: Used to determine the functional groups in compounds.

3.3. Biological Studies

3.3.1. Antioxidant Activity

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage cells. Oxidative stress, caused by an imbalance between free radical production and antioxidant defenses, is implicated in various pathological conditions, including cardiovascular diseases, cancer, neurodegenerative disorders, and aging (Vergara-Jimenez, M et al. 2017).

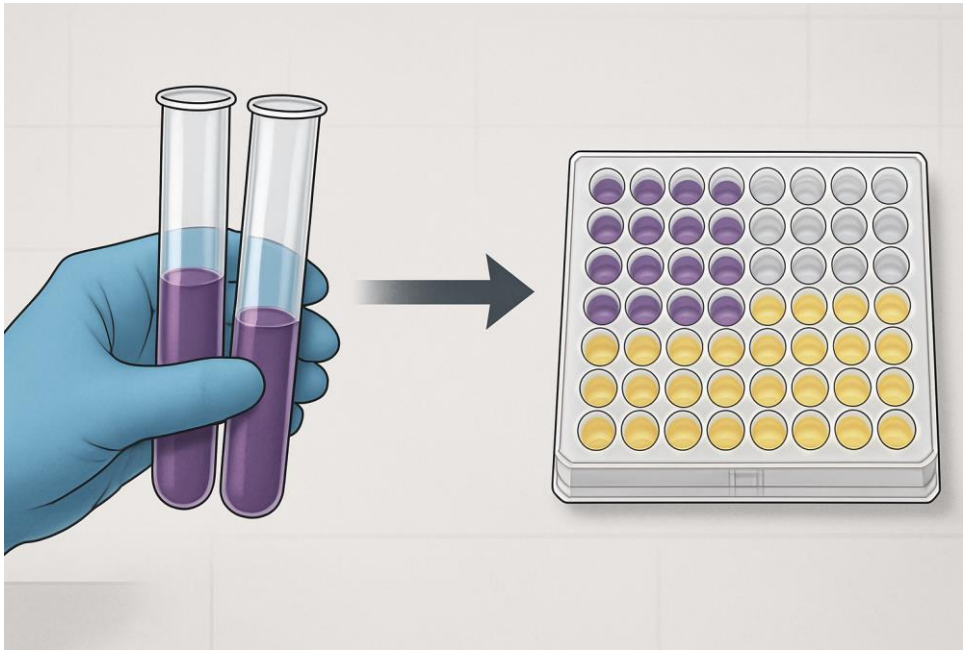


Figure 3.2. DPPH antioxidant assay showing the color change from purple to yellow as antioxidants neutralize the DPPH radical

3.3.1.1. Mechanisms of Antioxidant Activity

Antioxidants can act through several mechanisms:

1. Direct scavenging of free radicals: Antioxidants can donate electrons or hydrogen atoms to neutralize free radicals, converting them into stable, non-radical products.

2. Chelation of metal ions: Some antioxidants can bind to metal ions such as iron and copper, preventing them from participating in reactions that generate free radicals.

3. Inhibition of pro-oxidant enzymes: Antioxidants can inhibit enzymes that generate reactive oxygen species (ROS), such as NADPH oxidase and xanthine oxidase.

4. Enhancement of antioxidant enzymes: Some compounds can increase the activity or expression of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

5. Regeneration of other antioxidants: Some antioxidants can regenerate other antioxidants that have been oxidized, maintaining the overall antioxidant capacity of the system.

3.3.1.2. Methods for Evaluating Antioxidant Activity

Several methods are used to evaluate the antioxidant activity of plant extracts and compounds:

1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay: This is one of the most widely used methods for evaluating antioxidant activity. DPPH is a stable free radical with a deep purple color. When an antioxidant is added, DPPH is reduced to DPPH-H, resulting in a color change from purple to yellow. The degree of discoloration indicates the scavenging potential of the antioxidant compound.

2. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Assay: This method is based on the ability of antioxidants to reduce the ABTS radical cation, which is blue-green in color. The reduction results in a loss of color, which is measured spectrophotometrically.

3. FRAP (Ferric Reducing Antioxidant Power) Assay: This method measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which form a colored complex with TPTZ (2,4,6-tripyridyl-s-triazine).

4. ORAC (Oxygen Radical Absorbance Capacity) Assay: This method measures the ability of antioxidants to protect a fluorescent probe from oxidative degradation by peroxyl radicals.

5. Total Phenolic Content (TPC): This method, using the Folin-Ciocalteu reagent, measures the total phenolic content in a sample, which often correlates with antioxidant activity.

6. Total Flavonoid Content (TFC): This method measures the total flavonoid content in a sample, which also often correlates with antioxidant activity.

3.3.2. Antibacterial Activity

Antibacterial activity refers to the ability of a substance to inhibit the growth of bacteria or to kill bacteria. This activity is crucial in the development of antimicrobial agents for treating bacterial infections and in the preservation of food and other products (Rahman, et al. 2009)

Figure 3.3. Antibacterial activity testing showing inhibition zones around antibiotic discs in petri dishes with bacterial cultures

3.3.2.1. Mechanisms of Antibacterial Activity

Antibacterial agents can act through several mechanisms:

1. Inhibition of cell wall synthesis: Some antibacterial agents interfere with the synthesis of peptidoglycan, a key component of bacterial cell walls, leading to cell lysis and death.

2. Disruption of cell membrane integrity: Some compounds can disrupt the bacterial cell membrane, causing leakage of cellular contents and eventually cell death.

3. Inhibition of protein synthesis: Some antibacterial agents bind to bacterial ribosomes, preventing the synthesis of proteins essential for bacterial growth and survival.

4. Inhibition of nucleic acid synthesis: Some compounds interfere with the synthesis of DNA or RNA, preventing bacterial replication and transcription.

5. Inhibition of metabolic pathways: Some antibacterial agents inhibit specific metabolic pathways that are essential for bacterial growth and survival.

3.3.2.2. Methods for Evaluating Antibacterial Activity

Several methods are used to evaluate the antibacterial activity of plant extracts and compounds:

1. Disc Diffusion Method (Kirby-Bauer Test): This method involves placing paper discs impregnated with the test substance on an agar plate inoculated with the test bacteria. If the substance has antibacterial activity, a clear zone of inhibition appears around the disc, indicating the inhibition of bacterial growth.

2. Well Diffusion Method: Similar to the disc diffusion method, but instead of discs, wells are created in the agar and filled with the test substance.

3. Broth Dilution Method: It involves preparing serial dilutions of the test substance in a liquid medium, inoculating with the test bacteria, and determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

4. Agar Dilution Method: This method involves incorporating the test substance into the agar medium at different concentrations, inoculating with the test bacteria, and determining the MIC.

5. Time-Kill Assay: This method measures the rate at which a test substance kills bacteria over time, providing information on the bactericidal or bacteriostatic nature of the substance.

The antibacterial and antioxidant activities of Moringa oleifera vary among different parts of the plant and are influenced by factors such as geographical location, cultivation conditions, harvesting time, and extraction methods. The antibacterial and antioxidant compounds in Moringa oleifera often work synergistically, enhancing the overall antibacterial efficacy of the plant extracts. This synergistic effect is a crucial consideration in the development of antibacterial and antioxidant agents derived from Moringa oleifera.

3.3.3. Other Biological Activity

In addition to antioxidant and antibacterial activities, Moringa oleifera exhibits several other biological activities that contribute to its medicinal properties. Its extracts have demonstrated effectiveness in reducing inflammation by inhibiting pro-inflammatory mediators and in improving glycemic control by stimulating insulin secretion and reducing oxidative stress (Jaiswal et al., 2009; Mbikay., 2012). Additionally, its antioxidant compounds contribute to inhibiting the proliferation of cancer cells and inducing apoptosis, particularly in breast, colon, and liver cancer (Berkovich et al., 2013).

Moringa also exhibits hepatoprotective effects by enhancing detoxification enzymes and preventing drug-induced liver toxicity (Fakurazi et al., 2008). Moreover, it helps reduce cholesterol levels and blood pressure, thereby supporting cardiovascular health (Chumark et al., 2008). The therapeutic efficacy of Moringa oleifera varies depending on the plant part used and the extraction method, making it a valuable candidate for the development of multi-targeted natural therapies.

3.4. Challenges and Future Prospects

Despite the significant progress in understanding the bioactive compounds and biological activities of Moringa oleifera, several challenges remain, and there are numerous opportunities for future research and development.

3.4.1. Challenges

3.4.1.1. Standardization of Extracts

One of the major challenges in the study and use of Moringa oleifera is the standardization of extracts. The content of bioactive compounds in the plant can vary significantly depending on factors such as geographical location, cultivation conditions, harvesting time, and extraction methods. This variability can lead to inconsistent biological activities and therapeutic effects.

Developing standardized extraction methods and quality control parameters is essential for ensuring the consistency and efficacy of Moringa oleifera products. This includes identifying marker compounds for standardization, establishing acceptable ranges for their content, and developing validated analytical methods for their quantification.

3.4.1.2. Bioavailability and Metabolism

Another challenge is understanding the bioavailability and metabolism of the bioactive compounds in Moringa oleifera. Many of these compounds, particularly phenolics and flavonoids, have limited bioavailability due to poor absorption, extensive metabolism, or rapid elimination.

Research on the pharmacokinetics of these compounds, including their absorption, distribution, metabolism, and excretion, is needed to optimize their therapeutic use. This includes studying the effects of different formulations and delivery systems on bioavailability, as well as investigating the role of gut microbiota in the metabolism and bioactivity of these compounds.

3.4.1.3. Clinical Evidence

While numerous in vitro and animal studies have demonstrated the biological activities of Moringa oleifera, clinical evidence is still limited. More well-designed clinical trials are needed to confirm the efficacy and safety of Moringa oleifera products in humans, to determine optimal dosages, and to identify potential interactions with drugs or other supplements.

These clinical studies should focus on specific health conditions where Moringa oleifera shows the most promise, such as diabetes, hypertension, and inflammatory disorders, and should include diverse populations to account for potential genetic and environmental factors that may influence the response to the plant's bioactive compounds.

3.4.2. Future Prospect

3.4.2.1. Advanced Extraction and Analysis Techniques

The development and application of advanced extraction and analysis techniques offer promising prospects for the study of bioactive compounds in Moringa oleifera. Techniques such as supercritical fluid extraction, microwave-assisted extraction, and

ultrasound-assisted extraction can improve the efficiency and selectivity of extraction, while advanced analytical techniques such as LC-MS/MS, GC-MS/MS, and NMR can provide more detailed information on the chemical composition of the plant.

These techniques can help identify new bioactive compounds, elucidate their structures, and quantify their content in different parts of the plant. They can also help understand the changes in the plant's chemical composition under different conditions, such as stress, disease, or different cultivation practices.

3.4.2.2. Nanotechnology and Drug Delivery

Nanotechnology offers promising prospects for enhancing the bioavailability and efficacy of bioactive compounds from Moringa oleifera. Nanoformulations, such as nanoparticles, nanoemulsions, and nanoliposomes, can protect these compounds from degradation, enhance their absorption, and target their delivery to specific tissues or cells.

Research on the development and evaluation of these nanoformulations is an exciting area for future studies. This includes investigating the effects of different nanocarriers on the stability, release, and bioactivity of the plant's compounds, as well as assessing the safety and efficacy of these nanoformulations in appropriate models.

3.4.2.3. Systems Biology and Network Pharmacology

Systems biology and network pharmacology approaches offer promising prospects for understanding the complex interactions between the bioactive compounds in Moringa oleifera and biological systems. These approaches can help elucidate the molecular mechanisms underlying the plant's biological activities, identify key targets and pathways, and predict potential therapeutic applications.

Research using these approaches can involve integrating data from genomics, proteomics, metabolomics, and bioinformatics to create comprehensive models of the plant's effects on biological systems. These models can guide the development of more effective and targeted therapeutic strategies based on Moringa oleifera.

