

الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

جامعة فرحات عباس سطيف 1

University of Ferhat Abbas – Setif 1

Faculty of Natural and Life Sciences



كلية علوم الطبيعة و الحياة

DERARTEMENT OF BIOCHIMISTRY

N°...../SNV/2025

THESIS

Presented by

Ben yahia Aicha

Mechahougui Maroua

Mekarni Aziza

For the fulfillment of the requirements for the degree of

Master

Field: Biological Sciences

Special field: Applied Biochemistry

TOPIC

Unveiling the antioxidant and immunomodulatory potential

of *Silybum marianum* seed extract.

Presented in/...../2025

JURY:

Chair: AMRAUOI Nacer

MCA. UFA Setif 1

Supervisor: BOUTEFAHA Zineddine

MCB. UFA Setif 1

Examiner: CHENITI Wafa

MAA. UFA Setif 1

Laboratory Of Applied Biochemistry

2024-2025

ACKNOWLEDGMENTS

We would like to begin by expressing our profound gratitude to **Allah Almighty**, whose infinite mercy, guidance, and blessings have accompanied us throughout this journey. It is through His will that we have found the strength, clarity, and determination to bring this work to completion.

We would like to express our heartfelt appreciation to our supervisor, **Mr. BOUTEFAHA Zineddine**, for his continuous support, valuable guidance, and constant encouragement. His expertise, constructive feedback, and availability at every stage of this research have played a vital role in shaping the quality of this work. We are truly grateful for his endless trust and encouragement.

Our sincere appreciation goes to **Professor BELATTAR Noureddine**, head of the laboratory, for offering an excellent research environment and for providing us with the necessary facilities which greatly contributed to the success of this study.

We wish to sincerely thank **Mr. AMRAOUI Nacer**, the Chair of the Jury, for honoring us with his presence and for the careful attention he gave to the evaluation of this work. His thoughtful remarks and valuable suggestions are deeply appreciated.

We're also thankful to the examiner, **Ms. CHENITI Wafa**, for her careful review, thoughtful comments, and valuable suggestions which helped improve the final version of this thesis. Her time and efforts reviewing our thesis are greatly valued.

work is the result of many efforts and much support. We are grateful to everyone who contributed in one way or another to the success of this study.

May Allah bless you all.

DEDICATION

I dedicate this work to those who have always been my greatest support and most profound sources of inspiration:

*To Abii, **Madani**,*

For your wisdom, thoughtful guidance, and unwavering support. You have always been and will always remain my role model in life. Your presence is a constant source of strength and motivation. May God bless and protect you always.

*To Mamy, **Nouara**,*

For your unconditional love, tenderness, and endless encouragement. You are the light that brightens my path. May God watch over you and shower you with his blessings.

*To my beloved sisters, **Meriem** and **Manar**, For your steadfast support and companionship through every joy and challenge. Your presence in my life is an invaluable treasure, and I cherish every moment we share.*

*To my friends: **Aziza**, **Aisha**, **Kawther**, **Chahrazed**, **Aya**, and **Djouhaina**, For your sincere friendship, uplifting words, and all the unforgettable memories we've created together. You have enriched my life in countless ways.*

Thank you all for believing in me and standing by my side throughout this journey.

*With all my love, **Maroua***

DEDICATION

*To the roots of my soul my mother **Habiba**, the heartbeat of my world, thank you for your
endless sacrifices and love.*

*My father **Cherif**, a pillar of strength, your love shaped the light in me, thank you for
unwavering support, and boundless love.*

*To my sisters **Amina**, **Assia**, and **Meriem** you are the laughter in my storms, the echoes of my
childhood, and the harmony in my chaos.*

*To my extended family Grandpa may Allah bless his soul, thanks for encouraging
me. Grandma , My aunts , And the whole family your presence is the quiet force that lifted
me when I forgot I had wings.*

*To the friends gifted by university days thank you for the memories stitched with late-night
talks, shared dreams, and the courage to keep going.*

*To **Aziza**, thank you for being here.*

*And to myself the dreamer, the fighter, the fire. Thank you for believing when no one was
watching, for writing your own story, and for being the poem in a world of prose.*

AICHA

DEDICATION

First and foremost, I am deeply grateful to Allah, the source of strength, guidance, and endless mercy. None of this would have come to pass without his grace.

