Ministry of Higher Education and Scientific Research Larbi Tebessi University-Tebessa-

Faculty of Exact Sciences, Natural Sciences and Life Sciences Department of Applied Biology

Domain of Natural and Life Sciences

Thesis

Presented for the Partial Fulfillment of the Requirements for the Master's.

Degree In: Biological Sciences

Option: -Plant Ecophysiology.
- Animal Ecophysiology.
-Animal production.

Entitled:

Azolla Caroliniana cultivation for animal's feed

Presented by:

Ahlem TAIB.	Ecophysiology Plant.
Dounia MALLOUK.	Ecophysiology Plant.
Abd Elhakim BESKRI.	Ecophysiology Animal
Ouail SOULTANI.	Ecophysiology Animal
Saif eddine KHNISSI.	Production Animal.

Before the Jury:

Name and First name	Grade	Etablissment	Quality
Djalleb Sihem	Senior Lecturer A	Univ. Chikh Larbi Tbessi Tebessa	**Presidant**
Guenez Radjaa	Senior Lecturer A	Univ. Chikh Larbi Tbessi Tebessa	**Supervisor**
Bouabida Hayette	Profissor	Univ. Chikh Larbi Tbessi Tebessa	**Supervisor**
Sghir Hanan	Senior Lecturer A	Univ. Chikh Larbi Tbessi Tebessa	**Examinaer**


بسم الله الرحمن الرحيم

THANK

Acknowledgments

First and foremost, we express our deepest gratitude to **Almighty God**, the source of strength, peace, and inspiration, who granted us the ability and perseverance to complete this work. All praise is due to **Allah**, through whose grace good deeds are accomplished and goals are achieved. May peace and blessings be upon the best of creation, our Prophet **Muhammad (peace be upon him)**.

We extend our sincere appreciation to everyone who supported and stood by us throughout our academic journey. Your encouragement has played a vital role in the realization and success of this research.

We would like to express our heartfelt thanks to our **supervisor**, **Dr. Guenez Radja**, for her invaluable guidance, constructive feedback, and continuous support. Her expertise and dedication were instrumental in shaping the direction of our research and deepening our understanding of the biological sciences. We are truly grateful for her unwavering mentorship.

Our warmest thanks also go to **Prof. Bouabida Hayette** for her ongoing support, insightful advice, and valuable suggestions, which significantly enriched the quality of this work.

We express our sincere appreciation to **Assistant Head of Laboratory, Ms. Ben Zaïm Karima**, for her assistance, technical support, and dedication throughout the various stages of this project.

We are honored to extend our deepest respect and thanks to the **members of the thesis examination committee** for accepting to evaluate our work and for their constructive remarks and scientific insights.

Our gratitude also goes to all the **professors of the Department of Natural and Life Sciences – Division of Biological Sciences**, whose teachings and expertise have guided us throughout our academic formation.

Special thanks to the **University Business Incubator Management** and to all the **professors and administrative staff** who offered their support, resources, and encouragement during the completion of this project.

هداء ملوك دنيا

باسم الله الرحمن الرحيم

﴿**وَقُلْ رَبِّ زِدْنِي عِلْمًا** ﴾ سورة طه114

الحمد لله الذي علم الإنسان ما لم يعلم، وبنعمته تتمّ الصالحات، وبتوفيقه نُذلل الصعاب ونصل إلى أعلى المراتب. وفي لحظة طال انتظارها، لحظة تُختصر فيها مشاعر الفخر والتعب والسهر، أُهدي هذا الإنجاز المتواضع: إلى نفسي...يا من سَهرتِ الليالي، تحدّيتِ الظروف، ولم تستسلمي أمام العوائق، لكِ أقول: تعِبتِ.. نعم، ولكنكِ وصلتِ وفي دربِ المجدِ قد شمختِ فيا نفسُ ابتهجي.. فهذا التاجُ حقًا قد لبستِ

إلى أبي الغالي(**ملوك الطيب)** يا من كان دعاؤك لي طريقًا للنجاح، وسندك قوتي كلما ضعفت، وابتسامتك دافعًا لي للاستمرار. يا نبض القلب يا منبع فخر واعتزاز لك الفضل بعد الله في كل إنجاز.

إلى أمي الحنونة (براي نعيمة) يا نبع الحنان، ويا من صنعتِ من صبرك دربًا مضيئًا أمشي عليه، فكل حرف كتبته، كان بدعائك ودمعتك الخفية. أمي يا سر النور في دنياي يا شمعة عمري ودفء خطاي لولاكِ بعد الله ما وصلت، وما عرفت طعم النجاح إلى عائلتي الحبيبة... إخوتي الأعزاء، ومنهم سامي... أخواتي الغاليات، ومنهن سماح، إكرام...أنتم سندي وفرحي، كنتم الجدار الذي استندت عليه في كل لحظة ضعف، لكم كل الامتنان من أعماق القلب.

الى الاستاذة الفاضلة الدكتورة **قنز رجاء**

التي كانتْ نبراسًا يُضيء دروب العلم، وبصمةً خالدةً في مسيرتي الأكاديمية. إليكِ أهدي هذا الجهد المتواضع، عرفانًا بفضلكِ، وشكرًا على عطائكِ الذي لا ينضب، وصبركِ الذي كان سندًا لي في أصعب اللحظات. لقد كنتِ أكثر من مُعلمة، كنتِ مُرشدةً ومُلهمةً، علّمتني ليس فقط العلم، بل الإنسانية والتفاني. فجزاكِ الله خيرًا، وجعل عطاءكِ في ميزان حسناتكِ. شكري وتقديري لكِ لا تفيه الكلمات، فلقد كنتِ الموجّه والداعم، المعلمة والمُلهمة.

إلى صديقاتي الرائعات...

أحلام تايب، مباركية روميسة، ربوش زوليخة، جباري وفاء...كنتنّ الأخوات في قلب الغربة، والرفيقات في درب العلم، ضحكاتكنّ خففت عني الكثير. واليكم منّي خالص الشكر على دعمكم، وصدقتكم، ومرافقتكم لي في هذه الرحلة.

إلى الأستاذ القدير علي العربي علمك نبع ننهل منه، لك مني جزيلا الشكر والتقدير. وإلى كل من ساندني، بكلمة، بدعاء، بابتسامة...

لكم جميعًا أقول:

نجاحي اليوم هو ثمرة حبكم، ودعمكم، وإيمانكم بي فلكم كل المحبة... وكل الامتنان... وكل فخر يحمل اسمي وإياكم

دنيا ملوك

إهداء تايب أحلام

بعد بسم الله شكر لله عز وجل اولا على نعمه وفضله وتوفيقه لي في مسيرتي الدراسية، الذي مكنني من إتمام هذا العمل واتمامه على أكمل وجه الحمد لله دائما وأبدا.

أهدي هذا الإنجاز إلى نفسي...

التي عملت بجد وتفانٍ لتحقيق هذا الحلم وإلى روحي التي لم تيأس أبدًا، أهدي هذا العمل كتعبير عن فخري بنفسي وشكرا وتقديرا لي على الصبر والمثابرة التي أظهرتها خلال فترة الدراسة وأنا فخورة جدا بنفسي على ما حققته، وسأسعى دائمًا لتحقيق المزيد ان شاء الله."

إلى **والدي** عزيز قلبي...

الذي طالما كان داعما وسند لي ومسند اتكئ عليه في أصعب اوقاتي وفيها كلها ودائما ما اجده ثابتا لا يميل عني ولا يمل اهديك كل كلمة وكل حرف لك راجية من الله عز وجل ان يتقبله صدقة جارية في ميزان حسناتك اليك شكرا كثيرا والدي

تايب معمر.

الي **والدتي** روح الروح...

التي كانت داعمتي وملهمتي على ان اكون اليوم هنا وفي هذا المكان بينكم شكرا لك أمي أنك جعلتي مني شخص ناجحا ذو خلقا حسنا يقوم اليوم بتكريمك بهذا العمل وهذا الانجاز كهدية لك ولوالدي على ما سهرتي وما داعيتي لي من ايام ولياليي

شكرا جزيلا أمي **تايب خضرة**

اشكر جميع افراد عائلتي على دعمهم المستمر وتشجيعهم لي طوال فترة دراستي كل من اخوتي **محمد** و**حمزة** واخواتي **هناء ايمان فاتن** وخاصة اختي **تايب هناء** على دعمها لي بشتى انواع الاعانة في فترة انجازي هذا المشروع " واخص بالذكر براعم العائلة المدلل **شهاب** و توأم الروح **زينب والاء**.

اشكر **زوجي** و**عائلته** على دعمهم وحسن استقبالهم وتشجيعهم لي في هذه الفترة ."

إلى أستاذتي الفاضلة **فنز رجاء...**

التي كانت لي قدوة في العلم والاخلاق، التى من لم تبخل على بعلمها وتوجيهاتها السديدة، اهدي لك هدا العمل كعربون شكر ووفاء، وامتنان لا يوفيه الكلام، اسأل الله ان يجعل هذا العمل في ميزان حسناتك شكرا.

الى **صديقاتي...**

كل من ملوك دنيا ومباركية رميسة وربوش زوليخة وجباري وفاء شكرا لكم على دعمكم ومساندتكم لي في هذه الرحلة الأكاديمية واعانتكم في انجاز هده المدكرة وعلى مشاركتكم لي في لحظات الفرح والتحديات التي جعلت منكم اعز اصدقاء لي ومسندا في وقت الحاجة شكرا جزيلا لكم دمتم لي خيرت الاصدقاء.

ختاما

أتمنى أن يكون هذا العمل إضافة قيمة في مجال تخصصي، وأن يكون خالصًا لوجه الله تعالى أشكر كلمن ساهم في إنجاح هذا العمل، وأسأل الله تعالى أن يجعله في ميزان حسناتنا جميعًا.

تايب احلام

إهداء بسكري عبد الحكيم

بسم الله الرحمن الرحيم

الحمد لله أولًا وآخرًا، ظاهراً وباطنًا، الذي بنعمته تتمّ الصالحات، الحمد لله الذي وفق وسدّد، وعلّم الإنسان ما لم يعلم،

والصلاة والسلام على خير خلق الله، سيدنا محمد، منارة العلم، وقدوة السائرين على طريق الحق والمعرفة.

إلى **والديَّ العزيزين،**

يا من غرستما في نفسي الإيمان، وزوّدتماني بقوة الصبر والعزيمة، يا من كنتم ولا زلتم رمز التضحية والبذل، كل حرف في هذه الصفحات هو ثمرة من غراس أيديكما، ودعواتكما، فجزاكما الله عني خير الجزاء، وأطال الله في أعماركما في طاعته ورضاه.

إلى عائلتي الكريمة، إخوتي وأخواتي، ومن كان دعاؤه وابتسامته ووقوف الصادق دعمًا يُقدّر بثمن، لكم مني أصدق مشاعر الامتنان. والتقدير أسأل الله أن يبارك فيكم ويجمعنا دائمًا على الخير والمودة.

