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THEME

Development of Bio-Derm Triple-Action: A Natural Formulation Combining Sun Protection, Skin Tone Enhancement, and Radiance — Study and Development

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بسم الله الرحمن الرحيم

DEDICACES

I dedicate this modest work to my beloved parents, may Allah preserve them and grant them long life, in sincere appreciation of their immense sacrifices, unwavering support, and wise guidance, which have profoundly shaped my academic journey.

I also dedicate this work to my dear family, in recognition of their constant encouragement, heartfelt prayers, and continuous support throughout the completion of this thesis.

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Fatma Zahra

DEDICACES

All praise is due to Allah for the joy of achievement, and all praise is due to Him at the beginning and the end.

As Allah says:

"***And the last of their supplication will be: 'All praise is due to Allah, the Lord of the worlds.'***" (***Qur'an*** 7:206)

With love and gratitude, I dedicate the fruit of my graduation and success:

To the one who adorned my name with the most beautiful titles, who supported me without limits, gave without expecting anything in return, and taught me that success is a struggle wielded by knowledge and learning—my pillar and support after Allah, my pride and honor: my father, whose guidance has always been a beacon lighting my path.

To the one whom Allah has placed paradise beneath her feet, who embraced my heart before my hands, and eased the hardships I faced with her prayers—my tender heart, the source of my strength, and my paradise in this life: my mother, who taught me patience, determination, and loyalty in every step of my life.

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Abstract

This study aimed to develop and scientifically evaluate a multifunctional cosmetic formulation named BIO NOFA, based on a synergistic composition of niacinamide, zinc oxide, and Moringa oleifera seed oil, in order to investigate its antioxidant, ultraviolet-protective, antimicrobial, and antibiofilm properties, in addition to assessing its biological safety.

The physicochemical properties of the formulation were evaluated. The yield of Moringa oleifera seed oil was 38.97%, while the yield of the niacinamide–zinc oxide mixture was 11%. The formulation exhibited a pH ranging from 5 to 5.5, which is suitable for skin applications and supports its compatibility for cosmetic use. The antioxidant activity was assessed using the FRAP assay, and the results demonstrated a considerable reducing capacity, reflecting the presence of biologically active compounds with free radical scavenging properties. These findings are consistent with the scientific literature highlighting the role of phytochemical compounds in enhancing antioxidant protection.

The sun protection factor (SPF) was evaluated using an in vitro method, yielding a value of 57.97, indicating a high capacity to absorb or scatter ultraviolet radiation, particularly due to the presence of zinc oxide, a well-known photoprotective agent.

The formulation also exhibited antibacterial activity against both Gram-positive and Gram-negative bacterial strains, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. The MIC and MBC values ranged between 20 and 40 mg/mL, with the highest sensitivity observed for Staphylococcus aureus (20 mg/mL). In most cases, MIC and MBC values were identical (MBC/MIC = 1), confirming both inhibitory and bactericidal effects. In addition, the formulation demonstrated a notable ability to inhibit biofilm formation, highlighting its potential in preventing microbial adhesion.

The toxicity test using Artemia salina indicated an acceptable level of biological safety, supporting its biocompatibility for topical application.

Overall, the results suggest that BIO NOFA represents a promising multifunctional cosmetic formulation combining antioxidant activity, photoprotective capacity, and antimicrobial effects, with an acceptable safety profile, supporting its potential development and application in advanced skincare formulations.

Key words: -Moringa oleifera seed oil -Zinc oxide -Niacinamide -Antioxidant activity Ferric Reducing -Antioxidant Power (FRAP) assay -Antibacterial activity -Toxicity -Tyrosinase -

الملخص

هدفت هذه الدراسة إلى تطوير وتقييم علمي لمستحضر تجميلي متعدد الوظائف تحت اسمBIO NOFA ، قائم على تركيبة متآزرة تضم النياسيناميد، وأكسيد الزنك، وزيت بذورMoringa oleifera ، وذلك بهدف دراسة خصائصه المضادة للأكسدة، والواقية من الأشعة فوق البنفسجية، والمضادة للميكروبات، والمثبطة لتكوين الأغشية الحيوية، بالإضافة إلى تقييم سلامته الحيوية.

تمت دراسة الخصائص الفيزيائية-الكيميائية للمستحضر فكان مردود زيت بذور المورينغا (38.97%) ومردود مزيج النياسيناميد مع أكسيد الزنك (11%)، وقيمة pH بلغت للمستحضر (5_5.5)، وهي قيمة مناسبة للتطبيقات الجلدية، مما يعزز ملاءمته للاستعمال التجميلي. كما تم تقييم النشاط المضاد للأكسدة باستخدام اختبارFRAP ، وأظهرت النتائج قدرة اختزالية معتبرة، تعكس وجود مركبات حيوية فعالة ذات خصائص كاسحة للجذور الحرة، وهو ما يتوافق مع ما ورد في الأدبيات العلمية حول أهمية المركبات الفيتوكيميائية في تعزيز الحماية المضادة للأكسدة.

تم تقييم الفعالية الواقية من أشعة الشمس (SPF) بطريقةin vitro ، حيث بلغ معامل الحماية الشمسي 57.97، مما يدل على قدرة عالية على الامتصاص أو التشتت للأشعة فوق البنفسجية، خاصة بفضل وجود أكسيد الزنك كمادة واقية معروفة بفعاليتها الضوئية.

كما أظهر المستحضر نشاطًا مضادًا للبكتيريا ضد سلالات موجبة وسالبة الغرام، من بينها Staphylococcus aureusو Escherichia coliو Pseudomonas aeruginosaو Bacillus subtilis، مع قيم MIC و) MBC تراوحت قيم MIC بين 20 و40mg/ml ، مع تسجيل أعلى حساسية لدى .(20 mg/ml) Staphylococcus aureus كما تساوت قيم MIC و MBCفي معظم الحالات (MBC/MIC = 1) تؤكد فعاليته التثبيطية والقاتلة للبكتيريا. إضافة إلى ذلك، تم تسجيل قدرة ملحوظة على تثبيط تكوين الأغشية الحيوية، مما يعزز إمكاناته في الوقاية من الالتصاق الميكروبي.

أظهر اختبار السمية باستخدام Artemia salina درجة أمان حيوية مقبولة، مما يدعم توافقه الحيوي للاستعمال الموضعي .

بشكل عام، تشير النتائج إلى أن مستحضر BIO NOFA يمثل صيغة تجميلية واعدة متعددة الوظائف، تجمع بين النشاط المضاد للأكسدة، والحماية الضوئية، والنشاط المضاد للميكروبات، مع درجة أمان مناسبة، مما يدعم إمكانية تطويره وتطبيقه في مجال العناية الجلدية المتقدمة.

الكلمات المفتاحية: -زيت بذور مورينغا اولوفيرا -اوكسيد الزنك -النياسيناميد -النشاط المضاد للاكسدة -اختبار (frap) -النشاط المضاد للبكتيريا -السمية -التيروزيناز -عامل الوقاية من الشمس(spf)

Résumé

Cette étude avait pour objectif de développer et d'évaluer scientifiquement un produit cosmétique multifonctionnel nommé NOFA BIO, basé sur une formulation synergique comprenant la niacinamide, l'oxyde de zinc et l'huile de graines de Moringa oleifera, afin d'étudier ses propriétés antioxydantes, sa protection contre les rayons ultraviolets, son activité antimicrobienne et son effet inhibiteur sur la formation de biofilms, tout en évaluant sa sécurité biologique.

Les propriétés physico-chimiques du produit ont été étudiées : le rendement en huile de graines de moringa était de 79,83 %, le rendement du mélange niacinamide-oxyde de zinc était de 11 %, et la valeur du pH du produit variait entre 5 et 5,5, une plage appropriée pour les applications cutanées, renforçant ainsi sa compatibilité pour un usage cosmétique. L'activité antioxydante a été évaluée à l'aide du test FRAP, et les résultats ont montré une capacité réductrice significative, reflétant la présence de composés bioactifs efficaces possédant des propriétés de piégeage des radicaux libres, ce qui est en accord avec les données de la littérature scientifique sur l'importance des composés phytochimiques dans la protection antioxydante.

L'efficacité de protection solaire (SPF) a été évaluée par une méthode in vitro, donnant un facteur de protection solaire de 53,83, indiquant une capacité élevée à absorber ou disperser les rayons ultraviolets, en particulier grâce à la présence de l'oxyde de zinc en tant qu'agent photoprotecteur reconnu pour son efficacité.

Le produit a également montré une activité antibactérienne contre des bactéries à Gram positif et Gram négatif, notamment Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa et Escherichia coli. Les valeurs de MIC variaient entre 0,2 et 0,02 mg/mL, avec la plus grande sensibilité observée pour Staphylococcus aureus (MIC = 0,02 mg/mL). Dans la plupart des cas, les valeurs de MIC et MBC étaient équivalentes (MIC/MBC = 1), confirmant à la fois son effet inhibiteur et bactéricide. De plus, une capacité notable à inhiber la formation de biofilms a été observée, renforçant son potentiel dans la prévention de l'adhésion microbienne.

Le test de toxicité utilisant Artemia salina a montré un niveau de sécurité biologique acceptable, confirmant sa biocompatibilité pour un usage topique.

En résumé, les résultats indiquent que le produit BIO NOFA représente une formulation cosmétique prometteuse et multifonctionnelle, combinant activité antioxydante, protection photique, activité antimicrobienne et sécurité appropriée, soutenant ainsi son potentiel de développement et d'application dans le domaine des soins cutanés avancés.

Mots clés: -Huile de graines de -Moringa oleifera -Oxyde de zinc -Niacinamide -Activité antioxydante Essai du pouvoir -antioxydant réducteur du fer (FRAP) -Activité antibactérienne -Toxicité -Tyrosinase -Facteur de protection solaire (SPF)

Content

Experimental Analysis

CHAPTER I:Materials and methods

CHAPTER II:Results and discussion

Liste of Abbreviation

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
IC50	Half Maximal Inhibitory Concentration
v/v	Volume per Volume
°C	Degrees Celsius
cm	Centimeter
cm²	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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INTRODUCTION

Introduction

edicinal plants have been recognized for centuries for their therapeutic value and continue to serve as a rich source of bioactive compounds for the

Development of natural remedies and skincare products. In recent decades, global demand for natural products has increased significantly, driven by growing awareness of the limitations of conventional chemical products 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, and natural products have increasingly become a focus of modern cosmetic industries.

In Algeria, a country rich in biodiversity and ethnobotanical heritage, medicinal plants are still widely used in both traditional and modern practices, particularly in rural areas where access to cosmetic products may be limited.

Among the promising medicinal plants that have attracted researchers' attention is Moringa oleifera, known as the "miracle tree," due to its diverse bioactive components and its traditional and modern medical and cosmetic applications (Leone et al., 2015; Anwar et al., 2007). Moringa seeds contain a high oil content rich in essential fatty acids, phenolics, flavonoids, and tocopherols, which provide the skin with antioxidant, moisturizing, and tissue-regenerating properties (Vergara-Jimenez et al., 2017; Mbikay, 2012; Rockwood et al., 2013).

In addition to Moringa seed oil, niacinamide (Vitamin B3) plays a key role in skincare, helping to strengthen the skin barrier, even out skin tone, reduce pigmentation, and improve elasticity (Bissett et al., 2005). Furthermore, zinc oxide (ZnO) is an effective ingredient for protection against UVA/UVB radiation, reduces irritation, and has mild antibacterial properties, making it a valuable addition to protective cosmetic formulations (Moghassemi & Hadjizadeh, 2014).

Based on these properties, the present study aims to develop a triple-action cosmetic product combining Moringa seed oil, niacinamide, and zinc oxide, with the goal of achieving an integrated effect that includes:

Skin hydration and cell regeneration

Even skin tone and enhanced skin barrier function

Protection against UV radiation and reduced irritation Thesis Problematic

This study seeks to answer the following key questions:

To what extent does the product exhibit antioxidant properties due to Moringa seed oil? Does niacinamide contribute to improving skin tone and strengthening the skin barrier when applied topically?

Does zinc oxide provide effective protection against UV radiation and reduce skin irritation?

Does combining these three components in a single product lead to significant improvements in skin texture, hydration, and tone compared to using each ingredient individually?

Thesis Structure

To address these questions, the thesis is divided into two main parts: Theoretical Part – three chapters:

Chapter 1: General background and literature review on Moringa seed oil and its cosmetic properties.

Chapter 2: General study of active ingredients in skincare products, with a focus on niacinamide and zinc oxide, and a review of their mechanisms of action.

Chapter 3: Chemical analysis and biological evaluation of the triple-action product, focusing on antioxidant and antibacterial activities, inhibitory mechanisms, biofilm prevention, and UV protection.

Experimental Part – two chapters:

Chapter 1: Description of materials, experimental methods, and protocols used to evaluate the efficacy of the triple-action product.

Chapter 2: Presentation and discussion of results, including antioxidant assays, sun protection factor (SPF) evaluation, antibacterial tests, and skin safety assessments.

FIRST PART

Bibliographic synthesis

CHAPTER I
Scientific Foundations of Moringa oleifera and Its Seed Oil: From Phytochemical Composition to Cosmetic Applications

PART 1: General overview of Moringa Oleifera

1.1. Introduction

This section presents Moringa oleifera as a versatile multipurpose tree recognized for its medicinal, nutritional, and agricultural significance. It emphasizes the growing global interest in this species, particularly in resource-limited regions, owing to its high adaptability and well-documented health- promoting properties.

Figure 1.1. Moringa oleifera tree (Permamercy Tanzania, n.d.)

1.2 Moringa Oleifera

Moringa oleifera is a perennial tree characterized by rapid growth and strong resistance to drought, and it belongs to the Moringaceae family. It is widely recognized for its remarkable nutritional value and diverse therapeutic properties. Known by several common names, including the drumstick tree, horseradish tree, and ben oil tree, it is often described as the "Miracle Tree" or the "Tree of Life" because of its broad range of uses and its importance in both traditional practices and modern applications (Islam et al., 2021).

The importance of this species lies in the fact that almost all of its parts—such as the leaves, seeds, pods, flowers, and roots—are edible and exhibit medicinal properties. This exceptional characteristic makes M. oleifera one of the most versatile and beneficial plant species worldwide.

In recent years, M. oleifera has attracted increasing scientific attention, mainly due to its high content of essential nutrients and its richness in bioactive compounds with strong biological activity. As a result, it has become a major focus of research in the areas of functional foods, nutraceuticals, and phytomedicine. Moreover, its ability to adapt to a wide range of

climatic conditions and its high tolerance to arid environments make it a strategically important crop for sustainable cultivation, particularly 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

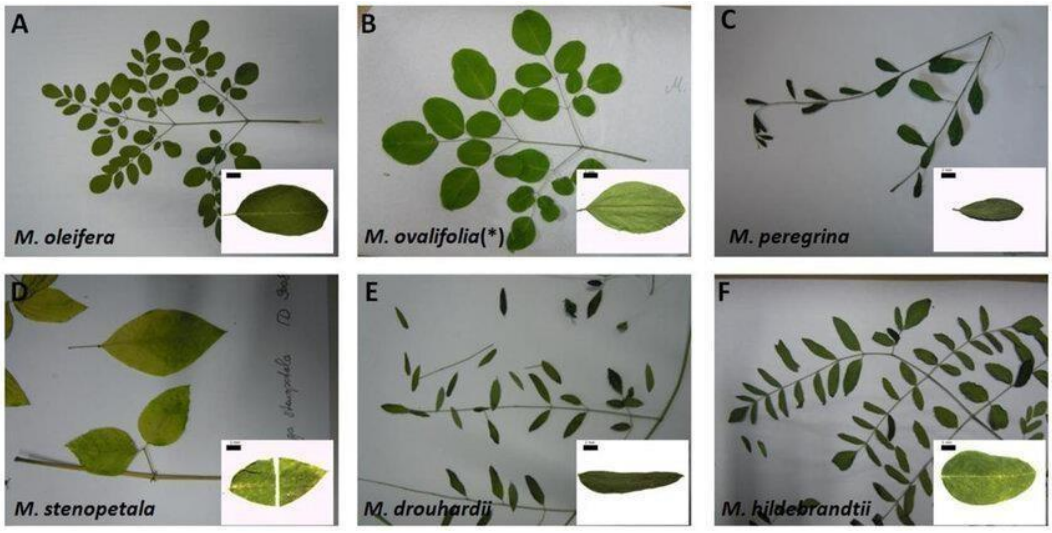


Figure 1.2. Comparison of leaf appearance of Moringa species (Wetters et al., 2024)

Table 1.2 list of moringa species throughout the world

Species	Country	Trivial name
M. arborea Verdcourt	Kenya, Somalia	–
M. borziana Mattei	Kenya, Somalia	–
M. concanensis Nimmo	India	–
M. drouhardii Jumelle	Southern Madagascar	–
M. hildebrandtii Engler	Southwest Madagascar	**Hildebrandt's Moringa**
M. longituba Engler	Kenya, Southeast Ethiopia, Somalia	**Moringa tubiflora**
M. oleifera Lam.	India	**Horseradish, Ben-oil Drumstick, Kelor**
M. ovalifolia Dinter ex Berger	Namibia, Southwest Angola	**Phantom Tree, Ghost Tree, African Moringa**
M. peregrina Forssk. ex Fiori	Red Sea, Arabia, Northeast Africa	**Ben tree, wispy-needled Yasar tree, Wild drumstick tree, Yusor, Al Yassar, Al Ban**
M. pygmaea Verdcourt	North Somalia	–
M. rivae Chiovenda	Kenya, Ethiopia	**Swanjehro**
M. ruspoliana Engler	Kenya, Ethiopia, Somalia	–
M. stenopetala (Baker f.) Cufodontis	**Kenya, Southwest Ethiopia, Somalia**	**Cabbage tree, Haleko, Shelqada, Shiferaw**

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.

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 (Shutterstock)

·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 (Floreo, 2024)

·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 (Altibbi, n.d.)

·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 (Morten, 2025)

· 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 (Wyss Samen und Pflanzen AG, 2024)

· 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 (Team Ethique, 2021)

· Roots

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

Figure 1.9. Moringa roots (IndiaMART, n.d.)

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

1.7. Ecology of Moringa oleifera

Moringa oleifera has significant ecological value due to its ability to adapt to a wide range of climatic conditions and soil types, as well as its resilience to unfavorable environmental conditions. It is considered a fast-growing, high-yield plant that requires less water compared to many other crops. Furthermore, Moringa oleifera is recognized for its ability to enhance soil fertility through atmospheric nitrogen fixation, thereby reducing dependence on chemical fertilizers. Owing to its deep root system, it also contributes to soil stabilization and the prevention of erosion. These characteristics make Moringa oleifera a promising plant for the rehabilitation of degraded soils and the conservation of natural resources (Marcu et al., 2012).

