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# Master's thesis

Presented by

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## Master

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## TOPIC

# Evaluation of the antioxidant potential and acute toxicity of *Stipa tenacissima* L.

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# *Dedication*

To the one who embraced me with his tenderness, supported me with patience, to the one who was both a father and a friend and a steadfast support, to my dear father ***Kamel***.

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From the little girl who grew with you**,** ***Malak*****.**

# *Dedication*

بِسْمِ ٱللهِ الرَّحْمٰنِ الرَّحِيم

﴿ وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ ﴾ ــ ٱلضُّحَى، الآيـة ٥ ــ

**“And your Lord is going to give you, and you will be satisfied.” — Qur'an, Surah Ad-Duhaa (93:5)18:42**

To begin with, all praise and thanks are due to Allah, the Most Merciful, the Most Compassionate — Without His infinite mercy and guidance, none of this would have been possible.

I dedicate this humble work…
To my beloved **parents**,
To my father ***Laid***, whose strength, wisdom, and quiet sacrifices have been the foundation of my perseverance, And to my mother ***Yamina***, the beating heart of our home, whose unconditional love, prayers, and tenderness have been my constant source of courage and comfort.
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This work holds not just my name, but the reflection of your love, prayers, and sacrifices.
From the depths of my heart: thank you.

Yours, ***Nesrine***

## *DEDICATION :*

**"يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍ"**

"Allah will raise those who have believed among you and those who were given knowledge by degrees."

(Surah Al-Mujadila, 58:11)

All praise and gratitude belong to Allah, the Most High, who honored me with the pursuit of knowledge and surrounded me with His endless mercy. By His will alone, this work has come to fruition.

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**"رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ"**

"My Lord, enable me to be grateful for Your favor which You have bestowed upon me and upon my parents and to do righteous work of which You approve."

(Surah Al-Ahqaf, 46:15)

With profound humility and eternal gratitude , *Djihad*

# List of Abbreviations

**ALT:** Alanine Aminotransferase

**AQ:** Aqueous

**AST:** Aspartate Aminotransferase

**ATP:** Adenosine triphosohate

**BHT:** Butylated hydroxytoluene

**CREA:** Creatinine

**D1:** Dose 1 of extract

**D2:** Dose 2 of extract

**DPPH:** 2,2-diphenyl-1-picrylhydrazyl

**EC50:** Effective concentration at 50%

**EDTA:** Ethylene Diamine Tetra-Acetic acid

**EQ:** Quercetin equivalent

**EtOH:** Ethanol

**FRAP**: Ferric Reducing Antioxidant Power

**GAE:** Gallic acid equivalent

**GPx:** Glutathione Peroxidase

**IC50:** Inhibitory Concentration 50

**LD50:** Lethal Dose 50

**LYMPH:** Lymphocytes

**OECD:** Organisation for Economic Co-operation and Development

**PAL/ALP:** Alkaline Phosphatase

**PLT:** Platelets

**RBC:** Red Blood Cells

**RNS:** Reactive Nitrogen Species

**ROS:** Reactive Oxygen Species

***S. tenacissima* L:** *Stipa tenacissima* L

**SD**: Standard Deviation

**SEM:** Standard Error of the Mean

**SOD:** Superoxide Dismutase

**TGO:** Transaminase Glutamo-Oxaloacétique

**TGP:** Transaminase Glutamo-Pyruvique)

**TPC:** Total Phenolic Content

**TRIG:** Triglyceride

**WBC**: White Blood Cells

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# الملخص

تهدف هذه الدراسة للتقييم الكمي الفيتو كيميائي، والخصائص المضادة للأكسدة، بالإضافة إلى دراسة السمية الحادة لنبتة *Stipa tenacissima* L.، وهي نبتة متوطنة وموجودة على نطاق واسع في الجزائر، ومعروفة محليًا باسم "الحلفاء". وعلى الرغم من استعمالاتها التقليدية المتعددة والمهمة، إلا أن القليل جدا من الأبحاث العلمية التي تناولت تأثيراتها العلاجية الحيوية.تم تحضير ثلاثة مستخلصات للجزء العلوي للنبات: مستخلص مائي، ومستخلص كحولي، ومستخلص هيدروكحولي. ثم تَمَّ قياس محتوى المركبات الفينولية والفلافونويدات الكلية، تلاه تحديد النشاط المضاد للأكسدة باستخدام طريقتين DPPH وFRAP.أظهرت نتائج التحليل النوعي أن الجزء الهوائي غني بالفينولات، الفلافونويدات، التربينويدات، الدباغ، الكينونات والصابونينات. وقد أكدت نتائج التحاليل الكمية ذلك، حيث كان المستخلص المائي الأغنى بالفينولات (97.02 mg GAE/g) والفلافونويدات (50.45 mg EQ/g)، كما أظهر أعلى قدرة مضادة للأكسدة عبر اختبار DPPH($IC_{50}$ = 0.062 mg/mL) وFRAP (امتصاص أعلى عند 700 nm) مقارنة بباقي المستخلصات.اما بالنسبة لدراسة السمية حيث تم إعطاء مجموعتين من الفئران جرعتين من المستخلص المائي عن طريق المريء (2 و5 غ/كغ) فلم تُسجل أي حالة وفاة، وكانت الجرعة المميتة $LD_{50}$ أكبر من 5000 ملغ / كغ، لكن ظهرت علامات طفيفة على السمية عند الجرعة العالية، تمثلت في ارتفاع إنزيمي الكبد ALT وAST، والتهاب كبدي معتدل في الفحص النسيجي، دون وجود ضرر كبير على الكلى.

