الجمهورية الجزائرية الديمقراطية الشعبية وزارة التعليم
العالي والبحث العلمي

Setif-1 University Ferhat Abbas
Faculty of Nature and Life Sciences

جامعة سطيف-1 فرحات عباس
كلية علوم الطبيعة والحياة

DEPARTMENT OF BIOCHEMISTRY N°……/Bioch/SNV/2025

MEMORY

Presented by

BOUHARIZI Ouail Imad Eddine

DEROUICHE Mostefa

This thesis is for the purpose of obtaining for the degree of

MASTER

Field: Biological Sciences

Special filed: Applied Biochemistry

TOPIC

Phytochemical profiling, antioxidant, and anti-inflammatory activities of *Moringa oleifera* Lam.: an ethnopharmacological and experimental study

Presented publically in: **03/06/2025**

Jury:

Chair: CHAREF Noureddine	**Pr. Setif 1. UFA**
Supervisor: KARBAB Ahlem	**MCB. Setif 1. UFA**
Examiner: HARACHE Rabiaa	**MAB. Setif 1. UFA**

Laboratory of Applied Biochemistry
2024-2025

Acknowledgements

In the name of Allah, the most gracious and the most merciful

All praises to Allah, the almighty, to whom we owe our success and all the chances that madefrom us the persons we are to day

We would like to express our sincere gratitude for our supervisors

Pr. CHAREF Noureddine and **Dr. KARBAB Ahlem** for all his support and valuable advicethat helped us all along the realization of this important work, and for the precious knowledgethey shared with us.

We would like to express our sincere gratitude for our examiner **Dr. HARACHE Rabiaa** for accepted to jury our memory.

DEDICATION

قال الله تعالى:

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾ [المجادلة: 11]

Today, we celebrate not just an ending, but a new beginning

I would like to dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, ***Derouiche Mouloud*** *and* ***Derouiche Dalila*** *whose words of encouragement and push for tenacity ring in my ears.*

To my brothers ***Zaki*** *and* ***Amir*** *and especially to my sister* ***Soumia****.*

Without forgetting my binome, ***Bouharizi Ouail Imad Eddine****, for his moral support, his patience, and his understanding throughout this project*

Here's to the friends who became family and the professors who became mentors …

To all who believed in me, offered

DEDICATION

قال الله تعالى:

﴿يَرْفَعِ اللهُ الهذِينَ آمَنُوا مِنْكُمْ وَالهذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾]المجادلة: 11[

And so, the five-year journey has come to an end.

To you, dearest Mother Bouchaoui Nadjet may Allah have mercy on your soul.

I carried your dream in my heart, and by the grace of God, through perseverance, sleepless nights, and unwavering effort, I have made it real

To my Father Bouharizi Lafia may God preserve you.You are the one who shaped me with your sacrifices, who raised me with care and strength. This success is as much yours as it is mine.

Bouharizi Randa my sister, my second mother. in the times of darkness, you kept your candle burning for me, never letting the light fade. Thank you, and my gratitude extends to your husband as well. May God protect you both and bless your bond.

To my brother, Al-Bachir I'll simply say: my shield, my strength, my steady back in my teenage years. May God keep you and always make you my pillar of support.

To the youngest, my brother Achref I hope I have become a role model you can look up to.

I will never forget my classmate and study partner, Mostefa Derouiche Thank you for your understanding, your dedication, and your patience. Thank you for standing by me through all the stress and pressure. May the bond of brotherhood between us last forever.

To my dear friends Yasser and Aymen,

And to everyone I met at university both men and womenThank you for being such wonderful people in my life.

Ouail Imad Eddine

ملخص

تُعد .*Moringa oleifera* L من النباتات الطبية المستخدمة في الطب التقليدي لعلاج العديد من الأمراض. هدفت هذه الدراسة
إلى تقييم الجوانب الإثنوفارماكولوجية بالإضافة إلى النشاط المضاد للأكسدة والمضاد للالتهابات. تم إجراء مسح إثنوفارماكولوجي لجمع معلومات مفصلة حول
الاستخدامات التقليدية (55.4%) لأجزاء النبات المختلفة، الأمراض المعالجة، وطرق الاستخدام. إلى جانب توثيق المعارف التقليدية، تم إجراء تحاليل كيميائية
لمستخلص جاف من أوراق .*M. oleifera* L أظهرت نتائج تحليل
المركبات الثانوية مستويات مرتفعة من البوليفينولات والفلافونويدات والعفصات المثفلة، بقيم بلغت 59...2 ± 2..2
ميثروغرام مثافئ حمض التانيك، 4.24 ± 2..2 ميثروغرام مثافئ الثيرسيتين، و44.45 ± 4.44 ميثروغرام مثافئ الثاتيشين لكل غرام من المستخلص الجاف. في
اختبار النشاط المضاد للأكسدة باستخدام اختبارDPPH ، أظهرت النتائج قدرة عالية على تثبيط الجذور الحرة بقيمة IC_{50} تساوي .2.4 ± 2.24 ملغ/مل. كما
أظهرت الاختبارات المخبرية لقياس تثبيط تحلل البروتينات نشاطًا مضادًا للالتهاب يعتمد على الجرعة، حيث وصلت نسبة التثبيط إلى 5..2. ± 2.22% عند
تركيز . ملغ/مل. أما في الدراسة الحيوانية باستخدام وذمة الأذن المحفزة بالزيلين في الفئران، فقد تم تسجيل تثبيط كبير للالتهاب عند جرعة 2 ملغ/مل، حيث بلغت نسبة
التثبيط .5.2. %. وأخيرًا، أظهرت نتائج اختبار السمية الحادة أن المستخلص آمن حتى عند جرعات تصل إلى
2 غرام/كلغ من وزن الجسم. تدعم هذه النتائج الاستخدامات التقليدية لنبات *M. oleifera* علميًا وتبرز إمثانياته الواعدة كمصدر
طبيعي لمضادات الأكسدة والعوامل المضادة للالتهابات. وتُوصى بإجراء المزيد من الدراسات لعزل وتحديد المركبات النشطة المسؤولة عن هذه التأثيرات.

الكلمات المفتاحية :- *Moringa oleifera* Lam. ، دراسة إثنوفارماكولوجية، النشاط المضاد للأكسدة، النشاط المضاد
للالتهابات، البوليفينولات.

Abstract

Moringa oleifera Lam. is a medicinal plant used in traditional medicine to cure various ailments. This study aimed to evaluate the ethnopharmacological study, antioxidant and anti-inflammatory activities. An ethnopharmacological survey was conducted to gather detailed information regarding the traditional usage (55.4%) of different plant parts, diseases treated, and routes of administration. In addition to traditional knowledge documentation, phytochemical analyses of a dried extract (DE) of *M. oleifera* leaves were performed. Quantification of secondary metabolites revealed high levels of polyphenols, flavonoids and condensed tannins with a values of 255.79 ± 6.82 µg gallic acid equivalent, 4.64 ± 0.52 µg quercetin equivalent, and 41.47 ± 1.41 µg catechin equivalent dry extract, respectively. In antioxidant activity using the DPPH radical scavenging assay, the results demonstrated a potent free radical inhibition with an IC_{50} of 0.15 ± 0.01 mg/mL. *In vitro* assays measuring the inhibition of protein denaturation revealed dose-dependent anti-inflammatory activity of the extract, achieving 82.57 ± 0.00% inhibition at 8 mg/mL. *In vivo* study using xylene-induced ear edema in mice showed a significant inhibition of inflammation at a dose of 2 mg/mL, with inhibition percentages reaching 87.65%. Finally, the findings of the acute toxicity experiment demonstrated that extract is safe and that even at dosages as high as 2 g/kg DW. These findings provide strong scientific support for the traditional uses of *Moringa oleifera* and highlight its promising potential as a source of natural antioxidants and anti-inflammatory agents. Further investigation is warranted to isolate and characterize the specific active compounds responsible for these effects.

Key words: *Moringa oleifera* Lam., ethnopharmacological study, antioxidant activity, anti-inflammatory activity, polyphenols.