Table 1.3 les exigences écologiques de la plante Moringa oleifera (Fuglie, 2001)

FACTEUR	EXIGENCE
CLIMAT	Tropical à subtropical, températures moyennes de 25-35°C, précipitations annuelles de 600-1200 mm
SOL	Bien drainé, fertile, pH de 6,0-9,0
LUMIERE	Plein soleil à mi-ombre
PH	Le Moringa tolère une grande plage de pH (de 5 à 9), et pousse assez bien dans les milieux alcalins jusqu'à un pH de 9.
EAU	Besoins en eau modérés, tolérance à la sécheresse
HUMIDITE	Tolérance à l'humidité élevée

1.8. Origin and Geographical Distribution of Moringa Oleifera

Moringa oleifera originates from the Agra and Oudh regions, located in northeastern India, south of the Himalayan mountain range. In the early 20th century, it was introduced to Eastern Africa through significant maritime trade and commercial exchanges of that period (Foidl, 2001)

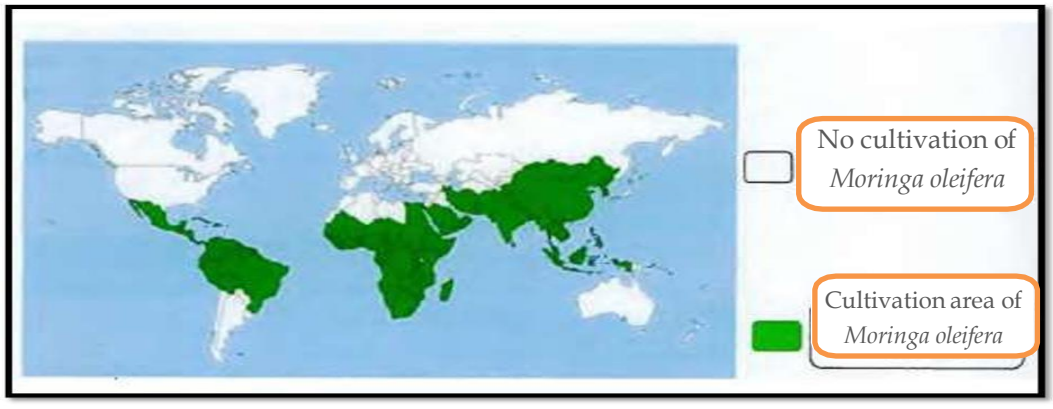


Figure 1.10. Cultivation areas of Moringa oleifera (Rongead, 2014)

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 characterized by a high content of nutrients, antioxidants, and phytochemical compounds (Table 3), particularly β-carotene, iron, and antinutritional factors. Its leaves are a high-quality vegetable and are considered among the most nutritionally valuable tropical leafy vegetables (Yang et al., 2008).

A large number of reports, both scientific and popular, now document the nutritional qualities of Moringa oleifera. Its leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas

(Figure 6). The protein quality of Moringa is comparable to that of milk and eggs. Indeed, the nutritional properties of this plant are now well established, and there is little doubt that its consumption provides substantial health benefits (Fuglie, 1999).

Table 1.4 Nutritional values of the M. oleifera plant (Data for 100 g of dried leaves Broin, 2005).

Minerals (mg)	Amount	Amino Acids (mg)	Amount
Calcium	2100	Arginine	**1600**
Copper	1	Histidine	**530**
Iron	27	Leucine	**2050**
Potassium	1300	Lysine	**1200**
Magnesium	405	Methionine	**370**
Phosphorus	310	Phenylalanine	**1400**
Manganese	8	Threonine	**1080**
Selenium	2.6	Tryptophan	**580**
Zinc	2.6	Valine	**1400**
Molybdenum	0.5	Isoleucine	**1140**
Sodium	100		
Fatty Acids (mg)	Amount	Vitamins (mg)	**Amount**
C16:0	530	Vitamin A	**14300**
C18:0	70	Vitamin B	**850**
C18:1	60	Vitamin B1	**264**
C18:2	170	Vitamin B2	**205**
C18:3	11400	Vitamin C	**220**
		Vitamin E	**130**

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. (Leone et al., 2015)

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.(Islam et al., 2021)

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. (Warra, 2014)

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. (Bichi,2013)

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).

The Role of Moringa in Deep Skin Hydration

Moisturizing extracts: The leaves and seeds of Moringa oleifera contain fatty acids, vitamins, and antioxidants that help retain water within the skin.

Enhancement of the skin barrier: Moringa contributes to strengthening the skin's protective barrier, thereby reducing moisture loss through the upper layers of the skin.

Sustained hydration: Regular use of formulations containing Moringa significantly and continuously increases the moisture levels in the skin (Álvarez-Román et al., 2020).

PART 2:Essential oils: Moringa Oleifera seed extract oil.

1.1. Introduction to Essential Oil

SSENTIAL oils are concentrated plant extracts that contain volatile chemical compounds responsible for the characteristic aroma of plants. These oils are usually

obtained from different parts of the plant such as flowers, leaves, seeds, peels, or roots, using various extraction methods, the most common of which include steam distillation, cold pressing, and solvent extraction. Essential oils are complex mixtures of natural compounds, including terpenes, terpenoids, and phenolic compounds, which give them their distinctive aromatic and biological properties.

The chemical composition of essential oils varies depending on several factors such as plant species, environmental conditions, geographical location, growth stage, and extraction method, leading to significant differences in their biological activity. Essential oils have been used since ancient times in traditional medicine and aromatherapy, and they continue to attract increasing scientific interest due to their antioxidant, antimicrobial, and anti-inflammatory properties.

Figure 1.11. Various essential oil bottles with herbs (France Bleu, n.d.)

In addition to their role in human applications, essential oils play important ecological functions in plants, including defense against insects and pathogenic organisms, attraction of pollinators, and involvement in chemical interactions between plants. Today, essential oils are widely used in the pharmaceutical, cosmetic, food, and agricultural industries, making them an important subject of scientific research and industrial application.

1.2. Essential Oil of Moringa Oleifera Seed

Moringa oleifera seed oil is classified as a fixed oil rather than a true essential oil because it is mainly composed of fatty acids rather than volatile aromatic compounds. Nevertheless, the oil contains a small fraction of volatile compounds that contribute to its characteristic aroma and some of its biological properties (Leone et al., 2016; Stohs & Hartman, 2015). The oil is extracted from the seeds of the Moringa oleifera tree, which are known for their high oil yield, typically ranging from approximately 35 to 40% of the seed weight (Anwar et al., 2007).

The fatty acid composition of Moringa oleifera seed oil is dominated by oleic acid (C18:1), a monounsaturated fatty acid that generally represents about 65–75% of the total fatty acids (Tsaknis et al., 1999; Leone et al., 2016).

Other fatty acids are also present in smaller proportions, including:

- palmitic acid (C16:0)
- stearic acid (C18:0)
- behenic acid (C22:0)
- arachidic acid (C20:0)
- and linoleic acid (C18:2) (Tsaknis et al., 1999; Idris et al., 2020).

This distinctive fatty acid profile, particularly the high oleic acid content, contributes to the oxidative stability of the oil and enhances its suitability for nutritional, pharmaceutical, and cosmetic applications (Anwar et al., 2007; Stohs & Hartman, 2015).

In addition to fatty acids, Moringa oleifera seed oil contains several bioactive compounds that contribute to its functional properties. These include:

- tocopherols (vitamin E): which act as natural antioxidants and protect the oil from oxidative degradation (Leone et al., 2016; Stohs & Hartman, 2015).
- The oil also contains phytosterols :such as β-sitosterol,
- stigmasterol, and campesterol, which are associated with beneficial biological activities (Anwar et al., 2007; Stohs & Hartman, 2015).

Furthermore, small quantities of phenolic compounds and carotenoids have been reported in the oil, contributing to its antioxidant potential and characteristic color (Leone et al., 2016; Kumar & Sharma, 2020).

Although present in relatively small amounts, the volatile fraction of Moringa oleifera seed oil contains several compounds responsible for its mild aroma, including:

- aldehydes such as hexanal,
- 2-hexenal, octanal, and nonanal, as well as ketones

– and certain terpenoid compounds (Leone et al., 2016; Stohs & Hartman, 2015).

From a physicochemical perspective, Moringa oleifera seed oil is typically described as a pale yellow to golden liquid with a mild nut-like aroma. It has a relatively high smoke point (around 200 °C), low viscosity, and good spreadability, characteristics that make it particularly suitable for cosmetic and dermatological formulations (Anwar et al., 2007; Idris et al., 2020).

1.3. Extraction Methods

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

1.3.1. Cold Pressing

Cold pressing is a mechanical method for extracting vegetable oils that relies on applying pressure to the seeds to release the oil without the use of external heat. This method preserves the physical and chemical properties of the oil, including its nutritional value, aromatic compounds, and natural color. Moringa seed oil obtained through cold pressing is considered high-quality and is widely used in premium cosmetic and culinary applications.

The extraction process typically involves a series of steps, including cleaning and sorting the seeds, removing the outer seed coats, grinding the seeds into a paste, and then pressing the paste using specialized mechanical presses. After pressing, the oil is filtered to remove impurities and solid particles.

Although the yield of oil obtained by this method is lower compared to solvent extraction—typically ranging between 25–30% of the seed weight—the final quality of the oil is superior in terms of chemical stability and nutritional properties. (Mat Yusoff, 202, Boukandoul et al., 2018)

Figure 1.12. Cold pressing extraction method (Geramitcioski et al., 2018)

1.3.2.Solvent Extraction

The solvent extraction method involves the use of organic solvents, such as hexane, to dissolve the oil from the seed material. The general procedure typically includes the following steps:

- Grinding the seeds to increase their surface area.
- Mixing the ground seeds with the solvent to allow oil dissolution. Separating the solvent-oil mixture from the remaining seed residue. Evaporating the solvent to recover the extracted oil.
- Refining the oil to remove any residual solvent and impurities.

Compared to cold pressing, solvent extraction generally yields a higher amount of oil (up to 35–40% of the seed weight). However, there are concerns regarding possible solvent residues in the final product, even after refining. This technique is commonly applied in industrial-scale production and for oils intended for non-food applications. (Anwar et al., 2019)

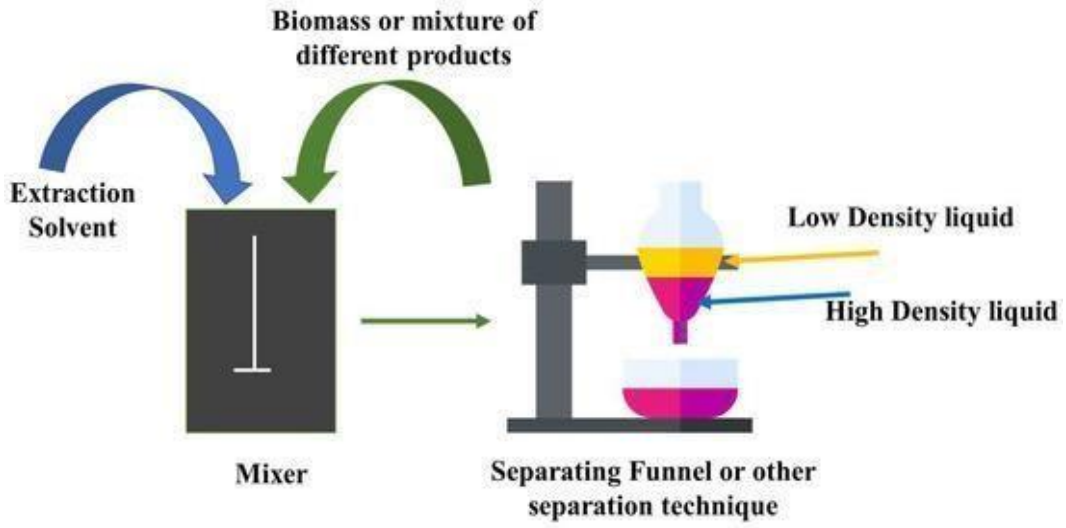


Figure 1.13. Schematic diagram of the solvent extraction method (Usman et al., 2023)

1.3.3.Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction, particularly using carbon dioxide (CO_2), is a modern technique that provides several advantages for the extraction of Moringa seed oil. In this method, CO_2 is pressurized and heated to a supercritical state, where it exhibits the properties of both a liquid and a gas. The supercritical CO_2 acts as a solvent, effectively extracting oil from the seed material. Upon releasing the pressure, the CO_2 returns to its gaseous state, leaving behind pure oil.

SFE with CO_2 offers several benefits:

- It operates at relatively low temperatures, preserving heat-sensitive bioactive compounds.

- CO_2 is non-toxic and leaves no solvent residues in the oil.
- Extraction parameters can be adjusted to selectively target specific compounds. The method produces a high-quality oil free from solvent residues.

However, the technique has some limitations:

- The equipment is expensive.
- The method may be less economically feasible for large-scale industrial production compared to conventional solvent extraction. (Zhang et al., 2014; Zhang et al., 2021)

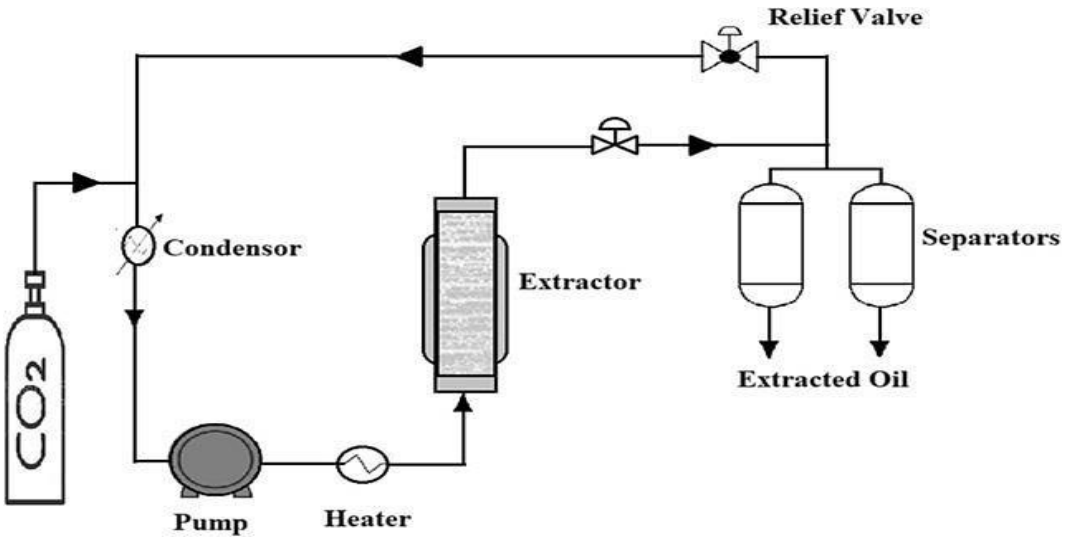


Figure 1.14. Supercritical CO2 extraction method (Gaber et al., 2018)

1.3.4.Aqueous Enzymatic Extraction

Aqueous enzymatic extraction (AEE) is an environmentally friendly alternative to conventional solvent extraction. This technique employs water and specific enzymes to break down the cell walls of the seed material, facilitating the release of oil. The procedure generally includes the following steps:

1. Grinding the seeds and mixing them with water to form a uniform slurry.
2. Adding enzymes (such as cellulases, hemicellulases, and proteases) to degrade the cell walls.
3. Incubating the mixture at optimal temperature and pH conditions for enzyme activity.
4. Separating the oil from the aqueous phase and the residual solid matter.
5. Purifying the oil to remove impurities and obtain the final product.

This approach eliminates the need for organic solvents, making it environmentally sustainable and suitable for food-grade oil production. However, it generally yields less oil than solvent extraction and requires careful control of the extraction parameters to achieve optimal efficiency. (Yusoff et al., 2016)

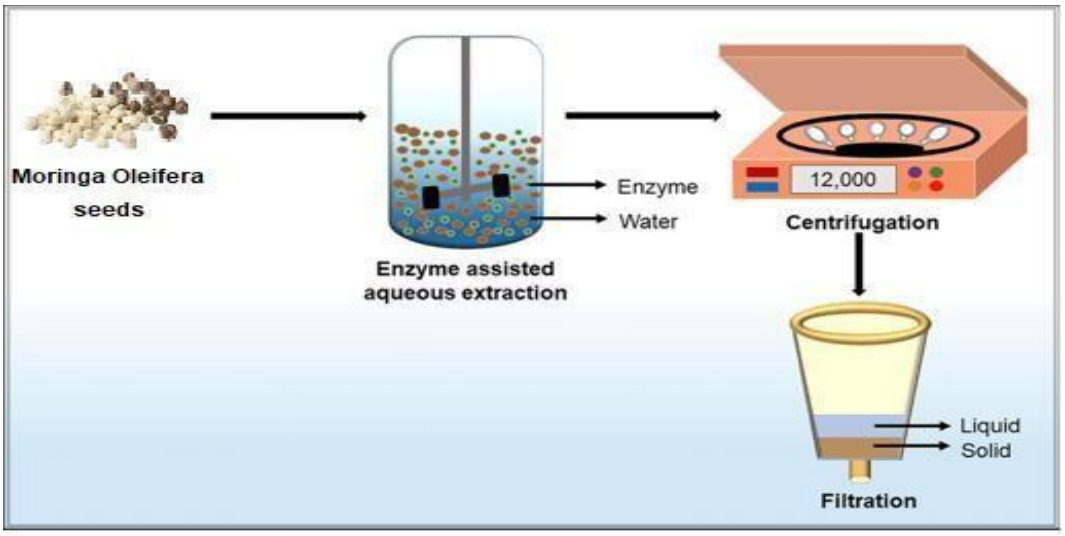


Figure 1.15. Enzymatic Extraction Method (Nigam et al., 2021)

1.4. Biological Activities

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

1.4.1. Antioxidant Activity

Moringa oleifera seed oil is rich in natural antioxidants, including tocopherols, phenolic compounds, and carotenoids, which are capable of neutralizing free radicals and preventing oxidative damage. These antioxidant components protect the oil from rancidity and contribute to its potential health benefits, whether consumed or applied topically (Wang et al., 2017).

Research has demonstrated that Moringa seed oil can scavenge free radicals, inhibit lipid peroxidation, and protect against oxidative stress-induced cellular damage. The antioxidant activity is commonly assessed using assays such as DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP).

The strong antioxidant properties of Moringa seed oil make it particularly valuable in anti-aging skincare formulations, as oxidative stress is a major factor in skin aging. The oil can help shield the skin from environmental stressors, including UV radiation and pollution, thereby supporting skin health and resilience.

1.4.2. Antimicrobial Activity

Moringa oleifera seed oil has shown significant antimicrobial activity against a range of bacteria and fungi. This activity is primarily attributed to certain fatty acids and other bioactive compounds present in the oil. Research indicates that the oil can inhibit the growth of both Gram-positive bacteria (e.g., Staphylococcus aureus) and Gram-negative bacteria (e.g., Escherichia coli), as well as some fungal species (e.g., Candida albicans).

The antimicrobial properties of Moringa seed oil make it a valuable ingredient in formulations designed to treat skin infections, acne, and other conditions related to microbial overgrowth. Additionally, the oil is employed in natural preservative systems for cosmetics and personal care products (Ruttarattanamongkol & Petrasch, 2015).

1.4.3. Anti-Inflammatory Activity

Moringa oleifera seed oil has demonstrated notable anti-inflammatory properties in multiple studies. The oil is capable of inhibiting the production of pro-inflammatory mediators and reducing inflammation in various experimental models. This activity is largely attributed to several bioactive compounds, including oleic acid, which is well- known for its anti-inflammatory effects.

The anti-inflammatory properties of Moringa seed oil make it a valuable component in formulations designed to treat 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).

1.4.4.Moisturizing and Emollient Properties

Moringa oleifera seed oil is well-recognized for its excellent moisturizing and emollient properties. The oil can penetrate the skin easily, delivering deep hydration without leaving a greasy residue. It also helps strengthen the skin barrier, prevents water loss, and improves skin elasticity and smoothness (Athikomkulchai et al., 2020).

These moisturizing effects are primarily attributed to the oil's fatty acid composition, particularly its high oleic acid content. Oleic acid is highly compatible with the skin's natural oils and contributes to restoring and maintaining the skin's lipid barrier.

CHAPTER II

Biological Role and Dermatological Applications of Niacinamide and Zinc Oxide

Chapiter II: Biological Role and Dermatological Applications of Niacinamide and Zinc Oxide

2.1 Introduction of vitamin

Vitamins are a class of organic compounds that are essential for human health and survival, yet they cannot be synthesized by the human body in adequate amounts and therefore must be obtained through the diet. Historically, the concept of vitamins emerged from early nutritional research identifying specific dietary factors that prevented deficiency diseases, which highlighted their critical role in maintaining physiological functions. Although the definition of vitamins has evolved over time, they remain recognized as micronutrients required in small quantities to support normal growth, development, and metabolic integrity. Different vitamins vary considerably in chemical structure and biological function, yet collectively they contribute to vital processes such as energy metabolism, immune function, and the maintenance of cellular and organ health. Modern definitions increasingly emphasize their necessity for long-term health beyond merely preventing classical deficiency diseases.

