PEOPLE' S DEMOCRATIC REPUBLIC OF ALGERIA

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THEME

Influence of geographical location and environmental factors on the phytochemical composition, antioxidant activity, anti-inflammatory activity and antibacterial activity of the hydroethanolic extracts of *Moringa oleifera lam.* Seeds from three distinct regions of Algeria.

Defended on 07/07/2025

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Dedication

With love and gratitude, this Thesis is dedicated to:

My Father, Mr. CHEROR ISAAC BROKONY, whose unwavering support and belief have been a lighthouse through every storm, your support, Love, prayers and belief in me illuminated my path during difficult times.

My Mother, Mrs. CHEMUTAI JULIET CHEROR, whose endless love, support, prayers, sacrifices and encouragement have become the cornerstone of who I am and the source of my courage and peace.

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And to my dear friends, thank you for reminding me to laugh; to breathe and to keep going, your presence has been a beautiful reminder that I was never alone on this journey.

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support, encouragement and sacrifices, my friends, myself for the hard work and the patience, and every individual who has participated in the realization of this research work. Your efforts, which have been fundamental to my progress and success, have not gone unnoticed.

Abstract

The present study investigates the influence of geo-environmental heterogeneity on the phytochemical constitution and biological efficacy of the hydroethanolic extract of *Moringa oleifera lam.* seeds harvested from three climatically divergent Algerian regions: Mostaganem (Mediterranean coastal), Adrar (hyper-arid), and Tamanrasset (mountainous hyper-arid Sahara).

Various methods like DPPH radical scavenging assay, protein inhibition assay and well diffusion assay were used to determine the antioxidant activity, Anti-inflammatory activity and antibacterial activity respectively. Quantitative methods like Follin ciocalteau assay, vanillin-HCl assay, Ferric chloride assay and Aluminum chloride assay were used to determine the total phenolic content, condensed tannins, hydrolysable tannins, and total flavonoid content respectively. A comprehensive comparative analysis revealed significant regional disparities (significance determined using ANOVA, $p < 0.05$) in physicochemical and phytochemical traits. Seeds from Mostaganem exhibited the highest dry matter content ($99.25 \pm 0.32\%$), mineral matter ($6.44 \pm 0.39\%$), and the lowest organic matter ($93.55 \pm 0.35\%$), coupled with the phytochemical profile, total phenolic content (TPC: 34.83 ± 4.33 mg GAE/g DW), condensed tannins (CT: 7.25 ± 0.29 mg CE/g DW), and hydrolysable tannins (HT: 2.29 ± 0.07 mg GAE/g DW), with Tamanrasset having the highest phytochemical profiles (TPC: 37.76 ± 2.22 mg GAE/g DW), (TFC: 6.04 ± 0.89 mg QE/g DW), and (CT: 12.26 ± 0.77 mg CE/g DW). Antioxidant capacity, evaluated via DPPH radical scavenging assay, demonstrated a dose-dependent inhibition, with the lowest IC_{50} observed in Tamanrasset (2.13 ± 0.05 mg/mL), outperforming Mostaganem (2.64 ± 0.08 mg/mL) and Adrar (3.25 ± 0.10 mg/mL). Protein denaturation inhibition assay revealed potent anti-inflammatory activity, particularly in Mostaganem ($49.72 \pm 2.22\%$) and Adrar ($49.26 \pm 1.65\%$) samples, closely approximating the reference drug diclofenac ($52.96 \pm 1.22\%$). Antibacterial tests against *Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis, Klebsiella pneumonia, Salmonella paratyphi* with *Staphylococcus aureus and Escherichia coli* demonstrating superior inhibition zones in Tamanrasset and Adrar extracts (15.3 ± 0.5 mm and 13.6 ± 0.4 mm, respectively), indicating robust antimicrobial potential. Multivariate principal component analysis (PCA) underscored

the strong correlation between environmental stressors (temperature, altitude, and edaphic factors) and the biosynthesis of bioactive metabolites.

These findings emphasize that eco-climatic determinants substantially modulate the therapeutic capacity of *Moringa oleifera*, making regional provenance a critical factor in standardizing raw materials for phytopharmaceutical and nutraceutical applications.

Keywords: *Moringa oleifera*, Phytochemical composition, Antioxidant activity, Anti-inflammatory potential, hydro ethanol, Geographical variation.

Résumé

La présente étude examine l'influence de l'hétérogénéité géoenvironnementale sur la constitution phytochimique et l'efficacité biologique des graines de *Moringa oleifera* récoltées dans trois régions algériennes climatiquement divergentes: Mostaganem (côtière méditerranéenne), Adrar (hyper-aride) et Tamanrasset (Sahara montagneux hyper-aride).

Différentes méthodes telles que l'essai de piégeage des radicaux DPPH, l'essai d'inhibition des protéines et l'essai de diffusion en milieu gélosé ont été utilisées pour déterminer respectivement l'activité antioxydante, l'activité anti-inflammatoire et l'activité antibactérienne. Des méthodes quantitatives telles que l'essai de follin ciocalteau, l'essai de vanilline-HCl, l'essai au chlorure ferrique et l'essai au chlorure d'aluminium ont été utilisées pour déterminer respectivement la teneur totale en phénols, les tanins condensés, les tanins hydrolysables et la teneur totale en flavonoïdes.Une analyse comparative complète a révélé des disparités régionales significatives (Significatifs déterminé à l'aide d'ANOVA, $p < 0,05$) dans les caractéristiques physico-chimiques et phytologiques. Les graines de Mostaganem ont montré la plus haute teneur en matière sèche (99,25 ± 0,32 %), matière minérale (6,44 ± 0,39 %) et la plus faible matière organique (93,55 ± 0,35 %), DPPH associé au profil phytochimique, au contenu total en phénols (TPC : 34,83 ± 4,33 mg GAE/g DW), aux tannins condensés (CT : 7,25 ± 0,29 mg CE/g DW) et aux tannins hydrolysables (HT : 2,29 ± 0,07 mg GAE/g DW), avec Tamanarasset ayant les profils phytochimiques les plus élevés (TPC : 37,76 ± 2,22 mg GAE/g DW), (TFC : 6,04 ± 0,89 mg QE/g DW) et (CT : 12,26 ± 0,77 mg CE/g DW). La capacité antioxydante, évaluée par essai de capture des radicaux DPPH, a démontré une inhibition dépendante de la dose, avec la plus faible IC_{50} observée à Tamanrasset (2,13 ± 0,05 mg/ml), surpassant Mostaganem (2,64 ± 0,08 mg/ml) et Adrar (3,25 ± 0,10 mg/ml). Le test d'inhibition de la dénaturation des protéines a révélé une puissante activité anti-inflammatoire, en particulier dans les échantillons de Mostaganem (49,72 ± 2,22 %) et d'Adrar (49,26 ± 1,65 %), approchant de près le médicament de référence diclofénac (52,96 ± 1,22 %). Les tests antibactériens contre *Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis, Klebsiella pneumonia, Salmonella paratyphi avec Staphylococcus aureus* et *Escherichia coli* ont démontré des zones d'inhibition supérieures dans les extraits de Tamanrasset et d'Adrar (15,3 ± 0,5 mm et 13,6 ± 0,4 mm, respectivement), indiquant un potentiel antimicrobien robuste. L'analyse en composantes principales multivariée (PCA) a souligné la forte corrélation

entre les stress environnementaux (température, altitude et facteurs édaphiques) et la biosynthèse des métabolites bioactifs.

Ces résultats soulignent que les déterminants éco-climatiques modulent considérablement la capacité thérapeutique de *Moringa oleifera*, faisant de l'origine régionale un facteur critique dans la standardisation des matières premières pour les applications phytopharmaceutiques et nutraceutiques.

Keywords : *Moringa oleifera*, composition Phytochimique, Activité Antioxydant, hydroethanolique, potentielle Anti-inflammatoire, variatiMOon Géographique

ملخص

تبحث الدراسة الحالية تأثير التباين الجغرافي-البيئي على التركيب الكيميائي النباتي والفعالية البيولوجية للمستخلص الهيدروإيثانولي من بذور *Moringa oleifera lam*. التي تم حصادها من ثلاث مناطق جزائرية متباينة مناخياً: مستغانم (الساحل المتوسطي)، أدرار (شديدة الجفاف)، وتمنراست (الصحراء الجبلية شديدة الجفاف) .

تم استخدام طرق مختلفة مثل اختبار إزالة الجذور الحرة DPPH واختبار تثبيط البروتين واختبار الانتشار الجيد لتحديد النشاط المضاد للأكسدة والنشاط المضاد للالتهابات والنشاط المضاد للبكتيريا على التوالي. استخدمت طرق كمية مثل اختبار Follin ciocalteauواختبار الفانيلين HCl-واختبار كلوريد الحديديك واختبار كلوريد الألومنيوم لتحديد المحتوى الفينولي الكلي والتانينات المكثفة والتانينات القابلة للتحلل والمحتوى الكلي للفلافونويد على التوالي. كشف تحليل مقارن شامل عن تباينات إقليمية كبيرة (تم تحديد الأهمية باستخدامANOVA ، ($p < 0.05$ في الخصائص الفيزيائية والكيميائية والنباتية. أظهرت بذور موستغانيم أعلى محتوى من المادة الجافة (99.25 ± 0.32٪) والمواد المعدنية (6.44 ± 0.39٪) وأدنى محتوى من المواد العضوية (93.55 ± 0.35٪)، إلى جانب الملف الكيميائي النباتي والمحتوى الفينولي الإجمالي :TPC) 34.83 ± 4.33مجم/GAE جم(DW ، والتانينات المكثفة 0.29 ± 7.25 :CT) مجم/CE جم(DW ، والتانينات القابلة للتحلل 0.07 ± 2.29 :HT) مجم/GAE جم(DW ، مع تمانراست التي تتمتع بأعلى ملامح كيميائية نباتية 37.76 :TPC) 2.22 ±مجم/GAE جم(DW ، 0.89 ± 6.04 :TFC)مجم/QE جم(DW ، و 0.77 ± 12.26 :CT) مجم/CE جم DW).أظهرت القدرة المضادة للأكسدة، التي تم تقييمها من خلال اختبار إزالة الجذور الحرةDPPH ، تثبيطًا يعتمد على الجرعة، حيث لوحظت أقل قيمة IC_{50} في تمنراست (2.13 ± 0.05 مجم/مل)، متفوقة على مستغانم (2.64 ± 0.08 مجم/مل) وأدرار (3.25 ± 0.10 مجم/مل). أظهر اختبار تثبيط تحلل البروتين نشاطًا مضادًا للالتهابات قويًا، خاصة في عينات مستغانم (49.72 ± 2.22٪) وأدرار (49.26 ± 1.65٪)، وهو ما يقترب كثيرًا من الدواء المرجعي ديكلوفيناك (52.96 ± 1.22٪). أظهرت الاختبارات المضادة للبكتيريا ضد *Pseudomonas aeruginosa* و *Bacillus cereus* و *Proteus mirabilis* و *Klebsiella pneumonia* ، *Salmonella paratyphi*مع *Staphylococcus aureus* و Escherichia coli أظهرت مناطق تثبيط فائقة في مستخلصات تمنراست وأدرار (15.3 ± 0.5 مم و 13.6 ± 0.4 مم على التوالي)، مما يشير إلى إمكانات مضادة للميكروبات قوية. أكد تحليل المكونات الرئيسية المتعددة المتغيرات (PCA) على وجود علاقة قوية بين عوامل الإجهاد البيئي (درجة الحرارة والارتفاع والعوامل التربة) والتخليق الحيوي للمستقلبات النشطة بيولوجيًا .

تؤكد هذه النتائج أن العوامل البيئية والمناخية تؤثر بشكل كبير على القدرة العلاجية لمورينغا أوليفيرا، مما يجعل المنشأ الإقليمي عاملاً حاسمًا في توحيد المواد الخام المستخدمة في التطبيقات الصيدلانية النباتية والمغذية.

الكلمات المفتاحية*Moringa oleifera* :، التركيب الكيميائي النباتي، النشاط المضاد للأكسدة، القدرة المضادة للالتهابات، الهيدرو إيثانول، التباين الجغرافي.

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List Of Acronyms

A

ASE: Accelerated Solvent Extraction

ALT: Alanine AminoTransferase

AEMO: Aqueous Extract of *Moringa oleifera*

AST: Aspartate aminotransferase

ATCC: American Type Culture Collection

B

Bcl-2: B-cell Lymphoma 2

Bax: Bcl-2 Associated X protein

BC: Before Christ

BHT: Butylated HydroxyToluene

C

CA: Caffeic Acid

CCl4: Carbon Tetrachloride

CVDs: Cardiovascular Diseases

CHS: Chalcone Synthase

CoA: Co-enzyme A

CTs: Condensed Tannins

COX-2: Cyclooxygenase-2

CE: Catechin Equivalent

D

DPPH: 2, 2-DiPhenyl-1-PicrylHydrazyl

DNA: Deoxy Ribonucleic Acid

DM: Dry Matter

DA: Daltons

E

VEGF: Endothelial Growth Factor

F

FPG: Fasting Plasma Glucose

FRAP: Ferric Reducing Antioxidant Power

FA: Ferulic Acid

FC: Follin ciocaltaeu

G

GAE: Gallic acid equivalents

H

IC_{50}: Half Maximal Inhibitory Concentration

HaCaT: Human Adult Catastrophic Transformed Keratinocytes

HEK-293: Human Embryonic Kidney 293

HPAEC: Human Pulmonary Artery Endothelial Cells

HCl: Hydrochloric Acid

I

iNos: Inducible Nitric Oxide Synthase

IKBα: Inhibitor of Kappa B alpha

IL: Interleukin

$FeCl_3$: Iron Chloride

L

LD_{50}: Lethal Dose

LIF: Leukemia Inhibitory Factor

M

MVA: Mevalonic Acid

MAE: Microwave Assisted Extraction

MM: Mineral Matter

MDM: Monocyte-Derived Macrophages

MO: *Moringa oleifera*

N

NFKB: Nuclear Factor Kappa B

NSAIDs: Non-steroidal anti-inflammatory Drugs.

O

OM: Organic Matter

P

POD*:* Peroxidase

PBMC: Perpheral Blood Mononuclear Cells

PAs: Phenolic Acids

PAL: Phenylalanine Ammonia Lyase

MDA: Monoaldehyde

PPO: Polyphenol Oxydase

PMs: Primary Metabolites

PCA: Principal component analysis

Q

QE: Quercetin Equivalent

R

RSA: Radical Scavenging Activity

RAW364.7: Rapidly Adherent White cells, clone 364.7

ROS: Reactive Oxygen Species

RT-PCR: Real Time - Polymerase Chain Reaction

RNA: Ribonucleic Acid

S

SMs: Secondary Metabolites

Na_2CO_3: Sodium Bicarbonate

NaOH: Sodium hydroxide

$NaNO_2$: Sodium Nitrate

SFE: Supercritical Fluid Extraction

SPSS: Statistical package for social sciences

T

TAC: Total Antioxidant Capacity

TV: Tumor Volume

TW: Tumor Weight

TPC: Total Phenolic Content

TFC: Total Flavonoid Content

U

UAE: Ultrasound-Assisted Extraction

UK: United Kingdom

USA: United States of America

UV: Ultraviolet

W

WHO: World Health Organization

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CHAPTER 3: Materials and Methodology

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General introduction

Introduction

Moringa oleifera (*M. oleifera*), frequently referred to as the "tree of life" or "miracle tree¨, is native to the sub-Himalayan regions of northern India and Pakistan (**Pareek *et al.,* 2023; Outani *et al.,* 2023)**. Due to its fast growing and drought-resistant properties, *M. oleifera* is widely cultivated across all the subtropical and tropical climates including African nations. *M. oleifera* has drawn global attention to farmers and researchers for its extensive medicinal and non-medicinal advantages, including traditional medicine, water purification and food supplements. The leaves, fruits, stems, flowers, immature pods and seeds have been reported to contain high nutritional value and phytochemical content, including Vitamins (A, B and C), minerals like calcium and iron, protein, flavonoids, tannins, saponins, phenolic acids and alkaloids among others which is the main reason for its popularity **(Anwar *et al.,* 2007; Leone *et al.,* 2015).** Its biological properties, including anticancer, antimicrobial, antioxidant, anti-inflammatory and hepatoprotective activity have been attributed to its extensive phytochemicals **(Vergara-Jimenez *et al.,* 2017).** Consequently, the plant has emerged as a principal contender in the formulation of natural therapeutic agents and nutraceutical products.

Recent studies imply that the phytochemical content and profiles of *M. oleifera* may vary depending on environmental conditions including temperature, altitude, soil composition and rainfall, which may influence the accumulation of secondary metabolites despite their well-documented medical advantages **(Li *et al.,* 2020; Bhuker et al., 2023).** Moreover, since the biological properties are attributed to phytochemical composition, their variation may influence the overall therapeutic potential of *M. oleifera* **(Saini *et al.,* 2016; Vergara-Jimenez *et al.,* 2017).** Despite the numerous literature on *M. oleifera*'s pharmacological properties, there remains a significant gap in the research regarding how environmental factors affect the plants phytochemical and biological properties in particular geographical areas, especially in Algeria. Most of the research focuses on *M. oleifera* found in the Asian or sub-Saharan nations, but there is a limited literature on the North African populations of the plant.

Given the fact that Algeria is one of the largest countries in Africa, having a variety of environments including arid, semi-arid and Mediterranean coastal regions, *M. oleifera* cultivated in various regions may have varied phytochemical and biological profiles. Therefore, it is important to comprehend how environmental conditions, both biotic and abiotic, may influence the phytochemical profiles for effective use in health-based applications of *M. oleifera*. Lack of knowledge concerning this may be a risk factor in the low quality and less

effective use of *Moringa*-based products, compromising scientific research and commercial standards.

The primary research problematic this study examines is the gap regarding how environmental factors may influence the phytochemical composition, antioxidant, anti-inflammatory and the antimicrobial activity of *M. oleifera*, given the fact that, there is scarcity of comparative studies assessing this question from multiple regions in Algeria.

This concern is especially relevant to researchers, local communities, traditional doctors and industries when considering the standardization of *M. oleifera* plant and *Moringa*-based products for medicinal or nutritional purposes. Variations in phytochemical concentration may have an impact on both the plant health benefits and its consistency in the pharmaceutical and food industries, affecting their reliability as a natural remedy. As a result, this study aims to investigate the influence of environmental conditions by adopting a comparative study on the phytochemical content, antioxidant, anti-inflammatory and the antimicrobial activities of *M. oleifera* from the three different regions of Algeria.

This research is divided into two main sections. The first section comprises bibliographic synthesis which is divided into two chapters: chapter 1 introduces the knowledge of medicinal plants and their biological potential, and chapter 2 focuses on the identification and the ethnopharmacology of *M. oleifera* plant. The Second Section is divided into two chapters: Chapter 3 presents the materials, and the methods used in the study and chapter 4 presents the results and the discussion, and lastly the conclusion of the study.

SECTION 1: Bibliographic Synthesis

CHAPTER 1:

Medicinal plants and their biological potential.

