

الجمهورية الجزائرية الديمقراطية الشعبية

Democratic and Popular Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة مولاي الطاهر، سعيدة

University of Saida "Dr Tahar Moulay"



كلية العلوم الطبيعية والحياة

Faculty of Natural and Life Science

قسم البيولوجيا

Department of Biology

Dissertation for obtaining the master's degree in Biological Sciences

option: Biochemistry

Theme:

Comparative evaluation of the biological activated of *Ephedra alata* and *Aquilaria malaccensis* extracts and their mixture

Presented by: **LACHI Yasmina**

MESKINE Ahlem

Defended on: June 21, 2026

Before the jury composed of:

President:	Dr. DIDAUI Hayat	MCA	University of Saida -Dr Tahar Moulay
Examiner:	Dr. ZERAGUI Benkadour	MCB	University of Saida -Dr Tahar Moulay
Supervisor:	Dr. SIDI IKHLEF Amel	MCB	University of Saida -Dr Tahar Moulay

University year 2025/2026

الجمهورية الجزائرية الديمقراطية الشعبية

Democratic and Popular Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة مولاي الطاهر، سعيدة

University of Saida "Dr Tahar Moulay"



كلية العلوم الطبيعية والحياة

Faculty of Natural and Life Science

قسم البيولوجيا

Department of Biology

Dissertation for obtaining the master's degree in Biological Sciences

option: Biochemistry

Theme:

Comparative evaluation of the biological activated of *Ephedra alata* and *Aquilaria malaccensis* extracts and their mixture

Presented by: **LACHI Yasmina**

MESKINE Ahlem

Defended on: June 21, 2026

Before the jury composed of:

President:	Dr. DIDAOUI Hayat	MCA	University of Saida -Dr Tahar Moulay
Examiner:	Dr. ZERAGUI Benkadour	MCB	University of Saida -Dr Tahar Moulay
Supervisor:	Dr. SIDI IKHLEF Amel	MCB	University of Saida -Dr Tahar Moulay

University year 2025/2026

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Acknowledgements

*First of all, we thank **ALLAH**, the master of the heavens and the earth, who gave us the will, the strength, and the health to carry out and complete this modest work.*

"Thank you, our dear parents, for everything"

*Our deepest gratitude goes first and foremost to our supervisor **Dr Sidi Ikhlef Amel**, who agreed to guide our work and gave us all the attention and patience necessary to accomplish it. We also wish to thank her for her availability and valuable advice*

*We also wish to thank **Dr. Didaoui Hayat** and **Dr. Zeragui Benkadour** who honored us by accepting to examine and evaluate this modest work.*

*we extend our sincerest thanks to **Mr. Otman, Ms. Hajira**, head of the biology laboratory. Our gratitude also goes to all the staff and the teachers of the Faculty Sciences*

Finally, A big thank you to all those who helped us, directly or indirectly in the completion of this work

Dedication

*Before everything, I thank **Allah**, the Merciful, for granting me the courage, strength, and patience needed to complete this project*

*I dedicate this thesis **to myself**, for all the sacrifices, efforts, and struggles I endured throughout this journey. To myself, who faced doubt, exhaustion, and moments when everything seemed impossible, yet still found the strength to continue. To every hidden tear behind a smile, every sleepless night spent working in silence, and every inner battle no one could see. This thesis is not just an academic work; it is a reflection of my patience, determination, and perseverance to never give up on my dreams.*

- ❖ ***To my beloved parents**, Words cannot express the depth of my gratitude for you. Thank you for every heartfelt prayer, every sacrifice you made for me, and every unconditional love you have given me. You are the source of my strength and the reason for every step I have taken in this journey.*
- ❖ ***To my dear mother**, To the heart that loved me before seeing me, and to the hands that held me whenever I was about to fall. To the one who stayed awake for me, prayed for me in every prayer, and carried my pain before her own. Mother, if I were to give you the world, it would never be enough to repay even a part of what I owe you. You are not just part of my success you are my success. May Allah protect you and grant you a long and healthy life.*
- ❖ ***To my dear father**, To the first man I believed in for his strength and safety, the one who has always been a source of support, protection, and reassurance throughout my life. Thank you for being my strength and my pride. May Allah bless you, grant you good health, and extend your life.*
- ❖ ***To my dear brother**, you are not just a brother, but a piece of my soul and my support in difficult times. Your presence alone gives me strength.*
- ❖ ***To my dear friend and partner**, Ahlem, thank you for being a companion throughout this journey and for your true support in both joyful and difficult moments.*
- ❖ ***To my dear friends**: Sara, Bouchra, Hafsa, Nihad, Hadjer, and Rania, thank you for every moment of support, love, and sincerity we shared.*

Finally, to everyone who stood by me, with a kind word, a sincere prayer, or a small gesture of help, I extend my deepest gratitude.

Yasmina



Dedication

*First and foremost, I thank **Allah** for all the blessings He has bestowed upon me throughout the preparation of this thesis and during my years of study,*

*I also dedicate this thesis **to myself**, in recognition of the effort, courage, and perseverance I have demonstrated throughout my academic journey.*

*This journey was not always easy, but I demonstrated patience, determination, and enthusiasm to overcome difficulties and pursue my goals without giving up. Despite moments of doubt and exhaustion, I always remained confident in my abilities. By grace of **Allah**, I am now proud to have completed this thesis.*

and I dedicate this modest work to all those who are dear to me:

- ***To my dearest father**, thank you for all the sacrifices, advice, and efforts you have made to give me the best possible future. Your strength, support, and trust have been my greatest source of motivation. May God grant your health, happiness, and a long life.*
- ***To my dearest mother**, your love, your tenderness, your sacrifices, and your prayers have always lit my way. Thank you for always believing in me and for supporting me at every stage of my life. May God protect you and keep you by my side.*

I dedicate this thesis to you with all my love, respect, and immense gratitude.

- ***To my dear sisters and brothers**, who have always supported and encouraged me throughout my years of study, may God protect them and grant them happiness and good fortune.*
- *To my partner in this project and my dear friend **Yasmina**, who has accompanied me in this work, I would like to express my deep gratitude and appreciation for all her efforts and for the trust she has placed in me until we successfully completed this thesis.*
- *as well as to my dear friends **Sara, Bouchra, Nihed, Hadjar, Hadil, and Rania**, who have always encouraged me, and to whom I wish even greater success.*

To all those who contributed, directly or indirectly, to the completion of this thesis

Ahlem



Abstract

Abstract

Medicinal plants are widely used for therapeutic purposes because they contain bioactive secondary metabolites with important pharmacological properties, making them valuable sources of medicinal compounds. This study aimed to investigate the qualitative phytochemical composition, as well as the antioxidant, anti-inflammatory, and antimicrobial activities of *Ephedra alata*, *Aquilaria malaccensis*, and their binary mixture. Phytochemical screening revealed the presence of several secondary metabolites, including alkaloids, flavonoids, anthocyanins, terpenoids, and coumarins in both extracts. Catechic tannins were detected in *A. malaccensis*, whereas gallic tannins were found in *E. alata*. Biological activity assessment revealed promising pharmacological properties for the studied extracts. *A. malaccensis* exhibited the highest total antioxidant capacity (112 mg AAE/g extract), while the mixture showed the strongest DPPH radical scavenging activity ($IC_{50} = 93.09 \pm 2.59 \mu\text{g/mL}$), suggesting possible synergistic interactions between phytochemicals. Regarding anti-inflammatory activity, *A. malaccensis* demonstrated the greatest efficacy, showing the lowest IC_{50} value in the bovine serum albumin denaturation assay and the highest erythrocyte membrane protection percentage. Antibacterial evaluation also indicated that *A. malaccensis* generally exhibited stronger activity against the tested bacterial strains compared with *E. alata* and the mixture. Overall, the findings confirm the significant biological potential of both plants, with *A. malaccensis* showing the most promising pharmacological properties and the mixture exhibiting synergistic effects.

Keywords: medicinal plant; *Ephedra alata*; *Aquilaria malaccensis*; binary mixture; Biological activity

Résumé :

Les plantes médicinales sont largement utilisées à des fins thérapeutiques en raison de leur richesse en métabolites secondaires bioactifs possédant d'importantes propriétés pharmacologiques, ce qui en fait des sources précieuses de composés médicinaux. Cette étude avait pour objectif d'évaluer la composition phytochimique qualitative ainsi que les activités antioxydante, anti-inflammatoire et antimicrobienne de *Ephedra alata*, *Aquilaria malaccensis* et de leur mélange binaire. Le criblage phytochimique a révélé la présence de plusieurs métabolites secondaires, notamment des alcaloïdes, des flavonoïdes, des anthocyanes, des terpénoïdes et des coumarines dans les deux extraits. Les tanins catéchiques ont été détectés chez *Aquilaria malaccensis*, tandis que les tanins galliques ont été identifiés chez *Ephedra alata*. L'évaluation des activités biologiques a révélé des propriétés pharmacologiques prometteuses pour les extraits étudiés. *A. malaccensis* a présenté la capacité antioxydante totale la plus élevée (112 mg EAA/g d'extrait), tandis que le mélange a montré la plus forte activité de piégeage du radical DPPH ($IC_{50} = 93,09 \pm 2,59 \mu\text{g/mL}$), suggérant l'existence d'interactions synergiques possibles entre les composés phytochimiques. Concernant l'activité anti-inflammatoire, *A. malaccensis* a démontré la meilleure efficacité, en présentant la valeur d' IC_{50} la plus faible lors du test de dénaturation de l'albumine sérique bovine ainsi que le pourcentage le plus élevé de protection de la membrane érythrocytaire. L'évaluation antibactérienne a également montré que *A. malaccensis* exerçait généralement une activité plus importante contre les souches bactériennes testées comparativement à *E. alata* et au mélange. Dans l'ensemble, les résultats confirment le potentiel biologique significatif des deux plantes, *A. malaccensis* présentant les propriétés pharmacologiques les plus prometteuses, tandis que le mélange a mis en évidence des effets synergiques intéressants.

Mots-clés : plantes médicinales, *Ephedra alata*, *Aquilaria malaccensis*, Mélange binaire, Activité biologique

ملخص

تُستخدم النباتات الطبية على نطاق واسع لأغراض علاجية نظرًا لغناها بالمستقبلات الثانوية النشطة بيولوجيًا، والتي تمتلك خصائص دوائية مهمة مما يجعلها مصادر قيمة للمركبات العلاجية. هدفت هذه الدراسة إلى تقييم التركيب الكيميائي النباتي النوعي، بالإضافة إلى الأنشطة المضادة للأكسدة والمضادة للالتهاب والمضادة للميكروبات لنبات *الإيفيرا/اللاتا* و *الأكويلاريا مالاكسيس* وخليطهما الثنائي أظهر التحري الكيميائي النباتي وجود العديد من المستقبلات الثانوية، بما في ذلك القلويدات، الفلافونويدات، الأنثوسيانينات، التربينويدات والكومارينات في كلا المستخلصين. كما تم الكشف عن التانينات الكاتيشية في نبات *الأكويلاريا مالاكسيس*، بينما تم التعرف على التانينات الغاليكية في نبات *الإيفيرا/اللاتا*.

أظهر تقييم الأنشطة البيولوجية خصائص دوائية واعدة للمستخلصات المدروسة. فقد سجل نبات *الأكويلاريا مالاكسيس* أعلى قدرة مضادة للأكسدة كلية (112 ملغ مكافئ حمض الأسكوربيك/غرام من المستخلص) في حين أظهر الخليط أعلى نشاط لالتقاط الجذر الحر ($IC_{50} = 93.09 \pm 2.59$ DPPH ميكروغرام/مل مما يشير إلى احتمال وجود تأثيرات تآزرية بين المركبات الكيميائية النباتية. في IC_{50} وفيما يتعلق بالنشاط المضاد للالتهاب، أظهر نبات *الأكويلاريا مالاكسيس* أفضل فعالية، حيث سجل أقل قيمة اختبار تثبيط تمسخ ألبومين مصل الأبقار، بالإضافة إلى أعلى نسبة حماية لغشاء كريات الدم الحمراء كما أظهر التقييم المضاد للبكتيريا أن نبات *الأكويلاريا مالاكسيس* يمتلك عمومًا نشاطًا أكبر ضد السلالات البكتيرية المختبرة مقارنةً بنبات *الإيفيرا/اللاتا* وخليط الثنائي. بشكل عام، تؤكد النتائج إمكانات البيولوجية المهمة لكلا النباتين، حيث أظهر نبات *الأكويلاريا مالاكسيس* الخصائص الدوائية الأكثر واعدة، بينما أبرز الخليط الثنائي تأثيرات تآزرية مثيرة للاهتمام.

الكلمات المفتاحية: *الإيفيرا/اللاتا*، *الأكويلاريا مالاكسيس*، الخليط الثنائي، النشاط البيولوجي.

List of Abbreviations and symbol:

- **% PI:** Percentage inhibition
- ***A. malaccensis* / AM:** *Aquilaria malaccensis* (Plant species)
- **AAE:** Ascorbic Acid Equivalents.
- **Abs:** Absorbance
- **ABTS:** 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
- **AlCl₃:** Aluminum Chloride
- **ATCC:** American Type Culture Collection
- ***B. cereus*:** *Bacillus cereus*
- ***B. subtilis*:** *Bacillus subtilis*
- **Bax:** Bcl-2-associated X protein
- **BHA:** Butylated Hydroxyanisole
- **BHT:** Butylated Hydroxytoluene
- **BSA:** Bovine Serum Albumin
- **CAT:** Total Antioxidant Capacity
- **COX:** Cyclooxygenase
- **D control:** Diameter of the control
- **D sample:** Diameter of the sample zone
- **D:** Diameter
- **DM:** Dry Matter
- **DMSO:** Dimethyl Sulfoxide
- **DPPH:** 2,2-diphenyl-1-picrylhydrazyl.
- ***E. alata*:** *Ephedra alata*
- ***E. coli*:** *Escherichia coli*
- **EAC:** Cathechique Acid equivalent
- **EAG:** Gallic Acid Equivalent
- **EtOH:** Ethanol
- **FeCl₃:** Ferric Chloride
- **FPP:** Farnesyl Pyrophosphate
- **GAE:** Gallic Acid Equivalent
- **GC:** Gas Chromatography
- **GC–MS:** Gas Chromatography–Mass Spectrometry
- **H₂O₂:** Hydrogen Peroxide
- **H₂SO₄:** Sulfuric Acid
- **HCl:** Hydrochloric Acid
- **HEPG2:** Hepatocellular Carcinoma G2
- **IC₅₀:** Inhibitory Concentration 50%
- **IL-1β:** Interleukin 1 Beta.
- **IL-6:** Interleukin-6
- **LAPRONA:** Natural Products Laboratory
- **LOX:** Lipoxygenase
- **LPS:** stands for Lipopolysaccharide

- **MAPK/MAPKs:** Mitogen-Activated Protein Kinases.
- **MBC:** Minimum Bactericidal Concentration
- **MCF-7:** Michigan Cancer Foundation-7
- **ME:** Methanolic Extract
- **MeOH:** Methanol
- **MIC:** Minimum Inhibitory Concentration.
- **MNHN:** National Museum of Natural History (Paris).
- **MS:** Mass Spectrometry
- **NaCl:** chloride sodium
- **NaNO₂:** Sodium Nitrite
- **NaOH:** Sodium Hydroxide
- **NF- κ B:** Nuclear Factor kappa B
- **NO:** Nitric Oxide
- **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs
- **p:** Weight
- **P21:** Cyclin-Dependent Kinase Inhibitor 1
- **P3C:** prostate cancer 3
- **P53:** Tumor Protein
- **PBS:** Phosphate Buffered Saline
- **PC3:** prostate cancer
- **PEC:** Phenylethyl Chromone.
- **pH:** potential hydrogen
- **PSE:** pseudoephedrine
- **QE:** Quercetin Equivalent
- **ROS:** Reactive Oxygen Species
- **rpm:** Revolutions Per Minute
- ***S. aureus:*** *Staphylococcus aureus*
- **SD:** Standard Deviation
- **SEM:** Standard Error of Mean
- **TAC:** Total Antioxidant Capacity
- **TFC:** Total Flavonoid Content
- **TLR4:** Toll-Like Receptor 4
- **TNF- α :** Tumor Necrosis Factor alpha
- **TPC:** Total Phenolic Content
- **USA:** United American Staite
- **UV:** Ultraviolet
- **WHO:** World Health Organization

Figure List:

Figure 1: treatise on Materia medica in Arabic translation	5
Figure 2: Soxhlet extraction apparatus	9
Figure 3: <i>Aquilaria malaccensis</i> Lam	18
Figure 4: the types of agarwood sesquiterpene skeleton and their corresponding fragrance profiles ..	24
Figure 5: General port of <i>ephedra alata</i> subsp alanda Batt §Trab A; Flowering branch B and blooming flower C	29
Figure 6: Geographic distribution of ephedra in the world	30
Figure 7: chemical structure of <i>Ephedra</i> alkaloids.....	32
Figure 8: general diagram of analyses carried out.....	38
Figure 9: Bark (A) and powder(B) of <i>A. malaccensis</i>	39
Figure 10: Stem(A) and Powder(B) of <i>E. alata</i>	39
Figure 11: Steps of maceration.....	40
Figure 12: Reduced from pf the DPPH° radical	44
Figure 13: Photographs illustrating the experimental procedure.....	50
Figure 14: Quercetin calibration curve.....	55
Figure 15: flavonoid content of <i>A. malaccensis</i> and <i>E. Alata</i> . Values are expressed as mean $\pm$ SEM; <i>A. Malaccensis</i> vs <i>E. Alata</i> (*: $p < 0,05$).....	56
Figure 16: Inhibition percentage of ascorbic.....	57
Figure 17: Inhibition percentage of <i>A. malaccensis</i>	58
Figure 18: Inhibition percentage of <i>E. alata</i>	58
Figure 19: Inhibition percentage of Mixture	59
Figure 20: Comparison of the percentage of inhibition between <i>A. malaccensis</i> , <i>E. alata</i> and Mixture	59
Figure 21: inhibitory concentration 50 of extracts plants and their mixtures. Values are expressed as mean $\pm$ SEM ($p \geq 0.05$)	60
Figure 22: Calibration curve of ascorbic acid. Each point represents the mean of three experiments.	62
Figure 23: Total antioxidant capacity of extracts plants and their mixtures	62
Figure 24: Comparison of the percentage of inhibition between <i>A. malaccensis</i> , <i>E. alata</i> and Mixture. Each point represents the mean of three experiments.	64
Figure 25: inhibitory concentration 50 of extracts plants and their mixtures	65
Figure 26: Percentage of membrane protection induced by the plant extracts and their mixture. Values are expressed as mean $\pm$ SEM standard vs <i>A. malaccensis</i> (**: $p < 0.01$), standard vs <i>E. alata</i> (***: $p < 0.001$), <i>E. alata</i> vs <i>A. malaccensis</i> and mixture (**: $p < 0.01$).....	67
Figure 27: Inhibition zones induced by the plant extracts and their mixture.	69

Table List:

Table 1: genetic structure of major classes of phenolic compound.....	13
Table 2 : genetic structure of major classes of flavonoids.....	14
Table 3: classification of <i>Aquilaria malaaccensise</i>	17
Table 4: Aromatic compounds from agarwood	20
Table 5: chemical structure of 2-(2-phenylethyl) chromones in agarwood.....	22
Table 6: classification of <i>Ephedra alata</i>	28
Table 7: Origin of the strains used for the evolution of antimicrobial activity.....	48
Table 8: Aspect, colors and yields of extract <i>A. malacensis</i> and <i>E. alata</i>	53
Table 9: phytochemicals screening <i>A. malaccensis</i> and <i>E. alata</i>	54

Table of Contents

Table of Contents

Abstract	I
Résumé	II
ملخص	III
List of Abbreviations and symbol	
Figure List	
Table List	
Introduction	1

Theoretical part

Chapter I: Medicinal plant

1. Medicinal plant.....	4
1.1 Definition	4
1.2. History	4
2. Types of medicinal plants.....	6
2.1 Wild plants	6
2.2 Cultivated plants.....	6
3. Different parts used	6
4. Extraction Methods	7
4.1 Maceration.....	7
4.2 Tisane or infusion	7
4.3 Decoction.....	8
4.4 Soxhlet extraction.....	8
5. Secondary metabolites in medicinal plants	9
5.1 Alkaloids.....	10
5.2 Polyphenols	10
5.2.1 Phenolic Acids	11
5.2.2 Stilbenes	11
5.2.3 Coumarins	12
5.2.4 Lignans	12
5.2.5 Quinones.....	12
5.2.6 Tannins	13
5.2.7 Flavonoids	14

Table of Contents

5.3 Terpenes.....	15
5.4 Saponins	15

Chapter II: *Aquilaria malaccensis* & *Ephedra alata*

1. <i>Aquilaria malaccensis</i>	17
1.1 Taxonomic and botanic description	17
1.2 Geographical distribution and economic Value	19
1.3 Chemical Composition.....	19
1.3.1 Aromatic group.....	19
1.3.2 Chromone	21
1.3.3 Sesquiterpenoids.....	23
1.4 Agarwood utilization	25
1.4.1 Anti-inflammatory	26
1.4.2 Antimicrobial.....	26
1.4.3 Anti-tumor	26
1.4.4 Other pharmacological effects.....	27
2. <i>Ephedra alata</i>	27
2.1 Taxonomy and botanical description.....	28
2.2 Geographical distribution	30
2.3 Chemical composition.....	31
2.3.1 Lipids and Polysaccharides	31
2.3.2 Alkaloids	31
2.3.3 Flavonoids and Phenolic Compounds	32
2.4 Biological Activities	33
2.4.1 Antioxidant Activity	33
2.4.2 Antimicrobial Activity.....	33
2.4.3 Anti-Inflammatory Activity.....	34
2.4.4 Cytotoxic and Anti-Tumor Activities	34
2.4.5 Other Activities.....	35

Experimental part

Chapter III: Materials and Methods

1. Objective of study	38
2. Plant materiel and Extract Preparation.....	38
3. Phytochemical screening.....	40
3.1 Preparation of ethanolic extract	40
3.2 Preparation of the aqueous extract.....	41

Table of Contents

3.3 Maceration of plant material with sulfuric acid (H ₂ SO ₄)	42
4. Quantification of flavonoids.....	43
5. Antioxidant activity	43
5.1 DPPH° radical scavenging method	43
5.2. Total antioxidant capacity.....	45
6. Anti-inflammatory activity	45
6.1 Bovine serum albumin (BSA) denaturation inhibition test	46
6.2 Red blood cell (erythrocyte) membrane stabilization test.....	47
7. Antimicrobial activity.....	48
8. Statistical analysis	51

chapter IV: Resultant and Discussion

1. Extraction Yield.....	53
2. Qualitative Phytochemical Analysis.....	54
3. Total flavonoid content.....	55
4. Evaluation of antioxidant activity	56
4.1 Antiradical activity by DPPH	56
4.2 Total antioxidant capacity (TAC) assay.....	61
5. Anti-inflammatory activity	64
5.1 Bovine serum albumin denaturation inhibition (BSA) test	64
5.2 Erythrocyte membrane stabilization test	66
6. Antibacterial activity	68
Conclusion and perspective.....	73
Reference bibliography	76
Appendices	90

Theoretical part



General Introduction

Introduction:

For millennia, human societies have relied on natural resources from their environment to prevent, manage, and treat various diseases. Among these resources, medicinal plants represent an essential source of raw materials for the discovery of novel molecules with therapeutic potential. They constitute one of the primary reservoirs of bioactive compounds and form the foundation of phytotherapy, which involves the use of plant-derived products for the prevention and treatment of different health disorders (**Jdaidi *et al.*, 2023**).