إلى أصدقائي الصادقين،

أنتم من خففتم عني ثقل الأيام، من كنتم بجانبي بالكلمة الطيبة، والرفقة الحسنة، والدعاء في الغيب، لكم كل الشكر، ولكم مكانة لا تزول في القلب.

وإلى **أستاذتي** الفاضلة،

من كانت مثالًا للعلم، والخلق، والصدق في التوجيه، جزاكِ الله عنّا خير الجزاء، وشكرًا لك على كل لحظة منحتِنا فيها من علمك، وكل نصيحة كان لها أثر في مسيرتنا، نسأل الله أن يجعل ذلك في ميزان حسناتك، وأن يرفع قدرك في الدنيا والآخرة.

وختامًا،

الى كل من ساهم، ولو بكلمة أو دعوة أو دعم صامت،اهدي هذا الجهد المتواضع، وأسأل الله أن يكون خالصًا لوجهه الكريم، نافعًا لعباده.

بسكري عبد الحكيم

إهداء سلطاني وائل

إلى من كانت دعواتهم سرّ توفيقي ونجاحي...إلى من ساندني في أحلك الظروف والليالي إلى من شجعني ودفعني نحو الأمام وآمن بي ومددني بالقوة والمحبة

إلى والديّ العزيزين **سلطاني زهير وفطوم**، نبع الحنان والأمان، وإلى إخوتي **لؤي ونور الإسلام وأميرة**

إلى خالتي وأستاذتي وامي الثانية **سلطاني إلهام** وإلى كل أفراد عائلتي، من ساندوني بصمت ومحبة، أهديكم بمحبة هذا العمل الجميل لكم مني كل الامتنان والعرفان.

إلى حبيبتي الغالية، رفيقة الروح ومصدر الإلهام في لحظات اليأس والتعب، شكرًا لأنك كنتِ النور في طريقي، والسند في كل مراحل هذه الرحلة أهديك هذه المذكرة، ففي كل صفحة منها أثرٌ من حبك ودعمك.

وإلى صديقي العزيز **بسكري عبد الحكيم**، رفيق الدرب، من شاركني لحظات التعب والفرح، شكرًا على كل كلمة مشجعة، وكل وقفة صادقة، كنت دومًا أكثر من مجرد صديق.

وإلى أستاذتي الفاضلة **قنز رجاء**،

التي كانت لي خير مُوجهة وملهمة، شكراً لصدق توجيهك، لرحابة صدرك، ولحرصك على ان نكون أفضل. سيبقى دعمك العلمي والإنساني محفورًا في قلبي.

وإلى كل من آمن بي ودفعني للاستمرار...

هذه المذكرة ثمرة تعب، ودليل امتنان، وبداية لمسيرة أرجو أن تكون مباركة.

سلطاني وائل

اهداء خنيسي سيف الدين

﴿ **وَقُلْ رَبِّ زِدْنِي عِلْمًا** 114﴾ سورة طه.

الحمدلله الدي بنعمته تتم الصالحات,و بتوفيقه تتحقق الغايات,الحمدلله عدد ما خطت الاقلام,و عدد ما رفعت الاعلام.

الى نفسي...

شكرا لي,على الصبير حين كان الطريق صعبا,و على العزيمة التي لم تخب رغم التعب,كنت قويا رغم كل شي.

الي ابي الغالي...

يا من غرست في قلبي معنى الطموح,و بنيت هدا المجد البسيط,ابي يا منبع الامان و الحنان اهدى اليك تعبي,و نجاحي,و الامتنان, وجودك في حياتي نعمة, و دعاؤك طوق نجاحي.

الي امي الحبيبة...

امي,يانبع الحنان,يا من حملتني بدعائك و خوفك و صبرك,كنت السند و الظل و النبض,لك في قلبي كل الامتنان و الحب.

الي استادتي االمؤطرة قنز رجاء...

لك مني اسمى ايات الشكر و الامتنانو التقدير,فقد كنت خير مرشد,و خير مشرق,وخير من منحني الامل و الدعم طوال هدا المشوار.

الى كل من كان له اثر في مسيرتي,بكلمة,بدعاء,بابتسامة... شكرا من القلب,فبكم كانت الرحلة اجمل و اخف.

خنيسي سيف الدين

Abstract

This study investigates the physical, chemical, and medicinal properties of *Azolla caroliniana*, focusing on its potential applications in agriculture, animal feed, and pharmaceuticals. The research was conducted through field and laboratory experiments at the Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa.

Field Study:

The experiment took place in a controlled greenhouse using a cultivation basin measuring 2.5 m × 1 m. The water was carefully monitored for purity and enriched with micronutrients essential for nitrogen fixation. Over a period of three months, a rapid increase in biomass was observed within the first 15 days, demonstrating the high growth efficiency of *Azolla caroliniana*. The plant's symbiotic relationship with *Anabaena azollae* enabled atmospheric nitrogen fixation, which enhances soil fertility and reduces dependence on chemical fertilizers, thereby supporting sustainable agriculture.

Laboratory Study – Rabbit Feeding Trial:

Fifteen rabbits were divided into three groups: Group 1 (control) received traditional feed; Group 2 was given Azolla extract (1 ml per 100 ml water); and Group 3 was fed fresh Azolla directly. The feeding trial lasted 30 days, with body weights recorded every 3 days. Statistical analysis using ANOVA and t-tests revealed significant differences in weight gain among the groups, confirming the nutritional benefits of *Azolla* supplementation.

Blood Analyses:

Blood tests measured total protein, glucose, and lipid levels, showing marked improvements in the supplemented groups, thus confirming the positive nutritional value of *Azolla* as a natural feed supplement.

Chemical Analyses:

Extraction yielded 7 ml of essential oil from 100 g of plant material and 50 ml of ethanolic-aqueous extract from 70 g using a rotary evaporator. Qualitative phytochemical screening identified biologically active compounds including

flavonoids, proteins, saponins, tannins, sterols, mucilages, and compounds with mild narcotic properties, indicating promising pharmaceutical potential.These results highlight the versatile benefits of *Azolla caroliniana* in sustainable agriculture, animal nutrition, and alternative medicine, positioning it as a rich and multifunctional plant with significant promise for both human and animal applications.

Keywords: *Azolla caroliniana*, essential oils, ethanolic extract, chemical analysis, dietary supplement

Resumé

Cette étude porte sur les propriétés physiques, chimiques et médicinales d'*Azolla caroliniana*, en mettant l'accent sur ses applications potentielles en agriculture, alimentation animale et en pharmacie. La recherche a été réalisée à travers des expériences sur le terrain et en laboratoire à la Faculté des Sciences Exactes et des Sciences Naturelles et de la Vie de l'Université de Tebessa.

Étude sur le terrain :

L'expérience a eu lieu dans une serre contrôlée, utilisant un bassin de culture mesurant 2,5 m × 1 m. L'eau utilisée a été soigneusement contrôlée pour sa pureté et enrichie en micronutriments essentiels à la fixation de l'azote. Sur une période de trois mois, une augmentation rapide de la biomasse a été observée dès les 15 premiers jours, démontrant la grande efficacité de croissance d'*Azolla caroliniana*. La relation symbiotique de la plante avec *Anabaena azollae* permet la fixation de l'azote atmosphérique, ce qui améliore la fertilité du sol et réduit la dépendance aux engrais chimiques, soutenant ainsi une agriculture durable.

Étude en laboratoire – Essai d'alimentation chez le lapin :

Quinze lapins ont été divisés en trois groupes : le groupe 1 (contrôle) a reçu une alimentation traditionnelle ; le groupe 2 a reçu un extrait d'*Azolla* (1 ml pour 100 ml d'eau) ; le groupe 3 a été nourri directement avec de l'*Azolla* fraîche. L'essai d'alimentation a duré 30 jours, avec un suivi du poids corporel tous les 3 jours. L'analyse statistique par ANOVA et tests t a révélé des différences significatives de gain de poids entre les groupes, confirmant les bénéfices nutritionnels de la supplémentation en *Azolla*.

Analyses sanguines :

Des analyses sanguines ont mesuré les taux de protéines totales, de glucose et de lipides, montrant des améliorations marquées dans les groupes supplémentés, confirmant ainsi la valeur nutritionnelle positive de l'*Azolla* comme complément alimentaire naturel.

Analyses chimiques :

L’extraction a permis d’obtenir 7 ml d’huile essentielle à partir de 100 g de matière végétale et 50 ml d’extrait éthanolique-aqueux à partir de 70 g grâce à un évaporateur rotatif. Le dépistage phytochemique qualitatif a révélé la présence de composés biologiquement actifs tels que flavonoïdes, protéines, saponines, tanins, stérols, mucilages et composés aux propriétés narcotiques modérées, indiquant un potentiel pharmaceutique prometteur. Ces résultats soulignent les bénéfices polyvalents d’*Azolla caroliniana* en agriculture durable, nutrition animale et médecine alternative, positionnant cette plante comme une ressource riche et multifonctionnelle, avec un fort potentiel pour les applications humaines et animales.

Mots-clés : *Azolla caroliniana*, huiles essentielles, extrait éthanolique, analyses chimiques, complément alimentaire

الملخص

تتناول هذه الدراسة الخصائص الفيزيائية والكيميائية والطبية لنبات الأزلّا كارولينيانا(*azolla carolini*
(*ana* تتناول هذه الدراسة الخصائص الفيزيائية والكيميائية والطبية لنبات الأزلّا كارولينيانا(*Azolla carolini*
(*ana*، مع التركيز على تطبيقاته المحتملة في الزراعة، تغذية الحيوان، والصناعات الدوائية. أُجريت الأبحاث من خلال تجارب ميدانية ومخبرية في كلية العلوم الدقيقة وعلوم الحياة بجامعة تبسة.

:الدراسة الميدانية

أُجريت التجربة في بيت زجاجي مسيطر عليه باستخدام حوض زراعة بقياس 2.5 م ×1م. تمت مراقبة نقاء المياه بدقة وتم إثراؤها بالعناصر الدقيقة الضرورية لعملية تثبيت النيتروجين. خلال فترة ثلاثة أشهر، لوحظت زي ادة سريعة في الكتلة الحيوية خلال أول 15 يومًا، مما يدل على كفاءة نمو عالية لنبات الأزلّا كارولينيانا. تساعد ال علاقة التعايشية مع البكتيريا *أنابينا أزولّا* على تثبيت النيتروجين الجوي، مما يعزز خصوبة التربة ويقلل الاعتماد على الأسمدة الكيميائية، داعماً بذلك الزراعة المستدامة.

الدراسة المخبرية – تجربة تغذية الأرانب

تم تقسيم 15 أرنبًا إلى ثلاث مجموعات: المجموعة الأولى (الضابطة) تلقت التغذية التقليدية؛ المجموعة الثان ية تلقت مستخلص الأزلّا (1 مل لكل 100 مل ماء)؛ والمجموعة الثالثة تم إطعامها مباشرة بالأزلّا الطازجة. استم رت التجربة لمدة 30 يومًا، وتم تسجيل أوزان الجسم كل 3 أيام. أظهرت التحليلات الإحصائية باستخدام اختبار ANOVAواختبار t فروقات معنوية في زيادة الوزن بين المجموعات، مما يؤكد الفوائد الغذائية للمكمل بالأزلّا.