1.1. Historical review of medicinal plants' usage.

From time immemorial, man has been depending on Mother Nature for all his basic needs and plant diversity always attracted his curiosity. Man's preliminary interest in plants started from his need for food, shelter and protection. Then he sought among them the remedies for injuries and diseases, and this resulted in the science of medicine **(Rahman *et al.,* 2018).** Ever since ancient times, in the search of rescue for their disease, the people searched for drugs in nature. Since at the time, there was not sufficient information either concerning the cause of the illness or concerning which plant and how it could be utilized as a cure, everything was based on first-time experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts **(Rahman *et al.,* 2018).** The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake **(Petrovska, 2012).**

The father of Botany Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium" Plant Etiology and "De Historia Plantarium" Plant History. In the books, he classified more than 500 medicinal plants known at the time. **(Pelagic, 1970; Kelly, 2009).** Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. In the description of the plant toxic action, Theophrast underscored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants**. (Katic,1958; Bazala, 1943).**

Until the mid-19th century, plants were the major therapeutic agents used by humans, and their role in medicine remains relevant today **(Camejo-Rodrigues *et al.,* 2003).** In the mid-19th century, the residents of Hawaiian Islands were attacked by the infection of Mycobacterium leprosy, which is the known causal organism for the disease also called Hansen's disease or leprosy. During those days, the plant *Hydnocarpus kurzi* (Chalmogra) was brought to the islands, where 14-month trial of intravenous injection of chalmogra oil was conducted on the infected patients. The treatment showed astonishingly positive results, wherein half of the patients recovered from the grip of the disease **(Parascandala, 2003).**

By the late 19th and early 20th century, references to cannabis and cannabis extracts/tinctures appear in many pharmacopoeia in Europe and North America **(Kalant, 2001).** However, in the early 20th century, the use of cannabis for treatment of disease began to fall out of favor as Western medicine began to focus on isolated chemical entities, often synthetic, for pharmacotherapy. With the new Law on Drugs and Medical Devices dated September 2007 and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs **(Miranda, 2021)**.

1.2. Definition of medicinal plants.

In 1967, the phrase "medical plants" was coined to refer to the study of hallucinogenic plants. Any species of plant whose parts; flowers, leaves, roots, stems, fruits, or seeds—are used directly or in some form as medicine to cure an illness or condition is considered a medicinal plant **(Miranda, 2021)**.

Numerous chemical molecules with a range of structures and functions that have significant biological activities and are associated with numerous advantageous qualities, including antimicrobial, anticancer, antiviral, antioxidant, and enzyme inhibitory effects, as well as anti-aging, anti-inflammatory, antihypertensive, neuroprotective, and anticoagulant effects, can be found in medicinal plants. **(Ali *et al.,* 2019; Lesellier *et al.,* 2021).**

Medicinal plants are of great importance worldwide, both when used alone and as a supplement to traditional medication (Figure 1).

1.2.1. Herbal medicine

The use of medicinal plants to cure illness is known as herbal medicine, phytotherapy, herbalism, or phytomedicine. Henri Lelcerc, a French physician, coined the word "phytotherapy" in 1993 **(Satyajit and Lutfun 2018).** Traditional medicine, which includes herbal medications, has been defined by the World Health Organization (WHO) as encompassing therapeutic approaches that have been in use for centuries or more before modern medicine developed and spread **(Kumar and Shukla, 2013).**

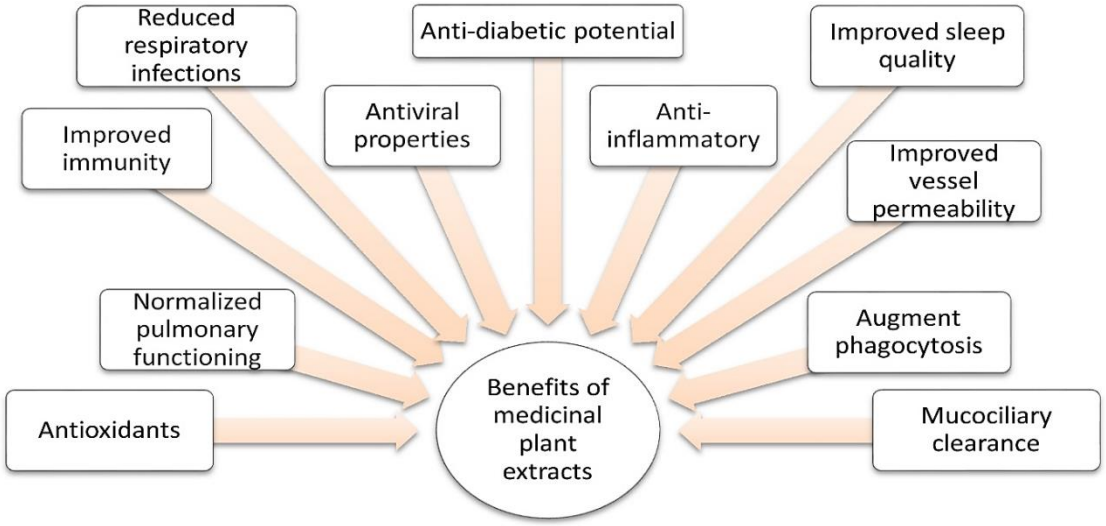

Figure 1: The benefits of the medicinal plant extracts used in phytotherapy **(Kalyniukova *et al., 2021***

Herbs can be used alone or in conjunction with one or more other herbal plants to make tea through infusion; it's common practice to occasionally add non-herbal supplements like vitamins and minerals.

When health care professionals help patients choose phytotherapies from the marketplace, it is important to be aware that the active ingredient and the marker constituent of a phytotherapeutic agent are not always the same chemical. Ultimately, the processes for determining source material selection, marker constituent standardization, active constituent and cofactor concentrations, and medication assembly can vary by both product and manufacturer. Because most herbal medicines are not covered by either government or commercial medical insurances, the variances identified increase the importance of being familiar with product quality and safety resources before recommending phytotherapeutic products for general use that are potentially costly and may have indeterminate efficacy **(Falzon and Balabanova, 2017).**

1.2.2. Herbal medicine in Algeria

Algeria is the largest country in the Mediterranean basin, Africa, and the Arab region with a total area of almost 2.4 million km2 and 1,600 of coastline. In addition to a diversified climate, Algeria is characterized by a rich flora consisting of 4,000 taxa, 917 genera, and 131 families. Moreover, owing to its ancient history as one of the first cradles of Homo sapiens and civilization in the world, Algeria possesses an important and rich cultural diversity **(Mohammed *et al.,* 2013).**

In Algeria, plants occupy an important place in traditional medicine, which itself is widely used in various health areas. Old and recent publications reveal that many medicinal plants are used for the treatment of many diseases. Algeria benefits from a very diverse climate, plants grow in abundance in the coastal, mountainous and Saharan regions. These plants are potential natural remedies, which can be used in curative and preventive treatment. In recent years, traditional phytotherapy has spread throughout the country, plants and plant mixtures are used for the treatment of all kinds of diseases: diabetes, rheumatism, slimming and incurable diseases. The capital alone housed the largest number with 199 stores, followed by the wilaya of Sétif (107), Bechar (100) and El Oued with 60 stores **(Mohammed *et al.*, 2013).**

There are various medicinal plants that are used as therapeutic agents in Algeria (Table 1).

Table 1 : Few selected lists of new therapeutic uses recorded in the southwest of Algeria **(Belhouala and Benarba, 2021)**

Botanical name	**Part used**	**New uses**	**Preparation methods**	**Previously reported uses**
Silybum marianum (L.) Gaertn	Leaves	breast cancer and legs cancer	Raw	Biliary, liver disorders, and degenerative necrosis,
Prunus persica (L.) Batsch.	Leaves	cancer limb swelling	Raw infusion	Cough, constipation, and menstruation absent
Inula helenium L	Capitulum	breast cancer and legs cancer	Raw	Hematomas, relief of bruises, joint pains, rheumatism, and gastrointestinal
Calendula arvensis M. Bieb.	Capitulum	pneumonia	Decoction	Burns, varicose veins, eczema, fungus, warts, and wounds
Artemisia campestris L.	Leaves	scorpion sting	Raw	Digestive troubles, gastric ulcer, and menstrual pain
Clinopodium nepeta (L.) Kuntze.	Aerial part	Colon	Decoction	ailments, abdominal pain,

1.3. Pytochemistry and bioactive (secondary metabolites) of medicinal plants.

1.3.1. Phytochemistry

It will be recalled that in the food chain, plants are referred to as the producers because they had the ability to trap energy from sunlight, harness and assemble some basic units which transform through some chemical process into complex high energy-yielding compounds that

are readily available to organisms. Their generosity became overwhelmingly and practically complex to comprehend immediately. A field must emerge "phytochemistry." Phytochemistry is the study of chemicals or phytochemical compounds produced by plants, particularly the secondary metabolites (SM). Knowledge of phytochemistry is essential in the: **(Egbuna *et al*., 2018).**

- Search for the discovery of new drugs and repurposing of existing ones
- Characterization and standardization of traditional herbal drugs in the crude form
- Assessment of the toxicity levels of plants
- Understanding of plant physiology, biosynthetic pathways, and metabolomics
- Identification and classification of plants
- Study of inter and intraspecific chemical variability within plants
- Biotechnology and genetic engineering for the optimization and synthesis of classic compounds
- Plant pathology

1.3.2. Generalities of bioactive compounds.

The history of bioactive compounds is clearly illustrated by the ancient use of herbal plants. Bioactive chemicals were unknown to people in the past, but their applications were sufficiently varied in many contexts. All living things, from single-celled bacteria to multicellular plants, use a variety of chemical compounds to survive **(Ramawat *et al.,* 2009).** The usage of plant metabolites dates to 2600 Before Christ (BC), and during the next 4,000 years, secondary metabolites derived from plants were mostly utilized for food, medicine, and poisoning. A new era in secondary metabolite study began in 1806 when morphine, the first naturally occurring substance extracted from the opium poppy (Papaver somniferum), was discovered **(Twaij and Hasan, 2022).**

Previously, metabolites were identified as metabolic waste or detoxifying products, and their biological functions were mostly unknown because of their often-low concentrations in plants. Over the past forty years, knowledge regarding secondary metabolites once recognized for their harmful effects on animal cells but now recognized for their ecological significance and many other advantages has grown **(Weinberg, 1962; Twaij and Hasan 2022).**

1.3.3. Classification and biosynthesis of common phytochemicals.

1.3.3.1. Classification of the common phytochemicals

Two kinds of metabolites are produced by plant cells. Proteins, lipids, and carbohydrates are examples of primary metabolites (PMs) that have a direct role in growth and metabolism. Photosynthesis produces PMs, which are also involved in the synthesis of cell components **(Ramawat *et al.,* 2009).**

When necessary, nutrients are present in a growth medium, PMs production starts during the active growth phase, also known as the trophophase. Plant PMs, such as respiratory and photosynthetic enzymes, are also involved in the synthesis of energy. Additionally, PMs are the primary constituents of the fundamental cell structures, including proteins for cytoskeletons, peptidoglycan and chitin for cell walls, and phospholipids for cell membranes. Additionally, nucleic acid primary metabolites make up DNA and RNA, which store and transfer genetic information. However, through the mechanisms of pathogen detection and signal transduction, PMs carry out their roles as signal molecules that initiate the defense response. Peptides, biogenic amines, steroid hormones, auxins, gibberellins, and other substances make up signalling molecules like hormones and other growth factors **(Pawlikowski, 2010).**

Most natural products are classified as secondary metabolites since they are substances that are formed from primary metabolites including fatty acids, carbohydrates, and amino acids **(Ramawat *et al.,* 2009).** Usually, plants produce their bioactive chemicals as secondary metabolites **(Bernhoft, 2010).** By enabling them to interact with their environment, SMs a class of molecules other than primary metabolites are thought to assist plants improve their overall capacity for survival and overcome local obstacles. To put in another way, secondary metabolites are those that are frequently produced in a phase that follows growth, have no role in growth (though they might have a survival function), are produced by specific limited taxonomic groups of microorganisms, have peculiar chemical structures, and are frequently created as mixtures of closely related members of a chemical family **(Ramawat *et al.,* 2009).**

Bioactive compounds in medicinal plants can be roughly divided into three classes based on their chemical structure and biosynthetic pathway: Plant volatiles, sterols, carotenoids, saponins, and glycosides are examples of **terpenoids**. Flavonoids, phenolic acids, lignin, lignans, coumarins, stilbenes, and tannins are examples of **phenolic compounds**. Alkaloids, glucosinolates, and cyanogenic glycosides are examples of **nitrogen-containing compounds (Elshafie *et al.,* 2023).**

Atropine (used to treat cardiac arrhythmias and ulcers), codeine and morphine (used as analgesics), camptothecin (antineoplastic action), ergotamine (used to cure migraines), and quinine (used to treat malaria) are a few bioactive compounds that are significant for world health **(*Cordeiro et al.* 2022).**

1.3.3.2. Biosynthesis of common phytochemicals.

The synthesis of various classes of secondary metabolites from primary metabolites is presented in schematic form in Figure 2.

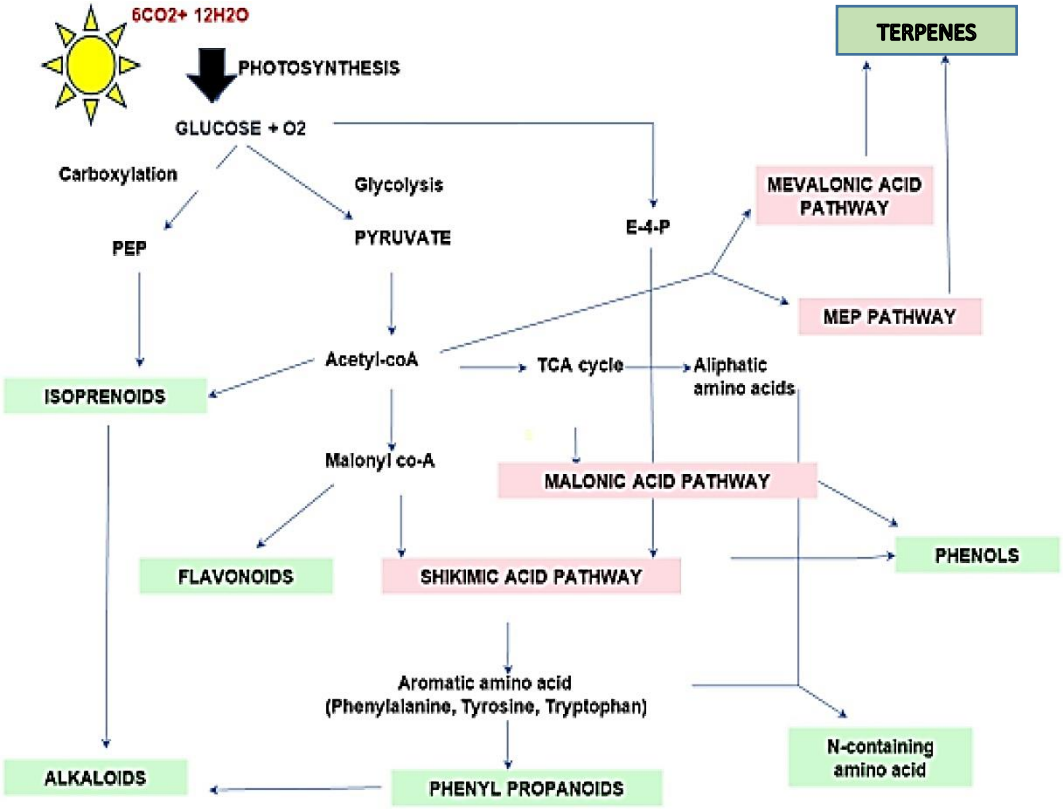

Figure 2: Principal biosynthetic pathways leading to synthesis of secondary metabolites (**Kajla *et al.*, 2023**)

1.3.4. Overview of the common SMs

1.3.4.1. Terpenoids

The primary class of SMs found in plants, terpenes or terpenoids, are derived from acetyl Co-enzyme A (CoA) or glycolytic intermediates. They exhibit a great deal of structural diversity (more than 50,000 distinct structures) and are lipid soluble. Terpenes are the primary constituents that give plants their scent and physiological effects. They are also the fundamental building blocks of many complex phytohormones, sterols, and pigments. Most of the terpenes

function as defensive poisons and herbivore deterrents, while others have a pleasant scent and attract pollinators **(Jane *et al.*, 2021).**

The number of carbons generated by the isoprene units they contain determines their classification. The building blocks of terpenoids, which are gaseous hydrocarbons with the chemical formula C_5H_8, are these isoprene units. The classification of the naturally occurring terpenoid is displayed in Table 2 **(Kamran *et al.*, 2022).** With a few chosen cyclic structures displayed in figure 3, they are divided into six basic classes: tocopherols, taxanes, ingenanes, artemisinins, sterols, and cannabinoids. Numerous biological functions, such as anti-inflammatory, anti-allergic, anti-cancer, and anti-microbial qualities, have been demonstrated for some of them. In addition to their environmental importance, they have enormous economic worth and a variety of uses in several industries, such as the food, pharmaceutical, cosmeceutical, and chemical sectors **(Câmara *et al.*, 2024).**

Table 2 : Classification of terpenoids **(Kamran *et al.*, 2022).**

Class	**Number of atoms**
Monoterpenoids	C10
Sesquiterpenoids	C15
Diterpenoids	C20
Triterpenoids	C30

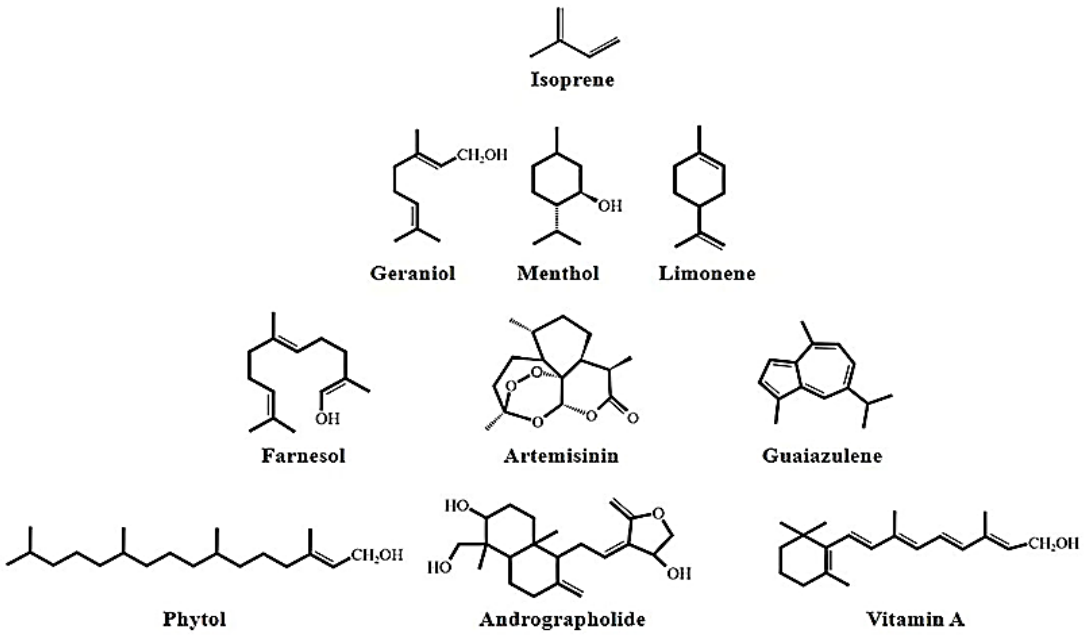

Figure 3: cyclic structures of terpenoids **(Fan *et al.*, 2023)**

Terpenes are not produced by a straightforward biosynthetic process. Usually, there are two biosynthetic pathways: MEP (2C-methylerythritol 4-phosphate) and MVA (mevalonic acid) (figure 2). Six stages of enzymatic processes make up the MVA pathway, which supplies the mitochondria with precursors for triterpenoids such ubiquinones and brassinolide, as well as sesquiterpenes and phytosterols. The seven enzymatic stages that make up the MEP route primarily serve as substrate sources for carotenoids, monoterpenes, diterpenes, and the byproducts of their breakdown (cytokinin's, gibberellins, chlorophyll, tocopherols, and plastids) **(Li *et al.,* 2023).**

1.3.4.2. Phenolic compounds (PCs)

A broad class of secondary metabolites found in many higher plant organs, including fruits, vegetables, grains, legumes, nuts, and spices, phenolic compounds are crucial for several physiological functions, including plant quality, color, flavor, and stress tolerance. Phenolic compounds' inherent anti-inflammatory, anti-carcinogenic, anti-microbial, and antioxidant properties are currently a focus of much research and application. Several types of phenolic compounds share a common chemical structure consisting of an aromatic ring with one or more hydroxyl substituents. Flavonoids, phenolic acids, tannins, stilbenes, and lignans are the primary families of phenolic compounds. **(Zhang *et al.,* 2022).** Different classes are shown in figure 4 and the structural identification of some phenolic compounds are provided in figure 5 respectively **(Alara *et al.,* 2021).**

