



DERARTEMENT OF BIOCHEMISTRY

N°...../SNV/2025

THESIS

Presented by

Bensedira Manar

Benallal Malak

For the fulfillment of the requirements for the degree of

Master

Field: Biological Sciences

Special field: Immunology

TOPIC

**Antioxidant and antiarthritic activities
of the methanolic extract of *Anthemis arvensis*.**

Presented on/...../2025

JURY:

Chair: BOUTEFAHA Zineddine

MCB. UFA Setif 1

Supervisor: AMRAOUI Nacer

MCA. UFA Setif 1

Examiner: MADOUY Soraya

MCA. UFA Setif 1

Laboratory of Applied Biochemistry

2024-2025

Acknowledgments

First and foremost, we express our deepest gratitude to **Allah** the Almighty, who gave us the courage and determination to complete this work.

We extend our sincere thanks to our thesis supervisor **Dr. Amraoui nacer**, for his guidance, patience, and trust throughout this research work. His valuable advice, expertise, and unwavering support have been invaluable and greatly contributed to the completion of this project.

Thanks also to the members of our thesis defense committee for agreeing to be part of the jury. Their remarks and suggestions were precious and helped us improve the quality of our work.

We would like to express our deepest gratitude to **Pr. Filaouine Sofiane**, God's mercy be upon him. Specialist at the Mother and Child Hospital, for his invaluable support throughout our work. His kindness was instrumental in the successful completion of this thesis. It is with great sadness that we acknowledge his passing. We honor his memory and the significant impact he had on our academic journey. His legacy will remain forever in our hearts.

We wish to thank all of **Nesrine, Dalia, Selma, Amira, Khalil, Nour, Hamza** and **Najat** for agreeing to be interviewed as part of this study. Their feedback enriched this work and allowed us to question and reorient our thesis problem.

A special thank you to our classmates and friends, especially **Bouthaina**, for their encouragement, stimulating intellectual exchanges, and for all the moments shared.

We cannot forget our families, for their unwavering support and for always believing in us. Their encouragement was our refuge and motivation throughout our academic journey.

Finally, we would like to thank all the people who, directly or indirectly, contributed to the realization of this thesis. Whether through enriching discussions, advice, or simply by their presence, their contribution was precious.

This thesis is the result of both collective and personal work, and we are deeply grateful to all those who accompanied

Dedication

I would like to dedicate this thesis to my wonderful parents, **Chirine** and **Hamza**, whose unconditional love, support, and sacrifices have been the foundation of my success. To my sister **Djoumana** and brother **Iyed**, thank you for your constant encouragement and for always being there when I needed you.

I am also deeply grateful to my friends, with a special mention to my partner **Manar**, whose motivation and positivity kept me going during challenging times.

This achievement is a testament to all of you who believed in me and helped me along this way.

Malak

Dedication

I dedicate this work to the one who planted in my heart the love of knowledge and hard work.

To my dear parents, who never withheld anything from me.

To my loving mother, **Berkahoum**, the symbol of sacrifice and generosity, and to my father, **djelloul**, my support in this life.

To my sisters, **Marwa** and **Zineb**, who were always by my side, encouraging me through every step, and to my dear brother **Bilal**

Thank you all for being part of my life.

Also, to all my friends especially **Malak**, **Bouthaina** and to everyone I love.

Manar

Abstract

Anthemis arvensis has been traditionally used as a medicinal herb for the treatment of many diseases. However, the antiarthritic activity of *A. arvensis* has still not been demonstrated. The present study was conducted to evaluate the antioxidant and antiarthritic activities of the methanol extract of *A. arvensis*. The total phenolic and flavonoid contents were determined using Folin–Ciocalteu's reagent and aluminium chloride assays, respectively. While, the antioxidant capacity was determined using DPPH test. The antiarthritic activity was evaluated using complete freund's adjuvant induced arthritis model. Male wistar rats were immunized by intradermal injection of 0.1 mL of CFA into subplantar region in the right hind paw, and were orally treated daily with diclofenac 4 mg/kg and the extract of *A. arvensis* (200 and 400 mg/kg) for 24 days. Body weight, hind paw swelling, arthritic score, spleen and liver index, and ESR were evaluated. The methanol extract of *A. arvensis* showed a yield of 19,5%. The total phenolic and flavonoid contents was $74,54 \pm 5,27$ μg GAE/mg and $17,54 \pm 0,76$ μg QE/mg of dry extract, respectively. While the antioxidant activity represents a half-maximal inhibitory concentration (IC_{50}) of 24 ± 2 $\mu\text{g}/\text{ml}$. In the *in vivo* study, the animals treated with *A. arvensis* showed significant improvement in joint swelling, arthritic score and ESR compared with AIA group. However, the treatment with the plant extract didn't show any significant effect on body weight and spleen and liver indices. In conclusion, these results support the use of *A. arvensis* in folk medicine in the treatment of rheumatic diseases.