2.2. Definition of vitamin B3

Vitamin B3, scientifically known as niacin, is a water-soluble vitamin that belongs to the B- complex group and is considered an essential nutrient indispensable for maintaining normal metabolic functions in the human body.

Niacin occurs primarily in two biologically active forms: nicotinic acid and nicotinamide (niacinamide). Vitamin B3 plays a central role as a precursor for the synthesis of the coenzymes nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), which are involved in numerous oxidation–reduction reactions essential for energy production from carbohydrates, fats, and proteins, as well as for DNA repair and cellular signaling processes (Encyclopaedia Britannica, n.d.; Kirkland, 2018).

In humans, limited amounts of niacin can be synthesized endogenously from the amino acid tryptophan; however, this synthesis is insufficient to meet physiological requirements, making dietary intake necessary to maintain normal metabolic homeostasis (Encyclopaedia Britannica, n.d.). Deficiency of vitamin B3 leads to pellagra, a condition classically characterized by the clinical triad of dermatitis, diarrhea, and neurological disturbances, underscoring the critical importance of niacin for human health (Kirkland, 2018).

2.3 Chemical Structure of Vitamin B3

- Vitamin B3 is chemically known as niacin and is primarily represented by nicotinic acid.
- Nicotinic acid is classified as a pyridine derivative, and its molecular structure consists of an aromatic pyridine ring.
- The pyridine ring contains a carboxyl group (–COOH) attached at the third position, which determines its chemical and acidic properties.
- The molecular formula of vitamin B3 (nicotinic acid) is $C_6H_5NO_2$.
- The molecular weight of nicotinic acid is approximately 123.11 g/mol.
- This chemical structure forms the basis of the biological role of vitamin B3 as a precursor for the synthesis of the enzymatic cofactors NAD^+ and $NADP^+$, which are essential for cellular oxidation
- reduction reactions (National Center for Biotechnology Information [NCBI], 2024)

2.4. Forms of Vitamin B3 (Niacin)

Vitamin B3 consists of several chemically related compounds, known as vitamers, which act as precursors for NAD^+ synthesis, a key cofactor in cellular oxidation–reduction reactions (Orlandi, 2020; PubMed, 2019).

2.4.1Nicotinic Acid (Nicotinic Acid / Niacin):

- The classic dietary form.
- Converted in the body to NAD^+ and $NADP^+$.
- Participates in energy metabolism and redox reactions.
- High doses may cause skin flushing (Orlandi, 2020; PubMed, 2019).

2.4.2 Nicotinamide Riboside (Nicotinamide Riboside / NR):

- A newer form, highly effective as a precursor for NAD^+.
- Supports cellular energy production.
- Studied for its potential roles in healthspan and aging (Balancing, 2022).

2.4.3Niacinamide (Niacinamide / Nicotinamide):

- The amide form of vitamin B3, in which the carboxyl group (–COOH) of nicotinic acid is replaced by a carboxamide group (–$CONH_2$) (Orlandi, 2020; PubMed, 2019).
- Acts as a direct precursor for NAD^+ and $NADP^+$, enabling essential redox reactions and cellular energy metabolism.
- Does not cause flushing, unlike nicotinic acid.
- Widely used in nutritional supplements, pharmaceutical preparations, and dermatological applications, including skin aging and pigmentation control (Boo, 2021).
- Summary: All forms of vitamin B3 contribute to maintaining cellular NAD^+ levels, ensuring proper metabolic function and supporting overall health (Orlandi, 2020; Balancing, 2022; Boo, 2021; PubMed, 2019)

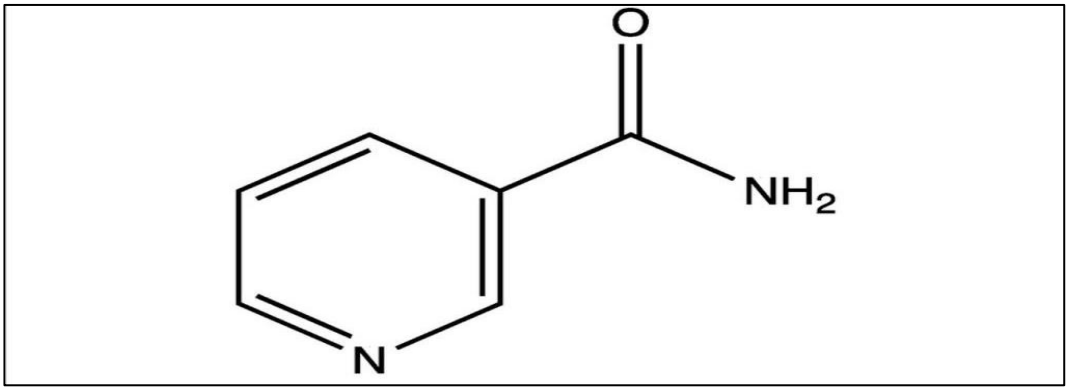


Figure 2.1. The 2D structure of niacinamide

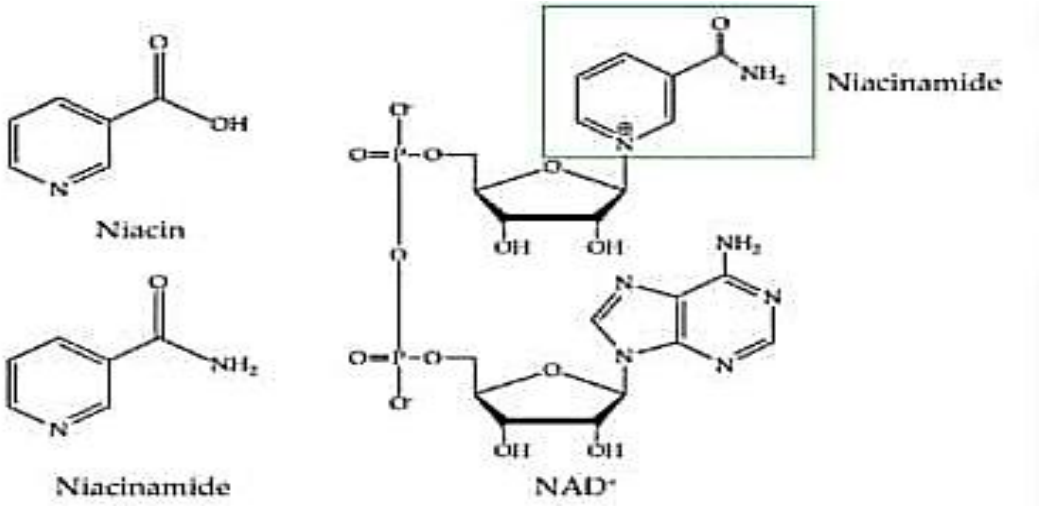


Figure 2.2. Molecular structures of niacin and niacinamide in the vitamin B3 complex and their molecular constitutive role in NAD+ synthesis. NAD+, nicotinamide adenine dinucleotide.

2.5. Niacinamide Concentrations Used

2.5.1Very Low Concentrations (≤ 0.1%):

Very low concentrations of niacinamide are used in certain dermatological formulations intended to provide mild activity or to support the skin barrier, particularly in products designed for sensitive skin or for long-term use. Safety assessments have demonstrated that these concentrations are well tolerated and are not associated with significant adverse cutaneous effects (Cosmetic Ingredient Review Expert Panel, 2005).

2.5.2Commonly Used Concentrations in Dermatological Formulations (up to 3%):

Niacinamide is commonly incorporated into creams, lotions, and spray formulations at concentrations of up to 3%, where it contributes to improving skin hydration, enhancing epidermal barrier function, and reducing transepidermal water loss. These concentration ranges are among the most extensively evaluated with respect to safety in dermatological pharmaceutics (Cosmetic Ingredient Review Expert Panel, 2005).

2.5.3Clinically Effective Concentrations (4–5%):

Clinical studies indicate that moderate concentrations of niacinamide, particularly within the range of 4–5%, are the most effective for improving signs of skin aging, regulating hyperpigmentation, and supporting overall skin functional homeostasis. This concentration range is therefore widely adopted in cosmeceutical and pharmaceutical– cosmetic formulations with functional therapeutic objectives (Boo, 2021).

2.5.4High Concentrations (> 5%):

Although commercial products containing niacinamide at concentrations exceeding 5% are available on the market, published clinical data regarding the safety and efficacy of these higher levels remain limited. Consequently, such concentrations are not generally considered optimal in dermatological pharmaceutics when compared with moderate concentrations that are supported by stronger scientific and clinical evidence (Boo, 2021).

2.6. Pharmaceutical, Cosmeceutical, and Paracosmeceutical Forms of Niacinamide (Nicotinamide)

2.6.1. Topical Creams and Lotions

Topical creams and lotions are among the most widely used forms of niacinamide in both pharmaceutical and cosmeceutical products. These emulsions allow for even distribution of the active ingredient over the skin surface, enhance barrier function, improve hydration, and help reduce signs of aging (e.g., fine lines and uneven pigmentation).

Clinical evidence supports their tolerability and efficacy in long-term daily use for improving skin appearance and function (Boo, 2021; Marques et al., 2024).

2.6.2. Topical Gels

Niacinamide can also be formulated into gel systems, which possess a non-greasy texture and are suitable for oily and acne-prone skin. These formulations allow rapid absorption and efficient penetration into epidermal layers, maintaining cutaneous barrier integrity while providing adequate bioavailability (Vesicular Nanocarrier Gel System with Niacinamide, 2025; Applied Sciences, 2025).

2.6.3Serums and Light Emulsions

Serum formulations of niacinamide, often combined with other actives such as peptides or antioxidants, are common in cosmeceutical skincare due to their high concentration and rapid penetration. These products enhance skin brightness, reduce inflammation, and support barrier repair. Clinical research indicates measurable benefits in skin hydration and complexion when used consistently (Boo, 2021; Cosmetics, 2025).

2.6.4Penetration-Enhanced Formulations

Advanced solvent systems and formulation enhancers have been explored to improve niacinamide’s transdermal delivery. These strategies increase diffusion through the stratum corneum without disrupting barrier function, enhancing therapeutic efficacy in topical applications (Niacinamide: a review on dermal delivery strategies, 2025).

2.6.5Nanocarrier-Based Topical Formulations

Nanocarrier-based delivery systems, such as vesicular carriers and nanoemulsions, enhance penetration, prolong residence time in the epidermis, and allow controlled release. These systems are particularly valuable for cosmeceutical and dermatological applications targeting skin aging, hyperpigmentation, and oxidative stress (Vesicular Nanocarrier Gel System with Niacinamide, 2025; Boo, 2021).

2.6.6Cosmeceutical Emulsions and Complex Formulations

Niacinamide is frequently used in cosmeceutical emulsions combined with functional ingredients (e.g., collagen, ceramides, plant extracts) to enhance skin health beyond basic hydration. These products are designed for stability, safe pH, desirable texture, and improved barrier support (Cosmetics, 2025; Marques et al., 2024)

2.7. Nicotinamide Metabolism as a Key Regulator of Skin Hydration and Barrier Function

The metabolism of niacinamide (nicotinamide) within skin cells represents a fundamental process in maintaining skin health and barrier function. Niacinamide is primarily metabolized through the salvage pathway to generate nicotinamide adenine dinucleotide (NAD^+), an essential coenzyme involved in cellular energy production, regulation of redox reactions, and DNA repair mechanisms (Covarrubias et al., 2021; Revollo et al., 2004). In epidermal cells, the availability of NAD^+ derived from niacinamide metabolism supports keratinocyte activity and differentiation, which are crucial for preserving the integrity of the stratum corneum.

Furthermore, studies have demonstrated that niacinamide, through its indirect metabolic effects mediated by NAD^+, enhances the synthesis of structural skin lipids such as ceramides, leading to a reduction in transepidermal water loss (TEWL) and an improved capacity of the skin to retain moisture, thereby promoting skin hydration (Boo, 2021; Sjöberg et al., 2025). In addition, the support of NAD^+-dependent cellular repair processes contributes to the maintenance of skin structure and the attenuation of oxidative stress, which positively affects skin elasticity and barrier function (Covarrubias et al., 2021).

Consequently, the beneficial effects of niacinamide on skin hydration are not limited to its topical action but are also closely associated with the products of its cellular metabolism, particularly NAD^+, which sustain cellular energy metabolism, reinforce the skin barrier, and

regulate water homeostasis within the epidermis. This highlights the close relationship between niacinamide metabolism and skin hydration and health (Boo, 2021; Sjöberg et al., 2025.

2.8. Uses of Niacinamide in Cosmetics

2.8.1Enhancing skin hydration and barrier function:

- Niacinamide reduces transepidermal water loss (TEWL) and increases moisture content in the stratum corneum.
- Stimulates ceramide and keratin production, strengthening the skin barrier and reducing water loss.
- Studies have shown that creams containing 2–5% niacinamide improved skin hydration, smoothness, and elasticity after 4 weeks of regular application (Gehring, 2004; MDPI, 2024; Ong & Goh, 2024).

2.8.2Reducing oxidative stress and combating signs of aging:

- Exhibits antioxidant properties, protecting against free radical damage caused by UV exposure and environmental pollutants.
- Helps minimize fine lines and improve skin texture with regular use (MDPI, 2024; Ong Goh, 2024).

2.8.3Evening skin tone and reducing surface pigmentation:

- Inhibits melanosome transfer from melanocytes to keratinocytes, reducing age spots and superficial hyperpigmentation.
- Clinically documented in studies using niacinamide-containing creams (Hakozaki, 2002; Załęcki, 2025; Ong & Goh, 2024).

2.9.3Safe for daily use and compatible with various formulations:

_Can be included in creams, serums, and lotions without causing irritation at recommended concentrations (Gehring, 2004; Ong & Goh, 2024).

2.10 Uses of Niacinamide in Cosmeceuticals

2.10.1Anti-aging and wrinkle reduction:

- Enhances collagen and elastin production and improves extracellular matrix integrity, reducing wrinkles and fine lines.
- Mitigates glycation-related and protein-aging effects, supporting skin health from within (Boo, 2021; MDPI, 2024; Ong & Goh, 2024).

2.10.2Reducing skin pigmentation:

- Improves skin tone and reduces dark spots clinically.
- Used for melasma and post-inflammatory hyperpigmentation (MDPI, 2024; PubMed, 2024; Ong & Goh, 2024).

2.10.3Anti-inflammatory properties:

_Soothes redness and reduces irritation from various skin conditions.

_Beneficial for sensitive and acne-prone skin (MDPI, 2024; Boo, 2021; Ong & Goh, 2024).

2.10.4 Regulating sebum production:

_Reduces excessive sebum, helping control shine and supporting oily and acne-prone skin (MDPI, 2024; Ong & Goh, 2024).

2.10.5 Enhancing skin biological functions:

_Improves cellular energy processes and skin physiological functions, such as cell regeneration and metabolic support, making it a highly effective cosmeceutical ingredient (Boo, 2021; MDPI, 2024; Ong & Goh, 2024).

2.11 Introduction

Zinc Oxide (ZnO) is a white inorganic compound considered one of the most versatile materials in the fields of cosmetics, pharmacy, and pharmaceutical industries. This compound is characterized by its chemical stability and unique physical properties, such as the ability to absorb ultraviolet radiation, antioxidant resistance, and possessing antibacterial and anti-

inflammatory properties. These characteristics make zinc oxide an essential component in a wide range of medical and cosmetic applications, from sunscreens to moisturizing and soothing creams (Raghupathi, K. R., Koodali, R. T., & Manna, A. C., 2018).

2.12. Definition of zinc oxide

Zinc Oxide (ZnO) is a white inorganic compound considered one of the most versatile materials in the fields of cosmetics, pharmacy, and pharmaceutical industries. This compound is characterized by its chemical stability and unique physical properties, such as the ability to absorb ultraviolet radiation, antioxidant resistance, and possessing antibacterial and anti-inflammatory properties.

These characteristics make zinc oxide an essential component in a wide range of medical and cosmetic applications, from sunscreens to moisturizing and soothing creams (Raghupathi, Koodali, & Manna, 2018; Sirelkhatim et al., 2015).

2.13. Properties of Zinc Oxide

Zinc oxide is an inorganic compound with the chemical formula ZnO. It appears as a white powder that can turn into a pale yellow, odorless substance and is insoluble in water. ZnO is considered safe in case of fire and relatively inert upon contact with the human body (Ruddick, 1984). It is widely used as an additive in various materials and products, including rubber, plastics, ceramics, glass, cement, paints, and batteries (Ruddick, 1984). Naturally, zinc oxide occurs as the mineral zincite, which ranges in color from yellow to red and often contains manganese; however, it is largely produced synthetically (Ruddick, 1984).

Zinc oxide is a direct wide bandgap semiconductor (3.37 eV) that is transparent in the visible and near-infrared regions, with an exciton binding energy of 60 meV (Wahab et al., 2013). Due to its unique properties, ZnO has been applied in a variety of fields, including light-emitting diodes (LEDs) (Lee et al., 2012), lasers (Abreu Fernandes et al., 2013), piezoelectric transducers and varistors, photocatalytic degradation of environmental contaminants (Chankhanittha & Nanan, 2021), solar cells (Kim et al., 2014), and chemical sensors (Alias et al., 2013)

Table 1.1 – Some basic properties of ZnO (Rivastava, Gusain & Sharma, 2013)

Property	Value
Molecular Mass	81.37 g/mol
Crystal structure	Wurtzite
Density	5.606 g/cm³
Melting Point	1975 °C
Boiling Point	2360 °C
Solubility in water	0.16 g/100 mL
Thermal Conductivity	0.6 ; 1–1.2
Energy gap	3.37 eV
Exciton binding energy	60 mV

2.14. Applications of Zinc Oxide in the Cosmetic and Para-Cosmetic Field

2.14.1. Sunscreens:

Zinc oxide is used as a physical ultraviolet (UV) protective agent, as it reflects and scatters both UVA and UVB radiation. This action helps protect the skin from sunburn, premature aging, and UV-induced cellular damage. ZnO is particularly suitable for sensitive skin when compared to chemical UV filters.

2.14.2. Soothing and Anti-Inflammatory Agent:

Zinc oxide possesses anti-inflammatory properties, which makes it an effective ingredient in skin-soothing creams, especially in cases of redness, irritation, and skin rashes.

Therefore, it is widely used in para-cosmetic products with therapeutic purposes.

2.14.3. Skin Protection and Healing

ZnO contributes to accelerating skin healing and acts as a protective barrier against harmful external factors. For this reason, it is used in formulations designed for damaged skin, protective creams, and infant skin-care products.

2.14.4. Antibacterial Properties

The referenced article indicates that zinc oxide exhibits antibacterial activity, which helps limit the growth of certain microorganisms on the skin surface. This property makes it beneficial in products intended for skin prone to mild inflammatory conditions.

2.14.5.Use in Cosmetic Products

Zinc oxide is incorporated into certain cosmetic formulations, such as powders and creams, where it improves the overall appearance of the skin, reduces shine, enhances formulation stability, and provides an additional protective effect.

anti-aging skincare formulations, as oxidative stress is a major factor in skin aging. The oil can help shield the skin from environmental stressors, including UV radiation and pollution, thereby supporting skin health and resilience.

CHAPTER III

Phytochemical Characterization and Biological Evaluation

Chapiter III: Phytochemical Characterization and Biological Evaluation

3.1Introduction

ORINGA 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 :

3.2.1. Moringa Oleifera

3.2.1.1. 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.