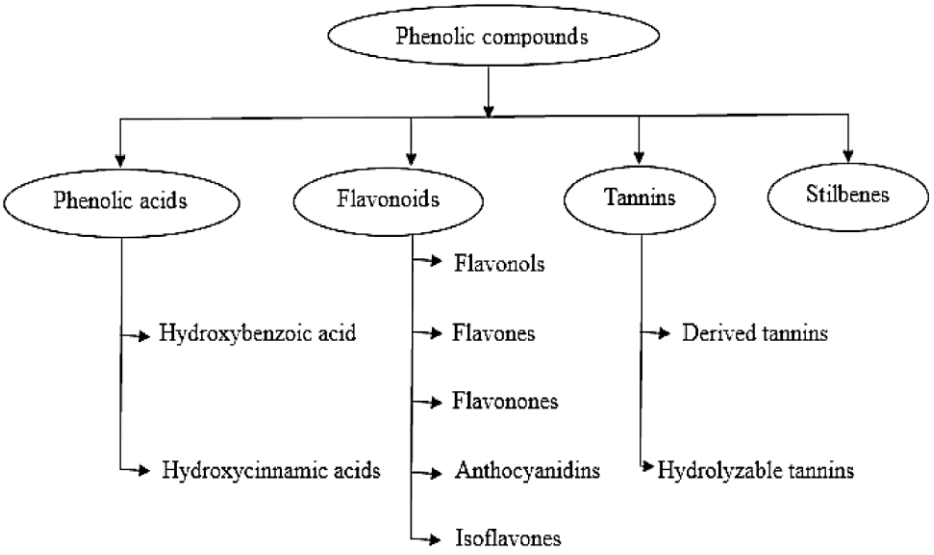

Figure 4: Main classes of phenolic compounds **(Alara *et al*., 2021).**

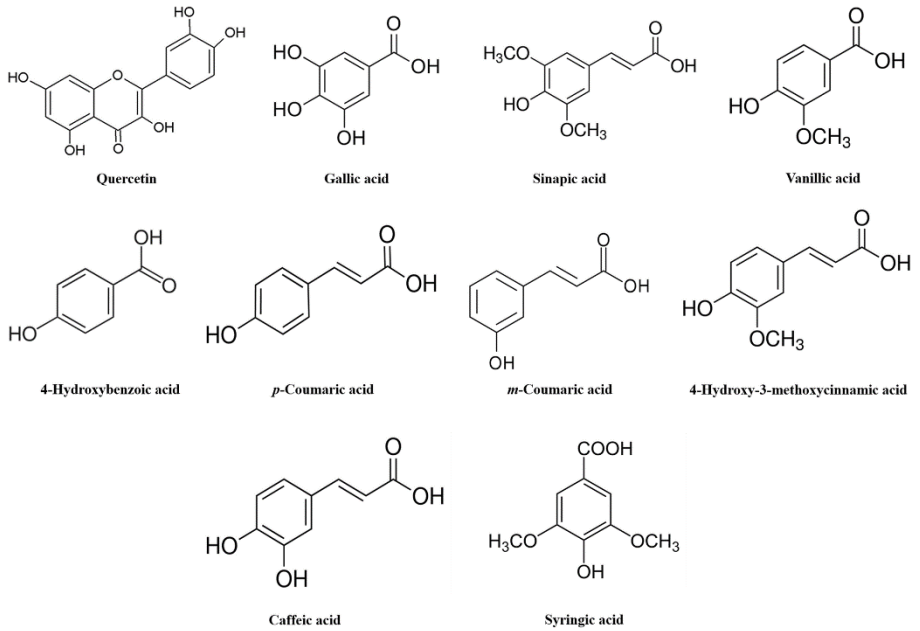

Figure 5: Structural identification of some phenolic compounds found in *Moringa oleifera* leaves **(Alara *et al.*, 2021).**

1.3.4.2.1. Flavonoids

One type of secondary metabolite that is abundant in plants is flavonoid, which gives them their color, flavor, and aroma. The chemical structure of flavonoids contains hydroxyl groups, which may have an impact on their bioavailability and biological activity in the human body. Two benzene rings (A and B) connected by a three-carbon pyran ring (C) make up the fundamental chemical structure of flavonoids. The antioxidant potential of flavonoids can vary depending on the location of the B-ring, as well as the quantity and position of hydroxy groups on it **(Shi *et al.*, 2022).**

Subclasses of flavonoids include anthocyanidins, isoflavonoids, flavones, flavanones, and flavanols (catechins and proanthocyanidins) (Fig. 6). The presence or absence of a double bond at position 4 of the C ring, a double bond between C2 and C3, and the hydroxyl groups in ring B determine this classification **(Mutha *et al.*, 2021).**

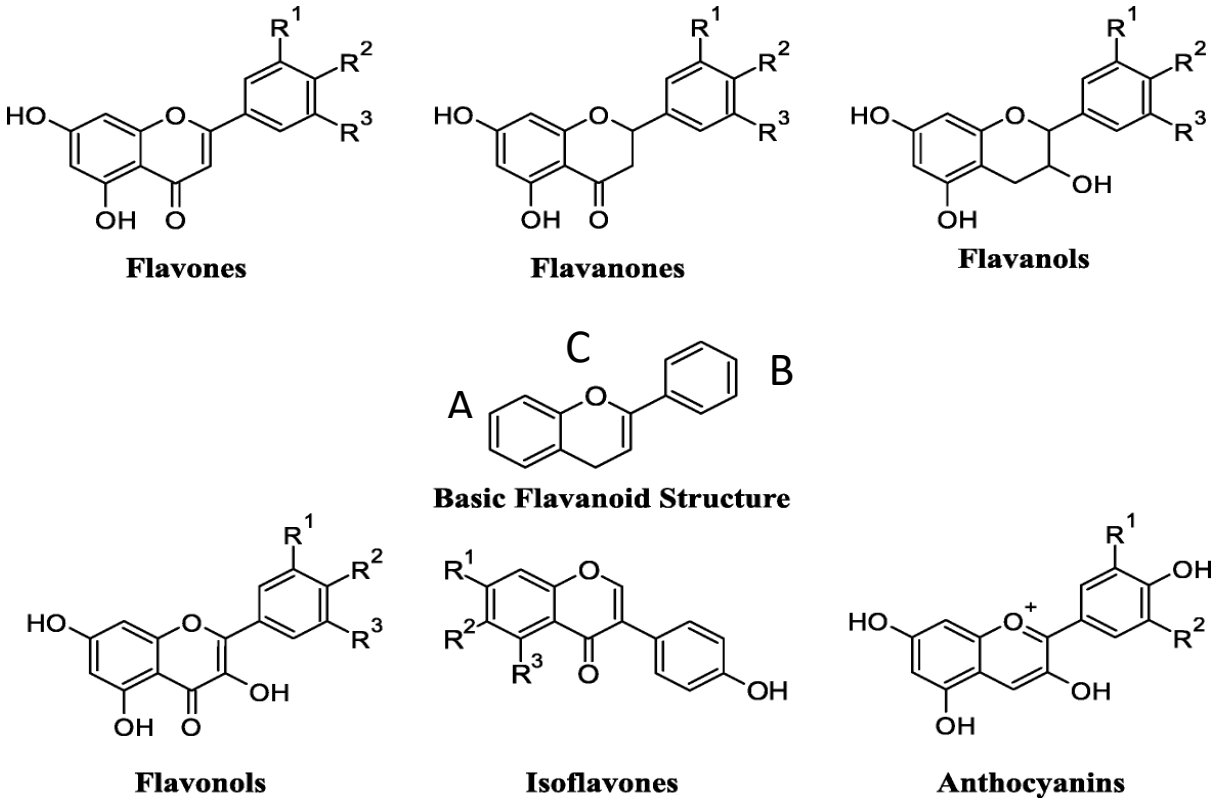

Figure 6: Chemical structures of various flavonoids **(Mutha *et al.*, 2021).**

1.3.4.2.2. Stilbenes

Many distantly related plant species contain stilbenes, a tiny class of secondary metabolites that are polyphenolic. These substances function as phytoalexins, which are essential for both plant defense against phytopathogens and plant adaptability to abiotic environmental conditions **(Valletta *et al.*, 2021).** Natural stilbene compounds from a variety of plant species, including rhubarb, blueberries, mulberries, cranberries, grapes, and soybeans, serve as evidence of these (**Klusska *et al.*, 2023).** Stilbene phytoalexins are mostly derived from either pinosylvin (found in pine plants, for example) or resveratrol (3,5,4′-trihydroxy trans-stilbene), which are found in peanut and grapevine plants, respectively **(Teka *et al.*, 2022).**

1.3.4.2.3. Tannins

Tannins are distinctive phenolic compounds with a broad molecular weight range of 500 Da to 30,000 Da. Tannins are typically categorized as hydrolysable tannins and condensed tannins **(Lang *et al.*, 2023).** The classification of the tanins is given in figure 7 **(Pizzi, 2021).**

Astringent, Reactive Oxygen Species (ROS) reduction, anti-mutagenic, antiviral, and antimicrobial properties are based on tannins' ability to interact with proteins and denaturize them in vertebrate herbivores. These natural compounds, which are found in fruits, vegetables, cereals, and seeds, have a wide range of applications in human health, plant defense, development, and plant growth **(Melo *et al.*, 2023).**

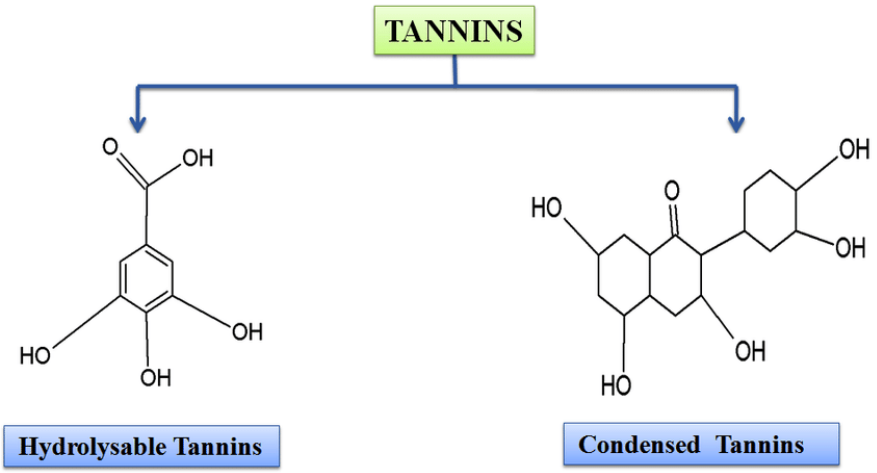

Figure 7: Classiffication of the Tanins **(Ghosh, 2015).**

1.3.4.2.4. Phenolic acids (PAs)

Phenolic acids (PAs) are found ubiquitously in almost all food plants, such as fruits, vegetables, cereals, etc., mostly ester-bound to carbohydrates in the form of O-glycosides. Among the most important substances of the PAs are the hydroxybenzoic acids and hydroxycinnamic acids, which biosynthetically derive from cinnamic acid or p-coumaric acid by hydroxylation and methylation. Some of the most typical representatives of PAs include gallic acid, syringic acid, caffeic acid (CA), ferulic acid (FA), and sinapic acid **(Schefer *et al.*, 2021).** The molecular structures of phenolic acids are represented in fig.8. PAs emerge as promising therapeutic agents due to their antitumor, antimicrobial **(Heleno *et al.*, 2015),** antioxidant and anti-inflammatory properties **(Singh and Panda 2024)**

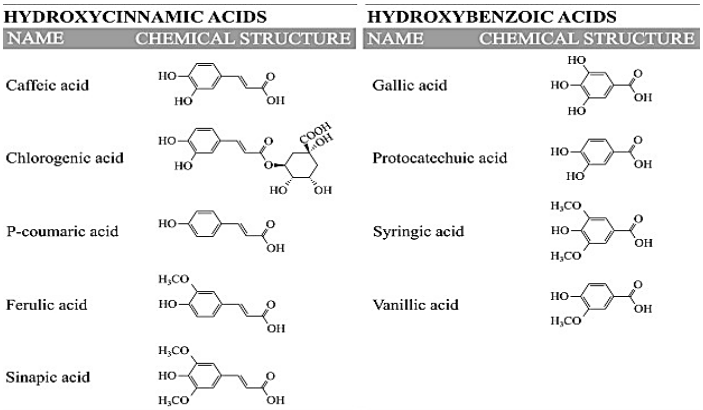

Figure 8: Molecular structure of phenolic acids **(Heleno *et al.*, 2015).**

1.3.4.2.5. Lignans

Natural plant phenols called lignans are produced biosynthetically from phenylpropanoids **(Zhang *et al.,* 2014).** Flaxseed, cereal, whole wheat, vegetables, select fruits including cherries and strawberries, and tea all contain high levels of phytolignans. They are also found in the human body's sperm, prostate, saliva, urine, and plasma. Plant parts that contain them include leaves, roots, seeds, stems, and fruits **(Polat and Gulcin, 2021).** Numerous lignans have anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer, and antioxidant qualities **(Jang *et al.,* 2022).**

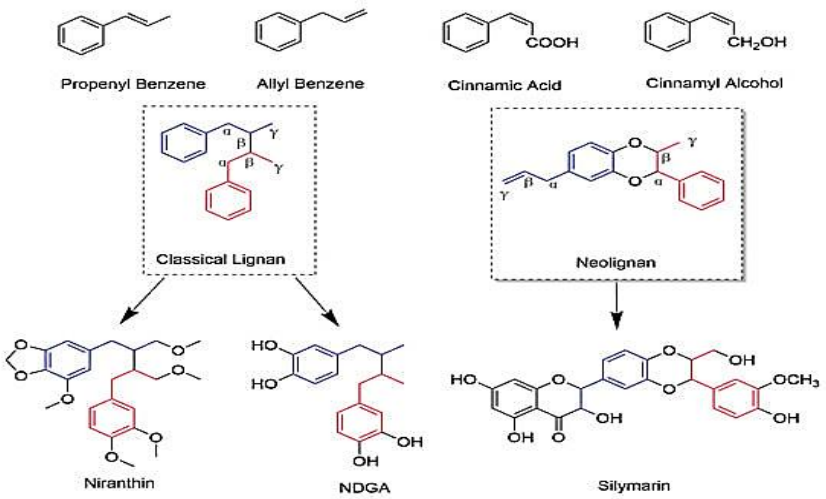

Figure 9: The different monomers and classification of Lignans **(Cui *et al.,* 2020).**

1.3.4.3. Nitrogen-containing compounds

1.3.4.3.1. Alkaloids

Most of the largest class of phytochemicals are made up of alkaloids, a huge class of organic substances that exist naturally. Alkaloids' alkaline characteristics and therapeutic effects are primarily due to their nitrogen atoms **(Faisal *et al.,* 2023).** Pyrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, terpenoids, and steroids are typical chemical classifications for them. Alkaloids are typically crystalline, colorless, and non-volatile **(Dhyani *et al.,* 2022).** It is challenging to enumerate the structural traits of every alkaloid due to their many structural classifications. Figure 10 contains the comprehensive structural formula **(Rui *et al.,* 2022).**

The Arabic name al-Qali, which is associated with the plant from which soda was first extracted, is where German scientist Carl F. W. Meissner got the term "alkaloid" from in 1819. Alkaloids are low-molecular-weight substances that make up around 20% of secondary

metabolites found in plants. More than 12,000 different plant species have yielded alkaloids thus far **(Zandavar and Babazad, 2023).**

Alkaloids show numerous pharmacological actions on human health such as anti-cancer, anti-inflammatory, Anti-malarial, Anti-microbial, Anti-hypertensive, Anti-diabetic, Antioxidant. Alkaloids immediately act on the central nervous system in the human body and impact nucleic acid, DNA (Deoxy Ribonucleic acid), RNA (Ribonucleic acid), membrane permeability and proteins **(Rajput *et al.*, 2022).** While they are largely generated from amino acids, alkaloids can be found in seeds, roots, stems and leaves **(Olofinsan *et al.*, 2023)** of higher plant groups such Amaryllidaceae, Apocynaceae, Papaveraceae, Asteraceae, Solanaceae, Rutaceae, Fabaceae, and Rubiaceae **(Aryal *et al.*, 2022).**

Numerous studies on the medicinal characteristics of various alkaloids derived from plants have been conducted.

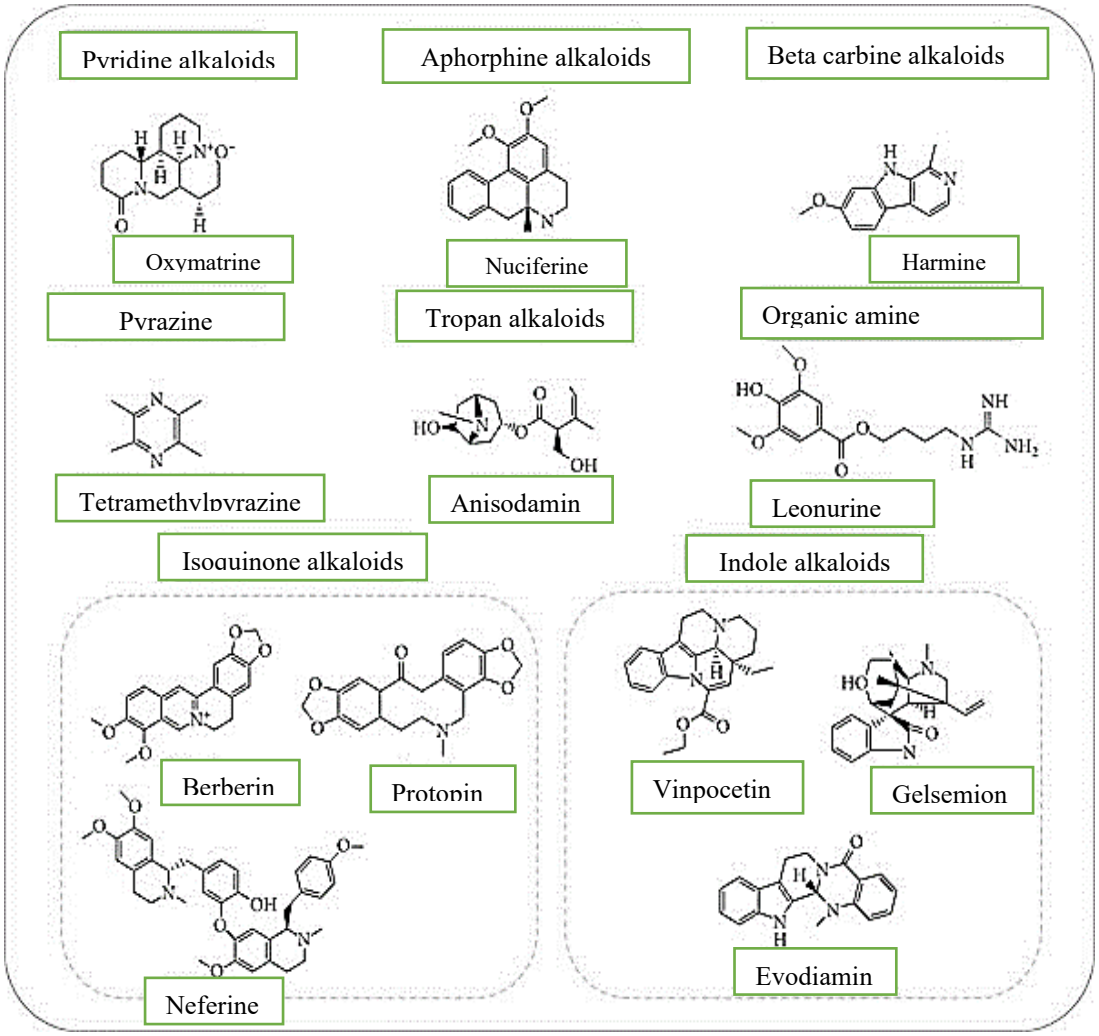

Figure 10: Structural formula of different classifications of Alkaloids **(Rui *et al.*, 2022)**

1.4. Extraction methods of secondary metabolites

Using selective solvents and a regular procedure, extraction is the process of isolating the medicinally active mixture of numerous naturally occurring active chemicals that are typically found inside plant materials (tissues) **(Stéphane *et al.,* 2021).** Pre-washing, drying or freeze-drying plant materials, grinding to produce a homogenous sample, and frequently enhancing the kinetics of analytic extraction and sample surface contact with the solvent system were all part of the basic operation. Proper activities must be made to ensure that possible active ingredients are not lost, altered or destroyed during the preparation of the extract from plant samples **(Sasidharan *et al.,* 2011).**

Phytochemicals have traditionally been extracted using a variety of solvents, and researchers typically use a dried plant powder to extract bioactive molecules while simultaneously removing the influence of water. The polarity of the solute of interest determines which solvents are employed to extract biomolecules from plants. The solute will dissolve correctly in a solvent with a similar polarity to the solute. It is possible to limit the quantity of similar compounds in the intended yield by using several solvents in succession. A few common solvents have the following polarities, ranked from least to most polar: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water **(Altemimi *et al.,* 2017).** Table 3 lists the primary solvents used in the extraction of plants and herbs.

Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing such as characterization, isolation, separation and purification. Several of the commonly used extraction methods are discussed below:

Table 3: Solvents used for active component extraction **(Fonmboh *et al.,* 2020)**

WATER	ETHANOL	METHANOL	CHLOROFORM	ACETONE	ETHER
Polyphenols Tanins Lectins Terpenoids Anthocyanins	Tannins Polyphenols Sterols Polyacetylenes Terpenoids Flavonol	Anthocyanins Tanins Totarol Terpenoids Saponins Xanthoxyllines	Terpenoids Flavonoids	Phenol flavonols	Alkaloids Fatty acid Coumarin Terpenoic
Starches	Alkaloids	Quassinoids			
Saponins		Phenones Polyphenols Lactones Flavones			

1.4.1. Maceration

For a long time, maceration was a common and low-cost homemade method for making tonic. Additionally, this method was employed to extract active ingredients and essential oils from plant sources. The maceration process typically involves several extraction phases. To properly mix the powdered components with the solvent, the whole or coarsely powdered crude medication is ground to improve its surface area. Menstruum, the proper solvent, is added to a closed tank to complete this operation. To recover the maximum amount of occluded solution, the solvent is then strained off and the solid residue from the extraction process, known as marc, is pressed.

The strained solvent and the resulting pressed-out liquid are combined and filtered to remove any remaining impurities. By providing additional solvent to the menstruum, frequent agitation during maceration increases the extraction yield by (1) promoting diffusion and (2) separating concentrated solutions from the sample surface **(Srivastava *et al.*, 2021).**

1.4.2. Infusion

For plant parts with thinner cell walls, such as flowers, leaves, and thin aerial portions, as well as some fruit after it has been pre-crushed, infusion is a technique of extraction that yields biologically active principles that contain active thermostable and cold-soluble chemicals. This is accomplished by adding either boiling or cold water to the vegetable material and then boiling the mixture. The recipient is afterwards covered and left at room temperature for 10 to 15min, with intermittent shaking.

After the product is filtered, diluted solutions of the easily soluble components of crude medications are produced. The most basic aqueous extractive solutions are infusions, which are made from vegetable raw materials with particularly loose tissues, such as entire aerial sections of the plant, flowers, leaves, and young stems. For up to 12 hours, the infusions are made as needed. Because extraction in water promotes the formation of bacterial mass, it is kept cold **(Rodino and Butu, 2019).**

1.4.3. Percolation

When making tinctures and fluid extracts, this is a method most employed to extract active components. The most common type is a percolator, which is a thin, cone-shaped vessel that is open at both ends (Figure 11). After moistening the solid ingredients with a suitable quantity of the designated menstruum and letting them stand for around four hours in a tightly sealed container, the mass is packed, and the percolator's top is sealed. The combination is left to

macerate in the closed percolator for twenty-four hours after more menstruum is added to create a shallow layer above the mass **(Singh, 2008).**

The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three

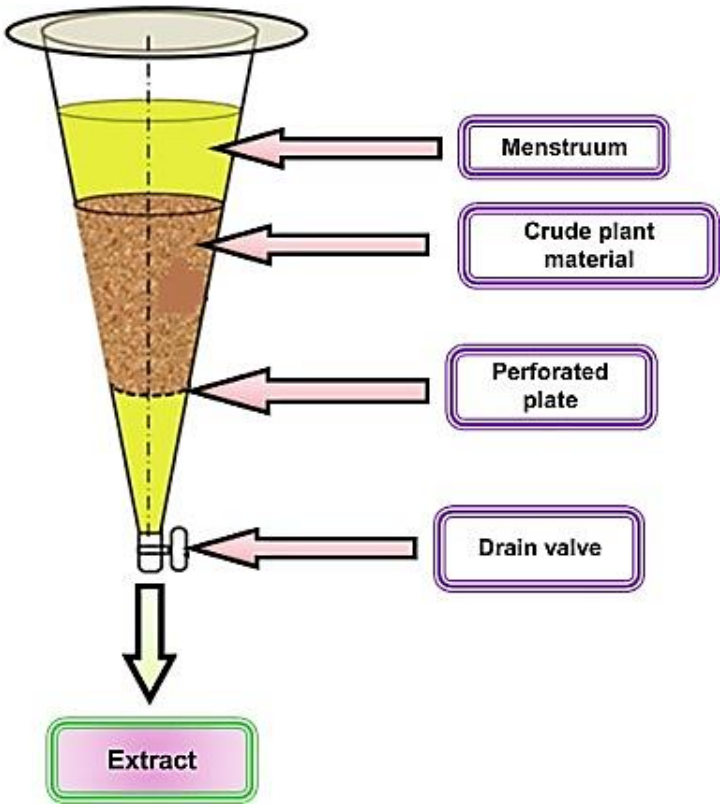

Figure 11: Schematic diagram of the extraction bioactive molecules using percolation **(Mukherjee, 2019)**

quarters of the required volume of the finished product. The marc is then pressed, and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by

The solvent passes through the non-moving solid material and extracts the soluble active constituents. One advantage of this method is that the solid material requires little mechanical treatment because it does not need to move in the percolator while the product passes on the solution. Moreover, since self-filtration takes place, there is minimum content of fine solid particles in the extract **(Tandon and Rane, 2008).**

1.4.4. Decoction

A great method for removing water-soluble ingredients that won't be harmed by heat is decoction. Decoction is a technique that uses water to remove active compounds from material

(Fig. 12). In this process, the components are boiled with water to create the liquid preparation. When dealing with tough, fibrous plants, barks and roots, and plants that contain water-soluble chemicals, the decoction is the preferred technique **(Mathews *et al.*, 2024).**

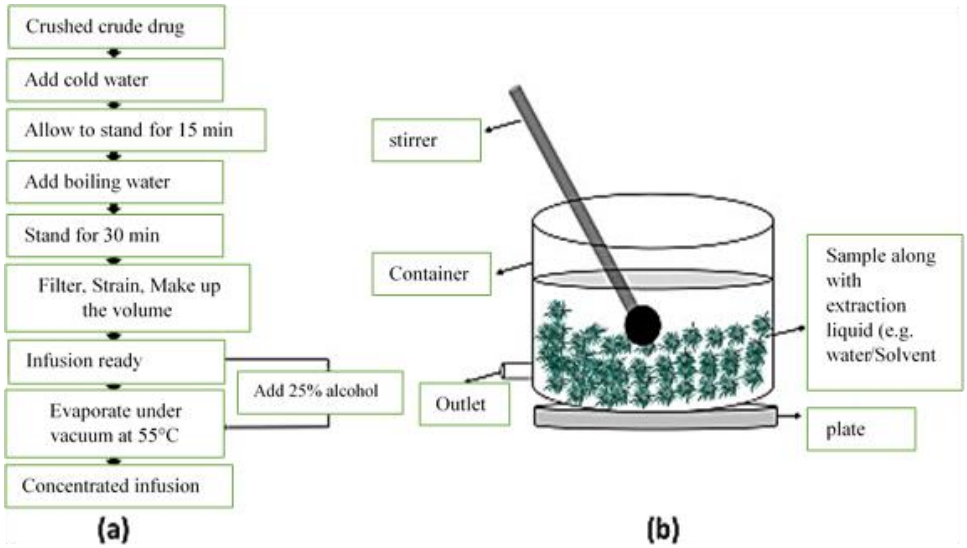

Figure 12: (a) and (b) Flow chart and diagram of preparation of infusions using decoction **(Mathews *et al.*, 2024).**

1.4.5. Other extraction methods

Other methods such as accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE), Soxhlet extraction or hot continuous extraction, Microwave assisted extraction (MAE), hydro distillation (water distillation, water and steam distillation and direct steam distillation) and Ultrasound-assisted extraction (UAE) or sonication extraction are also being used in the extraction of plant materials. Despite their effectiveness, these techniques are less common because they are expensive. However, the growing economic importance of bioactive substances and products containing them could eventually lead to the development of increasingly complex extraction techniques **(Rasul, 2018).**

CHAPTER 2

identification and ethno pharmacology of Moringa Oleifera lam. Plant

Preamble

Moriga oleifera Lam., *(M.oleifera)* which is commonly known as the "tree of life" or "miracle tree" **(Pareek *et al.,* 2023)** is categorized as a significant herbal plant because of its extensive medicinal and non-medicinal advantages. *M.oleifera* is also known by its regional names, including Horseradish tree, Drumstick tree, Benzolive tree, Kelor tree, Mlonge tree, Marango tree, Saijihan tree, Sajna tree, and Mulangay tree and it was given the "Botanical of the Year in 2007″ award by the National Institutes of Health **(Gharsallah *et al.,* 2023).**

Two of this plant's most significant attributes are its anti-inflammatory and antioxidant qualities among the many other proven qualities **(Vezza *et al.,* 2023).**

2.1. The taxonomical classification.

Moringa belonging to the Moringaceae family is one of the 13 species which includes: *MO, M. stenopetala, M. concanensis, M. drouhardii, M. arborea, M. hildebrandtii, M. longituba, M. rivae, M. ruspoliana, M. borziana, M. ovalifolia, M. peregrine, and M. pygmaea* **(Anzano *et al.,* 2021).** It can be classified as follows:

Table 4: Taxonomical classification of *Moringa oleifera* **(Chukwuebuka, 2015, Pareek *et al.,* 2023)**

kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	*Moringa*
Species	*Oleifera*

2.2. Morphological description

M.oleifera is a small, slender, deciduous, perennial tree that reaches a height of roughly 10 meters. Its stem and branches are corky and brittle, and its branches droop. The feathery, pale green, tri-pinnate, compound leaves are 30 to 60 cm long. They have numerous tiny leaflets that are 1.3 to 2 cm long and 0.6 to 0.3 cm wide. The terminal leaflets are obovate and slightly

larger, while the lateral ones are slightly elliptic. The fragrant, white or creamy-white flowers are carried in sprays and measure 2.5 cm in diameter. The stamens are yellow, and the pods are pendulous, brown, triangular, separating lengthwise into three sections when dry, and holding around 20 seeds lodged in the pith. The pod has nine ribs on each end and the seeds are dark brown with three papery wings **(Anzano *et al.*, 2021).** Figure 13 shows the *M.oleifera* plants parts and their uses.

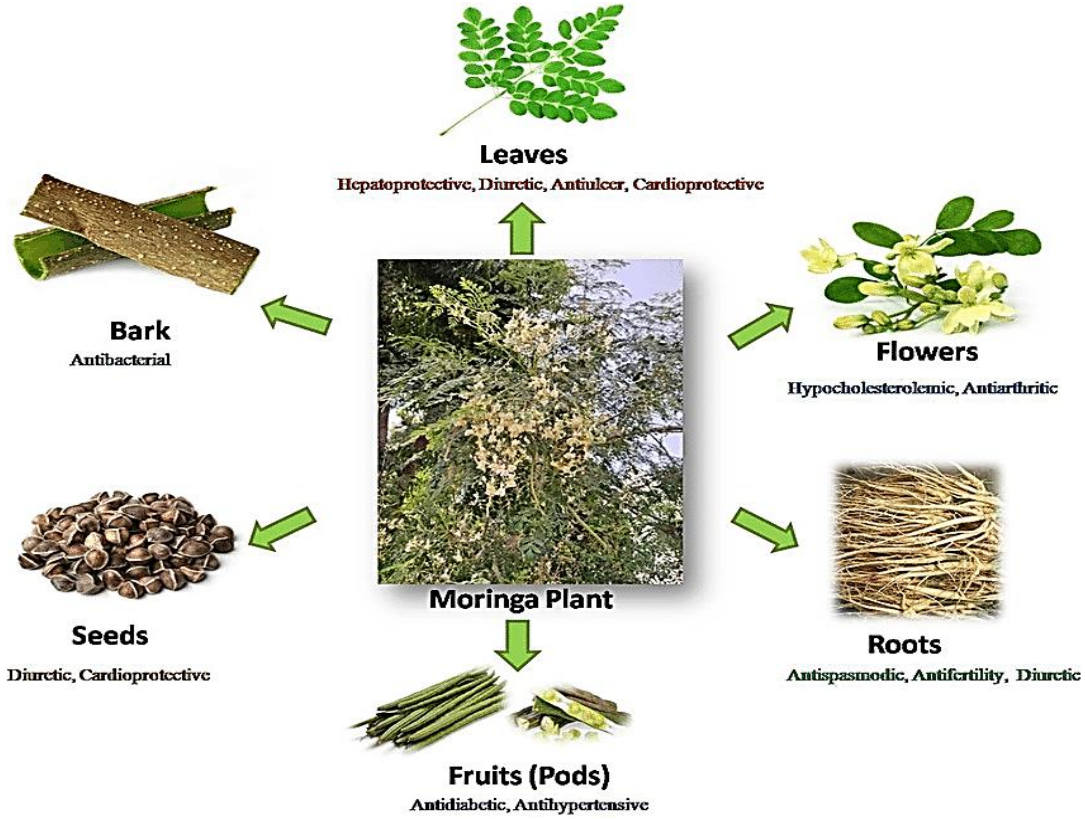

Figure 13: *M.oleifera* plant parts and functions **(Patil *et al.*, 2022).**

When fully grown, unripe green pods are grayish, fibrous, and somewhat meaty. The seeds have wings are trigonal, and have a blackish hue. The *M.oleifera* tree thrives in temperatures between 25°C and 35°C, direct sunlight, 500 meters above sea level, and soil that is slightly acidic to alkaline **(Sonewane *et al.*, 2022).**

2.3. Geographical distribution and habitat.

M.oleifera is indigenous to northern India and Pakistan's sub-Himalayan areas. All tropical and subtropical climates have seen its introduction, and many African nations have seen it naturalize. Ethiopia, the Pacific Islands, Florida, Sudan, the Caribbean, the Philippines, West Africa, South Africa, Asia, and Latin America are among the places where it is widely grown.

As a result, it is highly well known and goes by many names worldwide. The generic name comes from the Tamil name morunga **(Outani *et al.*, 2023).**

However, because of extensive research on its nutritional value, it has been introduced to many regions of the world and is now found and grown in nearly all tropical nations. This has been made feasible by the unique growing conditions of *M.oleifera*, which allows it to thrive in tropical and sub-tropical nations with dry to moist tropical climates and 760–2500 mm of precipitation annually (figure 14). In an area capable of producing 38,000 hectares of crop pods, India alone produced up to 1.1 to 1.3 million tons of the plants annually **(Azlan *et al.*, 2022).**

Figure 14: World map distributions of *M.oleifera*. World map images were obtained and modified from Outline World Map (free access) **(Azlan *et al.*, 2022)**.

2.4. Ethno-medicinal and traditional uses.

Since ancient times, people all throughout the world have incorporated *M.oleifera* into their diets because of its significant medicinal benefits. The plant has been used for millennia to make a variety of medications that are believed to have ethno-medical qualities to treat illnesses **(Sivakumar, 2024).** Relevance to ethno-pharmacology: Every portion of *M.oleifera* such as flowers, leaves, roots, fruits (pods), seeds, stem bark, and gum has therapeutic value and can be used to treat a wide range of conditions, including as blood pressure, fever, asthma, wounds, cough, arthritis, skin infections, diabetes, epilepsy, and bodily aches and weakness. Additionally, moringa has a strong power to combat fatal illnesses like HIV/AIDS infections, chronic anemia, cancer, malaria, and bleeding **(Popoola and Obembe, 2013).**

The above corresponds with **(Stevens *et al.*, 2013)** who reported that, most respondents (98.9%) said they had either used or witnessed the use of *Moringa* plants for culinary and therapeutic purposes. Most Nigerians were aware of the *Moringa* plant and used its leaves, in particular, for a variety of culinary and medical uses. In particular, the leaves were utilized as a vegetable in soup, salad, and tea making; these leaves were also used medicinally to treat ear infections (71.8%), fever (78.7%), blood pressure (64.7%), and diabetes mellitus (65.2%)

2.4.1. Phyto-chemistry of *Moringa oleifera*

Phenolic compounds, Carotenoids, alkaloids, tannins, glucosinolates, saponins, isothiocyanates, folates, and fatty acids are the most important bioactive compounds of *M.oleifera* **(Pop *et al.*, 2022). (Paikra *et al.*, 2017)** reported that the major phytochemical constituents are found in different parts of *M.oleifera* as shown in Table 5.

M.oleifera, abundant in vitamins, minerals, proteins, amino acids (methionine, tryptophan, lysine, and cysteine), and other phenolics, is very suited for human consumption **(Chhikara *et al.*, 2021).**

Table 5: phyto-constituents of *Moringa oleifera* **(Paikra *et al.*, 2017).**

Sr. No	Plant part	Extract	Phytoconstituents
1	Leaves	Aqueous and alcoholic	Niazirin and Niazirinin – nitrile glycosides,4-[(4′-O- acetylalpha- L-rhamnosyloxy) benzyl isothiocyanate, Niaziminin A, and Niaziminin B, three mustard oil glycosides,
2	Seeds	Aqueous and Hydro-alcoholic	Methionine, cysteine, 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate, Moringine,
3	Pods	Hydro-alcoholic	Isothiocyanate, nitrites, thiocarbamates,O-(1heptenyloxy) propyl undecanoate, O-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate,
4	Bark	Alcoholic	4-(alpha-L- rhamnopyranosyloxy) benzylgiucosinolate.
5	Flowers	Hydro-alcoholic	D-glucose, quercetin, isoquercetin, kaemopherol, kaempferitin and ascorbic acid, protein, D-mannose.
6	Roots	Alcoholic	Moringine, moringinine, spirachin, 1,3-dibenzyl urea, alpha- phellandrene, p-cymene,
7	Stem	Aqueous and Hydro-alcoholic	4-hydroxyl mellein, vanillin, octacosonoic acid, beta- sitosterone and beta- sitosterol.

2.5. Pharmacological properties of *Moringa oleifera Lam.*

2.5.1. Antioxidant activity.

By regulating GSH levels, the plant's alcoholic extract decreased glucose-induced cataractogenesis in isolated goat eye lenses. Alpha-tocopherol and butylated hydroxytoluene (BHT) have not been found to be as effective antioxidants as myricetin, which is extracted from *Moringa* seeds. In Human Embryonic Kidney 293 (HEK-293) cells, *M.oleifera* leaf extract and substances including isoquercetin, astragalin, and crypto-chlorogenic acid can reduce ROS. Additionally, moringa helps lower fasting plasma glucose (FPG) concentrations and plasma monoaldehyde (MDA) levels in healthy volunteers as compared to those who are administered warm water **(Pareek *et al.*, 2023)**.