Resumé

Moringa oleifera L. est une plante médicinale utilisée en médecine traditionnelle pour traiter diverses affections. Cette étude avait pour objectif d'évaluer les données ethnopharmacologiques ainsi que les activités antioxydantes et anti-inflammatoires. Une enquête ethnopharmacologique a été menée afin de recueillir des informations détaillées sur l'utilisation traditionnelle (55.4%) des différentes parties de la plante, les maladies traitées et les voies d'administration. En plus de la documentation des connaissances traditionnelles, des analyses phytochimiques d'un extrait sec (ES) des feuilles de *M. oleifera* ont été réalisées. La quantification des métabolites secondaires a révélé des teneurs élevées en polyphénols, flavonoïdes et tanins condensés, avec des valeurs respectives de 255.79 ± 6.82 µg équivalent acide gallique, 4.64 ± 0.52 µg équivalent quercétine, et 41.47 ± 1.41 µg équivalent catéchine par gramme d'extrait sec. Lors de l'évaluation de l'activité antioxydante à l'aide du test de piégeage du radical DPPH, les résultats ont montré une puissante inhibition des radicaux libres avec un IC_{50} de 0.15 ± 0.01 mg/mL. Les essais in vitro mesurant l'inhibition de la dénaturation des protéines ont révélé une activité anti-inflammatoire dépendante de la dose, atteignant 82.57 ± 0.00% d'inhibition à 8 mg/mL. L'étude in vivo, utilisant un œdème de l'oreille induit par le xylène chez la souris, a montré une inhibition significative de l'inflammation à une dose de 2 mg/mL, avec des pourcentages d'inhibition atteignant 87.65%. Enfin, les résultats de l'étude de toxicité aiguë ont démontré que l'extrait est sûr, même à des doses aussi élevées que 2 g/kg de poids corporel. Ces résultats apportent un solide soutien scientifique à l'usage traditionnel de *Moringa oleifera* et mettent en lumière son potentiel prometteur en tant que source naturelle d'antioxydants et d'agents anti-inflammatoires. Des études complémentaires sont nécessaires pour isoler et caractériser les composés actifs responsables de ces effets.

Mots-clés: *Moringa oleifera* L., ethnopharmacological study, antioxidant activity, anti-inflammatory activity, polyphenoles.

List of abbreviations

CE: Catechin equivalent

DE : Dry extract.

DPPH: 1,1-diphényl 1-picrylhdrazyle.

DW: Dry weight.

GE: Gallic acid equivalent.

LD_{50}: Lethal dose

ME: Methanolic extract.

MO: *Moringa olifeira*

QE: Quercetin equivalent.

SD: Standard deviation.

SEM: Standard error of mean.

TFC: Total flavonoids content.

TPC: Total phenolics content.

IC_{50}: Half maximum inhibitory concentration.

List of figures

List of tables

Table of contents

Introduction

1 Introduction

Plants have supported human life for centuries not just by providing food, but also by offering healing through their medicinal properties. Today, there's a growing interest in studying plants and their natural compounds, especially because many traditional remedies are still used in local communities and may offer new ways to treat modern diseases (Malheiros *et al*, 2017). Thanks to scientific and technological advancements, researchers are now able to better understand how these plants work, particularly their potential to reduce inflammation and improve health (Malheiros *et al*, 2017; Zhu *et al*, 2018).

Medicinal plants are especially valuable because they often contain antioxidants and other bioactive compounds that can help prevent or manage chronic conditions that are difficult to cure (Do et al., 2014). Exploring these plants is not just about learning what's inside them it's the first step toward creating new natural treatments that may be safer and more accessible (Singh *et al*, 2017).

Many of these natural substances, whether used as pure compounds or in plant extracts, have been shown to help control inflammation, which plays a role in countless health problems (Zhang *et al*, 2019). For generations, people have turned to nature to relieve symptoms like pain, fever, and joint issues. These remedies often work by blocking or slowing down the body's inflammatory responses (Yuan *et al*, 2006).

Natural anti-inflammatory compounds like quercetin, allicin, terpenes, polyphenols, and flavonoids are now being studied more closely for how they might help with inflammation in the skin, joints, heart, lungs, brain, and digestive system (Maione *et al*, 2016; Howes, 2018). While the benefits of medicinal plants are promising, we still don't fully understand their potential risks especially when it comes to their toxic effects (Mordenis, 2019). That's why continued research is essential, not only to unlock their healing potential but also to ensure they're safe to use.

A medicinal plant is a plant whose organs (such as leaves, bark, or fruits) possess healing properties due to their active compounds intended to produce a pharmacological effect (Chevallier, 2001). For centuries, medicinal plants have been used as remedies for human diseases because they contain components with therapeutic value (Nostro *et al.*, 2000).

Introduction

Medicinal plants play a crucial role in pharmacological research and drug development. Their constituents are used directly as therapeutic agents and serve as raw materials for drug synthesis or as models for pharmacologically active compounds (Ameenah, 2006). However, the uncontrolled use of medicinal plants can lead to severe, sometimes fatal, intoxications (Fouché *et al.*, 2000).

The aim of this study was to evaluate the pharmacological potential and safety profile of Moringa oleifera through a comprehensive investigation combining ethnopharmacological data and experimental analyses. Specifically, the study sought to :

1. Document the traditional uses of Moringa oleifera through an ethnopharmacological survey.
2. Quantify key phytochemical constituents, including total polyphenols, flavonoids, and condensed tannins.
3. Assess the *in vitro* antioxidant activity of the plant extract using standard radical scavenging assays.
4. Evaluate both *in vitro* and *in vivo* anti-inflammatory activities to determine its therapeutic relevance in inflammation-related conditions :
5. Perform acute toxicity test to establish the safety margin and potential toxicological effects of the extract.

This integrative approach aims to scientifically validate traditional uses of *Moringa oleifera*, contribute to its pharmacological characterization, and support its potential development as a natural therapeutic agent.

Review

Of

Literature

2 Phytotherapy

The term phytotherapy originates from Greek and is composed of two words: "Phyto," meaning plant, and "therapy," meaning treatment. Together, they refer to treatment through plants (Baba Aissa, 2000). Phytotherapy is the study and use of medicinal plants for therapeutic purposes. It remains one of the oldest forms of disease treatment, relying on centuries of observation, which modern analysis continues to validate (Beloud, 2001).

3 Biological activities

3.1 Anti-inflammatory

In vivo anti-inflammatory test is a scientific method used to evaluate the effectiveness of substances such as plant extracts or synthetic drugs in reducing inflammation within a living organism, typically animal models like rats or mice. These tests are crucial for understanding how potential therapeutic agents perform in complex biological systems. *In vivo* anti-inflammatory assays involve inducing an inflammatory response in an animal and then administering the test substance to observe its effects on inflammation. Plant-derived compounds: Curcumin, Quercetin, Resveratrol, Boswellic acids. Examples of plants: Turmeric (*Curcuma longa*), Grapes (*Vitis vinifera*), Boswellia tree (*Boswellia serrata*), (*Moringa olifeira).* Many natural compounds exhibit anti-inflammatory activity. For example, curcumin from turmeric inhibits IKK/NF-κB signaling and cytokine production. Flavonoids (quercetin, catechins) and terpenoids (boswellic acids, resveratrol) suppress inflammatory enzymes (COX, LOX) and cytokines. Fatty acid–derived mediators (resolvins, protectins from omega-3 oils) actively promote resolution of inflammation. These natural anti-inflammatory agents are being studied for their therapeutic potential.

3.2 Anti-oxidant activity

Antioxidant activity is the ability to neutralize reactive oxygen/nitrogen species (ROS/RNS) and prevent oxidative damage. ROS such as superoxide, hydroxyl radical) are highly reactive molecules from normal metabolism; in excess they damage lipids, proteins and DNA. Antioxidants maintain redox balance by scavenging ROS or enhancing their breakdown. Plant-derived compounds: Flavonoids (Quercetin, Catechins), Carotenoids, Polyphenols

Examples of plants: Green tea (*Camellia sinensis*), Blueberries *(Vaccinium spp*), Carrots (*Daucus carota*).

3.3 Antiviral activity

Antiviral activity encompasses agents that inhibit viral infection, replication or spread. Innate antiviral defences include interferons and restriction factors, while therapeutic antivirals target viral enzymes. Interferons (IFNs): Type I IFNs (IFN-α/β) and type III IFN (IFN-λ) are cytokines produced by host cells in response to viral infection, and they "mediate antiviral activity" by inducing hundreds of antiviral proteins. Plant-derived compounds: Epigallocatechin gallate (EGCG), Glycyrrhizin, Baicalin. Examples of plants: Green tea (*Camellia sinensis*), Licorice root (*Glycyrrhiza glabra*), Skullcap (*Scutellaria baicalensis*)

3.4 Antimicrobial activity

Antimicrobial activity refers to the ability of a substance to kill or inhibit microorganisms such as bacteria, fungi, and protozoa, thereby preventing infection. Plant-derived compounds: Allicin, Eugenol, Berberine, Thymol and Cinnamaldehyde, Examples of plants: Garlic (*Allium sativum*), Clove (*Syzygium aromaticum*), Goldenseal (*Hydrastis canadensis*), Thyme (*Thymus vulgaris*), Cinnamon (*Cinnamomum verum*).