3.4.2.4. Sustainable Production and Quality Control

Sustainable production and quality control of Moringa oleifera products are crucial for meeting the growing demand while ensuring their efficacy and safety. This includes developing sustainable cultivation practices, optimizing harvesting and processing methods, and implementing rigorous quality control measures.

Research on the effects of different cultivation practices on the content of bioactive compounds, the development of non-destructive methods for quality assessment, and the establishment of quality standards for different types of products are important areas for future studies.

In summary, Moringa oleifera is a plant of exceptional medicinal and nutritional value, containing a wide array of bioactive compounds that contribute to its diverse biological activities. The study of these bioactive compounds and their biological activities has provided valuable insights into the plant's traditional uses and has opened new avenues for its application in modern medicine, nutrition, and other fields.

The bioactive compounds in Moringa oleifera, including phenolics, flavonoids, alkaloids, saponins, and isothiocyanates, exhibit various biological activities, such as antioxidant, antibacterial, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and cardiovascular protective activities. These activities are often the result of synergistic effects among multiple compounds, highlighting the importance of studying the plant as a whole rather than focusing solely on isolated compounds.

Despite the significant progress in understanding the bioactive compounds and biological activities of Moringa oleifera, several challenges remain, including the standardization of extracts, understanding the bioavailability and metabolism of bioactive compounds, and gathering more clinical evidence. However, these challenges also present opportunities for future research and development, such as the application of advanced extraction and analysis techniques, the use of nanotechnology for drug delivery, the adoption of systems biology and network pharmacology approaches, and the development of sustainable production and quality control methods.

In conclusion, Moringa oleifera represents a valuable resource for the development of natural products with various health benefits. Continued research on its bioactive compounds and biological activities will contribute to a better understanding of its therapeutic potential and will guide its optimal use in promoting health and preventing disease.

Second part

Experimental Analysis

Chapter 1

Materials and methods

1.1. Introduction

THIS chapter presents the comprehensive methodological framework employed in the experimental investigation of Moringa oleifera seed oil's biological properties and therapeutic potential. The study design integrates multiple analytical approaches to evaluate the oil's physicochemical characteristics, biological activities, and wound healing efficacy. Beginning with the careful selection and preparation of plant material, bacterial strains, and animal models, this chapter details the systematic procedures followed to ensure scientific rigor and reproducibility. The extraction method chosen for obtaining Moringa seed oil prioritizes the preservation of bioactive compounds, while subsequent analyses focus on determining its physical constants and biological activities, including antioxidant capacity, sun protection factor, antibacterial properties, and enzyme inhibition potential. The in vivo wound healing study employs a well-established animal model to assess the oil's therapeutic efficacy compared to commercial treatments. Each methodological approach is described with sufficient detail to enable replication, with clear protocols, measurement parameters, and statistical analysis techniques. This experimental framework provides the foundation for the subsequent results and their interpretation, contributing to the scientific understanding of Moringa oleifera seed oil's potential applications in pharmaceutical, cosmetic, and therapeutic contexts.

1.2. Materials

1.2.1. Plant Material

The plant material used in this study consisted of dried *Moringa oleifera* seeds, which were obtained from the local market. After collection, the seeds underwent a thorough cleaning process that involved manual removal of the outer shells to obtain the inner seeds containing the oil. The dehulled seeds were then stored under appropriate conditions until used for oil extraction.

Figure 1.1. *Moringa oleifera* seeds used in the study

1.2.2. Bacterial Strains

The bacterial strains used in the antibacterial study are reference strains obtained from the Pasteur Institute in Algeria. They were stored at 5°C in tubes containing nutrient agar.

Table 1.1 The microbial species used during the experiment

Gram Type	Strains	Reference	Origin
Gram+	Bacillus subtilis	ATCC6633	The Pasteur Institute of Algiers
	Staphylococcus aureus	ATCC10237	
Gram-	Escherichia coli	ATCC25922	
	Pseudomonas aeruginosa	ATCC27853	

1.2.3. Animal Material

Nine male Wistar rats, 10 weeks old and weighing between 170 and 265 g, were obtained from the animal facility of the Pasteur Institute in Algeria. The animals were housed under standardized environmental conditions (12 hours light/12 hours dark cycle), relative humidity of 65.3% and ambient temperature of (25 ± 2°C) for a one-month acclimatization period.

The rats had free access to water and food and were maintained on a standard diet according to Southon et al. (1984). After the acclimatization period, the animals were randomly divided into three experimental groups and housed in polypropylene cages, each equipped with a label holder indicating the batch number and the treatment assigned.

The experiment was conducted over a period of 20 days, during which the designated treatment protocols were applied according to the study objectives.

1.3. Methods

1.3.1. Extraction Method

The oil from Moringa oleifera seeds was extracted using the cold-press method with a mechanical oil press specifically designed for vegetable oils. This solvent-free, non-thermal technique is widely recognized for its ability to produce pure oil without the introduction of chemical residues or degradation of bioactive compounds. It ensures the preservation of the oil's natural composition and enhances its chemical stability (Siddhuraju & Becker, 2003; Manzoor et al., 2007).

Figure 1.2. Used Cold Press Oil Machine

After extraction, the oil was collected and stored in tightly sealed dark glass bottles, kept in a cool, dry place away from light and heat sources to maintain its physicochemical stability and minimize oxidative degradation (Anwar & Bhanger, 2003).

Figure 1.3. Extracted Moringa oleifera seed oil

1.3.2. Determination of Physical Constants of the Oil Sample

The color and viscosity of the oil extracted from Moringa seeds were described, the pH was measured in the laboratory, and both the density and the percentage yield of the oil were calculated:

1.3.2.1. Determination of Extraction Yield

The extract yield was calculated using the following formula (Falleh et al., 2008):

$$R(\%) = 100 \times M_{ext} / M_{sc}$$

Where: R is the yield in (%). M_{ext} is the mass of the extract after solvent evaporation in (g). M_{sc} is the dry mass of the plant in (g).

1.3.2.2. pH Measurement

Measuring the pH is essential, as it can have significant implications for the therapeutic efficacy of topical preparations. It may influence both the physical stability of the oil and the chemical stability of the active ingredients.

For skin care applications, the pH of topical formulations should ideally range between 4 and 7 to ensure compatibility with the skin barrier (Lambert et al., 2006).

The pH was measured directly in the laboratory using a digital pH meter. The reading was obtained by immersing the pH electrode into the extracted Moringa seed oil, as illustrated in the accompanying image.

1.3.3. Biological Activities

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

The ferric reducing antioxidant power (FRAP) assay is a spectrophotometric method used widely to evaluate the antioxidant potential of compounds based on their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. During the FRAP reaction, Fe^{3+} ions are associated with a specific ligand TPTZ (tripyridyl-s-triazine-2,4,6), forming a colorless [Fe^{3+}-TPTZ] complex. The method relies on the reduction of a ferric-tripyridyl triazine [Fe^{3+}-TPTZ] complex to its ferrous form [Fe^{2+}-TPTZ] in the presence of antioxidants that donate electrons under specific acidic conditions:

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

In this study, the antioxidant activity of Moringa oleifera seed oil was assessed by FRAP assay according to the (Benzie et al.1996) method with some modifications, and using ascorbic acid as the primary reference standard due to its well-characterized redox properties ($Fe^{3+} \Rightarrow Fe^{2+}$) and high electron-donating capacity.

- *Experimental Protocol:*

a) Sample preparation: A series of standard ascorbic acid solutions diluted in distilled

water to establish a calibration curve and Moringa oleifera seed oil solutions diluted in dimethyl sulfoxide (DMSO) were prepared, all at different and known concentrations.

b) Protocol:

1. 1000 µl of sample of each test solution was taken (500 µl for ascorbic acid).
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 (ascorbic acid) using the following formula:

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

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 1.4. 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 Moringa oleifera seed oil was determined in vitro using UV-Visible spectrophotometry, following the method originally developed by Mansur et al. (1986).

➢ *Experimental Protocol:*

a) Sample preparation:

- A mixture was prepared by dissolving 1 ml of Moringa oleifera seed oil in 3 ml of DMSO (dimethyl sulfoxide). DMSO was selected as the solvent due to its ability to dissolve lipophilic compounds and its transparency in the UV range.
- The solution was gently vortexed by a vortex device for 3 minutes until fully homogeneous.

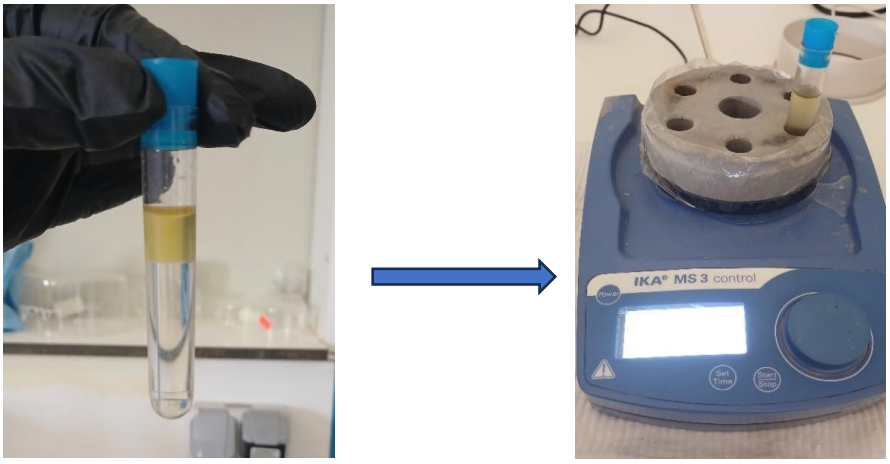


Figure 1.5. SPF sample preparation

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.

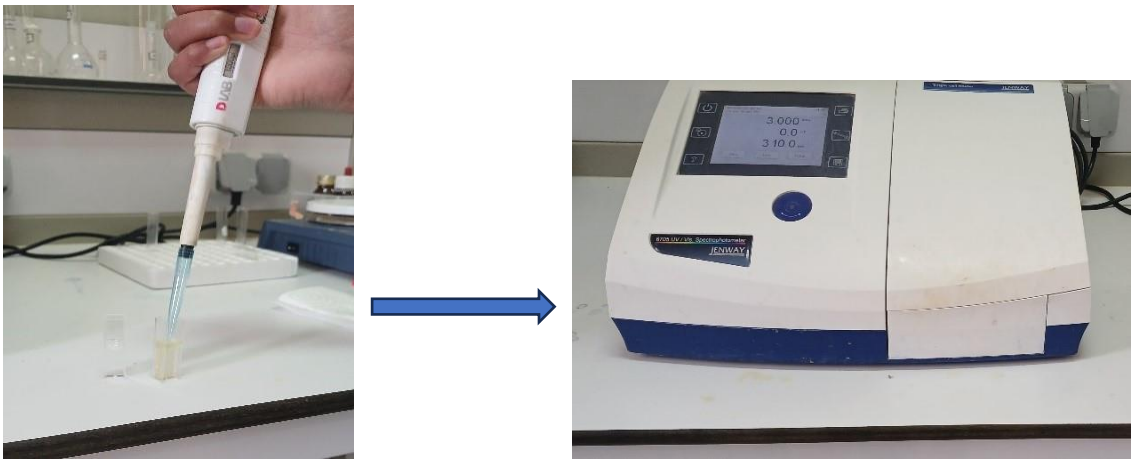


Figure 1.6. Measurement of the solution absorbance

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)

Table 1.2 displays the normalized product function utilized in SPF calculation, based on the constants determined by (Sayre et al. 1979).

Table 1.2 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**.

Table 1.3 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. Study of Antibacterial Activity

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

- *MIC and MBC*

The MIC test refers to the lowest concentration of the oil that visibly inhibits bacterial growth after the incubation period, whereas the MBC is defined as the lowest concentration that results in complete bacterial cell death without regrowth. The MBC/MIC ratio is used as an additional indicator of the nature of the effect, where a value

of 1 suggests a bactericidal effect (Wayne, 2010).

In this study, both tests were applied to assess the efficacy of Moringa seed oil against four different bacterial isolates using:

a) Broth Microdilution Assay: The broth microdilution assay is a standardized method for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against a variety of bacteria and yeast strains. The assay is performed by the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI) (Qaiyumi, 2007; Weinstein, 2018; Wayne, 2010; PA, 2002).