In previous studies, the extract's reducing power and scavenging ability were assessed using four in vitro antioxidant assays: ferric reducing antioxidant power (FRAP), metal chelating activity, total antioxidant capacity (TAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Metal chelating ferrous ion activity had the highest antioxidant activity (7.05 ± 0.2 µg/mL), followed by 2.54 ± 0.01 µg/mL for FRAP, 3.90 ± 0.02 µg/mL for DPPH, and 1.50 ± 0.02 µg/mL for TAC, according to the antioxidant study, which also showed that the half-maximal inhibitory concentration (IC_{50}) and percentage (%) inhibition were dose dependent. Thus, the methanol extract of MO leaf has great potential as an antioxidant **(Agrawal *et al.*, 2025).**

2.5.2. Anti-inflammatory activity.

Carrageenin injections were used to create subcutaneous edema in the rat paw to study the anti-inflammatory effects of an aqueous decoction of the root in rats weighing 120 and 160 grams. Following treatment with *M.oleifera* decoction, the rat-paw volume was measured one, three, and five hours after the carrageenin injection and compared at the same intervals. The outcomes were contrasted with indomethacin's anti-inflammatory effects in the same experimental model **(Rode *et al.*, 2022).**

Male albino rats have been used to study the anti-inflammatory properties of aqueous and ethanolic (90 percent) extracts of *M.oleifera* leaves. Within two hours of the challenge, both extracts exhibited their peak activity. In the first hour after carrageenan injection, the aqueous

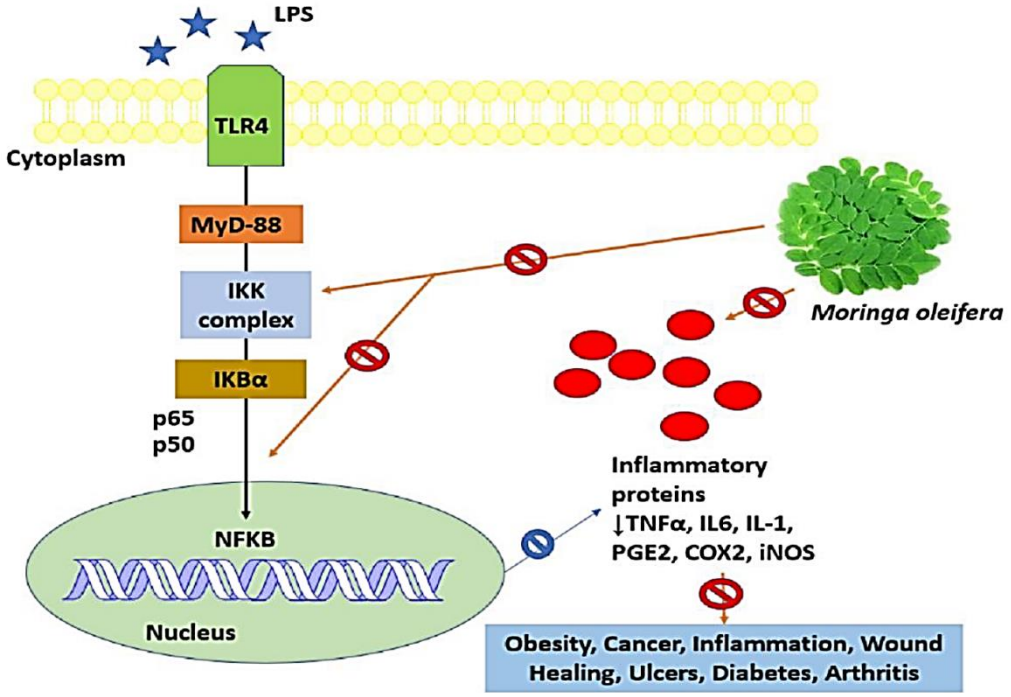

Figure 15: how *Moringa oleifera* performs its biological properties **(Rode *et al.*, 2022).**

extract caused considerable edema suppression, like that seen with Ibuprofen. The results confirmed the anti-inflammatory effect of the plant. The edema suppression action exhibited by the drug may be due to the inhibitory effects on the release of histamine, 5-hydroxytryptamine, and kinin-like substances, which are reported to be released from mast cell degradation during the first hour of carrageenan-induced artificial paw edema **(Rode *et al.*, 2022).**

As an oxidative and inflammatory marker, *M. oleifera* prevents NFKB (nuclear factor kappa B) (figure 15) inhibition by blocking the Inhibitor of Kappa B alpha (IKBα) phosphorylation. By blocking the nuclear translocation and dimerization of IkBα and NFKB, it inhibits the production of inflammatory proteins like cyclooxygenase-2 (COX-2), interleukin-6 (IL6), tumor necrosis factor (TNFα), and inducible nitric oxide synthase (iNos). This reduces inflammation and cures other conditions like obesity, arthritis, cancer, diabetes, and ulcers **(Rode *et al.*, 2022).**

2.5.3. Antibacterial and antifungal properties

The antibacterial activity of *M.oleifera* is attributed to phytochemicals, including flavonoids, tannins, steroids, benzyl glucosinolate, benzyl isothiocyanate, alkaloids, and saponins. The standard strains of *Staphylococcus aureus* (ATCC25923) and *Klebsiella pneumoniae* (ATCC35637) were inhibited by methanolic extracts of *M.oleifera* at concentrations of 20%, 40%, and 60% by 16, 18, and 20 mm in diameter. On the *Proteus vulgaris* (NCTC8196) strain, aqueous extracts of *M.oleifera* (20%, 40%, and 60%) exhibited inhibitory effects of 18, 19, and 21 mm in diameter. There was also antibacterial efficacy against clinically isolated *Escherichia coli, S. aureus*, and *Staphylococcus saprophyticus* from patients with urinary tract infections **(Sonewane *et al.*, 2022).**

At a dosage of 15 mg/ml, *M.oleifera* leaf extracts in water showed antifungal efficacy against *Penicillium spp*. (13.0 ± 0.2 mm) and ppetroleumextracts against *Mucor spp.* (12.0 ± 0.2 mm). Fungal infections *Candida albicans* (10.0 ± 0.1 mm), *Penicillium spp.* (9.1 ± 0.1 mm), and *Mucor spp.* (9.1 ± 0.3 mm) were suppressed by ethanol extracts **(Raj *et al.*, 2011).**

2.5.4. Anticancer activity

According to a study by **(Barhoi *et al.,* 2021),** quinic acid, octadecanoic acid, hexadecanoic acid (palmitic acid), α-tocopherol (Vitamin E), and ɣ-sitosterol were shown to be the main bioactive components in the aqueous extract of *M.oleifera* (AEMO). The treatment of AEMO lengthened the life span of tumor-bearing mice and decreased their tumor weight (TW) and tumor volume (TV). Concluding that AEMO has enormous potential to stop the growth of tumors.

Conducting the investigation to assess *M.oleifera* leaf extracts' in vitro anticancer properties. In a dose-dependent way, the extracts from *M.oleifera* leaves dramatically reduced the proliferation of the human cancer cell line A549. According to morphological investigations, the extract of moringa leaves induced apoptosis, as evidenced by nuclear fragmentation, chromatin condensation, blebbing, and cell shrinkage. Under different treatment circumstances, quantitative RT-PCR (Real Time - polymerase Chain Reaction) studies of the genes Bcl-2 associated X protein (Bax), and B-cell Lymphoma 2 (Bcl-2) revealed aberrant expression profiles. According to vitro research, this study shows that *M.oleifera* leaves may be able to inhibit the proliferation of cancer cells and could be used as the main drug to treat cancer. While creating novel food ingredients and improving human health **(Bhadresha *et al.*, 2022).**

Methanol extract from drumstick leaves demonstrated strong cytotoxicity against B16-F10 mouse melanoma cell lines in anticancer experiments, with an IC_{50} of 127.12 ug/ml. When compared to untreated and normal Kojic acid, 1 mg of *M.oleifera* leaf methanol extract showed anti-proliferation at 36.54% in cell cycle study **(Umme *et al.*, 2025).**

2.5.5. Hepatoprotective and Nephroprotective activity

The hepatoprotective and antioxidant properties of *M.oleifera* leaf extract were examined in relation to Carbon Tetrachloride (CCl4) induced hepatotoxicity in rats. According to the results, the antioxidant and hepatoprotective properties of *M.oleifera* leaves may be linked to their ability to scavenge free radicals. This could be because the extract contains flavonoids and total phenolics, or because the purified compounds kaempferol, quercetin, and β-sitosterol were separated from the ethanol extract of *M.oleifera* leaves **(Singh *et al.*, 2014).**

The amazing potential of *Moringa oleifera* leaves to lessen drug-induced liver and kidney damage in animal models is supported by scientific findings. Numerous investigations have clarified *M.oleifera* 's hepatorenal protective potential against a range of pharmaceutical drugs, including acetaminophen, gentamicin, pyrazinamide, rifampicin, and isoniazid. The main source of its quality protection is its leaves. More precisely, when rats were given *M.oleifera* leaf extract, their blood levels of important indicators like urea, creatinine, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase dropped as a result. These results were supported by histological analyses, which demonstrated that patients treated with *M.oleifera* showed a considerable decrease in drug-induced hepatic and renal system damage. Additionally, *M.oleifera* roots and flowers have significant hepatoprotective qualities against acetaminophen-induced hepatotoxicity in both aqueous and alcoholic extracts. This is demonstrated by decreased levels of bilirubin, alkaline phosphatase, and serum transaminases (alanine aminotransferase and aspartate aminotransferase), indicating a complex protective mechanism provided by *M.oleifera* **(Divya *et al.*, 2024).**

2.5.6. Cardiovascular properties

Hyperlipidemia, hyperglycemia, and hypertension are risk factors for cardiovascular diseases (CVDs) that are prevented and improved by the bioactive substances. *M.oleifera* preparations might be pure components, complete extracts, or isolated metabolites. Research indicates that certain phytochemical substances, such as quercetin and N, α-L-rhamnopyranosyl vincosamide, have anti-inflammatory, anti-apoptotic, and antioxidant

molecular functions. These enhance cardiac contractility and guard against harm to the heart's structural integrity. These substances also have endothelium-protective and vasorelaxant properties **(Alia *et al.,* 2022).**

2.5.7. Toxicity

M.oleifera was not cytotoxic at the pharmacological dose utilized, according to in vitro experiments conducted on human pulmonary artery endothelial cells (HPAEC), Rapidly Adherent White cells, clone 364.7 (RAW364.7) cells, Human Adult Catastrophic Transformed Keratinocytes (HaCaT), human Monocyte-Derived Macrophages (MDM), human Perpheral Blood Mononuclear Cells (PBMC). However, one study by **(Araújo *et al.,* 2013)** demonstrated that whilst the diluted extracts were not cytotoxic, the coagulant *M.oleifera* lectin (cMol) from seed aqueous extracts was possibly cytotoxic in PMBC **(Louisa *et al.* 2022).**

To determine potential toxicity, a multidisciplinary method entailed a thorough examination of many plant sections using animals through leaves, seeds, and stem bark. In one part of the investigation, female albino Wistar rats who were not pregnant were given an aqueous methanol extract of *M.oleifera*. After a randomly chosen cohort was given the extract orally at a dose of 2000 mg/kg, blood samples were taken for examination. Levels of the vital indicators aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin were regularly tracked. A good safety rating for the provided dose was indicated by the studies, which showed that the lethal dose in female Wistar rats surpassed 2000 mg/kg via aqueous extract **(Adedapo *et al.,* 2009).** Studies have indicated encouraging potential for using *M.oleifera* seed extract for nutritional purposes, despite the short-term negative effects that have been noted. To lower the risk of adverse effects, the study's acute manifestations highlight the necessity of careful dosage considerations and improved formulation techniques **(Divya *et al.,* 2024).**

All test groups received the plant extract graded dosages up to 3 g/kg body weight, and the rats were monitored for symptoms of toxicity and death for 60 days following the administration of the extract. When administered orally to rats, the extract was determined to be nearly non-toxic, and its Lethal Dose (LD_{50}) value exceeded 3 g/kg body weight. Rats were given the lowest dose levels viz. 100, 200, and 400 mg/kg body weight orally throughout the experiment **(Singh *et al.,* 2014)**.

2.5.8. Fertility/antifertility effects

There is increasing evidence of the progressive fall in human fertility in both industrialized and developing countries, with a varying prevalence. The prevalence of infertility, for example, is estimated to be 6% in the United States of America (USA), 10%–15% in the United Kingdom (UK), and 20%–46% in sub-Saharan Africa. The continent's overall infertility rate is 41.91% for both males and females, with male infertility accounting for 22.26% of the total **(Mohlala *et al.,* 2023).** Studies have shown that dietary supplementation with *Moringa oleifera* extract improved semen quality, fertility, and hatchability in aged broiler breeder roosters **(Ghadimi *et al.,* 2024).**

In a recent study, female Wistar rats were used to test the antifertility effects of ethanol extracts of *Moringa oleifera* leaves in female Wistar rats with chemically induced decidualization, the effects of the extracts at concentrations of 100, 250, and 500 mg/kg on fertility, implantation, decidualization, and local cytokine signalling were assessed. Intraluminal injection was used to create artificial decidualization. female rats were ovariectomized and given injections. Female rats in the control group received 0.5% gum acacia for five to nine days, while three groups of six rats each received 100, 250, and 500 mg/kg of ethanol extract. When compared to the control group, *Moringa oleifera* at doses of 250 and 500 mg/kg resulted in defective implantation, which might have been caused by defective decidualization. Additionally, studies on artificial decidualization showed a dose-dependent decrease in weight gain, progesterone, and estradiol levels. This, in turn, resulted in a decrease in the expression of several local cytokines, including cyclooxygenase-2 (COX-II), leukemia inhibitory factor (LIF), vascular endothelial growth factor (VEGF), and IL-11. There are several facets of implantation that are impacted by these cytokines and the uterine receptors for them. Progesterone and estrogen levels in uterine tissue were analyzed, and the results indicated a dose-dependent decrease in the treated group relative to the control group. Thus, it was discovered that *Moringa oleifera* extracts caused anti-fertility activity by disrupting implantation and the process of decision-making, possibly because of their anti-estrogenic and anti-progestogenic properties. They proposed that more research was required to ascertain its possible contraceptive effects and to pinpoint the active ingredients causing the anti-implantation effect **(Agrawal *et al.,* 2018).**

2.6. Environmental influence on the phytochemical composition of medicinal plants

Plants respond to changes in their environment, which can be the result of biotic or abiotic activity. Numerous research has investigated the impacts of abiotic stress on plants, including

how it affects both primary and secondary metabolism and recent studies reveal the complexity of understanding global change's effects on plant secondary metabolites **(Jamieson *et al.*, 2017; Prinsloo and Nogemane, 2018).**

2.6.1. Environmental Influence on the Contents of Bioactive Compounds

Various environmental factors such as temperature, carbon dioxide, lighting, ozone, soil water, salt, and fertility, either stimulate or impede plant growth and development. Thus, the adaptation of plant shape, anatomy, and physiological processes to changes in biotic and abiotic conditions may influence the accumulation of secondary metabolites. Climate and ecological changes might impact the synthesis of secondary metabolites **(Li *et al.*, 2020; Pant *et al.*, 2021).**

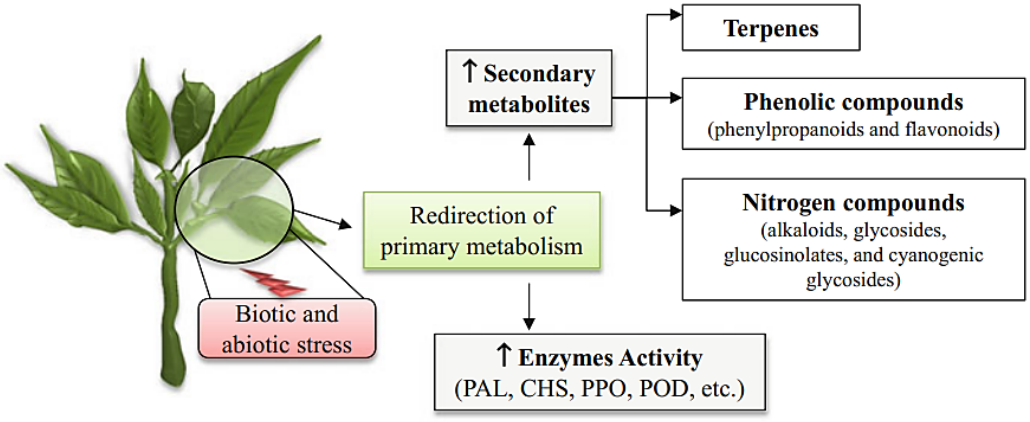

Figure 16: figure showing how plants respond to their environment, and how it affects the synthesis of secondary metabolites **(Borges, 2O17)**

Plants respond to biotic and abiotic stress by redirecting their core metabolism, leading to increased enzyme activity and secondary metabolite synthesis. Enzymes such as PAL, CHS, Polyphenol Oxidase (PPO), and Peroxidase (POD) play a crucial role in the plant response. Secondary metabolites are categorized into three groups: terpenes, phenolic chemicals, and nitrogen compounds ***(*Borges, 2017*).***

ROS, including superoxide radicals and hydrogen peroxide, are created in response to stress and act as signalling molecules. ROS activate antioxidant defense mechanisms and signal transduction pathways, which aid in stress adaptation **(Sharma, 2024).** Plants use enzymatic mechanisms such as peroxidases, superoxide dismutase, and catalases to eliminate ROS, as well as non-enzymatic secondary metabolism compounds. To maintain a healthy ROS balance without harming cells, a combination of enzymatic and non-enzymatic processes is essential. Under stress, plants increase enzymatic activity to synthesize secondary metabolism

compounds, including phenylalanine ammonia lyase (PAL) (EC 4.1.3.5) and chalcone synthase (CHS) (EC 2.3.1.74). These enzymes play a crucial role in flavonoid synthesis and can be affected by environmental stress. The PAL enzyme produces phenols and lignans, which are crucial for plant defense. It is the first enzyme in the phenylpropanoids pathway, catalyzing the deamination of L-phenylalanine to produce trans-cinnamic acid. This acid is an intermediary in the biosynthesis of phenolics, which helps plants scavenge ROS during stress **(Dixon *et al.* 1992).** Phenylpropanoids play an important part in plants' reactions to abiotic and biotic stressors, such as herbivory and pathogen infection **(Borges, 2017).**

2.7. Current status

Because of its many uses, *Moringa* is a plant that can be utilized in a wide range of pharmacological activities and their related formulations, biomedical applications, and the production of fish, poultry, and animals. Numerous studies carried out in China, Brazil, Nigeria, and India between 2019 and 2022 have produced an invaluable resource for scholars everywhere. Following a thorough investigation, it was shown that *M.oleifera* has developed to provide numerous advantages to humans. This plant is ideal for both human and animal consumption due to its abundance of minerals and phytoconstituents. It has emerged as a pharmacological option for the creation of formulations like wound healing, anti-cancer, and anti-aging, among others, because of its potent antioxidant qualities. In addition to being appropriate for human consumption, *M.oleifera* can be used as a fertilizer. In addition to its advantages, taking high amounts of it can have serious toxic and abortifacient effects **(Pareek *et al.*, 2023)**

CHAPTER 3:

Materials and Methodology

3.1. Research problematic

For both scientific and practical reasons, it is crucial to comprehend how *Moringa oleifera*'s phytochemical composition and antioxidant activity vary depending on the environment. Especially in Algeria with scarcity of comparative literature on how much *Moringa'*s phytochemical composition and its biological properties vary according to the environment (climate, soil type, altitude and geographical location). Understanding this is a critical factor in standardizing raw materials and moringa based products for phytopharmaceutical and nutraceutical applications.

3.2. Objectives of the study

The objectives of this study were to quantify the concentrations of key bioactive compounds: total phenolic compounds, total flavonoid compounds, condensed tannins, and hydrolysable tannins, antioxidant, antimicrobial and anti-inflammatory activities, in *Moringa oleifera lam.* plant collected from the three different regions of Algeria and to investigate how geographical regions influence its biological activity.