3.5 Analgesic activity

Analgesic activity refers to pain relief. Analgesics are drugs that alleviate pain without causing loss of consciousness. They act on different pathways of pain signaling. NSAIDs: Aspirin, ibuprofen and related drugs inhibit COX enzymes (reducing prostaglandin-mediated pain/inflammation). NSAIDs "decrease pain" (and lower fever) and at higher doses reduce inflammation. Plant-derived compounds: Salicin, Capsaicin, Menthol Examples of plants: Willow bark (*Salix spp*.), Chili peppers (*Capsicum spp*.), Peppermint (*Mentha piperita*)

3.6 Anticancer activity

Anticancer activity involves killing or inhibiting the growth of malignant (tumor) cells. Such agents typically induce apoptosis, DNA damage, or inhibit proliferation of cancer cells. Plant-derived compounds: Curcumin, Vincristine, Camptothecin, Taxol (Paclitaxel), Examples of plants: Turmeric (Curcuma longa), Madagascar periwinkle (Catharanthus roseus), Camptotheca (Camptotheca acuminata), Pacific yew (Taxus brevifolia).

4 Toxicity test

A toxicity test is a scientific procedure used to assess the adverse effect s of substances such as chemicals, pharmaceuticals, or environmental pollutants on living organisms. These tests are crucial for determining the potential risks associated with exposure to various substances, ensuring safety for humans, animals, and the environment. Toxicity testing involves exposing biological systems (cells, tissues, or whole organisms) to a substance and observing for harmful effects. The primary objectives are to :

- Identify potential health hazards.
- Determine safe exposure levels.
- Understand dose-response relationships.
- Comply with regulatory safety assessments**.**

These tests can be conducted in various ways, including:

- *In vitro*: Using cultured cells or tissues to assess cellular responses.
- *In vivo*: Using whole organisms (rodents, fish) to observe systemic effects.
- *In silico*: Employing computational models to predict toxicity based on chemical structure and known data.

5 *Moringa oleifera* Lam.

According to Jed et al. (2005), Moringa oleifera leaves are used as a medicinal and nutritional plant. The addition of M. oleifera leaf powder to human diets has been shown to be beneficial. A number of antinutritional factors have also been identified in leaves (tannins, phenolic compounds, etc.), which have antinutritional effects as a general introduction due to their action on proteins and polysaccharides, which impairs their absorption by the body and inhibits the activity of digestive enzymes (Gonçalves et al., 2010).

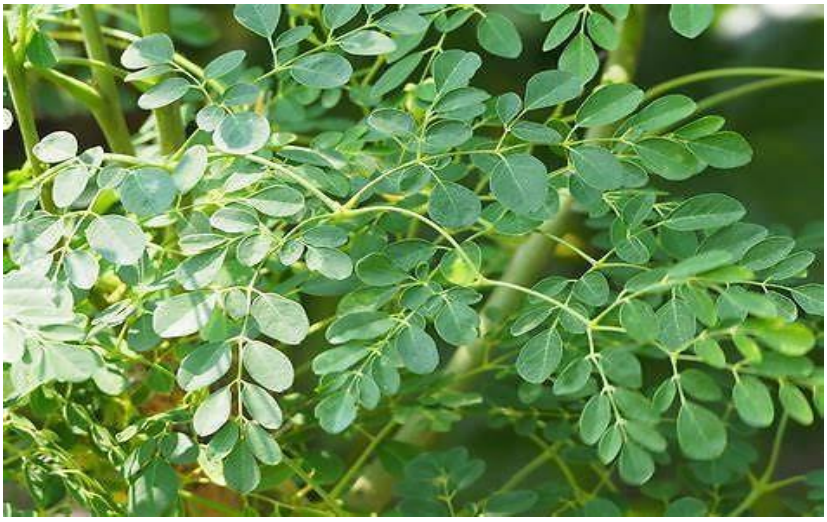

Figure 4*: Moringa olifeira* Lam *leaves*

5.1 Botanical classification

The following is the botanical classification of *Moringa oleifera :*

- **Kingdom:** Plantae
- **Superdivision** : Spermatophyta
- **Division** : Magnoliophyta
- **Class** : Magnoliopsida
- **Subclass** : Dilleniidae
- **Order** : Capparales
- **Family** : Moringaceae

5.2 Geographical distribution

Global Distribution of *Moringa oleifera* originally native to the Indian subcontinent, this plant has been introduced and cultivated in numerous regions worldwide

- **Africa**: Extensively grown in countries such as Kenya, Tanzania, Uganda, Ethiopia, Sudan, Nigeria, Ghana, and Senegal.
- **Asia**: Cultivated in nations including India, Pakistan, Bangladesh, Nepal, Sri Lanka, the Philippines, and Indonesia.
- **Americas:** Found in parts of Central America, the Caribbean, and northern regions of South America.
- **Oceania**: Grown in areas like Fiji, New Caledonia, and Vanuatu.
- **United States**: Cultivation has been initiated in states like Florida, Arizona, and California.
- Moringa thrives in semiarid, tropical, and subtropical climates, particularly in well-drained sandy or loamy soils. It is drought-resistant and does not tolerate freezing temperatures.

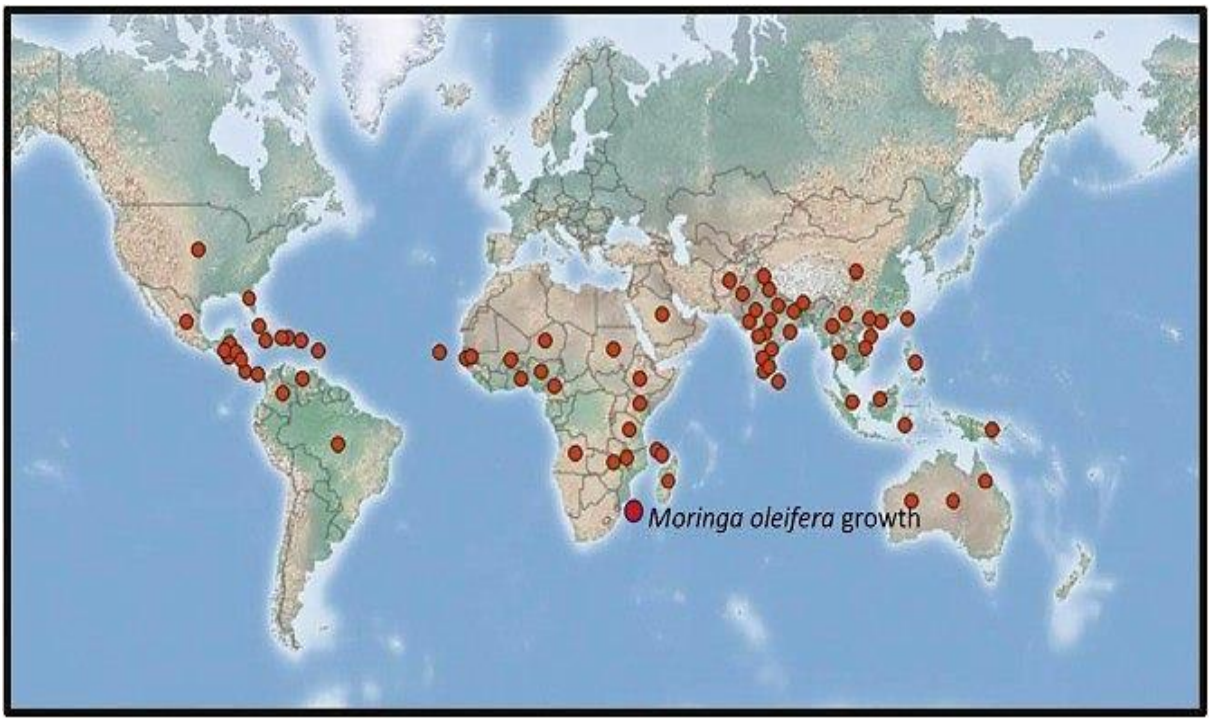

Figure 5*:* Represente the geograpic distribution of *M. olifeira (*Bhattacharya *et al., 2019)*

5.3 Traditional uses of *Moringa oleifera*

5.3.1 Nutritional supplement

Moringa leaves are highly nutritious, containing vitamins, minerals, and antioxidants. In many traditional systems of medicine, moringa leaves are consumed as a dietary supplement to boost overall health and well-being.

5.3.2 Treatment of digestive disorders

Traditional medicine systems across different regions have utilized moringa for treating various ailments, ranging from digestive disorders and skin conditions to inflammatory diseases and infectious illnesses.