Secondary metabolites are organic compounds synthesized by plants from primary metabolites such as amino acids and carbohydrates through various metabolic pathways (**Makhloufi, 2013**). Although these compounds are not directly involved in basic growth and development processes and are therefore often considered non-essential for plant survival (**Levasseur-Garcia *et al.*, 2013**), they play fundamental roles in plant adaptation and defense. Secondary metabolites contribute to responses against biotic and abiotic stresses, improve reproductive success, and participate in defense mechanisms against pathogens and herbivores, attraction of pollinators, and allelopathic interactions (**Guillon, 2010**). Furthermore, these compounds have considerable importance for human applications due to their use as pharmaceuticals, dyes, flavoring agents, antibiotics, herbicides, and therapeutic substances (**Merzougui and Tadj, 2015**). Plant secondary metabolites are generally classified into several major groups, including phenolic compounds, saponins, alkaloids and other nitrogen-containing compounds, and terpenoid compounds. These classes contain numerous bioactive molecules associated with important pharmacological activities.

Ephedra alata is widely used in traditional medicine for the treatment of various disorders. This species contains numerous secondary metabolites, particularly alkaloids, among which ephedrine is considered one of the principal active compounds. Ephedrine contributes not only to the pharmacological properties of the plant but also to certain toxicological effects (**Kebeli, 2016**). Previous studies have reported that high doses of ephedrine may induce hepatotoxicity associated with extensive hepatic necrosis (**Song *et al.*, 2023**). Despite these concerns, *E. alata* has demonstrated therapeutic potential in the management of several conditions, including cardiovascular disorders, hypotension, obesity, common cold, asthma, fever, diabetes mellitus, and cancer (**Ben Guedouad and Taleb, 2021**).

Species belonging to the genus *Aquilaria* are shade-tolerant trees that naturally grow in forest understories. When these trees are injured, exposed tissues become susceptible to colonization by environmental microorganisms, particularly fungi. In response to this biotic stress, the tree produces a modified resinous wood rich in secondary metabolites through complex interactions with invading microorganisms (Naziz *et al.*, 2019). This resinous material, commonly known as agarwood or oud, is characterized by its dark coloration resulting from oxidizable compounds and is enriched in oleoresins containing chromones and sesquiterpenes, compounds associated with numerous pharmacological properties (Naef, 2011; Mohamed *et al.*, 2010).

Considering the therapeutic importance of medicinal plants and the biological activities associated with their phytochemical constituents, the objective of the present study was to perform a phytochemical characterization of *A. malaccensis* and *E. alata* ethanolic extracts and to investigate the potential synergistic effects of their combination. In addition, antioxidant, anti-inflammatory, and antimicrobial activities were evaluated to determine their pharmacological potential.

This work is organized into two main parts:

- **The first part** provides a bibliographic overview of medicinal plants and presents a literature review focused on the selected species.
- **The second part** describes the experimental methodology, including the materials and methods employed, and presents and discusses the obtained results.
- **Finally**, the manuscript concludes with a general conclusion and future research perspectives.



Chapter I:

Medicinal plants

1. Medicinal plant:

1.1 Definition:

Medicinal plants are defined as plants that contain active components with therapeutic properties, used in various medical systems to treat, diagnose, and prevent diseases. These plants have been an integral part of human health practices for centuries, with their use documented in ancient texts and traditional medicine. The importance of medicinal plants lies not only in their historical applications, but also in their potential for the development and synthesis of modern medicines. The precise definition of medicinal plants indicates that about one-third of all medicinal preparations are derived from plants, highlighting their importance as sources of biologically active substances with therapeutic effects (**Dilafroz, 2023**).

pharmacological firms utilize herbal-based goods, which are a centuries-old source of bioactive substances with pharmacological significance, to formulate a variety of medical medicines. The use of phototherapeutic compounds has grown dramatically in recent years. 80% of people worldwide rely on Phyto therapeutic products, according to the World Health Organization (WHO). Due to the presence of unique phytochemical ingredients that cause physiological reactions in the human body, therapeutic flora is considered significant. Sterols, alkaloids, tannins, phenolic compounds, flavonoids, and saponins are some examples of these bioactive chemical components. Because of their ease of use and wide range of therapeutic effects, herbal-based pharmaceuticals have a distinct edge over conventional medication. Additionally, their incidence of side effects is much lower than that of chemotherapeutic medicines. About 426 biomes encompass a multitude of distinct habitats, making them some of the richest centers of phylogenetic resources in the world. Among the 18,665 flowering species, only 3,000 plants have been used (**Arpita and Bharadvaja, 2017**).

1.2. History:

Since the earliest stages of human existence, plants have been used for medicinal purposes, with prehistoric hunter-gatherers relying on them not only for food but also for healing. Archaeological evidence indicates that as early as 35,000 years ago, Cro-Magnon people were familiar with plants such as chamomile, yarrow, nettle, flax, poppy, and valerian (**Chevalier, 2017**). Early written records from ancient China, Egypt, and Mesopotamia reveal that therapeutic knowledge was already well developed 3,000 to 4,000 years ago, with remedies derived from minerals, animals, and especially plants (**Cantau, 2011**). During the Middle Ages, following the fall of the Roman Empire, Europe experienced a decline in

knowledge until the Arab world played a key role in preserving and advancing medical sciences, leading to a new intellectual revival (**Bruneton, 2001**). By the 12th century, the Crusades facilitated exchanges between Europe and the Middle East, promoting the resurgence of trade, particularly in Italy, including medicinal plants and spices. Over time, this exchange enriched traditional knowledge, allowing communities to develop diverse plant-based remedies; until the 20th century, villages commonly used local plants in the form of infusions, ointments, or oils to treat minor ailments. Today, advances in chemistry and pharmacology have improved our understanding of plant active compounds, their therapeutic effects, and their mechanisms of action, confirming the long-standing importance and diverse uses of medicinal plants (**Cantau, 2011**). (**figure 1**)



Figure 1: treatise on Materia medica in Arabic translation (**Chevalier, 2017**).

1.2. Utilizations of medical plant in Algeria:

Algeria is the largest country bordering the Mediterranean Sea, covering an area of 2,381,741 km². It is characterized by remarkable plant biodiversity, particularly in medicinal species, which are especially abundant in the Kabyle and play an important role in traditional practices and popular cultural heritage (**Sahi, 2016**). The use of medicinal plants remains widespread across the country, especially in rural areas, as in other Maghreb countries, where ancestral knowledge is preserved by older generations, notably through the preparation of herbal infusions. The Hoggar region alone contains more than 300 of the countries over 600 medicinal plant species, with more than 25% still used in traditional medicine. However, this valuable natural heritage is increasingly threatened by climate change, particularly drought, as well as unsustainable human activities (**Ibn Sina and Bounab Triki, 2017**).

2. Types of medicinal plants:

2.1 Wild plants:

Wild medicinal plants are those that grow naturally in their native habitats without human intervention, relying on natural ecological processes for their growth and reproduction. They are typically found in forests, grasslands, deserts, or mountainous regions, where they are exposed to varying climates, diverse soil types, pests, and competition. Due to these environmental stresses, their growth may be slower compared to cultivated plants.

The types of wild plants used as medicinal herbs vary in classification and morphology, yet they play a crucial role in providing health services, especially in developing countries. For generations, wild plants have been recognized for their medicinal properties in treating both internal and external ailments and have served as alternative remedies for various diseases. For example, in Ethiopia, certain wild plants are used to treat tuberculosis, in South Africa, they serve as fever reducers and treatments for sexually transmitted diseases; in China, wild plants are used as antiviral agents, for internal diseases, and as herbal remedies (**Damhuri *et al.*, 2023**).

2.2 Cultivated plants:

Cultivated medicinal plants are deliberately grown and managed by humans, typically in farms, gardens, or controlled environments, for medicinal, commercial, or research purposes. The practice of cultivating medicinal plants has a long history, aimed at facilitating their collection and use while ensuring consistent quality. A key goal of cultivation is to produce raw materials that are rich in pharmacologically active substances. The value of a medicinal plant is not determined solely by the quantity of its biomass, but also by the preservation and concentration of biologically active compounds that are used to produce extracts or drugs. For instance, when preparing tinctures or extracts from valerian roots and rhizomes, it is crucial that the raw material contains sufficient valepotriates, free valeric acid, and essential oils.

While the biosynthesis of active compounds occurs naturally in wild plants, cultivation allows better control over these processes. By managing growth conditions and intervening in the plant's metabolic pathways, it is possible to enhance the production of specific bioactive substances and direct their synthesis in desired ways (**Dalir *et al.*, 2025**).

3. Different parts used:

Medicinal plants refer to species of the plant kingdom whose parts such as flowers, leaves, roots, stems, fruits, or seeds are used or prepared as medicine to treat conditions or diseases.

The selection of a particular plant part for medicinal use depends largely on the specific bioactive compounds required and the intended method of preparation. Leaves are the most

used, accounting for approximately 31% of usage, as they often contain the highest concentrations of active ingredients; examples include *Aloe vera* for skin care, *Ginkgo biloba* for cognitive health, and *Thyme* for respiratory support. Roots, representing about 12% of usage, serve as reservoirs of potent medicinal compounds and are used in plants such as *Ginseng* for anti-inflammatory and energy-boosting effects, *Devil's Claw* for joint pain, and *Ginger* for digestive and anti-inflammatory purposes. Bark, comprising roughly 8% of use, is often processed into infusions or powders, with Willow bark being a classic example due to its pain-relieving properties derived from salicylic acid. Flowers, which account for 6% of usage, are frequently used in teas and extracts, such as *Chamomile* for its calming and anti-inflammatory effects, and *Hibiscus* for antioxidant benefits. Fruits and seeds also serve important medicinal roles, including *Garlic* bulbs as a natural antibiotic, Senna seeds as a laxative, and Fennel seeds for digestive support. Finally, the whole plant is used in about 28% of cases, particularly in traditional medicine for holistic treatments. Together, these diverse plant parts allow herbal medicine to target a wide range of health conditions effectively (Miranda, 2021; USFS, 2025).

4. Extraction Methods:

4.1 Maceration:

The term “maceration” refers to the softening of plant material in the process of extraction. Maceration is a solid–liquid extraction method in which ground plant materials are immersed in a suitable solvent within a closed container, and the mixture is periodically shaken or agitated. The plant–solvent mixture is typically left at room temperature for 2–7 days, depending on the specific bioactive compounds targeted for extraction. During this period, the plant tissues soften and the cell walls break down, allowing the release of intracellular phytoconstituents into the solvent. Once the extraction is complete, the solvent containing the dissolved bioactive compounds is separated from the solid plant residue. To maximize yield, the remaining plant material is often pressed to recover the solvent still trapped within, though in some cases this step is replaced by filtration or centrifugation (Khongthaw *et al.*, 2023; Martínez-Moreno *et al.*, 2024).

4.2 Tisane or infusion:

Tisane or infusion extraction is a method used to extract bioactive compounds from plant materials that are readily soluble. In this process, the plant material, which easily releases its phytoconstituents when in contact with organic solvents, is soaked in a specific volume of hot or cold solvent for approximately 15 minutes. After soaking, the mixture is allowed to cool for around 45 minutes, followed by filtration to separate the liquid extract from the plant residues.

This method is considered safe and effective for obtaining crude plant extracts and is officially recognized by the Indian Pharmacopoeia for the extraction of crude drugs (**Remya *et al.*, 2020; Pandiselvam *et al.*, 2023**).

4.3 Decoction:

Decoction is a water-based extraction method used to obtain active compounds from medicinal plants. In this process, plant materials are boiled in a measured volume of water for a specified duration, typically ranging from 15 minutes to 2 hours, followed by cooling and filtration. Decoction is particularly effective for extracting water-soluble, heat-stable, and tough bioactive phytoconstituents. Tender plant parts such as leaves, flowers, roots, and young stems are generally boiled for about 15 minutes, whereas harder materials like branches and tree bark may require up to an hour of boiling. The resulting herbal preparations are usually consumed orally and remain a common practice among traditional health practitioners in formulating traditional herbal medicines. However, this method has certain limitations: it lacks selectivity, especially for water-insoluble compounds, often produces lower yields, and requires large volumes of water, which may raise safety and environmental concerns (**Pandiselvam *et al.*, 2023**).

4.4 Soxhlet extraction:

Soxhlet extraction is a method used to obtain bioactive compounds from plant materials using a Soxhlet apparatus (**Figure 02**), which allows continuous exposure of the plant to a warm solvent for higher yields. In this process, the plant material is placed in a thimble holder, while the solvent in a distillation flask is heated to produce vapor. The vapor condenses and repeatedly passes through the plant material, dissolving the desired phytoconstituents. When the thimble reaches capacity, the solvent is siphoned back into the flask, and the cycle continues. After extraction, the bioactive compounds are typically concentrated using rotary evaporation. While efficient, Soxhlet extraction has limitations, including long processing times (6–48 hours), large solvent requirements, and high temperatures (65–100°C), which can degrade heat-sensitive compounds, reducing the potency and biological activity of the extract (**Antony and Farid, 2022; Thilakarathna *et al.*, 2023**).

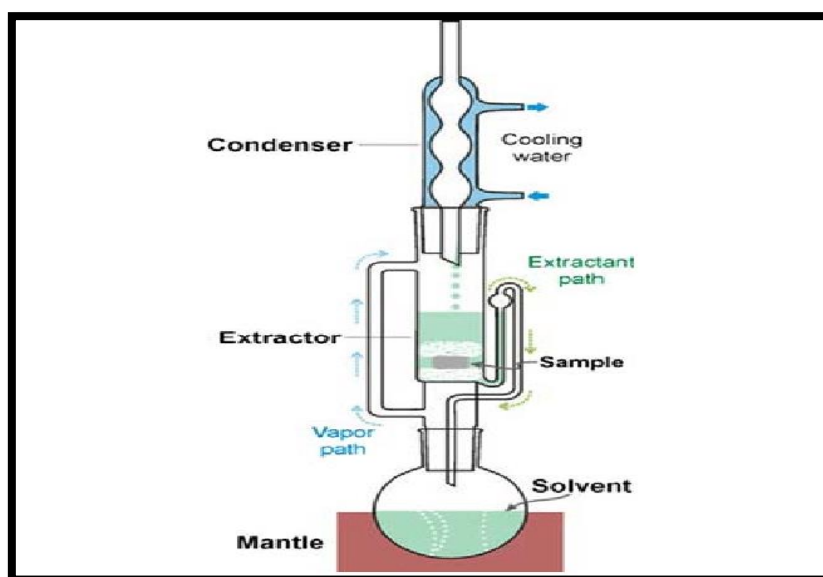


Figure 2: Soxhlet extraction apparatus (Dabbs *et al.*, 2006).

Modern extraction techniques with enhanced efficiency and selectivity are increasingly replacing traditional methods in laboratory and industrial settings. This shift is driven by limitations of conventional approaches, such as the lack of high-performance, reliable methods for ensuring the purity and standardization of herbal medicines. Traditional solvent extraction relies on solvent choice, heat, and/or agitation to enhance the solubility and transfer of target bioactive compounds, but it often requires long extraction times, which can compromise heat-sensitive phytoconstituents. In contrast, novel methods such as microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, subcritical water extraction, and ultrasound-assisted extraction have received significant research attention over the past two decades. When properly applied, these techniques can provide more efficient and standardized extraction processes, ensuring higher quality herbal medicines for global markets. However, a major limitation in many African settings is the lack of studies evaluating these advanced methods for traditional formulations, leaving their suitability for extracting bioactive phytoconstituents in such medicines largely unexplored (Chaves *et al.*, 2020; Jha and Sit, 2022).

5. Secondary metabolites in medicinal plants:

Traditionally, plant metabolism has been classified into primary and secondary metabolism, although current research shows a strong interaction between these two systems, making this distinction increasingly less clear. Primary metabolic pathways occur in all plant species and operate throughout most stages of the plant life cycle because they are essential for survival and growth. These pathways produce fundamental small molecules such as simple sugars, amino

acids, and fatty acids, which are widely distributed in the plant kingdom and serve as precursors for the synthesis of macromolecules including polysaccharides, proteins, nucleic acids, and lipids that form plant structures and support development. In contrast, secondary metabolism is not universal and often occurs only at specific developmental stages, in specialized organs or tissues, or under environmental conditions. Secondary metabolites are derived from the main metabolic routes of carbohydrates, lipids, and nitrogen metabolism and give rise to a wide variety of compounds such as phenolics, terpenoids, and alkaloids, many of which play important ecological roles and contribute to the medicinal properties of plants (**Palazon and Alcalde, 2025**).

5.1 Alkaloids:

Alkaloids are a diverse group of low-molecular-weight compounds characterized by the presence of nitrogen atoms, usually incorporated within heterocyclic rings, which contribute to their strong biological activity and typically bitter taste. Structurally, most alkaloids consist of one or more carbon rings containing nitrogen and are generally derived from amino acids. However, variations in their biosynthetic origin across different plant families lead to differences in the position of the nitrogen atom within the ring system, resulting in a wide diversity of chemical structures and biological functions. To date, more than 10,000 naturally occurring alkaloids have been identified in over 300 plant species (**Zhao *et al.*, 2015**).

Alkaloids play an essential role in both human medicine and in an organism's natural defense. Alkaloids make up approximately 20% of the known secondary metabolites found in plants. In plants, alkaloids protect plants from predators and regulate their growth. Therapeutically, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents. Well-known alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine (**Heinrich *et al.*, 2021**).

5.2 Polyphenols:

Polyphenols are a large group of secondary metabolites that play an important role in plant defense, particularly in scavenging reactive oxygen species such as hydrogen peroxide. They are among the most abundant compounds in higher plants after carbohydrates and exhibit a wide structural diversity, ranging from simple phenolic molecules to complex polymers like lignin. Structurally, phenolic compounds are characterized by the presence of at least one hydroxyl group directly attached to an aromatic (phenyl) ring. Based on their chemical structure, they are classified into several groups, including phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids. These compounds are mainly

synthesized through the shikimic acid and phenylpropanoid pathways. Polyphenols are well known for their significant biological activities, including antiviral, immunomodulatory, and anti-inflammatory effects. In humans, they exhibit strong antioxidant properties and contribute to the prevention of diseases associated with oxidative stress, such as cardiovascular disorders, neurodegenerative diseases, and cancer. However, their health benefits depend on their specific type, the quantity consumed, and their bioavailability (**Kisiriko *et al.*, 2021; Sun and Shahrajabian, 2023**).