التحاليل الدموية

تم قياس البروتين الكلي، والجلوكوز، ومستويات الدهون في الدم، وأظهرت النتائج تحسناً واضحاً في المجم وعات المكملة، مما يؤكد القيمة الغذائية الإيجابية لنبات الأزلّا كمكمل غذائي طبيعي.

التحاليل الكيميائية

تم استخراج 7 مل من الزيت العطري من 100 جم من المادة النباتية، و50 مل من المستخلص الإيثانولي ال مائي من 70 جم باستخدام جهاز التبخير الدوراني. كشفت الفحوصات الكيميائية النوعية عن وجود مركبات نشطة بيولوجيًا مثل الفلافونويدات، والبروتينات، والصابونينات، والعفص، والستيرولات، والمخاطيات، ومركبات ذات خصائص مخدرة خفيفة، مما يدل على إمكانات دوائية واعدة.

تُبرز هذه النتائج الفوائد المتعددة لنبات الأزلّا كارولينيانا في الزراعة المستدامة، تغذية الحيوان، والطب البد يل، مما يجعله نباتًا غنيًا ومتعدد الوظائف ذو إمكانات كبيرة للتطبيقات البشرية والحيوانية.

الكلمات المفتاحية: الأزولّا كارولينيانا، الزيوت العطرية، المستخلص الإيثانولي، التحليل الكيميائي، المكمل الغذائي.

Table of Contents

Acknowledgment

Dedication

Dedication

Dedication

Dedication

Dedication

Résumé

Abstract

ملخص

Introduction : **1**

Chapter I: Azolla Caroliniana **2**

1.Definition of *Azolla caroliniana* plant (discovery and habitat) **3**

2.Taxonomic classification of the *azolla caroliniana* plant: **4**

2.1. Types of azolla plant: **4**

2.1.1. Azolla filiculoides: **05**

2.1.2. Azolla pinnata **05**

2.1.3. Azolla mexicana **05**

2.1.4. Azolla microphylla **05**

2.1.5. Azolla rubra **05**

***2.2. Azolla caroliniana* plant morphology (structure and external form)** **06**

2.3. Morphological Characteristics: **06**

2.3.1. Size: **06**

2.3.2.Roots 07

2.3.3. Symbiotic Feature: 07

3. Uses of *Azolla caroliniana* plant 08

3.1. Agricultural Applications 08

3.1.1. Green Manure 08

3.1.2. Animal Feed 08

3.2. Environmental Applications 09

3.2.1. Water Purification 09

3.2.2. Methane Reduction in Rice Fields 09

3.2.3. Desertification Control 09

3.3. Medicine and Pharmacy (Future Prospects) 09

4. General benefits of the *Azolla caroliniana* plant: 10

4.1. Key Benefits: 10

4.1.1. Improves Soil Fertility: 10

4.1.2. High-Protein, Sustainable Natural Feed: 10

4.1.3. Water Purification: 10

4.1.4. Reduction of Agricultural Costs: 10

4.1.5. Support for Permaculture and Sustainable Farming: 10

4.1.6. Scientific and Research Value: 11

4.1.7. Potential Beyond Feed and Fertilizer 11

Chapter II : Materials and Methods 11

1. Materials and Methods 12

1.1. Objective 13

1.2. Plant Material 13

1.2.1. Method of Cultivation 14

1.2.2. Extraction of Essential Oils 14

1.2.3. Extraction Process 15

1.3. Quantitative Analysis 16

1.3.1. Determination of Total Phenols 16

1.3.2. Determination of Total Flavonoids 16
1.3.3. Preparation of the Hydro-Ethanolic Extract 17
1.3.4. Maceration 17
1.3.5. Filtration 17
1.3.6. Determination of Hydro-Ethanolic Extract Yield 17
1.4. Phytochemical Screening 18
1.4.1. Qualitative Analysis 18
a. Phytochemical tests, or screening, 18
b. Flavonoids 18
c. Saponosides 18
d. Anthocyanins 19
e. Proanthocyanidols 19
f. Mucilages 19
g. Narcotics 19
h. Proteins 20
i. Sterols and Triterpenes 20
j. Alkaloids 20
k. Tannins 21
l. Anthraquinones 21
1.5. Animal Material 21
1.5.1. Administration of *Azolla caroliniana* Extract 22
1.5.2. Rabbit Care and Feeding 21
2. Monitoring of Weight Variation 24
3. Blood Sample Collection 24
4. Statistical Analysis of Results 25
Chapter III 27
Chqpter III. Results and Discussion 27
1. Results 28
1.1. Cultivation 28

1.2. Phytochemical Screening 29

1.3. Physiological Parametres 31

a. Effect on Body Weight 31

b. Effect of the Plant and Extract of *Azolla caroliniana* on Blood Parameters 32

c. Effect of the Plant and Extract of *Azolla caroliniana* on Biochemical Parameters 33

2. Discussion 34

2.1 Discussion 34

2.2. Nutritional Impact on Growth: 35

2.3. Biochemical Effects and Organ Protection: 35

Implications for Sustainable Animal Nutrition: 35

2.4. Limitations and Future Directions: 35

2.5. Discussion 36

2.6. Nutritional Impact on Growth: 36

Conclusion 38

Bibliographie 40

Figures liste

Figure01: *Azolla Caroliniana (*B.C.Ghosh,2018).....03

figure02: Types of *Azolla caroliniana* plant (Don Loarie, 2020).....06

Figure03: Morphological characteristics of *Azolla Caroliniana* (Jan De Veri, 2018).....07

Figure04: The green approach of Azolla for different applications (Ting, 2024).....08

Figure05: General benefits of the *Azolla caroliniana* plant. (Mathur, 2024).....11

Figure06: *Azolla caroliniana* in a greenhouse pool (Personal Photo, 2025).....14

Figure07: Extraction of Essential Oils (Personal Photo, 2025).....16

Figure08: Yield of hydro-ethanolic extract from *Azolla caroliniana*. (Personal photograph, 2025).....18

Figure09: Rabbits housed in the animal facility during the experiment. (Personal photograph, 2025).....22

Figure10: Preparation and oral administration of *Azolla caroliniana* extract to rabbits using gavage. (Personal photograph, 2025).....23

Figure11: Distribution of rabbits by groups and treatments. (Personal photograph, 2025)...24

Figure12: Blood sample collection from rabbits via cardiac puncture. (Personal photograph, 2025).....25

Figure13:*Azolla caroliniana* crop cultivated under controlled conditions. (Personal photograph, 2025).....28

Figure14: Effect of the plant and the extract of *Azolla caroliniana* on weights body...31

Figure15: Effect of the plant and the extract of *Azolla caroliniana* on hematological parameters.....33

List Table

Table 01: Classification of *Azolla caroliniana* **(Asghar, 2022)**.....01

Table 02: Apparatus, Organic Materiale, For Cultivation.....14

Table 03: Apparatus, Glassware, and Products Used for Extraction.....15

Table 04: Distribution of rabbits by groups and treatment protocols.....23

Table 05. Phytochemical tests Extracts of *Azolla caroliniana*.....29

List of Abbreviations

AG : Gallic Acid

VS : Erythrocyte Sedimentation Rabbits (ESR)

$AlCl_3$: Aluminum Chloride

ASLO : Antistreptolysin O

$CHCl_3$: Chloroform

CRP : C-Reactive Protein

EAG : Gallic Acid Equivalent

ECAT : European Concerted Action on Thrombosis

EDTA : Ethylenediaminetetraacetic Acid

EQ : Emotional Quotient

ER : Escorteur Rapide (Rapid Escort) - *Note: This abbreviation seems out of context for a scientific paper, consider if it's truly relevant.*

$FeCl_3$: Ferric Chloride

FNS : Complete Blood Count (CBC)

GB : White Blood Cells (WBC)

GC : Glucocorticoids

GR : Red Blood Cells (RBC)

H_2SO_4 : Sulfuric Acid

HCl : Hydrochloric Acid

HGB: Hemoglobin

HTC : Hematocrit

IFNγ : Interferon gamma

KOH : Potassium Hydroxide

MC : Crohn's Disease

MCH : Mean Corpuscular Hemoglobin

MCHC : Mean Corpuscular Hemoglobin Concentration

MPV : Mean Platelet Volume

MS : Dry Matter

Na_2CO_3 : Sodium Carbonate

NaOH : Sodium Hydroxide

NH_4OH : Ammonium Hydroxide

OMS : World Health Organization (WHO)

PGE : Prostaglandin

PLT : Platelets

PMN: Polymorphonuclear Leukocytes

PNN : Polymorphonuclear Neutrophils

UV : Ultraviolet Radiation

VGM : Mean Corpuscular Volume (MCV)

Introduction



Introduction

In light of the environmental and economic challenges faced by modern societies, there is an increasing need to explore sustainable and innovative solutions based on the intelligent and responsible use of natural resources. Among the promising plant organisms attracting growing interest from researchers and investors is *Azolla caroliniana*, a small aquatic fern belonging to the family Azollaceae. It grows floating on the surface of stagnant or slow-moving waters such as swamps, canals, and flooded fields, and is especially notable for its unique symbiotic relationship with *Anabaena azollae*, a nitrogen-fixing cyanobacterium. This association makes *Azolla* an important natural source of biological nitrogen, traditionally used for centuries in rice cultivation (Peters & Meeks, 1989).

Azolla caroliniana is characterized by rapid vegetative growth, high biomass productivity, and the ability to thrive without intensive agricultural inputs (Lumpkin & Plucknett, 1982). These traits, combined with its rich nutritional content proteins, amino acids, vitamins, and minerals make it an attractive low-cost feed alternative for animals such as poultry, rabbits, fish, and cattle (Becerra *et al*., 1995; Alalade & Iyayi, 2006). Its use helps reduce dependence on industrial feed and supports local agricultural production (Pillai *et al*., 2005).

What further distinguishes *Azolla* is its versatility and multidisciplinary potential. Recent studies have demonstrated its capacity to contribute to various fields beyond agriculture. Its phytochemical composition includes bioactive compounds such as flavonoids, tannins, and saponins with antioxidant, antimicrobial, and anti-inflammatory properties (El-Gendy *et al*., 2021; Della Greca *et al*., 2009), positioning it as a promising natural source for pharmaceutical and nutraceutical products. Other research highlights its role in water purification (Reddy & D'Angelo, 1997), biofuel production (Pandey & Sree, 2015), and even in cosmetics (Van Hove & Lejeune, 2002).

Despite these well-documented benefits, *Azolla caroliniana* remains significantly underutilized in many parts of the world, particularly in regions such as Algeria, where conventional agricultural practices still rely heavily on expensive chemical fertilizers and industrial feed. This underutilization is largely due to a lack of localized research, limited awareness of its potential, and the absence of innovative

business models (Wagner, 1997; UNEP, 2011).

This presents a growing concern: although *Azolla* offers scientifically proven advantages in agriculture, animal nutrition, medicine, and environmental management, its full potential remains untapped. The key challenge is how to transform this natural resource into a practical and profitable solution adapted to local socio-economic and environmental conditions.