*To my parents, my family and especially my dear siblings **Abdelkader, Zakaria, Sofiane, Nour el islem**, , and to **Zineddine**, who may be miles apart, but remains always in the heart.*

*To my twin and other half, **Salaheddine** , your unspoken support carried me. You've been my safe place and my guiding light in the darkest moments. In your strength, I found my own .*

This victory is yours as much as it is mine

To the little voice in me that never gave up, thank you for holding on, for whispering even when everything felt too heavy, for reminding me that I was capable and for pushing forward even when things felt impossible.

*And last but not least, to those who **truly love Aziza**.*

AZIZA

Abstract

Silybum marianum or milk thistle, is a medicinal plant belonging to the Asteraceae family. In this study, the methanolic extract of the seeds of *Silybum marianum* was studied through the quantification of its total phenolic and flavonoid contents using spectrophotometric methods. Three tests were carried out to evaluate the antioxidant activity of the seed extract, namely DPPH free radical scavenging, total antioxidant capacity (TAC), and anti-hemolytic activity assays. Additionally, the immunomodulatory effect has been evaluated *in vivo* using mouse models. The seed extract exhibited a high phenolic content of 213.62 μg GAE/mg and a total flavonoid content of 25.86 μg RE/mg of the extract. Evaluation of antioxidant activity using DPPH assay revealed an IC_{50} value of 34.24 ± 0.3 $\mu\text{g}/\text{ml}$, reflecting remarkable free radical scavenging activity. Moreover, the seed extract exhibited an $\text{A}_{0.50}$ value of 41.09 ± 5.3 $\mu\text{g}/\text{ml}$ in the total antioxidant capacity (TAC) assay and demonstrated a noticeable protective effect against hemolysis in the anti-hemolytic assay. At a concentration of 1000 $\mu\text{g}/\text{ml}$, the extract achieved a protection of 77.29%, comparable with that of quercetin (81.76%). Furthermore, the immunomodulatory treatment with the extract resulted in significant immunostimulation of antibody production in mice immunized with sheep red blood cells. These findings indicate that the seeds of *Silybum marianum* are rich in phenolic compounds and possess significant antioxidant and immunomodulatory effects. Thus, they could be used in the pharmaceutical field as a promise source of bioactive molecules with therapeutic properties.

Keywords: *Silybum marianum*, polyphenols, antioxidant activity, anti-hemolytic activity, immunomodulatory effect.

ملخص

Silybum marianum، أو شوك الحليب، هو نبات طبي ينتمي إلى فصيلة النجمية (Asteraceae). في إطار هذا البحث، تمت دراسة المستخلص الميثانولي لبذور *Silybum marianum* عن طريق تحديد محتواه الكلي من المركبات الفينولية والفلافونويدات باستخدام طرق التحليل الطيفي. تم إجراء ثلاثة اختبارات لدراسة النشاط المضاد للأكسدة لهذا المستخلص والمتمثلة في اختبار تثبيط الجذور الحرة (DPPH) واختبار القدرة الكلية المضادة للأكسدة (TAC) وكذلك اختبار النشاط المضاد لانحلال كريات الدم الحمراء. كما تم تقييم التأثير المناعي للمستخلص في الجسم الحي باستخدام نماذج الفئران. أظهرت النتائج احتواء مستخلص البذور على نسبة عالية من المركبات الفينولية بلغت 213.62 ميكروغرام مكافئ حمض الغاليك/مغ، ومحتوى كلي من الفلافونويدات بلغ 25.86 ميكروغرام مكافئ روتين/مغ من المستخلص. من ناحية أخرى كشف تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPH عن قيمة IC_{50} بلغت 34.24 ± 0.3 ميكروغرام/مل، مما يبين قدرة كبيرة على إزاحة الجذور الحرة. أما اختبار القدرة الكلية المضادة للأكسدة (TAC) فقد كشف عن قيمة $A_{0.50}$ بلغت 41.09 ± 5.3 ميكروغرام/مل. وفي اختبار مقاومة الانحلال الدموي، بيّن مستخلص البذور تأثيراً وقائياً ملحوظاً ضد انحلال كريات الدم الحمراء، إذ أظهر المستخلص عند تركيز 1000 ميكروغرام/مل حماية قُدرت بنسبة 77.29% وهي نسبة مقاربة لتلك المُحصَّل عليها مع الكيرسيتين (81.76%). بالإضافة إلى ذلك، أدت المعالجة بالمستخلص إلى تحفيز ملحوظ في إنتاج الأجسام المضادة لدى الفئران المُمنَّعة بكريات الدم الحمراء للأغنام. تثبت هذه النتائج أن بذور *Silybum marianum* غنية بالمركبات الفينولية وتمتلك خصائص مضادة للأكسدة ومحفزة للمناعة مما يجعلها مصدراً واعداً للجزيئات النشطة حيويّاً ذات الخصائص العلاجية في المجال الصيدلاني.

