الجمهورية الجزائرية الديمقراطية الشعبية

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جامعة فرحات عباس، سطيف1


**DEPARTMENT OF BIOCHEMISTRY**

N° ...../SNV/**2025**

# Thesis

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**For the fulfillment of requirements for diploma of**

**Master**

**Field:** Biology

**Speciality:** Applied biochemistry

## Topic

# Evaluation of the antioxidant and anti-inflammatory activity of *Styphnolobium japonicum* extracts

Presented publically in: 24/ 06 /2025

**Jury**

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**2024- 2025**

# Acknowledgements

At the very beginning, we are very grateful to **Professor Karima SAFFIDINE** for always supporting us, providing guidance and encouraging us throughout this project. At every point in our research, her knowledge, kindness and helpful suggestions were very important to us making our research better. We continue to appreciate all the support and belief in our skills that she showed us.

Thank you, **Professor Sabah CHERMAT** for offering to review this thesis and we are gratefully indebted to her for her very valuable comments on this thesis.

We are very thankful to **Doctor Thoraya GUEMMAZ** for agreeing to review our thesis, for her constant encouragement and excellent advice.

We are very grateful to **Professor Abderahmane BAGHINI** for allowing us to carry out our work in the Laboratory of Applied Biochemistry. We are very thankful for his willingness to look over what we do and his helpful comments. Because of his mentorship, our academic development has improved.

The team at the Laboratory of Applied Biochemistry is thanked for creating a friendly and cooperative atmosphere. We would not have accomplished as much without your help.

# Dedication

**To my beloved family,**

**Your love and support have been the foundation of my journey.**

**To my mother, whose strength, kindness, and prayers have carried me through every challenge. Thank you for being my guiding light.**

**To my sister, my confidante and friend, thank you for standing by me with patience and encouragement.**

**To my dear friends Amna and Maroua, your friendship, support and belief in me have meant the world. Thank you for being there through every step.**

**Imane**

# Dedication

**To those whose silent presence guided me, whose love carried me, and whose prayers lifted me when I needed it most...**

**To my beloved mother Sadika, the source of endless love, whose prayers lifted me through every challenge and whose strength inspired my own**

**To my dear father Kamel a symbol of perseverance and dedication, who taught me the value of patience and hard work.**

**To my precious sister Lina, my companion and joy, who stood by me with love and encouragement.**

**To my supportive brother Rami, whose presence gave me strength and comfort.**

**And to Oussama my soulmate, my safe place, and my greatest blessing. Thank you for believing in me even when I doubted myself.**

**Amira**

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## ملخص

ركز عملنا على تحديد كمية عديدات الفينول الاجمالية و الفلافونوييدات و العفص وتقييم النشاط المضاد للأكسدة والمضاد للالتهابات في مستخلصات بذور *Styphnolobium Japonicum*: مستخلص خام : CrE و كسور أسيتات الإيثيل: EAES والمائية: AqES; ولحاء الساق( الكسورأسيتات الإيثيل: EAEB و المائية : AqEB. أظهرت النتائج أن إجمالي محتويات متعددات الفينول و مركبات الفلافون و العفص تراوحت على التوالي من 12.59 إلى 210.16 ملغم من مكافئ حمض الغاليك/غرام، و 3.55 إلى 9.89 ميكروغرام من مكافئ الكيرستين/غرام، و 2.42 إلى 11.56 ميكروغرام من مكافئ الكاتشين/ميلليغرام .وقد تم الحصول على أعلى كميات من هذه المركبات الفينولية في مركبات EAES و EAEB. تمت دراسة إمكانات مضادات الأكسدة في المختبر للمستخلصات المختلفة باستخدام عدة طرق كان لدى EAES أعلى قدرة مضادة للأكسدة في التقاط الجذور الحرة ABTS و بيروكسيد الهيدروجين مع $IC_{50}$ بمقدار 0.05 ± 1.19ميكروغرام/مل و 0.49 ± 20.21 ميكروغرام/مل على التوالي؛ وأعلى قدرة ارجاعية مع $EC_{50}$ بمقدار 0.23 ± 13.86ميكروغرام/مل .من ناحية أخرى، أظهرت جميع المستخلصات قدرة عالية المضادة للأكسدة الكلية (TAC)وكان أكثرها فعالية هو EAES مع EC50 يبلغ 0.46 ± 12.61 ميكروغرام/ملل. بالإضافة إلى ذلك، تم تقييم القدرة المضادة للالتهابات في المستخلصات المختلفة باستخدام اختبار تثبيط تمزق الألبومين بتركيزات 2 و 1و 0.5 و 0.25 ملغم/ملل. كان هذا النشاط المضاد للالتهاب معتمدً على الجرعة وتراوح بين 10.64 و .%91.51 وأظهر EAES أعلى نشاط مع تثبيط تمزق الألبومين بنسبة 91.51% عند 2 ملغم/ملليتر، وانخفضت هذه النسبة تدريجياً إلى 54.36%عند 0.25 ملغم/ملليتر، يليه CrES بمعدل تثبيط تراوح بين 71.75 و 67.59%.