3.2.1.2. Classification of Secondary Metabolites

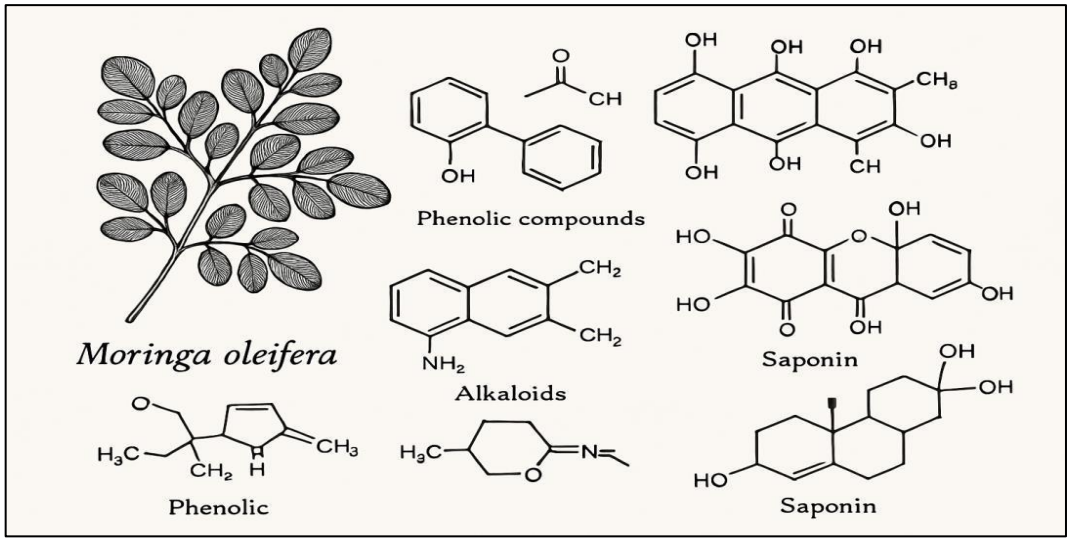


Figure 3.1. Major bioactive compounds found in Moringa oleifera, including phenolic compounds, alkaloids, and saponins, with their molecular structures

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:

3.2.1.2.1. 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.

3.2.1.2.2. 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.

3.2.1.2.3. 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.

3.2.1.2.4. 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.

3.2.1.2.5. Terpenes

Terpenes are a large and diverse group of organic compounds derived from isoprene units (C5H8). 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.

3.2.1.2.6. 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.

3.2.2. Zinc

Zinc-containing bioactive products are widely used in dermatology and cosmetic skincare due to their antibacterial, anti-inflammatory, sebum-regulating, and UV- protective properties. Compounds such as zinc oxide (ZnO), nano zinc oxide (nano ZnO), zinc gluconate, zinc amino acid complexes, and zinc sulfate have demonstrated efficacy in managing acne, reducing inflammation, and supporting overall skin health. Their topical application is supported by multiple preclinical and clinical studies, confirming their safety and effectiveness in cosmetic and dermatological formulations (Topical ZnO- NPs systematic review, 2025; Gupta et al., 2024; Zinc(II) complexes, 2021; Gupta et al., 2014; Zinc and other bioactive compounds, 2021).

Table 1 – Bioactive Zinc Products in Cosmetic & Dermatological Applications

Bioactive Zinc Product	Chemical Form / Type	Bioactive Role	Common Applications	References
Zinc oxide	ZnO	Anti-inflammatory, UV protection, wound healing	Sunscreens, protective creams	**(Gupta et al., 2014; Zinc and other bioactive compounds, 2021)**
Nano zinc oxide	nano ZnO	Enhanced antibacterial, anti-inflammatory, UV protection	Advanced sunscreens, acne creams	**(Topical ZnO-NPs systematic review, 2025)**
Zinc gluconate	Zn gluconate	Anti-inflammatory, sebum regulation	Anti-inflammatory creams, acne treatments	**(Gupta et al., 2024)**
Zinc amino acid complexes	Zn + glycine/histidine	Antibacterial, skin- compatible, anti-acne	Acne gels and creams	**(Zinc(II) complexes, 2021)**
Zinc sulfate	**ZnSO4**	**Anti-inflammatory, sebum balancing**	**Anti-inflammatory topical creams, acne treatments**	**(Gupta et al., 2014)**

The classification of zinc-containing bioactive products in the table highlights their multifunctional roles in skincare formulations, with nano-engineered zinc oxide and zinc amino acid complexes demonstrating enhanced antibacterial and anti-inflammatory effects suitable for acne-prone and photo-exposed skin. These findings support the inclusion of both conventional and nano-formulations of zinc compounds as key ingredients in dermatological and cosmetic products (Topical ZnO-NPs systematic review, 2025; Gupta et al., 2024; Zinc(II) complexes, 2021; Gupta et al., 2014; Zinc and other bioactive compounds, 2021).

3.2.3. Vitamin B3

Table 2 – Bioactive Vitamin B3 Products in Cosmetic & Dermatological Applications

Bioactive product	Chemical formula	Bioactive properties	Pharmaceutical / Cosmeceutical relevance
Niacin (Nicotinic acid)	C6H5NO2	Precursor of NAD+ and NADP+	Treatment of niacin deficiency; lipid-lowering agent
Niacinamide (Nicotinamide)	C6H6N2O	Anti-inflammatory; antioxidant; DNA repair	Acne, hyperpigmentation, anti-aging formulations
Nicotinamide riboside (NR)	C11H15N2O5+	Bioavailable NAD+ precursor	Nutraceuticals; metabolic health
Nicotinamide mononucleotide (NMN)	C11H15N2O8P	Immediate NAD+ precursor	Anti-aging and pharmaceutical research
NAD+	C21H27N7O14P2	Central redox coenzyme	Metabolic and aging-related research
NADP+	C21H28N7O17P3	Antioxidant and anabolic pathways	Oxidative stress protection

3.3. Biological Studies in:

3.3.1. Moringa Oleifera

3.3.1.1. 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).

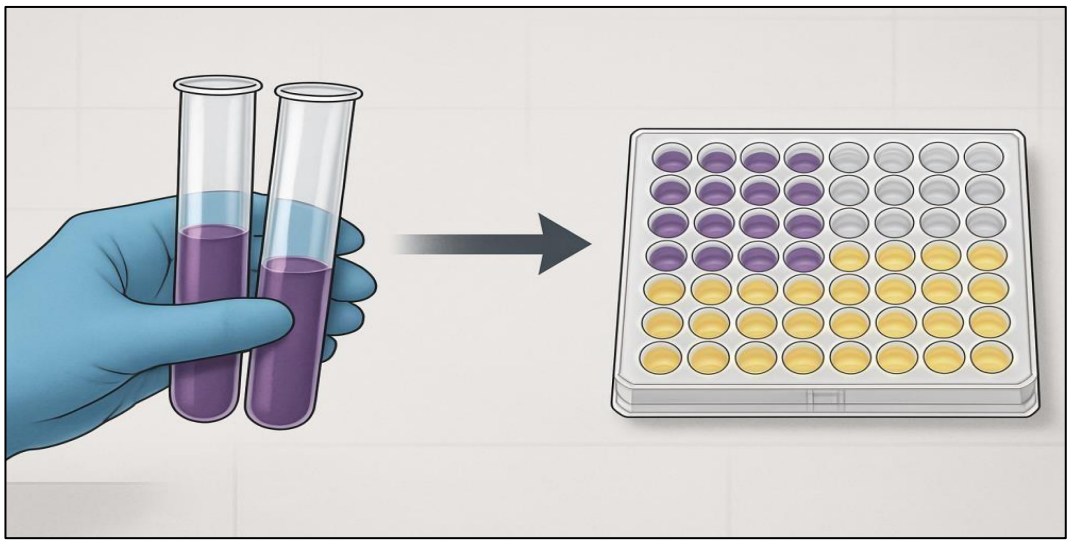


Figure 3.2. DPPH antioxidant assay showing the color change from purple to yellow as antioxidants neutralize the DPPH radical

3.3.1.1.1 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. 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

3.3.1.2.1. 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. Niacinamide (Nicotinamide) Biological Activities

3.3.2.1. Antioxidant Activity

Niacinamide is a water-soluble form of vitamin B3 with potent antioxidant properties. It reduces oxidative stress by inhibiting reactive oxygen species (ROS) production and restoring cellular redox balance. Oxidative stress is implicated in various pathological conditions, including skin aging, inflammation, and metabolic disorders. Niacinamide protects human keratinocytes and other cell types against oxidative damage, helping maintain cellular integrity (Pan et al., 2019; Veselý et al., 2023).

3.3.2.1.1. Mechanisms of Antioxidant Activity

1. Direct scavenging of free radicals by donating electrons or hydrogen atoms, neutralizing ROS.
2. Enhancement of endogenous antioxidant enzyme activity (such as superoxide dismutase and catalase).
3. Inhibition of pro-oxidant pathways that generate ROS, e.g., NADPH oxidase-dependent processes.
4. Regeneration of other antioxidants and restoration of total cellular antioxidant capacity (Veselý et al., 2023).

3.1.1.1.1. Methods for Evaluating _Antioxidant Activity

- Measurement of ROS levels in cells exposed to oxidative stress.
- Lipid peroxidation assays to assess oxidative damage.
- Enzyme activity assays for antioxidant enzymes (SOD, CAT, GPx).
- DPPH or ABTS radical scavenging in vitro assays (Pan et al., 2019; Veselý et al., 2023).

3.3.2.2. Antibacterial Activity

Niacinamide exhibits antimicrobial properties, particularly against skin-associated bacteria such as Cutibacterium acnes and Staphylococcus aureus. It contributes to controlling bacterial growth by affecting microbial cell cycles and altering the local skin environment, including sebum regulation (Horovitz et al., 2024).

3.3.2.2.1. Mechanisms of Antibacterial Activity

1. Interference with bacterial cell cycle and replication.
2. Disruption of membrane integrity and metabolic pathways essential for bacterial growth.
3. Indirect antimicrobial effects through enhancement of skin barrier function and sebum regulation (Horovitz et al., 2024).

3.3.2.3. Other Biological Activities

Niacinamide also contributes to skin barrier repair, anti-inflammatory activity, and regulation of lipid metabolism. It enhances cellular energy production via NAD^+ pathways, reduces hyperpigmentation, and improves overall skin health (Veselý et al., 2023).

3.3.3. Zinc Oxide (ZnO)

3.3.3.1. Antioxidant Activity

Zinc oxide exhibits antioxidant potential in skin applications primarily through its indirect protective role against oxidative stress induced by environmental factors such as ultraviolet

(UV) radiation. By acting as a barrier to harmful UV rays, ZnO reduces the generation of reactive oxygen species (ROS) in epidermal tissues, thereby minimizing oxidative damage and associated inflammatory reactions in the skin (Rezzani, 2022).

3.3.3.1.1. Mechanisms of Antioxidant Activity

The mechanisms underlying ZnO's antioxidant effects in dermatological formulations include:

1. Physical scattering and absorption of UV radiation, which limits ROS formation.
2. Attenuation of oxidative stress-related inflammatory signaling in keratinocytes.
3. Supporting local homeostasis of cellular antioxidant defense systems by maintaining zinc ion availability within skin layers (Rezzani, 2022).

3.3.3.2. Antibacterial Activity

Zinc oxide demonstrates antibacterial activity in dermatological contexts, inhibiting the growth and colonization of common skin pathogens. This activity enhances the protective efficacy of topical products designed for compromised or irritated skin, contributing to infection prevention and improved wound care (Rezzani, 2022).

3.3.3.2.1Mechanisms of Antibacterial Activity

The antibacterial mechanisms of ZnO in skin applications include:

1. Disruption of microbial cell membrane integrity due to localized zinc ion interactions.
2. Generation of ROS at the microenvironmental level, which impairs bacterial DNA and protein functions.
3. Interference with essential bacterial metabolic pathways, leading to growth inhibition (Rezzani, 2022).health

SECOND PART

Experimental Analysis

CHAPTER I

Materials and methods

SECOND PART

CHAPTER I: Materials and Methods

Materials

1.1. Introduction

HIS 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. The Complex(niacinamide and zinc oxide)

Figure 1.2. Commercial Niacinamide 10% + Zinc 1% Serum (The Ordinary®) Used as the Aqueous Phase in the Formulation

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 1.3. 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 1.4. 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):

$(\%) = 100 \times Mext / Msc$

Where: R is the yield in (%). $Mext$ is the mass of the extract after solvent evaporation in (g). Msc 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. Preparation of the cosmetic product(moringa oil, niacinainamide, zinc oxide)

1.3.3.1. Preparation of the Oil Phase

1. 30 mL of the selected plant oil was transferred into a sterile beaker.

2. 4 mL of Polysorbate 80 was addad as the emulsifier .

3. The Mixture was stirred for 30 seconds until a clear and homogeneous

1.3.3.2. Mild Heating

1. the oil phase was heated only to 40°C.to enhance emulsion stability.

2. the aqueous phase (serum) was not heated to preserve its properties.

1.3.3.3. Emulsification Process

1. 50 mL of Niacinamide 10% + Zinc 1% serum was placed in a clean beaker.

2. the oil phase was added slowly in a thin stream under continuous stirring.

3. A mini mixer was used to Homogenize the mixture for 4 minutes until a uniform milky emulsion is formed.

1.3.3.4. Cooling and Packaging

1. The emulsion was Allowed to cool to room temperature.

2. the final product was transferred into an airtight container.

3. The container was gently shaken to ensure homogeneity before use (Dănilă et al., 2024)

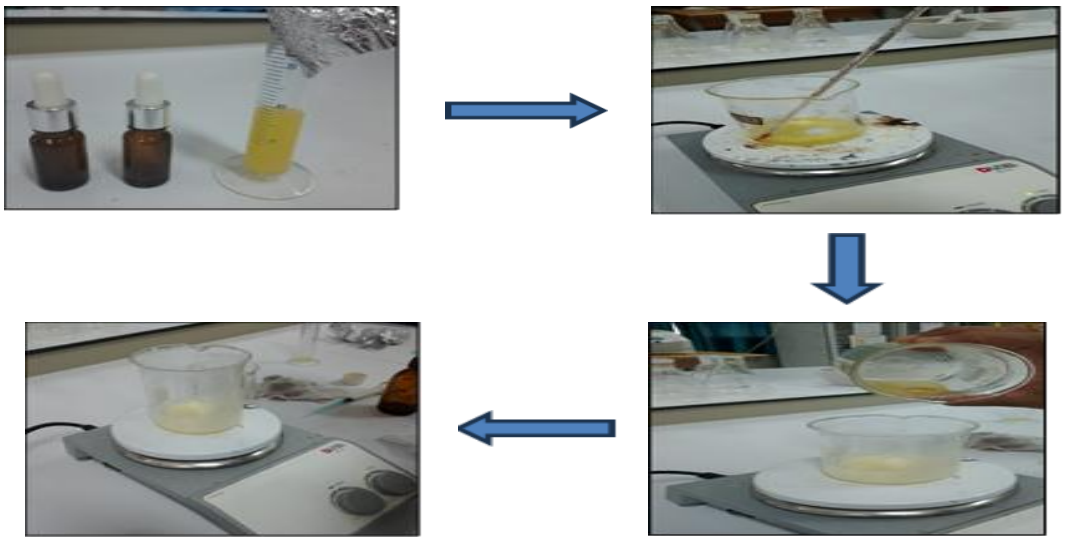


Figure 1.5. steps for the Preparation of the cosmetic product

1.3.4.Biological Activities

1.3.4.1. 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 product 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 product 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.(Makoto Oyaizu, 1986)

c) Quantification and expression of antioxidant capacity: The antioxidant capacity of the sample (prodect) was then expressed as a percentage relative to the reducing power of the standard antioxidant (ascorbic acid) using the following formula

$FRAP\ inhibition\ percentage$ (%) = $[(Acontrol - Asample) / Acontrol] \times 100$

where:

$Inhibition$ (%) : The percentage of free radicals scavenged or neutralized by the sample.

$Acontrol$: The absorbance of the control solution (without antioxidant).

$Asample$: The absorbance of the solution containing the sample or antioxidant extract.

Figure 1.6. Samples of solutions prepared for the FRAP experiment

1.3.4.2. 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 prodect 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 prodect 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.

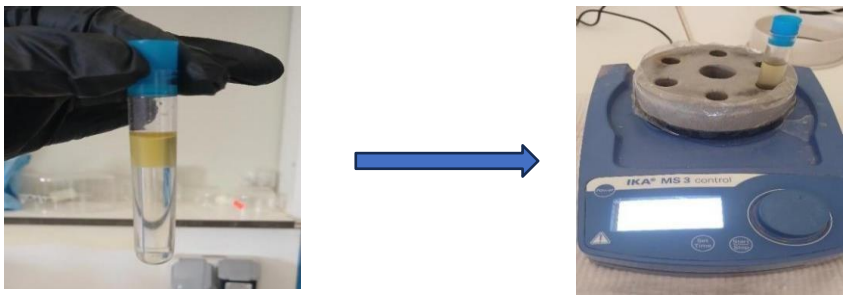


Figure 1.7. SPF sample preparation

b) spectrophototometric meqsurement: 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.

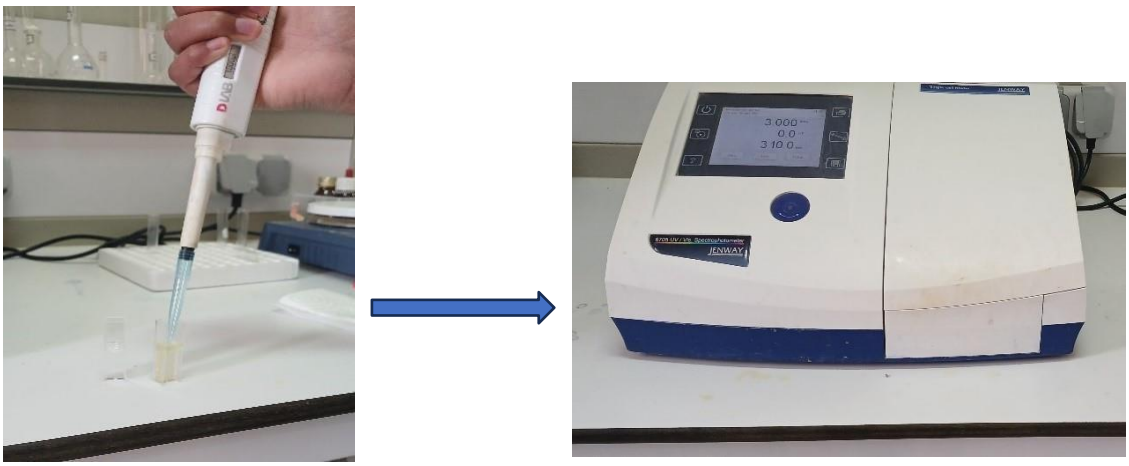


Figure 1.8. Measurement of the solution absorbance

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+

1.3.4.3. 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 Cosmetic product 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 Cosmetic product 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, Cosmetic product solution is prepared by dissolving the Cosmetic product 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 Cosmetic product 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).

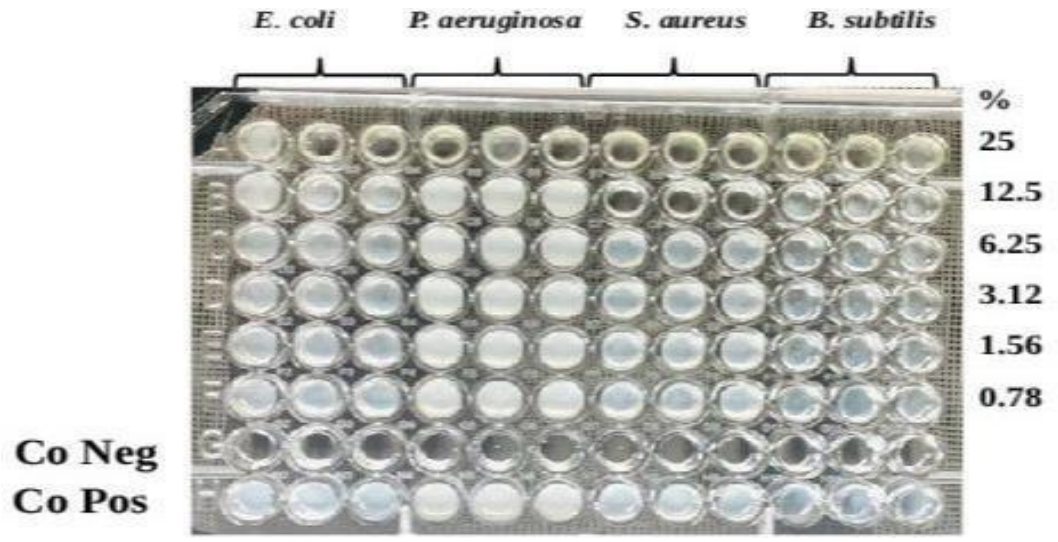


Figure 1.9. Photo of the broth microdilution method used to assess the antimicrobial activity of the Cosmetic product against bacterial strains

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

- Protocol:

Initially, various concentrations of the essential Cosmetic product 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 product , 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 Cosmetic product 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 Cosmetic product on Biofilm Formation**

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

The effect of the Cosmetic product 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 (prodect) on biofilm formation, overnight bacteria cultures were diluted and transferred to a 96-well microtiter plate. A subinhibitory

Concentration of product was added ,and the plate was incubated at 37C° 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].