5.2.1 Phenolic Acids:

Phenolic acids are a class of polyphenols characterized by the presence of a carboxylic acid group in addition to the basic phenolic structure, and they are broadly classified into two main groups: hydroxybenzoic acids and hydroxycinnamic acids (**Table 01**). Hydroxybenzoic acids possess a C6–C1 skeleton and are commonly found conjugated with small organic acids, sugars, or structural components of plant cells. Representative examples include gallic, syringic, protocatechuic, p-hydroxybenzoic, vanillic, gentisic, and salicylic acids; for instance, gallic acid occurs in cloves (*Eugenia caryophyllata*), while protocatechuic acid is present in coriander (*Coriandrum sativum*), star anise (*Illicium verum*), and dill (*Anethum graveolens*). Their derivatives include compounds such as syringaldehyde and vanillin, the latter being a component of vanilla. In contrast, hydroxycinnamic acids are based on a C6–C3 skeleton and are frequently linked to molecules such as quinic acid or glucose. Major examples include caffeic, p-coumaric, ferulic, and sinapic acids; caffeic acid is found in parsley (*Petroselinum crispum*), sage (*Salvia officinalis*), and ginger (*Zingiber officinale*), whereas p-coumaric acid occurs in oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and basil (*Ocimum basilicum*). Important derivatives of hydroxycinnamic acids include rosmarinic acid (an ester of caffeic acid), caftaric acid (formed from caffeic and tartaric acids), and chlorogenic acid, an ester of caffeic and quinic acids (**Kisiriko *et al.*, 2021**).

5.2.2 Stilbenes:

Stilbenes are based on 1,2-diphenylethylene, which has a C6–C2–C6 skeleton. They are often hydroxylated (stilbenoids) and can be found as aglycones, monomers, oligomers or glycosylated derivatives. They can also be methylated or prenylated among other modifications. *Trans*-resveratrol is the most common stilbene and is a precursor for many larger stilbenes. Medicinal plants belonging to *Eucalyptus* and *Pinus* genera are common sources of stilbenes (**Kisiriko *et al.*, 2021**).

5.2.3 Coumarins:

Coumarins are benzopyrone derivatives occurring in all plant parts but mainly found in the flowers of plants belonging to several families. They may occur in a free or glycosylated state and are divided into six categories, namely simple coumarins such as coumarin and esculetin; furanocoumarins such as psoralen and imperatorin; dihydrofuran coumarins such as anthogenol and felamidin; pyranocoumarins such as grandivittin and pseudocordatolide C; phenylcoumarins such as isodispar B and disparinol D; and bicoumarins such as dicoumarol (Kisiriko *et al.*, 2021).

5.2.4 Lignans:

Lignans are a large group of naturally occurring secondary metabolites derived from the shikimic acid pathway, characterized by a basic structure composed of two or more phenylpropanoid units. They are formed from monomers such as cinnamic acid and its derivatives and are classified as “classical lignans” when the linkage occurs at the β - β' (8-8') position, while other linkage patterns give rise to “neolignans.” Lignans are widely distributed throughout the plant kingdom and can be found in various plant parts, including roots, stems, leaves, seeds, and resins, with families such as *Lauraceae*, *Annonaceae*, and *Schisandraceae* being particularly rich sources. These compounds exhibit a broad range of pharmacological activities, including antitumor, antioxidant, antibacterial, immunosuppressive, and antiasthmatic effects (Cui *et al.*, 2020).

5.2.5 Quinones:

Quinones are ubiquitous biological pigments found in a range of living organisms (bacteria, fungi, higher plants, and in few animals). They exist in nature in many forms such as benzoquinones, naphthoquinones, anthraquinones, and polycyclic quinones. For example, the K vitamins (phylloquinone) are naphthoquinones (Devi and Mehendale, 2014).

As vitamins, they represent a class of molecules preventing and treating several illnesses such as osteoporosis and cardiovascular diseases. Quinones, by their antioxidant activity, improve general health conditions. Many of the drugs clinically approved or still in clinical trials against cancer are quinone related compounds. Quinones have also toxicological effects through their presence as photoproducts from air pollutants. Quinones are fast redox cycling molecules and have the potential to bind to thiol, amine and hydroxyl groups (El-Najjar *et al.*, 2011).

5.2.6 Tannins:

Tannins are high molecular weight polyphenolic compounds known for their ability to complex proteins and their astringent taste that deters insects, birds and herbivores from feeding on plants. They are found in most plant parts including the bark, wood, leaves, fruits and roots and can be synthesized as a defensive mechanism in response to pathogen attack and abiotic stresses such as UV radiation. Several medicinal plant species belonging to the genera *Acacia*, *Terminalia*, *Eucalyptus*, *Pinus*, *Betula* and *Quercus* are abundant sources of tannins. Based on their structures, tannins in plants can be classified into mainly the hydrolysable tannins and the condensed tannins, also known as proanthocyanidins. Hydrolysable tannins are built based on gallic acid and are divided into the gallotannins and ellagitannins (**Kisiriko et al., 2021**). Tannins can reduce the risk of cancer and neurodegenerative diseases, such as cardiovascular diseases and Alzheimer's, respectively (**De Melo et al., 2023**). (Table1)

Table 1: genetic structure of major classes of phenolic compound (**Sun and Shahrajabian, 2023**).

Phenolic compound	Examples	Molecular Formula
Hydroxybenzoic acids	Gallic acid Protocatechuic acid	$C_7H_6O_5$ $C_7H_6O_4$
Hydroxycinnamic acids	<i>p</i> -coumaric acid Caffeic acid Ferulic acid Sinapic acid	$C_9H_8O_3$ $C_9H_8O_4$ $C_{10}H_{10}O_4$ $C_{11}H_{12}O_5$
Coumarins	Umbelliferone Esculetin Scopoletin	$C_9H_6O_3$ $C_9H_6O_4$ $C_{10}H_8O_4$
Stilbenes	Resveratrol Piceatannol Pterostilbene	$C_{14}H_{12}O_3$ $C_{14}H_{12}O_4$ $C_{16}H_{16}O_3$
Condensed tannins or proanthocyanidins	Procyanidin B1	$C_{30}H_{26}O_{12}$
Lignan	Sesamin	$C_{20}H_{18}O_6$

5.2.7 Flavonoids:

Flavonoids are a highly abundant group of secondary metabolites found in plants, fruits, and seeds, where they contribute to color, aroma, and flavor. In plants, they play essential roles in regulating growth, attracting pollinators, and providing protection against biotic and abiotic stresses. They also function as signaling molecules, UV filters, and scavengers of reactive oxygen species (ROS), thereby enhancing tolerance to environmental stresses such as drought, heat, and cold. In humans, flavonoids are associated with numerous health benefits due to their diverse bioactivities, including anti-inflammatory, anticancer, antioxidant, cardioprotective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral effects. Structurally, flavonoids are characterized by a 15-carbon skeleton (C6–C3–C6) consisting of two aromatic rings (A and B) linked by a three-carbon heterocyclic pyran ring (C). Their biological activity and bioavailability are largely influenced by the number and position of hydroxyl groups, particularly on the B ring, which determine their antioxidant capacity by enabling electron donation and free radical stabilization. Based on structural variations, flavonoids are classified into several major groups, including flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins (**Table2**). Owing to their potent antioxidant properties, flavonoids are widely utilized in the food, cosmetic, and pharmaceutical industries (**Dias *et al.*, 2021**).

Table 2 : genetic structure of major classes of flavonoids (**Sun and Shahrajabian, 2023**).

Flavonoids	Examples	Molecular Formula
Flavones	Apigenin Luteolin Chrysin	C ₁₅ H ₁₀ O ₅ C ₁₅ H ₁₀ O ₆ C ₁₅ H ₁₀ O ₄
Flavanols	Kaempferol Quercetin Isorhamnetin	C ₁₅ H ₁₀ O ₆ C ₁₅ H ₁₀ O ₇ C ₁₆ H ₁₂ O ₇
Flavanones	Naringenin Eriodictyol Hesperetin	C ₁₅ H ₁₂ O ₅ C ₁₅ H ₁₂ O ₆ C ₁₆ H ₁₄ O ₆
Flavanols Anthocyanidin		C ₁₅ H ₁₄ O ₂ C ₁₅ H ₁₁ O ⁺
Flavanonols	Taxifolin Aromadendrin	C ₁₅ H ₁₂ O ₇ C ₁₅ H ₁₂ O ₆
Flavan-3-ols	Gallocatechin Catechin	C ₁₅ H ₁₄ O ₇ C ₁₅ H ₁₄ O ₆
Isoflavones	Genistein Daidzein Formononetin	C ₁₅ H ₁₀ O ₅ C ₁₅ H ₁₀ O ₄ C ₁₆ H ₁₂ O ₄

5.3 Terpenes:

Terpenes, also known as isoprenoids, are a large and diverse group of plant secondary metabolites involved in growth, development, and defense. They represent the most structurally diverse class of natural compounds, with more than 30,000 types identified to date. Chemically, terpenes are built from isoprene units (C_5H_8), and their general formula follows the pattern $(C_5H_8)_n$, where n indicates the number of linked units this is known as the isoprene or C5 rule. These units can be arranged in linear “head-to-tail” chains or form cyclic structures, resulting in a wide variety of molecular forms. Based on the number of isoprene units, terpenes are classified into different groups, including monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), and triterpenes (C_{30}) (**Zhao *et al.*, 2015**). Terpenes have a significant role in treating various types of diseases, in many studies in vitro and vivo using as anticancer agents, antimicrobial, anti-inflammatory, antioxidants, antiallergic, neuroprotective, anti-aggregator, anti-coagulation, sedative and analgesic (**Masyita *et al.*, 2022**).

5.4 Saponins:

Saponins are the bioactive compounds that predominately originate from plants and generally occur as polycyclic triterpenes or steroids with glycosides. Chemically, saponin can be divided into two groups, the triterpenoidal saponins and the steroidal saponins. Steroidal saponins contain 27 carbon atoms which form their core structures, such as spirostan. Triterpenoidal saponins or their nor-derivatives contain 30 carbon atoms which form their core structures, such as tetracyclic dammarans and pentacyclic oleanans (**Zhao *et al.*, 2015**), and are noted for their multiple biological activities Fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects have all been observed (**Juang and Liang, 2020**).



Chapter II:

Aquilaria malaccensis
& *Ephedra alata*

1. *Aquilaria malaccensis*:

Aquilaria is defined as a genus of trees within the *Thymelaeaceae* family, known for producing fragrant resin-infused wood, particularly from wounded trees of *Aquilaria* species. This wood, possesses various phytochemical constituents and exhibits significant pharmacological properties (Liang *et al.*, 2022). The *Aquilaria* genus of trees grows in the understory and prefers shade. The tree's trunk, which has silvery-white bark and can reach a diameter of sixty centimeters, can reach heights of over forty meters (Ansel, 2019). Lamarck first described the *Aquilaria* genus (species *malaccensis*) in 1783, Hutchinson and Hou reviewed it in 1924 and 1960, respectively (Lee *et al.*, 2013).

Agarwood, oud, and gaharu are some of the names of the highly valuable fragrant heartwood used for nontimber purposes and produced by the *Aquilaria* species. These “woods of the Gods” have been used and traded for thousands of years for making perfume, which continue to be used in cultural and religious ceremonies, and for the production of incense sticks and fragrance products. It is also used in traditional medicine and has been recorded in the Ayurvedic medicinal text, the Susruta Samhita of prehistoric times, and in the Sahih Muslim that dates to the 8th century (Shivanand *et al.*, 2022).

1.1 Taxonomic and botanic description:

A. malaccensis also called *Aquilaria agallocha*, *Aquilaria secundaria*, *Aquilaria agallochum*, *Aquilaria ovata*, *Aquilaria moluccensis* in English named Agar wood or Aloewood or Malacca Eaglewood (Sarma *et al.*, 2015), in French Bois d’agar or bois d’aigle de Malacca in Arabic خشب العود and in Algerian language named عود غريس or العود الهندي. (Table3)

Table 3: classification of *Aquilaria malaaccensise* (USDA, 2025).

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Malvales
Family	<i>Thymelaeaceae</i>
Genus	<i>Aquilaria</i>
Species	<i>Aquilaria malaccensis</i> Lam

Agarwood is a medium- to large-sized evergreen tree that can reach heights of 20 to 40 meters. Its trunk, which may grow up to 180 cm in diameter, is generally straight and unbranched, supported by prominent buttress roots that can rise up to 2 meters high. The bark is smooth and whitish in appearance. The tree bears simple, alternate leaves with elliptical blades. Its small, fragrant flowers are pale green to yellowish and typically occur in clusters in the leaf axils. The fruits are green, ovoid capsules that, upon ripening, split into two halves to release seeds suspended on long appendages. These seeds are ovoid, blackish brown in color, and densely covered with reddish-brown hairs (Ali *et al.*, 2016) (Figure 3).



Figure 3: *Aquilaria malaccensis* Lam (Mustaqim, 2021).

The tree usually begins flowering and fruiting at around 6–7 years of age, typically during the dry season. In the Malesian region, flowering occurs between July and August, followed by fruit maturation from November to December. A mature tree can produce approximately 1.5 kg of seeds in a favorable year, with an estimated yield of up to 19,000 seeds per season. Seed germination generally begins within 7 to 15 days after sowing. Most seedlings disperse within a radius of about 5 meters from the parent tree. The seeds are recalcitrant, meaning their viability declines rapidly when their moisture content decreases (Ali *et al.*, 2016).

1.2 Geographical distribution and economic Value:

This species is native to Assam, Bangladesh, Borneo, East Himalaya, Malaya, Myanmar, Philippines, Sumatera, Thailand, Vietnam; Bhutan; India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura); Singapore, while its presence in Sarawak needs confirmation. In Indonesia, the range distribution of *A. malaccensis* is in the western part of the country i.e., Sumatra and Kalimantan. However, based on the herbarium, this species has also been collected from small islands near Sumatra e.i: Bangka, Belitung, Karimun and Singkep (CITES, 2025).

Generally, *A. malaccensis* can be found in forests from lowland up to submontane ecosystems at the elevation 0-1000 m. It grows in areas with 20-33 °C temperature, 60-100% of humidity, 56-75% light intensity, 0-50% slope, 6.4-7 of soil pH, and 10-75% of soil humidity, with rain intensity 1.200-2.000 mm/year. This species may grow in rocky, sandy, or calcareous soils, well-drained slopes, and ridges, or areas near swamps. This species can also occur as an emergent tree. *A. malaccensis* starts to produce flowers and seeds at > 20 cm in diameter, or between 6 - 10 years after planting. They are reported to produce thousands of seeds during one fruiting period. *A. malaccensis* produce fruits from sapling stage at < 10 cm diameter (CITES, 2025).

High-quality agarwood can be sold at US \$100,000 per kg, while those of poor quality can only be sold at US \$100 per kg. As a high-quality and high-grade agarwood essential oil, it can even be sold for US \$1500 per 11.7 g. The higher the grade of agarwood, the richer the layers of aroma. The best agarwood fragrance is mellow and sweet, full of penetration and persistence, and the powdery waxy material on the surface can be scraped off and kneaded into a ball. Its aroma is regarded as a symbol of high quality. There are various kinds of volatile components, each with a unique aroma (Wang *et al.*, 2021).

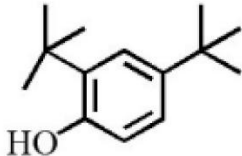
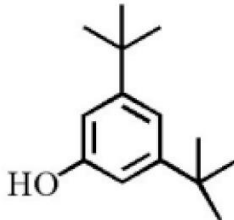
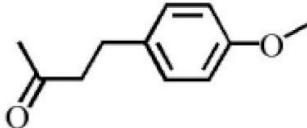
1.3 Chemical Composition:

1.3.1 Aromatic group:

Aromatic volatile compounds constitute another important component of agarwood. Among them, benzylacetone is widely recognized as a key marker compound of its aromatic profile. Analytical studies using GC–MS have shown that chloroform is an effective solvent for the extraction and separation of these aromatic constituents. A variety

of aromatic compounds have been isolated from agarwood volatile oil, with benzyl acetone often representing the highest proportion, reaching up to 19.51%. The composition and relative abundance of these compounds can vary significantly among different species. For instance, agarwood from Malaysia has been reported to contain a higher proportion of aromatic compounds compared to other species, with 4-phenyl-2-butanone accounting for up to 32.1% of the volatile oil. Additional compounds identified in agarwood include benzyl acetone, 2,4-di-tert-butylphenol, 3,5-di-tert-butylphenol, and 4-methoxyphenylacetone (**Table 4**). These compounds have been structurally characterized using spectroscopic techniques and have shown no cytotoxic activity against various cell lines (**Wang *et al.*, 2021**).

Table 4: Aromatic compounds from agarwood (**Wang *et al.*, 2021**).

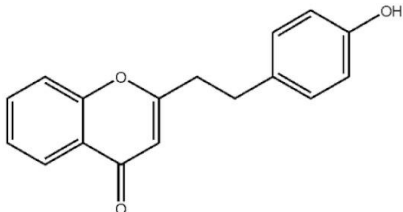
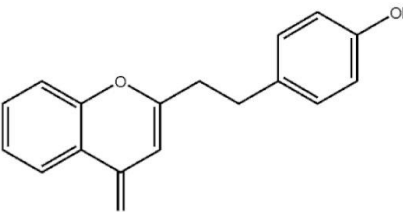
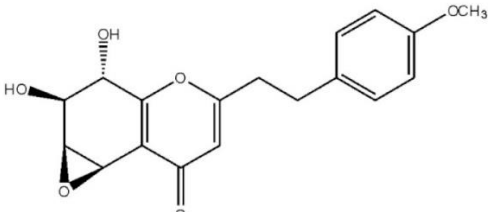
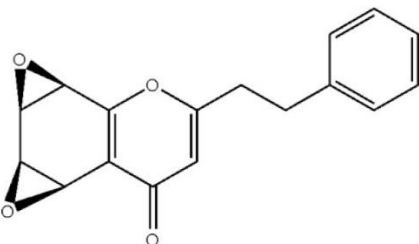
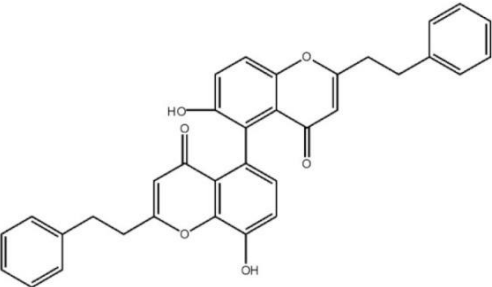
Name	Contents (%)	Chemical Structure
Benzylacetone	0.95	
2,4-Di-tert-butylphenol	4.15	
3,5-Di-tert-butylphenol	2.70	
4-Methoxyphenylacetone	0.95	

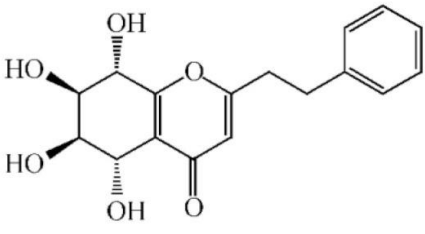
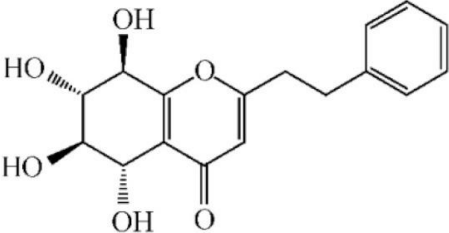
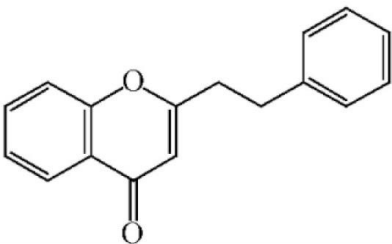
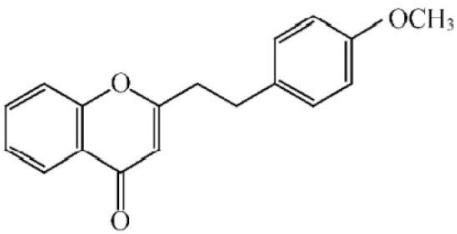
1.3.2 Chromone:

Chromone derivatives are other major constituents of agarwoods. They have been obtained from only a few plant species, including *Eremophila georgei*, *Bothriochloa ischaemum* (Gramineae), and agarwoods originating from *Aquilaria* spp. (Thymelaeaceae). 2-(2-Phenylethyl) chromone derivatives are the characteristic components of agarwoods, and more than 40 such derivatives have been found in agarwoods belonging to different species (**Table 5**). Depending on the molecular skeleton, chromones can be divided into 2-(2-phenylethyl) chromones, 5,6,7,8-tetrahydro-2-(2-phenylethyl) chromones, diepoxy-tetrahydro-2-(2-phenylethyl) chromones, and associated chromones (**Gao et al., 2019**).