5.3.3 Management of diabetes and blood sugar control

In Asia, it is commonly used to treat diabetes and high blood pressure. moringaoleifera.org

5.3.4 Anti-inflammatory and pain relief roperties

Moringa olifeira is renowned for its rich nutritional content, including vitamins, minerals, proteins, and antioxidants, which contribute to its diverse health-promoting effects.

5.3.5 Treatment of skin conditions

Moringa oleifera is a perennial plant broadly used in South Asia and Africa as a traditional folk medicine to treat many ailments such as paralysis, helminthiasis, sores, and skin infections.

5.3.6 Water Purification

For thousands of years, ground Moringa seeds have been used to purify dirty water

5.4 *Moringa oleifera* in Algeria

In Algeria, *Moringa oleifera* has been introduced primarily in the arid and semi-arid southern regions, where its cultivation is being explored for both nutritional and medicinal purposes:

- Bechar Province: Research conducted in the Tabelbala area has demonstrated that Moringa leaves possess significant antioxidant and antimicrobial activities. Extracts have shown effectiveness against various pathogenic microbes, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

- Ghardaïa Region: Studies have focused on optimizing germination techniques for Moringa seeds. Methods such as seed scarification and soaking have been evaluated to improve germination rates, indicating the plant's adaptability to the local climate.
- Southwestern Algeria (Oued Bechar): Investigations into the antibacterial properties of Moringa seed extracts have revealed their potential in combating drug-resistant pathogens found in wastewater, highlighting the plant's environmental and health benefits.

These studies underscore the potential of Moringa oleifera as a valuable resource in Algeria's efforts to promote sustainable agriculture and address health challenges in arid regions.

5.5 Toxicity

During the gestational period, *Moringa olifeira* may cause abortion, but not at doses under 30 mg/kg. MO alleviates postpartum depression in mice and has galactagogue effect Postpartum depression negatively impacts the offspring's body weight, but this effect is remedied by MO. Postpartum depression causes an increase in aggression and a lack of maternal care in mice. MO impacts gestational parameters at high doses.

5.6 Previous research studies on *Moringa oleifera*

The previous research studies on *Moringa oleifera* showed:

- **Chand Raza *et collaborators* (2024),** present the *in vivo* study using methanolic and ethanolic seed extracts. The mice underwent six behavioral tests (open field test, stepping test, pole climb test, stride length test, beam walk test, tail suspension test) to evaluate Parkinson's-like motor symptoms. The baseline behavior was similar across groups before treatment. ToxTrac analysis showed higher locomotor activity in control mice compared to rotenone-treated ones.

- **Sharad Bissa et collaborators (2024),** used Soxhlet extracts of leaves, flowers, pods, and seeds screened against eight pathogenic bacterial strains.The petroleum ether extract of leaves showed the highest activity against *E. coli, B. subtilis, and P. putida.*

- **Ahmed et collaborators (2021),** nvestigated ethanolic and water extracts of leaves using the DPPH radical scavenging test. Results showed antioxidant activity with a significant presence of polyphenolic compounds. Traditional uses: Nutritional, medicinal, and other health benefits.

- **Wiwit Denny Fitriana et collaborators (2016),** used solvent extracts from leaves, analyzed using DPPH and ABTS radical scavenging assays. Found that natural antioxidants from Moringa can enhance the body's system for scavenging free radicals.

- **Charlette Tiloke et collaborators (2013),** applied aqueous leaf extract (166.7 μg/mL) on cancerous human alveolar epithelial cells. Oxidative stress was evaluated using TBARS, glutathione assays, comet assay, and caspase 3/7 and 9 activities. A significant increase in ROS and DNA damage, with decreased glutathione levels, was observed.

- **Sangeeta Chandrashekar et collaborators (2020),** extracted bioactive peptides from seeds using ethanol delipidation, centrifugation, and 5% chloroform extract preparation. Found higher protein concentration in crude extracts and purified coagulant protein induced cell aggregation in six bacterial strains.

- **Kokoette Bassey *et collaborators* (2022),** sequential solvent extraction of seeds using hexane, dichloromethane, methanol, acetone, and water. The hexane extract yielded the highest mass (24.04%). The study identified Moringa seed oil as nutritionally rich.

MATERIALS AND METHOD

6 Materials

6.1 Plant material

Dried leaves of *Moringa oleifera* were obtained from a local herbalist, leaves were identified based on morphological characteristics and cleaned to remove dust and foreign particles, after that they were ground into a fine powder using a mechanical grinder and stored in containers at room temperature until further use.

Figure 6: Dried *Moringa olifeira* leaves

6.2 Animals

Mice weighing 30-42 g were purchased from institut pasteur d'Algerie. The animals were acclimatized in the pet shop at a tempurature of 25-27°C, relative humidity of 50-62%, and light/dark cycles of 12h prior to the start of the expirements. All animal experimental protocols performed in this study were approved by the ethics committe of the Algerian association of sciences in animal. Experimentation under Law No 88-08/1988, which describes guidelines for veterinary medical for veterinary medical for veterinary medical for for veterinary medical for veterinary medical activities and animal health protection (N° JORA : 004/1988)

6.3 Ethnopharmacological survey

In order to identify and establish a list of plants used in traditional medicine due to their pharmacological properties, an ethnobotanical survey was conducted according to Karbab et al. (2021) with few modifications. The current study was carried out for information about

different parts, treated diseases and route of applications of *M. olifeira* according to the traditional medicine due to their constituents compounds used in pharmacological. The plant *M. olifeira leaves* was purchased from a trusted herbalist.

6.4 Questionnaire and sampling

The present work aims to organize such overall information on medicinal plants along to the differents parts *Moringa oleifera* and its relation with therapeutic usefulness in 21 centry traditional medicine. All the participants (villagers civilians and university students and teachers, traditional healers and herbalists) were native to the study area. A total of 101 informants (40 males and 61 female) of different ages which ranged between 20 to 65 years old participated in this survey. In addition, all citizens in urban and farming areas were questioned about their knowledge on *Moringa oleifera*. We used a website to collect all the infermations drive. This is some of our result shaped on charts.

7 Methods

7.1 Extraction

Approximately 300 g of fine powder of the *Moringa oleifera* leaves was employed for the extraction.The powdered plant material was first macerated in petroleum ether for days at room tempurature with occasional stirring, then the filtered extract was evaporated and dried in an oven to remove all the solvant.

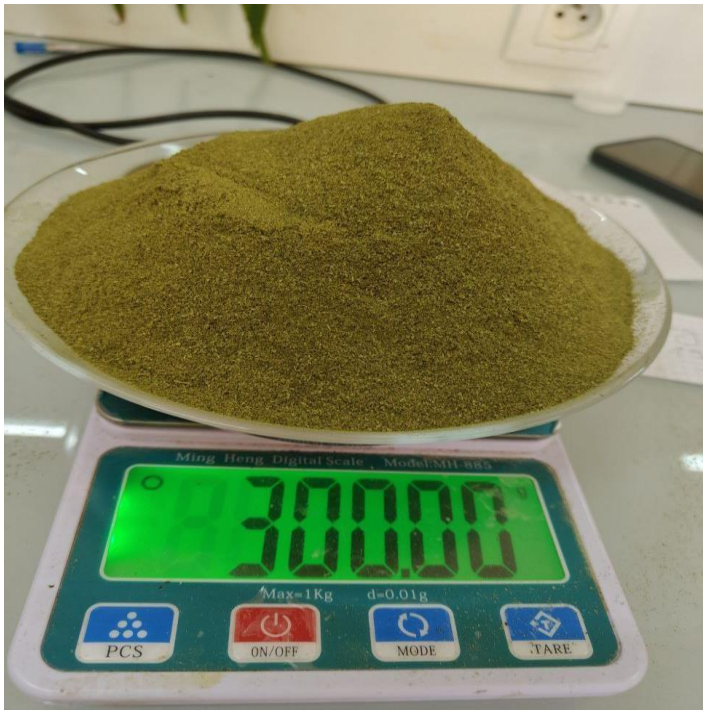

Figure 7: Represente fine pouder of *Moringa olifeira* leaves used in maceration

The sediment was immersed in methanol for days. At this time,a series of freezing at 4c° and agitation were conducted alternating daily to improve extraction efficiency. The mixture was then filtered through filter paper and the methanolic extract obtained was put in the oven to evaporate the the solvant (methanol) completely. The resulting brut extract was collected and stored at 4°C for future use.

Figure 8*:* represente the ethanolic extract before and after collection

7.2 Quantitative phytochemical analysis

7.2.1 Determination of total polyphenols

The extract's total phenolic content was evaluated using the Folin-Ciocalteu technique (Karbab *et al*., 2020). To begin, 100 µL of extract was combined with 1 mL of Folin-Ciocalteu's reagent for 4 minutes before adding 400 µL of 7.5% aqueous Na_2CO_3. The solution's absorbance was measured at 765 nm after 2 hours of incubation. Polyphenolic content was measured as µg gallic acid equivalent (GE) per mg dried extract (DE). Total polyphenols in extracts were measured using a gallic acid standard curve.

