الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي و البحث العلمي

University of Setif 1 Ferhat ABBAS
Faculty of Natural and Life Sciences

جامعة ، سطيف 1، فرحات عباس
كلية علوم الطبيعة و الحياة

Université Ferhat ABBAS Sétif 1

DEPARTMENT OF BIOTECHNOLOGY

N°..... ...../SNV/**2026**

DISSERTATION

Presented by

Seklouli Ichrak

Abid Nour El Yakine Asma

To obtain the diploma of

MASTER

Branch of study: Biotechnology

Specialty: Biotechnology

THEME

Assessment of the In Vitro Hemolytic Effect of Atriplex halimus on Erythrocytes from Different Mammalian Species

DEFENSE JURY:

PRESIDENT:	**FERDJIOUI SIHEM**	**MCA. UFA Sétif 1**
SUPERVISOR:	**BETTIHI SARRA**	**MCA. UFA Sétif 1**
EXAMINER:	**BENBERINIS SOUMIA**	**MCA. UFA Sétif 1**

ACADEMIC YEAR: 2025/2026

Abstract

Atriplex halimus L. is a halophytic medicinal plant widely distributed in the Mediterranean region and traditionally used for its numerous therapeutic properties. Rich in bioactive compounds such as polyphenols, flavonoids, tannins, and saponins, this species exhibits both protective and potentially cytotoxic activities. The present study aimed to evaluate the in vitro hemolytic effect of different extracts of *Atriplex halimus* on erythrocytes from different mammalian species and to compare their effects according to the extraction method and the type of blood cells used.Leaves of *Atriplex halimus* were subjected to three extraction procedures: hydroalcoholic extraction, decoction, and infusion. Human, mouse, and rat erythrocytes were isolated, washed, and prepared as 2% suspensions. Hemolytic activity was assessed by incubating erythrocytes with different extract concentrations (0–300 µg/mL) at 37°C for 30 minutes. After centrifugation, the released hemoglobin was quantified spectrophotometrically at 540 nm. The hydroalcoholic extraction yielded 30.8%, whereas decoction and infusion presented extraction yields of 16.28%. The results demonstrated differences in hemolytic responses according to both the extraction method and the erythrocyte species. The hydroalcoholic extract exhibited the lowest hemolytic activity and appeared to exert a membrane-stabilizing effect, probably due to its high content of antioxidant compounds such as polyphenols and flavonoids. In contrast, the aqueous extracts (decoction and infusion) showed a more pronounced hemolytic activity, which may be associated with the presence of saponins and other water-soluble constituents capable of interacting with erythrocyte membranes. Furthermore, human and rodent erythrocytes displayed different sensitivities to the tested extracts. In conclusion, *Atriplex halimus* possesses both protective and hemolytic properties depending on the extraction method and erythrocyte type. These findings contribute to a better understanding of the interactions between the phytochemical constituents of this medicinal plant and red blood cell membranes and provide useful information regarding its biological safety and potential therapeutic applications.

Keywords: Atriplex halimus, hemolysis, erythrocytes, blood, decoction, infusion, phytochemicals, mammalian species.