Keywords: AIA, *Anthemis arvensis*, Antiarthritic, CFA, DPPH.

المخلص

تم استخدام *Anthemis arvensis* تقليدياً كنبات طبي لعلاج العديد من الأمراض، غير أن نشاطه المضاد للتهاب المفاصل لم يُتم دراسته بعد. تهدف هذه الدراسة إلى تقييم النشاط المضاد للأكسدة والمضاد للتهاب المفاصل الرثوي للمستخلص الميثانولي لنبات *A. arvensis*. تم تحديد المحتوى الكلي من عديدات الفينول والفلافونويدات باستخدام اختبار Folin–Ciocalteu's واختبار كلوريد الألمنيوم على التوالي. أما القدرة المضادة للأكسدة فقد تم تقييمها باستخدام اختبار DPPH. تمت دراسة النشاط المضاد للتهاب المفاصل باستخدام نموذج التهاب المفاصل المحفّز بواسطة مادة مساعد فروند الكامل. تم تحفيز التهاب المفاصل لدى ذكور الجرذان من نوع *wistar* عن طريق حقن 0.1 مل من مساعد فروند الكامل في باطن قدم الرجل اليمنى الخلفية للجرذان، وتم علاجهم عن طريق الفم يوميًا بجرعة 4 ملغ/كغ من الديكلوفيناك ومستخلص نبات *A. arvensis* (200 و 400 ملغ/كغ) لمدة 24 يومًا. تم تقييم النشاطية المضادة للتهاب المفاصل عن طريق تقدير وزن الجسم، وتورم القدم الخلفية، درجة التهاب المفاصل، مؤشر الطحال والكبد، بالإضافة إلى سرعة ترسيب الكريات الحمراء. أظهر المستخلص الميثانولي للنبات مردودًا بلغ 19.5%. قدر المحتوى الكلي من عديدات الفينول والفلافونويدات بـ 5.27 ± 74.54 ميكروغرام مكافئ حمض الغاليك/ملغ و 0.76 ± 17.54 ميكروغرام مكافئ كيرسيتين/ملغ من المستخلص الجاف على التوالي. أما النشاطية المضادة للأكسدة فقد قدرت بحساب التركيز المثبط لـ 50% من جذور DPPH والذي قدر بـ 2 ± 24 ميكروغرام/مل. وفي الدراسة على الحيوان، أظهرت الحيوانات المعالجة بالمستخلص تحسنًا ملحوظًا في تورم القدم الخلفية، درجة التهاب المفاصل وسرعة ترسيب الكريات الحمراء مقارنةً مع مجموعة الشاهدة. في حين أن المعالجة بالمستخلص النباتي لم تُظهر تأثيرًا ملحوظًا على وزن الجسم أو مؤشر الطحال والكبد. تدعم هذه النتائج الاستخدام التقليدي للنبات في الطب الشعبي لعلاج الأمراض الروماتيزمية.

الكلمات المفتاحية: *Anthemis arvensis*, AIA، CFA، مضاد للتهاب المفاصل، DPPH.