- **Protocol:**

First, the bacterial and yeast suspensions are prepared. For bacteria, the strains are cultured on Mueller-Hinton agar (MHA) and then inoculated into cation-adjusted Mueller-Hinton broth (MHB). The cultures are incubated until visibly turbid and then diluted to a turbidity corresponding to 0.5 McFarland (1.5×10^8 CFU/mL) using BioMerieux DensiCHEK Plus for VITEK 2 Systems.

Next, Moringa oleifera seed oil solution is prepared by dissolving the oil in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml. The solution is then homogenized by vortexing for 1 minute.

The microtiter plate is set up by adding 100 µL of Moringa oleifera seed oil solution to each well. Then, 50 µL of the bacterial or yeast suspension is added to each well. A growth control (no antibiotic, no xenobiotic) and a sterile control (MHB only) are included for all isolates (Schwalbe et al., 2007).

The microtiter plate is incubated at 37°C for 18-24 hours. After incubation, the MIC is determined as the lowest concentration of plant extract that inhibits the growth of the bacteria (Wayne, 2010).

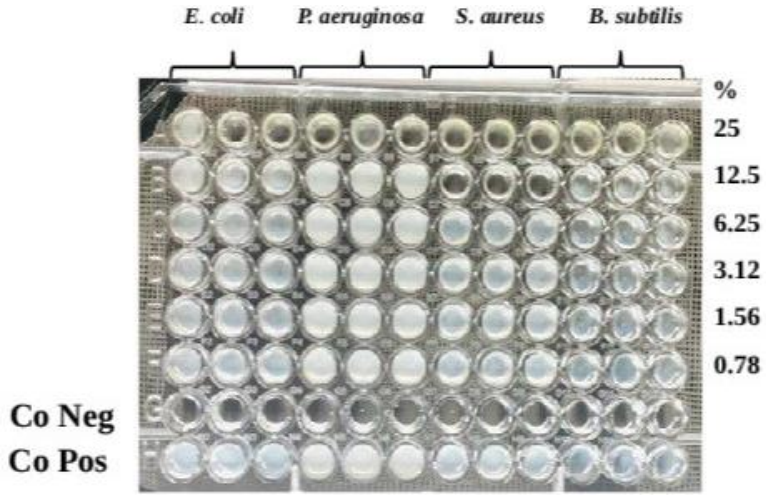


Figure 1.7. Photo of the broth microdilution method used to assess the antimicrobial activity of the oil against bacterial strains

b) MBC by spot test method: The spot test method for determining the Minimum Bactericidal Concentration (MBC) using microplates with volatile essential oils involves a series of concise steps.

- **Protocol:**

Initially, various concentrations of the essential oil are prepared in a broth medium across multiple wells of a microplate. A standardized bacterial suspension is then introduced to each well, followed by incubation under appropriate conditions to allow for bacterial growth.

Afterward, a small aliquot from each well is spotted onto agar plates (3 µL) devoid of the Moringa seed oil, and incubated again on Sabouraud dextrose agar for yeast and MHA for bacteria. The absence of bacterial growth on these plates indicates the bactericidal activity of the plant extract at specific concentrations.

The MBC is thus identified as the lowest concentration of the plant extract at which no visible bacterial growth occurs, signifying the effective killing of the bacteria. This method is particularly useful for efficiently assessing the bactericidal properties of Moringa seed oil against various bacterial strains in a high-throughput manner (Suppi et al., 2015; Wayne, 2010).

All experiments were conducted in triplicate to ensure the statistical reliability of the results.

➢ *Effect of Oil on Biofilm Formation*

This test evaluates the ability of Moringa seed oil to prevent or reduce the formation of bacterial biofilms, which play a key role in antibiotic resistance and chronic infection.

The effect of the oil at a sub-inhibitory concentration (Sub-MIC) on biofilm formation was evaluated using the 96-well microtiter plate assay, a standard method for quantifying surface-attached bacterial cells through crystal violet staining (Crystal Violet Assay).

To investigate the effect of oil (EXT) on biofilm formation, overnight bacteria cultures were diluted and transferred to a 96-well microtiter plate. A subinhibitory concentration of oil (EXT) was added, and the plate was incubated at 37°C for 24 hours without agitation. To quantify the biofilm cells, the culture supernatant was discarded, and the wells were washed twice with phosphate-buffered saline (PBS) to remove nonadherent cells. The wells were air-dried, and the surface-attached cells were stained with 200 µl of 0.1% crystal violet for 30 minutes. The crystal violet was removed, and the plate was washed with water. The stained biofilm cells were determined at 570 nm with the microplate reader after adding dimethyl sulfoxide (DMSO) [1, 2].

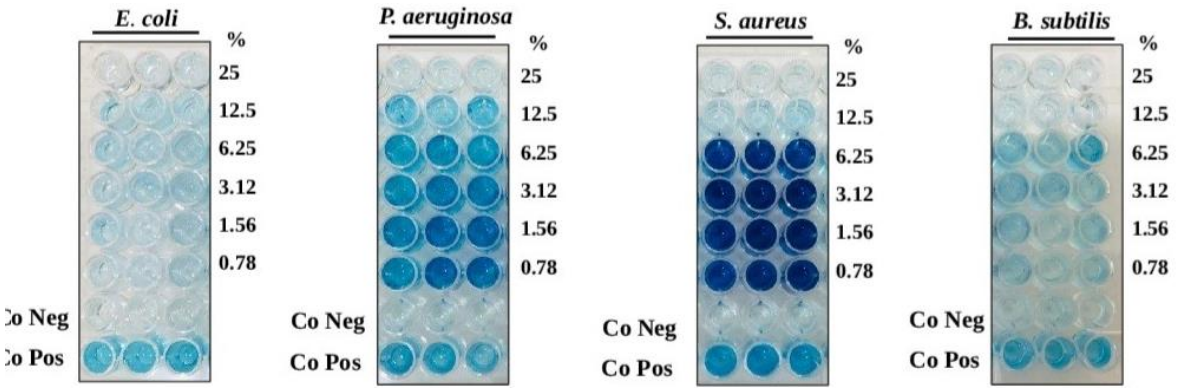


Figure 1.8. Effect of the tested Moringa seed oil on biofilm formation in four bacterial strains (E. coli, P. aeruginosa, S. aureus, B. subtilis) at different concentrations

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of EXT that inhibited bacterial growth. To determine the minimum bactericidal concentration (MBC), 5 µL of each diluted suspension was spotted on MHB plates and incubated at 37°C for 12 h [3].

➢ *Enzyme Inhibition*

In the context of skin health, protease enzymes catalyze proteolysis by breaking peptide bonds. Their excessive activity leads to tissue degradation and the loss of the skin's fundamental protein structure (Lopez-Otin & Bond, 2008), primarily involving:

- Collagen, which is mainly targeted by collagenase enzyme (MMP-1 and MMP-8).
- Elastin, which is broken down by elastase (MMP-12).

This degradation results in the loss of skin elasticity and the appearance of aging signs (Fisher et al., 1996; Quan et al., 2009).0 its cosmetic potential in protecting the skin's key structural proteins – collagen and elastin.

- **<u>Experimental Protocol:</u>**

Assessment of protease inhibition by plant oil emulsions using a casein agar diffusion assay:

A food-grade vegetable oil was emulsified in phosphate buffer (0.01 M, pH 7.5) using 1% Tween-20 as a surfactant to prepare four different concentrations (10%, 20%, 30%, and 50% v/v). The emulsions were vortexed for 2 minutes and sonicated for 5 minutes to ensure homogeneity and stability before use.

a) Caseinolytic Agar Plate Assay

To assess the protease inhibitory potential of the vegetable oil, casein agar plates were prepared by dissolving 1% casein and 1% agar in phosphate buffer (pH 7.5), followed by sterilization by autoclaving. After solidification in sterile Petri dishes, wells (6 mm diameter) were created using a sterile cork borer.

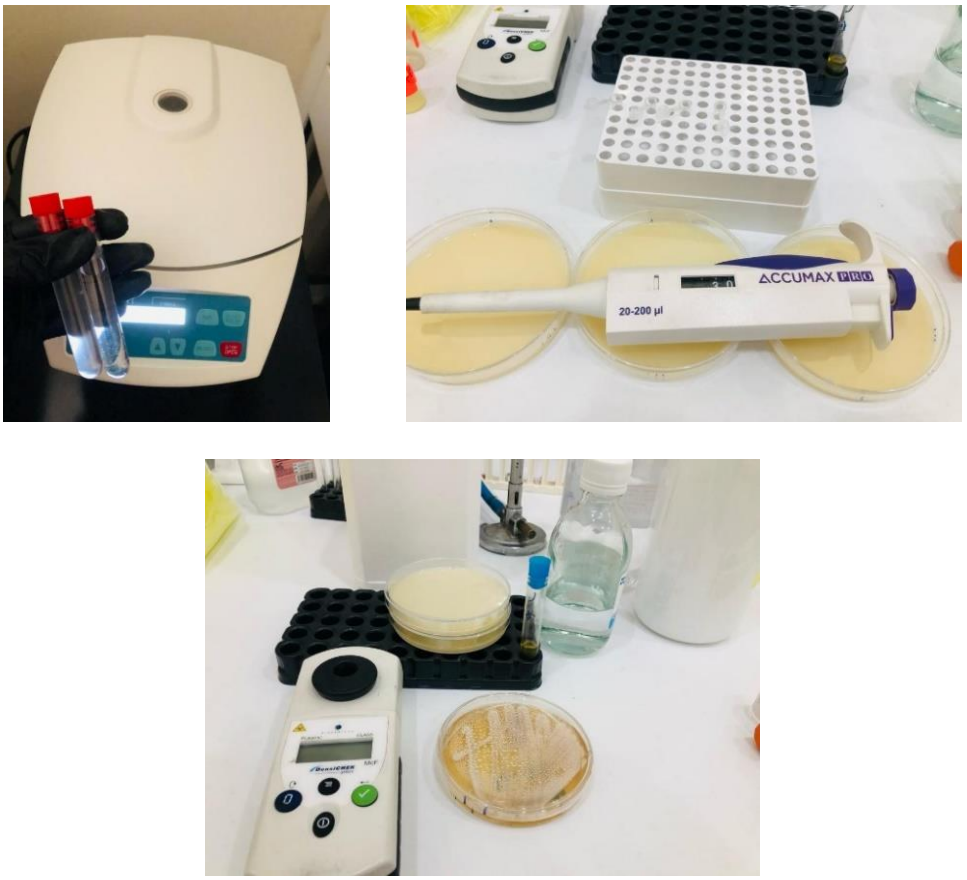


Figure 1.9. Experimental Setup for Enzyme Inhibition Analysis

For each assay: - The vegetable oil emulsions were pre-incubated with trypsin solution (0.1 mg/mL, equivalent to 1000 U/mL) in a microplate at varying volume-to-volume ratios for 10 minutes at room temperature. - After incubation, 5 µL of each mixture was introduced into peripheral wells around the central well. - The central well in each plate was filled with 5 µL of trypsin solution alone. - For the negative control, 5 µL of trypsin solution was mixed with 5 µL of distilled water and placed into a separate well.

Plates were then incubated at 37°C for 18 hours. The enzymatic activity was halted by flooding the surface with 10% trichloroacetic acid (TCA), followed by gentle washing with distilled water and air drying at room temperature.

b) Quantification of proteolytic inhibition:

Zones of casein hydrolysis, visible as clear halos around the wells, were photographed and analyzed. Instead of relying solely on the diameter, the area of proteolysis was calculated for more precise quantification using the formula:

$$Area\ (mm^2) = \pi \times \left(\frac{D}{2}\right)^2$$

Where D is the diameter (in mm) of the clear zone.

The percentage inhibition of proteolytic activity was then calculated as follows:

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

where $A_{control}$ is the average area of the clear zone caused by trypsin alone. A_{sample} is the average area in the presence of the oil sample.