الكلمات المفتاحية: *Silybum marianum*، عديدات الفينول، النشاط المضاد للأكسدة، النشاط المضاد لانحلال الدم، النشاطات المعدلة للمناعة.

Resumé

Silybum marianum, ou chardon-Marie, est une plante médicinale appartenant à la famille des Asteraceae. Dans cette étude, l'extrait méthanolique des graines de *Silybum marianum* a été étudié à travers la quantification de ses teneurs totales en composés phénoliques et en flavonoïdes à l'aide de méthodes spectrophotométriques. Trois tests ont été réalisés pour évaluer l'activité antioxydante de l'extrait de graines notamment le test de piégeage du radical libre (DPPH), la capacité antioxydante totale (TAC), et l'activité anti-hémolytique. D'autre part, l'effet immunomodulateur a été évalué *in vivo* chez des modèles murins. L'extrait de graines a présenté une teneur élevée en composés phénoliques (213,62 µg EAG/mg) et une teneur totale en flavonoïdes de 25,86 µg ER/mg d'extrait. L'évaluation de l'activité antioxydante par le test de DPPH a révélé une valeur d'IC₅₀ de 34,24 ± 0,3 µg/ml, indiquant une capacité remarquable de piégeage des radicaux libres. Concernant le test de la capacité antioxydante totale (TAC), l'extrait a montré une valeur d'A_{0.50} de 41,09 ± 5,3 µg/ml. Lors du test anti-hémolytique, un effet protecteur notable contre l'hémolyse a été observé. En effet, avec la concentration de 1000 µg/ml, l'extrait a montré une protection de 77,29 %, comparable avec celle de la quercétine (81,76%). En outre, l'évaluation de l'effet immunomodulateur de l'extrait a entraîné une stimulation significative de la production d'anticorps chez les souris immunisées avec des globules rouges de mouton. Ces résultats indiquent que les graines de *Silybum marianum* sont riches en composés phénoliques et possèdent des propriétés antioxydantes et immunomodulatrices importantes. Elles pourraient ainsi être utilisées dans le domaine pharmaceutique comme source prometteuse de molécules bioactives aux propriétés thérapeutiques.

Mots-clés : *Silybum marianum*, polyphénols, activité antioxydante, activité anti-hémolytique, effet immunomodulateur.

LIST OF ABBREVIATIONS

A_{0.50} : Concentration for 0,5 of Absorbance

BHT: Butylated Hydroxytoluene

DNA: Deoxyribonucleic Acid

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DTH: Delayed-Type Hypersensitivity

EDTA: Ethylenediaminetetraacetic Acid

GPx: Glutathione Peroxidase

GR: Glutathione Reductase

GST: Glutathione S-Transfirase

IC₅₀: Inhibitory concentration for 50% of activity

Ig: Immunoglobulin

MDA: Malondialdehyde

MHC: Major Histocompatibility Complex

NMRI: Naval Medical Research Institute

PBS: Phosphate-Buffered Saline

RBCs: Red Blood Cells

RNS: reactive Nitrogen Species

ROS: Reactive Oxygen Species

SOD: Superoxide Dismutase

SRBCs: Sheep Red Blood Cells

LIST OF FIGURES

Figure 1. <i>Silybum marianum</i> morphological features : (a) entire plant, (b) seeds, and (c) dried head flower.	3
Figure 2. Major constituents of silymarin	5
Figure 3. Gallic acid standard curve for the determination of total flavonoid content.	12
Figure 4. Rutin standard curve for the determination of total flavonoid content.	13
Figure 5. (a) Oral gavage administration of the methanolic seed extract and (b) intraperitoneal injection of sheep red blood cells (SRBCs) in mice.....	15
Figure 6. Protective effects of <i>Silybum marianum</i> seed extract against oxidative erythrocyte damage induced by H ₂ O ₂	20
Figure 7. Effect of <i>Silybum marianum</i> seed extract on antibody titer in mice.	21