Résumé

Anthemis arvensis a été traditionnellement utilisée comme plante médicinale pour le traitement de nombreuses maladies. Cependant, son activité antiarthritique n'a pas encore été démontrée. La présente étude visait à évaluer les activités antioxydante et antiarthritique de l'extrait méthanolique d'*A. arvensis*. La teneur totale en phénols et en flavonoïdes ont été déterminées respectivement par l'utilisation de Folin–Ciocalteu et par l'essai au chlorure d'aluminium, respectivement. Tandis que, La capacité antioxydante a été évaluée à l'aide du test DPPH. L'activité antiarthritique a été étudiée à l'aide du modèle d'arthrite induite par l'adjuvant complet de Freund. Des rats Wistar mâles ont été immunisés par injection intradermique de 0,1 mL de ACF dans la région sous-plantaire de la patte postérieure droite et ont été traités par voie orale pendant 24 jours avec du diclofénac (4 mg/kg) ou l'extrait de *A. arvensis* (200 et 400 mg/kg). Les paramètres évalués comprenaient le poids corporel, le gonflement de la patte postérieure, le score arthritique, les indices de la rate et du foie, ainsi que la vitesse de sédimentation (VS). L'extrait méthanolique d'*A. arvensis* a présenté un rendement de 19,5 %. La teneur totale en phénols et en flavonoïdes étaient respectivement de $74,54 \pm 5,27$ µg GAE/mg et $17,54 \pm 0,76$ µg QE/mg d'extrait sec. L'activité antioxydante correspondait à une concentration inhibitrice médiane (IC₅₀) de 24 ± 2 µg/mL. Dans l'étude *in vivo*, les animaux traités avec *A. arvensis* ont montré une amélioration significative du gonflement articulaire, du score arthritique et de VS par rapport au groupe témoin. Alors que, le traitement par l'extrait de la plante n'a pas montré d'effet significatif sur le poids corporel, les indices de la rate et du foie. En conclusion, ces résultats soutiennent l'utilisation d'*A. arvensis* dans la médecine traditionnelle pour le traitement des maladies rhumatismales.

Mots clés : ACF, AIA, *Anthemis arvensis*, antiarthritique, DPPH.

List of abbreviations

AIA: Adjuvant induced arthritis.

AP-1: Activator Protein-1.

BHT: Butylated-hydroxy-toluene.

CFA: Complete Freund's adjuvant.

COX: Cyclooxygenase.

CSs: Corticosteroids.

DMARDs: Disease modifying antirheumatic drugs.

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl.

ESR: Erythrocyte sedimentation rate.

HSF1: Heat Shock Factor 1.

IC₅₀: Half-maximal inhibitory concentration.

MAPKs: Mitogen-activated protein kinases.

ONOO⁻ : Peroxynitrite anion.

NSAIDs: Non-steroidal anti-inflammatory drugs.

PADs: Peptidyl arginine deiminases.

SD: Standard deviation.

SEM: Standard error of mean.

List of figures

Figure 1: Pathogenesis of Reumatoid Arthritis.	7
Figure 2: <i>A. arvensis</i>	12
Figure 3: Extract preparation steps. A: Dried plant powder; B: Agitation; C: Filtration; D: Drying; E: The final extract.	16
Figure 4: Exampels of colors observed inFolin Ciocalteu assay for total polyphenols assay.	17
Figure 5: Calibration curve of Gallic acid.....	17
Figure 6: Exampels of colors observed in aluminum chloride assay for total flavonoids assay.	17
Figure 7: Calibration curve of Quercetin.....	17
Figure 8: Exemples of colors observed in the DPPH assay for the antioxidant activity....	19
Figure 9: Gavage of a rat with <i>A. arvensis</i>	20
Figure 10: Measurement of the thickness of the paw.....	19
Figure 11: Arthritic score during the experiment. A: Score 0; B: Score 1; C: Score 2; D: Score 3; E: Score 4.....	20
Figure 12: Determination of sedimentation rate (ESR).....	22
Figure 13: DPPH scavenging activity of <i>A. arvensis</i> extract compared to BHT.	22
Figure 14: Effect of various doses of <i>A. arvensis</i> on paw thickness in adjuvant induced arthritis.....	25
Figure 15: Effect of various doses of <i>A. arvensis</i> on arthritis scores in adjuvant-induced arthritis.....	26
Figure 16: Effect of <i>A. arvensis</i> extract on: A/ the spleen indices and B/ liver indices in adjuvant induced arthritis.	27
Figure 17: Effect of <i>A. arvensis</i> extract on ESR in adjuvant induced arthritis..	28

List of tables

Table 1: RA classification.	8
Table 2: Effect of <i>A. arvensis</i> extract on body weight changes in AIA.	24