Therefore, this study aims to explore the scientific valorization and economic potential of *Azolla caroliniana* under local conditions—specifically in the Tebessa region by analyzing its physicochemical properties, biological activity, and possible applications in agriculture, animal feed, and pharmacology. At the same time, it seeks to evaluate the feasibility of developing a sustainable startup centered around this plant, aligned with global shifts toward green economy principles (UNEP, 2011).

In this context, the study focuses on evaluating the benefits of the rare *Azolla caroliniana* plant by examining its applications in agriculture, fodder, and pharmaceuticals. The experiments were conducted at the University of Tebessa, Faculty of Natural and Life Sciences, where *Azolla caroliniana* was cultivated for three months with daily monitoring. For the fodder application, trials were carried out on an animal model (rabbits), which were administered an extract and fresh *Azolla caroliniana* daily for 30 days, with daily monitoring to record any changes.

This work is divided into two main parts:

- The first part (Chapter 1) is devoted to a literature review of the *Azolla caroliniana* plant.
- The second part (Chapters 2 and 3) presents the experimental methodology, obtained results, their interpretation, and discussion.
- Finally, the study concludes with a general conclusion and prospects for future research.

Chapter I :

Azolla caroliniana



5.Definition of *Azolla caroliniana* plant (discovery and habitat):

Azolla caroliniana is a small free-floating aquatic fern that thrives on the surface of stagnant or slow- moving freshwater bodies. It is distinguished by its rapid vegetative reproduction and its remarkable ability to form dense mats, covering the water surface in a short time (Wagner, 1997; Van Hove & Lejeune, 2002).

Although Azolla has been known for centuries, systematic scientific study began in the 18th century due to growing interest in its unique biological features. The plant's significance largely stems from its symbiotic relationship with the cyanobacterium Anabaena azollae, which resides in its leaf cavities and fixes atmospheric nitrogen into bioavailable forms for the plant and surrounding environment (Peters & Meeks, 1989; Becking, 1979).

The species *Azolla caroliniana* was first documented in the United States in 1783, and was formally classified by the French naturalist Jean-Baptiste Lamarck (Lamarck, 1783). Since then, it has gained attention not only for its botanical interest but also for its ecological and agricultural benefits. Today, it is cultivated in several regions of the world and used in rice paddies, animal feed systems, and water management projects (Lumpkin & Plucknett, 1980; Watanabe, 1992)



Figure 01: *Azolla Caroliniana (*B.C.Ghosh,2018).

2.Taxonomic classification of the *azolla caroliniana* plant:

Azolla caroliniana is a non-flowering vascular plant, belonging to a primitive group of plants that reproduce through spores instead of seeds. It is part of the group known as pteridophytes, which are characterized by vascular tissue but lack flowers and seeds (Smith *et al.*, 2006). The plant exhibits traits typical of early-evolved ferns, yet it has also developed unique adaptations, including a symbiotic relationship with nitrogen-fixing cyanobacteria (*Anabaena azollae*).

Table 01: Classification of *Azolla caroliniana* **(**Zimmerman**, 1989)**

Kingdom:	Plantae.
Division:	Pteridophyta (Ferns).
Class (Sect):	Polypodiopsida.
Order (Rank):	Salviniales.
Family:	Azollaceae.
Genus:	Azolla.
Species:	*Azolla caroliniana* Willd.

As a member of the order Salviniales, *Azolla caroliniana* is grouped with aquatic ferns that display heterospory, producing both microspores and megaspores (Tryon and Tryon, 1982). The genus Azolla is notable for its rapid growth, ecological importance, and the ability to colonize freshwater environments worldwide (Wagner, 1997)

2.1. Types of azolla plant:

In addition to *Azolla caroliniana*, the genus *Azolla* includes five other main species, each characterized by specific morphological traits, environmental preferences, and geographic distributions. These species vary in size, leaf structure, growth rate, and temperature or salinity tolerance, which makes them suitable for

different agricultural, ecological, and industrial applications (Van Hove & Lejeune, 2002; Wagner, 1997).

2.1.1. Azolla filiculoides:

This is the most commonly used species in sustainable agriculture due to its ability to fix nitrogen efficiently. It is widely cultivated in temperate regions and is known for its tolerance to relatively low temperatures (Lumpkin & Plucknett, 1980)

2.1.2. Azolla pinnata

Known for its rapid growth, *A. pinnata* is extensively distributed across tropical and subtropical Asia and Africa. It is particularly favored in rice cultivation systems in countries such as India, China, and the Philippines (Watanabe, 1992)

2.1.3. Azolla mexicana

Native to Central America, this species thrives in freshwater environments. It is adapted to moderate temperatures and is often found in ponds, lakes, and irrigation systems (Becerra *et al*., 1995)

2.1.4. Azolla microphylla

Characterized by very small leaves, (*A). microphylla* grows in warm and humid climates. Its compact structure allows for efficient light absorption and rapid mat formation (Lumpkin & Plucknett, 1980)

2.1.5. Azolla rubra

Found in Australia and New Zealand, this species is notable for its resistance to cold and tolerance of slightly saline water, making it suitable for marginal aquatic environments (Stone, 1981)

Each species has distinct ecological adaptations, and understanding these differences is essential for selecting the appropriate Azolla species for local climatic conditions and desired applications in agriculture, aquaculture, or environmental remediation.



Figuer 02: Types of *Azolla caroliniana* plant (Don Loarie, 2020).

6.*2. Azolla caroliniana* plant morphology (structure and external form):

Despite its small size, *Azolla caroliniana* exhibits a complex morphological structure that supports its high ecological adaptability and biological efficiency. Its anatomy reflects both its aquatic lifestyle and its symbiotic relationship with nitrogen-fixing cyanobacteria.

2.3. Morphological Characteristics:

2.3.1. Size:

The plant typically measures between 1 and 2.5 cm in length, forming dense floating mats on the water surface (Wagner, 1997)

6.1.1. Color:

Its fronds are bluish-green under normal conditions but turn reddish when exposed to high light intensity or direct sunlight due to the increased production of anthocyanin pigments (Lumpkin and Plucknett, 1980)

6.1.2. Leaves (Fronds):

The plant consists of overlapping small leaves, covered with a waxy layer that helps repel water and maintain buoyancy. These leaves are arranged in two lobes, an upper lobe (which floats on water) and a lower lobe (which may be submerged) (Van Hove and Lejeune, 2002)

2.3.2. Roots:

From the ventral side, Azolla produces numerous filamentous roots, which serve primarily for nutrient and water absorption rather than anchorage, as the plant floats freely (Peters and Meeks, 1989)

2.3.3. Symbiotic Feature:

The most distinctive morphological trait of Azolla is the presence of specialized cavities within its dorsal leaf lobes, which house colonies of the cyanobacterium Anabaena azollae. This endosymbiont is capable of biological nitrogen fixation, converting atmospheric nitrogen (N_2) into forms usable by plants, thus giving Azolla an ecological advantage in nitrogen-deficient environments (Becking, 1979)

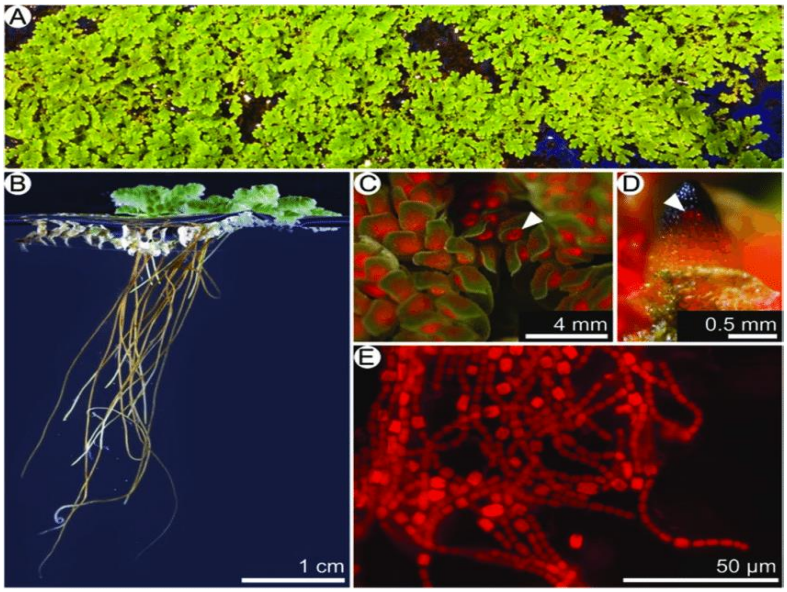


Figure 03 : Morphological characteristics of *Azolla Caroliniana* (Jan De Veri, 2018).

7. Uses of *Azolla caroliniana* plant:

The multifunctional nature of *Azolla caroliniana* makes it a promising organism for a wide range of applications. Its ecological, agricultural, and biomedical potential has been the focus of numerous research efforts around the world (Van Hove and Lejeune, 2002; Wagner, 1997)

7.1. Agricultural Applications

7.1.1. Green Manure:

Azolla forms a symbiotic relationship with *Anabaena azollae*, enabling it to fix atmospheric nitrogen and enrich the soil naturally. When incorporated into the soil, it increases nitrogen content, improving fertility and reducing dependence on synthetic fertilizers (Watanabe, 1992; Lumpkin and Plucknett, 1980)

7.1.2. Animal Feed:

Rich in protein, amino acids, and vitamins, Azolla is a valuable supplementary feed for various animals:

- ✓ **Cows:** Enhances milk yield and improves digestion
- ✓ **Poultry:** Promotes faster growth rates and better feed conversion (Alalade and Iyayi, 2006).
- ✓ **Fish**: Acts as a sustainable and cost-effective protein source in aquaculture (Becerra *and al.*, 1995)

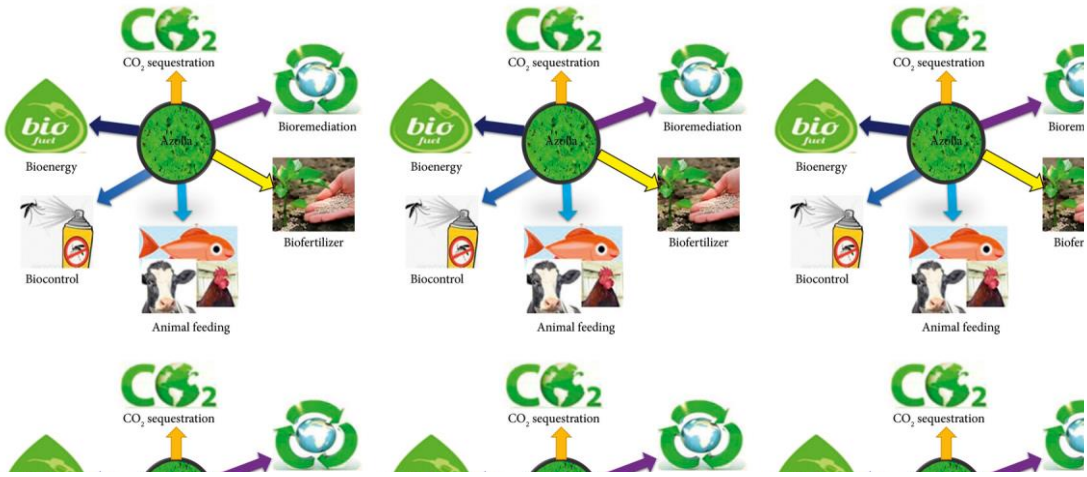


Figure 04 : The green approach of Azolla for different applications (Tingj, 2024).