1.3.4. In Vivo Study

Our experimental study was designed to evaluate the wound healing effect of Moringa oleifera seed oil using an animal model of cutaneous injury and scars. The experimental protocol was adapted from several previous studies, with modifications tailored to our objectives.

1.3.4.1. Animals Preparation

Nine healthy adult rats were selected following a thorough examination to rule out any injuries or abnormal behavior. No fasting or water restriction was imposed prior to the experiment. Each animal was identified using an indelible marker on the tail, and the rats were housed in clean cages with access to standard food and water ad libitum under controlled environmental conditions (temperature, light).

Figure 1.10. Experimental Animals (rats) Stock

1.3.4.2. Wound Induction

Before wound creation, we carefully shaved the dorsal area of each rat using sterile scissors to obtain a clean and exposed surface. The exposed skin was then disinfected to ensure aseptic conditions.

We manually created a linear full-thickness incision approximately 2 cm in length along the mid-dorsal line using a sterile surgical blade. The procedure was performed without the use of anesthesia due to its short duration, minimally invasive nature, and was carried out swiftly to limit discomfort. We selected the dorsal area to minimize the risk of licking or self-inflicted injury and to allow for optimal visual monitoring of the wound healing process throughout the study.

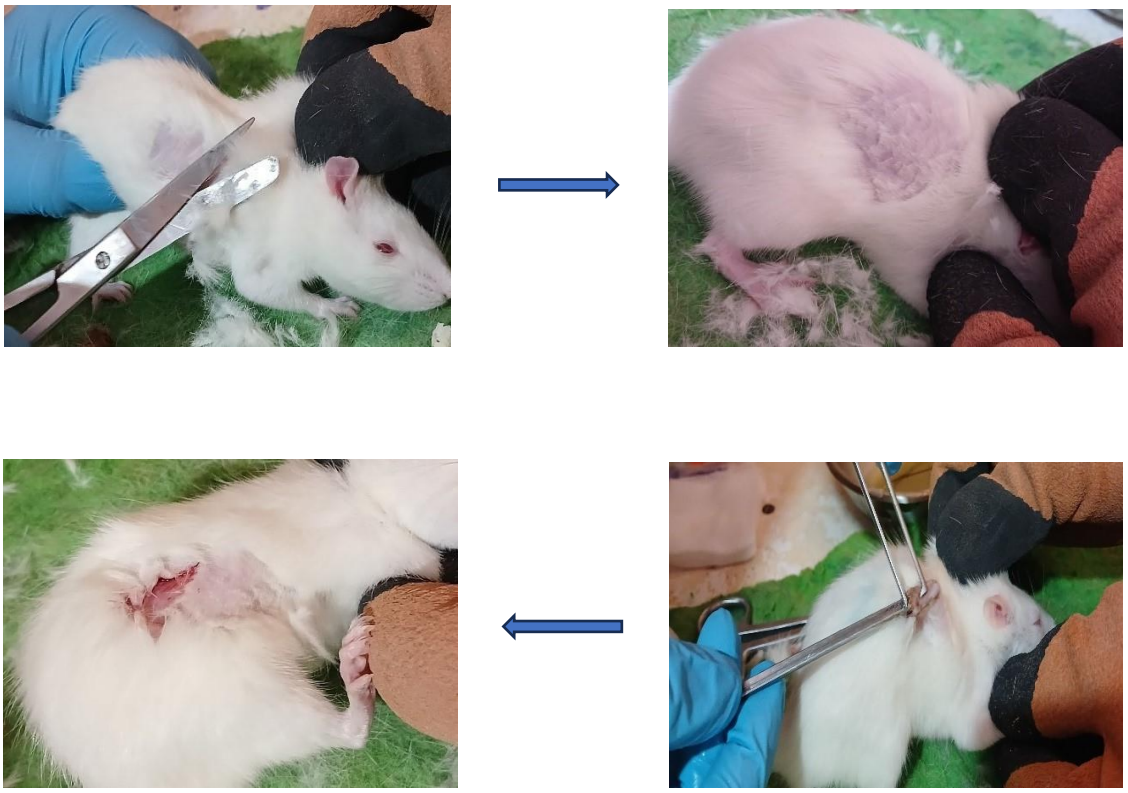


Figure 1.11. Illustration of wound induction

We closely monitored all rats following the incision to detect any signs of pain or distress. No adverse effects or signs of infection were observed during or after the procedure.

1.3.4.3. Group Distribution

To evaluate the wound healing and the disappearance of scars effect of Moringa oleifera seed oil, it was compared to a commercial treatment.

We randomly divided the nine rats into three experimental groups, with three animals in each group:

- Group 1 (Moringa oil-treated group): received topical application of Moringa seed oil.
- Group 2 (Reference group): treated with MEBO ointment, a commercially available wound-healing cream.
- Group 3 (Control group): did not receive any topical treatment and served as the untreated control.

1.3.4.4. Application of Treatments

The treatment began two hours after wound induction. We applied the respective treatment every other day for 20 days. Prior to each application, we gently cleaned the wound area using sterile saline solution to maintain hygiene and ensure optimal absorption of the treatment.

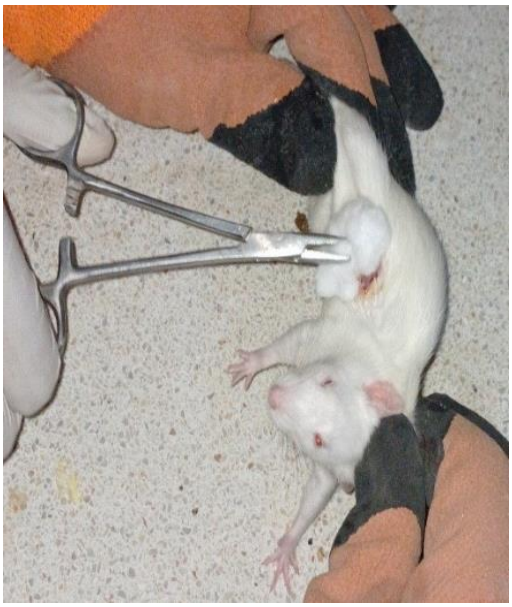


Figure 1.12. Illustration of Treatment Procedures

All rats were kept under identical housing and feeding conditions throughout the experiment, and the progression of wound healing was regularly observed and recorded.

1.3.4.5. Expression of Results

Wound healing progression was assessed every other day throughout the 20-day treatment period. For each rat, the length of the wound was measured on days D1, D3, D5, D7, D9, D11, D13, D15, D17, D20 using a sterile millimeter ruler. Measurements were recorded in centimeters and used to generate healing progression curves.

To quantify the healing process, the percentage of wound contraction was calculated using the following formula:

$$\%\, Wound\ contraction\ = \frac{Initial\ wound\ length\ on\ D_o - \ Wound\ length\ on\ D_n}{Initial\ wound\ length\ on\ D_o} \times\ 100$$

This method allowed for an objective and continuous evaluation of wound healing over time. No clinical scoring system was used, as direct length measurement was considered more accurate and reliable for the type of wound induced.

1.3.4.6. Two-way ANOVA Analysis – Wound Healing

A two-way ANOVA with interaction was performed to study the effects of time and type of treatment on wound healing. The three treatment groups analyzed were:

(1) Moringa seed oil;

(2) MEBO ointment;

(3) Control group.

The dependent variable was the wound surface area in (cm^2) measured at different time points (day 1 to day 20).

In summary, the methodological approach outlined in this chapter represents a comprehensive framework for evaluating the multifaceted properties of Moringa oleifera seed oil. By employing a combination of in vitro and in vivo techniques, this study addresses key questions regarding the oil's physicochemical characteristics, biological activities, and therapeutic potential. The cold-press extraction method was selected to preserve the oil's natural bioactive compounds, while subsequent analyses were designed to provide quantitative assessments of its antioxidant capacity, photoprotective properties, antimicrobial efficacy, and enzyme inhibition potential. The in vivo wound healing model, with its carefully controlled experimental conditions and objective measurement parameters, offers valuable insights into the oil's therapeutic applications. Throughout the experimental process, standardized protocols were followed to ensure reliability and reproducibility, with appropriate controls and statistical analyses incorporated to validate the findings. This methodological framework not only supports the current investigation but also establishes a foundation for future research exploring additional properties and applications of Moringa oleifera seed oil in various fields, from cosmetics to pharmaceutical development.

Chapter 2

Results and discussion

2.1. Introduction

THIS chapter presents the comprehensive findings of our experimental investigation into the biological properties and therapeutic potential of Moringa oleifera seed oil. The results are organized to systematically address the key research questions regarding the oil's physicochemical characteristics, antioxidant capacity, photoprotective properties, antimicrobial efficacy, and wound healing potential. Beginning with the determination of physical constants, which establish the baseline properties of the extracted oil, the chapter progresses through increasingly complex biological assays that reveal the oil's multifaceted activities. The antioxidant evaluation using the FRAP assay provides insights into the oil's capacity to neutralize free radicals, while the SPF determination highlights its remarkable photoprotective potential. The antibacterial investigations, encompassing MIC, MBC, biofilm inhibition, and enzyme inhibition assays, demonstrate the oil's efficacy against various pathogens and its potential mechanisms of action. Finally, the in vivo wound healing study offers compelling evidence of the oil's therapeutic efficacy in promoting tissue repair and regeneration. Throughout the chapter, the experimental results are contextualized within the broader scientific literature, with discussions that explore the underlying mechanisms, comparative efficacy, and potential applications of Moringa oleifera seed oil in pharmaceutical, cosmetic, and therapeutic contexts.

2.2. Results

2.2.1. Determination of Physical Constants of the Oil Sample

The physical and chemical properties of Moringa oleifera seed oil were evaluated to establish its baseline characteristics. The results are summarized in Table 1.

Table 2.1 Physical and chemical properties of Moringa oleifera seed oil

Parameter	Value
Color	Dark yellow
Viscosity	Heavy
Density	1.95 g/ml
pH	4.11
Percentage yield	38.97%

The extracted Moringa oleifera seed oil exhibited a dark yellow color with heavy viscosity. The oil had a density of 1.95 g/ml and a slightly acidic pH of 4.11, which falls within the optimal range (4-7) for skin compatibility as suggested by (Lambert et al. 2006). The extraction process yielded 38.97% oil from the dried seeds, indicating an efficient extraction method.

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

The antioxidant capacity of Moringa oleifera seed oil was evaluated and compared to that of ascorbic acid using the ferric reducing antioxidant power (FRAP) assay. Based on absorbance readings obtained from a series of previously prepared diluted solutions with known concentrations, calibration curves were generated for both Moringa seed oil and ascorbic acid (used as a reference antioxidant) by plotting absorbance (measured at 700 nm) against concentration (µg/ml).

The calibration curve of the Moringa seeds oil exhibited a linear relationship with the regression equation $y = 0.2813x + 0.0813$ and a coefficient of determination $R^2 = 0.9763$, indicating excellent linearity. Similarly, the ascorbic acid standard showed a regression line of $y = 0.0068x + 0.1937$ with $R^2 = 0.991$, confirming the reliability and accuracy of the assay for both compounds.

From the constructed curves, the EC_{50} values were determined (Table 2.2). The EC_{50} (Effective concentration 50%) is defined as the concentration of the substance required to achieve 50% of its maximal antioxidant activity and serves as a key indicator of antioxidant potency.

Table 2.2 EC_{50} values for Moringa seed oil and ascorbic acid in the FRAP assay

Reducing Power	A 0.5 (µg/ml)
Moringa seeds oil	0.873 ± 0.030
Ascorbic acid	0.5243 ± 1.10

These values show that ascorbic acid possesses a higher antioxidant capacity than the oil, as indicated by its lower EC_{50} value.