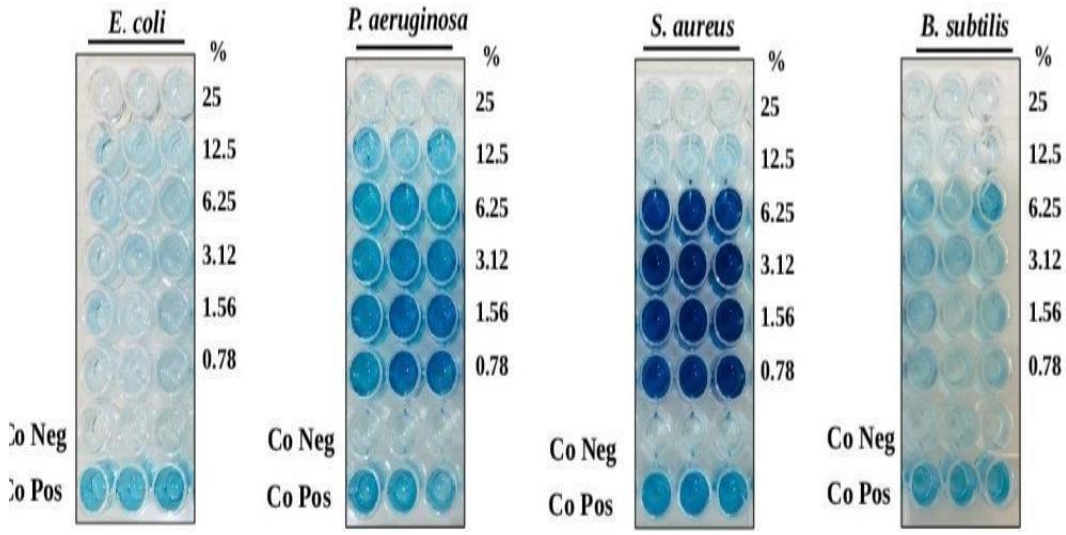


Figure 1.10. Effect of the tested Cosmetic product on biofilm formation in four bacterial strains (E. coli, P. aeruginosa, S. aureus, B. subtilis) at different concentrations

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].

❖ **Cytotoxicity test**

Cytotoxicity testing is an in vitro evaluation used to measure the effect of a substance on cell viability, growth, and biological function, aiming to determine whether the substance causes cell damage or death upon exposure. This assessment is typically conducted using cell cultures under controlled laboratory conditions and is considered a fundamental tool in pharmaceutical and environmental toxicology for estimating the preliminary level of toxicity before proceeding to animal or clinical studies. The test primarily relies on measuring decreased cell viability or increased cell death as a result of exposure to the compound, providing an important indicator of the safety or potential hazard of the substance at the cellular level (Open Access Pub, n.d.).

- Protocol:

Cytotoxicity assessment using Artemia salina lethality bioassay (cosmetic product in DMSO)

- The cytotoxic potential of the tested cosmetic product was evaluated using the Artemia salina lethality bioassay, which is widely used for preliminary toxicity screening.
- The procedure was adapted from published methods with minor adjustments to suit a 96-well microplate format and to ensure good reproducibility.
- Artemia salina cysts were hatched in artificial seawater prepared by dissolving

- 35 g/L of commercial sea salt in distilled water, and then incubated under continuous light with gentle aeration at room temperature (approximately 25 °C) for 24–48 h (Rajabi et al., 2015). Actively swimming nauplii were collected and used for the assay.
- Stock solutions of the cosmetic product were prepared in dimethyl sulfoxide (DMSO) and then diluted with artificial seawater to obtain final test concentrations of 50, 25, 10, and 5% (v/v), while keeping the final DMSO concentration at a non-toxic level.
- In each well of a sterile 96-well microplate, 100 µL of seawater containing about
- 20 viable nauplii was added, followed by the appropriate volume of the cosmetic product solution and artificial seawater to reach the desired concentration.
- The plates were incubated at room temperature under constant light for 24 h. After incubation, the number of surviving larvae was counted under a stereomicroscope, and nauplii were considered dead when no movement was observed. The survival percentage was calculated as:

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

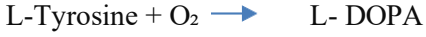
All treatments were carried out in triplicate. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control to verify assay sensitivity, while seawater containing the same concentration of DMSO without cosmetic product served as the negative control (Nguta et al., 2012).

1.3.4.4. Tyrosinase test

The tyrosinase assay is an in vitro method used to evaluate the ability of a substance to inhibit the activity of the tyrosinase enzyme, a copper-containing enzyme that plays a key role in the process of melanogenesis by catalyzing the oxidation of tyrosine to DOPA and subsequently to DOPA-quinone. This assay is commonly based on measuring changes in absorbance resulting from the formation of oxidation products, with L- tyrosine or L-DOPA frequently employed as reaction substrates. The results are expressed as the percentage of enzyme activity inhibition (%) or by calculating the IC_{50} value, allowing the assessment of the anti-melanogenic potential of natural or synthetic compounds, particularly in cosmetic, pharmaceutical, and dermatological applications (Fan et al., 2021)

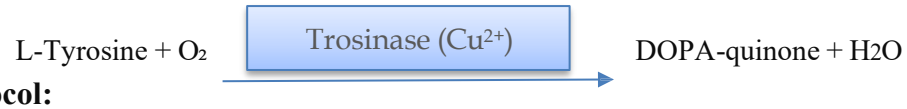
- **The half equations:**

1. Hydroxylation (Monophenolase activity):



2. Oxidation (diphenollase activity)

The overall equation:



- Protocol:

1-Preparation of Buffer Solutions (Tampon Solutions)

- Two buffer solutions were prepared at pH 6.8 and pH 7.0 as described below. Preparation of pH 6.8 Buffer
- First, two stock solutions were prepared:
- Solution A:
- A mass of 8.890 g of $Na_2HPO_4 \cdot 2H_2O$ (molecular weight = 177.99 g/mol) was accurately weighed and dissolved in 500 mL of distilled water.
- Solution B:
- A mass of 1.56 g of $NaH_2PO_4 \cdot 2H_2O$ (molecular weight = 156.01 g/mol) was accurately weighed and dissolved in 100 mL of distilled water.
- To obtain the buffer solution at pH 6.8, 49 mL of Solution A was mixed with 51 mL of Solution B.
- The pH was measured using a pH meter. If the pH value was confirmed to be 6.8, distilled water was added to adjust the final volume to 200 mL.
- The solution was then stirred for approximately 10 minutes.
- The resulting buffer had a concentration of 100 mM. However, for the purposes of this study, a 50 mM buffer solution was required; therefore, the volume was doubled by adding an equal volume of distilled water (e.g., 200 mL of distilled water).
- Note: The pH can be adjusted, if necessary, by adding a few drops of NaOH solution. 2/Preparation of the Enzyme Solution:
- A mass of 1 mg of the enzyme was dissolved in 250 µL of pH 6.8 buffer.
- The enzyme solution was then aliquoted into five Eppendorf tubes, each containing 50 µL, and stored in a freezer until use.
- At the time of use:

2000 µL of pH 6.8 buffer was added to 50 µL of enzyme solution when an alcoholic solvent was used (control).

- 3000 µL of pH 6.8 buffer was added to 50 µL of enzyme solution when water was used as the solvent (control).

3/Preparation of L-DOPA Solution:

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

thoroughly until complete dissolution.

- Experimental Procedure

The experimental steps were carried out according to the protocol described above:

150 µl (tampon pH=6.8)

+

10 µl extract or control

+

20 µl Enzyme

(3-minute hug with vibration Spectro) 10-minute hug under 37°c

↓

20 µl L-DOPA

(Kinematic reading Spectro) Kinetic reading 475nm(Sigma-Aldrich, n.d.)

Figure 1.11: Excerpts from the Tyrosinase Test Procedures

Effects of Product Concentration on Skin Hydration, Radiance, and Sun Protection: Two-way ANOVA Analysis

A two-way analysis of variance (Two-way ANOVA) was applied to analyze the data obtained from the in vitro assays related to the evaluation of the moisturizing, skin- radiance–enhancing, and sun-protective properties of the studied cosmetic product. The concentration of the product was considered the first independent factor, while the type of biological assay was considered the second independent factor, with the interaction effect between both variables also examined on the measured quantitative outcomes.

The results demonstrated a statistically significant effect associated with concentration, indicating a dose-dependent biological response of the formulation. Furthermore, significant differences were observed between the performed assays, reflecting the variation in the measured parameters related to skin hydration indicators, radiance enhancement, and photoprotective activity. In addition, the interaction analysis revealed a

combined effect between the two factors, suggesting that the efficacy of the product varies according to its concentration and the specific biological activity assessed.

These findings confirm that the cosmetic product, composed of three active ingredients, exhibits consistent biological performance within the applied in vitro models, with strong statistical support for its moisturizing, skin-enhancing, and photoprotective properties. Statistical significance was established at $p < 0.05$, and all analyses were conducted according to standardized procedures to ensure reliability and accuracy of the results.

CHAPTER II

Results and discussion

CHAPTER II: Results and discussion

2.1. Introduction

This chapter presents the comprehensive findings of the experimental investigation conducted to evaluate the biological properties and therapeutic potential of the formulated cosmetic preparation. The results are systematically organized to address the principal research questions concerning the physicochemical characteristics of the formulation, its antioxidant capacity, photoprotective properties, antimicrobial efficacy, and its ability to enhance skin radiance and appearance.

The chapter begins with the determination of the physical constants, which establish the fundamental properties of the formulation, and then progresses to increasingly complex biological assays that elucidate its multifunctional activities. The assessment of

antioxidant activity using the FRAP assay provides insight into the formulation's capacity to neutralize free radicals, while the determination of the Sun Protection Factor (SPF) highlights its significant photoprotective potential.

The antibacterial investigations—including the determination of the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), biofilm inhibition assay, and tyrosinase inhibition assay—demonstrate the product's effectiveness against various pathogens and reveal its possible mechanisms of action. Finally, the in vivo evaluation of skin radiance enhancement and tone uniformity provides compelling

evidence of the formulation's therapeutic efficacy in promoting tissue repair and regeneration.

Throughout this chapter, the experimental findings are contextualized within the broader scientific literature, with discussions exploring the underlying mechanisms, comparative effectiveness, and potential pharmaceutical, cosmetic, and therapeutic applications of the natural cosmetic formulation.

2.2. Results

2.2.1. Determination of Physical Constants of the cosmetic product:

The physical and chemical properties of cosmetic product were evaluated to establish its baseline characteristics. The results are summarized in Table 1

Table 2.1 : Physical and chemical properties of the cosmetic product:

Paramètre	Moringa oleifera	The complex (Zinc oxide; Niacinamide)
Color	Dark yellow	**Clear to slightly cloudy**
Viscosity	Heavy	**Low**
Density	1.95 g/ml	**Approximately 1 g/ml**
pH	4.11	**5.0 – 6.5**
Percentage yield	**38.97%**	**11%**

The table demonstrates clear differences between Moringa oleifera oil and the complex (Zinc oxide + Niacinamide) in terms of their physicochemical properties. Moringa oil is characterized by a dark yellow color, high viscosity, and a higher density (1.95 g/mL), reflecting its lipid-based nature. In contrast, the complex appears clear to slightly cloudy, with low viscosity and a density of approximately 1 g/mL, indicating its semi-aqueous nature and ease of topical spreadability.

Regarding pH, Moringa oil recorded a value of 4.11 (moderately acidic), whereas the pH of the complex ranged between 5.0 and 6.5, which is close to the physiological skin pH. Additionally, the percentage yield was higher for Moringa oil (38.97%) compared to the complex (11%). These findings confirm the distinct physicochemical characteristics of each component, supporting their functional complementarity within the final formulation..

2.2.2. Antioxidant Activity: FRAP Assay (FerricReducing Antioxidant Power Assay)

The activity percentage versus concentration curve obtained from the FRAP assay shows an increasing trend for both samples (the cosmetic product and ascorbic acid), indicating a direct proportional relationship between concentration and reducing power.

For the cosmetic product, the linear regression equation was: $y = 0.9436x + 0.1978$, with a coefficient of determination $R^2 = 0.9532$, indicating good linear fit and a strong correlation between concentration and activity percentage. The calculated IC_{50} value was 52.81 µg/mL, representing the concentration required to achieve 50% of the maximal reducing activity.

In the case of ascorbic acid, the regression equation was: y = 0.9112x + 12.757, with R^2 = 0.9483, also reflecting a high degree of linearity. The recorded IC_{50} value was 40.88 µg/mL, which is lower than that of the cosmetic product.

Comparatively, the lower IC_{50} value of ascorbic acid indicates higher reducing efficiency (stronger antioxidant capacity) at lower concentrations, which is expected given its role as a well-established reference standard in the FRAP assay. In contrast, the cosmetic product exhibited appreciable antioxidant activity, although lower than that of ascorbic acid, confirming its considerable but comparatively moderate reducing potential.

Overall, the high R^2 values (≈ 0.95) reflect the reliability of the results and the accuracy

of the linear regression model used to estimate IC_{50}, thereby supporting the validity of the statistical approach applied in evaluating antioxidant efficacy.

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

Reducing Power	A 0.5 (µg/ml)
Cosmetic product	**0.873 ± 0.030**
Ascorbic acid	**0.5243 ± 1.10**

These values show that ascorbic acid possesses a higher antioxidant capacity than Cosmetic product, as indicated by its lower EC50

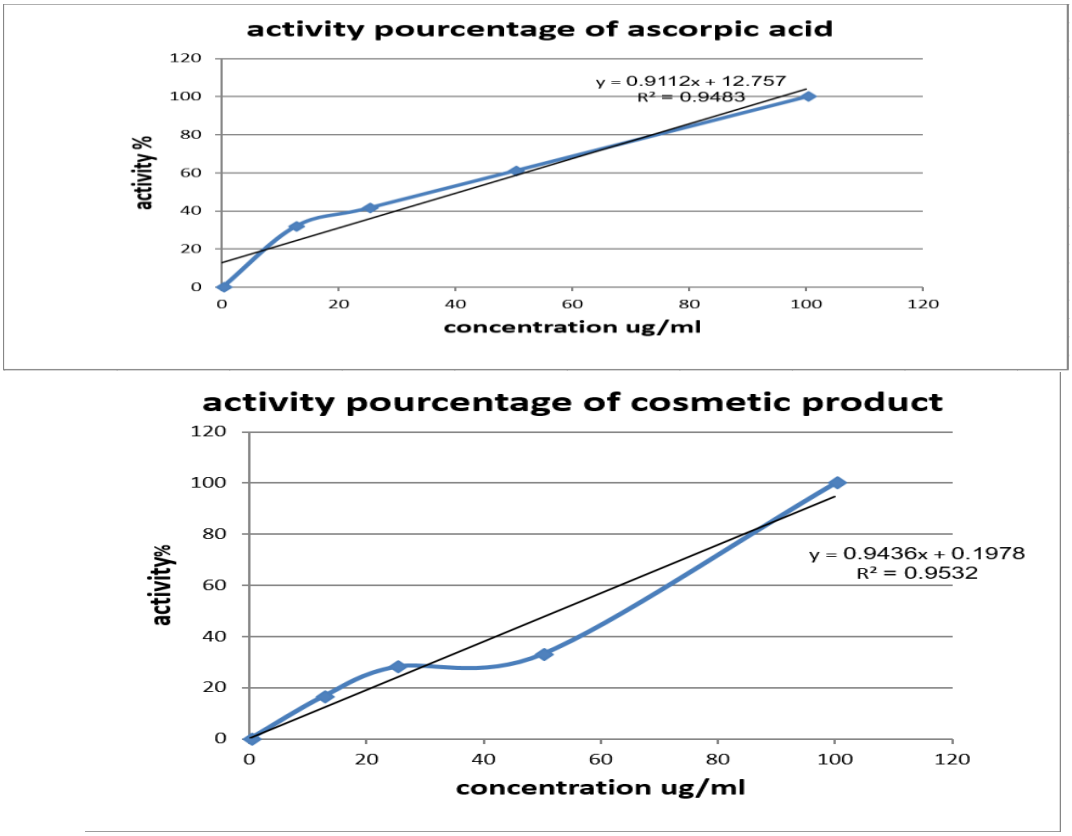


Figure 2.1. FRAP calibration curve for The cosmetic product

2.2.3. SPF Test

In our study, we evaluated the Sun Protection Factor (SPF) of the cosmetic formulation using an in vitro UV spectrophotometric method proposed by Mansur et al. (1996).

This method is based on measuring the absorbance (Abs) of the sample within the harmful ultraviolet (UV) radiation range, particularly the ultraviolet B (UVB) region (290–320 nm).

The absorbance results were as follows:

Table 2.3 Absorbance values of the formulation at different wavelengths

Wavelength λ (nm)	Absorbance (Abs)
290	**1.216**
295	**1.017**
300	**0.648**
305	**0.958**
310	**0.728**
315	**0.530**
320	**0.711**

Ultraviolet (UV) spectrophotometric analysis of the cosmetic formulation was performed within the UVB region (290–320 nm), which is primarily responsible for erythema (skin redness) and sunburn. The recorded absorbance values at different wavelengths are presented in Table 2.3.

The results demonstrate that the formulation exhibits a distinct absorption capacity across the entire UVB range, indicating its ability to absorb harmful ultraviolet radiation. A relatively high absorbance was recorded at 290 nm (A = 1.216) and 295 nm (A = 1.017), suggesting strong absorption in the lower portion of the UVB spectrum, where erythemogenic radiation is most intense.

At 300 nm, the absorbance decreased to 0.648, followed by a slight decline at 305 nm (A = 0.518). However, absorbance increased again at 310 nm (A = 0.718), indicating a secondary absorption contribution within the mid-UVB region. At 315 nm and 320 nm, the absorbance values reached 0.530 and 0.711, respectively, reflecting sustained absorption capacity up to the upper limit of the UVB spectrum.

Overall, the absorption curve displays a broad and continuous spectral profile within the UVB range, a characteristic essential for effective photoprotection. The presence of significant absorption across the 290–320 nm interval supports the potential protective efficacy of the formulation against UVB-induced skin damage. This spectral behavior is consistent with

formulations exhibiting measurable Sun Protection Factor (SPF) values, thereby confirming the contribution of the active ingredients to reducing ultraviolet radiation penetration.

The non-linear variation in absorbance values across different wavelengths may be attributed to the intrinsic absorption properties of the active compounds present in the formulation, as well as to possible synergistic interactions among them.

2.2.3.1. Comparison of SPF

To provide practical insight into the photoprotective performance of the cosmetic formulation, it was compared with a well-known high-protection commercial product, Avène High Protection Cream SPF 50+, which is considered one of the leading formulations in the European skincare market.

Table 2.4: Comparison of Sun Protection Factor (SPF) values between the cosmetic formulation and the commercial sunscreen.:

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

Product	SPF
Cosmetic product	**57 ± 2**
Avène	**50± 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 table illustrates the efficacy of the formulation against four reference bacterial strains through MIC, MBC, and the MBC/MIC ratio. All strains exhibited a bactericidal effect, with an MBC/MIC ratio of 1 for E. coli, S. aureus, and B. subtilis, indicating that the formulation can directly kill bacteria rather than merely inhibiting their growth.