7.2. Environmental Applications

7.2.1. Water Purification

Azolla caroliniana is capable of absorbing heavy metals, nutrients, and toxins from contaminated or stagnant water bodies, making it useful in phytoremediation and wastewater treatment (Reddy and D'Angelo, 1997)

7.2.2. Methane Reduction in Rice Fields:

By forming a mat on flooded rice fields, Azolla reduces methane emission by blocking light and suppressing methanogenic bacteria (Roger and Ladha, 1992)

7.2.3. Desertification Control:

Azolla can help retain soil moisture and protect the land from erosion when cultivated in shallow water systems, acting as a living mulch in arid zones (Becking, 1979).

7.3. Medicine and Pharmacy (Future Prospects)

Recent studies have revealed that *Azolla caroliniana* contains bioactive compounds such as:

- Antioxidants
- Antibacterial agents
- Anthocyanins and flavonoids

These natural constituents show potential therapeutic effects, and ongoing research is investigating their applications in:

- Infection treatment
- Immune system enhancement
- Cancer prevention (Della Greca and *al*., 2009; Rahman and *al*., 2013)

While still under clinical evaluation, the use of Azolla extracts in nutraceuticals and herbal medicine holds promising potential for the future of sustainable healthcare solutions.

8. General benefits of the *Azolla caroliniana* plant:

Azolla caroliniana is increasingly recognized as a multi-functional plant resource with a wide range of applications in agriculture, ecology, and science. Its unique characteristics make it a valuable component in sustainable development strategies, especially in regions seeking eco-friendly and low-cost solutions (Wagner, 1997; Van Hove and Lejeune, 2002).

8.1. Key Benefits:

8.1.1. Improves Soil Fertility:

Thanks to its symbiotic relationship with *Anabaena azollae*, Azolla fixes atmospheric nitrogen, enriching soil nitrogen content naturally and enhancing overall soil productivity (Lumpkin and Plucknett, 1980).

8.1.2. High-Protein, Sustainable Natural Feed:

Containing 25–35% crude protein on a dry weight basis, Azolla serves as an affordable and renewable feed supplement for livestock, poultry, and fish (Becerra *and al*., 1995; Alalade and Iyayi, 2006).

8.1.3. Water Purification:

The plant has demonstrated strong potential in phytoremediation, removing heavy metals, excess nutrients, and toxins from contaminated water sources (Reddy and D'Angelo, 1997).

8.1.4. Reduction of Agricultural Costs:

By serving as both green manure and animal feed, Azolla significantly reduces reliance on commercial fertilizers and industrial feed, thereby lowering the total cost of production (Watanabe, 1992)

8.1.5. Support for Permaculture and Sustainable Farming:

Its rapid growth, soil-enhancing properties, and ecological compatibility make Azolla an effective tool in permaculture systems and integrated farming (Becking, 1979).

8.1.6. Scientific and Research Value:

Due to its nitrogen-fixing capacity, rapid biomass production, and symbiotic biology, Azolla is a valuable model organism for studies in plant physiology, microbial ecology, and environmental sciences (Peters and Meeks, 1989)

8.1.7. Potential Beyond Feed and Fertilizer:

The benefits of *Azolla caroliniana* extend into human nutrition, pharmaceutical applications, and biotechnology, as its bioactive compounds continue to attract attention for their antioxidant and therapeutic properties (Della Greca and al., 2009; Rahman and al., 2013)



Figure 05 : General benefits of the *Azolla caroliniana* plant. (Mathur, 2024).

Chapter II: Materials and Methods

2. Materials and Methods

2.1. Objective

The main objective of this study is to investigate the natural and chemical composition of the *Azolla caroliniana* plant in order to evaluate its potential applications in agriculture, animal nutrition, health, medicine, and pharmacy. Specifically, the research aims to:

1. Assess the nutritional value of *Azolla* as a natural, fast-growing fodder by studying its effects on animal growth, weight gain, and overall health using a rabbit feeding experiment.
2. Analyze the plant's bioactive compounds through the extraction of oils and plant concentrates, with the goal of identifying their usefulness in the development of natural products, dietary supplements, and essential oils.
3. Contribute to the understanding of *Azolla*'s versatility and explore its potential role in enhancing productivity and sustainability in various fields.

2.2. Plant Material

2.2.1. Method of Cultivation

Azolla caroliniana was imported from the Tunisian region and cultivated under controlled conditions within the air-conditioned greenhouses of the college. The cultivation was carried out in a well-equipped basin measuring 2.5 meters in length and 1 meter in width. The plant was maintained for a period of three months, during which various cultivation techniques and good agricultural practices were applied to support optimal growth and ensure successful production.

A significant increase in biomass was observed in less than 15 days, highlighting *Azolla*'s remarkable growth rate and its strong potential as a fast-cultivating crop. Furthermore, due to its nitrogen-fixing capability, *Azolla* contributes to improved soil fertility when used as green manure, thereby reducing dependence on synthetic chemical fertilizers.

The table below lists the materials and instruments utilized during the cultivation process.

Table 02: Apparatus, Organic Materiale, For Cultivation.

Apparatus	Organic Materials
Pool.	Azolla
Rake.	Water
Iron digging tool	
Black plastic cover	
Watering pipe.	
Wooden stirring tool	



Figure 06: *Azolla caroliniana* in a greenhouse pool (Personal Photo, 2025).

2.2.2. Extraction of Essential Oils

We used *Azolla caroliniana* as our plant material, as described in the previous chapter.

To facilitate the extraction of essential oils from *Azolla caroliniana,* two pre-treatment operations were performed: drying and grinding.

- ✓ **Drying**: The *Azolla caroliniana* was dried in the shade, away from humidity, for two weeks (15 days) at ambient temperature.
- ✓ **Grinding**: The dried *Azolla caroliniana* was then ground into very small pieces using a mortar. This ground material was stored in sealed glass bottles, protected from light and heat, until ready for use.

The table below presents the various materials and instruments used for the extraction procedure.

Table 03: Apparatus, Glassware, and Products Used for Extraction

Apparatus	Glassware and Other Equipment	Solvent and Solute
Balance (Scout Pro)	500 ml Graduated Cylinder	Distilled Water
Clevenger-type Hydro-distiller	50 ml Beaker	Acetone
Heating Mantle	5 ml Glass Vial	
Pyrex Glass Flask (2000 ml)	Wash Bottle	
A column	Funnel	
A condenser	Aluminum foil	
A collector		

2.2.3. Extraction Process

One hundred grams (100 g) of dried *Azolla* caroliniana plants were placed into a 2000 ml round-bottom flask, and 1000 ml of distilled water was added. The mixture was then brought to a boil for three hours (Bouguerra *and al.*, 2019). A heating mantle ensured homogenous heat distribution within the flask. The steam generated from the heated flask was condensed in a condenser. Since the essential oil has a lower density, it separated from the water in a glass collector. The essential oil was then collected in an amber vial and stored under refrigeration until ready for use.




Figure 07 : Extraction of Essential Oils (Personal Photo, 2025).

1.3. Quantitative Analysis

1.3.1. Determination of Total Phenols

Polyphenols were measured by spectrophotometry, using the Folin- Ciocalteu method (1927). A mixture of 0.2 ml of the plant's *hydro-ethanolic extract* and 1 ml of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) was incubated for 5 minutes. Subsequently, 800 µl of sodium carbonate (Na_2CO_3) at a concentration of 75 g/L were added. The mixture was then incubated for 1 hour in the dark. Three tests were performed for each sample, and the absorbance was measured at 765 nm against a blank. The polyphenol concentration was determined using a calibration curve prepared with different concentrations of gallic acid (0-0.2 g/ml) under the same conditions and following the same protocol as for the extracts (Clementine *and al*., 2012).

1.3.2. Determination of Total Flavonoids:

Total *flavonoids* were determined using the *aluminum chloride* (AlCl3) method (Jeridane *and al*., 2006). A mixture of 0.5 ml of extract and 0.5 ml of AlCl3 solution (2% in *methanol*) was prepared, then kept in the dark at room temperature for 10 minutes. The absorbance was measured at 430 nm using a UV spectrophotometer. The flavonoid concentration was determined by a calibration curve, prepared using different concentrations of quercetin, following the same conditions and steps as for the extract.

1.3.3. Preparation of the Hydro-Ethanolic Extract:

Ethanolic extraction is a commonly used method to extract bioactive substances from medicinal plants. It involves solubilizing and extracting the active components from the plant using ethanol as a solvent. Studies have shown that ethanol extraction yields extract rich in phenolic compounds with interesting biological activity, including antibacterial and antioxidant properties. This extraction technique was used in accordance with the guidelines of (Hadjadj ,2017).

1.3.4. Maceration

Macerate 70 g of *Azolla caroliniana* powder with 500 ml of a distilled water/ethanol mixture (800/200 v/v) in a beaker wrapped in aluminum foil. Then, stir the mixture using a magnetic stirrer for 40 minutes.

1.3.5. Filtration

Filter the obtained macerate using Whatman No. 1 paper. Use 500 ml of the ethanol/distilled water solution to recover the residue from the previous maceration and stir it for a full day. Repeat the initial filtration with this second maceration.

1.3.6. Determination of Hydro-Ethanolic Extract Yield

The yield refers to the mass of the extract obtained after solvent evaporation. It's expressed as a percentage relative to the initial mass of the plant material used for extraction. To calculate the yield, you need to know the initial mass of the dry plant material and the final mass of the crude extract, and then determine their ratio as a percentage.

The hydro-ethanolic extraction yield is calculated using the formula below (Boucherit, 2014):

$$\mathbf{Y\% = (Me / Ms) \times 100}$$

Where:

- Y%: Yield percentage
- Me: Mass of the dry extract in grams

- Ms: Mass of the sample in grams



Figure 08: Yield of hydro-ethanolic extract from *Azolla caroliniana*. (Personal photograph, 2025)

2.4.Phytochemical Screening

2.4.1. Qualitative Analysis

m. Phytochemical tests, or **screening**,

They are qualitative analyses aimed at identifying the main groups of chemical compounds present in plant organs. These are specific physicochemical reactions used to detect the presence of certain chemical constituents. Two methods are generally employed for carrying out these tests.

n. Flavonoids

The detection of flavonoids was performed using the cyanidin reaction**.** To do this, 5 ml of the extract was mixed with 5 ml of 10% sulfuric acid (H_2SO4), and then a few milliliters of ammonium hydroxide (NH_4OH) were added. The presence of anthocyanins is confirmed if the solution, initially intensified by acidification, turns a blue-violet color in a basic medium.

o. Saponosides

Saponins were detected using the foam test, which was performed on a 1% decoction. One hundred milliliters of this decoction were divided among ten test tubes, numbered 1 to 10. Each tube was then filled with distilled water to a total volume of 10 ml and shaken vigorously for 15 seconds. After a 15-minute resting period, the foam

height was measured. A foam index greater than 100 indicates a positive reaction, signifying a high content of saponosides (Harborne,2005).