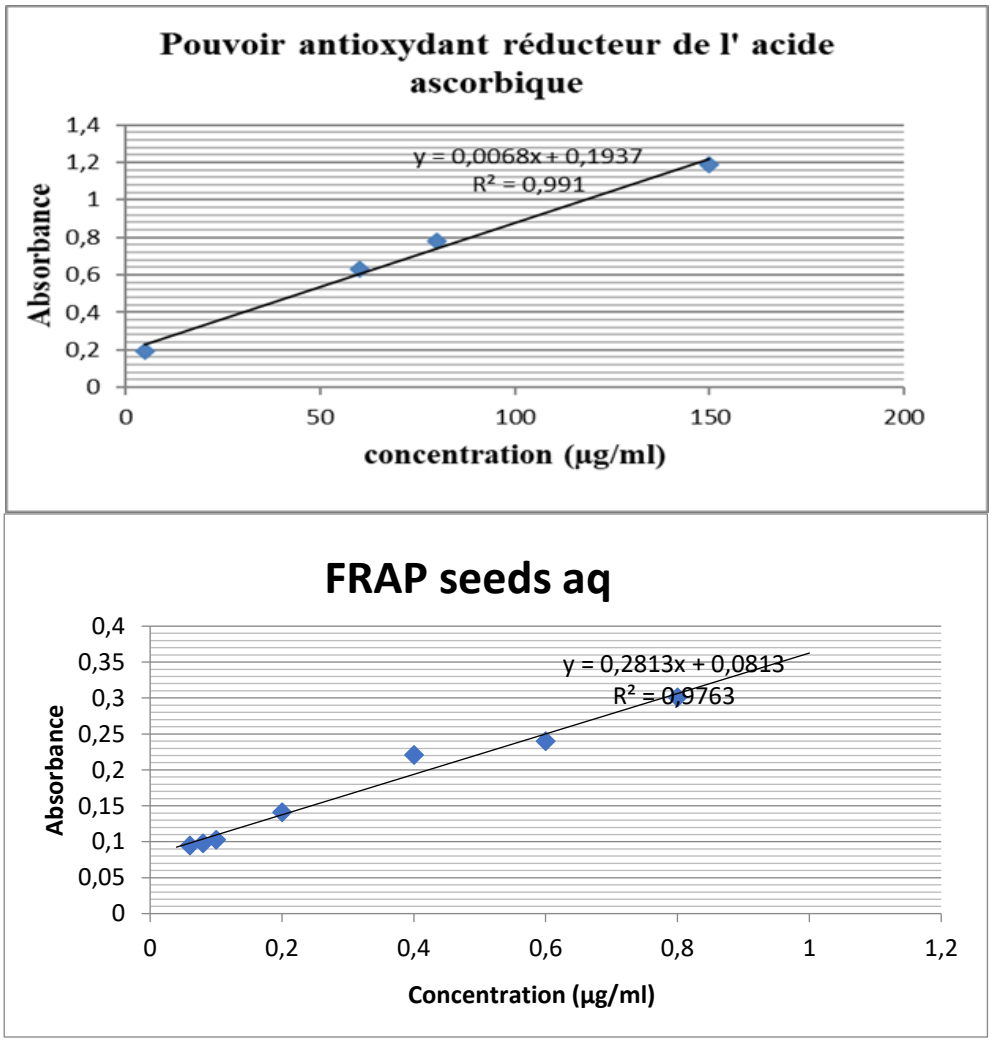


Figure 2.1. FRAP calibration curve for *Moringa oleifera* seed oil

2.2.2.1. Statistical Analysis

To assess the significance of the difference between the two EC_{50} values, a statistical analysis was performed as shown in **Error! Reference source not found.**.

The EC_{50} values of the two samples were statistically compared. Each bar represents the mean EC_{50} value for the respective substance, and the vertical lines above each bar indicate the standard deviation, representing the variability in the measurements.

This analysis revealed a statistically significant difference between oil and vitamin C, denoted by an asterisk (*), with a confidence level of $p < 0.05$. This implies that the variation observed is unlikely to be due to random chance or experimental variability. However, it is important to note that while valid, the difference is not substantial because a single asterisk (*) statistically indicates a difference that is not large; it reflects a moderate difference in antioxidant capacity between the two samples.

This means that while ascorbic acid demonstrates superior antioxidant capacity, Moringa oleifera seed oil still exhibits considerable antioxidant potential.

2.2.3. SPF Test

In our study, we evaluated the sun protection factor (SPF) of Moringa oleifera seed oil using an in vitro spectrophotometric method proposed by Mansur et al. (1996). This method is based on measuring the absorbance (Abs) of the sample in the harmful ultraviolet (UV) radiation, particularly the ultraviolet B (UVB) range (290-320 nm). The absorbance results were as follows:

Table 2.3 Absorbance values of Moringa oleifera seed oil at different wavelengths

Wavelength λ (nm)	Absorbance (Abs)
290	1.79
295	2.89
300	2.64
305	3.00
310	3.00

Upon analyzing the data, it was observed that the absorbance value peaked at 305 nm and then stabilized with no significant further increase. According to Olejnik et al. (2016) and Kaur & Saraf (2010), it is scientifically acceptable to include only absorbance values up to the wavelength at which a plateau is reached, as stabilized absorbance beyond this point does not contribute significantly to increased UV protection and is therefore excluded from the SPF calculation.

Based on the above data, the SPF of Moringa oleifera seed oil was calculated to be SPF = 73.25, which qualifies as very high protection according to international classification standards. These standards recognize SPF > 50 as offering the highest level of protection against UV (UVB).

2.2.3.1. Comparison of SPF

To provide practical insight into the photoprotective performance of Moringa oleifera seed oil, we compared it with a well-known high-protection commercial product, Avène high protection cream SPF +50, a leading formulation in the European skincare market:

Table 2.4 Comparison of SPF values between Moringa seed oil and commercial sunscreen

Product	SPF
Moringa oleifera seeds oil	73 ± 2
Avène	41 ± 4

These findings clearly demonstrate that Moringa seed oil offers superior photoprotective efficacy compared to the commercial sunscreen tested, with lower variability in the measurements (± 2 vs ± 4), suggesting greater spectral stability.

2.2.4. Antibacterial Activity

2.2.4.1. MIC and MBC Test

The antimicrobial activity of the Moringa oleifera seed oil was evaluated through determination of its MIC, MBC, and corresponding MBC/MIC ratios against four bacterial strains. The results revealed that Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis exhibited the highest susceptibility to the oil, with both MIC and MBC values recorded at 25%, yielding an MBC/MIC ratio of 1. In contrast, Staphylococcus aureus showed comparatively lower sensitivity, requiring a higher concentration of 50% for both MIC and MBC. Notably, all tested strains demonstrated an MBC/MIC ratio of 1, suggesting a bactericidal effect of the oil across the board. Among the panel, E. coli, P. aeruginosa, and B. subtilis were the most responsive to the oil treatment, indicating a broader and more potent antibacterial profile compared to S. aureus.

Table 2.5 MIC, MBC, and MBC/MIC ratio of Moringa oleifera seed oil against different bacteria

Bacteria strains (n = 3)	MIC (%)	MBC (%)	MBC/MIC
Escherichia coli	25	25	1
Pseudomonas aeruginosa	25	25	1
Staphylococcus aureus	50	50	1
Bacillus subtilis	25	25	1

2.2.4.2. Effect on Biofilm Formation

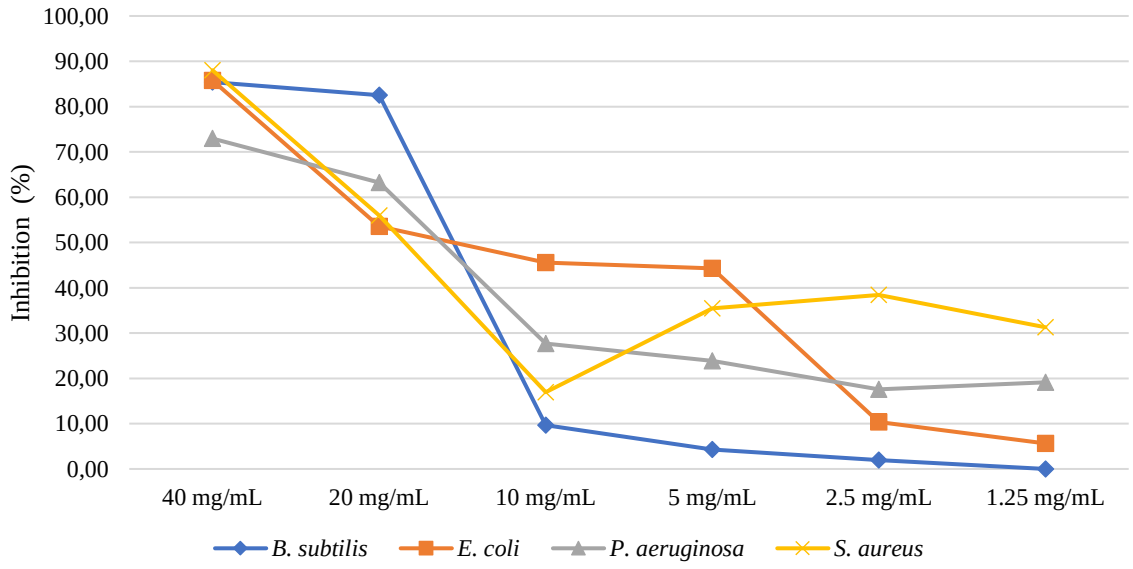


Figure 2.2. Concentration-dependent inhibition of biofilm formation by Moringa seed oil across different bacterial strains

The inhibitory effect of the tested Moringa seed oil (EXT) on biofilm formation was concentration-dependent across all examined bacterial strains. As shown in Figure 2, Bacillus subtilis exhibited the highest sensitivity to the oil, with biofilm formation inhibition exceeding 85% at 40 mg/mL and remaining notably suppressed even at lower

concentrations. Escherichia coli followed closely, showing strong inhibition above 80% at the highest concentration and maintaining moderate inhibition down to 5 mg/ml. Pseudomonas aeruginosa and Staphylococcus aureus demonstrated comparatively lower susceptibility, with maximum inhibition levels not exceeding 70% and displaying minimal decline across decreasing concentrations. Overall, B. subtilis emerged as the most responsive strain, highlighting the potential of the oil in targeting resilient biofilms, particularly in Gram-positive bacteria.

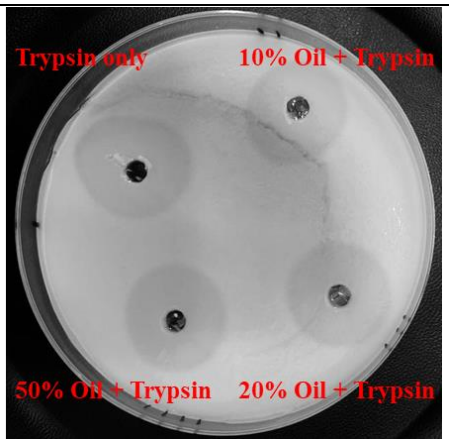
2.2.4.3. Dose-dependent Inhibition of Proteolytic Activity

The results from the triplicate assays demonstrate a clear dose-dependent inhibitory effect of the vegetable oil on proteolytic activity. The data from three independent experiments are presented in Table 2.6, Table 2.7 and Table 2.8.

Based on the mean inhibition zone areas, the samples can be ranked in descending order of inhibitory efficacy as follows: 50% Oil + Trypsin (68.30 mm^2), 20% Oil + Trypsin (84.52 mm^2), 10% Oil + Trypsin (150.72 mm^2), and Control (Trypsin only) (195.29 mm^2). Among the tested concentrations, the 50% oil emulsion exhibited the highest inhibitory effect, reducing the mean proteolysis zone area by approximately 65% compared to the control. These findings indicate a significant and concentration-dependent protease inhibition potential of the tested oil.