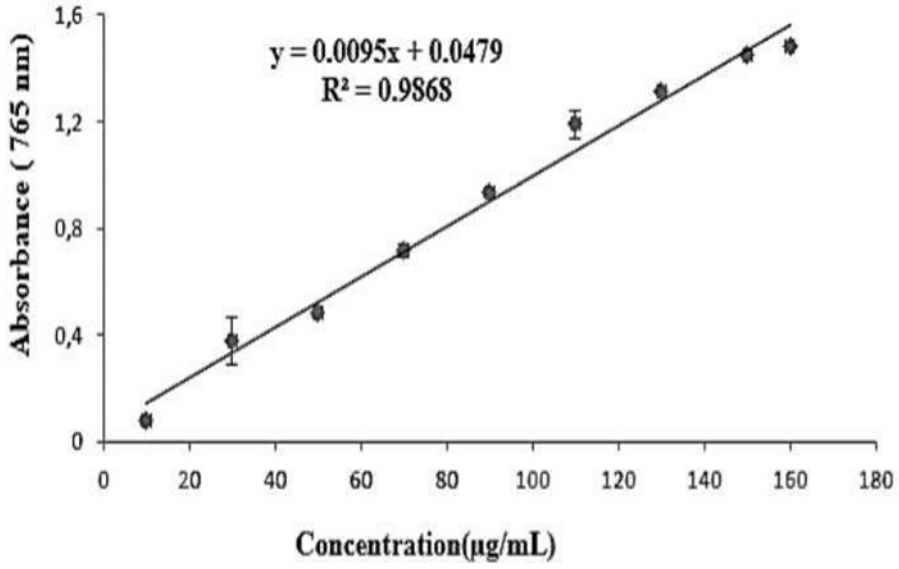

Figure 9:Standard curve of gallic acid for determination of total polyphenols.

7.2.2 Determination of total flavonoids

The total flavonoid content was determined using the aluminum chloride technique (Karbab *et al*., 2020). This approach involved adding 500 µL of extract to 1 mL of 2% aluminum chloride solution. After 10 minutes of incubation, the mixture's absorbance at 430 nm was measured in comparison to a methanol blank. Total flavonoids were reported as µg of quercetin equivalent (QE) per mg dried extract (DE). The amount of total flavonoids in extracts was evaluated using a standard curve of quercetine.

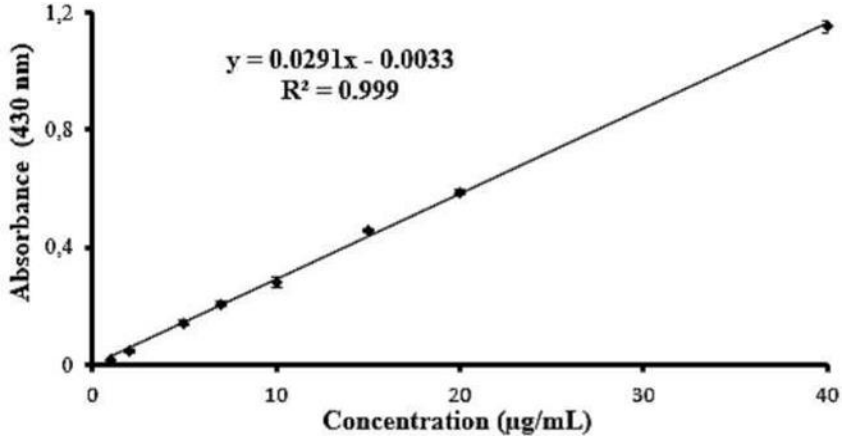

Figure 10:standard curve of quercetine for determination of total flavonoids

7.2.3 Determination of tannins

When condensed tanins react with vanillin after being linked to phenol rings, they tend to generate a red-colored complex (Nagy *et al*, 2021; Schofiel *et al*., 2001) The measurement

of condensed tannins was carried out as described in Amari *et al*, (2023). We added 375µL of 4% vanilin solution to 250 µL of our extract. The mixture was then incubated for 20 minutes at ambient temperature. The absorbances were measured at 500nm from the standard curve of various catechin concentrations. The quantity of condensed tannins can be determined.

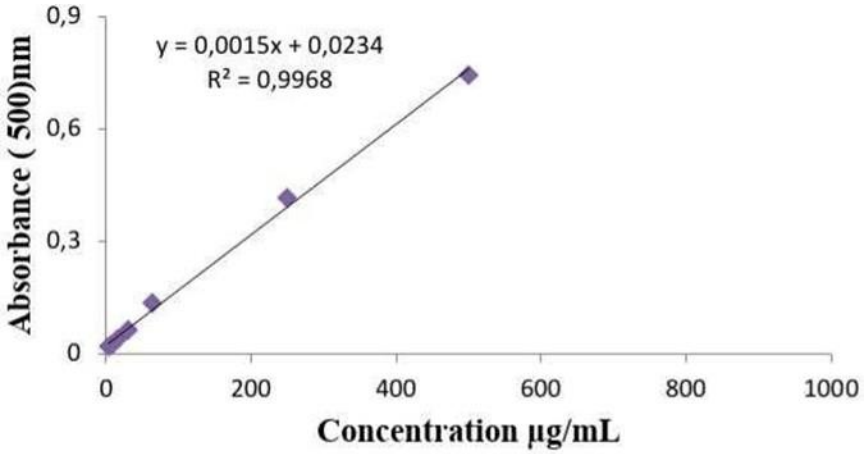

Figure 11:standard curve of catechin for detection of condensed tannins.

7.3 Bioactivity Screening

7.3.1 In vitro antioxidant activity (DPPH Radical-scavenging assay)

Using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, the extracts' ability to scavenge free radicals was evaluated by detecting the drop in the DPPH maximum absorbance at 517 nm (Charef *et al*, 2015). In this procedure, 675 µL of 4% DPPH was combined with 50 µL of different extract/standard concentrations. After the sample was incubated for 30 minutes at room temperature in the dark, its absorbance was measured at 517 nm. Positive control, butylated hydroxytoluene (BHT), was employed. Equation (1) was utilized in the computation of the scavenging capacity.

$$I\% = (Ablank - Atest /Ablank) \times 100. \quad (1)$$

Where Ablank = absorbance of the solution except the tested sample, and Atest = absorbance of the tested sample

7.3.2 Anti-inflammatory activity

7.3.2.1 *In vitro* evaluation of the anti-inflammatory activity

The effect of the *Moringa olifeira* Lam. leaves on protein denaturation was used to assess their anti-inflammatory potential. This procedure was modified from the Akkouche *et al*. (2012) approach. After being cleansed and washed, eggs were broken. After the white and

yolk separated, the chalazae were removed as needed. Using a test tube, the egg white volume was measured. The buffer solution Tris-HCl (20 mM, pH 6.87) was then added to generate a diluted solution of 1:100. After agitating the liquid for ten minutes, a strip agase was used for filtering. Two milliliters of 1% egg white suspension, two milliliters of the extract, or four milliliters of aspirin as a standard (4 and 16mg) was combined, and it spent 15 minutes being incubated in a water bath at 70 °C. At 650 nm, absorbance was measured following cooling. The following equation was used to compute the percentage inhibition of protein denaturation:

$$\% \text{ inhibition of denaturation} = 100 \times (AC - AS)/AC \quad (2)$$

where AC = absorption of the control sample, and AS = absorption of the test sample.

7.3.2.2 In vivo investigations of the anti-inflammatory activity

7.3.2.2.1 Xylene-induced ear edema

Using the protocol described by Delaporte *et al.*, (2004), the anti-inflammatory properties of *Moringa olifeira* extract were examined in mice with xylene-induced ear edema. Six mice were separated into a group.To generate edema in mice, 30 µL/ear of xylene was applied topically then the extract. Ear thickness was measured with a digital caliper before and 2 hours after the application. The inhibition percentage of ear edema was calculated using Equation (3).

$$\text{Inhibition percentage } (\%) = 100 \times (Dn–D)/\ Dn, \quad (3)$$

where D = the difference of ear edema thickness in the treated group and Dn = the difference of ear edema thickness in the negative group.

7.4 Acute toxicity study

Three mice were given our extract at a dose of 2000 mg/kg. For 15 days, we suppressed their behavior and harmful symptoms. Mice's ability to survive suggests a non-toxic effect. With few adjustments, we used the (Ugwah-Oguejiofor *et al.*, 2019) approach.