When the foam height reaches 1 cm in a given tube, the foam index (Idm) is calculated using the following formula:

Foam Index

$$\textbf{(Idm) = (10 / N) × } 10^{-2}$$

Where **N** represents the number of the tube in which the foam reaches exactly 1 cm in height.

p. Anthocyanins

Two milliliters of a 5% infused extract were mixed with 2ml of (2N) hydrochloric acid. The presence of anthocyanins is revealed by the appearance of a pink-red coloration, which turns blue-violet after the addition of ammonia (Senhaji and *al.*, 2005).

q. Proanthocyanidols

Two milliliters of an infusion containing proanthocyanidols were mixed with 2 ml of concentrated hydrochloric acid. The mixture was then heated in a water bath for 5 minutes. The appearance of a red coloration indicates a positive reaction, confirming the presence of proanthocyanidols (Senhaji *and al*., 2005).

r. Mucilages

One milliliter of a 10% decoction was placed into a test tube, then mixed with 5 ml of absolute ethanol. After shaking, the mixture was left to rest for about ten minutes. The appearance of a flocculent precipitate confirmed the presence of mucilages in the plant sample (Amadou, 2005).

s. Narcotics

For the detection of narcotic substances, 0.5 g of plant powder was placed into a test tube and mixed with 5 ml of petroleum ether. After shaking for 15 minutes, the ethereal phase was decanted into a capsule and evaporated to dryness in a water bath. Then, 3 to 4 drops of a 5% KOH solution in alcohol were added. The presence of tetrahydrocannabinols was evidenced by a violet coloration (Diallo, 2005).

t. Proteins

Protein detection was carried out according to the Lowry method. To 1 ml of plant extract, 5 ml of solution A (prepared from 98 ml of 2% $NaCO_3$ in 0.1 N NaOH, 1 ml of 1% copper sulfate, and 1 ml of potassium sodium tartrate) were added. After shaking and a 10-minute rest, 0.5 ml of Folin reagent was incorporated. The mixture was vigorously shaken and then left to rest for 30 minutes. The appearance of a blue coloration indicates the presence of proteins(Koné, 1998).

u. Sterols and Triterpenes

Sterols and triterpenes were identified using the Liebermann-Burchard reaction. Two grams of plant powder were macerated in 40 ml of ether for 24 hours. After filtration, the extract was reduced to 20 ml. Ten milliliters of this extract were evaporated to dryness in a water bath. The residue was then dissolved in 1 ml of acetic anhydride, followed by 1 ml of chloroform ($CHCl_3$). The resulting solution was divided into two test tubes: one served as a control, and to the other, 2 ml of concentrated sulfuric acid (H_2SO_4) was added. The presence of sterols and triterpenes is confirmed by the appearance of reddish-brown or purplish rings at the contact zone between the liquids, as well as a discoloration of the supernatant phase turning green or violet (Harborne, 2005).

v. Alkaloids

Alkaloids were identified using general specific reagents for their characterization, notably (potassium iodomercurate). For the analysis, a solution was prepared by adding 10 g of dried plant powder to 50 ml of 10% H_2SO_4. After agitation, the mixture was macerated for 24 hours at room temperature, then filtered through filter paper and rinsed with distilled water, yielding 50 ml of filtrate. Characterization was then performed by precipitation. In two test tubes, 1 ml of filtrate was introduced, followed by 5 drops of Mayer's reagent in the first tube, while a third tube served as a control without reagent. After 15 minutes, the presence of alkaloids was confirmed by the formation of a yellowish-white precipitate in the first tube, and an orange precipitate in the control tube (Harborne, 2005).

w. Tannins

The presence of tannins was demonstrated by a reaction with a 1% ferric chloride ($FeCl_3$) solution, with a few drops of $FeCl_3$ being added. This reaction produces a precipitate or a greenish or blue-black coloration. Gallic tannins were identified by the Stiasny reaction: 30 ml of infused solution were mixed with 15 ml of Stiasny's reagent (composed of 10 ml of 40% formalin and 5 ml of concentrated HCl). The mixture was then kept in a water bath at 90 °C for approximately 15 minutes. After filtration, the filtrate was saturated with 5 g of powdered *sodium acetate*. Subsequently, 1 ml of a 1% $FeCl_3$ solution was added drop by drop. The presence of gallic tannins was confirmed by the formation of precipitates (Harborne, 2005).

x. Anthraquinones

Free anthraquinones were detected using the Bornsträger test. For this, 10 ml of *chloroform* were added to 1 g of plant powder. The mixture was heated in a water bath at 60 °C for 3 minutes, then hot-filtered and adjusted to 10 ml. Subsequently, 1 ml of diluted NH_4OH was added to 1 ml of the obtained chloroformic *extract* and shaken. The presence of free anthraquinones was indicated by the appearance of a more or less intense red color.

1.5. Animal Material

1.5.1. Rabbit Care and Feeding

For this experiment, we used 15 male rabbits, each weighing approximately 2,500 grams at the beginning of the study and aged 8 weeks. The animals were obtained from the Pasteur Institute of Algiers and were selected due to their common use in various fields of experimental research.

The study was conducted over a 60-day period, which was structured into three distinct phases:

- 15 days of acclimatization,
- 15 days of nursing and stabilization,
- 30 days of treatment.

All procedures were carried out under controlled conditions at the Animal facility of the Department of Biology, within the Faculty of Exact Sciences, Natural and Life Sciences, at the University of Tebessa.

Environmental parameters were carefully regulated, with a stable ambient temperature maintained at 25°C, and a natural photoperiod of 12 hours light / 12 hours dark.

The rabbits were housed in polyethylene cages lined with wood shavings, and organized into three experimental groups, each consisting of five animals. Each cage was properly labeled to indicate the corresponding group. Animals received a standard diet, and fresh water was provided daily. To ensure hygienic conditions, cages were cleaned regularly, and bedding was changed every two days.

For individual identification, each rabbit was marked on the tail using a permanent marker.

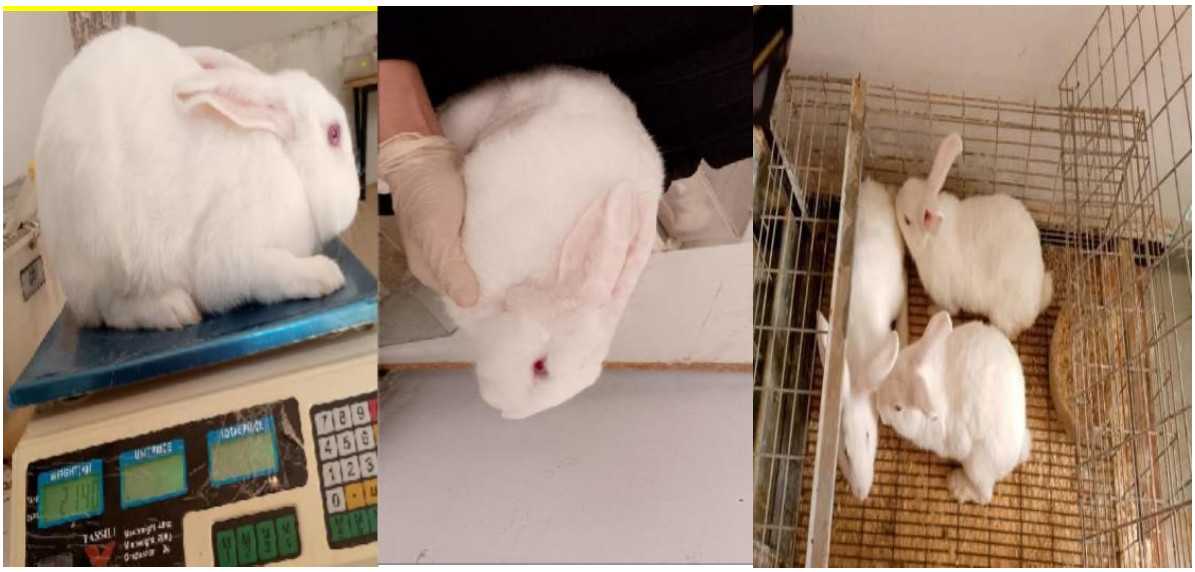


Figure 09 : Rabbits housed in the animal facility during the experiment. (Personal photograph, 2025)

1.5.2. Administration of *Azolla caroliniana* Extract

The extract solution was administered orally to the rabbits at a dose of 1 ml per 100 g of body weight, based on their average weight of approximately 2,500 g. Administration took place on the day of the experiment, following an overnight fasting

period.

The 20 rabbits were randomly assigned into four groups, each consisting of five animals. Treatments were given orally according to the specified protocols for each group, as detailed in Table 5.



Figure 10 : Preparation and oral administration of *Azolla caroliniana* extract to rabbits using gavage. (Personal photograph, 2025)

Table 04: Distribution of rabbits by groups and treatment protocols.

Lot 1	Control Group, no treatment
Lot 2	Group for the (1 ml of concentrated *Azolla caroliniana*)
Lot 3	Group for *Azolla caroliniana*

As part of evaluating *Azolla caroliniana* as a potential animal feed, a feeding trial was conducted on 15 rabbits, each weighing approximately 2,500 grams. The rabbits were divided into three groups:

- **Group 1 (Control):** Fed exclusively with traditional feed.
- **Group 2:** Received *Azolla* in concentrated form, administered daily at a dose of 1 ml of concentrate per 100 ml of pure water.
- **Group 3:** Fed directly with fresh green *Azolla caroliniana*.

The experiment lasted for 30 days, with rabbit weights recorded every 3

days. Results demonstrated that rabbits fed fresh *Azolla caroliniana* exhibited the highest average weight gain of 610 grams, followed by those receiving the concentrated extract, which gained 330 grams. The control group showed the lowest gain, at 178 grams.

Additionally, blood tests conducted at a private medical analysis laboratory revealed a moderate increase in blood protein, glucose, and lipid levels among the treated groups, indicating potential positive effects of *Azolla* supplementation on metabolic health.



Figure 11: Distribution of rabbits by groups and treatments. (Personal photograph, 2025)

2. Monitoring of Weight Variation

Throughout the treatment period, the body weights of the rabbits were regularly measured and recorded at predetermined intervals to monitor growth and assess the effects of the different feeding regimens.

3. Blood Sample Collection

At the conclusion of the experiment, the rabbits were anesthetized using xylazine, and blood samples were collected via cardiac puncture using hematocrit tubes. Blood samples collected in tubes containing EDTA were utilized for hematological analyses, including complete blood count parameters such as red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), platelets (PLT), and hemoglobin (HGB).

Blood collected in dry tubes was used for serological analyses to assess

inflammation markers, specifically C-reactive protein (CRP) and antistreptolysin O (ASLO). All samples were carefully labeled and transported on the same day to a private analytical laboratory for the determination of the specified inflammatory markers.