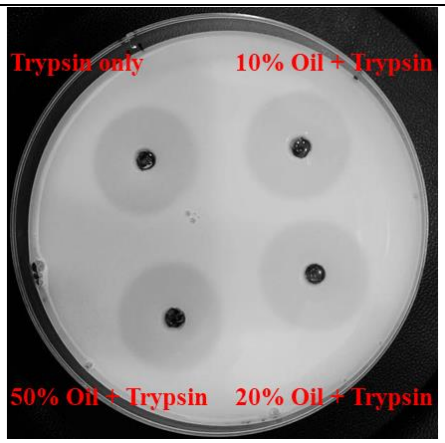
By inhibiting trypsin (used here as a model Serine protease), Moringa seed oil demonstrates a protective potential that can be associated with its ability to suppress matrix metalloproteinases (MMPs) responsible for the degradation of collagen and elastin. Thus, it may help preserve the structural integrity of the skin and support key functions such as anti-aging (Quan et al., 2009).

Table 2.6 First experiment - Proteolytic inhibition by Moringa seed oil



Zone	Estimated Diameter (mm)	Estimated Area (mm^2)
Control (Trypsin only)	17.87 mm	250.81 mm^2
10% Oil + Trypsin	16.25 mm	207.28 mm^2
20% Oil + Trypsin	14.62 mm	167.90 mm^2
50% Oil + Trypsin	13.65 mm	146.26 mm^2

Table 2.7 Second experiment - Proteolytic inhibition by Moringa seed oil



Sample	Estimated Diameter (mm)	Estimated Area (mm^2)
Trypsin only	14.71 mm	169.93 mm^2
10% Oil + Trypsin	12.22 mm	117.21 mm^2
20% Oil + Trypsin	10.72 mm	90.26 mm^2
50% Oil + Trypsin	9.47 mm	70.49 mm^2

Table 2.8 Third experiment - Proteolytic inhibition by Moringa seed oil

Sample	Estimated Diameter (mm)	Estimated Area (mm^2)
Trypsin only	14.50 mm	165.13 mm^2
10% Oil + Trypsin	12.75 mm	127.68 mm^2
20% Oil + Trypsin	11.00 mm	95.03 mm^2
50% Oil + Trypsin	9.25 mm	67.20 mm^2

2.2.5. Wound Observation

2.2.5.1. Evaluation of Wound Healing in Rats

The macroscopic progression of wound healing was evaluated in rats subjected to three different conditions. Observations were recorded at specific time points: days 3, 7, 9, 12, 15, and 20 post-wounding.

(1) Untreated Group: The wound remained largely open and thick by day 7, with marked erythema, serous exudate, and visible necrotic tissue. The wound edges were clearly defined at this stage. By day 13, a moderate reduction in wound surface area was observed, with persistent signs of inflammation and less pronounced redness; the wound edges became indistinct, suggesting the beginning of tissue contraction. On day 17, a more significant reduction in wound size was noted, accompanied by mild erythema. By day 20, the wound was fully closed, with partial hair regrowth and a visible residual scar.

(2) Treated with MEBO Ointment Group: This group showed more rapid healing. By day 5, the wound exhibited a significant decrease in size and depth, along with noticeable erythema, inflammation, and serous exudate. The wound edges remained clearly demarcated. By day 9, there was substantial reduction in the wound area and the presence of granulation tissue, although the edges became irregular. By day 15, the wound had almost completely closed, leaving behind a mild scar. By day 20, skin regeneration was nearly complete, with full hair regrowth and the scar no longer visible.

(3) Treated with Moringa oleifera Seed Oil Group (applied every other day): This group demonstrated the most remarkable progression. By day 3, a slight reduction in wound size was observed, along with mild erythema and inflammation, minimal serous exudate, and well-defined wound margins. On day 7, the wound area had visibly contracted, with moderate redness and absence of exudate; the wound edges were no longer well-defined. By day 12, complete wound closure was achieved, the inflammation had resolved, and only a faint scar remained, indicative of effective tissue repair. From day 15 to day 20, progressive hair regrowth was observed, and by day 20, the scar had fully disappeared, reflecting successful and aesthetically favorable skin regeneration.

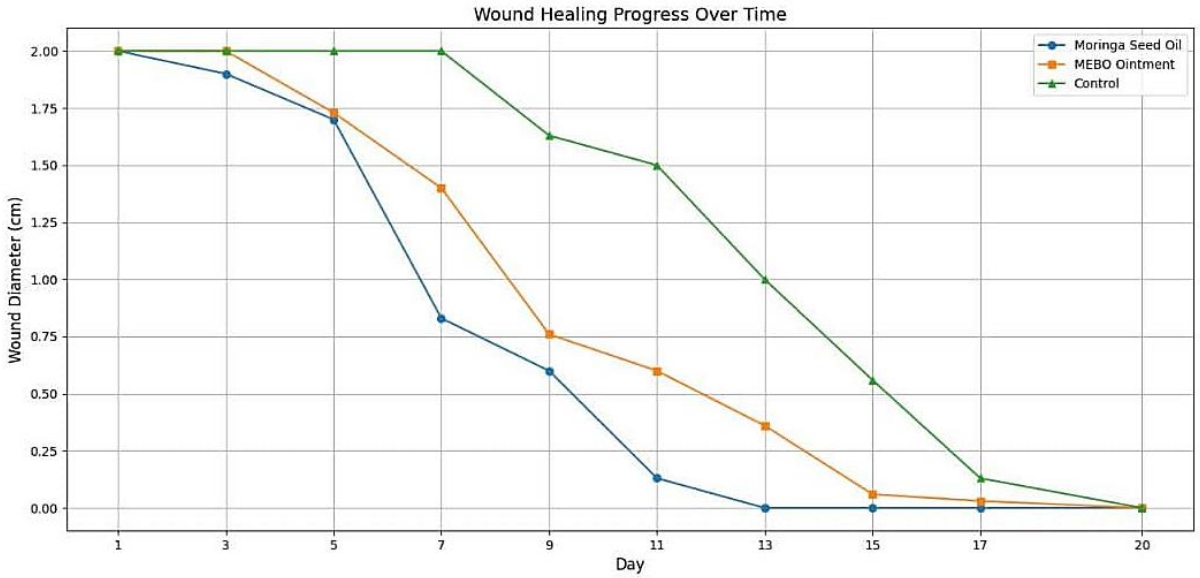


Figure 2.3. Graph showing wound healing progress over time for the three treatment groups

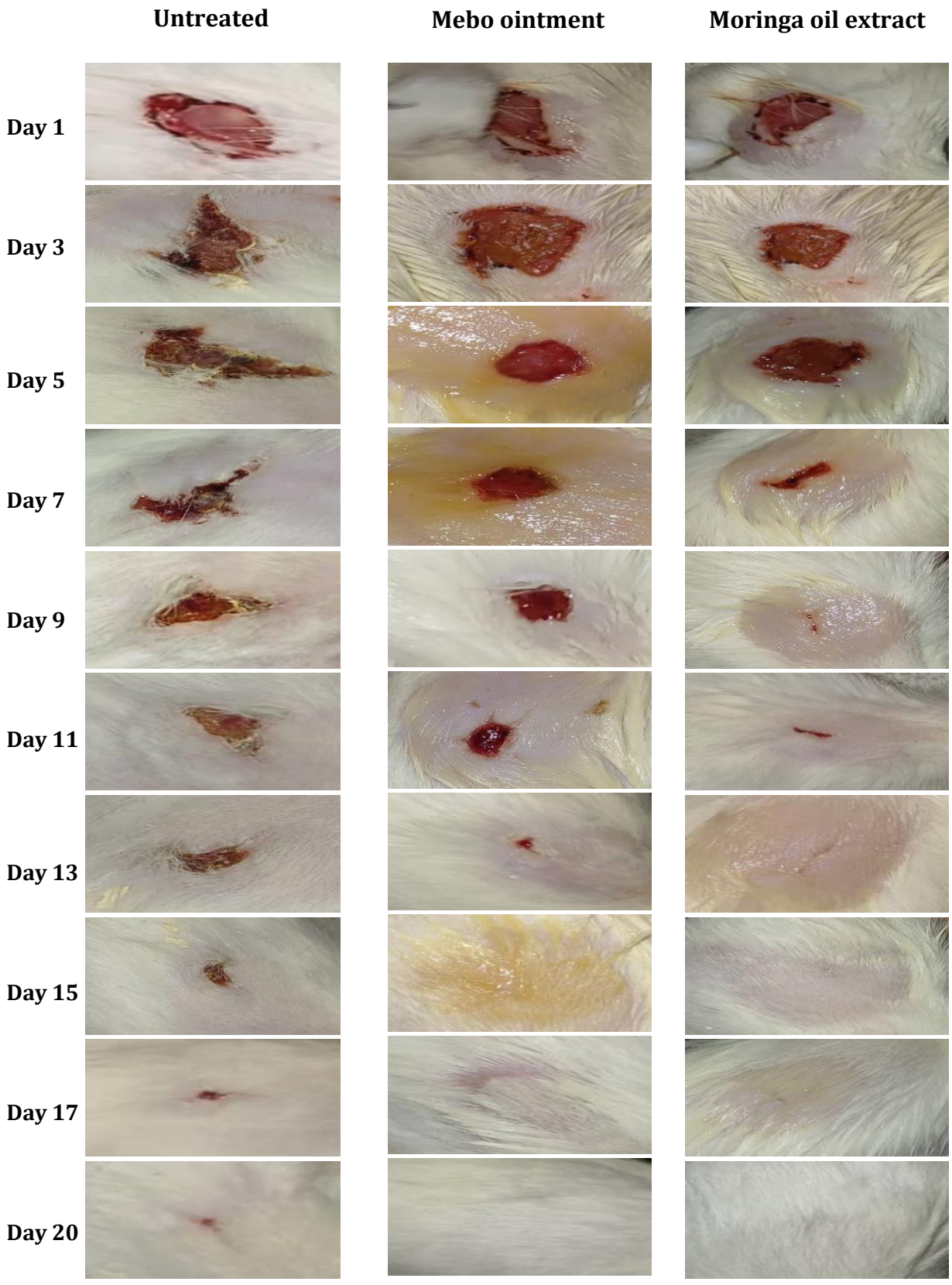


Figure 2.4. Burn appearance in different groups: Untreated, Mebo ointment; Moringa oil extract (original photo, 2025)

2.2.5.1. Statistical Analysis of the Effect of Moringa Seed Oil on Wound Healing

Our two-way ANOVA analysis revealed significant effects of both treatment type and time on wound healing, as well as a significant interaction between these factors. The treatment effect was highly significant ($F(2,60) = 159.41$, $p < 0.001$), demonstrating that the choice of treatment substantially influenced wound healing outcomes. The time effect was also highly significant ($F(9,60) = 356.55$, $p < 0.001$), confirming the expected progression of wound healing over the 20-day observation period. Importantly, the significant interaction between treatment and time ($F(18,60) = 14.35$, $p < 0.001$) indicates that the three treatments exhibited different healing patterns across the temporal dimension.

Moringa seed oil demonstrated superior efficacy in wound healing compared to both MEBO ointment and the control group. By day 7, wounds treated with Moringa seed oil showed a substantial reduction in surface area (mean = 0.83 ± 0.12 cm^2), while MEBO-treated wounds exhibited a more moderate reduction (mean = 1.40 ± 0.17 cm^2). In contrast, control wounds maintained their initial size (mean = 2.00 ± 0.00 cm^2) at this time point. The accelerated healing pattern of Moringa seed oil became even more pronounced by day 11, with wound areas reduced to a mean of 0.13 ± 0.06 cm^2 compared to 0.60 ± 0.36 cm^2 for MEBO ointment and 1.50 ± 0.10 cm^2 for control wounds.

Post-hoc analyses confirmed the statistical significance of these differences. Moringa seed oil treatment resulted in significantly smaller wound areas compared to the control group (mean difference = -0.57 cm^2, $p < 0.001$) across the study period. Similarly, MEBO ointment showed significantly improved healing compared to the control (mean difference = -0.39 cm^2, $p < 0.001$). The direct comparison between the two active treatments revealed a significant advantage for Moringa seed oil over MEBO ointment (mean difference = -0.18 cm^2, $p < 0.05$).