3.3. Description of the study regions

Four samples of the plant material were collected from three different regions of Algeria namely, Adrar, Mostaganem and Tamanrasset.

Tamanrasset

Tamanrasset (24,18231°N, 5,06109°E) is situated in the Sahara (figure 17), within the Ahaggar massif at an elevation of 1370 m above mean sea level and is about 65 km south of the Tropic of Cancer. This region predominantly experiences Mediterranean weather throughout the year, with an average annual temperature of 23.4°C. The hottest months are June and July, averaging 24.3°C, while the coldest months are December and January, averaging 6.0°C. It is characterized by rocky ground, an average annual precipitation of 42,9mm and 23% relative humidity **(Cuesta *et al,* 2008).**

Adrar

The research area is situated in Adrar, Algeria (27°53′N 0°17 W), which is approximately 700 km south of the capital city of Algiers in the central Sahara Desert (figure 17). Adrar has an arid desert environment, with scorching summers and mild winters. Average maximum

temperatures range from 22°C in January to 41°C in July. The average annual precipitation is around 18 mm, occurring predominantly during the winter months, the relative humidity is 24% and is characterized by sandy, Silty clayey soils **(Benatallah *et al.*, 2024).**

Mostaganem

Mostaganem is a coastal province located in North-West Algeria (figure 17), on the shore of the Gulf of Mostaganem, 80.7 kilometers east of Oran and 363 kilometers west of Algiers. The climate is characterized by an average temperature of 17.9 °C, saline halomorphic soil, meaning they are dominated by soluble salts, 387mm average annual precipitation and the relative humidity ranging from 60% - 70%.

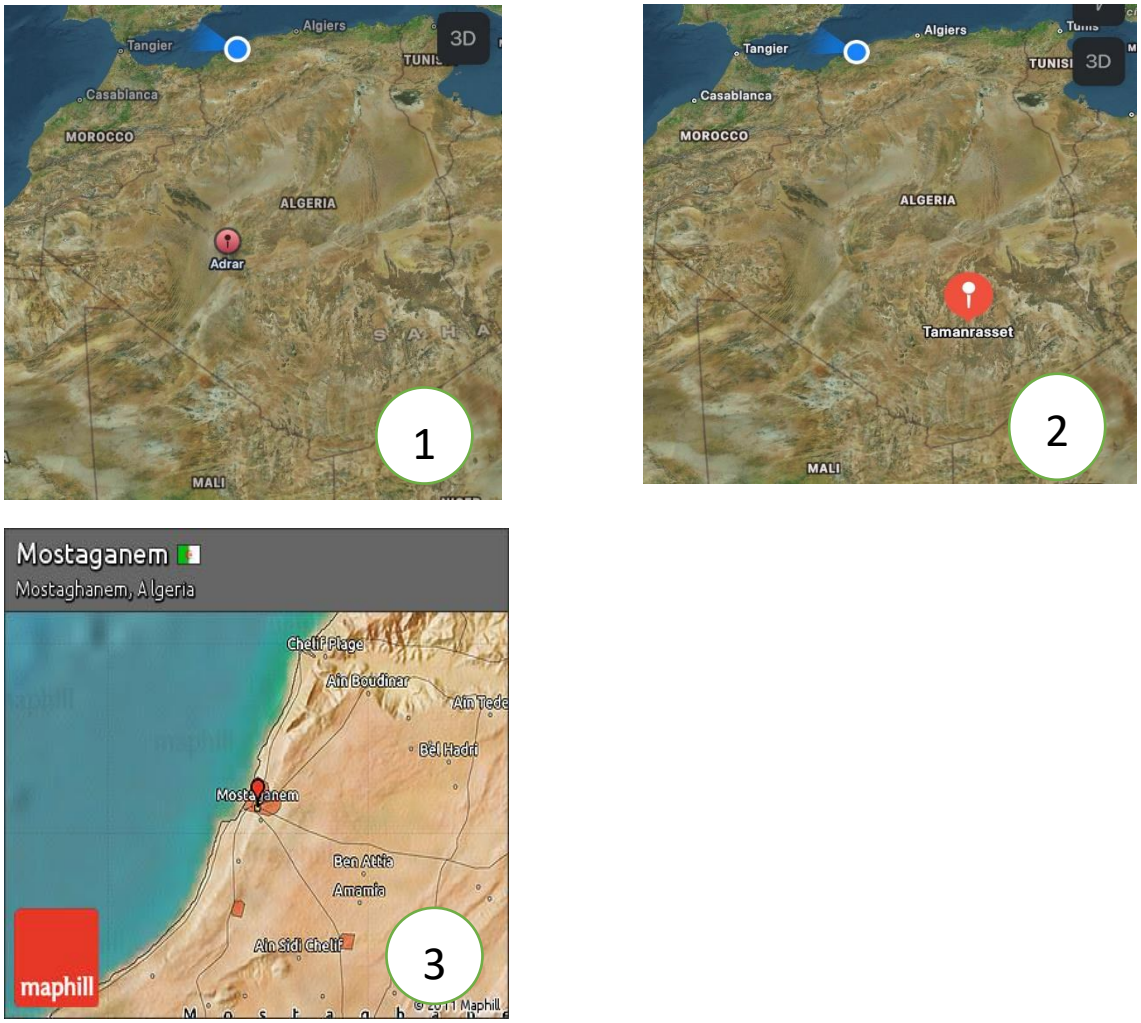

Figure 17: location of study regions; Adrar (1), Tamanrasset (2), and Mostaganem (3)
(Obtained from google maps on Wednesday, 4 June. 2025, 16:00H.

3.4. Materials

3.4.1. Plant Material

The good quality dry *Moringa oleifera lam.* seeds used in this study, aged three years were harvested from the three different regions/cities of Algeria namely, Mostaganem and Tamanrasset, Adrar [named MO1(10th, April 2025), MO2(21st, May 2025) and MO3(30th, May 2025) respectively according to the arrival time], the plant material was then transported to the Applied Plant Physiology laboratory, at the University of Mostaganem, SNV faculty where the studies were carried out. The plant material was kept at room temperature until used.

3.4.2. Microbial strains

The microbial strains tested in this study were obtained from the laboratory of Microbiology at the SNV faculty, Mostaganem University. The table below shows all the microbial strains used.

Table 6: The different microbial strains used and their references

Gram type	**Microbial strains**	**Reference**
Gram-negative strains	*Escherichia coli*	ATCC 25922
	Klebsiella pneumoniae	ATCC 13883
	Proteus mirabilis	ATCC 6051
	Salmonella paratyphi	ATCC 9150
	Pseudomonas aeroginosa	ATCC 27853
Gram-positive strains	*Staphylococus aureus*	ATCC 33862
	Bacillus cereus	ATCC 14579

3.4.3. Reagents and chemicals

96% Ethanol, 1,1-diphenyl-2-picrylhydrazil (DPPH), methanol, Folin-Ciocalteau (FC), 2% sodium bicarbonate (Na_2CO_3), gallic acid, vanillin, concentrated Hydrochloric acid (HCl), Catechin, Iron chloride ($FeCl_3$), Sodium nitrate ($NaNO_2$), Aluminum chloride ($AlCl_3$), and Sodium hydroxide (NaOH), phosphate saline solution, Egg, Diclofenac, were readily found at the laboratory.

3.5. Methodology

3.5.1. Study design

This study adopts a comparative experimental design to determine the influence of geographical location on the phytochemical content, antimicrobial, anti-inflammatory and the antioxidant activity of MO seeds. The study involves sample collection from the three different geographical regions, followed by standardized laboratory analysis to quantify and compare the chemical and biological properties of the plant material.

3.5.2. Preparation of *Moringa oleifera* seed powder

The bark for MO1 was removed manually to obtain the seed as shown in figure 18. The good quality MO1, MO2 and MO3 seeds were then ground separately into fine tan, beige powder (figure 20) using the ISO9001 multi-purpose blender. The flow chart for the preparation of MO powder is shown in figure 19.

Figure 18: MO seeds with bark (left) and exposed (right)

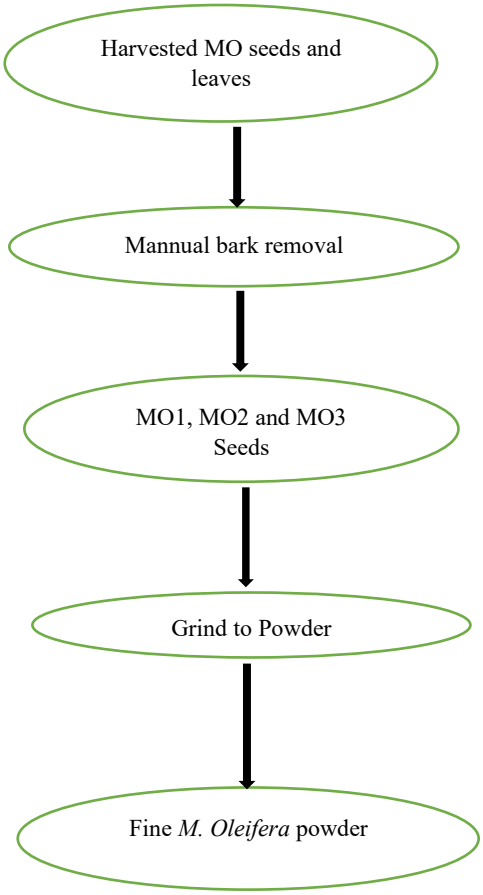

Figure 20: The flow chart of the preparation of MO powder.

Figure 19: MO1, MO2, and MO3 whole seed powder

3.5.3. Physico-chemical analysis of *Moringa oleifera seeds*

3.5.3.1. Dry matter content and the moisture content (AFNOR, 1985).

Dry matter refers to the non-aqueous component of a feed ingredient or diet **(Van Saun and Herdt 2014).**

To determine the dry matter (DM) content of the MO plant, 5 grams (g) of the ground powder of MO1, MO2, and MO3 introduced into three porcelain crucibles, each aliquot was placed in the Froilabo steam room at a temperature of 105°C for 24 hours for dehydration. After 24 hours, the crucibles were placed in the desiccator for 45 minutes and thereafter weighed. The amount of DM content was then calculated by finding the difference between fresh weight and dry weight, hence deducting the amount of water evaporated. The calculations were determined using the following equations.

Dry matter (g) = [(weight of the crucible + weight after drying) – weight of the empty crucible]

Calculation of the DM in percentages:

Dry matter (%) = [(DM (g) / sample mass (g)] *100

The humidity is therefore calculated according to the following formula:

Percentage of water (%) = [100 – percentage of the dry content] %

3.5.3.2. Mineral matter and organic matter (AFNOR, 1985).

A). Determination of the Mineral matter

To determine the mineral matter (MM) content and the organic matter (OM) content of the MO plant, the samples that were previously used to determine the dry matter and the moisture content were placed in a Carbolite Muffle oven at 550°C for 2 hours, with the aim of destroying the organic matter by incineration. The crucibles containing the ash content (figure 21) were put in the desiccator for 45 minutes and thereafter weighed.

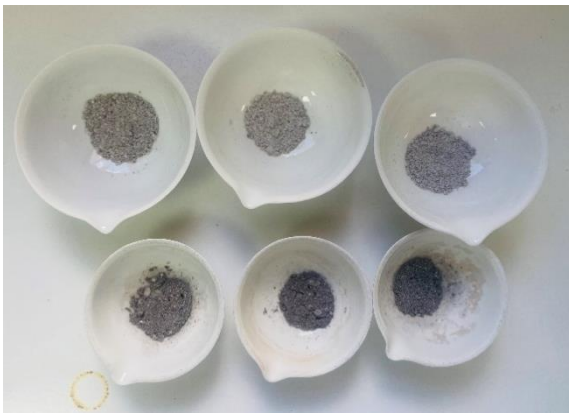

Figure 21: The ash content of MO plant after burning for 2 hours

The mineral matter of the MO plant powder sample was calculated by the following equations:

Mineral matter (g) = [weight of the crucible with ashes – weight of the empty crucible]

Calculation of the mineral matter content of MO plant in percentage (%):

Mineral matter (%) = [Mineral matter (g) / M1-M2] * 100

With:

M1: total mass of the crucible containing MO powder before burning (in grams).

M2: total mass of the crucible containing the ash after burning (in grams).

B). Determination of the organic matter

The OM content was determined by finding the difference between the percentage of the DM content and the percentage of the MM content.

Organic matter (%) = [100% - % Mineral matter]

3.5.4. Extraction of Phytochemicals of *Moringa oleifera* plant

Aqueous ethanolic extract of MO plant (Nurcahyo *et al*., 2020: Idris *et al.,* 2024)

To extract the bioactive compounds contained in the MO seeds, 10 g of *M. oleifera* (MO1, MO2, and MO3) powder was measured using the precision scale, and 200 ml of hydroethanolic solution was prepared. 100mls of the solution were added to 10 g of *M. oleifera* (10:1 v/w) powder in a beaker. The mixture was constantly stirred for 1 hour at room temperature using the VELP SCIENTIFICA heating magnetic stirrer and thereafter filtered using the Whatman No. 1 filter paper, and to the residue, the remaining 100mls of the aqueous ethanolic solution were added and stirred for another hour and thereafter filtered. The extract was kept in a flask covered with aluminum paper and stored at 4°C untilled used.

***Dilution of the Moringa oleifera plant extract (*Rahman, M *et al.,* 2009)**

In a series of test tubes labelled 20, 10, 5, 2.5, and 1.25 mg/ml of the extract, 2.5 ml of hydro ethanol (30% H_2O, 70% ethanol) was added, 2.5 ml of the previously prepared extract was added to the tube labelled 20 and stirred with an ISOLAB Laborgerate GmbH vortex, 2.5 ml of this solution was transferred using a syringe to the tube labelled 10 and stirred up, This process was repeated for the rest of the tubes, and three replicate of each tube were prepared.

3.5.5. Phytochemical Analysis of *Moringa oleifera* plant extract (quantitative analysis)

3.5.5.1. Determination of Total Phenolic Content (TPC)

Polyphenol content of the *M. oleifera* plant extract was determined using the Folin-Ciocalteau colorimetric method with a slight modification **(Singleton *et al.,* 1999).**

Principle

The basis for Folin-Ciocalteau (FC) colorimetry is a chemical reduction of the reagent, which is a combination of molybdenum and tungsten oxides. This approach was modified by

Singleton for wine analysis and was originally designed to quantify the phenolic amino acid tyrosine **(Waterhouse, 2002; Bärlocher and Graça, 2020).**

Protocol

In a beaker, 1 millilitre of FC reagent was diluted with 9 milliliters of distilled water making the stock FC solution, the beaker was covered with an aluminum foil for protection against light. In another beaker, Na2CO3 2% solution was prepared by dissolving 2 grams of Na2CO3 in 100 milliliters of distilled water.

Three replicates of the tubes each containing 100 microliters (µl) of the diluted extract was added 500 µl of FC reagent and left in the dark for 5 minutes, thereafter, 1.5 milliliters of Na2CO3 solution were added, homogenized and left in the dark for 1 hour. The blank solution was prepared with hydro ethanol in 500 µl of FC. After that, absorbance was measured using UV-Vis spectrophotometer at a wavelength of 765 nanometers (nm) in 1m quartz cuvettes.

The total polyphenol content was calculated as mean ± Standard Deviation (SD) using the standard curve of Gallic acid and the results were expressed as milligrams of Gallic acid equivalents (GAE) in 100 g of dry weight.

3.5.5.2. Determination of Total Flavonoid Content (TFC).

The total flavonoid content of *M. oleifera* plant extract was measured using the aluminum chloride colorimetric assay with a slight modification **(Zhishen *et al.*, 1999; Sulaiman and Balachandran, 2012).**

Preparation of products

10 milligrams (mg) of quercetin were dissolved in 10 ml of methanol. 1 gram of Sodium nitrate (NaNO2) was dissolved in 20 ml of distilled water; 2 g of Aluminum chloride (AlCl3) was dissolved in 20 ml of methanol and 0.5 g of Sodium hydroxide (NaOH) was dissolved in 50 ml of distilled water. All the beakers were covered with aluminum foil for protection against light.

Dilution of quercetin

In a series of test tubes, 0.8, 1.2, 1.6, 1.0, 0.24, 0.28, 0.32, 0.36, and 0.4 ml of previously prepared quercetin solution was added respectively, thereafter, 1.2, 0.8, 0.4, 9.0, 1.76, 1.72, 1.68, 1.64, and 1.60 ml of methanol was added respectively. The test was done in three replicates.

Protocol

500 µl of Quercetin solution or diluted *M. oleifera* seed extract was pipetted into a 15 ml test tubes, 1500 µl distilled water was added and thereafter, 150 µl of 5% Sodium nitrate (NaNO2) was added, the aliquot was left for 5 minutes, thereafter, 150 µl of 10% Aluminum chloride (AlCl3). After 6 minutes, 500 µl of (NaOH) and the volume was made up to 5 ml with distilled water. The solution was mixed well using a vortex and the absorbance was recorded against the blank at 510 nm using the UV-Vis spectrophotometer. Two blanks, one containing only methanol for measuring quercetin and the other containing hydro ethanol for measuring the sample were prepared.

The total flavonoid content was calculated as mean ± SD using the standard curve of quercetin and the results were expressed as milligrams of quercetin equivalents (QE) in 100 g of dry weight.

3.5.5.3. Condensed tannins determination

Proanthocyanidins, also known as condensed tannins (CTs), are a class of polyhydroxyflavan-3-ol oligomers and polymers that are connected by carbon-carbon (C-C) or C-O-C bonds between flavanol subunits, possessing the primary medical properties associated with tannins **(Schofield *et al.,* 2001; Bule *et al., 2020*).**

CTs content of *M. oleifera* plant extract was determined using the Vanillin-hydrochloric acid (HCl) assay with a slight modification **(Burns, 1971).** Vanillin-HCl assay has widely been used to quantify the CTs content or its monomeric content especially in sorghum grain **(Price *et al.,* 1978).** The CTs react with vanillin in an acidic medium yielding a colored product with an absorbance at 500nm **(Broadhurst and Jones, 1978).**

Protocol

Vanillin methanolic solution was prepared by dissolving exactly 4 grams of Vanillin in 100 milliliters of methanol, thereafter, to the test tubes containing 25 µl of the diluted *M. oleifera* seed extract each, 750 µl of Vanillin methanolic and 375 µl of concentrated HCL was added, stirred and left in the dark for 20 minutes. The blank used was a mixture of Vanillin methanolic solution, hydro ethanol and concentrated HCl. Density measurements at 500 nm were recorded using the UV-Vis spectrophotometer. Condensed tannins content was calculated as catechin equivalent from the calibration curve of standard catechin by plotting the absorbance versus concentration and expressed in mg CE/g dry weight.

3.5.5.4. Determination of hydrolysable tannins

The hydrolysable tannins in *M. oleifera* seed extract were determined by Ferric chloride method with a slight modification (**Mole and Waterman, 1987),** which has also been described by **Belem-Kabré *et al.,* 2021.**

Ferric chloride solution (10^{-2} M $FeCl_3$ in 10^{-3} M HCl) was prepared by dissolving exactly 0.41 g of $FeCl_3$ in 0.021 ml of HCl diluted with 250 ml of distilled water. In a series of 5 test tubes, 0.5 ml of the *M. oleifer*a seed extract was pipetted each with different concentrations ranging from 10.00, 5.00, 2.50, 1.25, and 0.625. Thereafter, 1.75ml of ferric chloride solution was added and mixed using a vortex, and left for 15 seconds in the dark, the blank that was used was hydro ethanol solution. The absorbance of *M. oleifera* seed extract was recorded at 660 nm using a UV-Vis spectrophotometer. The hydrolysable tannins content expressed in mg GAE/g dry weight was calculated by reference to the calibration line of Gallic acid.