Notably, P. aeruginosa displayed MIC and MBC values greater than 40 mg/ml, and the ratio was not determined (ND), reflecting relative resistance compared to the other strains. In contrast, S. aureus was the most sensitive, with MIC and MBC values of 20 mg/ml, while E. coli and B. subtilis showed moderate responses at 40 mg/ml. This suggests that the cosmetic product has a broad antibacterial spectrum but is less effective against Gram-negative resistant bacteria such as P. aeruginosa compared to Gram-positive strains.

- The analysis indicates that the cosmetic formulation exhibits bactericidal activity against most reference strains, with higher efficacy against S. aureus compared to E. coli and B. subtilis.

P. aeruginosa, however, showed relative resistance, demonstrating that the antibacterial spectrum of the product is broad but may be limited against certain resistant Gram-negative bacteria. These findings support the potential application of the oil as a natural antibacterial agent, with careful consideration of strain-specific differences in effectiveness.

Table 2.5 MIC, MBC, and MBC/MIC ratio of of cosmetic porduct against different bacteria

Bacteria strains (n = 3)	X			
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Antibacterial Effect
Escherichia coli ATCC 25922	40±0.00	40±0.00	1	**Bactericidal**
Pseudomonas aeruginosa ATCC 27853	>40±0.00	>40±0.00	ND	**Bactericidal**
Staphylococcus aureus ATCC 25932	20±0.00	20±0.00	1	**Bactericidal**
Bacillus subtilis ATCC 25973	**40±0.00**	**40±0.00**	**1**	**Bactericidal**

> Very high concentration, ND not determined.

2.2.4.2. Effect on Biofilm Formation

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate the effect of different concentrations of the plant oil on biofilm formation for each bacterial strain. When significant differences were observed, Tukey's post hoc test was applied to identify differences between individual concentrations. Data were expressed as mean ± standard deviation (SD) of three independent experiments. Differences were considered statistically significant at $p < 0.05$.

Results

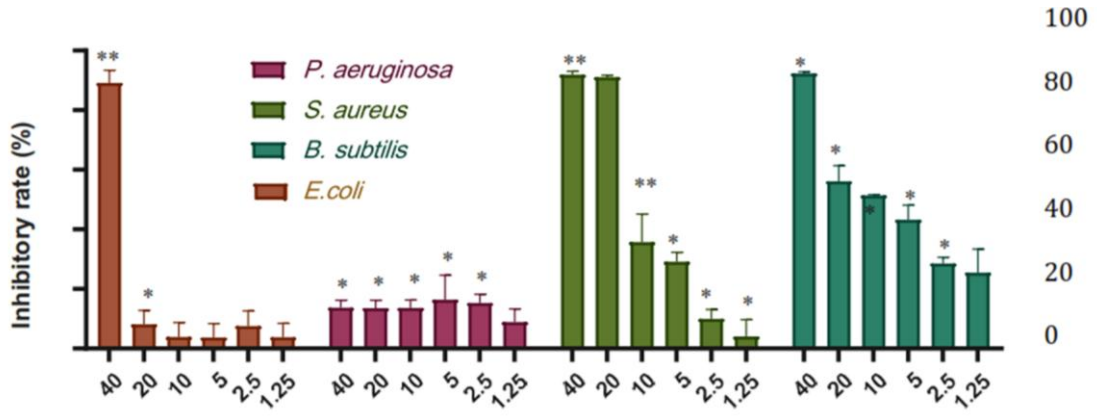


Figure 2.2. Comparative inhibition of biofilm formation by a product in Gram-positive and Gram-negative bacteria at increasing (v/v) concentrations.

Analysis of the Effect of the Cosmetic Product on Biofilm Formation:

The results presented in the graph illustrate the effect of different concentrations of the cosmetic product on the inhibition of biofilm formation in four bacterial strains: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis. The findings revealed a clear variation in the sensitivity of these strains to the different product concentrations.

For Escherichia coli, the highest inhibition rate was recorded at the concentration of 40 mg/mL, where the inhibition percentage reached a very high value compared with the other concentrations. In contrast, the inhibition rate decreased noticeably as the concentration decreased, indicating that the effectiveness of the product in reducing biofilm formation in this bacterium is strongly concentration-dependent.

In the case of Pseudomonas aeruginosa, the bacterium exhibited relatively lower sensitivity compared to the other strains, as the inhibition percentages remained moderate even at higher concentrations. This behavior may be explained by the strong ability of this bacterium to form complex biofilms and its advanced resistance mechanisms.

Conversely, Staphylococcus aureus showed a strong response to the cosmetic product, with very high inhibition rates recorded at the higher concentrations (40 and 20 mg/mL), followed by a gradual decrease as the concentration decreased. This indicates a notable effectiveness of the product in inhibiting biofilm formation in this strain.

Similarly, Bacillus subtilis exhibited relatively high inhibition levels at higher concentrations, with the highest inhibition rate observed at 40 mg/mL, followed by a gradual decrease with decreasing concentrations, reflecting the sensitivity of this strain to the components of the product.

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed the presence of statistically significant differences between some concentrations ($p < 0.05$), as indicated by the statistical symbols in the figure. These results suggest that the cosmetic product possesses promising antibiofilm activity against bacterial biofilm formation, with varying effectiveness depending on the bacterial strain and the concentration of the product used

2.2.4.3. Effect Cytotocicity

This table presents the survival percentages of Artemia salina larvae after exposure to the cosmetic product (from 5% to 50%), along with the control groups, at different time intervals (T0, T30, T60, T180, T360, and T24H).

Table 2.6: Cytotoxic Effects of the Cosmetic Product on Artemia salina Larvae Over Time.

Concentration	T0	T30	T60	T180	T360	T24H
Control Neg	100.00 ± 0.00	100.00 ± 0.00	96.67 ± 3.33	96.67 ± 3.33	93.33 ± 3.33	93.33 ± 3.33
Control Pos ($K_2Cr_2O_7$)	100.00 ± 0.00	46.67 ± 5.77	26.67 ± 5.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50%	100.00 ± 0.00	96.67 ± 3.33	90.00 ± 0.00	80.00 ± 0.00	66.67 ± 3.33	50.00 ± 0.00
25%	100.00 ± 0.00	96.67 ± 3.33	93.33 ± 3.33	86.67 ± 3.33	76.67 ± 3.33	66.67 ± 3.33
10%	100.00 ± 0.00	100.00 ± 0.00	96.67 ± 3.33	93.33 ± 3.33	86.67 ± 3.33	80.00 ± 0.00
5%	100.00 ± 0.00	100.00 ± 0.00	96.67 ± 3.33	96.67 ± 3.33	90.00 ± 0.00	86.67 ± 3.33

Analysis of the Cytotoxic Effects of the Cosmetic Product on Artemia salina:

The negative control showed consistently high survival rates (>93%) throughout the experiment, confirming that the test conditions and the presence of DMSO had no toxic effect on the larvae. The positive control ($K_2Cr_2O_7$), however, caused rapid larval death, demonstrating the sensitivity and effectiveness of the assay in detecting toxic substances.

For the cosmetic product, a gradual decrease in survival was observed with increasing concentration and exposure time. At 50%, survival dropped from 100% at T0 to 50% after 24 hours, whereas lower concentrations (5–10%) maintained high survival rates (>80%) over the same period. This pattern indicates a clear dose– response relationship, with toxic effects becoming more pronounced at higher concentrations.

-The cosmetic product appears relatively safe at low to moderate concentrations, showing limited toxicity only at high concentrations. This suggests it is suitable for preliminary cosmetic use, although further cellular or dermal evaluations are recommended to ensure full safety.

2.2.5. Tyrosinase Assay

The tyrosinase inhibitory activity of the cosmetic formulation was evaluated and compared with a sunscreen cream used as a reference. Both formulations exhibited a concentration-dependent increase in inhibition percentage within the tested range, indicating a biological response associated with increasing concentration.

For the cosmetic formulation, linear regression analysis yielded the equation:

$Y = 0.694x + 11.16$,

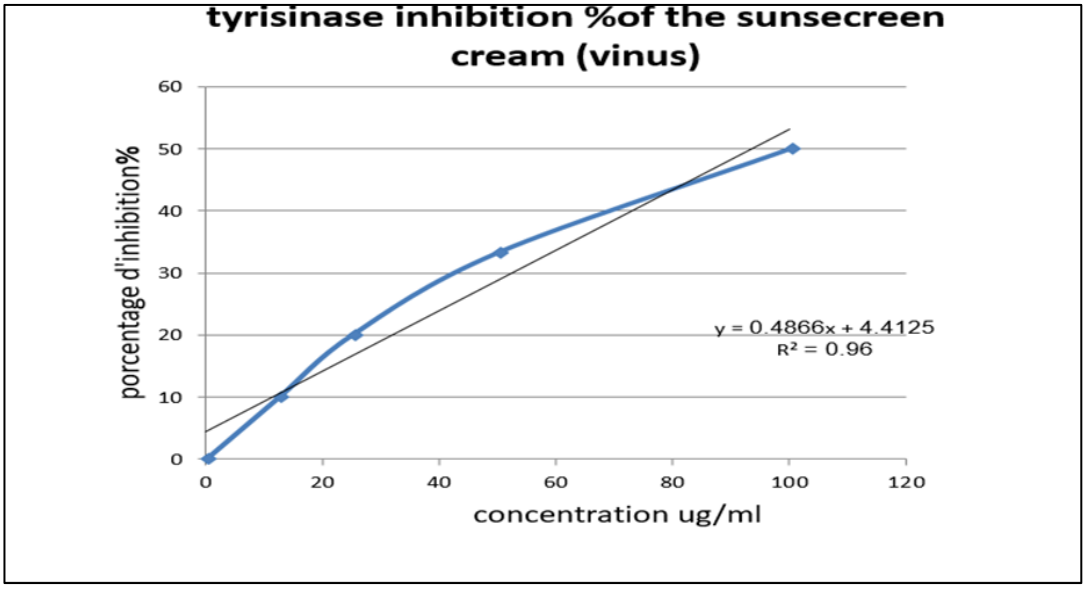
with a coefficient of determination $R^2 = 0.895$, indicating a good linear correlation between concentration and inhibition percentage. The calculated IC_{50} value was 55.96 µg/mL,

reflecting a pronounced inhibitory activity under the experimental conditions. In contrast, the reference sunscreen cream followed the regression equation:

$$Y = 0.486x + 4.412,$$

with a higher coefficient of determination ($R^2 = 0.96$), demonstrating excellent linearity. However, the recorded IC_{50} value was 93.80 µg/mL, indicating lower inhibitory efficacy compared to the tested formulation.

The comparison of IC_{50} values indicates that the cosmetic formulation possesses stronger tyrosinase inhibitory activity than the reference product, as evidenced by its lower IC_{50} value, reflecting greater efficiency in enzyme inhibition at lower concentrations



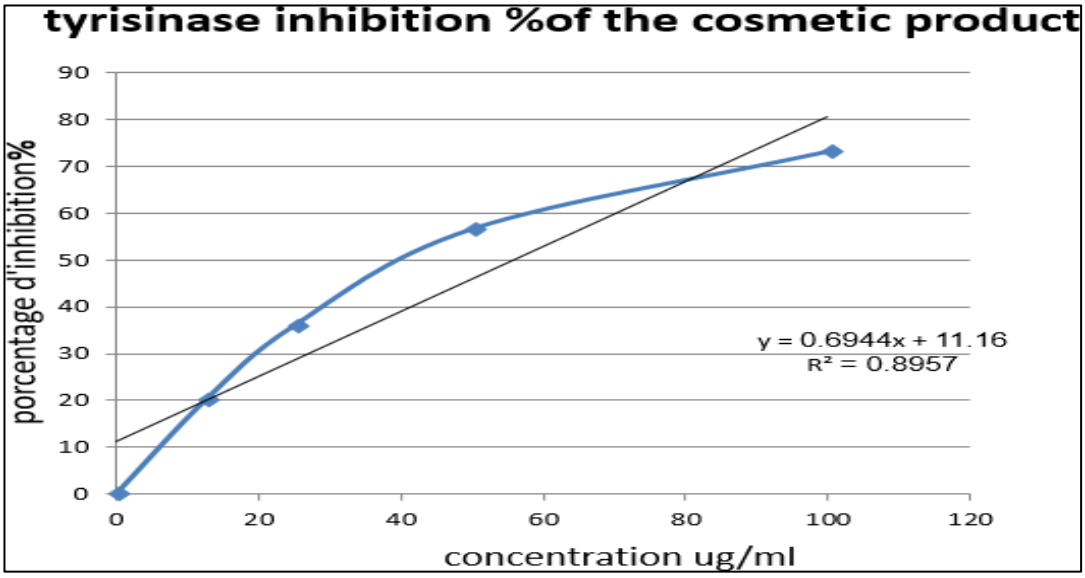


Figure 2.2. Dose–response curve in the tyrosinase assay

2.3. Discussion

2.3.1. Physicochemical Properties:

The results demonstrate clear differences between Moringa oleifera oil and the complex (Zinc oxide + Niacinamide) in terms of their physicochemical properties, reflecting the distinct nature of each component. Moringa oleifera oil is characterized by its dark yellow color, high viscosity, and elevated density, which are associated with its rich composition of unsaturated fatty acids, particularly oleic acid. This composition confers important moisturizing and antioxidant properties relevant to dermatological applications (Anwar et al., 2007; Leone et al., 2016).

In contrast, the complex exhibited a clear to slightly cloudy appearance, low viscosity, and a density close to 1 g/mL, indicating its semi-aqueous nature and ease of topical spreadability. Zinc oxide is a safe and stable mineral ingredient widely used in sunscreen formulations due to its protective properties, whereas niacinamide is known for improving skin barrier function, reducing inflammation, and enhancing skin tone uniformity (Draelos, 2013; Sivamani et al., 2014).

Regarding pH, Moringa oil showed an acidic value (4.11), while the pH of the complex ranged between 5.0 and 6.5, which is close to the physiological skin pH, thereby supporting its suitability for topical application (Lambers et al., 2006).

Concerning the percentage yield, it was higher for Moringa oil compared to the complex, which can be attributed to the extraction process and the actual plant oil content (Anwar et al., 2007).

2.3.2. Antioxidant Activity

The results of the FRAP assay demonstrated that cosmetic product preparation possesses significant reducing power, with a marked increase in activity at higher concentrations, confirming a dose-dependent relationship between concentration and antioxidant capacity. The Ferric Reducing Antioxidant Power (FRAP) assay is based on measuring the ability of compounds to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which serves as a direct indicator of the electron-donating capacity of the tested compound (Benzie & Strain, 1996).

The recorded antioxidant activity can be attributed to the presence of electron-donating compounds, particularly phenolic compounds or active plant constituents, which are considered among the main factors responsible for reducing activity in biological systems. Phenolic compounds are linked to their ability to stabilize free radicals through an aromatic ring structure containing hydroxyl groups, enhancing their effectiveness as potent antioxidants (Shahidi &

Ambigaipalan, 2015). Studies have also shown a positive correlation between total phenolic content and FRAP-measured activity, making this assay a reliable indicator for estimating total antioxidant capacity (Apak et al., 2016).

To further support the formulation, Niacinamide and Zinc Oxide can be incorporated into the preparation to enhance cosmetic and protective efficacy. Niacinamide (Vitamin B3) is known for its indirect antioxidant properties, as it contributes to supporting cellular DNA repair, reducing inflammation, and improving skin barrier function by promoting ceramide synthesis, thereby mitigating the adverse effects of oxidative stress. Additionally, it helps to even skin tone and reduce hyperpigmentation, complementing the antioxidant effects of the preparation.

Zinc Oxide, in addition to its role as a physical UV filter, limits the formation of free radicals generated by UV exposure, thereby reducing oxidative damage to skin cells. It also possesses soothing and anti-inflammatory properties that support barrier stability and reduce irritation associated with environmental stress.

From a cosmetic perspective, antioxidant properties are highly important for protecting the skin from oxidative stress induced by environmental factors, especially UV radiation, which contributes to skin aging and pigmentation changes. Antioxidants help reduce free radical formation, promoting skin radiance, uniform tone, and minimizing dullness (Halliwell, 2007).

Based on these findings, the FRAP results indicate that the Memwari preparation possesses reducing power that supports its potential cosmetic properties, including protection against oxidation, enhancement of skin radiance, improvement of color balance, and reinforcement of skin barrier function, thereby highlighting its value as a comprehensive formulation with antioxidant functionality in skincare applications.

2.3.3. SPF Test

Spectrophotometric analysis demonstrated that the formulation exhibited notable absorbance within the UVB range (290–320 nm), with the highest absorbance values recorded at 290 and 295 nm. This finding is particularly significant, as UVB radiation— especially within the 290–315 nm range—is the primary cause of erythema and direct DNA damage in keratinocytes (Sayre et al., 1979; Diffey, 2002).

According to the spectrophotometric method proposed by Mansur et al. (1996), the Sun Protection Factor (SPF) value is directly related to the measured absorbance within this spectral region after weighting by the erythemal action spectrum. Accordingly, the total recorded absorbance ($\Sigma A \approx 5.79$) suggests a considerable protective potential against UVB radiation, supporting the hypothesis that the formulation may contribute to reducing the biological effects of solar exposure.

Several studies have reported that plant extracts and oils rich in phenolic compounds and tocopherols possess natural UV-absorbing properties, attributed to the presence of conjugated electronic systems capable of absorbing light energy and converting it into less harmful forms (Kaur & Saraf, 2010; Nichols & Katiyar, 2010). In addition, antioxidant compounds play a complementary role in photoprotection by scavenging free radicals generated upon UV exposure, thereby mitigating oxidative stress in skin cells (Saewan & Jimtaisong, 2013).

The pronounced increase in absorbance at shorter UVB wavelengths (290–295 nm) is considered favorable, as these wavelengths carry higher photon energy and are more capable of inducing direct cellular damage (Diffey, 2002). This indicates that the formulation may provide dual protection: physical protection through UV absorption and biochemical protection through antioxidant activity, particularly if it contains phenolic- rich plant-derived components, as commonly found in vegetable oils.

However, it should be emphasized that in vitro SPF evaluation does not fully replicate real physiological skin conditions, as factors such as application thickness, film uniformity, photostability, and interactions with skin components significantly influence final efficacy (Osterwalder & Herzog, 2009). Therefore, although the present results indicate promising UVB absorbance, additional in vivo studies are required to confirm clinical effectiveness.

Overall, these findings support the potential inclusion of the formulation in multifunctional cosmetic products with photoprotective properties, particularly when combined with conventional UV filters to enhance protection efficiency and achieve

synergistic effects between spectral absorption and antioxidant activity.

2.3.4. Antibacterial Activity

The results of this study demonstrated that the cosmetic formulation exhibited effective antibacterial activity and biofilm inhibition, together with a relatively safe profile in the Artemia salina toxicity assay.

1/Antibacterial Activity (MIC / MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal:

Concentration (MBC) results showed clear antibacterial efficacy against most reference strains:

- Staphylococcus aureus ATCC 25932 exhibited the highest sensitivity, with MIC and MBC
- values of 20 mg/mL and an MBC/MIC ratio of 1, indicating a strong bactericidal effect.

- Escherichia coli ATCC 25922 and Bacillus subtilis ATCC 25973 showed MIC and MBC values of 40 mg/mL, with an MBC/MIC ratio of 1, confirming direct bactericidal activity.
- In contrast, Pseudomonas aeruginosa ATCC 27853 presented MIC and MBC values >40 mg/mL, showing relatively lower susceptibility, which is expected due to its intrinsic resistance mechanisms, including its outer membrane barrier and efflux systems (Lam et al., 2011).
- The antibacterial effect may be partially attributed to zinc oxide (ZnO), which disrupts bacterial membranes and induces the generation of reactive oxygen species (ROS) (Raghupathi et al., 2011). Additionally, Moringa oil, rich in phenolic compounds and fatty acids, may contribute to bacterial growth inhibition (Leone et al., 2015).