Résumé

Atriplex halimus L. est une plante médicinale halophyte largement répandue dans la région méditerranéenne et traditionnellement utilisée pour ses nombreuses propriétés thérapeutiques. Riche en composés bioactifs tels que les polyphénols, les flavonoïdes, les tanins et les saponines, cette espèce présente à la fois des activités protectrices et potentiellement cytotoxiques. La présente étude avait pour objectif d'évaluer l'effet hémolytique in vitro de différents extraits d'*Atriplex halimus* sur les érythrocytes de différentes espèces de mammifères et de comparer leurs effets en fonction de la méthode d'extraction et du type de cellules sanguines utilisées. Les feuilles d'*Atriplex halimus* ont été soumises à trois procédés d'extraction : l'extraction hydroalcoolique, la décoction et l'infusion. Des érythrocytes humains, de souris et de rats ont été isolés, lavés et préparés sous forme de suspensions à 2 %. L'activité hémolytique a été évaluée en incubant les érythrocytes avec différentes concentrations d'extraits (0 à 300 µg/mL) à 37 °C pendant 30 minutes. Après centrifugation, l'hémoglobine libérée a été quantifiée par spectrophotométrie à 540 nm. L'extraction hydroalcoolique a présenté un rendement de 30,8 %, tandis que la décoction et l'infusion ont montré des rendements de 16,28 %. Les résultats ont mis en évidence des différences de réponse hémolytique selon la méthode d'extraction et l'espèce des érythrocytes. L'extrait hydroalcoolique a montré la plus faible activité hémolytique et semble exercer un effet stabilisateur de la membrane, probablement en raison de sa forte teneur en composés antioxydants tels que les polyphénols et les flavonoïdes. En revanche, les extraits aqueux (décoction et infusion) ont présenté une activité hémolytique plus marquée, qui pourrait être liée à la présence de saponines et d'autres constituants hydrosolubles capables d'interagir avec les membranes érythrocytaires. Par ailleurs, les érythrocytes humains et ceux des rongeurs ont montré des sensibilités différentes vis-à-vis des extraits testés. En conclusion, *Atriplex halimus* possède à la fois des propriétés protectrices et hémolytiques qui dépendent de la méthode d'extraction et du type d'érythrocytes étudiés. Ces résultats contribuent à une meilleure compréhension des interactions entre les constituants phytochimiques de cette plante médicinale et les membranes des globules rouges et fournissent des informations utiles concernant sa sécurité biologique et ses potentielles applications thérapeutiques.

Mots-clés : *Atriplex halimus*, hémolyse, érythrocytes, sang, décoction, infusion, composés phytochimiques, espèces de mammifères.

الملخص

تُعد *Atriplex halimus* (القطف الملحي) نباتاً طبياً ملحياً واسع الانتشار في منطقة البحر الأبيض المتوسط ويُستخدم تقليدياً بفضل خصائصه العلاجية المتعددة. ويتميز هذا النبات بغناه بالمركبات النشطة بيولوجياً، مثل متعددات الفينول والفلافونويدات والتانينات والصابونينات، مما يمنحه خصائص وقائية وأخرى قد تكون سامة للخلايا. هدفت هذه الدراسة إلى تقييم التأثير الحالّ للدم في المختبر (In Vitro) لمختلف مستخلصات نبات *Atriplex halimus* على كريات الدم الحمراء لعدة أنواع من الثدييات، ومقارنة تأثيراتها حسب طريقة الاستخلاص ونوع الخلايا الدموية المستخدمة. تم تحضير ثلاثة أنواع من مستخلصات أوراق النبات، وهي: المستخلص الهيدروكحولي، والمغلي، والمنقوع. كما تم عزل كريات الدم الحمراء البشرية وتلك الخاصة بالفئران والجرذان وغسلها وتحضيرها على شكل معلقات بتركيز 2%. وتم تقييم النشاط الحالّ للدم من خلال حضن كريات الدم الحمراء مع تراكيز مختلفة من المستخلصات (0–300 ميكروغرام/مل) عند درجة حرارة 37°م لمدة 30 دقيقة. وبعد الطرد المركزي، تم قياس كمية الهيموغلوبين المنطلقة باستعمال المطيافية الضوئية عند طول موجي قدره 540 نانومتر. أظهرت عملية الاستخلاص الهيدروكحولي مردوداً قدره 30.8%، في حين بلغ مردود كل من المغلي والمنقوع 16.28%. وبينت النتائج وجود اختلافات في الاستجابة الانحلالية تبعاً لطريقة الاستخلاص ونوع كريات الدم الحمراء. فقد أظهر المستخلص الهيدروكحولي أقل نشاط حالّ للدم، ويبدو أنه يمتلك تأثيراً مثبتاً لغشاء الخلية، ويرجع ذلك على الأرجح إلى محتواه المرتفع من المركبات المضادة للأكسدة مثل متعددات الفينول والفلافونويدات. في المقابل، أظهرت المستخلصات المائية (المغلي والمنقوع) نشاطاً انحلالياً أكبر، وهو ما قد يرتبط بوجود الصابونينات ومركبات أخرى ذائبة في الماء قادرة على التفاعل مع أغشية كريات الدم الحمراء. كما أظهرت كريات الدم الحمراء البشرية وكريات دم القوارض درجات مختلفة من الحساسية تجاه المستخلصات المختبرة. و في الختام، يمتلك نبات *Atriplex halimus* خصائص وقائية وأخرى حالّة للدم تعتمد على طريقة الاستخلاص ونوع كريات الدم الحمراء المدروسة. وتسهم هذه النتائج في تحسين فهم التفاعلات بين المركبات الكيميائية النباتية لهذا النبات الطبي وأغشية كريات الدم الحمراء، كما توفر معطيات مهمة حول سلامته البيولوجية وإمكاناته العلاجية المحتملة.