7.5 Statistical analysis

Data were tested for significance using the student's t-test *in vitro*. The data were expressed as the mean ± standard deviation (SD) and the determinations were made in triplicate. To analyze the data from this inquiry, we used GraphPad Prism-5 when $p < 0.05$, differences were deemed significant.

RESULTS

AND

DISCUSSION

8 RESULTS AND DISSCUSION

8.1 Extractions and yield profile

The methanolic extraction of *Moringa oleifera* leaves by maceration yielded 4.32% (w/w), which is within the expected range for polar solvents extracting secondary metabolites such as polyphenols and flavonoids. Methanol is widely recognized for its efficiency in extracting a broad spectrum of phytoconstituents due to its polarity, and the current yield supports previous findings suggesting its effectiveness in isolating bioactive compounds from *Moringa* species (Anwar *et al*., 2007).

8.2 Total polyphenols, flavonoïds and condensed tannins contents

Quantitative tests were performed to determine the concentration of the phytochemicals in the extracts. Polyphenols, flavonoids and were quantified using spectrometric methods. The total phenolic contents (TPC), total flavonoids contents (TFC) condensed tanins of *Moringa olifeira* leaves were evaluated employing the Folin-Ciocalteu reagent, aluminum chloride, acid butanol-HCl assay. Results are 255.79 ±6.82 µg gallic acid equivalent (GE)/ mg DE (dried extract) and 4.64 ± 0.51 µg quercetin equivalent (QE)/ mg DE and 41.47 ± 1.41 mg catechin equivalents (CE)/g dry weight compared to Ankita Singh et al., (2024) they found that 18.32 ± 0.17mg/ml for the TPC content and 45.1± 0.27mg/ml for the TFC and 29.76 ± 0.97mg/ml for the tanins content. This variety may be explained by the difference in extraction protocols, plant maturity, geographical origin, and methodological differences. Overall, while our results confirm the presence of these important phytochemicals in *M. oleifera*, the variation across studies highlights the importance of standardizing extraction and quantification protocols to allow for more accurate comparisons.

Figure 12: Total polyphenols quantity test

8.3 *In vitro* antioxidant effect

The method used is DPPH Radical-scavenging assay many plants extracts exhibit efficient antioxidant properties due to their phyto-constituents, including phenolic acids and flavonoids (Barriada-Bernal *et al*, 2013). In the present study, reduction of DPPH• radicals can be significantly observed at 517 nm by the leaves extracts (LE) of *Moringa Olifeira* measured by DPPH• method the free radical scavenging activity (IC_{50}) of the leave extracts (LE). The free radical scavenging activity of methanolic extract (ME) the were showing an IC_{50}=0.15 ±0.01 mg/ml, This low IC_{50} indicates a high radical scavenging efficiency, comparable to standard antioxydants ascorbic acid,compared to (Wiwit Denny Fitriana, Taslim Ersam1, Kuniyoshi Shimizu, and Sri Fatmawati) they found IC_{50}= 49.30 µg/mL in DPPH assay. The difference in activity may be explained by the different solvants used in extraction.Methanol generally extracts a broader range of polar bioactive compounds compared to ethanol this sugsests that the methanol is more effective to isolate the bioactive compounds that are responsible on the antioxydante activity. However difference in extraction protocols, and assay conditions must also be considered when interpreting these results.

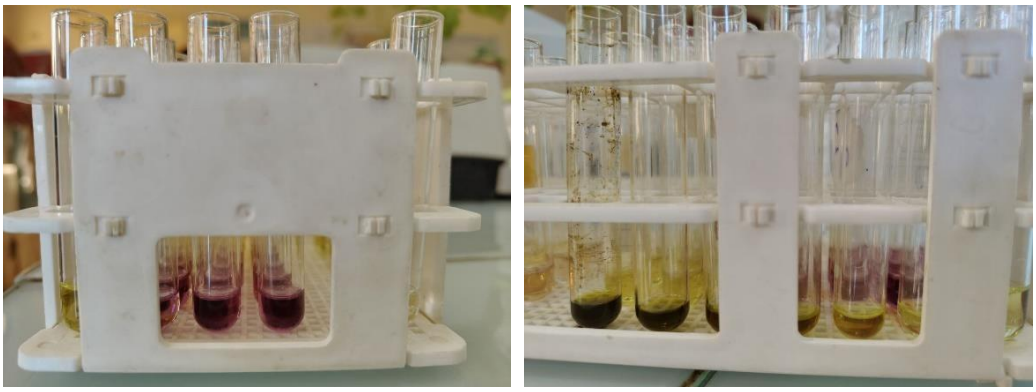

Figure 13:DPPH Radical-scavenging assay

8.4 Anti-inflammatory activity

8.4.1 *In vitro* evaluation of the anti-inflammatory activity

The anti-inflammatory activity of the plant extract was evaluated in vitro using the protein denaturation inhibition assay. This method assesses the extract's ability to inhibit heat-induced denaturation of egg proteins a key indicator of anti-inflammatory potential. The percentage inhibition of protein denaturation was measured at three different concentrations of the extract (2, 4, and 8 mg/mL) and compared to aspirin, tested at concentrations of 4,

08, and 16 mg/mL. All assays were performed in triplicate, and results are expressed as mean ± standard deviation (SD).

Table 1: *In vitro* anti-inflammatory activity.

Sample	Concentration (mg/mL)	% Inhibition (Mean ± SD)
Leaves extract	2	28.19 ± 1.32
	4	38.67 ± 2.15***
	8	82.57 ± 0.00ns
Aspirin	4	86.02 ± 5.91
	8	93.87 ± 0.31

8.4.2 *In vivo* anti-inflammatory activity

The anti-inflammatory activity of *Moringa oleifera* extract (2 mg/ear) was assessed using the xylene-induced ear edema model in mice and compared to the standard anti-inflammatory drug, indomethacin (0.5 mg/ear). The percentage inhibition of ear edema was calculated for each mouse, the results showed significant inhibition of inflammation with inhibition percentages reaching 87.65%, comparable to that of indomethacin (69.13–100% inhibition). The anti-inflammatory effect observed *in vivo* and *in vitro* may be attributed to the presence of phytochemicals such as flavonoids, tannins, and phenolic compounds, which have been reported to inhibit the release of inflammatory mediators. In conclusion, the significant inhibition of xylene-induced ear edema by *M. olifeira* extract supports its traditional use in treating inflammation and suggests its potential as a natural anti-inflammatory agent. Future studies should focus on isolating the active compounds and evaluating their specific mechanisms of action. In comparison with previous studies (Nworu *et al*. 2015) also demonstrated that the leaf extract of Moringa oleifera exhibits significant anti-inflammatory activity in a xylene-induced ear edema model in mice, with an inhibition rate ranging from 70% to 85%. This activity was mainly attributed to the presence of flavonoids and phenolic compounds in the extract.

Furthermore, Mbikay (2012) highlighted in a review that *M. oleifera* leaf extracts reduce inflammation and oxidative stress in animal models, emphasizing the key role of phenolic compounds in modulating inflammatory pathways.

In addition, Sreelatha and Padma (2009) showed that methanolic extracts of *M. oleifera* leaves inhibit inflammatory markers both in vitro and in vivo, further reinforcing the importance of flavonoids and other bioactive compounds in the observed anti-inflammatory effect.

These findings are consistent with the results of our study, which showed a significant inhibition of xylene-induced ear edema using *M. oleifera* extract at 2 mg/ear, comparable to that of indomethacin (0.5 mg/ear). This inhibition is likely due to the combined action of flavonoids, tannins, and phenolic compounds that are capable of suppressing the release of inflammatory mediators.

These studies confirm the potential of *Moringa oleifera* as a natural anti-inflammatory agent and support its traditional use for treating inflammatory conditions. Future research should focus on isolating the active compounds and evaluating their specific mechanisms of action

8.5 Toxicty test

An acute oral toxicity study was conducted to evaluate the safety profile of *Moringa oleifera* leaf extract *in vivo*. Three healthy adult mice were administered a single high oral dose of the extract. The animals were observed continuously for the first 4 hours post-administration and periodically over a 14-day period for any signs of toxicity, behavioral changes, or mortality.

Figure 14:acute oral toxicity test on mice.

Results and discussion

Throughout the observation period, no clinical signs of toxicity, such as piloerection, tremors, convulsions, lethargy, diarrhea, or abnormal behavior, were observed in any of the three mice. Furthermore, no mortality occurred during the study period.