2/Biofilm Inhibition:

The formulation demonstrated a marked inhibitory effect on biofilm formation, with differences observed between Gram-positive and Gram-negative bacteria:

- S. aureus and B. subtilis (Gram-positive) showed high inhibition rates (>90%) at the highest concentration (40% v/v), with sustained moderate inhibition at intermediate concentrations, indicating a stable dose-dependent response.
- Gram-negative bacteria (E. coli and P. aeruginosa) exhibited weaker responses; E. coli showed significant inhibition only at the highest concentration, whereas P. aeruginosa remained minimally affected across all tested concentrations.
- These findings suggest greater effectiveness against Gram-positive biofilm formation, potentially due to the ability of Moringa oil and ZnO to interfere with bacterial adhesion and quorum sensing signaling pathways (Donlan, 2002; Leone et al., 2015; Raghupathi et al., 2011).

3/ Biosafety Assessment (Cytotoxicity):

The Artemia salina assay indicated a relatively safe profile:

- High survival rates (>80%) were observed at low and medium concentrations over 24 hours.
- At the highest concentration (50%), a gradual decrease in survival was noted; however, 50% survival was still recorded after 24 hours, suggesting low to moderate toxicity.
- Compared with the positive control ($K_2Cr_2O_7$), which caused complete mortality within 180 minutes, the assay confirmed the sensitivity and reliability of the experimental model.
- These results support the relative safety of the formulation at concentrations relevant

- for topical application, consistent with the known safety profiles of its components (Draelos, 2006; Meyer et al., 1982; Solis et al., 1993).
- Overall Conclusion
- Collectively, the findings demonstrate that the cosmetic formulation possesses triple functionality:
- Broad-spectrum bactericidal activity, particularly against sensitive strains.
- Effective biofilm inhibition, especially in Gram-positive bacteria, reducing potential resistance development.
- Relative biosafety in preliminary toxicity testing, supporting its suitability for dermatological applications.

These results highlight the synergistic interaction among niacinamide, zinc oxide, and Moringa oil, combining antimicrobial efficacy, skin-protective properties, and acceptable safety, thereby enhancing the formulation's value as a multifunctional cosmetic product.

2.3.5. Tyrosinase test

The results of the tyrosinase inhibition assay showed that the developed cosmetic formulation exhibited noticeable inhibitory activity against the enzyme, with an IC_{50} value of approximately 55.96 µg/mL. The IC_{50} represents the concentration required to inhibit 50% of enzyme activity and is widely used to evaluate the effectiveness of compounds or formulations in reducing tyrosinase activity; lower IC_{50} values generally indicate stronger inhibitory potential (Pillaiyar et al., 2017).

This value suggests moderate inhibitory activity, which is considered acceptable for a multi-component cosmetic formulation rather than a pure compound. Natural extracts and cosmetic formulations typically display higher IC_{50} values compared with standard inhibitors such as kojic acid or ascorbic acid, due to the presence of multiple bioactive compounds acting through different mechanisms (Chang, 2009).

The observed activity may be attributed to the formulation's composition, which includes niacinamide, zinc oxide, and Moringa oleifera seed oil. Moringa seed oil contains phenolic compounds and antioxidants that may interact with the active site of tyrosinase and reduce melanin formation (Anwar et al., 2007). In addition, niacinamide contributes to reducing pigmentation by limiting the transfer of melanosomes from melanocytes to keratinocytes (Hakozaki et al., 2002), while zinc oxide (ZnO) provides antioxidant and UV-protective effects that may indirectly reduce melanin production (Mishra et al., 2017).

Overall, the tyrosinase inhibition observed for the formulation (IC_{50} = 55.96 µg/mL) may result from a synergistic effect among its components, suggesting its potential as a multifunctional cosmetic product for improving skin appearance and reducing hyperpigmentation (Pillaiyar et al., 2017; Draelos, 2018).

Conclusion

Conclusion

At the end of this work, a multi-component cosmetic formulation named **bioMorézinga** was developed and evaluated, based on a well-designed composition including niacinamide, zinc oxide, and Moringa seed oil, with the aim of investigating its biological effectiveness in the context of skin care, particularly with regard to improving skin hydration, promoting skin tone uniformity, enhancing radiance, and providing photoprotective properties.

The results obtained from the tyrosinase inhibition assay demonstrated the formulation's ability to interfere with the biological pathway responsible for melanin synthesis, thereby supporting its potential role in reducing hyperpigmentation and contributing to skin tone evenness.

Furthermore, the findings from the Sun Protection Factor (SPF) assessment indicated that the formulation provides a measurable level of photoprotection, which enhances its relevance in mitigating the harmful effects of solar radiation, a major contributor to oxidative stress and skin dullness.

In addition, the antioxidant assays revealed considerable activity, which can be attributed to the presence of bioactive compounds in Moringa seed oil, including phenolic compounds, tocopherols, phytosterols, and essential fatty acids.

These compounds contribute to the neutralization of free radicals and the reduction of oxidative stress, thereby supporting skin radiance and maintaining physiological balance.

Niacinamide plays a central role in strengthening the skin barrier by promoting ceramide synthesis and reducing transepidermal water loss, which positively affects skin hydration and structural integrity. Zinc oxide also contributes by providing protective and soothing properties, in addition to supporting defense against environmental oxidative stressors.

The synergy between these active ingredients, together with the positive outcomes obtained from the tyrosinase inhibition, SPF evaluation, antioxidant activity, biofilm inhibition, cytotoxicity testing, and antibacterial assays, confirms that these evaluations provided a clear scientific indication of the formulation's potential efficacy within the adopted experimental framework.

Accordingly, this study represents a comprehensive scientific step in the evaluation of a biologically based cosmetic product. However, further research, particularly future clinical studies, would be valuable to strengthen the scientific evidence, verify efficacy under real-life conditions, and assess dermal safety.

Moreover, long-term stability and shelf-life studies are recommended to ensure the preservation of the formulation's physicochemical and biological properties, thereby

supporting its potential industrial development in accordance with established regulatory standards.

Overall, these findings establish a promising scientific foundation for the development of a multi-functional cosmetic formulation that combines protection, hydration, and pigmentation regulation within an integrated system with strong future application potentialissues.

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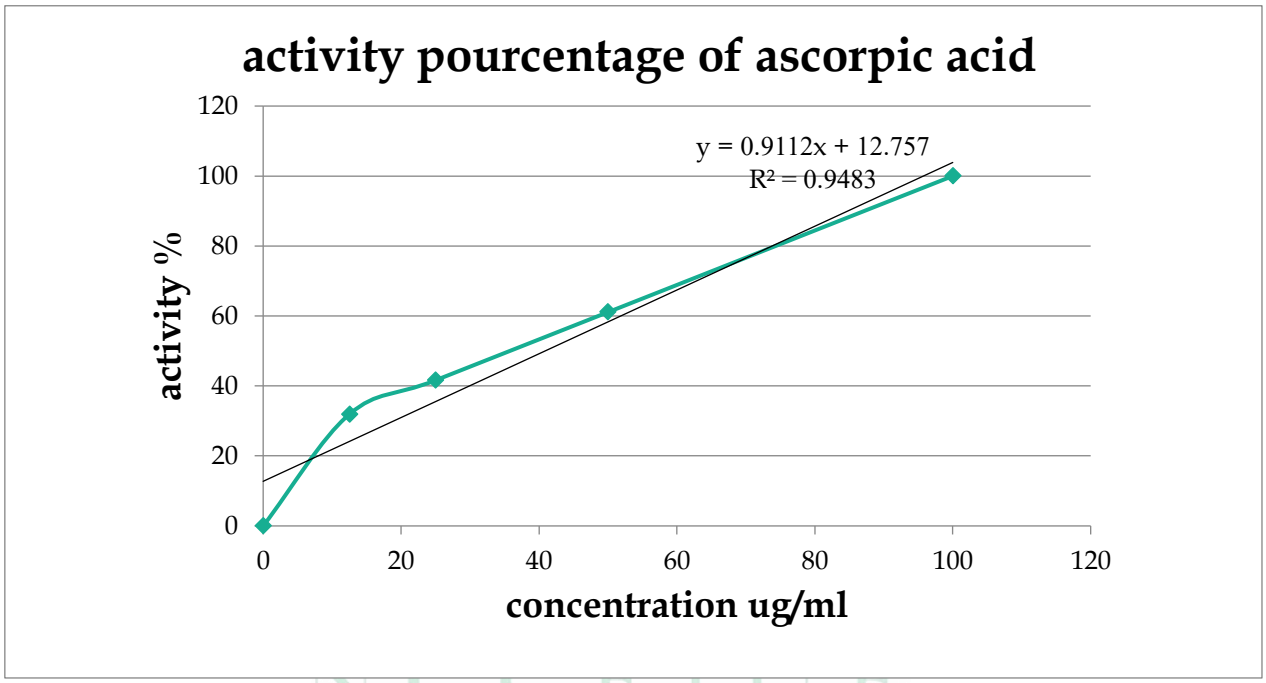
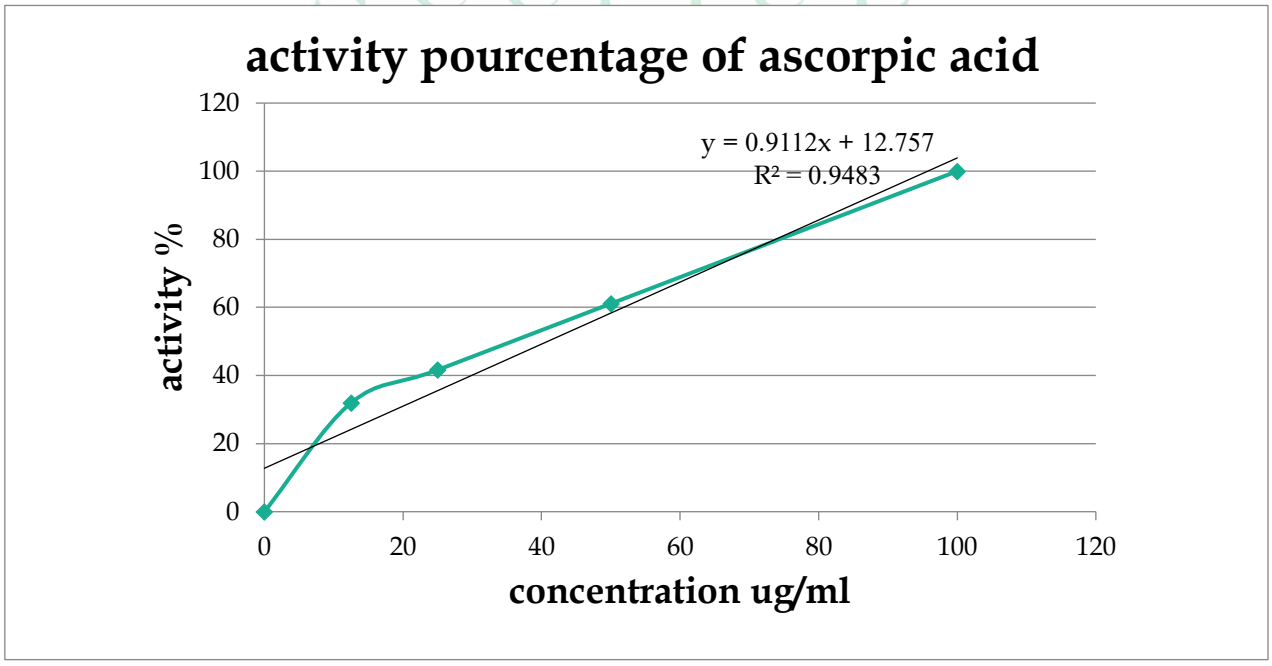
Annexes

Annexe 1.

Results statistical analysis of biofilm formation:

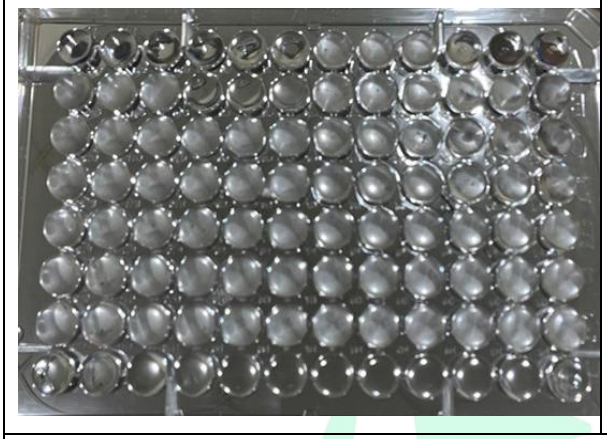
mg/ml	*E. coli*			*P. aeruginosa*			*S. aureus*			*B. subtilis*		
40	0.152	0.067	0.071	1.872	1.925	1.958	0.069	0.082	0.077	0.074	0.081	0.086
20	0.861	0.803	0.721	1.868	1.944	1.956	0.083	0.089	0.081	0.512	0.453	0.409
10	0.864	0.826	0.839	1.871	1.939	1.952	0.615	0.702	0.508	0.497	0.523	0.504
5	0.869	0.824	0.838	1.903	1.919	1.742	0.718	0.633	0.658	0.574	0.668	0.541
2.5	0.857	0.812	0.731	1.856	1.877	1.915	0.936	0.854	0.769	0.728	0.792	0.719
1.25	0.868	0.823	0.836	2.026	2.014	2.031	0.892	0.983	0.911	0.812	0.851	0.674
Control Neg	0.081	0.075	0.066	0.087	0.091	0.096	0.081	0.097	0.078	0.069	0.071	0.076
Control Pos	0.962	0.826	0.811	2.124	2.207	2.346	1.012	0.936	0.889	1.027	1.074	1.031





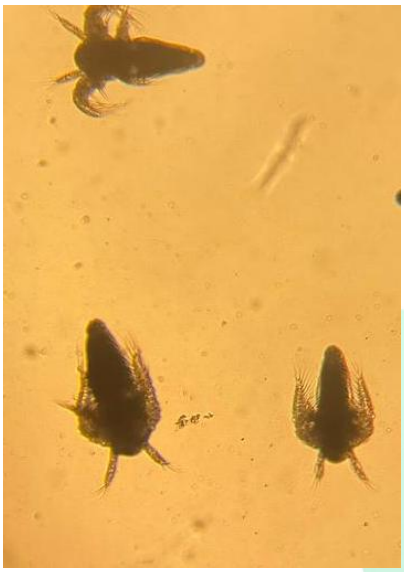
FRAP calibration curve for The cosmetic product and referenc (sunscreen -cream)

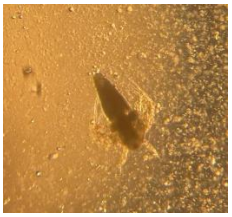
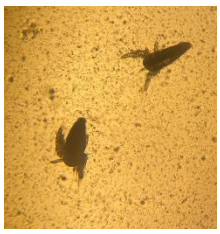
Fig. photo of broth microdilution method used for antimicrobial activity of **The cosmetic product Extract** against Bacteria strains.



Experimental steps to evaluate the toxicity of the BIONOFA product

Microscopic images illustrating the effect of the BIONOFA cosmetic formulation on larvae (toxicity assay).

	
Control Neg	Control Pos ($K_2Cr_2O_7$)

50% **25%** **10%** **5%**

Table 2.6. Effect of different volume/volume (v/v) concentrations of the product on bacterial biofilm formation, expressed as inhibitory rate (%) ± SD.

Concentration (v/v%)	E. coli	P. aeruginosa	S. aureus	B. subtilis
40	89.11 ± 4.27 **	13.73 ± 2.48 *	91.92 ± 1.09 **	92.30 ± 0.58 **
20	8.13 ± 4.64 *	13.53 ± 2.68 *	91.06 ± 0.67 **	56.10 ± 5.31 **
10	2.24 ± 7.09	13.62 ± 2.75 *	35.70 ± 9.44 **	51.34 ± 0.25 **
5	2.19 ± 6.71	16.40 ± 8.20 *	29.14 ± 3.19 **	43.15 ± 4.93 *
2.5	7.49 ± 5.05	15.31 ± 2.89 *	9.92 ± 3.16 *	28.54 ± 2.06 *
1.25	2.35 ± 6.65	8.93 ± 4.41	1.45 ± 9.10	25.44 ± 7.95

Data are presented as mean ± SD of three independent experiments.

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

*p < 0.05, **p < 0.01 compared to the positive control.

The cosmetic product showed a clear inhibitory effect on biofilm formation, and this effect depended on both the bacterial type and the concentration used. Among Gram- positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* were highly sensitive to the oil, showing strong biofilm inhibition above 90% at the highest concentration tested. Notably, *B. subtilis* also maintained moderate inhibition at intermediate concentrations, indicating a stable response to the treatment. In contrast, Gram- negative bacteria exhibited a weaker response. *Escherichia coli* showed marked inhibition only at the highest concentration, while *Pseudomonas aeruginosa* remained poorly affected across all concentrations. Overall, the results suggest that the plant oil is more effective against Gram- positive biofilm formation, with high concentrations acting as a strong biofilm inhibitor, whereas its activity against Gram-negative bacteria is limited.

الملحق الخاص بالمشروع :

Development of Bio-Derm triple-Action: A Natural Formulation Combining Sun Protection, Skin Tone Enhancement, and Radiance — Study and Development

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة الشهيد حمه لخضر-الوادي

مركز تطوير المقاولاتية لجامعة الوادي

الكلية/ المعهد: علوم الطبيعة والحياة

القسم: البيولوجية الجزيئية والخلوية

المستوى والتخصص ثانية مستر بيوكيمياء

مذكرة التخرج لنيل شهادة جامعية – مشروع مؤسسة اقتصادية

عنوان المشروع:

تطوير Bio-Derm Triple-Action: تركيبة طبيعية تجمع بين الحماية من أشعة الشمس، توحيد لون البشرة، والإشراقة — دراسة وتطوير

مقدمة في اطار الحصول على شهادة مؤسسة اقتصادية (مصغرة) في إطار القرار الوزاري 1275 المعدل و المتمم بالقرار الوزارى 008 المؤرخ فى 23 فيفرى 2025

Bio morézinga

السنة الجامعية: 2026 / 2027

الفهرس

أولا: فريق العمل والإشراف

1-فريق العمل

فريق المشروع	التخصص	الكلية/ المعهد	الخبرة/ الكفاءة في مجال المشروع
الطالب: بالخير فاطمة الزهراء	بيوكيمياء تطبيقية	علوم الطبيعة والحياة	مقبولة وقابلة للتطوير
الطالب: بسر نور الهدى	بيوكيمياء تطبيقية	علوم الطبيعة والحياة	مقبولة وقابلة للتطوير
الطالب:			
الطالب:			
الطالب:			
الطالب:			

2-فريق الإشراف

المشرف	التخصص	الكلية
المشرف الرئيسي: عبد المالك زعتر	علوم فلاحية	علوم الطبيعة والحياة
المشرف المساعد: ياسين بوراس	بيوكيمياء تطبيقية	علوم الطبيعة والحياة

ثانيا: تعريف المشروع: يكون النشاط وفقا للمدونات التي تحدد قائمة الأنشطة لكل هيئة تسجيل (المركز الوطني للسجل التجاري، الفلاحة، الصناعة التقليدية والحرف)؛

تحديد النشاط	قطاع النشاط	رمز النشاط (وفق مدونة الأنشطة للمركز الوطني للسجل التجاري cnrc)
صناعة مواد التجميل و التنظيف البدني	مستحضرات التجميل	104213

❖ في حالة نشاط مقنن، أو نشاط يحتاج إلى اعتمادات او تراخيص خاصة، يجب ذكرها وذكر الجهة المانحة لها

-السجل التجاري: يمنح من المركز الوطني للسجل التجاري

-مطابقة ومعايير السلامة الصحية لمستحضرات التجميل: تحت رقابة وزارة التجارة الداخلية وضبط السوق الوطنية ووزارة الصناعة

ثالثا: وصف فكرة المشروع:

ينغي ان يكون صاحب / أصحاب المشروع على دراية بكل تفاصيل المشروع وقادرين على تقديم وصف ملخص ودقيق عنه ، من خلال الإجابة بدقة على العناصر التالية :

ما هي منتجاتي أو خدماتي: (حدد بدقة منتجاتك. خدماتك): يتمثل المشروع في تطوير وإنتاج مستحضر تجميلي طبيعي متعدد الوظائف تحت اسم Bio-Derm Triple-Action، يجمع المنتج بين الحماية من الأشعة فوق البنفسجية، تحسين وتوحيد لون البشرة، وتعزيز إشراقتها، مما يجعله مناسبًا للاستخدام اليومي
يعتمد المستحضر على مكونات طبيعية وفعالة مثل أكسيد الزنك والزيوت النباتية والمركبات المضادة للأكسدة، بهدف توفير منتج آمن وفعال يتناسب مع مختلف أنواع البشرة ويواكب متطلبات سوق مستحضرات التجميل الطبيعية.