3.5.6. Determination of the biological properties of *Moringa oleifera* seeds

3.5.6.1. Antioxidant activity

The antioxidant property of the hydroethanolic seed extract of *M. oleifera* (MO1, MO2, and MO3) was determined using a 1,1-diphenyl-2-picryl hydrazyl (DPPH) technique. The 1,1-diphenyl-2-picrylhydrazil (DPPH) radical was identified by Goldschmidt and Renn in 1922, marking its centenary in 202, and developed by Blois **(Blois, 1958; Gulcin and Alwasel, 2023). Singh *et al.,* 2021** have reported that one free radical with the ability to take hydrogen from antioxidants is DPPH. This chemical is now frequently employed in the DPPH assay to measure the antioxidant activity of fruits, medicinal herbs, and other biological substrates. The chemical structure of DPPH is shown in figure 22.

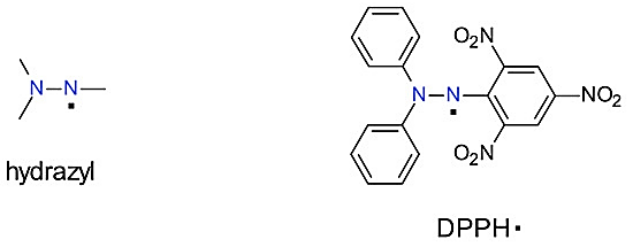

Figure 22: The chemical structure of DPPH **(Ionita, 2021)**

Principle of the DPPH assay

Several recent workers have adopted the original Blois method **(Molyneux, 2004).** The reduced form is created when a DPPH solution is combined with one of a chemical that may donate a hydrogen atom, losing its violet color (albeit the picryl group should still be present and giving off a faint yellow tint). Z° stands for the DPPH radical, and BH for the donor molecule, ZH is the reduced form of DPPH. The free radical **B°** will then react with another free radical produced by the parallel reaction. **(Molyneux, 2004).** The main reaction is.

Z° + BH = ZH + B°

With an absorbance at 517 in methanol, the color shift is tracked using spectrophotometry and used to calculate parameters for antioxidant qualities **(Mishra *et al.,* 2012).**

Protocol

According to the protocol used by **Baliyan *et al.,* 2022**, and **Gulcin and Alwasel, 2023**, 3 milligrams of DPPH were measured using a precision scale in a beaker and dissolved in 125 milliliters of methanol to make the stock solution of DPPH, the solution was protected from light with an aluminum foil and stirred for 5mins using the VELP SCIENTIFICA heating magnetic stirrer. 0.9 milliliters of the DPPH solution were added to 0.1ml of the diluted MO seed extract in a test tube and left in the dark for 1 hour. Two control solutions were prepared by mixing 0.1 milliliters of methanol in a test tube with 0.9 milliliters of the DPPH solution, and 0.1 milliliters of hydro ethanol with 0.9 milliliters of the DPPH solution respectively, Ascorbic acid was used as the standard and methanol solution was used as the blank. The experiment was done in triplicate, and the absorbance was therefore recorded at 517 nm using the JENWAY UV-VIS spectrophotometer. The following formula was used to calculate the radical scavenging activity (RSA) of MOS extracts:

Antioxidant activity (%) = [(Ac –As) ÷ Ac] × 100%

Where Ac is the absorbance of DPPH solution without the MOS extracts and as is the absorbance of samples.

3.5.6.2. Anti-inflammatory activity

Inhibition of protein denaturation

Protein denaturation assay was carried out according to the method described by (**Bentaiba *et al.*, 2023).** in a series of test tubes, 2000 µl of the diluted extract of *M. oleifera* plant at different concentrations, 2800 µl of phosphate saline with a PH of 6.4, and 200 µl of the egg albumin was added, the mixture was incubated in an oven at 37°C for 20 min and thereafter in a water bath heated at 70°C for 5 min. The control was prepared with hydro-ethanol, and the blank was phosphate saline. On the other hand, Diclofenac was prepared following the same protocol for reference (standard). The mixture was cooled at room temperature for 5 minutes and thereafter, the absorbance was read using the JENWAY UV-VIS spectrophotometer 6 at 660 nm, and the percentage of protein denaturation inhibition (PDI in %) was determined using the formula below:

$$\mathbf{PDI = (1- Aa/Ab) \times 100\ \%}$$

Where, Aa is the absorbance of control and Ab is the absorbance of the sample.

3.5.6.3. Anti-microbial activity

The extracts for MO1, MO2, and MO3 were dried in an oven at 40 °C for 24H to evaporate the ethanol which has an effect on the microorganisms. The antimicrobial activity was determined using the method described by **(Panova *et al.*, 2025),** which consists of pouring 21ml of the previously prepared liquid Mueller Hinton culture media on Petri dishes in a sterile zone, and inoculated with 100 µl of young culture of 18H of incubation with density adjusted to 10^7 CFU/ml (optical density ranging from 0.08-0.1), after hardening at room temperature, 4 wells per Petri dish were made, 100 µg (micro-gram) of each dried extract was completely dissolved in 1ml of distilled water, thereafter, 50 µl were pipetted into the wells. Petri dishes were then incubated at 37°C for 24H in an oven. Gentamicin (10 µg/disc) was used as positive control. The experiment was done in triplicate.

The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells. Diameters of 18 nm or more were considered sensitive, between 12 – 18nm,

were considered moderately sensitive and if the inhibition zone was from 0 – 12 nm, they were considered resistant.

3.6. Statistical analysis

All analysis was performed in triplicate and the data was organized and analyzed using the IBM SPSS (Statistical package for social sciences) statistics and all the results were recorded as mean ± SD) of all the triplicates. To identify the significance of differences from samples of *Moringa oleifera* collected from the three different regions, a one-way Analysis of Variance (ANOVA) was employed. With a p-Value of less than 0.05, the results were considered to have a significant difference. PCA was employed to study the correlations and patterns between phytochemical composition and DPPH across all the samples Graphs and tables were plotted using Microsoft Excell 2019 for enhanced visualization.

Chapter 4:

Results and Discussion

4.1. Physico-chemical analysis of *Moringa Oleifera* seeds

4.1.1. Dry matter, mineral matter, moisture, and organic matter content of *Moringa oleifera* seeds

The results of the physico-chemical composition of *Moringa oleifera* seeds from the three different regions (Adrar, Mostaganem and Tamanrasset) are presented in table 7. With Mostaganem seeds presenting the highest dry matter and ash content of 99.25 ± 0.32% and 6.44 ± 0.39% respectively

Table 7: physicochemical composition of *Moringa oleifera* seeds.

	***Moringa oleifera* Seeds**		
	Adrar	**Mostaganem**	**Tamanrasset**
Dry matter (%)	97.8 ± 0.34 b	99.25±0.32 a	95.26 ± 0.75 c
Ash (%)	3.89 ± 0.13 b	6.44 ± 0.39 a	4.77 ±0.25 b
Moisture (%)	2.2 ± 0.34 b	0.86 ± 0.5 c	4.73 ± 0.75 a
Organic matter (%)	96.1 ± 0.13 a	93.55 ± 0.39 b	95.22 ± 1.2 c

The proximate composition of *Moringa oleifera* seeds collected from three geographically and climatically distinct Algerian regions highlights substantial environmental influence on the biochemical traits of the seeds. These variations are not only statistically significant but biologically meaningful, providing insight into the adaptive physiology of *Moringa* across diverse arid and semi-arid ecosystems.

The highest dry matter content was recorded in seeds from Mostaganem (99.25 ± 0.32%), followed by Adrar (97.8 ± 0.34%) and Tamanrasset (95.26 ± 0.75%). This suggests that seeds from the Mediterranean coastal region of Mostaganem underwent a more complete maturation and desiccation process compared to those from arid and hyper-arid zones. Higher dry matter implies superior stability, lower water activity, and greater concentration of solids such as oils and proteins, making them more suitable for storage and industrial processing. These results are in line with reports by **(Saini *et al.*, 2016)**, who showed that seeds from *Moringa* trees grown in less stressful environments tend to accumulate more dry matter due to favorable metabolic conditions that enhance resource allocation to reproductive tissues.

Conversely, moisture content showed an inverse pattern, with Tamanrasset seeds retaining the highest amount (4.73 ± 0.75%) and Mostaganem seeds the lowest (0.86 ± 0.5%). Elevated

moisture in seeds from Tamanrasset likely reflects either incomplete dehydration at harvest or physiological adaptations to hyper-arid stress that affect water regulation. Such high moisture levels can reduce shelf life and increase susceptibility to microbial spoilage, necessitating additional post-harvest drying measures for industrial applications (**Gharsallah *et al.,* 2021**).

The ash content, a proxy for total mineral content, was highest in Mostaganem seeds (6.44 ± 0.39%), indicating a richer accumulation of essential minerals, potentially due to higher soil fertility and better water availability in coastal areas. Ash values from Adrar (3.89 ± 0.13%) and Tamanrasset (4.77 ± 0.25%) were significantly lower, consistent with the poor mineral content typically found in sandy and less fertile desert soils. This finding aligns with previous work by **(Saini *et al.,* 2016)**, who highlighted the nutritional richness of *Moringa* seeds, particularly their calcium, potassium, and phosphorus content, which vary greatly by growing location. High ash content increases the value of seeds for dietary supplementation and food fortification programs, especially in regions facing mineral deficiencies.

Organic matter content followed a different trend, with the highest levels in Adrar seeds (96.1 ± 0.13%), followed by Tamanrasset (95.22 ± 1.2%) and the lowest in Mostaganem (93.55 ± 0.39%). This result is somewhat paradoxical given that dry matter was highest in Mostaganem, but it may indicate a greater proportion of inorganic mineral content in Mostaganem seeds, reducing the relative organic fraction. The higher organic content in seeds from arid regions could reflect a physiological strategy to accumulate protective biochemical reserves, such as lipids, proteins, and phenolic compounds under environmental stress. This is supported by the review of **(Anzano *et al.,* 2021)**, who noted that *Moringa*'s secondary metabolites, including flavonoids, glucosinolates, and isothiocyanates, are often upregulated in response to drought and oxidative stress.

The differential seed composition can be attributed to the Eco geographical variability in climatic conditions. In Tamanrasset, located in the southern Sahara, high diurnal temperature ranges and low precipitation impose severe physiological stress on *Moringa* trees. Under such circumstances, slower growth, delayed seed maturity, and impaired nutrient uptake are common (**Nobossé *et al.,* 2018**). In contrast, Mostaganem, with its Mediterranean climate, offers moderate temperatures and greater soil fertility, enabling *Moringa* trees to complete their reproductive cycle under less abiotic stress, promoting more efficient nutrient partitioning into seeds.

The variation observed also has implications for pharmacological and nutraceutical uses. Seeds from all regions are likely to contain core bioactive compounds such as oleic acid, tocopherols, and sterols; however, their concentrations may differ. For example, **Gharsallah *et al.,* 2021)** highlighted that the phytochemical composition of *Moringa* seeds, especially the oil fraction, is highly influenced by environmental and processing conditions. Similarly, the study by **Anzano *et al.*, 2021)** emphasized that the bioavailability of antioxidants and other secondary metabolites varies significantly depending on location, with plants from arid zones showing enhanced accumulation of stress-induced phytochemicals like flavonoids and tannins.

Moreover, the ethnomedicinal significance of *Moringa* seeds is reinforced by their rich phytochemical profiles. Compounds such as moringinine, rhamnosides, and benzyl isothiocyanates, found in varying proportions across different ecotypes, contribute to *Moringa*'s reported antimicrobial, anti-inflammatory, and hepatoprotective activities (**Anzano *et al.,* 2021**; **Gharsallah *et al.,* 2021**). These bioactivities can be enhanced or diminished depending on agroecological variables, emphasizing the need for chemotypic and genotypic characterization alongside agronomic profiling.

The findings of this study support the strategic valorization of local *Moringa* populations based on region-specific seed traits. Seeds from Mostaganem, with superior dry matter and mineral content, are ideal for oil extraction, biodiesel applications, and mineral supplementation. Those from Adrar, with higher organic matter, may be better suited for nutraceutical and protein-rich food product development. This is consistent with global efforts to develop climate-resilient crops that maintain high nutritional value across diverse environments (**Saini *et al.,* 2016**).

The proximate composition of *Moringa oleifera* seeds from three Algerian regions reveals ecotype-specific biochemical profiles driven by local environmental factors. These differences are critical not only for optimizing nutritional and industrial applications but also for guiding conservation, domestication, and selective breeding programs for *Moringa* in North Africa and beyond.

4.2. Phytochemical composition of *Moringa oleifera* seeds

The results for the total phenolic content, total flavonoid content, Hydrolysable and condensed tannins are represented in figure 23

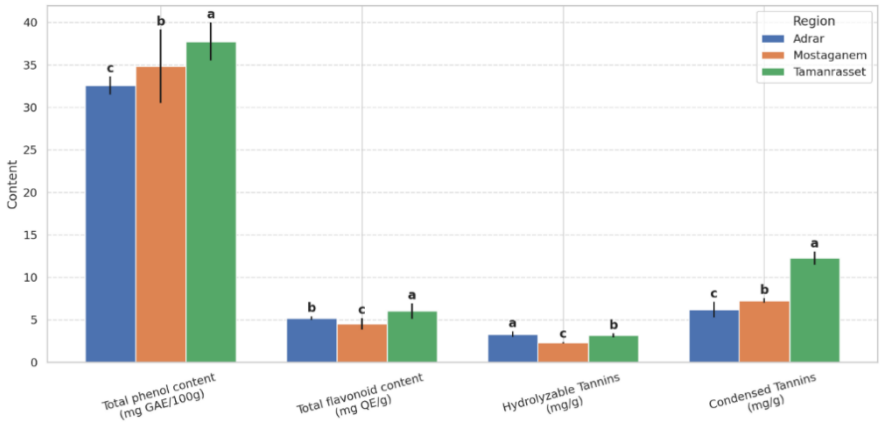

Figure 23: Phytochemical content of *Moringa oleifera* seeds from the three regions

The phytochemical composition of *Moringa oleifera* seeds from three Algerian regions demonstrates significant variability in total phenolic content (TPC), total flavonoid content (TFC), hydrolyzable tannins (HT), and condensed tannins (CT). These differences are statistically significant ($p < 0.05$), as indicated by non-overlapping superscript letters in the dataset.

Phenolic compounds are secondary metabolites known for their antioxidants, anti-inflammatory, and antimicrobial properties. In this study, TPC was significantly highest in Tamanrasset seeds (37.76 ± 2.22 mg GAE/100 g), intermediate in Mostaganem (34.83 ± 4.33 mg GAE/100 g), and lowest in Adrar (32.57 ± 1.07 mg GAE/100 g). The elevated TPC in Tamanrasset may reflect adaptive responses to abiotic stresses such as high UV exposure, poor soil fertility, and water scarcity. **(Nobossé *et al.*, 2018)** confirmed that *Moringa* plants subjected to harsher climates, such as tropical savannahs, tend to accumulate more phenolics and flavonoids as part of their protective mechanisms.

These values are within the general range observed in literature. For example, **Bennour *et al.* (2021)** reported TPC values for *Moringa oleifera* seeds from Algeria ranging from 30.72 to 38.10 mg GAE/g depending on extraction solvent, with ethanolic extracts yielding higher phenolic content. In contrast, optimized extractions, such as those using Soxhlet apparatus with methanol, can yield much higher phenolic concentrations; however, such values (e.g. 2027.07 mg GAE/100 g) reported in some studies may reflect methodological exaggeration or conversion differences and were not confirmed in the validated documents shared.

The higher TFC in Tamanrasset highlights again the role of environmental stress in enhancing the synthesis of protective flavonoids. These compounds play roles in UV absorption and oxidative stress mitigation. **(Popoola & Obembe, 2013)** and **(Gopalakrishnan *et al.,* 2016**) have described the importance of environmental cues in triggering flavonoid biosynthesis in *Moringa*.

Regarding tannins, HT was most abundant in Adrar (3.29 ± 0.34 mg GAE/g), followed by Tamanrasset (3.17 ± 0.23 mg GAE/g), and least in Mostaganem (2.29 ± 0.07 mg GAE/g). These results suggest that Adrar's hyper-arid conditions may specifically favor the synthesis of hydrolysable tannins. Such trends align with reports by **(Bennour *et al.,* 2021),** who showed that extreme environmental stress influences tannin production.

Condensed tannins (CT), on the other hand, were highest in Tamanrasset (12.26 ± 0.77 mg CE/g), followed by Mostaganem (7.25 ± 0.29 mg CE/g), and Adrar (6.18 ± 0.93 mg CE/g). These compounds, also known as proanthocyanidins, are synthesized along the flavonoid pathway and hence correlate strongly with TFC, as previously described by **(Nobossé *et al.,* 2018)** and **(Gopalakrishnan *et al.,* 2016).**

Environmental conditions, especially in Saharan zones like Tamanrasset, are key to shaping phytochemical profiles. High solar radiation and minimal rainfall are known to activate phenylpropanoid metabolism, leading to elevated production of antioxidant compounds, as described by **(Benkeblia, 2022)** and **(Gopalakrishnan *et al.,* 2016).**

The high standard deviation in Mostaganem's TPC (± 4.33) may indicate microenvironmental variability or differences in post-harvest handling, irrigation, or soil characteristics. As noted in **(Nobossé *et al.,* 2018),** such local factors can significantly influence metabolite profiles even within a single agroecological zone.

4.3. Biological properties of *Moringa oleifera* seeds

4.3.1. Antioxidant activity

Figure 24 presents the antioxidant activity (DPPH inhibition in %). The highest antioxidant activity was observed from seeds from Tamanrasset with (79.76 ± 0.92%), followed by Mostaganem and Adrar with DPPH inhibition of (74.00 ± 1.00%) and (60.21 ± 0.19%) respectively.

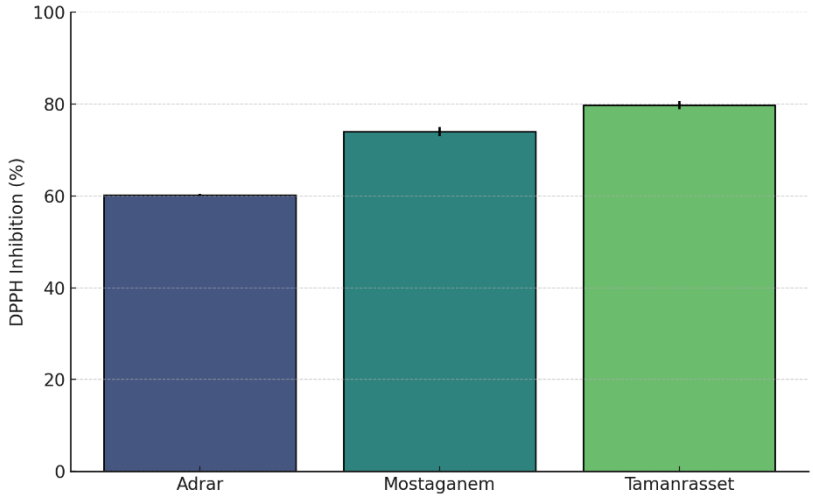

Figure 24: Antioxidant activity of Moringa oleifera seeds from three regions

Based on the DPPH radical scavenging activity results of *Moringa oleifera* seeds from Adrar (60.21 ± 0.19%), Mostaganem (74.00 ± 1.00%), and Tamanrasset (79.76 ± 0.92%), we observe statistically significant differences ($p < 0.05$) in antioxidant capacity among the regions. These variations closely correlate with the quantified levels of phytochemicals: total phenolic content (TPC), total flavonoid content (TFC), hydrolysable tannins (HT), and condensed tannins (CT).

The highest DPPH inhibition from the Tamanrasset seeds corresponds with their highest concentrations of total phenolics (37.76 ± 2.22 mg GAE/g), flavonoids (6.04 ± 0.89 mg QE/g), and condensed tannins (12.26 ± 0.77 mg CE/g). This result agrees with **Liang *et al.* (2019)**, who reported that Morin*ga* seeds with higher TPC and TFC exhibited stronger DPPH activity due to the free radical scavenging ability of phenolic hydroxyl groups.

Moreover, condensed tannins in Tamanrasset seeds, which were nearly double compared to Adrar and Mostaganem, likely enhanced antioxidant capacity, as proanthocyanidins are known to chelate metal ions and inhibit oxidative chain reactions. In support, **(Verma *et al.*, 2021)** also found that condensed tannins in *Moringa* contributed significantly to DPPH inhibition and overall antioxidant behavior.