Figure 12 : Blood sample collection from rabbits via cardiac puncture. (Personal photograph, 2025)

4. Statistical Analysis of Results

Data on rabbits' body weights and biological parameters were analyzed using SPSS software (version 7). Comparisons between groups were conducted using Student's t-test and one-way analysis of variance (ANOVA) to determine statistically significant differences. Results were considered significant at the appropriate confidence level, and post-hoc tests were applied where necessary to identify differences between specific groups.



Chapter III
Results and Discussion



1. Results

1.1. Cultivation

The cultivation of *Azolla caroliniana* was successfully established under controlled conditions, resulting in a stable and continuous biomass yield of approximately 6 kilograms every 15 days. This reliable and reproducible production cycle underscores the species' high growth rate and adaptability, confirming its suitability for large-scale propagation.

This achievement is particularly significant as it supports the sustainable production of a valuable natural forage resource. The consistent biomass output not only ensures a steady supply for agricultural use but also enhances the feasibility of incorporating *Azolla* into integrated farming systems. Moreover, the successful cultivation opens new avenues for its application in environmental management, such as soil fertility improvement through nitrogen fixation, and the development of eco-friendly agricultural practices.

These results highlight *Azolla caroliniana* as a promising candidate for addressing challenges related to sustainable animal feed production and environmental conservation, thereby contributing to increased productivity and resource efficiency.



Figure 13 :*Azolla caroliniana* crop cultivated under controlled conditions. (Personal photograph, 2025)

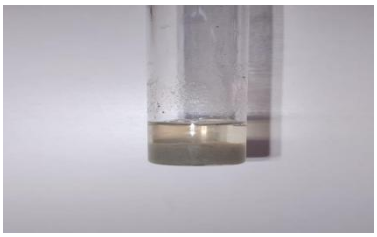
1.2. Phytochemical Screening

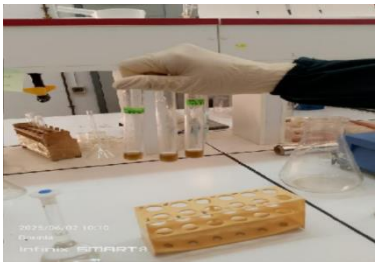
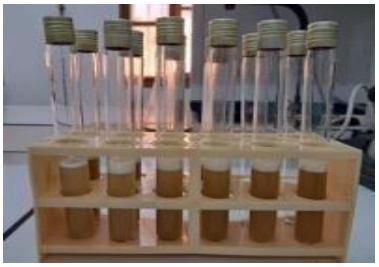
Azolla caroliniana is classified as a lower plant, belonging to a group of simple, non-vascular aquatic plants. It is important to note that this species is **non-flowering**, reproducing via spores rather than flowers. Phytochemical screening was conducted on extracts obtained from the entire plant, including leaves and roots.

Standard qualitative tests were performed to identify key bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and others. The results (summarized in Table 05) demonstrate that this lower plant contains a diverse range of bioactive substances, including essential oils, flavonoids, tannins, alkaloids, anthocyanins, saponins, and proteins. These findings highlight its significant potential for various therapeutic and pharmacological applications.

Table 05. Phytochemical tests Extracts of *Azolla caroliniana*

Phytochemical Tests	**Reagents**		**Final Result**
Flavonoids	Cyanidin Reaction		Pleasant
Essential Oil	Distilled Water +Azolla		**Odor**: Pleasant **Color**: Yellow
Sterols **Triterpenes**	Lieberman Reaction		**Yield %:** Presence 1.56

			
Tannins	$FeCl_3$		Presence
Mucilage	Ethanol		Presence

Anthocyanins	HCl (2N)		Presence
Saponosides	Foaming Index		Presence

1.3. Physiological Parameters

d. Effect on Body Weight

Quantitative analysis revealed statistically significant differences in body weight progression among the experimental groups. The control group showed an average body weight gain of +178 g. Rabbits that received the plant (*Azolla caroliniana*) exhibited a body weight gain of +610 g. In contrast, the group receiving the *Azolla caroliniana* extract showed a body weight gain of +330 g.

Throughout the experimental period, comparison of the mean values using the Student's *t*-test between the control and treated groups showed that both the plant and the extract of *Azolla caroliniana* significantly increased the rabbits' body weight starting from the third week (plant: $p = 0.038$; extract: $p = 0.046$); and in the fourth week (plant: $p = 0.032$; extract*:* $p = 0.044$). However, no effect was observed during the first and second weeks ($p > 0.05$).

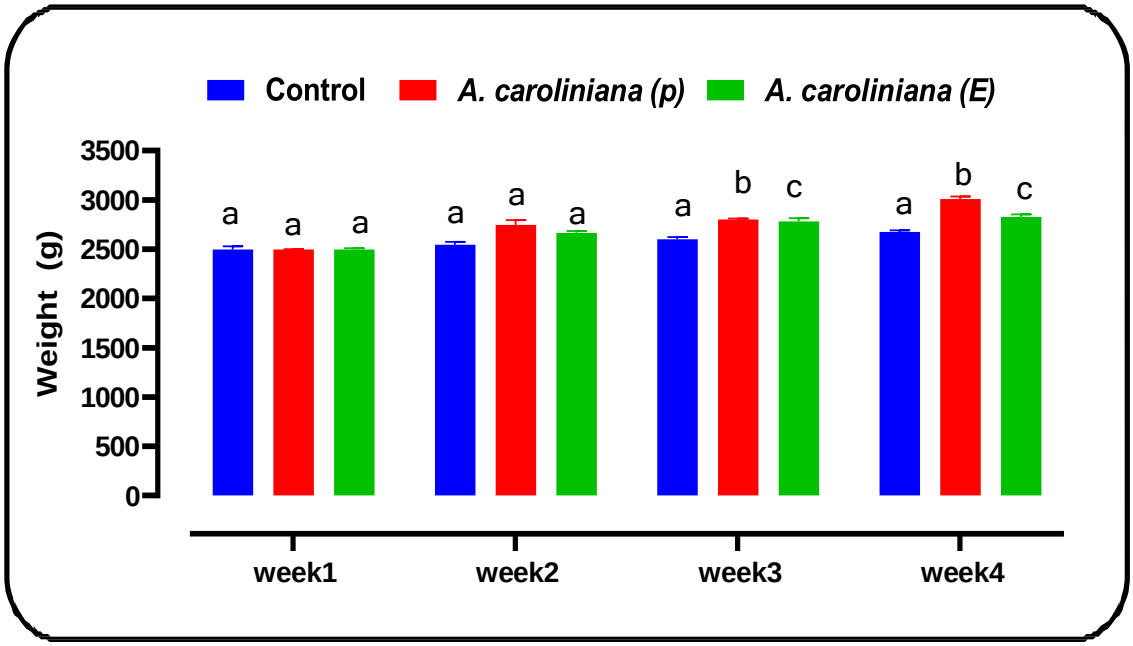


Figure 14 : Effect of the plant and the extract of *Azolla caroliniana* on weights body .

e. Effect of the Plant and Extract of *Azolla caroliniana* on Blood Parameters

As shown in Figure (14), no statistically significant differences were observed between the control group and the groups treated with the plant and extract of *Azolla caroliniana* ($p > 0.05$). red blood cell count platelets.

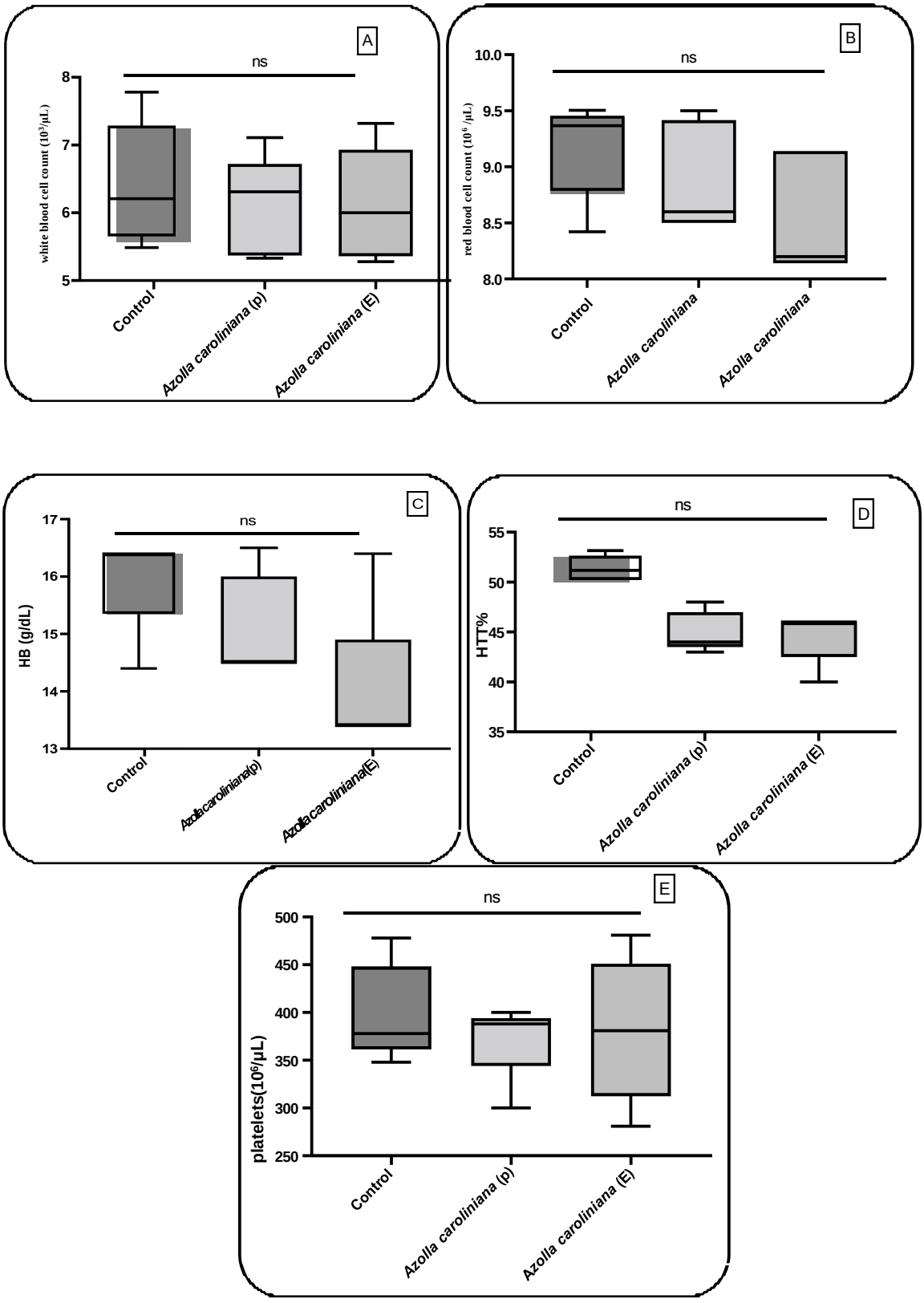


Figure 15 : Effect of the plant and the extract of *Azolla caroliniana* on hematological parameters .

f. Effect of the Plant and Extract of *Azolla caroliniana* on Biochemical Parameters

Table (05) shows that no statistically significant differences were observed between the control group and the groups treated with the plant and extract of *Azolla caroliniana* ($p > 0.05$) for the following biochemical parameters: blood glucose, cholesterol, triglycerides, CRP, ASLO, and urea. However, the treatment led to a significant decrease in: creatinine ($p = 0.045$; $p = 0.048$), AST ($p = 0.032$; $p = 0.028$), and ALT ($p = 0.046$; $p = 0.038$).