من سيشتري منتجاتي/ خدماتي (حدد زبائنك بدقة): -النساء من 18 إلى 45 سنة/الأشخاص الذين يعانون من مشاكل البشرة (صبغات، حب شباب، عدم توحيد اللون) /الأشخاص ذوو البشرة الحساسة/المهتمون بالمستحضرات الطبيعية والعناية بالبشرة/الأشخاص المعرضون بشكل كبير لأشعة الشمس ويحتاجون حماية جلدية

أين سيقام مشروعي وكيف تصل منتجاتي / خدماتي للزبائن :(بين مكان إقامة مشروعك وطرق التواصل مع زبائنك) سيقام المشروع في المنزل (أو ورشة صغيرة محلية) مع إمكانية التوسع لاحقًا إلى مخبر أو وحدة إنتاج صغيرة

_يتم تسويق وبيع المنتجات محليًا داخل المنطقة (الحي/البلدية/الولاية) /البيع عبر الصيدليات ومحلات مستحضرات التجميل/الترويج والبيع عبر وسائل التواصل الاجتماعي (Facebook، Instagram، TikTok) /إمكانية توصيل المنتجات للزبائن عبر التوصيل المحلي أو البريد/التواصل مع الزبائن يكون عبر الهاتف، الرسائل، وصفحات التواصل الاجتماعي.

كيف سيشتغل مشروعي (بين من سيقوم بتشغيل المشروع ومن سيساهم في ذلك): -سيتم تسيير المشروع من طرف صاحبتي المشروع بشكل أساسي (الإشراف، الإنتاج، والتسويق)/قد يتم الاستعانة بمساعد في مراحل الإنتاج والتغليف عند زيادة الطلب /الاستفادة من مختصين أو مخابر خارجية في حالة الحاجة إلى التحاليل أو تطوير التركيبات /التعاون مع مزودي المواد الأولية (الزيوت النباتية، المواد الفعالة، التغليف)/الاستعانة بخدمات التوصيل أو البريد لتوزيع المنتجات على الزبائن.

مبررات اختيارفكرة مشروعي (اشرح لماذا تم اختيار هذه الفكرة بالذات): -تزايد الطلب على منتجات العناية بالبشرة الطبيعية والآمنة/انتشار مشاكل البشرة مثل التصبغات وحب الشباب بسبب العوامل البيئية والتعرض للشمس/نقص المنتجات الطبيعية الفعّالة في السوق المحلي بأسعار مناسبة/توفر مواد أولية نباتية ذات خصائص علاجية وتجميلية (مثل زيت المورينغا)/الرغبة في تقديم منتج يجمع بين الفعالية والأمان للبشرة/إمكانية تحويل الفكرة إلى مشروع مربح وقابل للتطور مستقبلاً.

رابعا: دراسة السوق

1. **طبيعة وشدة المنافسة:** من هم المنافسون المباشرون وغير المباشرون؟

المنافسون	طبيعة المنافسة		شدة المنافسة		
	مباشرة	غير مباشرة	قوية	متوسطة	ضعيفة
L'Oréal		X		X	
المنتجات المنزلية التقليدية		X			X
Ordinary/Nivea		X	X		

أذكر استراتيجيات مؤسستك في التعامل مع منافسيها: .. تعتمد مؤسستنا على التميز في جودة المنتجات الطبيعية، والابتكار المستمر، والتسعير التنافسي لتعزيز مكانتها في السوق. كما تركز على التسويق الرقمي، وبناء ثقة الزبائن من خلال الشفافية وجودة الخدمة، إضافة إلى استهداف المهتمين بالعناية الطبيعية بالبشرة وتوسيع قنوات التوزيع عبر الصيدليات والمنصات الإلكترونية.

2. **حجم السوق المستهدف والمحتمل:**

- **حجم السوق المستهدف:** يمثل مجموعة الافراد أو المؤسسات والتي تقدم لها او تعرض عليها – منتجاتك/خدامتك. (يطلب تحديد الشريحة المستهدفة، تعدادها، مكان تواجدها، .....).
- يستهدف المنتج فئة النساء والرجال من 18 إلى 45 سنة، خاصة أصحاب البشرة الدهنية، الحساسة أو المعرضة للحبوب، والمهتمين باستعمال مستحضرات طبيعية للعناية بالبشرة. يتمركز السوق المستهدف في المدن والمناطق الحضرية عبر الصيدليات، محلات التجميل، ومنصات البيع الإلكتروني. أما السوق المحتمل فيشمل جميع مستعملي منتجات العناية بالبشرة الطبيعية داخل الجزائر مع إمكانية التوسع مستقبلاً إلى أسواق أخرى.
- **حجم السوق المحتمل:** السوق المحتمل هو مجموعة الافراد او المؤسسات التي تطلب أو يحتمل ان تطلب منتجاتك او خدماتك لإشباع حاجا تهم ورغباتهم. (من يشتري منتجاتنا/ خدماتنا؟ من وما الذي يحفزه على ذلك؟ أين يتواجدون؟ كم أعدادهم؟).

.. يتمثل السوق المحتمل في مستهلكي مستحضرات العناية بالبشرة الطبيعية وشبه الطبية، خاصة الفئة العمرية من 18 إلى 45 سنة من النساء والرجال الذين يعانون من مشاكل البشرة الدهنية، الحساسة أو المعرضة لحب الشباب. ويُحفَّز الطلب على المنتج بارتفاع الوعي بأهمية المكونات الآمنة والفعالة مثل النياسيناميد وأكسيد الزنك والزيوت النباتية الطبيعية. ويتمركز هذا السوق أساسًا في المناطق الحضرية وعبر منصات البيع الإلكتروني والصيدليات، مع قابلية نمو مرتفعة نتيجة التوسع المستمر لسوق مستحضرات العناية بالبشرة في الجزائر.

3. **تحليل SWOT:** نقاط القوة والضعف والفرص والتهديدات لمشروعي:

- **نقاط القوة:** كل العناصر (داخل المؤسسة) التي تشكل مصدر قوة لمشروعي (قد تكون: مهارات خاصة، موارد بشرية، موارد مادية، .....)
- **نقاط الضعف:** كل العناصر (داخل المؤسسة) التي تشكل نقطة ضعف أو تقف عائقا امام مشروعي حاليا أو مستقبليا
- **الفرص:** كل العناصر الخارجية التي لها أثر إيجابي على تنفيذ مشروعي أو تطوره في المستقبل
- **التهديدات:** كل العناصر الخارجية التي لها أثر سلبي على تنفيذ مشروعي أو تطوره في المستقبل

جدول تحليل SWOT

SWOT	عوامل تساعد في تحقيق الأهداف	عوامل تعطل تحقيق الأهداف
عوامل داخلية	**نقاط القوة** Strengths 1. تركيبة طبيعية فعالة (زيت المورينغا، النياسيناميد، أكسيد الزنك). 2. مناسب وآمن للبشرة الحساسة والدهنية. 3. يمنح ميزة تنافسية في سوق التجميل الطبيعي	**نقاط الضعف** Weaknesses 1. حداثة المشروع وقلة شهرة العلامة التجارية. 2. محدودية الإمكانيات الإنتاجية في المرحلة الأولى. 3. ضعف القدرات التسويقية قد يؤثر على سرعة الانتشار.
عوامل خارجية	**الفرص** Opportunities 1. تزايد الطلب على المنتجات الطبيعية للعناية بالبشرة. 2. ارتفاع الوعي الصحي لدى المستهلكين. 3. توسع التجارة الإلكترونية والصيدليات التجميلية.	**التهديدات** Threats 1. منافسة قوية من العلامات التجارية المحلية والدولية. 2. انتشار منتجات مشابهة ومتنوعة في السوق. 3.. تقلب أسعار المواد الأولية وصعوبة بناء الثقة بسرعة.

4. نموذج العمل التجاري BMC لمشروعي:

العملاء المستهدفون	العلاقة مع العملاء	القيمة المقترحة	الأنشطة الرئيسية	الشركاء الرئيسيون
.النساء والفتيات المهتمات بالبشرة .المصابون بالتصبغات والبقع .أصحاب البشرة الحساسة الشباب المهتمون بالعناية الحديثة. .كل الفئات من العمر(18-45)	تقديم إرشادات حول الاستعمال/تواصل عبر وسائل التواصل الاجتماعي/بناء الثقة عبر النتائج .العلمية/تعزيز الولاء عبر رضا العملاء **قنوات توزيع** .الصيدليات ومحلات التجميل .البيع الإلكتروني ووسائل التواصل .المعارض والأسواق المحلية التعاون مع مراكز التجميل	منتج طبيعي وآمن متعدد الوظائف تفتيح وتقليل التصبغات/مضاد أكسدة وبكتيريا/حماية من الأشعة فوق البنفسجية/بديل أقل ضررًا من المنتجات الكيميائية.	تطوير وتصنيع تركيبة تجميلية . طبيعية فعّالة مع إجراء اختبارات وتقييمات بيولوجية وكيميائية وفي ا.زيائية لضمان الجودة والفعالية لتسويق والترويج الإلكتروني للمن تج، ثم توزيعه ومتابعة رضا العملا ء **الموارد الرئيسية** تركيبة علمية مبتكرة/مواد فعالة مورينغا، نياسيناميد،) طبيعية ZnO) خبرة بحثية وعلمية/معدات التحضير .والاختبار/هوية تجارية وتغليف	مورّدو المواد الأولية الطبيعية (زيوت ومواد فعالة)/مخابر التحاليل والرقابة لضمان الجودة/الصيدليات ومحلات مستحضرات التجميل كمنافذ بيع/أطباء الجلد وخبراء التجميل للتوصية/شركات التغليف .والتسويق الرقمي

الإيرادات	التكاليف
.بيع مباشر للمنتج/ توزيع للصيدليات ومحلات التجميل .توسيع خط المنتجات مستقبلًا .شراكات مع مراكز التجميل	.المواد الأولية والمواد الفعالة/ التحاليل واختبارات الجودة .التغليف والتخزين/ التسويق والإعلانات .النقل والتوزيع / التصنيع والتجهيزات التقنية

خامسا: الدراسة التقنية

1. **احتياجات المشروع من الموارد البشرية:** حدد بدقة احتياجات مشروعك من الموارد البشرية

طبيعة المستخدمين	العدد	الكفاءات / الشهادات
المسير	01	شهادة جامعية في البيولوجيا او الكيمياء
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق، رؤساء الورشات...إلخ.)	02	خبرة في مستحضرات التجميل والتسيير
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	02	التحكم في التسيير والتنظيم
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	03	خبرة ميدانية بسيطة

2. **اختيار موقع إقامة المشروع: أين سيكون موقع المشروع ومبررات اختياره؟** (قدم المبررات اللازمة لاختيار موقع المشروع)

تم اختيار ولاية **EL OUED** (خاصة منطقة الشط الصناعية) كموقع لإنشاء المشروع نظرًا لما تتميز به من نشاط تجاري وحيوية اقتصادية تساعد على نجاح المؤسسة وتطورها. كما تشهد الولاية تزايدًا في اهتمام المستهلكين بمنتجات العناية بالبشرة والمستحضرات الطبيعية، خاصة المنتجات التي تتميز بالجودة

إضافة إلى ذلك، يساهم قرب المشروع من الصيدليات ومحلات التجميل في تسهيل عمليات التوزيع والتسويق والوصول إلى الفئة المستهدفة من الزبائن، مما يساعد على تعزيز انتشار المنتج داخل السوق المحلي. كما يوفر الموقع سهولة في النقل والتزوّد بالمواد الأولية، مع انخفاض نسبي في تكاليف الإيجار والتشغيل مقارنة ببعض الولايات الكبرى، وهو ما يساهم في تقليل تكاليف الانطلاق وتحقيق استقرار أفضل للمشروع في مراحله الأولى.

سادسا: تقدير تكاليف و إيرادات المشروع

1 - تقدير تكاليف المشروع

1.1 تقدير المبلغ الإجمالي للاستثمار: (تكلفة المشروع)

البيان	المبلغ (دج)
آلات ومعدات الإنتاج	1.200.000
تهيئة المحل	600.000
الأصول البيولوجية (الحيوانات- الأشجار...)	/
العتاد المتنقل	115.000
العتاد المكتبي	80.000
عتاد الإعلام الآلي	90.000
عتاد السمعي البصري	85.000
عتاد الاتصالات	35.000
تركيب التجهيزات	80.000
المصاريف العامة المتعلقة بالإنشاء	265.000
رأس المال العامل (المادة الأولية)	1.150.000
المبلغ الإجمالي للاستثمار (تكلفة المشروع)	**3.700.000دج**

2.1 تقدير مشتريات المشروع من المواد الأولية

قائمة المواد الاولية		السنة 1	السنة 2	السنة 3
المادة الاولية 1: بدور المورينغا	الكمية المشترات	25كغ	35كغ	45كغ
	سعر الوحدة	3333دج/كغ	3333دج/كغ	3333دج/كغ
	تكلفة شراء المادة الاولية 1	83.325دج	116.655دج	149.985دج
المادة الاولية 2: .السيروم(نياسيناميد/أوكسيد الزنك)	الكمية المشترات	30لتر	40لتر	50لتر
	سعر الوحدة	2000دج/لتر	2000دج/لتر	2000دج/لتر
	مبلغ المادة الاولية 2	60.000دج	80.000دج	10.000دج
المادة الاولية 3: مواد مساعدة(حافظة. تثبيت. تحسين قوام)	الكمية المشترات	40لتر	60لتر	80لتر
	سعر الوحدة	6000دج/لتر	6000دج/لتر	6000دج/لتر
	تكلفة شراء المادة الاولية 3	240.000دج	360.000دج	480.000دج
إجمالي مبالغ المشتريات من المواد الاولية		**383.325دج**	**556.655دج**	**729.985دج**

3.1 – تقدير تكاليف اليد العاملة

تسمية المنصب	عدد العمال			قيمة الاجر		
	السنة 1	السنة 2	السنة 3	السنة 1	السنة 2	السنة 3
المسير/ أو المسيرون	1	1	1	40.000دج	50.000دج	60.000دج
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق ، رؤساء الورشات...إلخ.)	2	2	3	45.000دج	50.000دج	60.000دج
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	1	1	1	35.000دج	40.000دج	45.000دج
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	3	4	5	30.000دج	35.000دج	40.000دج

- ينبغي ان لا يقل الاجر الشهري الصافي عن الحد الأدنى المضمون

4.1 تقدير التكاليف الخارجية للمشروع

البيان	السنة 01	السنة 02	السنة 03
مصاريف الكهرباء /الغاز /الماء	72.000دج	84.000دج	96.000دج
تكاليف الصيانة و التصليح	60.000دج	72.000دج	84.000دج
تكاليف الايجار	300.000دج	360.000دج	480.000دج
مصاريف تامين العتاد و المعدات	72.000دج	84.000دج	96.000دج
مصاريف التسويق (الدعاية والإشهار)	50.000دج	48.000دج	36.000دج
تكاليف التكوين والتدريب	30.000دج	35.000دج	40.000دج
مصاريف عامة (أعباء خارجية) أخرى	36.000دج	36.000دج	36.000دج
المجموع	**620.000دج**	**719.000دج**	**868.000دج**

5.1 تقدير تكاليف اهتلاك الاستثمارات

البيان	مدة الاهتلاك (بالسنوات)	قسط الاهتلاك السنوي (دج)
آلات ومعدات الإنتاج	5سنوات	120.000دج
العتاد المتنقل	5سنوات	23.000دج
العتاد المكتبي	5سنوات	16.000دج
عتاد الإعلام الآلي	5سنوات	18.000دج
عتاد السمعي البصري	4سنوات	21.250دج
عتاد الاتصالات	5سنوات	7.000دج
.....		
المبلغ الإجمالي		**182.021.25دج**

- يطبق الاهتلاك الخطي (تحدد مدة الاهتلاك وفقا لقرار وزارة المالية المؤرخ في 14 أفريل 2025 المعدل والمتمم للقرار الصادر بتاريخ 25 فيفري 2024 الذي يحدد مدة اهتلاك التثبيتات المطبقة لتحديد النتيجة الجبائية – الجريدة الرسمية عدد 29 لسنة 2025)

3-تقدير إيرادات المشروع

1.3 تقدير حجم المبيعات السنوية

البيان		السنة 1	السنة 2	السنة 3
المنتج / الخدمة 1: المنتج التجميلي متعدد الوظائف	الكمية المباعة	3000	6000	12000
	سعر الوحدة	1.800دج	1.900دج	2000دج
	قيمة مبيعات المنتج 1	5.400.000دج	11.400.000دج	24.000.000دج
المنتج / الخدمة 2:	الكمية المباعة			
	سعر الوحدة			
	قيمة مبيعات المنتج 2			
المنتج / الخدمة 3:	الكمية المباعة			
	سعر الوحدة			
	قيمة مبيعات المنتج 3			
إجمالي المبيعات		5.400.000	11.400.000	24.000.000

2 .3 حساب النتيجة السنوية

البيان	السنة 01	السنة 02	السنة 03
رقم الأعمال (المبيعات) ... (1)	3.600.000دج	7.700.000دج	16.400.000دج
المشتريات المستهلكة ... (2)	2.200.000دج	4.878.000دج	9.756.000دج
الأعباء الخارجية ... (3)	900.000دج	1.260.000دج	1.560.000دج
القيمة المضافة ... (4)= (1)-(2)-(3)	500.000دج	1.562.000دج	5.084.000دج
الأجور و الأعباء الاجتماعية ...(5)	420.000دج	660.000دج	840.000دج
إجمالي فائض الاستغلال ... (6) = (4)-(5)	80.000دج	902.000دج	4.244.000دج
الإهتلاكات و المؤونات....(7)	40.000دج	120.000دج	120.000دج
ناتج الاستغلال..... (8) = (6) - (7)	40.000دج	782.000دج	4.124.000دج
الضرائب على الشركات/ IFU .... (9)	7.600دج	148.580دج	783.560دج
صافي الدخل ..... (10) = (8) - (9)	32.400دج	633.420دج	3.340.440دج
تطوررقم الأعمال	**7.820.000دج**	**18.646.000دج**	**31.492.000دج**

الخاتمة : القيمة المضافة المتوقعة للمشروع على الاقتصاد المحلي والوطني

في ختام هذه الدراسة، يُتوقع أن يساهم مشروع إنتاج مستحضر تجميلي طبيعي في تحقيق قيمة مضافة على المستويين المحلي والوطني، من خلال دعم قطاع الصناعات التجميلية الطبيعية وتشجيع استغلال الموارد الطبيعية المحلية في إنتاج منتجات ذات جودة وفعالية. كما يساهم المشروع في خلق فرص عمل، وتنشيط الحركة الاقتصادية، ودعم المؤسسات الناشئة والمشاريع الصغيرة.

إضافة إلى ذلك، يساعد المشروع على تلبية الطلب المتزايد على مستحضرات العناية بالبشرة الطبيعية والآمنة، مما يعزز ثقة المستهلك بالمنتجات المحلية ويقلل من الاعتماد على المنتجات المستوردة. كما يمكن أن يشكل المشروع خطوة نحو تطوير صناعة تجميل وطنية قادرة على المنافسة وتحقيق قيمة اقتصادية مستدامة مستقبلًا.