Interestingly, Adrar samples had the highest hydrolysable tannins (3.29 ± 0.34 mg CE/g) but the lowest antioxidant activity, which emphasizes that not all tannin types contribute equally to radical scavenging. This observation is consistent with the findings by **(Nobossé *et al.*, 2018),** who noted that condensed tannins and polyphenols were stronger predictors of antioxidant activity than hydrolysable tannins in *Moringa oleifera*.

Environmental factors are also likely to contribute to these differences. Tamanrasset, being in a high-altitude Saharan region, is subject to stronger UV radiation and lower humidity. Such abiotic stressors are known to stimulate phenolic biosynthesis as part of the plant's defense system. This aligns with **(Bennour *et al.*, 2021),** who found that harsh climatic conditions in southern Algeria led to elevated phenolic profiles in *Moringa oleifera* seeds.

The principal component analysis (PCA) of our data confirms this biochemical correlation, with DPPH, TPC, TFC, and CT clustering in the same component, suggesting a shared role in defining antioxidant potential. Similar PCA results were presented by **(Gopalakrishnan *et al.*, 2016),** further confirming the chemical co-variation of these metabolites in *Moringa oleifera* under different environmental conditions.

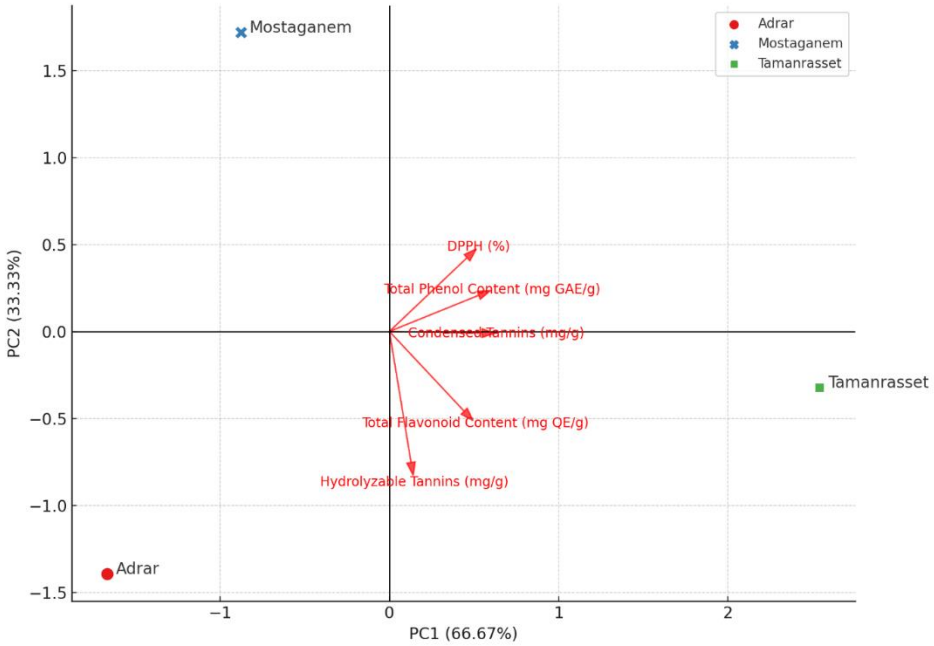

Figure 25: The principal component analysis (PCA), PC1/PC2 labels and % variance explained.

The principal component analysis (PCA) provided a robust visualization of the variance in antioxidant and phytochemical parameters of *Moringa oleifera* seeds collected from three Algerian regions: Adrar, Mostaganem, and Tamanrasset. The first two principal components

(PC1 and PC2) together explained 100% of the total variability which underscores the reliability of the biplot in distinguishing the samples based on their bioactive profiles.

PC1 was heavily loaded with total phenolic content (TPC), condensed tannins (CT), and DPPH antioxidant activity, with vectors aligned in the same direction and magnitude, reflecting a strong positive correlation. This association aligns with established literature highlighting phenolics and condensed tannins as major contributors to antioxidant potential. (**Liang *et al.*, 2019)** reported that the antioxidant capacity of *Moringa oleifera* seed extracts is significantly influenced by total phenol levels, while condensed tannins further enhance radical scavenging capacity due to their multiple hydroxyl groups that act as hydrogen donors.

Tamanrasset samples were positioned furthest along PC1, indicating their dominance in TPC (37.76 ± 2.22 mg GAE/g), CT (12.26 ± 0.77 mg/g), and DPPH activity (79.76 ± 0.92%). These values highlight this region as the most potent in antioxidant capacity, likely due to environmental adaptations that stimulate the biosynthesis of secondary metabolites under arid stress. **(Bennour *et al.*, 2021)** similarly found increased polyphenolic accumulation in *Moringa oleifera* leaves from harsh southern climates, reinforcing the role of the environment in modulating phytochemistry.

Adrar samples, on the opposite side of PC1, exhibited lower TPC and CT values, corresponding with the lowest DPPH activity (60.21 ± 0.19%). This suggests weaker antioxidant potential, potentially due to the extreme hyper-arid environment that may suppress polyphenol biosynthesis. **(Popoola and Obembe, 2013)** reported that intense environmental stress could either stimulate or limit secondary metabolite accumulation depending on the plant's tolerance threshold.

Mostaganem samples showed moderate loading along PC2, correlating more with total flavonoid content (TFC) and hydrolyzable tannins (HT). However, these variables were orthogonal to the DPPH vector, indicating a weaker relationship. Although flavonoids and hydrolyzable tannins contribute to antioxidant activity, their impact appears secondary compared to TPC and CT. **Shaban *et al.* (2020)** discussed this nuance, noting that not all flavonoids possess strong radical scavenging activity, as it depends on specific structural characteristics such as hydroxylation patterns and conjugation.

Furthermore, the PCA confirms that condensed tannins play a more dominant role than hydrolysable tannins in influencing antioxidant potential. **(Kumar *et al.*, 2022)** observed that condensed tannins contribute more significantly to antioxidant activities due to their higher

molecular weight and stronger electron-donating capacity compared to hydrolysable tannins, which are less stable under oxidative stress conditions.

The results collectively reinforce the notion that antioxidant capacity in *Moringa oleifera* seeds are primarily governed by total phenolics and condensed tannins, with regional environmental factors playing a critical role in shaping these bioactive profiles. This aligns with the findings of **(Nobossé *et al.*, 2018)** who established that solvent extracts rich in phenolics and condensed tannins from *Moringa* leaves and seeds exhibited the highest antioxidant scores across different assays.

Taken together, these results reinforce that Tamanrasset's *Moringa* seeds, rich in phenolics, flavonoids and condensed tannins, present the strongest antioxidant potential among the three regions. This is consistent with DPPH and ABTS assay outcomes in multiple studies that demonstrate a strong correlation between TPC/TFC and radical scavenging activity.

Finally, the rich phytochemical profile of Tamanrasset seeds supports their potential use in functional food and nutraceutical formulations, particularly for managing oxidative stress, cardiovascular health, and inflammatory conditions as reviewed by **(Fahey, 2005)** and **(Gopalakrishnan *et al.*, 2016).**

4.3.2. Anti-inflammatory activity

The anti-inflammatory activity of *Moringa* seeds is presented in figure 26. With moringa seeds from Mostaganem exhibiting the highest anti-inflammatory activity of (49.92 ± 2.22%), followed by Adrar and Tamanrasset with (49.26 ± 1.65%) and (41.25 ± 1.54%) respectively.

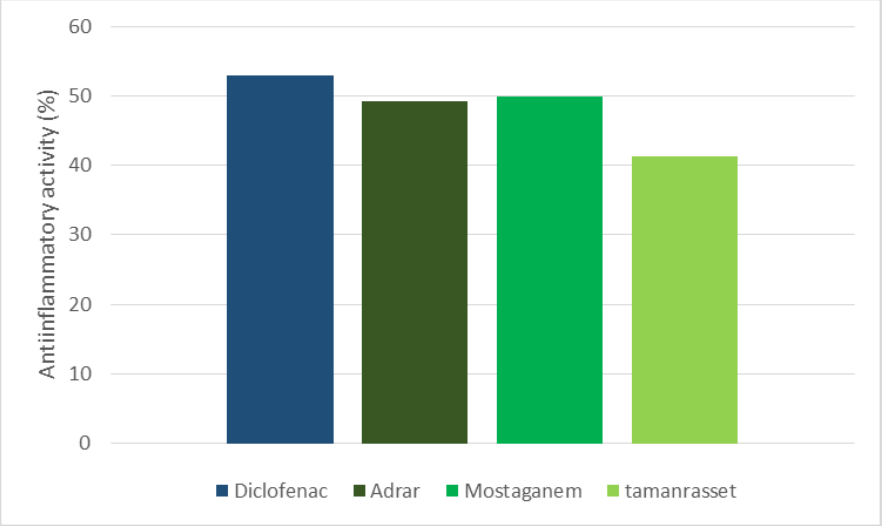

Figure 26: anti-inflammatory activity of Moringa oleifera seeds from the three regions

The anti-inflammatory activity of *Moringa oleifera* seed extracts from three Algerian regions demonstrated significant variability, as measured by protein denaturation inhibition. Tamanrasset seeds exhibited the lowest inhibition (41.25 ± 1.54%), while seeds from Adrar (49.26 ± 1.65%) and Mostaganem (49.92 ± 2.22%) had values statistically comparable to Diclofenac (52.96 ± 1.22%). This suggests that the anti-inflammatory potential of *Moringa* seeds from Adrar and Mostaganem is particularly promising and could be exploited as a natural alternative or complementary agent to conventional non-steroidal anti-inflammatory drugs (NSAIDs).

These findings align well with previous studies demonstrating the anti-inflammatory activity of *Moringa oleifera* seed extracts. **Xu *et al.,* (2019)** reported that *Moringa* extracts significantly inhibited nitric oxide (NO) production in LPS-stimulated macrophages, indicating their potential to suppress pro-inflammatory mediators. Similarly **Hamza (2010)** observed that *Moringa oleifera* seed extract ameliorated liver fibrosis and exerted significant anti-inflammatory effects in rats, linked to its phenolic and flavonoid content.

The relatively strong anti-inflammatory activity observed in Adrar and Mostaganem samples may be attributed to their higher contents of total phenolics (34.83–37.76 mg GAE/g), flavonoids (4.52–6.04 mg QE/g), and particularly condensed tannins (7.25–12.26 mg CE/g), which have been associated with anti-inflammatory responses **(Xu *et al.,* 2019; Almatrafi *et al.,* 2017).** These phenolic constituents are known to inhibit protein denaturation and modulate inflammatory pathways such as cyclooxygenase (COX) and NF-κB signalling (**Chumark *et al.,* 2008).** Moreover, hydrolyzable tannins and flavonoids present in higher levels in Tamanrasset and Adrar may exert additional effects by scavenging reactive oxygen species (ROS), which are key mediators of inflammation **(Xu *et al.,* 2019).**

The correlation observed between antioxidant markers (DPPH inhibition), and anti-inflammatory activity further reinforce the role of oxidative stress modulations in the inflammatory cascade. In this context, seeds from Tamanrasset, despite having the highest DPPH activity (79.76%), showed the lowest anti-inflammatory effect, suggesting that antioxidant capacity alone does not fully account for anti-inflammatory efficacy. This discrepancy implies that specific bioactive compounds, and not just total antioxidant activity, determine the modulation of inflammation.

Comparative literature also confirms regional variability in *Moringa* bioactivity. **Siddhuraju and Becker (2003)** showed that *Moringa* leaves from different agroclimatic regions vary

significantly in polyphenolic content and antioxidant activity, which could influence anti-inflammatory efficacy. Likewise **Gopalakrishnan *et al.* (2016)** highlighted the anti-inflammatory role of quercetin, kaempferol, and isothiocyanates present in *Moringa* seeds, compounds known to inhibit inflammatory mediators like TNF-α and IL-6.

4.3.3. Antibacterial activity

The results for the antimicrobial activity are demonstrated in figure 27. Staph aureus was observed to have a high sensitivity for all the extracts with Tamanrasset exhibiting the highest with (28.6 ± 0.4 mm) compared to the rest of the strains.

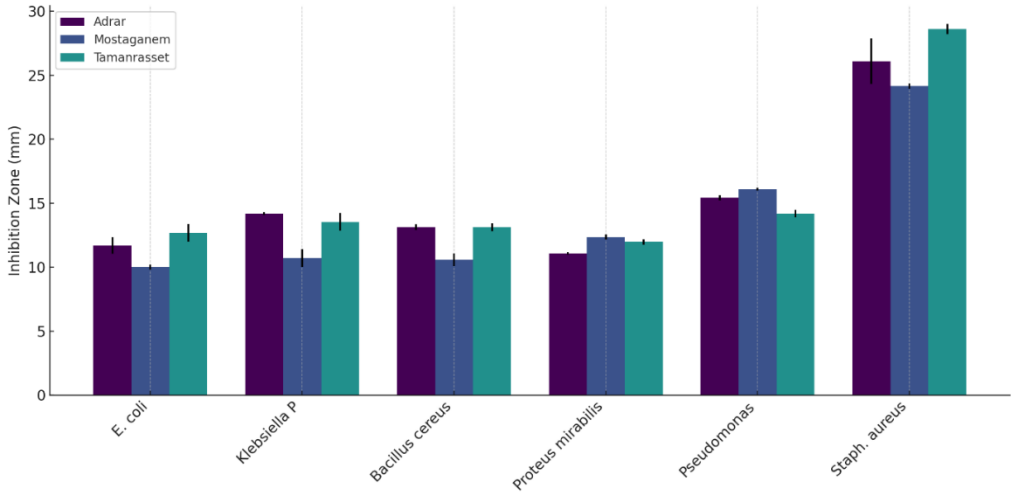

Figure 27: The antimicrobial activity of *Moringa oleifera* seeds

The antimicrobial activity of *Moringa oleifera* seed extracts varied notably among the three regions and across bacterial strains. The zone of inhibition measurements revealed that all extracts were particularly effective against *Staphylococcus aureus*, with the Tamanrasset extract showing the highest inhibition zone (28.6 ± 0.4 mm), followed by Adrar (26.11 ± 1.77 mm) and Mostaganem (24.17 ± 0.2 mm). This high sensitivity can be attributed to the Gram+ positive nature of *S. aureus*, which lacks the complex outer membrane that Gram-negative bacteria possess, making it more susceptible to the action of phenolic compounds and isothiocyanates present in *Moringa* seeds **(van den Berg & Kuipers, 2022; Anzano *et al.*, 2022).**

Conversely, the activity against Gram-negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* was generally lower, likely due to their lipopolysaccharide-rich outer membrane acting as a permeability barrier **(van den Berg & Kuipers, 2022).** However, *Pseudomonas aeruginosa* showed moderate susceptibility, particularly to Mostaganem's

extract (16.11 ± 0.1 mm), which could be related to differences in phytochemical composition, notably in total flavonoid and tannin contents **(Anzano *et al.,* 2022).**

Interestingly, *Bacillus cereus*, another Gram-positive bacterium, exhibited comparable sensitivity across all extracts (~13 mm), with slightly superior inhibition by the Adrar extract (13.14 ± 0.21 mm), suggesting a uniform efficacy of the antibacterial compounds in the seeds, particularly against spore-forming strains **(Salem *et al.,* 2021).**

The regional variation in activity may be correlated with differences in total phenolic and flavonoid content, as previously observed: Tamanrasset seeds displayed the highest phenolic and condensed tannin levels, which have been widely linked to antimicrobial efficacy due to their ability to disrupt microbial membranes, precipitate proteins, and chelate metal ions critical of microbial metabolism **(Anzano *et al.,* 2022; Shah *et al.,* 2024).**

These findings are in line with **(Flores *et al.,* 2021),** who demonstrated that *Moringa* seeds possessed strong antimicrobial activity against *S. aureus* and *E. coli*, with inhibition zones ranging between 12 and 25 mm. Likewise **Bichi *et al.* (2012)** confirmed that extraction method significantly affects antimicrobial efficacy, correlating activity with improved bioactive compound release.

Moreover, in a comparative study by **van den Berg & Kuipers (2022),** aqueous and methanolic extracts of *Moringa* seeds inhibited multiple pathogens, with activity more pronounced against *S. aureus* and *Bacillus subtilis*. The moderate activity against *Pseudomonas* observed here is noteworthy, as this pathogen is typically resistant to many herbal extracts, which align with the moderate but meaningful activity observed by **Anzano *et al. (*2022)** and **Salem *et al.* (2021).**

These data support the ethnopharmacological use of *Moringa oleifera* as a broad-spectrum antimicrobial, and they reinforce the importance of regional phytochemical profiling in selecting optimal seed sources for pharmaceutical or food preservation applications **(Shah *et al.,* 2024; Anzano *et al.,* 2022; Salem *et al.,* 2021).**

4.4. Conclusion

This study comparatively evaluated the influence of geographical regions and the environmental factors on the phytochemical composition, antioxidant, anti-inflammatory and antimicrobial activities of *Moringa oleifera* seeds harvested from three distinct regions of Algeria (Adrar, Mostaganem and Tamanrasset). The results demonstrated significant variations of the phytochemical and biological properties of *Moringa* across the three studied regions. Seeds from Tamanrasset showed the highest TPC, TFC and CTs values suggesting that, the harsh conditions like high UV exposure, poor soil fertility, and water scarcity may trigger abiotic stress of the plant which has been reported to directly influence the quantity of phytochemicals and hence their biological property. Contrary, the *moringa oleifera* seeds from Mostaganem and Adrar exhibited lower phytochemical composition which also affected their biological activity possibly due to the favorable environmental conditions and hence no abiotic stress to the *Moring*a plant.

These variations are crucial not only for optimizing nutritional and industrial applications but also for guiding conservation, domestication, and selective breeding programs for *Moringa* in North Africa and beyond. The aspect of regional phytochemical profiling is also important in selecting optimal seed sources for pharmaceutical or food preservation applications, potential use in functional food and nutraceutical formulations, particularly for managing oxidative stress, cardiovascular health, microbial and inflammatory conditions.

Despite its novelty and contributions, the study had some limitations like; only the seeds were used, only three regions were studied, soil analysis and the climate were not clearly analyzed, only in vitro studies were conducted. These limits the geographical region and further confirmation of the biological and phytochemical profiles.

These therefore lay a foundation for further studies into the relationship between the geographical, phytochemical profiles and biological properties of *Moringa* in Algeria and beyond. The proposed further studies may include, conducting in vivo studies to confirm the medicinal potential of *Moringa*, more regions with distinct geographical and climatic differences should be considered, different plant parts should be studied to compare the differences across the different parts of the plant, more extraction and analysis methods should be included to compare the differences and their effect on the medicinal properties of *Moringa* seeds.

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الجمهورية الجزائرية الديموقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة عبد الحميد بن باديس-مستغانم-

كلية علوم الطبيعة والحياة

تصريح شرفي خاص بالالتزام بقواعد النزاهة العلمية

لإنجاز البحث

أنا الممضي أدناه،

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عن : UGANDA GOVT KAMPALA

المسجل بكلية علوم الطبيعة والحياة / **قسم :** Biologie

شعبة : Sciences biologiques / **التخصص** : Biochimie appliqué

والمكلف بإنجاز مذكرة ماستر بعنوان :

Influence of geographical location and environmental factors on the phytochemical composition, antioxidant activity, anti-inflammatory activity and antibacterial activity of the hydroethanolic extracts of *Moringa oleifera lam.* Seeds from three distinct regions of Algeria.

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات العلمية والنزاهة الأك اديمية المطلوبة في إنجاز البحث ، وأتحمل المسؤولية الشخصية عن كل المحتوى المتضمن في ا لبحث المذكور أعلاه .

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إمضاء المعني

* ملحق القرار الوزاري رقم 933 المؤرخ في 28 جويلية 2016 الذي يحدد القواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.