Table 06: Effect of the Plant and Extract of *Azolla caroliniana* on Biochemical Parameters.

Biochemical Parameters	Control	*A. caroliniana*	*A. caroliniana Extract*
Blood Glucose (g/L)	0.96 ± 0.10^{a}	0.78 ± 0.06^{a}	0.74 ± 0.11^{a}
Creatinine	9.57 ± 0.47^{a}	7.78 ± 0.23^{b}	5.98 ± 5.23^{c}
Cholesterol	1.14 ± 0.09^{a}	1.34 ± 0.05^{a}	1.23 ± 0.21^{a}
Triglycerides	0.54 ± 0.095^{a}	0.51 ± 0.07^{a}	0.57 ± 0.14^{a}
AST (TGO)	37.60 ± 1.16^{a}	25.60 ± 5.25^{b}	24.20 ± 2.06^{c}
ALT (TGP)	39.60 ± 2.04^{a}	37.40 ± 11.20^{a}	30.60 ± 2.42^{c}
CRP	13.45 ± 2.04^{a}	13.42 ± 1.73^{a}	15.16 ± 2.58^{a}
ASLO	Negative	Negative	Negative
Urea	0.26 ± 0.03^{a}	0.28 ± 0.02^{a}	0.36 ± 0.01^{a}

2. Discussion

2.1. Discussion

The present study provides compelling evidence for the beneficial physiological and biochemical effects of *Azolla caroliniana* and its hydro-ethanolic extract when incorporated into the diet of New Zealand white rabbits. The significant increase in body weight observed in treated groups highlights *Azolla*'s potential as a highly nutritious and efficient natural feed supplement.

2.2. **Nutritional Impact on Growth:**

The observed weight gain, particularly the +610 g increase in rabbits fed fresh *Azolla* and +330 g in those receiving the extract, confirms the plant's richness in essential nutrients. These findings are consistent with earlier studies that identified *Azolla* as a rich source of essential amino acids, vitamins, and high-quality proteins which improve feed efficiency and promote growth in various livestock species (Leterme and al., 2009; Alalade and al., 2007). The delay in significant weight gain until after two weeks may reflect the time required for physiological adaptation and optimal nutrient absorption. This pattern suggests that while *Azolla* is effective, the full benefits on growth performance manifest after an initial adjustment period.

2.3. **Hematological Safety:**

The absence of significant changes in red blood cells (RBC) and platelets indicates that neither the whole plant nor its extract exerts hematotoxic effects. This is a critical finding supporting the safety profile of *Azolla* as a dietary supplement. Maintaining hematological homeostasis is essential for animal health, and our results corroborate previous reports by El-Sheikh and al. (2019) demonstrating the safety of plant-based feed additives in rabbits.

2.4. **Biochemical Effects and Organ Protection:**

Though parameters such as blood glucose, cholesterol, triglycerides, CRP, ASLO, and urea showed no significant changes, notable improvements were seen in markers related to kidney and liver function.

- *Creatinine Reduction:* The significant decrease in creatinine levels suggests enhanced renal function, which may be attributed to the antioxidant capacity of phenolic compounds found in *Azolla* (Jalali and al., 2020; Hassan and al., 2020). This antioxidant effect likely reduces oxidative stress and protects renal tissues from damage, thereby improving glomerular filtration rates. Given the importance of kidney health in maintaining overall metabolism, this protective effect is particularly valuable for animals under metabolic stress or exposed to nephrotoxic agents.

- *Hepatoprotective Effects:* The reduction in AST and ALT enzyme levels in treated groups provides evidence of *Azolla*'s hepatoprotective potential. Elevated levels of these enzymes are commonly associated with liver injury or inflammation. The results align with studies highlighting the anti-inflammatory and

antioxidant activities of aquatic plant extracts (Saygılı *and al*., 2012; Boulekbache *and al*., 2013). The bioactive compounds in *Azolla*, including flavonoids and tannins, may exert cytoprotective effects by neutralizing free radicals and modulating inflammatory pathways in hepatic cells.

2.5. **Implications for Sustainable Animal Nutrition:**

The dual benefits of growth promotion and organ protection position *Azolla caroliniana* as a promising sustainable feed resource. Its fast growth rate and ability to fix atmospheric nitrogen make it an eco-friendly alternative to conventional feed ingredients, potentially reducing reliance on costly and environmentally taxing feed crops. Moreover, the plant's bioactive compounds may contribute to improved animal health, reducing the need for synthetic additives or pharmaceuticals.

2.6. **Limitations and Future Directions:**

While this study demonstrates clear benefits, further research is warranted to fully characterize the mechanisms underlying *Azolla*'s effects. Dose-response studies are needed to optimize feeding levels for maximum efficacy and safety. Long-term feeding trials would help assess potential cumulative effects and ensure no delayed adverse outcomes. Additionally, isolating and identifying the specific bioactive compounds responsible for the observed effects could pave the way for targeted nutraceutical development.

Molecular studies examining the pathways modulated by *Azolla*'s compounds, especially regarding antioxidant and anti-inflammatory actions, would deepen our understanding and enable more precise applications in animal nutrition and health management.

IV Conclusion



Conclusion

This study supports the inclusion of *Azolla caroliniana* and its extract in rabbit diets to enhance growth performance and protect vital organs without compromising hematological or metabolic health. These findings contribute to the growing body of evidence highlighting Azolla's value as a sustainable, multifunctional feed additive with significant benefits for livestock production.

Azolla caroliniana is widely recognized as an alternative animal feed due to its rapid growth, high nutritional value, and richness in proteins and essential elements that promote muscle development. Beyond its agricultural benefits, *Azolla caroliniana* is also known for its numerous medicinal properties, which justify its potential applications in pharmaceutical fields.

This study evaluated the multiple advantages of *Azolla caroliniana*, including its high biomass production within a short period of approximately 15 days. Such rapid growth demonstrates the plant's ability to support agricultural productivity effectively.

In our rabbit experiments, oral administration of aqueous extracts of *Azolla caroliniana* at a dose of 1 gram per 100 milliliters of water resulted in an initial weight decrease within the first 4 days. However, subsequent treatment with both the extract and fresh Azolla led to a weight gain of approximately 330 grams over a 30-day period. Importantly, no signs of toxicity, mortality, or behavioral disturbances were observed throughout the study.

The experimental protocol also confirmed the richness of *Azolla caroliniana* in essential oils and other bioactive compounds. Based on these findings, we developed a nutritional supplement that enhances blood circulation and stimulates overall nutrition, highlighting the plant's potential as a natural and effective food alternative.

In conclusion, this preliminary experimental research underscores the agricultural significance of *Azolla caroliniana* as a promising fodder substitute and lays the foundation for future investigations, including:

- Production of nutritional supplements to support animal health and growth.
- Detailed qualitative and quantitative phytochemical analyses to identify and quantify key bioactive compounds.

Conclusion

- Extraction and characterization of essential oils from the plant.
- Evaluation of various administration routes and extension of treatment durations.
- Complementary in vitro studies to validate the observed biological effects.
- Targeted phytotherapeutic studies on local populations of Azolla caroliniana to explore regional variations and applications.

Bibliography

- Alalade, O. A., and Iyayi, E. A. (2006). Chemical composition and feeding value of Azolla meal for poultry. *International Journal of Poultry Science, 5*(2), 137–140.
- Becking, J. H. (1979). Environmental requirements of Azolla for use in tropical rice production. *IRRI Research Paper Series, No. 6*.
- Becerra, M., Preston, T. R., and Ogle, R. B. (1995). Azolla filiculoides as partial replacement for rice bran in diets of growing ducks. *Livestock Research for Rural Development, 7*(3).
- Della Greca, M., Monaco, P., Previtera, L., and Zarrelli, A. (2009). Biologically active compounds from Azolla filiculoides. *Journal of Natural Products, 72*(9), 1712–1715.
- Lumpkin, T. A., and Plucknett, D. L. (1980). Azolla: Botany, physiology, and use as a green manure. *Economic Botany, 34*(2), 111–153.
- Peters, G. A., and Meeks, J. C. (1989). The Azolla-Anabaena symbiosis: Basic biology. *Annual Review of Plant Biology, 40*(1), 193–210.
- Pillai, P. K., Premalatha, S., and Rajamony, S. (2002). *Azolla: Sustainable feed for livestock*. Indian Council of Agricultural Research.
- Rahman, M. A., and Hossain, M. T. (2013). Antioxidant activity of aquatic fern Azolla pinnata. *International Journal of Pharmaceutical Sciences and Research, 4*(2), 734–739.
- Reddy, K. R., and D'Angelo, E. M. (1997). Biological nitrogen fixation by Azolla and Anabaena in wetlands. *Biogeochemistry, 39*(1), 1–14.
- Smith, A. R., Pryer, K. M., Schuettpelz, E., Korall, P., Schneider, H., and Wolf, P. G. (2006). A classification for extant ferns. *Taxon, 55*(3), 705–731.
- Stone, R. D. (1981). Distribution and ecology of Azolla species in New Zealand and Australia. *New Zealand Journal of Botany, 19*(2), 113–120.
- Van Hove, C. (1989). *Azolla and its multiple uses with emphasis on Africa*. Food and Agriculture Organization of the United Nations (FAO).
- Van Hove, C., and Lejeune, A. (2002). The Azolla–Anabaena symbiosis: A review. *Botanical Review, 68*(2), 157–190.
- Wagner, G. M. (1997). Azolla: A review of its biology and utilization. *The Botanical Review, 63*(1), 1–26.

Conclusion

- Watanabe, I. (1992). Role of Azolla and blue-green algae in biological nitrogen fixation. In *Methods of Evaluating Nitrogen Fixation by Nodulated Legumes in the Field* (pp. 225–235). Springer.
- De Vries, S., and De Vries, J. (2018). Azolla: A model system for symbiotic nitrogen fixation and evolutionary developmental biology. Current advances in fern research, 21-46.
- Korsa, G., Alemu, D., and Ayele, A. (2024). Azolla plant production and their potential applications. *International Journal of Agronomy*, *2024*(1), 1716440
- Zimmerman, W. J., Lumpkin, T. A., and Watanabe, I. (1989). Classification of Azolla spp., section Azolla. *Euphytica*, *43*, 223-232.
- Prabakaran, S., Mohanraj, T., Arumugam, A., and Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Industrial Crops and Products*, *183*, 114942.
- https://upload.wikimedia.org/wikipedia/commons/8/80/Azolla_caroliniana0.jpg
- https://portsmouthnaturalhistory.co.uk/wpcontent/uploads/sites/4/2024/04/Fig.-1-1492x1200.jpg