Chromones (1,4-benzopyrone) are the known isomer of coumarin, which are derivatives of benzopyran with a substituted keto group in the pyran ring, are ubiquitously present in the Plant Kingdom, and are a part of the human and animal diet. The 2-(2-phenylethyl) chromones are the rare and uncommon group of chromones that possess phenylethylene at the C-2 position, which are reported to be abundantly available in agarwood resin. These 2-(2-phenylethyl) chromones have been isolated only from a few plant species, making it a rare compound extracted from plants such as agarwood that are responsible for the warm, balsamic, long-lastingly sweet odor of the burnt agarwood. They act as potential and remarkable pharmacological compounds containing various bioactivities such as antimicrobial, antiviral, anticancer, antitumor, anti-inflammatory, antioxidant, enzyme inhibition, antifeedant, anti-depressant, anti-obesity, and anti-hypersensitive properties, including antagonistic activity in melanin-concentrating hormone receptor-1 (**Shivanand et al., 2022**).

Table 5: chemical structure of 2-(2-phenylethyl) chromones in agarwood (Wang *et al.*, 2021).

Name	Chemical Structure
2-[2-(4-Hydroxyphenyl) ethyl] chromone	
5,6,7,8, -Tetrahydroxy-5,6,7,8-tetrahydro-2-[2-(4-methoxyphenyl) ethyl]-chromone	
<i>Rel</i> -(1 <i>AR</i> ,2 <i>R</i> ,3 <i>R</i> ,7 <i>bS</i>)-1 <i>a</i> ,2,3,7 <i>b</i> -Tetrahydro-2,3-hidydroxy-5[2-(4-methoxyphenyl) ethyl]-7 <i>H</i> -oxireno[f] benzophran-7-one	
Oxidoagarchromones A	
6,8'-Dihydroxy-2-2'-bis(2-phenylethyl)-4 <i>H</i> ,4' <i>H</i> -5,5'-bichromone-4,4'-dione	

Name	Chemical Structure
Agarotetrol	
Isoagarotetrol	
2-(2-phenylethyl) chromone	
2-[2-(4-methoxyphenyl) ethyl] chromone	

1.3.3 Sesquiterpenoids:

Sesquiterpenoids are the major constituents of agarwood, playing a crucial role in determining its quality and shaping its characteristic aroma in combination with 2-(2-phenylethyl) chromones. Their biosynthesis is derived from farnesyl pyrophosphate (FPP), which acts as the universal precursor. A wide range of sesquiterpene skeletons has been identified in agarwood, including eudesmane, guanine, eremophilane, agar furan,

agarospirane, cadinene, prezizaane, and humulene types. These compounds can also associate to form more complex structures such as dimers; for example, agarwood peroxide A has shown significant inhibitory effects on cancer cell proliferation and metastasis. In addition, sesquiterpenes may interact with phenylethyl chromones (PECs), contributing to important pharmacological activities. Numerous sesquiterpenoids have been isolated and characterized, providing a strong foundation for understanding the chemical composition and fragrance mechanisms of agarwood (**Yang *et al.*, 2026**).

With FPP as the precursor, the skeletons of agarwood sesquiterpenes can be divided into eight categories, including eudesmane-type (Eudesmanes), guaiane-type (Guaianes), eremophilane-type (Eremophilanes), agarofuran-type (Agarofurans), agarospirane-type (Agarospiranes), cadinane-type (Cadinanes), prezizaane-type (Prezizaanes), and humulene-type (Humulenes) (**Figure 4**). The fragrance profiles are diverse and rich, mainly including woody, sweet, cooling, floral, resinous, spicy, fruity, leathery, herbal, creamy, green, and nutty scents (**Yang *et al.*, 2026**).

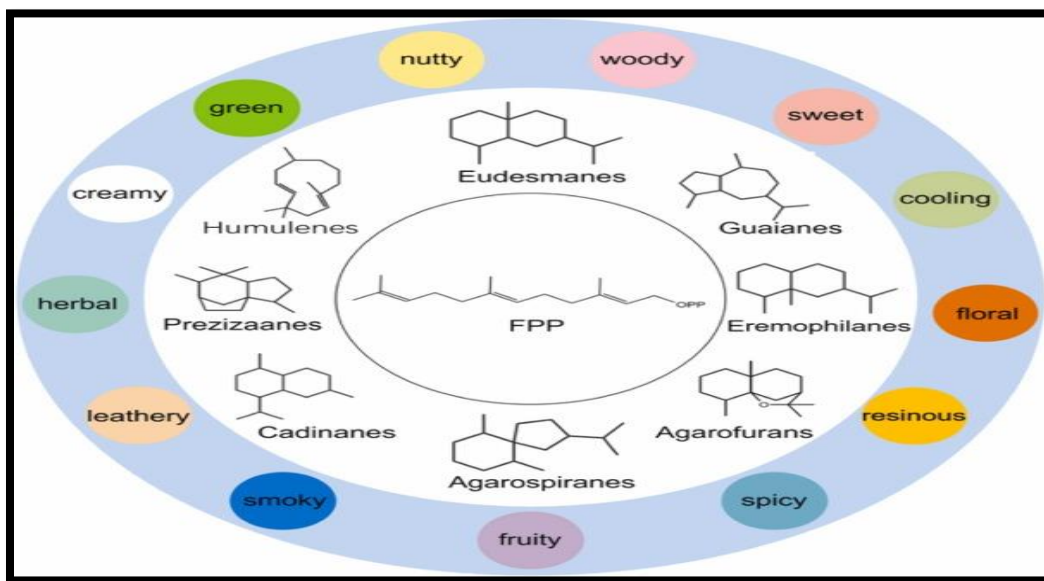


Figure 4: the types of agarwood sesquiterpene skeleton and their corresponding fragrance profiles (**Yang *et al.*, 2026**).

1.3.4 Flavonoids and Alkaloids:

A wide range of phytochemicals has been isolated from agarwood, including 31 flavonoids, notably flavonoid glycosides with demonstrated anti-inflammatory activity. Lignans and phenylpropanoids have also been identified, along with various phenolic acids such as *p*-methoxyphenyl propionic acid and 4-hydroxyphenylpropionic acid. In addition, alkaloids have been extracted from agarwood, further highlighting its chemical diversity. Benzophenones have also been isolated, including iriflophene, a compound shown to inhibit the respiratory burst of neutrophils (Wang *et al.*, 2021).

1.4 Agarwood utilization:

The use of agarwood dates to ancient civilizations and has been documented across many cultures. It was utilized by the Egyptians more than 3,000 years ago, and its significance was also recorded in the traditional texts of Chinese, Japanese, Korean, and Indian cultures, where it was valued for both culinary and medicinal purposes. Over time, the use of agarwood expanded throughout Southeast Asia including Laos, Cambodia, Vietnam, Thailand, Indonesia, and Malaysia driven by established trade routes and the growing demand for agarwood, particularly its highly prized essential oil (Ali *et al.*, 2016).

Agarwood has been used as a traditional aromatic perfume in many forms, from high-grade woodchips to personal perfuming before special occasions. It is sold as raw form (wood chips and pieces), as oil (both pure and blended with other fragrances), as perfume products, and in various other forms using small shavings of wood mixed with other fragrant ingredients (Ahmaed and Kulkarni, 2017).

Agarwood has been used for traditional medicine in Japan on account for its effectiveness as a sedative or tranquilizer in detoxifying the body and in maintaining stomach health. Agarwood has been used extensively in the incense industry more than in the medicinal sector in Japan, and from the 20–30% of Japan's total agarwood imports, it is estimated that approximately 20% of this amount comes from *A. malaccensis* (Ahmaed and Kulkarni, 2017).

1.4.1 Anti-inflammatory:

The anti-inflammatory potential of agarwood has been extensively investigated. Extracts of agarwood have demonstrated the ability to inhibit key inflammatory mediators, including cytokines, prostaglandins, and leukotrienes, which play central roles in the inflammatory response. This inhibitory activity suggests a significant capacity to reduce inflammation. Additionally, agarwood extracts may suppress the activity of enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for the synthesis of these mediators, thereby limiting the progression of the inflammatory cascade. Agarwood also appears to exert immunomodulatory effects by influencing the activity of immune cells involved in inflammation, helping to regulate immune responses. Furthermore, it may interact with important cellular signaling pathways, including NF- κ B and MAPKs, which are known to control the expression of inflammatory mediators, thus contributing to its overall anti-inflammatory activity (**Abd Rashed *et al.*, 2024**).

1.4.2 Antimicrobial:

Ethanollic extracts of agarwood leaves have demonstrated antibacterial and antifungal activities, largely attributed to the presence of bioactive compounds such as flavonoids and tannins. In addition, agarwood produces oud oil, which is rich in volatile constituents, including sesquiterpenoids and phenylethyl chromones, known for their antimicrobial properties. The antimicrobial effects of agarwood are thought to involve several mechanisms: disruption of microbial cell membranes leading to leakage of intracellular contents and cell death; inhibition of essential microbial enzymes, thereby impairing metabolic functions; antioxidant activity that may influence microbial survival and reduce oxidative stress; modulation of gene expression affecting microbial pathogenicity, replication, and survival; and interference with biofilm formation, which is often associated with increased resistance to antimicrobial agents (**Abd Rashed *et al.*, 2024**).

1.4.3 Anti-tumor:

Agarwood volatile oil contains a wide range of bioactive compounds with notable anti-tumor properties, exhibiting inhibitory effects against various cancer cell types. Among these, 2-(2-phenylethyl) chromone derivatives have been isolated and shown to possess cytotoxic activity against multiple human tumor cell lines. Extracts obtained using chloroform have also demonstrated significant anti-tumor activity, with inhibitory effects

observed across several cell lines at moderate concentrations. Furthermore, in vivo studies using essential oil have revealed its ability to suppress the growth of colon cancer cells, highlighting the therapeutic potential of agarwood-derived compounds in cancer treatment (Wang *et al.*, 2021).

1.4.4 Other pharmacological effects:

Agarwood exhibits a wide range of pharmacological effects, including respiratory, neurological, antioxidant, and cardiovascular benefits. It has been shown to relieve cough and asthma, with preparations such as Bawei Powder reducing cough symptoms and prolonging the latent period of induced coughing. Agarwood also demonstrates antidepressant and anxiolytic effects, as its essential oils and diterpenoids can inhibit depression and anxiety, partly by modulating gene expression and blocking the synaptic reuptake of serotonin and norepinephrine. Additionally, agarwood possesses significant antioxidant and anti-aging properties, with alcoholic extracts and volatile oils protecting against oxidative damage, neurodegeneration, and stress-induced cellular injury. In terms of cardiovascular health, agarwood formulations can enhance cardiomyocyte tolerance to hypoxia, inhibit phosphodiesterase activity, and provide protective effects against myocardial ischemia, highlighting its broad therapeutic potential (Wang *et al.*, 2021).

2. *Ephedra alata*:

Family *Ephedraceae* includes around 68 species in the genus *Ephedra* L. *Ephedra* is one of the largest genera of the *Ephedraceae* family, which is distributed in arid and semi-arid regions of the world. In the traditional medicine from several countries some species from the genus are commonly used to treat asthma, cold, flu, chills, fever, headache, nasal congestion, and cough. *Ephedra alata*, a medicinal plant belonging to the *Ephedraceae* family, has been traditionally used by Chinese and Arabian cultures for a variety of therapeutic purposes. This includes its application in treating allergies, asthma, inflammation, flu, headaches, and nasal congestion. *Ephedra*, also known as Ma-huang, is a shrub that has been familiar in China for over five centuries since its first discovery. Historically known as “Hoom” in ancient Iran, is one of the oldest medicinal plants used in traditional Persian medicine, with a legacy dating back over 2800 years. Deeply revered by Zoroastrian priests (Moghan), it was central to sacred rituals, where its stems were

crushed and consumed as a healing drink either pure or mixed with milk (**Faried *et al.*, 2018; González-Juárez *et al.*, 2020; Khattabi *et al.*, 2025**).

2.1 Taxonomy and botanical description:

Common English names of *Ephedra alata* include White Shrubby Horsetail and *Ephedra*. In North Africa and the Middle East, it is known by various local names such as Alanda, Alanda Mujanaa, Theel Maiz (or Telmiz), Anab Bahar, Ather, Jashia, Chdida (or Ššdida), La'lânda (or Laâlenda), Amatîr (or Amater), Andla, and Qodab (**Ouasti *et al.*, 2023**). (**Table 6**)

Table 6: classification of *Ephedra alata* (**Al-Snafi, 2017**).

Kingdom	Plant
Phylum	Tracheophyta
Class	Gnetopsida
Order	Ephedrales
Family	Ephedraceae
Genus	<i>Ephedra</i>
Species	<i>Ephedra alata</i>

It is a rare perennial shrub, classified as a nanophanerophyte, typically found in Saharan regions and reaching a height of 40 to 100 cm, often spreading wider than it grows tall (**Figure 5**). The stems are thin, rigid, yellow-green, and densely branched, with a diameter of about 1.5 mm, ending in sharp points and bearing small veins and ribs. They generally branch from the base and are jointed, with nodes spaced 4 to 6 cm apart, each bearing small, triangular, scale-like leaves arranged oppositely and showing a characteristic reddish-brown color. The plant produces unisexual flowers grouped into small cones, with male and female flowers usually on separate plants, although some individuals may bear both types. Tiny yellow-green fruits emit a strong pine scent and have an astringent taste. Ecologically, this species is highly effective at stabilizing wind-blown sand, but its high-quality charcoal, valued by Saharan communities, makes it vulnerable to overharvesting (**Al-Snafi, 2017; Hadjadj *et al.*, 2020**).

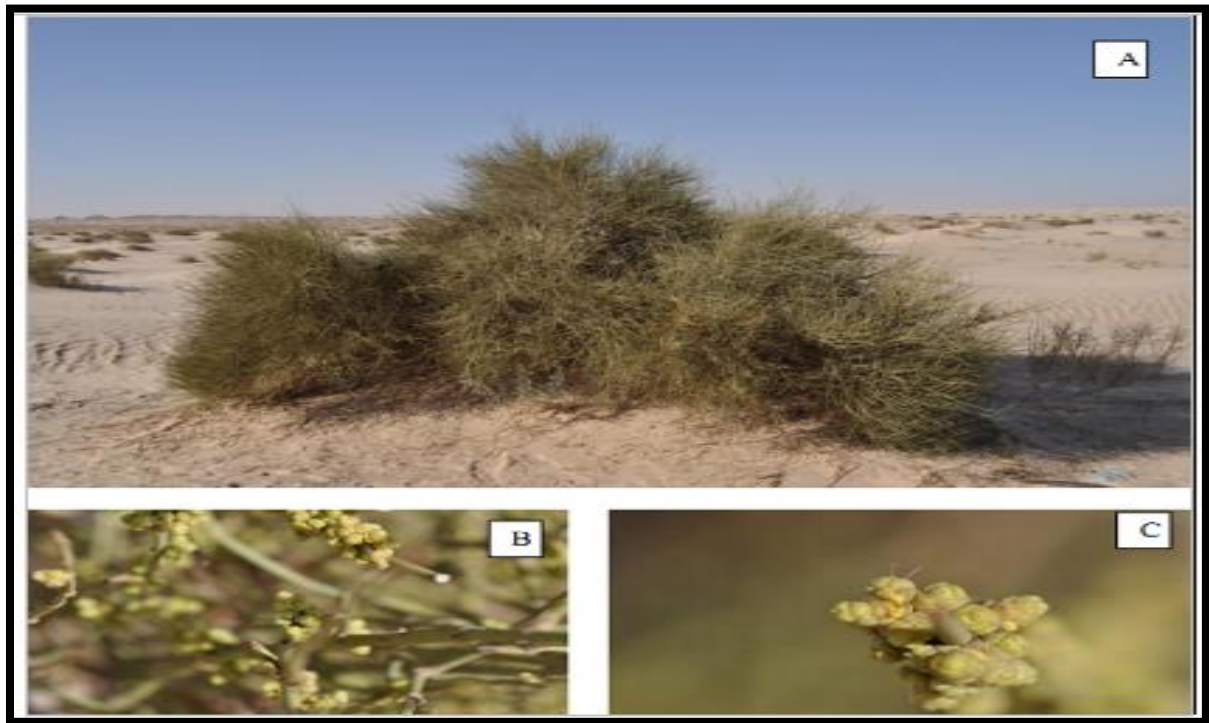


Figure 5: General port of *ephedra alata* subsp *alanda* Batt §Trab A; Flowering branch B and blooming flower C (Hadjadj *et al.*, 2020).

The branches are stout, opposite, grooved, and have a papillose and slightly rough (scabrous) surface. The leaves are small, opposite, elliptical ($1.8-3 \times 0.8-1.5$ mm), sparsely ciliate, and fused for more than half of their length. The reproductive structures consist of cones with broad, woody bracts and free scarious margins. Female cones are arranged in axillary clusters on straight peduncles and bear 4–5 (occasionally up to 6) pairs of bracts, which are orbicular with rounded apices and slightly lacerated margins; the outer bracts measure $4-5 \times 2-3$ mm, while the inner bracts are larger ($6-7.5 \times 3.5-4$ mm). The fruit is dry, creamy, cone-like, and borne on an erect peduncle. Seeds are flattened, ovoid, and brownish, measuring $6.5-8 \times 2.5-4$ mm, typically with one to two ovules per cone, and possess a straight micropylar tube of 2–3 mm. Male cones are also grouped in axillary clusters and contain numerous bracts (11–17 pairs), elliptical to obovoid in shape, with entire margins and rounded apices. The strobilus bracts are arranged in 6–12 pairs, obovate, and nearly uniform in size. The antherophores are 2.5–5 mm long, branched at the apex, and bear 3–6 anthers arranged in an umbellate form (Khalaf *et al.*, 2024).

2.2 Geographical distribution:

Ephedra alata, commonly known as “Alanda” in Arabic, belongs to the genus *Ephedra*, which is widely distributed across arid and semi-arid regions of Asia, Europe, northern Africa, southwestern North America, and South America (**González-Juárez *et al.*, 2020**). Thirteen *Ephedra* species occur in North America, while the genus overall ranges from the northern temperate zone including the Canary Islands, the Mediterranean region, Central Asia, and Shandong in China to the arid regions of the USA and Mexico, as well as the alpine areas of the Andes in South America (**Elhadeb *et al.*, 2020**). *Ephedra alata* is native to several Middle Eastern and North African countries, including Saudi Arabia, Egypt, Iran, Algeria, Lebanon, Jordan, Iraq, Libya, Tunisia, and Morocco (**Mohammed *et al.*, 2024**). In Algeria, it grows in the northern and western Sahara on sandy terrain, regs, sandy beds of wadis, the sands of the tropical zone, and the Hamada of Tinghert (**Hadjadj *et al.*, 2020**) (Figure 6).

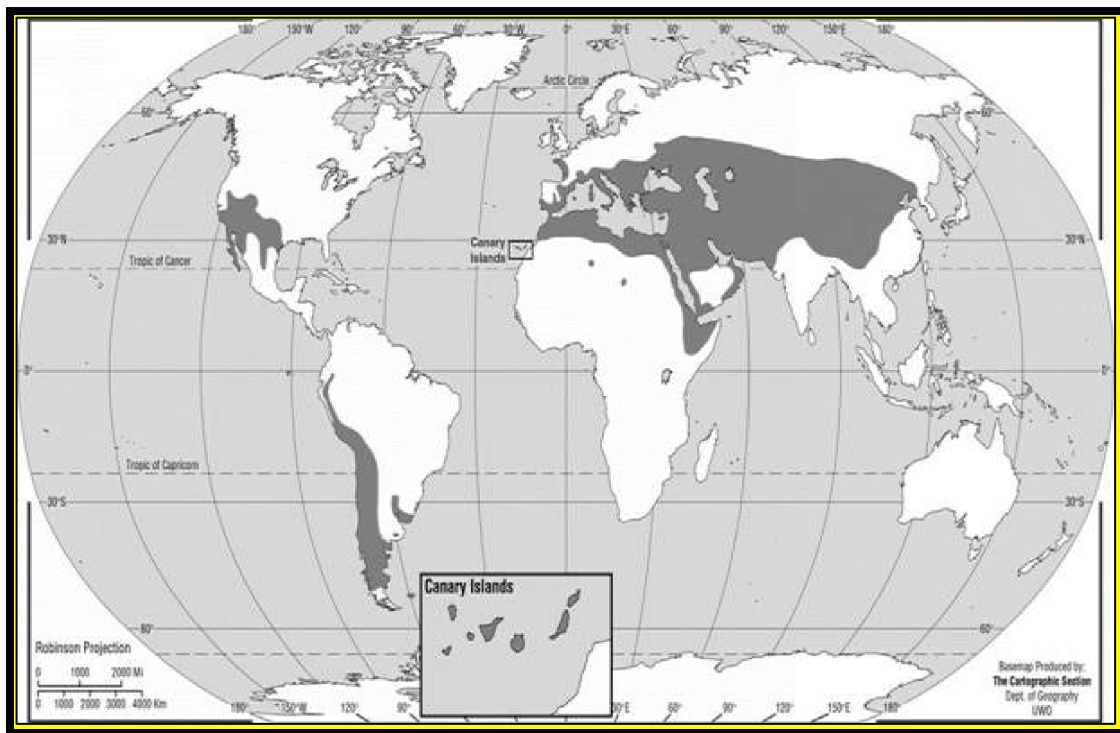


Figure 6: Geographic distribution of *ephedra* in the world (**Widad *et al.*, 2023**).