تُظهر هذه النتائج إمكانات نوع *Styphnolobium Japonicum* كمصدر قيم للمواد الكيميائية النشطة بيولوجيًا ذات الخصائص القوية المضادة للأكسدة والمضادة للالتهابات، وتدعم استخدامه التقليدي في علاج الأمراض المختلفة.



**الكلمات المفتاحية** : عديدات الفينول،لفلافونوييدات، العفص، ستيفنولوبيوم جابونيكوم، مضادات الأكسدة، مضادات الأكسدة، مضادات الالتهاب

## Abstract

Our work was focused on the determination of the polyphenols and flavonoids and tannins contents, the evaluation of the antioxidant and anti-inflammatory activity of *Styphnolobium Japonicum* extracts of seeds (CrE: crude extract, EAES: ethyl acetate and AqES: aqueous fractions) and stem bark (EAEB: ethyl acetate and AqEB: aqueous fractions). The results showed that the total polyphenol, flavonoid, and condensed tannin contents ranged from 12.59 to 210.16 mg GAE/g, 3.55 to 9.89 µg QE/mg and 2.42 to 11.56 µg CE/mg, respectively. The highest amount of these phenolic compounds were obtained for EAES and EAEB. In vitro antioxidant potential of different extracts has been investigated using several methods. EAES possesses the strongest antioxidant capacity in scavenging ABTS and hydrogen peroxide free radicals with an $IC_{50}$ of 1.19 ± 0.05 µg/mL and 20.21 ± 0.49 µg/mL, respectively; and the highest reducing power activity with $EC_{50}$ of 13.86 ± 0.23 µg/mL. Whereas, all the extracts have exhibited strong total antioxidant capacity (TAC) and the most effective was EAES with $EC_{50}$ of 12.61 ± 0.46 µg/mL. Moreover, the anti-inflammatory potential of different extracts was evaluated using the inhibition of albumin denaturation test at a concentration of 2, 1, 0.5 and 0.25 mg/mL. This anti-inflammatory activity was dose-dependent and varied from 10.64 to 91.51%. EAES showed the highest activity with albumin denaturation inhibition of 91.51% at 2 mg/mL which decreased gradually to 54.36% at 0.25 mg/mL, followed by CrES with an inhibition rate ranging from 71.75 to 67.59%.

These results demonstrate *Styphnolobium Japonicum* species' potential as a valuable source of bioactive chemicals with strong antioxidant and anti-inflammatory properties, and support its traditional use in treating various diseases.