The healing trajectory for each treatment revealed distinctive patterns. Moringa seed oil demonstrated the most rapid healing kinetics, with complete wound closure (0.00 ± 0.00 cm^2) achieved by day 13. MEBO ointment exhibited a more gradual healing progression, with near-complete closure by day 15 (0.07 ± 0.12 cm^2) and complete healing by day 20. The control group displayed a significantly delayed healing response, with wound reduction only beginning after day 7 and proceeding at a slower rate, reaching 0.57 ± 0.12 cm^2 by day 15 and 0.13 ± 0.06 cm^2 by day 17, before complete closure at day 20.

2.3. Discussion

2.3.1. Physicochemical Properties:

Moringa seed oil exhibited a high extraction yield of 38.97%, indicating a rich content of bioactive fatty acids, both saturated and unsaturated, which contribute to its anti-inflammatory and antimicrobial properties (Anwar et al., 2007).

The pH value (4.11) aligns well with the skin's natural surface pH (typically between 4 and 7), playing a key role in maintaining the skin barrier and preventing the overgrowth of pathogenic microbes (Lambers et al., 2006).

The oil's color, viscosity, and relatively high density (1.95 g/mL) reflect a concentrated presence of heavy bioactive constituents, supporting its stability and effectiveness in topical applications.

2.3.2. Antioxidant Activity

The FRAP (Ferric Reducing Antioxidant Power) assay conducted on Moringa oleifera seed oil revealed substantial reducing power, indicating a high concentration of antioxidant compounds. This result is a significant indicator of the oil's ability to neutralize free radicals—unstable molecules that contribute to oxidative stress, which plays a key role in the pathogenesis of chronic inflammation, premature aging, cardiovascular diseases, and cancer (Valko et al., 2007).

This antioxidant activity can be attributed to the presence of potent phenolic compounds such as catechin, rutin, chlorogenic acid, as well as vitamin E (alpha-tocopherol) and beta-carotene, all of which have been previously identified in Moringa seeds and oils (Saimi et al., 2016; Leone et al., 2015). These compounds exert their antioxidant effects through various mechanisms, including electron donation, metal chelation, and inhibition of oxidative chain reactions.

These findings are consistent with the study by (Verma et al. 2012), which reported high antioxidant activity of Moringa leaf extracts using FRAP and DPPH assays. This confirms that the oil retains a significant proportion of these bioactive compounds, especially when extracted using methods that preserve phenolic integrity.

From a therapeutic perspective, the strong antioxidant capacity of the oil is an important indicator of its potential efficacy in topical applications, such as promoting wound healing or protecting the skin from UV-induced oxidative damage, by limiting oxidative stress and supporting tissue repair (Mahmood et al., 2010).

2.3.3. SPF Test

Moringa oleifera seed oil exhibited considerable photoprotective ability, with the calculated Sun Protection Factor (SPF) reaching 73.25, according to the spectrophotometric method outlined by Mansur et al. (1986). This value clearly indicates the high efficacy of the oil in absorbing UVB radiation (290–320 nm), which is a primary cause of skin photodamage, actinic keratosis, and sun-induced skin cancers (Young, 2006).

According to the European Cosmetic Safety Agency classification, products with an SPF value exceeding 50 are categorized as "Very High Protection," highlighting the significant potential of this oil as a promising natural ingredient in sunscreen formulations.

This efficacy is attributed to the oil's composition, which is rich in polyphenolic compounds, flavonoids, and tocopherols—known for their UV-absorbing properties and their ability to mitigate oxidative damage caused by solar radiation (Díaz et al., 2020; Suhaimi et al., 2021). Previous studies have also indicated that certain plant oils, such as Moringa oil, exhibit relatively high natural SPF values due to their antioxidant content and bioactive compounds that interact with the UV spectrum (Olejnik et al., 2016).

2.3.4. Antibacterial Activity

The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays revealed that Moringa seed oil exhibits notable antibacterial activity against four bacterial strains. E. coli, P. aeruginosa, and B. subtilis all showed MIC and MBC values of 25%, with an MBC/MIC ratio of 1, indicating a bactericidal effect. However, S. aureus required a higher concentration (50%), suggesting a relative resistance.

These findings suggest that Moringa oil contains active compounds such as isothiocyanates, phenolics, and alkaloids, which interact with bacterial cell membranes, leading to their disruption (Peixoto et al., 2011; Rahman et al., 2009). The consistent MBC/MIC ratio of 1 across strains further confirms the bactericidal nature of the oil.

The oil also demonstrated concentration-dependent inhibition of biofilm formation, with the highest effect observed against Bacillus subtilis (over 86% inhibition at 40 mg/mL), followed by E. coli. The response was weaker in P. aeruginosa and S. aureus, likely due to differences in cell wall structure and biofilm composition between Gram-positive and Gram-negative bacteria.

Biofilms pose a significant challenge in antibiotic resistance. Natural oils like Moringa oil have the potential to penetrate or disrupt the biofilm matrix, enhancing their value as effective antimicrobial agents (Donlan & Costerton, 2002; Borges et al., 2016).

Additionally, trypsin inhibition assays (used as a model for protease activity) showed that Moringa oil reduced the proteolytic zone diameter in a concentration-dependent manner, with approximately 65% reduction at 50% oil concentration. This highlights the oil's ability to inhibit protein-degrading enzymes.

In the context of skin health, proteases such as collagenase (MMP-1, MMP-8) and elastase (MMP-12) contribute to the degradation of collagen and elastin, leading to skin aging and loss of elasticity (Fisher et al., 1996; López-Otín & Bond, 2008). Therefore, the inhibitory effect of Moringa oil on these enzymes suggests its potential in protecting structural skin proteins, positioning it as a promising ingredient in cosmetic formulations aimed at preventing skin aging.

2.3.5. In Vivo Evaluation

This study aimed to evaluate the effect of Moringa oleifera seed oil on skin wound healing in comparison to MEBO ointment and an untreated control group, using an experimental

model involving nine rats divided into three treatment groups. Wound diameter was measured periodically until day 20.

The results revealed a gradual and significant reduction in wound diameter in the rats treated with Moringa oil. For instance, by day 7, the average wound diameter in the Moringa group was 0.83 cm, compared to 1.4 cm in the MEBO group and 1.7 cm in the control group. This difference became more pronounced over time: complete healing (0 cm) was observed by day 13 in the Moringa group, whereas the MEBO group required until day 17, and the control group took the full 20 days to reach complete closure.

This accelerated healing in the Moringa group may be attributed to the bioactive profile of Moringa seed oil, which includes phenolic compounds, flavonoids, essential fatty acids (such as oleic acid), and potent antioxidants. These components contribute to enhanced tissue regeneration, reduced inflammation, and improved local blood circulation—collectively promoting faster progression through the three classical wound healing phases: inflammation, proliferation, and remodeling.

In contrast, MEBO ointment demonstrated moderate efficacy, reducing the wound diameter gradually from 2 cm on day 1 to 0.03 cm by day 17, and achieving complete healing by day 20. The control group exhibited significantly slower healing, with wound diameter remaining at 1.5 cm on day 11, 1.0 cm on day 13, and only decreasing to 0.1 cm by day 17.

The accelerated healing observed with Moringa seed oil—characterized by complete wound closure 7 days earlier than the control—represents a clinically relevant improvement in wound management. The early and rapid reduction in wound surface area between days 5 and 7 (from 1.70 ± 0.00 cm^2 to 0.83 ± 0.12 cm^2) suggests that Moringa seed oil may be particularly effective during the early proliferative phase of wound healing. This pronounced effect during a critical healing period could have important implications for reducing complications such as infection risk and scarring, which are directly correlated with healing duration.

The statistical robustness of these findings (with all comparisons significant at $p < 0.001$) provides strong evidence for the therapeutic potential of Moringa seed oil in wound treatment. While MEBO ointment—an established wound healing agent—demonstrated significant efficacy compared to the control ($p < 0.001$), the superior performance of Moringa seed oil ($p < 0.05$ vs. MEBO) warrants further investigation into its bioactive compounds and mechanisms of action.

These findings indicate that Moringa oil significantly enhances wound healing compared to both conventional treatment and no treatment. This is in line with previous studies, such as Dangi and Jolly (2021), which highlighted Moringa's tissue-regenerating and antimicrobial properties.

In summary, the in vivo wound healing study demonstrated the remarkable efficacy of Moringa oleifera seed oil in promoting tissue repair and regeneration. The oil

significantly accelerated the wound healing process compared to both the commercial MEBO ointment and the untreated control group, with complete wound closure achieved by day 13 in the Moringa-treated group, compared to day 20 in the control group.

The superior wound healing properties of Moringa seed oil can be attributed to several mechanisms:

1. **Anti-inflammatory effects**: The oil's ability to reduce inflammation, as evidenced by the decreased erythema and exudate in the treated wounds, likely contributes to faster resolution of the inflammatory phase of wound healing, allowing for earlier progression to the proliferative phase.

2. **Antimicrobial activity**: The demonstrated antibacterial properties of the oil may help prevent wound infection, a common complication that can significantly delay healing.

3. **Antioxidant properties**: The high antioxidant capacity of the oil, confirmed by the FRAP assay, may protect the wound environment from oxidative damage, supporting cellular proliferation and tissue regeneration.

4. **Protease inhibition**: The oil's ability to inhibit proteolytic enzymes may help preserve the extracellular matrix components necessary for proper wound healing and prevent excessive tissue degradation.

5. **Bioactive compounds**: The presence of various bioactive compounds in Moringa oil, including essential fatty acids, vitamins, and phytochemicals, may provide nutritional support for the regenerating tissues and stimulate cellular processes involved in wound repair.

The complete disappearance of scarring by day 20 in the Moringa-treated group is particularly noteworthy, as it suggests that the oil not only accelerates wound closure but also enhances the quality of the healed tissue, promoting more complete and aesthetically favorable regeneration.

These findings align with previous studies on the wound healing properties of Moringa extracts, such as those by (Rathi et al. 2006) and (Muhammad et al. 2013), which reported similar accelerated healing and improved tissue regeneration with Moringa preparations.

This study provides comprehensive evidence for the multifaceted biological activities of Moringa oleifera seed oil, supporting its potential applications in pharmaceutical, cosmetic, and therapeutic contexts. The oil demonstrated significant antioxidant capacity, though slightly lower than ascorbic acid, indicating its potential to combat oxidative stress and its associated pathologies. Its remarkable SPF value of 73.25 positions it as a promising natural ingredient for sun protection formulations, offering superior photoprotection compared to some commercial products.

The antibacterial investigations revealed potent activity against both Gram-positive

and Gram-negative bacteria, with particular efficacy against E. coli, P. aeruginosa, and B. subtilis. The oil's ability to inhibit biofilm formation, especially in B. subtilis, highlights its potential in addressing biofilm-associated infections, which are often resistant to conventional antibiotics. Additionally, the dose-dependent inhibition of proteolytic enzymes suggests applications in preventing protein degradation, particularly relevant for skin health and anti-aging formulations.

Perhaps most significantly, the in vivo wound healing study demonstrated the oil's superior efficacy in promoting tissue repair and regeneration compared to both a commercial wound healing product and untreated controls. The accelerated healing, complete wound closure by day 13, and absence of scarring by day 20 underscore the oil's potential as a natural wound healing agent.

These findings collectively support the traditional uses of Moringa oleifera in various healing applications and provide scientific validation for its integration into modern therapeutic formulations. Future research directions could include detailed mechanistic studies to elucidate the specific bioactive compounds responsible for each observed activity, optimization of formulations for specific applications, and expanded clinical trials to confirm efficacy and safety in human subjects.

General Conclusion

THIS study aimed to evaluate the physicochemical properties and biological activities of Moringa oleifera seed oil through a series of laboratory analyses and in vivo experiments, with the objective of exploring its potential applications in the field of skin care and dermatological treatments.

The findings demonstrated that Moringa seed oil possesses stable physicochemical characteristics, including a high content of unsaturated fatty acids, particularly oleic acid, which enhances the skin's hydration and helps restore its natural barrier function. These qualities make the oil highly suitable for use in nourishing and protective skincare products.

The FRAP antioxidant assay confirmed the oil's strong radical scavenging ability, supporting its role in combating oxidative stress, a key factor in skin aging, inflammation, and damage to structural proteins such as collagen and elastin.