2.3 Chemical composition:

2.3.1 Lipids and Polysaccharides:

The total lipid content in the aerial parts of *E. alata* is approximately 9.81 mg/g of fresh weight. Its fatty acid composition includes notable proportions of linoleic, arachidonic, arachidic, stearic, α -linolenic, and oleic acids. However, variations in fatty acid profiles have been reported across different studies, with some identifying oleic, palmitic, behenic, and linolelaidic acids as the predominant components, while others have highlighted γ -linolenic, linoleic, palmitic, and oleic acids as the major fatty acids. In addition to fatty acids, sphingolipids have also been detected in the methanolic extract of *E. alata* pulp, with hexadecasphinganine identified as the most abundant compound (**Dbeibia et al., 2022**). The yield of water-soluble polysaccharides extracted from *E. alata* stems is approximately 4%. These polysaccharides are predominantly composed of total carbohydrates (73.74%), while minor constituents include lipids, proteins, uronic acids, and ash. The monosaccharide composition mainly consists of glucose, galactose, and mannose, with smaller amounts of arabinose and gluconic acid (**Soua et al., 2020**).

2.3.2 Alkaloids:

All nitrogenous heterocyclic and basic organic compounds derived from amino acids are included in the alkaloid family (**Laccourreye et al., 2015**). They exhibit analgesic, laxative, anti-plasmodial, anticancer, and antirheumatic properties. The predominant and most widely used alkaloids found in ephedra are ephedrine and pseudoephedrine (**Yinyang et al., 2014**). Furthermore, norephedrine, nor pseudoephedrine, methyl ephedrine, and methyl pseudoephedrine are present in this plant (**Figure 07**).

Nagai isolated and characterized ephedrine for the first time in 1885. But this alkaloid was forgotten until Chen and Schmidt discovered it again in the early 1920s. Since then, ephedrine has grown to be a highly well-liked and successful medication for asthma, especially because it can be taken orally, unlike adrenaline, which was the conventional treatment for this condition (**Lee, 2011**). Due to its two stereogenic carbon atoms, pseudoephedrine (PSE) exists in four distinct diastereoisomers, making it one of the four stereoisomers of ephedrine. PSE is primarily used as a nasal decongestant, bronchodilator, and central nervous system stimulant. As a stereoisomer of ephedrine, it functions as a sympathomimetic amine with strong α -adrenergic receptor agonist activity, narrowing

blood vessels to reduce swelling in nasal passages (Głowacka and Wiela-Hojeńska, 2021).

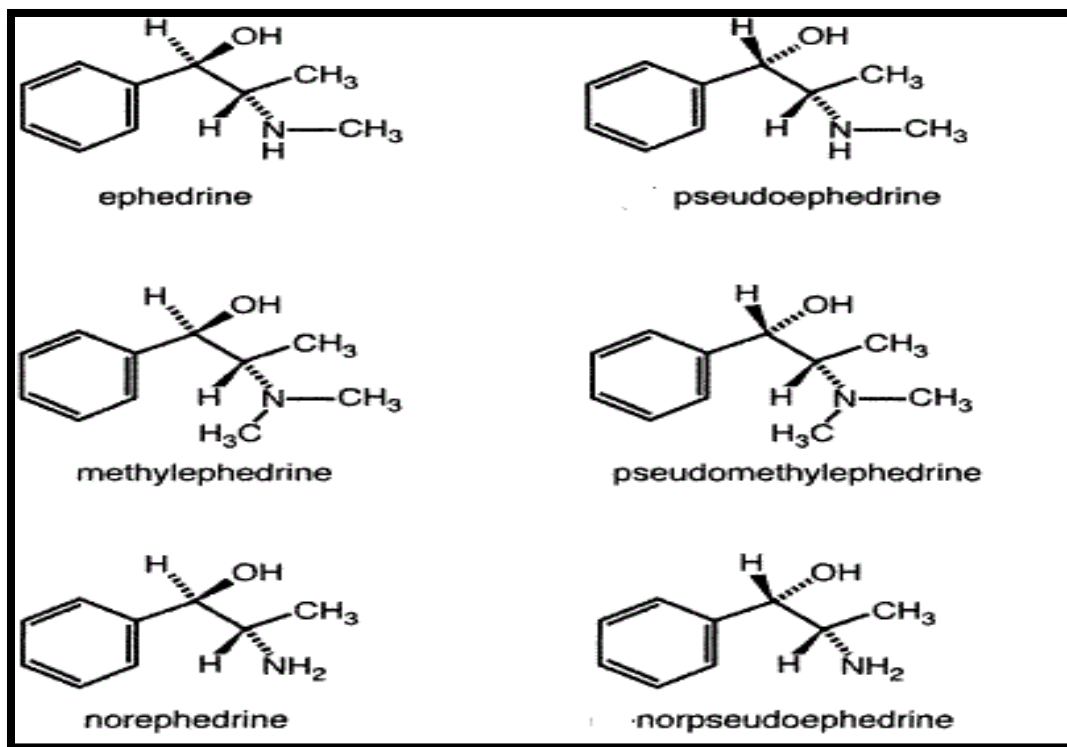


Figure 7: chemical structure of *Ephedra* alkaloids (Andraws *et al.*, 2005).

2.3.3 Flavonoids and Phenolic Compounds:

The phenolic compounds of *E. alata* vary depending on plant part and geographical origin. Methanolic extracts of seeds of some studies show relatively low levels of total phenols, flavonoids, and condensed tannins, whereas significantly higher contents of phenols and flavonoids have been reported in aerial parts in other research. Detailed phytochemical analysis of hydro methanolic extracts from aerial parts has identified a wide range of phenolic compounds, including phenolic acids (notably quinic acid as the major component), flavones, flavanols, flavan-3-ols, flavonoid glycosides, and flavanones. In seed extracts, the predominant compounds include quercetin, naringin, caffeic acid, epicatechin, quinic acid, rutin, and cirsiol. Additionally, minor flavonoids such as kaempferol and isorhamnetin derivatives have been detected in the pulp extract. Overall, *E. alata* is a rich source of diverse phenolic compounds with considerable variability depending on origin and plant part (Mighri *et al.*, 2019; Elhadef *et al.*, 2020).

2.4 Biological Activities:

2.4.1 Antioxidant Activity:

The antioxidant activity of *E. alata* has been widely attributed to its rich content of phenolic compounds, which are the main contributors to its bioactivity. A variety of phenolics have been identified, particularly phenolic acids such as quinic, chlorogenic, coumaric, trans-cinnamic, and gallic acids, as well as flavonoids including derivatives of quercetin, kaempferol, luteolin, myricetin, isovitexin, and herbacetin. In addition to phenolics, alkaloid extracts from the aerial parts exhibit significantly reducing power, with pseudoephedrine identified as the alkaloid and a precursor to ephedrine, one of the most active constituents. Polysaccharides extracted from the stems and essential oils have also demonstrated notable antioxidant activity, the latter being associated with compounds such as linalool, (Z)-3-tridecene, n-pentadecane, and 1,8-cineole. The antioxidant mechanisms of these bioactive compounds involve multiple pathways, including scavenging reactive oxygen species, inhibiting oxidative enzymes, modulating redox signaling pathways, and chelating transition metals (Al-Qarawi *et al.*, 2013; Carochio *et al.*, 2013; Soua *et al.*, 2020).

2.4.2 Antimicrobial Activity:

Extracts from *Ephedra* species, particularly *E. alata*, have demonstrated notable antimicrobial activity in various in vitro studies using methods such as agar diffusion and minimum inhibitory concentration (MIC) assays. These extracts exhibit bactericidal and fungicidal effects against a range of pathogenic and drug-resistant microorganisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida* species. The antimicrobial efficacy varies depending on the extraction method and solvent, with hydroalcoholic, methanolic, and acetonitrile extracts generally showing stronger activity than infusions or decoctions. Some extracts have been effective against methicillin-resistant strains, while others showed selective activity against specific bacteria such as *E. coli*. In addition, essential oils, alkaloid-rich extracts, and even biosynthesized nanoparticles derived from *Ephedra* have demonstrated significant antibacterial and antifungal properties. Overall, the antimicrobial potential of *Ephedra* species is influenced by extraction techniques, plant origin, and target microorganisms (Elhadeif *et al.*, 2020).

2.4.3 Anti-Inflammatory Activity:

Inflammation is a complex biological response of vascular tissues to mechanical, chemical, or endogenous damage, serving as a protective mechanism aimed at restoring or repairing injured tissue. During this process, macrophages are activated by stimuli such as bacterial lipopolysaccharides and interferon- γ , leading to the production of proinflammatory cytokines, including TNF- α , IL-1 β , IL-6, and interferon- α . These cytokines promote inflammatory responses through the release of mediators such as free radicals, prostaglandins, and excitotoxins, which help eliminate harmful agents and amplify immune reactions. Studies on *ephedra* have demonstrated significant anti-inflammatory properties. Proanthocyanidins, such as ephedrannins A and B, isolated from its roots, were shown to inhibit the transcription of TNF- α and IL-1 β in LPS-stimulated macrophages by blocking NF- κ B translocation and MAPK p38 phosphorylation. Additionally, polysaccharides such as ESP-B4 have exhibited anti-inflammatory effects by reducing inflammatory cell infiltration and decreasing cytokine production in models of lung inflammation. These compounds also show immunosuppressive activity, particularly in rheumatoid arthritis, by modulating the Toll-like receptor 4 (TLR4) signaling pathway (González-Juárez *et al.*, 2020).

2.4.4 Cytotoxic and Anti-Tumor Activities:

The hydroethanolic extract of *E. alata* aerial parts exhibits significant anticancer activity, including antiproliferative, pro-apoptotic, and cytotoxic effects, particularly against the human breast cancer cell line MCF-7. This extract is rich in bioactive flavonoids such as kaempferol and quercetin, which are known to inhibit tumor growth and target cancer stem cells. Experimental studies have confirmed its effectiveness, showing notable inhibitory activity against MCF-7 cells, with relatively low IC₅₀ values depending on the assay used (Soumaya *et al.*, 2020). Similar anticancer effects have been observed with methanolic extracts from different geographical origins, demonstrating cytotoxicity not only against breast cancer cells but also against liver (HEPG2) and prostate (PC3) cancer cell lines, with activities comparable to standard chemotherapeutic agents such as doxorubicin. Although some variability in potency exists among extracts, likely due to differences in origin and composition, *E. alata* consistently shows promising anticancer potential. Molecular analyses further indicate that its activity is associated with the

upregulation of key genes involved in apoptosis and cell cycle regulation, including Bax, p21, retinoblastoma protein, and p53 (Elshibani *et al.*, 2020; Bensam *et al.*, 2023).

2.4.5 Other Activities:

E. alata exhibits multiple pharmacological activities, including antidiabetic, anti-obesity, nephroprotective, hepatoprotective, antipyretic, and analgesic effects. Its antidiabetic activity is mainly linked to the inhibition of key digestive enzymes such as α -amylase, α -glucosidase, and lipase, largely due to polyphenols like flavonoids. The plant also shows anti-obesity potential through lipase inhibition and the action of compounds such as quinic acid, apigenin, quercetin, and rosmarinic acid (Tiss *et al.*, 2020). Nephroprotective and hepatoprotective effects have been observed, particularly against drug-induced organ damage, and are associated with alkaloids such as ephedrine and pseudoephedrine, as well as flavonoids (Sioud *et al.*, 2021). Additionally, *E. alata* demonstrates antipyretic and analgesic properties, likely mediated by the inhibition of prostaglandin synthesis and the presence of bioactive compounds including flavonoids and alkaloids (Tiss *et al.*, 2020). Overall, these activities highlight the broad therapeutic potential of *E. alata*.

Experimental part



Chapter III:

Material and method

1. Objective of study:

The experimental part was carried out at the biology laboratory of the University of Saida-Dr. Moulay Tahar, whose main objective is to study the antioxidant activity of the extract of Two plants *Aquilaria malaccensis* and *Ephedra alata*, also their combination. The phytochemical profile of each plant was evaluated, and their antioxidant, anti-inflammatory, and antimicrobial activities were investigated. The diagram below briefly explains the experiment (Figure8)

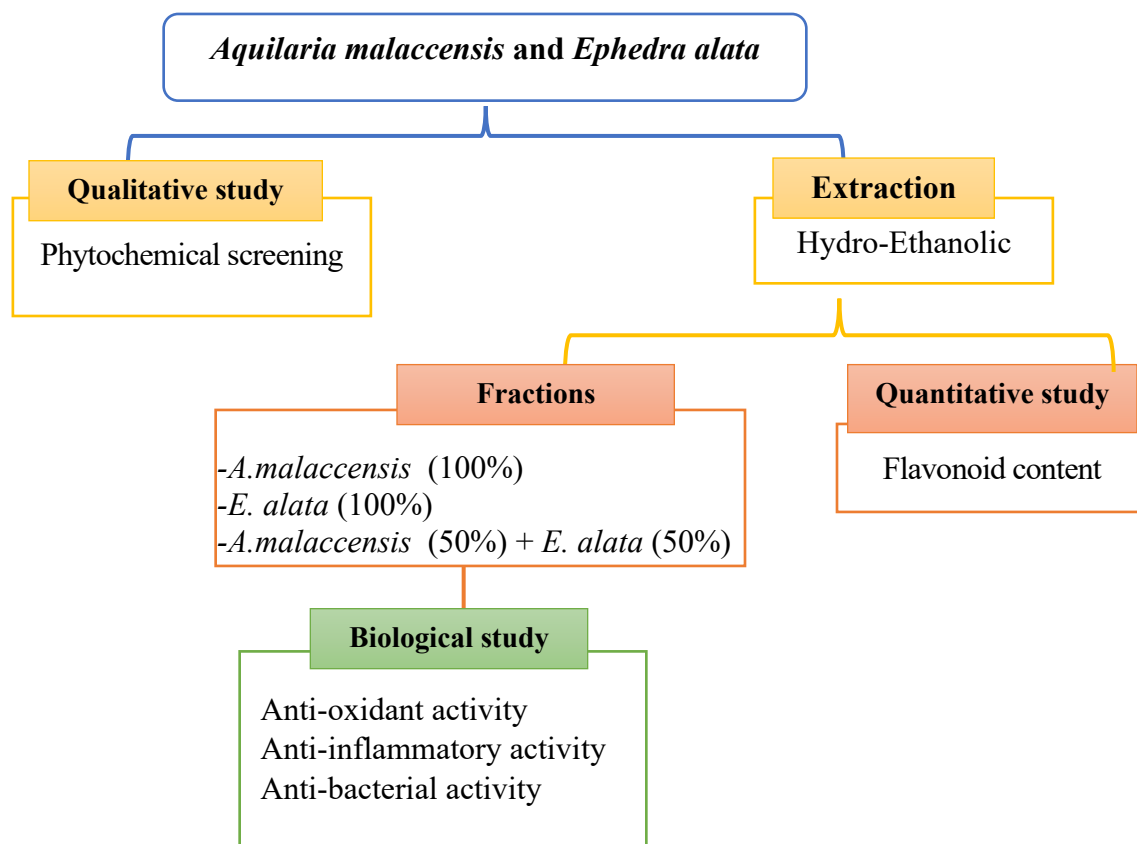


Figure 8: general diagram of analyses carried out

2. Plant materiel and Extract Preparation:

In January 2026, the plants were obtained from an herbalist in Saida. After being washed with water and air-dried, the stems of *Ephedra alata* and the bark of *Aquilaria malaccensis* were finely powdered using a blender (Figures 09 and 10).



Figure 9: Bark (A) and powder(B) of *A. malaccensis*



Figure 10: Stem(A) and Powder(B) of *E. alata*

For extraction, 20 g of the dried and powdered material from each plant were separately macerated in 200 mL of ethanol–water (7:3, v/v) for 72 h using a shaker at room temperature. The extracts were then filtered and concentrated using a rotary evaporator at 60°C (Bahri *et al.*, 2022).

The yield of the extracts is the ratio between the weight of the dry extract and the weight of the powdered plant used (Bahri *et al.*, 2022).

It is expressed as a percentage according to the following formula:

$$\text{Yield (\%)} = (P1/P2) * 100$$

P1: weight of the extract (g)

P2: weight of the plant material (g)

3. Phytochemical screening:

The qualitative phytochemical screening of the extracts was carried out to identify the main groups of chemical constituents (flavonoids, saponins, tannins, free quinones, coumarins, etc.), present in the extracts using colorimetric, precipitation reactions and observations under ultraviolet light. These tests were conducted according to the techniques described by (Harborne 1998 and Bruneton 1999).

3.1 Preparation of ethanolic extract:

Add 100 ml of 70% ethanol to 5g of plant powder in a 250ml Erlenmeyer flask. let it stir and macerate for 24 hours at the laboratory's room temperature. after filtration thru a filter paper, the ethanolic extract is subjected to the tests mentioned below:



Maceration



Filtration



Evaporation



Recuperation

Figure 11: Steps of maceration

- **Tannins:**

The presence of tannins is demonstrated by adding 1ml of the ethanolic extract, 2ml of water, and 2 to 3 drops of diluted FeCl₃ solution (1%). The appearance of a blue-black coloration indicates the presence of gallic tannins, while a green or blue-green coloration indicates the presence of catechin tannins (**Aiyegoro and Okoh, 2010**).

- **Flavonoids:**

The reaction for detecting flavonoids consists of treating 5ml of the ethanolic extract with 1ml of concentrated HCl and 0.5g of magnesium turnings. The presence of flavonoids is indicated if a pink or red color develops after 3 minutes (**Siddiqui et al., 2009**).

- **Sterols and triterpenes:**

It involves evaporating the ethanol extract corresponding to 10ml to dryness. Next, dissolve the obtained residue in a mixture of acetic anhydride/chloroform (5/5: V/V); then filter and treat the filtrate with a few drops of concentrated sulfuric acid (Liebermann-Burchardt reaction). If this reaction gives blue-green and green-violet colors, it indicates the respective presence of steroidal and triterpenes heterosides (**Aiyegoro and Okoh, 2010**).

- **reducing agents:**

Their detection involves treating 1ml of the ethanolic extract with distilled water and 20 drops of Fehling's solution, then heating. A positive test is revealed by the formation of a brick-red precipitate (**Kholkhal, 2014**).

3.2 Preparation of the aqueous extract:

In a monocol flask topped with a condenser. We proceeded with a decoction of 2 grams of plant powder with 100ml of distilled water, which we brought to a boil for 30 minutes. After cooling and filtration, the volume is adjusted to 100ml. The aqueous extract is subjected to the Following tests:

- **Saponins:**

Their presence is quantitatively determined by calculating the foam index. From the aqueous extract, prepare 10 tubes (1.3cm internal diameter) with 1, 2, ...10ml, the final volume being adjusted to 10ml with distilled water. Each of these tubes is vigorously

shaken in a horizontal position for 15 seconds. After a 15-minute rest in a vertical position, the height of the persistent foam is measured in centimeters. If it is close to 1 cm in the 10th tube, then the foam index is calculated using the following formula: The presence of saponins in the plant is confirmed with an index higher than 100 (**Dohou *et al.*, 2003**).

$$I = \text{Foam height (in cm) in the 10th tube} \times 5 / 0.0X$$

- **Starch:**

We heat 5ml of the aqueous extract with 10ml of a saturated NaOH solution in a water bath until boiling. Then add the iodine reagent. A positive test is revealed by the appearance of a blue-violet coloration (**Kholkhal, 2014**).

3.3 Maceration of plant material with sulfuric acid (H₂SO₄):

Add 2 grams of plant powder mixed with 50ml of H₂SO₄ diluted to half and distilled water into a 250ml Erlenmeyer flask. Let it stir and macerate for 24 hours at the laboratory's room temperature. After filtering the mixture and rinsing with water to obtain 50ml of filtrate, it is subjected to the following tests:

- **The alkaloids:**

Take two test tubes into which 1ml of the macerate is introduced. We added 5 drops of Mayer's reagent to tube no. 1 and 5 drops of Wanger's reagent to tube no. 2. the presence of turbidity or a precipitate, after 15 minutes, indicates the presence of alkaloids (**Siddiqui and Ali., 1997**).

- **Coumarins:**

A mass of 1 gram of plant powder is placed in a tube in the presence of a few drops of distilled water. The tubes are covered with paper soaked in diluted NaOH and brought to a boil. Any yellow fluorescence indicates the presence of coumarins after examination under ultraviolet light (**Aiyegoro and Okoh, 2010**).