All animals maintained normal feeding behavior and body weight, and there were no gross pathological changes noted at the end of the experiment. Based on these observations, the *Moringa oleifera* extract demonstrated no acute toxic effects at the administered dose under the study conditions. In comparison with previous studies, the present investigation demonstrated that the leaf extract of Moringa oleifera exhibited no signs of acute oral toxicity in mice, even at a high single oral dose. No abnormal clinical symptoms such as piloerection, tremors, convulsions, lethargy, diarrhea, or behavioral changes were observed during the 14-day observation period, and no mortality occurred. These findings are in full agreement with those reported by (Chivapat *et al*. 2013), who found no toxic symptoms or gross organ lesions in mice administered 20 g/kg of aqueous leaf extract.

Similarly, Awodele et al. (2012) observed no mortality in mice given oral doses ranging from 1000 to 3000 mg/kg, although mild transient lethargy was reported. Their study confirmed the low toxicity of *M. oleifera* leaf extract, with an estimated LD_{50} above 2000 mg/kg. (Odumeru *et al*. 2023) reported an estimated LD_{50} of 3900 mg/kg for ethanol-based leaf extract in Swiss albino mice, with no mortality observed at doses below this threshold.

On the other hand, some studies reported mild physiological effects at higher doses. (Adedapo *et al.,* 2011) estimated the LD_{50} of aqueous leaf extract in Wistar rats to be approximately 1585 mg/kg, though no significant hematological or biochemical alterations were noted. Similarly, (Daniel *et al*,2022) documented moderate toxicity following intraperitoneal injection of methanolic flower extract at doses up to 5000 mg/kg, with changes in red blood cell counts, hemoglobin levels, and leukocyte profiles.

Overall, our findings support the safety of *Moringa oleifera* leaf extract when administered orally, in alignment with existing literature that suggests low acute toxicity, particularly for aqueous extracts and in rodent models.

CONCLUSION

AND

PERSPECTIVE

Conclusion and Perspective

The present study demonstrated that the ethanolic extract of *Moringa oleifera* leaves possesses significant biological activities. The extract exhibited strong antioxidant potential, as evidenced by its low IC_{50} value in the DPPH assay, indicating its ability to scavenge free radicals and possibly prevent oxidative stress-related cellular damage.

Furthermore, the extract showed notable anti-inflammatory activity both in vitro and in vivo. In vitro assays revealed inhibition of key inflammatory mediators, while the xylene-induced ear edema model confirmed the extract's ability to reduce acute inflammation in vivo, supporting its traditional use in treating inflammatory disorders.

Importantly, the acute oral toxicity test revealed no signs of toxicity or mortality at the tested doses, suggesting that the ethanolic extract of *M. oleifera* is safe for oral administration in experimental animals.

Collectively, these findings highlight the therapeutic potential of *Moringa oleifera* ethanolic leaf extract as a natural source of antioxidants and anti-inflammatory agents, with a favorable safety profile. Further studies focusing on the isolation of active compounds and elucidation of their molecular mechanisms are recommended to support future pharmacological applications.

REFERENCE

References

Amari, S., Karbab, K., Charef, N., Arrar, L., Mubarak, M.S., 2023. Anti-urolithiatic, antibacterial, anti-inflammatory and analgesic effects of *Erica arborea* flowers and leaves hydromethanolic extracts: An ethnopharmacological study. Saudi Journal of Biological Sciences. 103785.

Ahmed, K. J., Jahan, I. A., Jahan, F., & Hossain, M. H. (2021). Antioxidant activities and simultaneous HPLC-DAD profiling of polyphenolic compounds from Moringa oleifera Lam. leaves grown in Bangladesh. Food Research, 5(1), 401–408.

Akkouche, A., Djerrou, Z., & Boumendjel, A. (2012). Anti-inflammatory activity of aqueous extract of microphylla. Journal of Ethnopharmacology, 144(1), 140–146.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: A food plant with multiple medicinal uses. Phytotherapy Research, 21(1), 17–25.

Awodele, O., Oreagba, I. A., Odoma, S., da Silva, J. A., & Osunkalu, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). Journal of Ethnopharmacology, 139(2), 330–336.

Baba Aïssa, F. (2000). Encyclopédie des plantes utiles: Flore d'Algérie et du Maghreb. Alger: Editions Librairie Moderne.

Barriada-Bernal, L. G., Barrera-Saldaña, H. A., & Salas-Villalobos, T. B. (2013). Antioxidant activity of plant extracts rich Thymelaea in phenolic compounds and flavonoids. Journal of Medicinal Plants Research, 7(14), 1019–1028.

Bassey, K. M., Mofokeng, M. M., & Mabusela, W. T. (2022). Chemical characterization and nutritional markers of South African Moringa oleifera seed oils. South African Journal of Botany, 144, 5749–5756.

Beloud, R. (2001). La phytothérapie traditionnelle en Algérie. Alger: Editions ENAG.

Bhattacharya, S., Pradhan, A., Saha, S., & Majumder, A. (2019). A review on technologies and their usage in solid waste monitoring and management systems: Issues and challenges. Environmental Technology & Innovation, 15, 100419.

Broadhurst, R. B., & Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture, 29(9), 788–794.

Chand Raza, S. M., Islam, M. T., & Uddin, M. S. (2024). In vivo study of Moringa oleifera seed extracts as potential sources of neuroprotection against rotenone-induced neurotoxicity. Plants, 13(11), 1479.

Chandrashekar, S., Venkat, R., & Harini, B. P. (2021). Identification and purification of potential bioactive peptides from Moringa oleifera seed extracts. Journal of Functional Foods, 85, 104638.

Charef, N., Aouadi, D., & Baghiani, A. (2009). Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chemistry, 112(2), 303–309.

Chevallier, A. (2001). Encyclopedia of Herbal Medicine. London: Dorling Kindersley.

Chivapat, S., Chavalittumrong, P., & Tunsaringkarn, T. (2013). Acute and chronic toxicity study of the water extract from Moringa oleifera Lam. leaves in rats. Journal of Health Research, 27(1), 57–64.

Delaporte, R. H., & Harborne, J. B. (2004). Evaluation of anti-inflammatory activity using xylene-induced mouse ear edema model. Phytotherapy Research, 18(2), 151–158.

Do, Q. D., Nguyen, H. T., & Vuong, Q. V. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3), 296–302.

Fouché, G., Venter, M., & van der Kooy, F. (2000). Toxicity studies of South African medicinal plants. Journal of Ethnopharmacology, 69(1), 105–111.

Fitriana, W. D., Tanjung, M., & Widjanarko, S. B. (2016). Antioxidant activity of Moringa oleifera extracts. Journal of Applied Pharmaceutical Science, 6(5), 297–301.

Gonçalves, A. L., & Lajolo, F. M. (2010). Evaluation of antinutritional factors in leaves of Moringa oleifera. Food Science and Technology International, 16(1), 25–31.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 27(1), 1–93.

Howes, M.-J. R., & Simmonds, M. S. J. (2018). Natural anti-inflammatory agents and their molecular targets. Phytotherapy Research, 32(1), 1–16.

Jed, W. P., & Sutherland, S. (2005). Moringa oleifera as nutritional and medicinal plant: current knowledge and future directions. Research Journal of Medicinal Plant, 1(2), 1450–1456.

Karbab, A., Charef, N., Zarga, M.H.A., Qadri, M.I., (2021). Mubarak, M.S., Ethnomedicinal documentation and anti-inflammatory effects of *n*-butanol extract and of four compounds isolated from the stems of *Pituranthos scoparius*: An *in vitro* and *in vivo* investigation. Journal of Ethnopharmacology. 267, 113488.

Karbab, A., Mokhnache, K., Ouhida, S., Charef, N., Djabi, F., Arrar, L., Mubarak, M,.S. (2020). Anti-inflammatory, analgesic activity, and toxicity of *Pituranthos scoparius* stem extract: An ethnopharmacological study in rat and mouse models. Journal of ethnopharmacology. 258, 112936.

Kokoette Bassey, M., Mofokeng, M. M., & Mabusela, W. T. (2022). Chemical characterization and nutritional markers of South African Moringa oleifera seed oils. South African Journal of Botany, 144, 5749–5756.

Maione, F., De Feo, V., & Mascolo, N. (2016). Anti-inflammatory compounds from natural sources and their effects on inflammation. Current Medicinal Chemistry, 23(5), 5376–5397.

Malheiros, A. F., & Oliveira, A. B. (2017). The ethnopharmacological approach to natural products research. Journal of Ethnopharmacology, 236, 236–246.

Mbikay, M. (n.d.). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review. Frontiers in Pharmacology, 24, 24.

Mordenis, R., & Smith, J. (2019). Potential toxicity of medicinal plant use. Toxicology Reports, 6, 423–428.