Additionally, the oil exhibited a high Sun Protection Factor (SPF) of 73.25, confirming its efficacy in absorbing UVB radiation and highlighting its potential as a natural sunscreen agent.

In terms of antibacterial activity, Moringa oil showed effective inhibition of several pathogenic strains commonly associated with skin infections, along with a demonstrated ability to reduce biofilm formation. These antimicrobial effects suggest it could be useful in preventing and managing skin disorders caused by microbial colonization.

The in vivo wound healing experiments reinforced these results, with Moringa oil significantly accelerating wound closure, improving tissue structure, and promoting collagen and elastin synthesis. These effects indicate its potential for use in therapeutic applications targeting skin regeneration and recovery.

Based on these comprehensive findings, it can be concluded that Moringa oleifera seed oil is a highly promising natural bioactive agent in the field of cosmetic and medical skin care. It offers a multifunctional profile, including protection, repair, and regeneration, that qualifies it for integration into a wide range of formulations aimed at improving skin health and appearance.

Further research is recommended to deepen the understanding of Moringa oil's mechanisms of action at the molecular level, optimize its extraction and formulation processes, and evaluate its long-term safety and efficacy through clinical trials. Moreover, studies comparing its performance with other established botanical oils and synthetic agents could strengthen its positioning as a reliable natural alternative in dermaceutical and cosmeceutical products.

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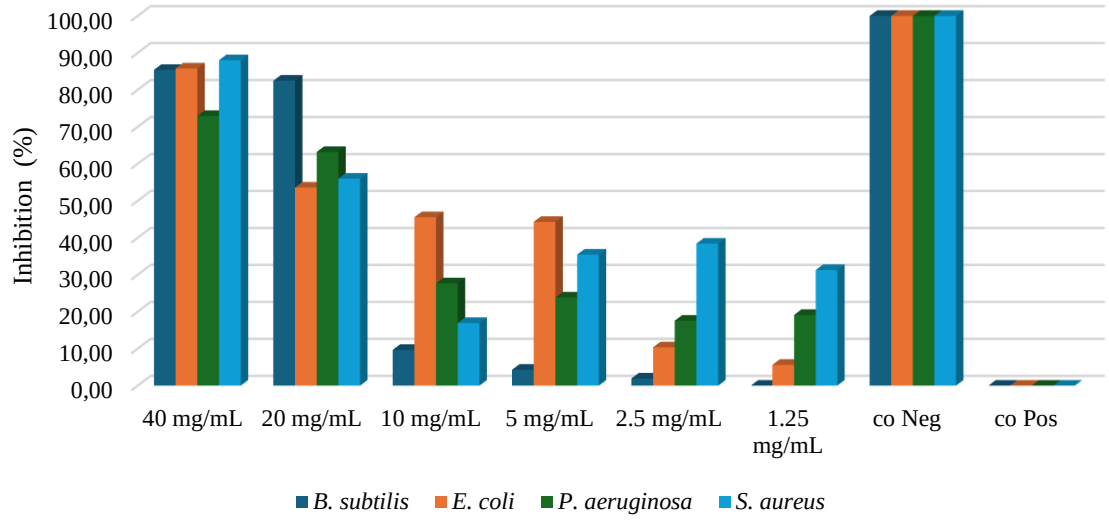
Annex

1) Results statistical analysis of biofilm formation:

%	E. coli			P. aeruginosa			S. aureus			B.subtilis		
25	0,166	0,15	0,16	0,156	0,156	0,156	0,443	0,540	0,454	0,16	0,133	0,123
12,5	0,187	0,156	0,228	0,49	0,471	0,56671	0,610	0,511	0,833	0,49	0,471	0,56671
6,25	1,035	0,95	0,96	0,564	0,664	0,564	1,320	1,310	1,210	0,76	0,611	1,511
3,12	1,08	1,03	1,01	0,611	0,611	0,611	1,511	1,513	1,020	0,87	0,81	0,560
1,56	1,097	1,05	1,05	0,977	0,991	0,981	1,560	1,387	1,430	0,7443	0,573	0,820
0,78	1,25	1,38	1,13	1,003	1,0443	1,0573	1,420	1,543	1,331	0,843	0,650	0,892
co Neg	0,155	0,160	0,154	0,121	0,143	0,135	0,120	0,121	0,122	0,101	0,120	0,109
co Pos	1,080	1,050	1,130	1,100	1,020	1,170	1,710	1,770	1,830	1,150	1,190	1,130



Concentration	B. subtilis	E. coli	P. aeruginosa	S. aureus
40 mg/mL	85,40	85,78	72,94	88,01
20 mg/mL	82,48	53,57	63,20	55,97
10 mg/mL	9,66	45,53	27,68	16,95
5 mg/mL	4,29	44,29	23,84	35,45
2.5 mg/mL	1,93	10,36	17,57	38,41
1.25 mg/mL	0,00	5,64	19,13	31,27
co Neg	100	100	100	100
co Pos	0	0	0	0

2) Results of the two-way ANOVA:

Source of variation	F	P-value	Significant
Treatment	159,41	< 0,001	Highly significant ***
Time	356.55	< 0.001	Highly significant ***
Interaction (treatment ×time)	14,35	< 0,0.001	Highly significant ***

3) Post-hoc comparisons between treatments:

Comparison	Mean difference	Significance
Moringa oil vs. Con	-0.57	Significant (<0.001)***
Mebo ointment vs. Control	-0.39	Significant (<0.001)***
Moringa oil vs. Mebo ointment	-0.18	Significant (<0.05)*

Time course of the three treatments

Data analysis shows that :

• Moringa seed oil: Starts with a wound surface area of 2 cm^2 on day 1, which rapidly decreases to 0 cm^2 by day 13.

• MEBO ointment: Also starts at 2 cm^2 on day 1, but healing is slightly slower, reaching 0 cm^2 by day 20.

• Control group: Healing is significantly slower. The surface area remains at 2 cm^2 until day 7, then gradually decreases to reach only 0 cm^2 by day 20.

Figure A.1. pH measuring device

Biofilm: Photos of experiments and relevant protocols

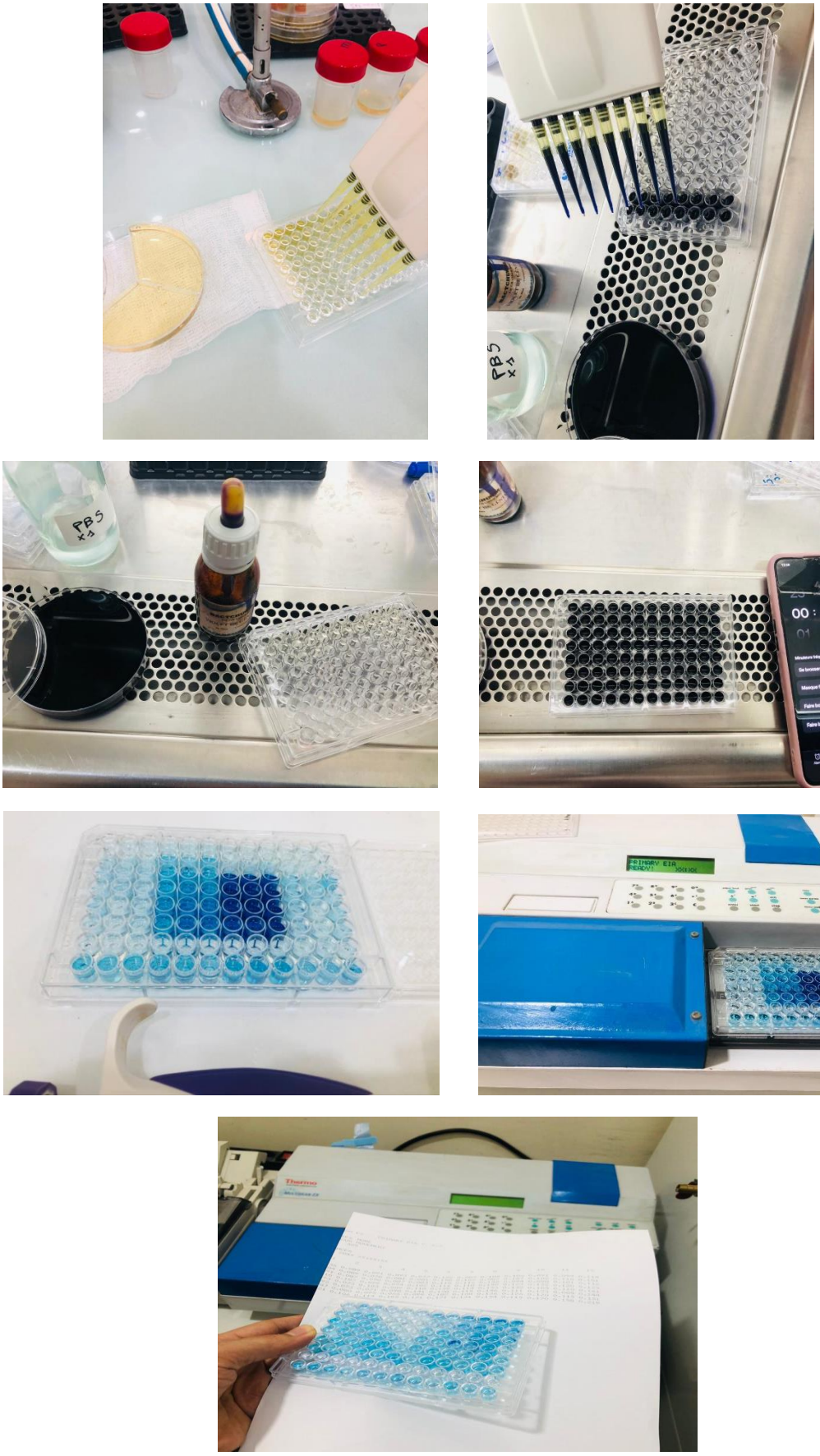
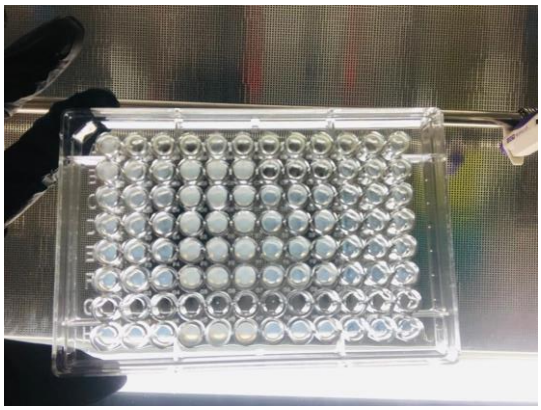


Figure A.2. Experimental steps to evaluate the effect of moringa seed oil on biofilm formation



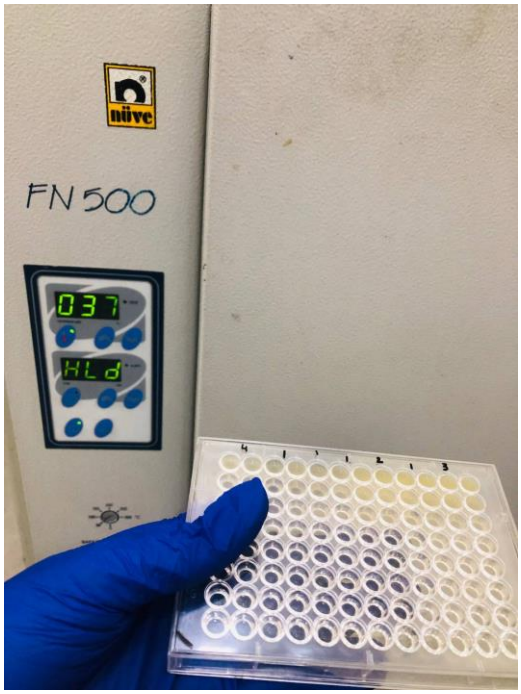


Figure A.3. Broth microdilution method to determine the antimicrobial activity (MIC/MBC) of Moringa seed oil.

Figure A.4. The Moringa seed oil extracted in this experiment