- **Anthocyanins:**

A volume of 2ml of aqueous infusion is added to 2ml of 2N HCl. the appearance of a pink-red color that turns blue-violet upon the addition of ammonia indicates the presence of anthocyanins (**Siddiqui *et al.*, 2009**).

4. Quantification of flavonoids:

A 0.5 mL sample solution was added to 2 mL of distilled water. Then, 0.15 mL of sodium nitrite (NaNO_2) was added to the tubes, and the mixture was thoroughly homogenized and allowed to stand for 5 minutes at room temperature. Subsequently, 0.15 mL of 10% (w/v) aluminium chloride (AlCl_3) was added, and the reaction mixture was left for an additional 6 minutes. After that, 1 mL of 1 M sodium hydroxide (NaOH) was added, and the solution was immediately mixed. The absorbance was measured at 510 nm after 10–15 minutes. Quercetin was used as a standard at different concentrations to construct a calibration curve, and the results were expressed as quercetin equivalents (QE, mg/g of extract) (Elazzouzi *et al.*, 2019).

5. Antioxidant activity:

The antioxidants are chemical compounds that, when present at very low concentrations in food and in the human body, reduce or prevent the oxidation process that leads to food spoilage and the spread of degenerative diseases in the body (Shahidi and Zhong, 2015). This process occurs by blocking the synthesis of free radicals by inhibiting them at the beginning of the reaction or by directly deactivating ROS (Desmier, 2016).

depend on several factors, the most important of which are structural properties, temperature, the properties of the substrate subjected to oxidation, concentration, and the physical state of the system (Shahidi and Zhong, 2011). Recently, methods for measuring antioxidants have made remarkable progress. Antioxidants have been used to neutralize certain types of oxidation products (scavenging activity against ROS and various types of free radicals). Among these measurements, which use direct measurement to transfer electrons or transfer a hydrogen atom from antigens to free radicals. Methods for measuring antioxidants are divided into spectroscopic methods (Shahidi and Zhong, 2015).

5.1 DPPH° radical scavenging method:

- Principle:

One of the first free radicals used to study the structure-antioxidant activity relationship of phenolic compounds is the chemical compound 2,2-diphenyl-1-picrylhydrazyl (α , α -diphenyl- β -picrylhydrazyl). It has an unpaired electron on an atom of

the nitrogen bridge; this delocalization causes the appearance of the characteristic violet color of the DPPH• solution (**Figure 12**). Measurement of the effectiveness of an antioxidant is done by measuring the decrease in the violet color, due to a recombination of the DPPH• radicals (**Cristina *et al.*, 2009**).

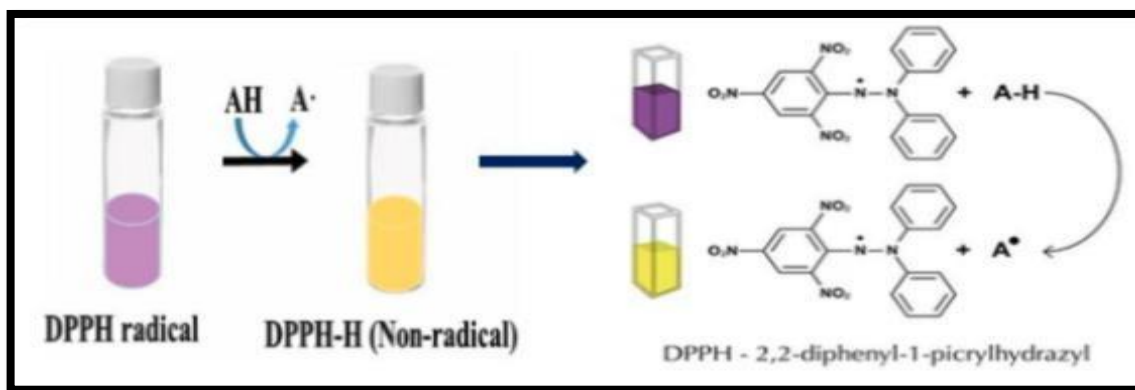


Figure 12: Reduced from pf the DPPH° radical (**Cesbron, 2020**).

- **Assay:**

The DPPH stock solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol and stored at 20°C until use. A series of different concentrations (0–1000 µg/mL) was prepared from a 1 mg/mL stock solution. For the assay, 2.7 mL of the DPPH solution was mixed with 300 µL of plant extracts or standard antioxidant (ascorbic acid) at various concentrations (0.2 mg/mL stock solution). The reaction mixtures were incubated for 30 minutes in the dark. The reduction of DPPH resulted in a color change from violet to yellow, and the absorbance was then measured at 517 nm using a spectrophotometer. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{D control} - \text{D sample}}{\text{D control}} * 100$$

Control D = absorbance of control

Sample D = absorbance of sample

The percentage of inhibition is then expressed by the IC₅₀ value, knowing that the IC₅₀ is the concentration of extract required to obtain 50% of the reduced form of the DPPH radical (Baslam *et al.*, 2023; Elazzouzi *et al.*, 2019).

5.2. Total antioxidant capacity:

- **Principle:**

Antioxidant capacity refers to the ability of various compounds in biological samples to scavenge free radicals, inhibit oxidation, and prevent disease, representing the overall activity of antioxidants within the human organism. This capacity can be evaluated through the assessment of endogenous or dietary components and is influenced by the presence of other compounds in the food matrix (Azzini and Maiani, 2015).

- **Assay:**

The total antioxidant capacity (TAC) of the plant extracts was evaluated using the phosphomolybdate assay. For sample preparation, 1 mg of each plant extract was dissolved in 1 mL of ethanol and vortexed to obtain a homogeneous solution. Ascorbic acid was used as the reference standard. A stock solution of ascorbic acid (1 mg/mL) was prepared, from which different concentrations were obtained by dilution. For the assay, 300 µL of plant extract was mixed with 3 mL of phosphomolybdate reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The test tubes were covered with aluminum foil and incubated at 95°C for 90 minutes. After incubation, the mixtures were cooled to room temperature, and the absorbance was measured at 695 nm using a spectrophotometer. A blank solution was prepared following the same procedure, replacing the plant extract with an equal volume of methanol. The total antioxidant capacity was expressed as micrograms of ascorbic acid equivalents (AAE) per milliliter (Ahmed *et al.*, 2015).

6. Anti-inflammatory activity:

Plant extracts exhibit potent anti-inflammatory activity primarily by neutralizing oxidative stress and suppressing key inflammatory signaling pathways. Rich in phytochemicals like flavonoids, polyphenols, and terpenoids, these extracts can block

the production of pro-inflammatory enzymes and cytokines, offering a natural approach to managing chronic and acute inflammation (Rasheed and Gruber, 2025).

6.1 Bovine serum albumin (BSA) denaturation inhibition test:

- **Principle:**

Protein denaturation is a mechanism involved in inflammatory processes, particularly in diseases such as rheumatoid arthritis. Compounds capable of preventing this denaturation have anti-inflammatory potential (Sarveswaran *et al.*, 2017). To evaluate the ability of extracts to stabilize bovine serum albumin subjected to heat stress, in comparison with a reference anti-inflammatory agent (Bailey-Shaw *et al.*, 2017).

- **Assay:**

A 5% (w/v) bovine serum albumin (BSA) solution was prepared, followed by the preparation of different extract dilutions. Ibuprofen was used as the reference standard and prepared at the same concentrations as the tested extracts. For the reaction mixture, a total volume of 2.85 mL was prepared containing 450 µL of 5% BSA solution, 1400 µL of phosphate buffer, and 1000 µL of either plant extract or ibuprofen. In the negative control, the extract was replaced with 1000 µL of distilled water, whereas in the positive control, 1000 µL of ibuprofen was used. The reaction mixtures were first incubated at 37°C for 15 minutes, then heated at 70°C for 5 minutes to induce protein denaturation. After cooling to room temperature, the absorbance was measured at 660 nm using a spectrophotometer (Castañeda-Corral *et al.*, 2025). The inhibition percentage was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

6.2 Red blood cell (erythrocyte) membrane stabilization test:

- **Principle:**

The erythrocyte membrane shares structural similarities with the lysosomal membrane. Anti-inflammatory compounds are capable of stabilizing these membranes and protecting them against lysis induced by hypotonic stress. Therefore, this assay was performed to evaluate the ability of the extracts to inhibit hemolysis of red blood cells under hypotonic conditions (Nurtamin *et al.*, 2018).

- **Assay:**

Blood was collected from a healthy volunteer who had not taken any anti-inflammatory drugs during the previous two weeks and immediately mixed with an equal volume of Alsever's solution containing 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl. The mixture was centrifuged at 3,000 rpm for 10 minutes, and the supernatant was discarded. The resulting pellet was washed three times with isotonic saline solution, after which a 10% (v/v) erythrocyte suspension was prepared. For the assay, the reaction mixture (total volume: 4.5 mL) consisted of 1 mL phosphate buffer, 2 mL hyposaline solution, 0.5 mL erythrocyte suspension, and 1 mL of plant extract. In the control group, the extract was replaced with 1 mL of distilled water. The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 3,000 rpm for 10 minutes. Finally, the absorbance of the supernatant was measured at 560 nm using a spectrophotometer (Nurtamin *et al.*, 2018). The inhibition percentage was calculated according to the following formula:

$$\text{Protection (\%)} = 100 - \frac{\text{DO sample}}{\text{DO control}} \times 100$$

7. Antimicrobial activity:

- **Principle:**

Antimicrobial activity refers to the ability of a substance, such as plants extracts, to inhibit the growth of or kill microorganisms, including bacteria and fungi, thereby demonstrating potential therapeutic effects against infectious diseases (Qadir *et al.*, 2020).

- **Microorganisms used in test:**

Table 7: Origin of the strains used for the evolution of antimicrobial activity

<u>Microorganisms</u>		<u>Gram</u>	<u>Code</u>	<u>Origin</u>
Bacteria	<i>Escherichia coli</i>	Negative	ATCC 8739	MNHN
	<i>Bacillus cereus</i>	Positive	ATCC 25921	LAPRONA
	<i>Bacillus subtilis</i>		ATCC 6633	LAPRONA
	<i>Staphylococcus aureus</i>		ATCC 6538	MNHN

LAPRONA: Natural Products Laboratory (University of Tlemcen); **MNHN:** National Museum of Natural History (Paris).

➤ ***Escherichia coli:***

It is a Gram-negative bacillus, non-spore-forming, facultatively anaerobic, generally motile thanks to flagella, its length varies from 2 to 6 μm , while its width is 1.1 to 1.5 μm . It is a normal saprophyte of the human intestinal tract and animals. It is one of the agents responsible for septicemia, suppuration, diarrhea, and even dysentery. (Jang, *et al.*, 2017).

➤ ***Staphylococcus aureus:***

These are Gram-positive cocci ranging in diameter from 0.5 to 1.5 μm , non-spore-forming, which tend to aggregate in pairs and are generally not. It is optional to analyze whether the samples are encapsulated or if the capsules are lime-based. *Staphylococcus aureus* is a common cause of postoperative infections and wounds, endocarditis, and food poisoning (Dworkin and Falkow, 2006).

➤ *Bacillus cereus*:

Bacillus cereus is a bacterium corresponding to Gram-positive bacilli. These bacilli are quite large, measuring 1 to 1.8 μm in diameter and 4 to 8 μm in length, and usually form short chains. The colonies can have very variable, yet distinctive shapes, which makes them easy to recognize. They are fairly large, 2 to 7 mm in diameter, with an irregular circular shape and a cream color (De Vos *et al.*, 2009).

➤ *Bacillus Subtilis*:

Bacillus Subtilis is a fast-growing Gram-positive bacterium whose cells are rod-shaped and generally measure between 2 and 6 μm in length and less than 1 μm in diameter. Its optimal growth temperature ranges from 30 to 35 °C, resulting in a growth period of 20 minutes. Under these growth conditions, the cells tend to form long chains connected by non-cleavable septal fragments. In the event of a nutritional deficit, the cells may undergo a type II nutritional differentiation process that results in the development of an endospore released by cell lysis (Errington *et al.*, 2020).

• **Assay:**

In this study, antibacterial compounds synthesized by the medicinal plants *Ephedra alata* and *Aquilaria malaccensis*, known for their therapeutic properties, were investigated. The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method on Petri dishes, according to the procedure described by (Perez *et al.*, 1990) and referenced by (Balouiri *et al.* 2016).

Mueller–Hinton agar was used as the culture medium and prepared by dissolving 19 g of agar in 500 mL of distilled water. The mixture was heated with continuous stirring until complete dissolution, sterilized by autoclaving at 121°C for 15 minutes, and then poured into sterile Petri dishes. All materials used in the experiment, including distilled water, culture media, tubes for bacterial suspensions, and micropipette tips wrapped in aluminum foil, were sterilized under the same autoclaving conditions.

For extract preparation, 0.2 g of *E. alata* and *A. malaccensis* extracts were separately dissolved in 2 mL of distilled water. Gentamicin was used as the reference antibiotic. The bacterial strains used in this study were *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, and *Escherichia coli*. These strains were cultured on nutrient agar plates and incubated at 37°C for 18–24 hours to obtain isolated colonies. Three to four well-isolated colonies were selected using a platinum loop and suspended in sterile physiological saline solution. The suspensions were homogenized using a vortex mixer, and their turbidity was adjusted to 0.5 McFarland standard.

For inoculum preparation, the bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24 hours. Distinct colonies were then suspended in 10 mL of sterile distilled water, and the inoculum density was adjusted by adding either bacterial culture or sterile saline solution when necessary.

The well diffusion assay was carried out under aseptic conditions. Approximately 20–25 mL of Mueller–Hinton agar was poured into each Petri dish to obtain a uniform thickness of about 4 mm and allowed to solidify (**Figure 13**). A sterile cotton swab was immersed in the bacterial suspension and streaked uniformly over the entire agar surface by rotating the plate at 60° angles to ensure homogeneous distribution of the inoculum. The edges of the agar surface were also swabbed. After allowing the plates to stand for 15 minutes at room temperature to facilitate bacterial adhesion, wells of 6 mm diameter were made using puncture. Subsequently, 20 µL of each extract was introduced into the wells. A well containing gentamicin served as the positive control, while distilled water was used as the negative control. Finally, the Petri dishes were incubated at 37°C for 24 hours.

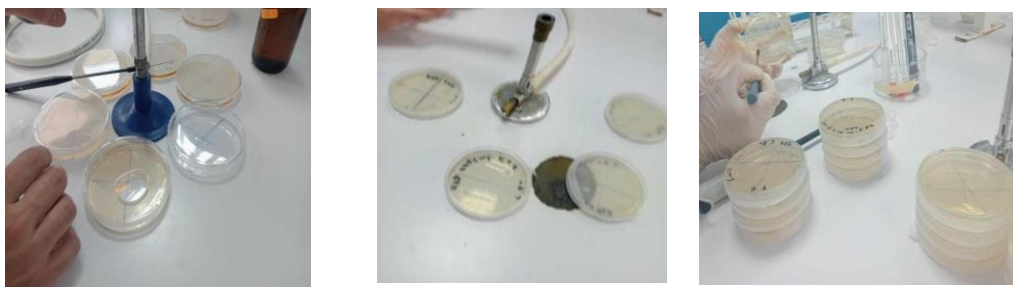


Figure 13: Photographs illustrating the experimental procedure (Personal photo)

The reading results was done by measuring the diameters of the inhibition halos around the wells. The strain having a zone of inhibition of diameter.

Range (mm)	Activity
< 8	Low
8–12	Moderate
12–20	Good
> 20	Very high

8. Statistical analysis:

The results are expressed as the mean (M) of three independent assays, accompanied by the standard error of the mean (SEM), and were calculated using Microsoft Excel. Comparison of multiple means was performed using one-way analysis of variance (ANOVA) for the two extracts and their mixture, followed, when appropriate, by the student–Newman–Keuls post hoc test. A p-value ≤ 0.05 indicates a significant difference, $p < 0.01$ indicates a very significant difference, and $p < 0.001$ indicates a highly significant difference. All statistical analyses were performed using Sigma Stat software.



Chapter IV:

Results and discussion

1. Extraction Yield:

The yields, physical characteristics, and colors of the extracts obtained from each plant are presented in the following table (Table8) . The results revealed differences in both appearance and extraction yield between the two extracts. The extract of *A. malaccensis* was obtained in paste form with a brown color, whereas the extract of *E. alata* appeared as a green powder. Regarding extraction efficiency, *A. malaccensis* produced the highest yield (26%), while *E. alata* showed the lowest yield (8.5%).

Table 8: Aspect, colors and yields of extract *A. malaccensis* and *E. alata*

Plants (parts)	Aspects	Colors	Yield
<i>Aquilaria malaccensis</i> (bark)	Pasty	Brown	26%
<i>Ephedra alata</i> (stem)	Powder	Green	8,5%

The results obtained in this study are partially consistent with previous findings. Regarding *A. malaccensis*, the observations agree with those reported by **Bouhaous (2022)**, who described the extract as brown in color with a pasty consistency and reported an extraction yield of 26%, similar to the yield obtained in the present study. However, lower extraction yields have been reported by other authors; **Halim et al. (2022)** obtained a yield of 10.7% from ethanolic leaf extracts of *A. malaccensis*, while **Hendra et al. (2016)** reported a yield of 9.2%, both considerably lower than our results.

For *E. alata*, the extraction characteristics are in agreement with **Bellahcene (2020)**, who described the extract as green with a powdery consistency. The extraction yield obtained in our study (8.5%) was also comparable, although slightly higher values were reported. In contrast, **Soumaya et al. (2020)**, reported a lower yield of 2.11% from the aerial parts of *E. alata*. These differences in extraction yields may be attributed to variations in plant parts used, extraction methods, solvent types, environmental conditions, and geographical origin of the plant material (**Mansour, 2014**).

2. Qualitative Phytochemical Analysis:

Table 09 presents the results of the phytochemical screening performed to determine the presence of various bioactive compounds, including phenolic compounds (tannins and flavonoids), saponins, sterols and triterpenes, alkaloids, coumarins, anthocyanins, starch, and reducing compounds in the analyzed plants parts

Table 9: phytochemicals screening *A. malaccensis* and *E. alata*

Phytoconstituents	Plants	
	<i>A. malaccensis</i>	<i>E. alata</i>
Alkaloids	+	+
Anthocyanins	+	+
Coumarins	+	+
Flavonoids	+	+
Reducing Compounds	-	-
Saponosides	-	-
Starch	-	-
Sterols and triterpenes	+	+
Tannins (Catcholic)	+	-
Tannins (Gallic)	-	+

+ (presence of phytoconstituents); - (absence of phytoconstituents).

Qualitative phytochemical analysis of *Ephedra alata* confirmed the presence of several secondary metabolites, including alkaloids, anthocyanins, coumarins, flavonoids, terpenoids, gallic tannins, and sterols/triterpenes. However, catechin tannins, reducing compounds, starch, and saponins were not detected. Similarly, the phytochemical profile of *Aquilaria malaccensis* showed results comparable to those observed for *E. alata*, with the main difference being the presence of catechin tannins instead of gallic tannins.

Our findings are generally consistent with those previously reported by **Bouhaus (2022)**, **Salah et al. (2022)**, **Kitana et al. (2017)** and **Jaradat et al. (2015)**. However, slight differences were observed, particularly in the study by **Jaradat et al. (2015)**, which reported the absence of tannins and steroids in *E. alata*. In general, phytochemical composition is strongly influenced by plant habitat and environmental conditions. According to **Firdiyani (2015)**, habitat characteristics may affect metabolite production,

while environmental factors such as altitude can significantly influence the chemical composition of plants (Herlina *et al.*, 2017).

3.Total flavonoid content:

The figure below represents the calibration curve of the quercetin standard used to determine the flavonoid content of our plant extracts.