ختامًا، تُبرز هذه النتائج غنى هذا النوع النباتي بالمركبات الثانوية الفعالة مثل الفلافونويدات، الصباغات، الكينونات، والتربينات، والتي تلعب دورًا أساسياً في آليات الدفاع البيولوجي. وتُعد *Stipa tenacissima* L مصدرًا نباتيًا واعدًا في تطوير منتجات طبيعية ذات فائدة علاجية مهمة، بشرط استعمالها باحتياطات صارمة.



**الكلمات المفتاحية**: التحليل الكمي الفيتوكيميائ، *Stipa tenacissima* L.، النشاط المضاد للأكسدة، السمية الحادة، الجرعة المميتة $LD_{50}$.

# Abstract

This study was conducted to evaluate the phytochemical composition, antioxidant potential, and acute toxicity profile of *Stipa tenacissima* L., an endemic plant widely distributed in Algeria and locally known as “Halfa”. Despite its various and significant traditional uses but limited scientific research has explored for its crucial therapeutic effects.

Three extracts from the aerial part of *Stipa tenacissima* L. were prepared: aqueous extract (decoction), ethanolic extract (maceration under reflux), and hydroalcoholic extract (cold maceration). The total polyphenol and flavonoid contents were quantified, followed by the evaluation of antioxidant activity using two methods: DPPH and FRAP.

Phytochemical screening revealed that the aerial part is rich in phenols, flavonoids, terpenoids, tannins, quinones, and saponins. Quantitative analysis confirmed this, showing that the aqueous extract contained the highest phenolic (97.02 mg GAE/g) and flavonoid content (50.45 mg EQ/g), and demonstrated the strongest antioxidant activity (DPPH $IC_{50}$ = 0.062 mg/mL) and highest reducing power in FRAP assay.

For the toxicity study, two doses of the aqueous extract (2 and 5 g/kg) were orally administered to groups of mice. No mortality was observed, so the lethal dose ($LD_{50}$) was greater than 5000 mg/kg, but slight signs of toxicity have appeared at the higher dose, including increased hepatic enzyme levels (ALT and AST) and moderate hepatic inflammation on histological examination, with no significant renal damage.

These results highlight the richness of this species in bioactive secondary metabolites such as flavonoids, tannins, quinones, and terpenes, which play an important part in biological defense processes.

In conclusion, *Stipa tenacissima* L. is a promising plant source for the production of natural compounds with significant therapeutic potential, provided that strict safety controls are ensured in its use.



**Keywords**: Phytochemical screening, *Stipa tenacissima* L., Antioxidant activity, Acute toxicity, $LD_{50}$.

# Résumé

Cette étude a été menée afin d'évaluer la composition phytochimique, le potentiel antioxydant ainsi que le profil de toxicité aiguë de *Stipa tenacissima* L., qui est une endémique plante largement répandue en Algérie et connue sous le nom « Halfa ». Malgré ses usages traditionnels variés et importants, peu de recherches scientifique ont été exploré ses effets thérapeutiques crucial.

Trois extraits de la partie aérienne de la plante *Stipa tenacissima* L ont été préparés : aqueux (décoction), éthanolique (macération sous reflux), et hydroalcoolique (macération à froid). Le dosage des polyphénols et flavonoïdes a été quantifié suivi par l'évaluation de l'activité antioxydant par deux méthodes DPPH et FRAP.

Le criblage phytochimique a révélé que la plante est riche en phénols, flavonoïdes, terpènoïdes, tanins, quinones et saponines. Ces résultats ont été confirmés par l'analyse quantitative, montrant que l'extrait aqueux contenait la plus forte teneur en polyphénols (97.02 mg GAE/g) et flavonoïdes (50.45 mg EQ/g), ainsi que la meilleure activité antioxydante ($IC_{50}$ DPPH = 0.062 mg/mL) et un pouvoir réducteur élevé dans le test FRAP.

Pour l'étude toxicologique, deux doses ont été administrées par voie orales (2 et 5 g/kg) de l'extrait aqueux à des groupes de souris. Aucune mortalité n'a été observée donc la dose létale ($DL_{50}$) était supérieure à 5000 mg/kg, mais légère signes de toxicité sont apparus à la dose élevée : augmentation des enzymes hépatiques ALT et AST, et inflammation hépatique modérée à l'examen histologique, sans atteinte rénale significative.

Ces résultats mettent en évidence la richesse de cette espèce en métabolites secondaires actifs tels que les flavonoïdes, les tanins, les quinones et les terpènes, qui jouent un rôle clé dans les mécanismes de défense biologique.

En conclusion, *Stipa tenacissima* L. représente une source végétale prometteuse dans le développement de produits naturels ont un intérêt thérapeutique important seulement sous réserve d'utilisation rigoureuse.

**Mots-clés :** Criblage phytochimique, *Stipa tenacissima* L., Activité antioxydant, Toxicité aiguë, $DL_{50}$