**Key words:** polyphenols, flavonoids, tannins, *Styphnolobium japonicum*, antioxidant, anti-inflammatory.

## Résumé

Notre travail a porté sur la détermination des teneurs en polyphénols, flavonoïdes et tanins, l'évaluation de l'activité antioxydante et anti-inflammatoire d'extraits de graines de *Styphnolobium Japonicum* (CrE : extrait brut, les fractions EAES : acétate d'éthyle et AqES: aqueuse) et d'écorce de tige (les fractions EAEB : acétate d'éthyle et AqEB : aqueuse). Les résultats ont montré que les teneurs totales en polyphénols, flavonoïdes et tannins condensés variaient respectivement de 12.59 à 210.16 mg GAE/g, 3.55 à 9.89 µg QE/mg et 2.42 à 11.56 µg CE/mg. Les taux les plus élevées de ces composés phénoliques ont été obtenues pour EAES et EAEB. Le potentiel antioxydant *in-vitro* des différents extraits a été étudié en utilisant plusieurs méthodes EAES possède la plus forte capacité antioxydante dans le piégeage des radicaux libres ABTS et le peroxyde d'hydrogène avec une $IC_{50}$ de 1.19 ± 0.05 µg/mL et 20.21 ± 0.49 µg/mL respectivement, et le pouvoir réducteur le plus élevé avec une $EC_{50}$ de 13.86 ± 0.23 µg/mL. En revanche, tous les extraits ont montré une forte capacité antioxydante totale (TAC) et EAES était l'extrait le plus efficient avec une $EC_{50}$ de 12.61 ± 0.46 µg/mL. De plus, le potentiel anti-inflammatoire de différents extraits a été évalué à l'aide du test d'inhibition de la dénaturation de l'albumine ; à une concentration de 2, 1, 0.5 et 0.25 mg/mL. Cette activité anti-inflammatoire était dose-dépendante et variait de 10.64 à 91.51 %. EAES a montré l'activité la plus élevée avec une inhibition de la dénaturation de l'albumine de 91.51 % à 2 mg/mL et qui a diminué progressivement jusqu'à 54.36 % à 0.25 mg/mL, suivie par CrES avec un taux d'inhibition allant de 71.75 à 67.59 %.

Ces résultats démontrent le potentiel de l'espèce *Styphnolobium Japonicum* en tant que source précieuse de substances chimiques bioactives dotées de fortes propriétés antioxydantes et anti-inflammatoires, et soutiennent son utilisation traditionnelle dans le traitement de diverses maladies.



**Mots clés** : polyphénols, flavonoïdes, tanins, *Styphnolobium japonicum*, antioxydant, anti-inflammatoire

# Abbreviations

**CrES :** crude extract of seeds
**EAES :** ethyl acetate extract of seeds
**AqES :** aqueous extract of seeds
**HeES :** hexane extract of seeds
**CrEB :** crude extract of bark
**HeEB :** hexane extract of stem bark
**EAEB :** ethyl acetate extract of stem bark
**AqEB :** aqueous extract of stem bark
**GAE :** Gallic Acid Equivalent
**QE :** Quercetin Equivalent
**CE :** Catechin Equivalent
**TAC :** Total Antioxidant Capacity
**BSA :** Bovine Serum Albumin
**ROS :** Oxygen reactive species
**RNS :** Nitrogen Reactive Species
**BHT :** Butyl hydroxytoluene
**BHA :** Butyl hydroxyanisole
**COX:** Cyclooxygenases enzyme
**MAPKs :** Mitogen-activated protein kinases
**NF-κB :** Nuclear factor kappa-light-chain-enhancer of activated B cells
**TNF-α :** Tumor Necrosis Factor alpha
**IL-1β :** Interleukine-1 bêta
**IL-6 :** Interleukine-6
**SOD :** Superoxide Dismutase
**CAT :** Catalase
**GPx :** Glutathion peroxidase
**GR :** Glutathion Reductase
**Prxs :** Peroxiredoxins
**NSAIDs :** Nonsteroidal Anti-inflammatory Drugs