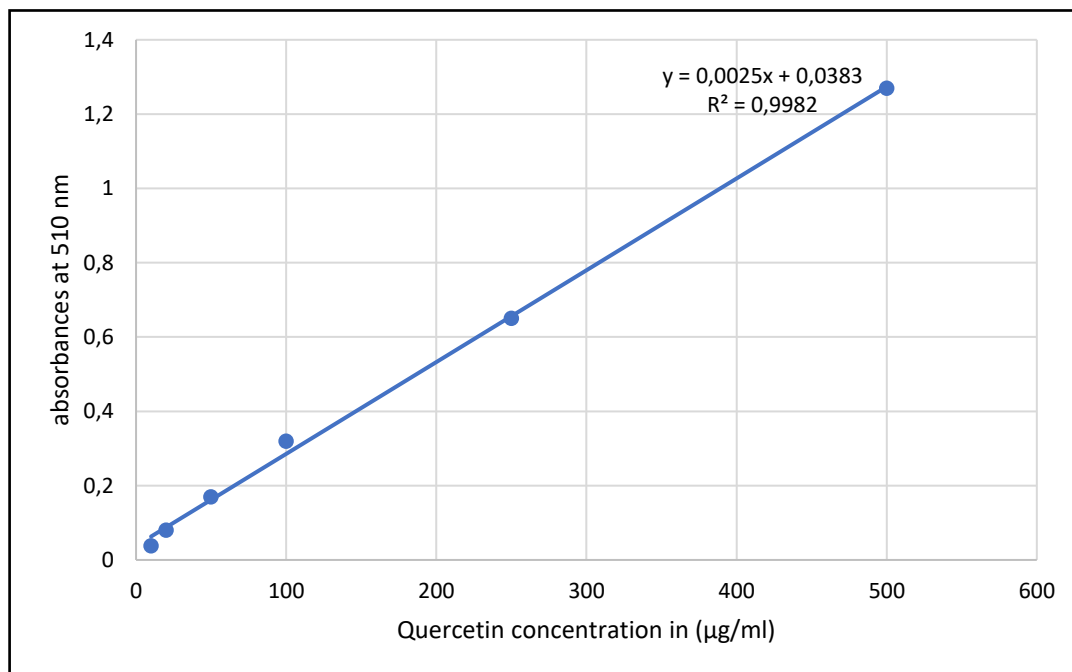


Figure 14: Quercetin calibration curve

The results of this assay showed that the *A. malaccensis* extract contained the highest flavonoid content, with a concentration of 39.61 ± 5.83 mg QE/g of extract, which was significantly higher ($p = 0.032$) than that of the ethanolic extract of *E. alata*, which contained 28.41 ± 0.742 mg QE/g of extract.

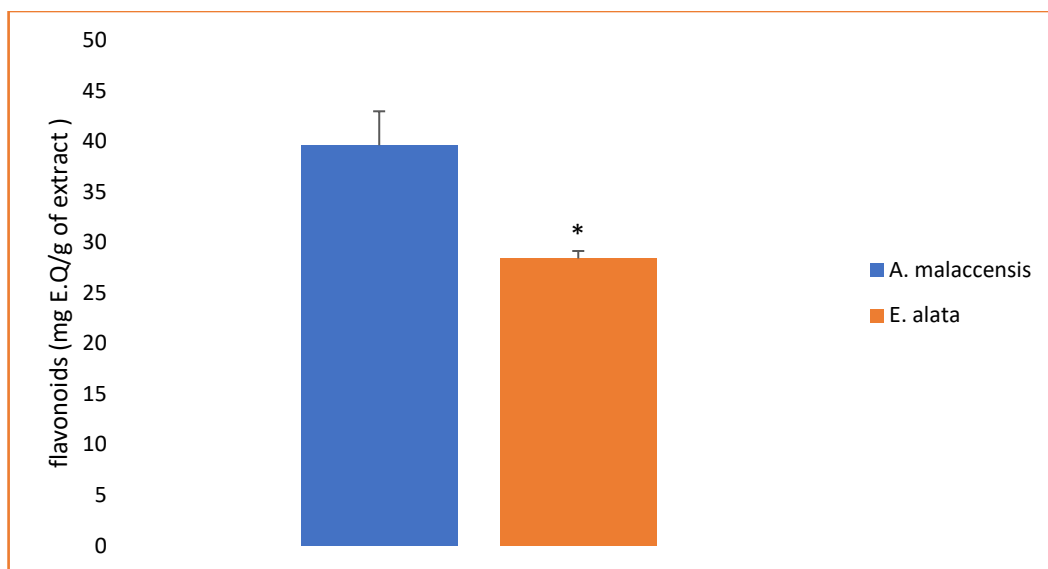


Figure 15: flavonoid content of *A. malaccensis* and *E. Alata*. Values are expressed as mean $\pm$ SEM; *A. Malaccensis* vs *E. Alata* (*: $p < 0,05$)

According to **Jaradat *et al.* (2015)**, *Ephedra alata* extracts from Palestine prepared by maceration using different organic solvents (ethanol, methanol, and acetone) showed flavonoid contents of 5.44 ± 0.625 , 54.66 ± 0.12 , and 0.519 ± 0.09 mg rutin equivalent/g extract, respectively. These findings indicate that flavonoid content may vary considerably depending on the extraction solvent used.

Ais and Boughazzel (2024) reported that *A. malaccensis* bark extracts contained flavonoids at a concentration of 1.98 ± 0.07 mg catechin equivalent/g extract, which is lower than the value obtained in our study. This difference may be attributed to the use of different reference standards for flavonoid quantification, as well as variations in plant material, extraction methods, and experimental conditions (**Trabelsi *et al.*, 2010**)

4. Evaluation of antioxidant activity:

4.1 Antiradical activity by DPPH:

Antioxidant activity appears to be the mechanism of action for a number of pharmaceutical products. We investigated the free-radical scavenging activity of extracts from *A. malaccensis* and *E. alata* by assessing their ability to neutralize the DPPH free radical. The absorbance values obtained were used to calculate the percentage inhibition

and to construct curves representing the relationship between inhibition percentage and the different tested concentrations.

Figure 16 presents the linear curve of the inhibition percentage of ascorbic acid used as a standard at concentrations ranging from 10 to 100 $\mu\text{g/mL}$. The results showed inhibition percentages varying between 6.28% and 94.2%.

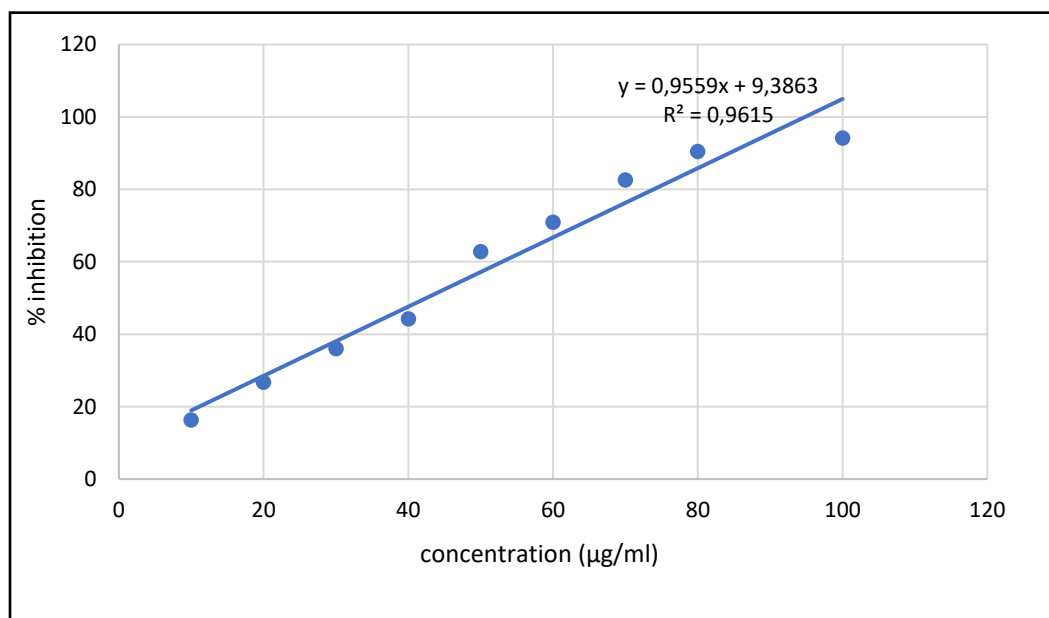


Figure 16: Inhibition percentage of ascorbic

Figures 17,18, and 19 present the inhibition percentage curves obtained for *A. malaccensis*, *E. alata*, and their mixture, respectively, at concentrations ranging from 5 to 250 $\mu\text{g/mL}$. The results demonstrated a concentration-dependent increase in inhibition activity, with inhibition percentages varying from 6.97% to 80.23% for *A. malaccensis*, 4.65% to 75.58% for *E. alata*, and 10.46% to 82.55% for their mixture.

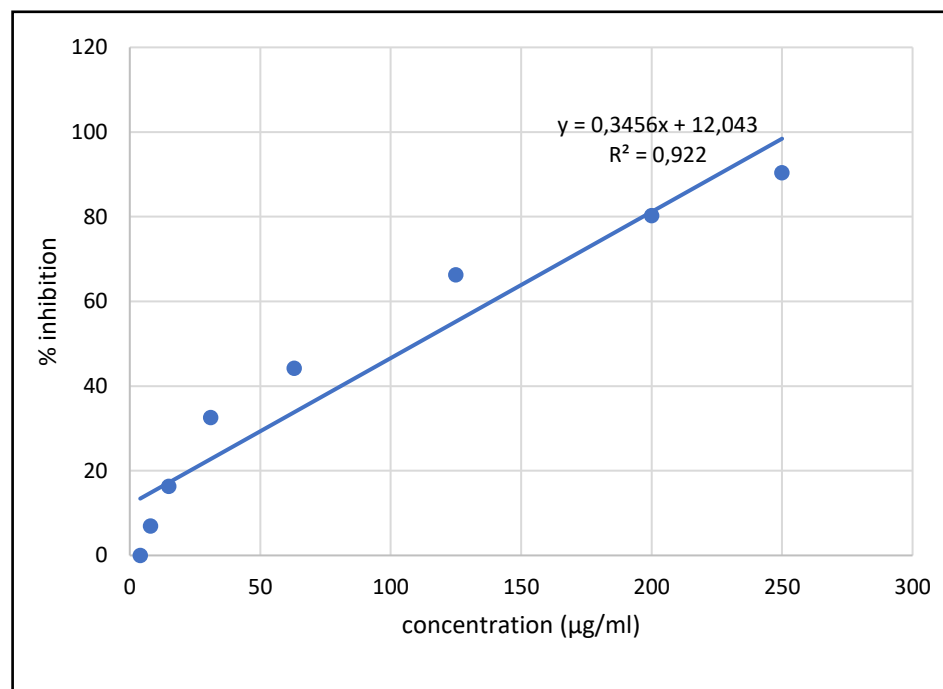


Figure 17: Inhibition percentage of *A. malaccensis*

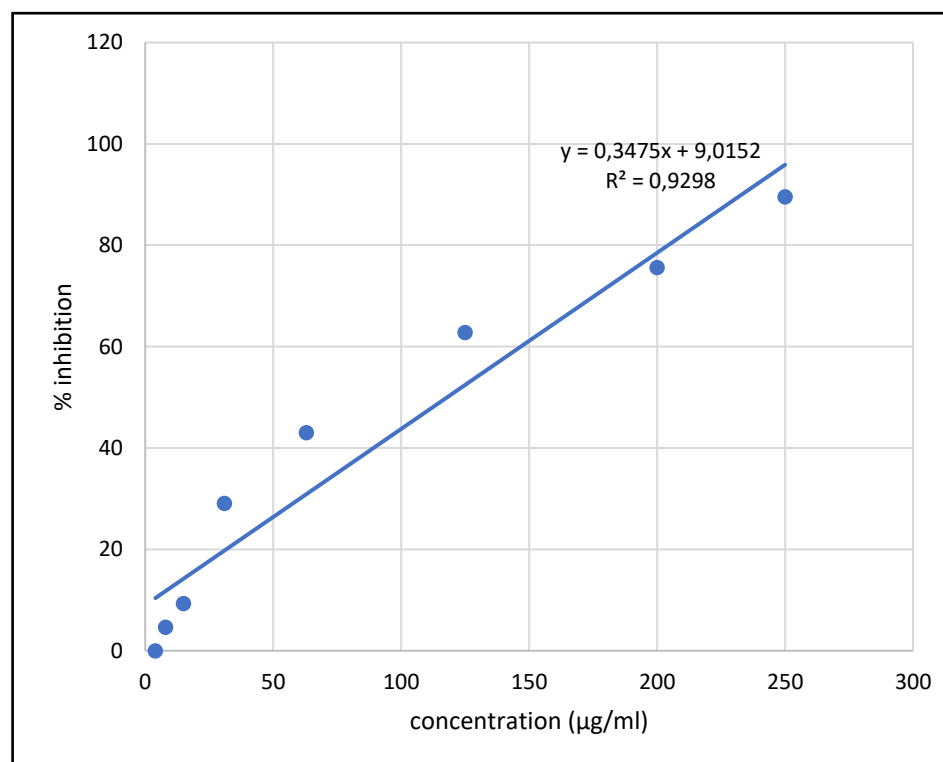


Figure 18: Inhibition percentage of *E. alata*

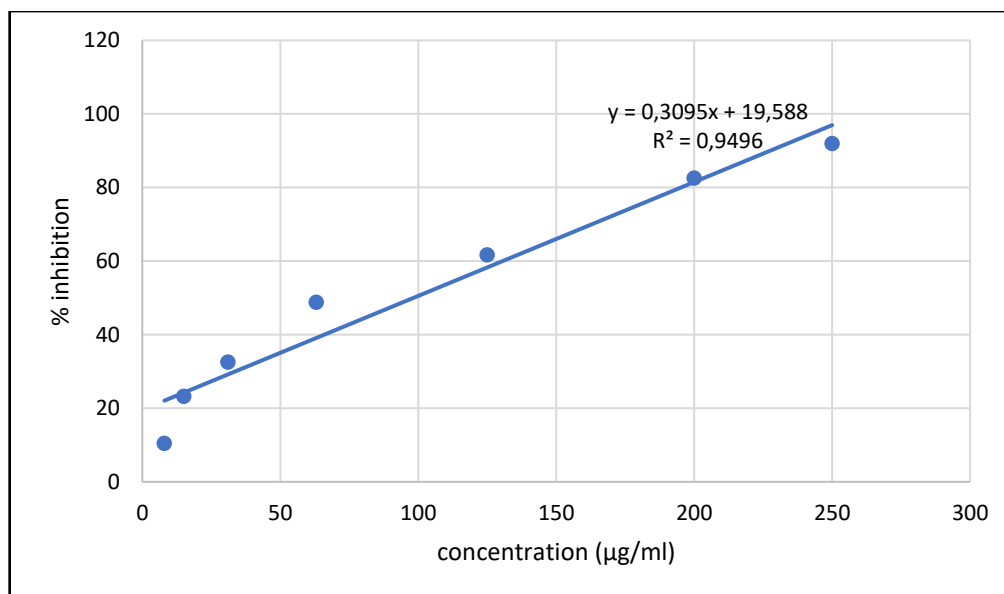


Figure 19: Inhibition percentage of Mixture

The figure below presents a comparison of the inhibition percentages of *A. malaccensis*, *E. alata*, and their mixture. The results show that *A. malaccensis* exhibited slightly higher inhibition percentages at lower concentrations, followed by the mixture and then *E. alata*. However, from a concentration of 30 µg/mL onward, the inhibition percentages became approximately similar among the two plant extracts and their mixture.

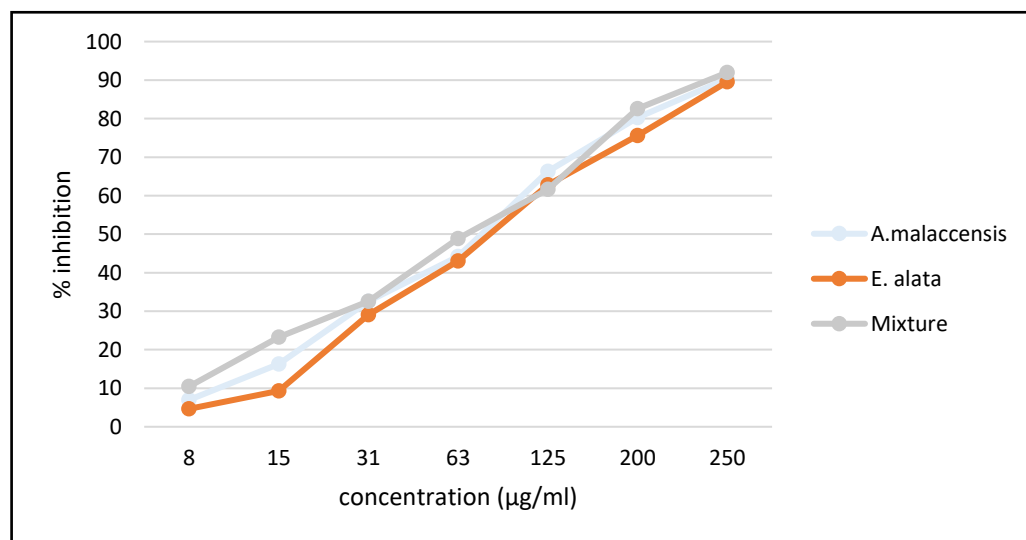


Figure 20: Comparison of the percentage of inhibition between *A. malaccensis*, *E. alata* and Mixture

Figure 21 presents the IC_{50} values obtained for ascorbic acid, *Aquilaria malaccensis*, *Ephedra alata*, and their mixture. Ascorbic acid showed an IC_{50} value of $42.5 \pm 4.9 \mu\text{g/mL}$. Among the plant extracts, *A. malaccensis* exhibited an IC_{50} of $99.7 \pm 5 \mu\text{g/mL}$, while *E. alata* showed a higher IC_{50} value of $116.06 \pm 0.94 \mu\text{g/mL}$. Furthermore, the mixture of both extracts presented an IC_{50} value of $93.09 \pm 2.59 \mu\text{g/mL}$.

Since lower IC_{50} values indicate stronger antioxidant activity, both plant extracts and their mixture exhibited lower antioxidant activity compared with ascorbic acid. Nevertheless, the mixture showed slightly better antioxidant potential than the individual extracts, followed by *A. malaccensis* and then *E. alata*. However, statistical analysis revealed no significant differences between the two extracts and their mixture.

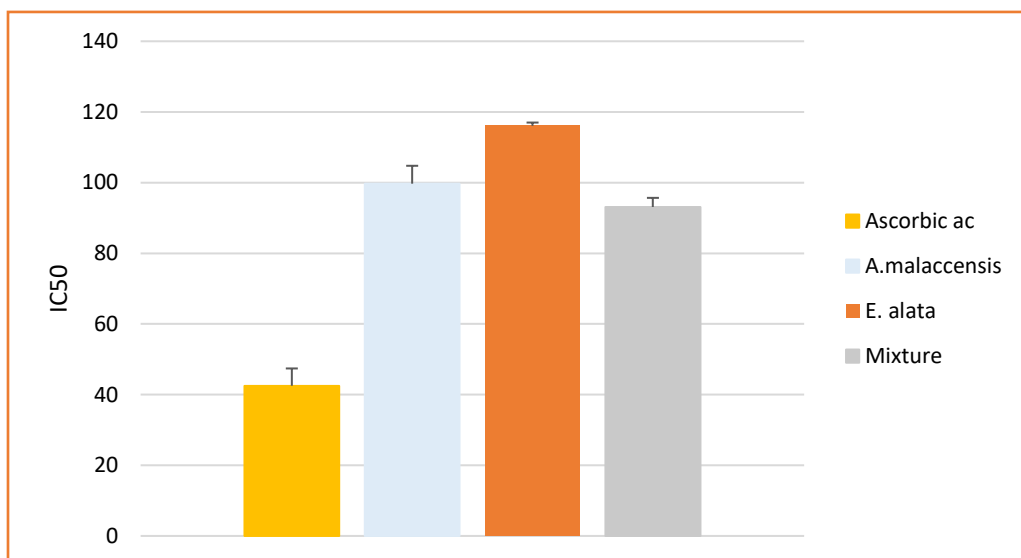


Figure 21: inhibitory concentration 50 of extracts plants and their mixtures. Values are expressed as mean $\pm$ SEM ($p \geq 0.05$)

Our findings are partially consistent with previous studies on the antioxidant activity of *A. malaccensis*. **Ridwanto *et al.* (2022)** reported that the methanolic bark extract of *A. malaccensis*, prepared by maceration for 48 hours, exhibited an IC_{50} value of $94 \mu\text{g/mL}$, which is relatively close to the value obtained in the present study. In contrast, **Halim *et al.* (2022)** reported a much lower IC_{50} value ($37.22 \mu\text{g/mL}$) for ethanolic extracts of *A. malaccensis*, indicating very strong antioxidant activity.

Previous studies by **Batubara *et al.* (2020)** reported the presence of glycosides, steroids/triterpenoids, flavonoids, and tannins in ethanolic extracts of *A. malaccensis*. These phytochemical compounds are known to contribute to antioxidant activity, which may explain the antioxidant potential observed in this plant.

The antioxidant activity obtained for *Ephedra alata* in the present study differs from values reported in previous investigations. **Ouartal *et al.* (2024)** reported an IC₅₀ value of 66.51 µg/mL for *E. alata* extract, indicating stronger antioxidant activity compared with our results. Similarly, **Jerbi *et al.* (2016)** reported high antiradical activity for the methanolic extract of *E. alata*, with an IC₅₀ value of 27 µg/mL.

In contrast, **Ziani *et al.* (2019)** found that *E. alata* extracts prepared as infusions, decoctions, and aqueous ethanolic extracts exhibited lower radical scavenging activity, with IC₅₀ values ranging from 450 to 550 µg/mL, which are considerably higher than the value obtained in our study. **Soumaya *et al.* (2020)** also reported antioxidant activity for ethanolic extracts of *E. alata* with an IC₅₀ value of 3 mg/mL. Therefore, the present findings suggest that *E. alata* possesses considerable antiradical activity, although its antioxidant potential varies widely depending on extraction methods and experimental conditions. Variations in antioxidant activity may be explained by differences in phytochemical composition, since antioxidant power can result from interactions among phenolic compounds, including synergistic, antagonistic, and additive effects (**Kurin *et al.*, 2012**).