الكلمات المفتاحية: Atriplex halimus، انحلال الدم، كريات الدم الحمراء، الدم، المغلي، المنقوع، المركبات الكيميائية النباتية، أنواع الثدييات.

List of Contents

Acknowledgements .....
Dedication.....
Abstract.....
Résumé .....
الملخص.....
List of Figures.....
List of Tables .....
Introduction .....
Part I: General presentation of *Atriplex halimus* 1
I.1. General Presentation of *Atriplex halimus*.....2
I.2. Classification.....3
I.3. Description botanique 3
I.3.1. Habit and stem 3
I.3.2. Leaves.....4
I.3.4. Fruits and seeds.....5
I.3.5. Physiology 5
I.3.6. Leaf anatomy 5
I.4. Ecological Description 5
I.5. Phytochemical Composition 6
I.5.1. Polyphenols (Phenolic Compounds).....6
I.5.2. Flavonoids.....6
I.5.3. Saponins.....7
I.5.4. Tannins.....7
I.5.5. Alkaloids and Glycosides 8
I.6. Traditional Uses and Applications.....8
I.6.1. Pastoral Use 8
I.6.2. Use in Human Nutrition.....8
I.6.3. Use in Traditional Medicine 9
I.6.4. Soil Conservation and Control of Land Degradation 9
I.6.5. Other Traditional Uses.....9
I.7. Main Pharmacological Activities 9
I.7.1. Antioxidant activity 9
I.7.2. Anti-inflammatory activity 10
I.7.3. Antibacterial activity.....10
I.7.4. Other activities (antidiabetic, anticancer) 10
Part II: Blood: targets and interactions 11
II.1. Blood Composition and Functions.....12

II.1.2. Formed elements 12
II.1.3. Plasma 13
II.1.4. Functions of blood: 14
II.1.4.1. Transportation 14
II.1.4.2. Regulation 14
II.1.4.3. Protection 14
II.2. Hemolysis Factors: Mechanisms and Anti-hemolytic Effects of Plant Extracts 15
II.2.1. Definition and Types of Hemolysis 15
II.2.2. Hemolysis factors 15
Part III : Effect of *Atriplex halimus* Extracts on Blood and Hemolysis 12
III.1. Direct hemolytic toxicity: 17
III.2. Protective and Antihemolytic Effects 17
III .2.1. Protective Effect (Membrane-Protective and Antioxidant Effects) 17
III .2.2. Antihemolytic Effect (Specific Protection of Erythrocytes) 17
Materials and Methods 17
I.1. Plant material 19
I.2. Biological material 19
I.3. Animal material 19
I.4. Preparation of leaf extracts of *Atriplex halimus* 20
I.4.1. Hydroalcoholic extraction 20
I.4.2. Decoction 21
I.4.3. Infusion ..... **Error! Bookmark not defined.**
I.5. Determination of extract yield 22
I.6. Preparation of physiological saline solution 22
I.7. Preparation of erythrocyte suspension 22
I.8. Preparation of the control range 23
I.9. Preparation of extracts 24
I.10. Hemolysis assay 24
Results and discussion 26
II.1. Yield table of *Atriplex halimus* 26
II.2. Calculation of percentage hemolysis 27
1.Control 27
1.1. Human control 27
1.2. Mouse control 28
1.3. Rat control 28
1.4. Human Control Graph 28
1.5. Mouse Control Graph 29
1.6. Rat Control Graph 29
2. Hydroalcoholic 29