Nostro, A., Germano, M. P., & D'Angelo, V. (2000). Medicinal plants: Antimicrobial activity. Journal of Ethnopharmacology, 71(1–2), 123–137.

Nworu, C. S., Akah, P. A., & Okoye, F. B. C. (2015). Anti-inflammatory and analgesic activities of Moringa oleifera leaf extract in rodents. African Journal of Traditional, Complementary and Alternative Medicines, 12(2), 109–115.

Odumeru, E. N., & Adegbite, A. (2023). Acute toxicity, phytochemicals, and nutrient composition of Moringa oleifera leaves, a plant used as a food supplement in the tropical region of Nigeria. Journal of Medicinal Plants Research, 17(3), 164–170.

Sharad, B. (2024). Moringa oleifera: A strong antimicrobial agent. International Journal of Microbiology Research, 5(2), 714–718.

Singh, A. K. (2017). Review on pharmacological potential of medicinal plants in modern health care. International Journal of Pharmacognosy and Phytochemical Research, 9(1), 21–32.

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods for Human Nutrition, 64(4), 303–311.

Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2013). The antiproliferative effect of Moringa oleifera crude aqueous leaf extract on cancerous human alveolar epithelial cells. BMC Complementary and Alternative Medicine, 13, 226.

Ugwah-Oguejiofor, C. J., Ugwah, M. O., & Ghasi, S. (2019). Acute and sub-chronic toxicity evaluation of herbal extracts. Journal of Ethnopharmacology, 235, 681–687.

Yuan, D. S., & Shen, Y. (2006). Anti-inflammatory activity of plant flavonoids. Journal of Ethnopharmacology, 104(1–2), 940–947.

Zhang, X. W., & Liu, Y. (2019). Antioxidant and anti-inflammatory effects of polyphenols. Food Chemistry, 275, 940–947.

Zhu, F. D., & Wang, Y. (2018). Advances in the studies of polyphenols from plant food and their effects on human health. Critical Reviews in Food Science and Nutrition, 58(12), 1809–1819

Abstract

Moringa oleifera L is a medicinal plant used in traditional medicine to cure various ailments. This study aimed to evaluate the ethnopharmacological study, antioxidant and anti-inflammatory activities. An ethnopharmacological survey was conducted to gather detailed information regarding the traditional usage (55.4%) of different plant parts, diseases treated, and routes of administration. In addition to traditional knowledge documentation, phytochemical analyses of a dried extract (DE) of *M. oleifera* leaves were performed. Quantification of secondary metabolites revealed high levels of polyphenols, flavonoids and condensed tannins with a values of 255.79 ± 6.82 µg gallic acid equivalent, 4.64 ± 0.52 µg quercetin equivalent, and 41.47 ± 1.41 µg catechin equivalent dry extract, respectively. In antioxidant activity using the DPPH radical scavenging assay, the results demonstrated a potent free radical inhibition with an IC_{50} of 0.15 ± 0.01 mg/mL. *In vitro* assays measuring the inhibition of protein denaturation revealed dose-dependent anti-inflammatory activity of the extract, achieving 82.57 ± 0.00% inhibition at 8 mg/mL. *In vivo* study using xylene-induced ear edema in mice showed a significant inhibition of inflammation at a dose of 2 mg/mL, with inhibition percentages reaching 87.65%. Finally, the findings of the acute toxicity experiment demonstrated that extract is safe and that even at dosages as high as 2 g/kg DW. These findings provide strong scientific support for the traditional uses of *Moringa oleifera* and highlight its promising potential as a source of natural antioxidants and anti-inflammatory agents. Further investigation is warranted to isolate and characterize the specific active compounds responsible for these effects.

Key words: *Moringa oleifera* L, ethnopharmacological study, antioxidant activity, anti-inflammatory activity, phytochemicals.

ملخص

تُعد *Moringa oleifera. L*من النباتات الطبية المستخدمة في الطب التقليدي لعلاج العديد من الأمراض. هدفت هذه الدراسة إلى تقييم الجوانب الإثنوفارماكولوجية بالإضافة إلى النشاط المضاد للأكسدة والمضاد للالتهابات. تم إجراء مسح إثنوفارماكولوجي لجمع معلومات مفصلة حول الاستخدامات التقليدية (55.4%) لأجزاء النبات المختلفة، الأمراض المعالجة، وطرق الاستخدام. إلى جانب توثيق المعارف التقليدية، تم إجراء تحاليل كيميائية نباتية لمستخلص جاف من أوراق *M. oleifera*.L أظهرت نتائج تحليل المركبات الثانوية مستويات مرتفعة من البوليفينولات والفلافونويدات والعفصات المثنفة، بقيم بلغت 59...2 ± 2..2 ميثروغرام مثافئ حمض التانيك، 4.24 ± 2..2 ميثروغرام مثافئ الثيرسيتين، و44.45 ± 4.44 ميثروغرام مثافئ الثاتيشين لكل غرام من المستخلص الجاف. في اختبار النشاط المضاد للأكسدة باستخدام اختبار DPPH ، أظهرت النتائج قدرة عالية على تثبيط الجذور الحرة بقيمة IC_{50}تساوي .2.4 ± 2.24 ملغ/مل. كما أظهرت الاختبارات المخبرية لقياس تثبيط تحلل البروتينات نشاطًا مضادًا للالتهاب يعتمد على الجرعة، حيث وصلت نسبة التثبيط إلى

5..2. ± 2.22% عند تركيز . ملغ/مل. أما في الدراسة الحيوانية باستخدام وذمة الأذن المحفزة بالزيلين في الفئران، فقد تم تسجيل تثبيط كبير للالتهاب عند جرعة 2 ملغ/مل، حيث بلغت نسبة التثبيط .5.2.%. وأخيرًا، أظهرت نتائج اختبار السمية الحادة أن المستخلص آمن حتى عند جرعات تصل إلى 2 غرام/كلغ من وزن الجسم. تدعم هذه النتائج الاستخدامات التقليدية لنبات *Moringa oleifera*علميًا وتبرز إمكانياته الواعدة كمصدر طبيعي لمضادات الأكسدة والعوامل المضادة للالتهابات. وتُوصى بإجراء المزيد من الدراسات لعزل وتحديد المركبات النشطة المسؤولة عن هذه التأثيرات.

الكلمات المفتاحية*Moringa oleifera. L* ، الدراسة الإثنوفارماكولوجية، النشاط المضاد للأكسدة، النشاط المضاد للالتهاب، المركبات الثيميائية النباتية.

Resumé

Moringa oleifera L. est une plante médicinale utilisée en médecine traditionnelle pour traiter diverses affections. Cette étude avait pour objectif d'évaluer les données ethnopharmacologiques ainsi que les activités antioxydantes et anti-inflammatoires. Une enquête ethnopharmacologique a été menée afin de recueillir des informations détaillées sur l'utilisation traditionnelle (55.4%) des différentes parties de la plante, les maladies traitées et les voies d'administration. En plus de la documentation des connaissances traditionnelles, des analyses phytochimiques d'un extrait sec (ES) des feuilles de *M. oleifera* ont été réalisées. La quantification des métabolites secondaires a révélé des teneurs élevées en polyphénols, flavonoïdes et tanins condensés, avec des valeurs respectives de 255,79 ± 6,82 µg équivalent acide gallique, 4,64 ± 0,52 µg équivalent quercétine, et 41,47 ± 1,41 µg équivalent catéchine par gramme d'extrait sec. Lors de l'évaluation de l'activité antioxydante à l'aide du test de piégeage du radical DPPH, les résultats ont montré une puissante inhibition des radicaux libres avec un IC_{50} de 0,15 ± 0,01 mg/mL. Les essais in vitro mesurant l'inhibition de la dénaturation des protéines ont révélé une activité anti-inflammatoire dépendante de la dose, atteignant 82,57 ± 0,00% d'inhibition à 8 mg/mL. L'étude in vivo, utilisant un œdème de l'oreille induit par le xylène chez la souris, a montré une inhibition significative de l'inflammation à une dose de 2 mg/mL, avec des pourcentages d'inhibition atteignant 87,65%. Enfin, les résultats de l'étude de toxicité aiguë ont démontré que l'extrait est sûr, même à des doses aussi élevées que 2 g/kg de poids corporel. Ces résultats apportent un solide soutien scientifique à l'usage traditionnel de *Moringa oleifera* et mettent en lumière son potentiel prometteur en tant que source naturelle d'antioxydants et d'agents anti-inflammatoires. Des études complémentaires sont nécessaires pour isoler et caractériser les composés actifs responsables de ces effets.

Mots-clés : *Moringa oleifera*.L, étude ethnopharmacologique, activité antioxydante, activité anti-inflammatoire, composés phytochimiques.