2.2. Mouse Hydroalcoholic Extract 30
2.3. Rat Hydroalcoholic Extract 30
2.4. Human Hydroalcoholic Graph 31
2.5. Mouse Hydroalcoholic Graph 31
2.6. Rat Hydroalcoholic Graph: 31
3.Décoction 32
3.1. Human decoction 32
3.2. Mouse decoction 32
3.3. Rat decoction 33
3.4. Human decoction graph 33
3.5 Mouse decoction graph 33
3.6. Rat decoction graph 34
4. Infusion 34
4.1. Human infusion 35
4.2. Mouse infusion 35
4.3. Rat infusion 35
4.4. Human infusion graph 36
4.5. Mouse infusion graph 36
4.6. Rat infusion graph 36
5. Comparison of the Human Model 36
6. Comparison of the Mouse Model 36
7. Comparison of the Rat Model 37
8. Discussion and Comparison with Similar Studies 37
a. Superiority of the Hydroalcoholic Extract and Membrane Stabilization 38
b. Cytotoxic/Hemolytic Effect of Aqueous Extracts (Decoction and Infusion) 38
c. Differential Sensitivity According to Species (Human vs Rodents) 38
Conclusion 26
General Conclusion 40
Bibliographic References 41

Introduction

General Introduction

Medicinal plants have constituted an important source of bioactive substances used in the prevention and treatment of numerous diseases for centuries. Despite the considerable advances in modern medicine, interest in natural products continues to grow due to their chemical diversity, accessibility, and wide range of pharmacological properties. According to the World Health Organization (WHO), a large proportion of the world's population still relies on traditional plant-based remedies to meet their healthcare needs. Among the medicinal plants widely used in arid and semi-arid regions is *Atriplex halimus* L., commonly known as "Guettaf". This halophytic species, belonging to the Amaranthaceae family, is widely distributed throughout the Mediterranean Basin, particularly in North Africa. It is recognized for its resistance to extreme environmental conditions and for its richness in secondary metabolites such as polyphenols, flavonoids, tannins, alkaloids, and saponins. These compounds are associated with various biological activities, including antioxidant, anti-inflammatory, antibacterial, antidiabetic, and anticancer effects. Blood is an essential biological tissue responsible for the transport of respiratory gases, nutrients, hormones, and metabolic waste products. Red blood cells, the main cellular components of blood, are particularly sensitive to chemical and physical insults that may alter the integrity of their membrane. Hemolysis, defined as the destruction of erythrocytes with the release of hemoglobin into the extracellular environment, represents an important indicator for assessing either the toxicity or the protective effect of various natural substances. In this context, investigating the effects of *Atriplex halimus* extracts on erythrocytes is of considerable scientific interest. Indeed, the coexistence in this plant of potentially protective compounds, such as polyphenols and flavonoids, and potentially hemolytic compounds, such as certain saponins, raises questions regarding their influence on the membrane stability of red blood cells. The main objective of this work is to evaluate the hemolytic and antihemolytic effects of different *Atriplex halimus* extracts obtained by infusion, decoction, and hydroalcoholic extraction. This study also aims to compare their activity on human, mouse, and rat erythrocytes in order to gain a better understanding of the interactions between the phytochemical constituents of the plant and blood cell membranes. To achieve this objective, this dissertation is organized into three main parts. The first part presents the botanical, phytochemical, and pharmacological characteristics of *Atriplex halimus*. The second part is devoted to blood, its composition, and the mechanisms of hemolysis. Finally, the third part presents the experimental results obtained, together with their analysis and discussion in light of the available scientific data.