4.2 Total antioxidant capacity (TAC) assay:

TAC is an important parameter used to evaluate the overall ability of biological samples or plant extracts to neutralize free radicals and prevent oxidative damage. Unlike assays that measure individual antioxidant compounds, TAC reflects the cumulative action of all antioxidants present in a sample, including phenolic compounds, flavonoids, vitamins, and other reducing agents. Higher total antioxidant capacity generally indicates a greater potential to scavenge reactive oxygen species and protect against oxidative stress-related damage. The antioxidant potential of plant extracts is strongly associated with their phytochemical composition, particularly their content of polyphenols and flavonoids (**Apak *et al.*, 2016**).

The figure below represents the calibration curve of ascorbic acid.

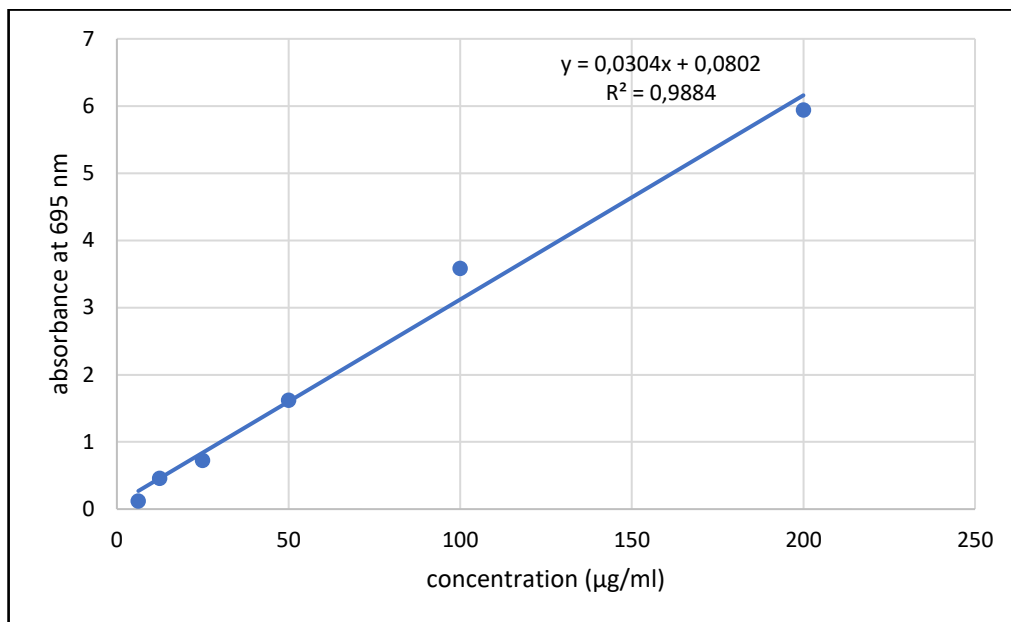


Figure 22: Calibration curve of ascorbic acid. Each point represents the mean of three experiments

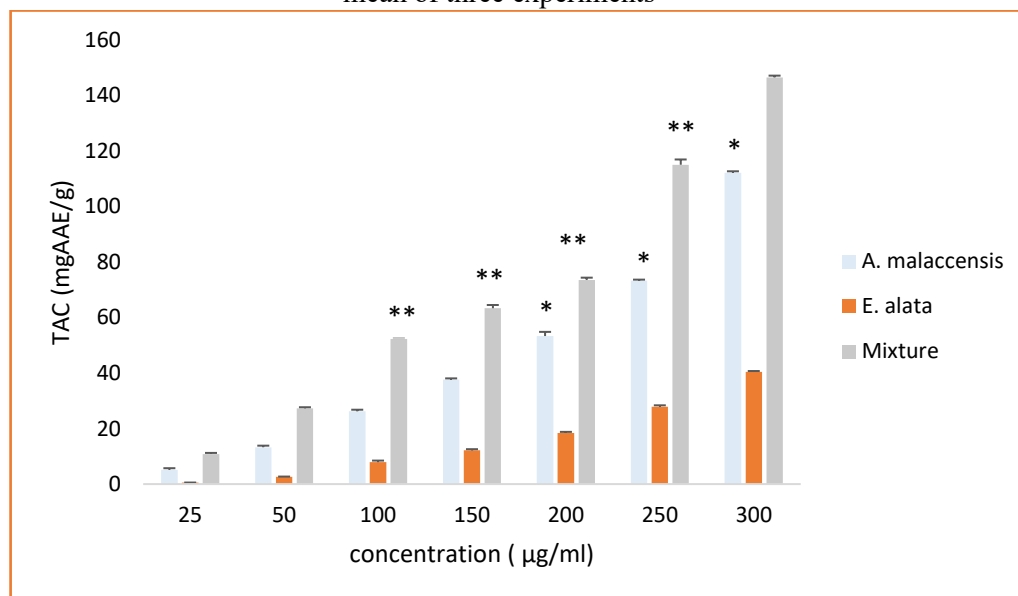


Figure 23: Total antioxidant capacity of extracts plants and their mixtures
Values are expressed as mean $\pm$ SEM E. alata vs A. malaccensis (*: $p < 0.05$), E. alata vs mixture (**: $p < 0.01$)

Figure23 presents the TAC, expressed as mg ascorbic acid equivalent per gram of extract (mg AAE/g), of the two plant extracts and their mixture at different concentrations ranging from 25 to 300 µg/mL. The results show that TAC increased with increasing

concentration for all tested samples. At the highest concentration (300 µg/mL), the mixture exhibited the highest TAC value (146.44 ± 0.66 mg AAE/g), followed by *A. malaccensis* (112.11 ± 0.55 mg AAE/g), while *E. alata* showed the lowest value (40.38 ± 0.34 mg AAE/g).

ANOVA analysis revealed no significant differences between the two plant extracts and their mixture at lower concentrations (25 and 50 µg/mL). However, at concentrations ranging from 100 to 300 µg/mL, the mixture exhibited significantly higher TAC values than *E. alata* ($p < 0.01$). Similarly, *A. malaccensis* showed significantly higher TAC values compared with *E. alata* ($p < 0.05$). No significant difference was observed between *A. malaccensis* and the mixture.

studies have shown that *A. malaccensis* extracts possess considerable antioxidant activity due to their richness in phenolic compounds and flavonoids. **Huda *et al.* (2009)**, reported that methanolic leaf extracts of *A. malaccensis* exhibited strong antioxidant activity, which was associated with the presence of flavonoids, triterpenoids, steroids, and other secondary metabolites. Furthermore, studies evaluating different extraction solvents demonstrated that ethanolic extracts generally exhibit higher antioxidant properties compared with aqueous or less polar solvents, highlighting the influence of solvent polarity on antioxidant capacity. The antioxidant potential of *A. malaccensis* is mainly attributed to its phytochemical composition, particularly polyphenols and flavonoids, which contribute to electron donation and free radical scavenging mechanisms (**Sumantri *et al.*, 2025**; **Mahmod *et al.*, 2017**).

The evaluation of total antioxidant activity demonstrated that antioxidant capacity is species-dependent and strongly influenced by the extraction solvent used. **Soumaya *et al.* (2020)** reported that the ethanolic extract of *E. alata* exhibited the highest total antioxidant capacity, reaching 280 mg GAE/g, which is considerably higher than the values obtained in our study. These differences may be attributed to variations in extraction conditions, solvent polarity, plant origin, environmental factors, and the methods used for antioxidant capacity determination.

5. Anti-inflammatory activity:

5.1 Bovine serum albumin denaturation inhibition (BSA) test:

To evaluate the anti-inflammatory activity of the studied plants, the method using BSA (Bovine Serum Albumin) protein was employed. The protective effect of *E. alata* and *A. malaccensis* extracts against the thermal denaturation of BSA.

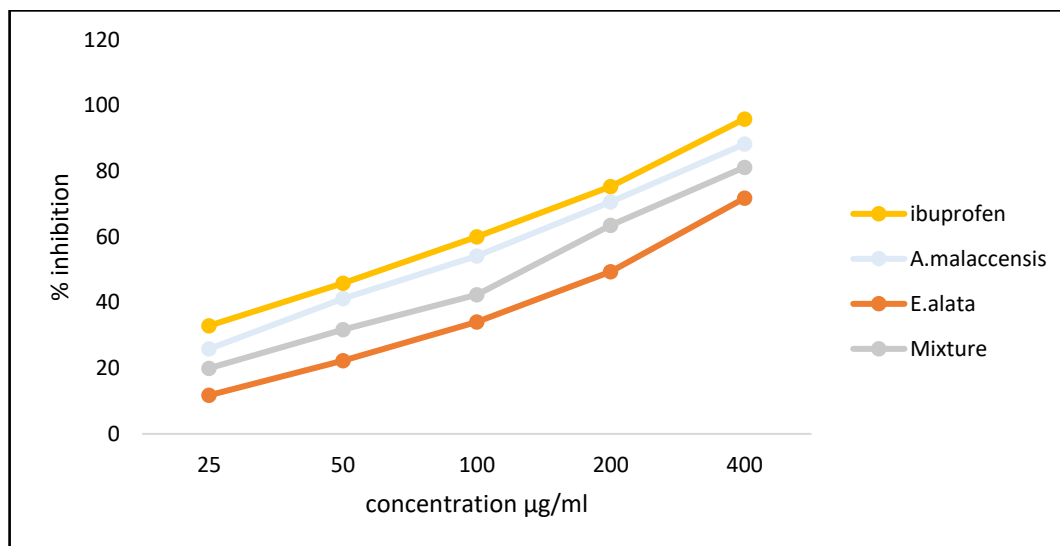


Figure 24: Comparison of the percentage of inhibition between *A. malaccensis*, *E. alata* and Mixture. Each point represents the mean of three experiments.

Figure 24 presents a comparison of the percentage inhibition exhibited by the two plant extracts and their mixture, as well as their comparison with the standard, over a concentration range of 25 to 400 µg/mL. Ibuprofen, a widely used nonsteroidal anti-inflammatory drug (NSAID), was used as a positive control and showed the highest anti-inflammatory activity, with inhibition percentages ranging from 32.94% to 95.88%.

Among the plant extracts, *A. malaccensis* demonstrated notable anti-inflammatory activity, with inhibition percentages ranging from 25.88% to 88.23%. *E. alata* showed lower activity, with inhibition values between 11.76% and 71.76%, while the mixture exhibited intermediate activity, ranging from 20.00% to 81.18%.

These results suggest that *A. malaccensis* possesses stronger anti-inflammatory potential compared with *E. alata* and the mixture.

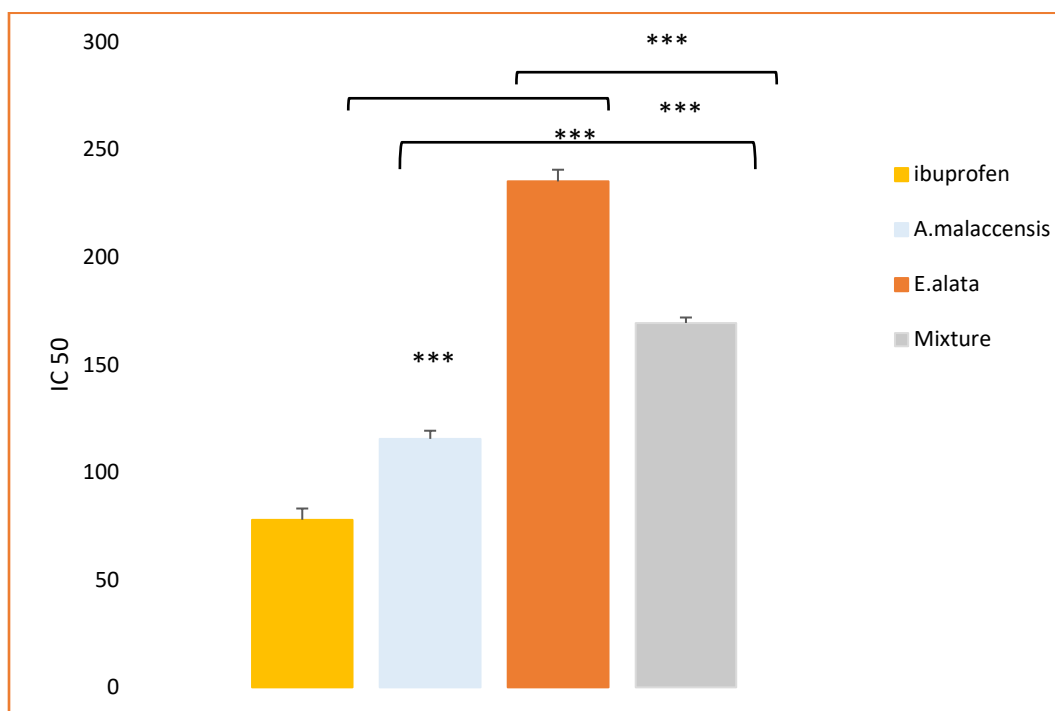


Figure 25: inhibitory concentration 50 of extracts plants and their mixtures
Values are expressed as mean $\pm$ SEM ibuprofen vs *E. alata* and *A. malaccensis* (***: $p < 0.001$), *A. malaccensis* vs *E. alata* and mixture (***: $p < 0.001$) and *E. alata* vs mixture (***: $p < 0.001$)

Figure 25 presents the IC₅₀ values of the plant extracts, their mixture, and the standard used for anti-inflammatory activity evaluation. Since lower IC₅₀ values indicate stronger anti-inflammatory potential, samples with lower concentrations required to inhibit 50% of the reaction are considered more active.

Ibuprofen exhibited the lowest IC₅₀ value ($77.92 \pm 5.23 \mu\text{g/mL}$), confirming its superior anti-inflammatory activity. Among the plant extracts, *A. malaccensis* showed an IC₅₀ value of $115.53 \pm 3.78 \mu\text{g/mL}$, indicating higher anti-inflammatory potential compared with *E. alata* ($235.20 \pm 5.45 \mu\text{g/mL}$) and the mixture ($169.38 \pm 2.56 \mu\text{g/mL}$). These findings suggest that *A. malaccensis* provides better protection against thermal denaturation of bovine serum albumin (BSA), reflecting stronger anti-inflammatory activity than *E. alata* and their mixture.

Statistical analysis revealed that the IC₅₀ value of ibuprofen was significantly lower ($p < 0.001$) than those of both plant extracts and their mixture, confirming its superior anti-

inflammatory activity. In addition, *A. malaccensis* exhibited a significantly lower IC₅₀ value ($p < 0.001$) compared with *E. alata* and the mixture, indicating stronger anti-inflammatory potential. Furthermore, the IC₅₀ value of *E. alata* was significantly higher ($p < 0.001$) than that of the mixture, suggesting lower anti-inflammatory activity.

Previous studies have demonstrated that both *Ephedra alata* and *Aquilaria malaccensis* possess anti-inflammatory properties, although their efficacy varies according to extraction conditions, phytochemical composition, and experimental models. **Ouartal *et al.* (2024)** reported that *E. alata* exhibited significant anti-inflammatory activity, with a low inhibition % value of 15.36 %. These findings support the results obtained in the present study and further confirm the anti-inflammatory potential of this species. Likewise, our results for *A. malaccensis* are comparable to those reported by **Rahman *et al.* (2016)**, who observed an inhibition percentage of 71.4%, confirming the ability of this species to inhibit inflammatory processes.

Analysis of the inhibitory concentration at 50% (IC₅₀) provides a more precise comparison of the relative efficacy of the tested samples. In the present study, *A. malaccensis* exhibited a lower IC₅₀ value compared with *E. alata*, indicating stronger anti-inflammatory activity. Similar trends have been reported in previous studies. According to **Danciu *et al.* (2018)**, extracts generally show IC₅₀ values ranging from 350 to 450 µg/mL, whereas *A. malaccensis* demonstrates lower IC₅₀ values ranging from approximately 85 to 200 µg/mL, reflecting greater inhibitory potential. Lower IC₅₀ values suggest that lower concentrations of extract are required to achieve 50% inhibition, indicating higher biological activity.

5.2 Erythrocyte membrane stabilization test:

The erythrocyte membrane stabilization assay is widely used as an in vitro method to evaluate the anti-inflammatory potential of plant extracts and bioactive compounds. This method is based on the principle that the erythrocyte membrane is structurally similar to lysosomal membranes; therefore, stabilization of red blood cell membranes against hemolysis can be considered an indicator of the ability of a substance to stabilize lysosomal membranes during inflammatory processes. During inflammation, lysosomal enzymes released from activated neutrophils contribute to tissue damage and the amplification of

inflammatory responses. Compounds capable of preventing membrane disruption may therefore reduce the release of these inflammatory mediators. In this assay, erythrocytes are exposed to hypotonic solution induced stress, and the degree of membrane protection is assessed by measuring hemoglobin release. A higher membrane stabilization percentage indicates stronger anti-inflammatory activity. The protective effects observed in plant extracts are frequently associated with the presence of phenolic compounds, flavonoids, tannins, and other secondary metabolites capable of interacting with membrane proteins and phospholipids, thereby enhancing membrane resistance to stress-induced lysis (Anosike *et al.*, 2012).

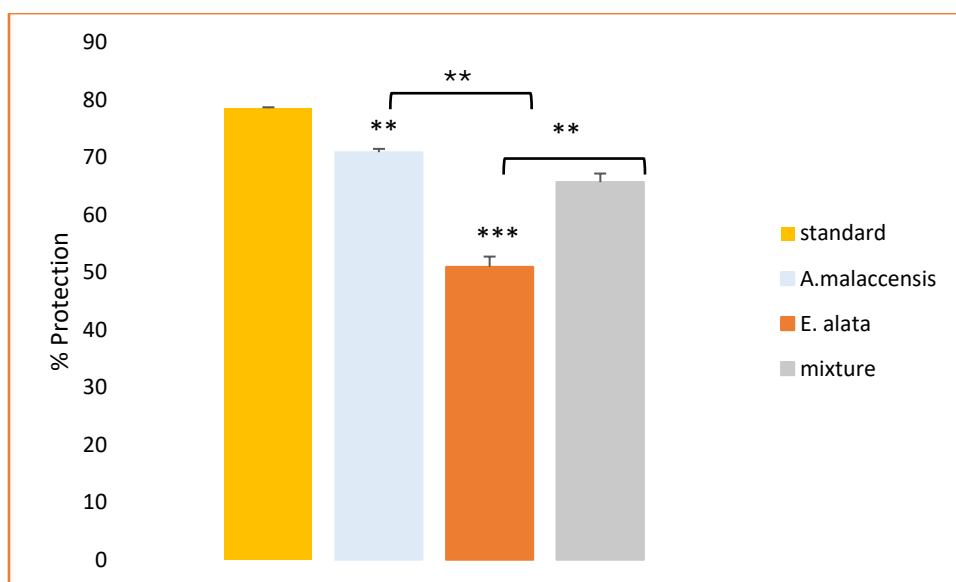


Figure 26: Percentage of membrane protection induced by the plant extracts and their mixture. Values are expressed as mean $\pm$ SEM standard vs *A. malaccensis* (**: $p < 0.01$), standard vs *E. alata* (***: $p < 0.001$), *E. alata* vs *A. malaccensis* and mixture (**: $p < 0.01$).

The mean membrane protection percentage of the standard drug, diclofenac sodium, was $78.55 \pm 0.17\%$, which was significantly higher than those observed for *A. malaccensis* ($70.90 \pm 0.58\%$, $p = 0.006$) and *E. alata* ($50.98 \pm 1.76\%$, $p < 0.001$). Furthermore, *E. alata* exhibited a significantly lower membrane protection percentage compared with both *A. malaccensis* and the mixture ($65.69 \pm 1.51\%$) ($p = 0.002$). No significant difference was observed between *A. malaccensis* and the mixture, suggesting comparable membrane-stabilizing effects between these two treatments.

Previous studies have demonstrated that *Ephedra alata* possesses significant anti-inflammatory activity through its ability to modulate inflammatory mediators and reduce inflammatory responses (Soumaya *et al.*, 2020). These findings are consistent with the results obtained in the present study, supporting the potential use of *E. alata* as a natural source of bioactive compounds with anti-inflammatory properties. The erythrocyte membrane stabilization assay demonstrated differences in anti-inflammatory potential among the tested extracts and their mixture. Diclofenac sodium exhibited the highest membrane protection percentage, confirming its effectiveness as a standard anti-inflammatory drug. Nevertheless, *A. malaccensis* and its mixture with *E. alata* also showed considerable membrane stabilization activity, whereas *E. alata* exhibited significantly lower protection. The higher membrane stabilization activity observed for *A. malaccensis* may be associated with its phytochemical composition, particularly its higher flavonoid and phenolic contents. Phenolic compounds, flavonoids, tannins, and terpenoids are known to interact with membrane proteins and phospholipid bilayers, thereby enhancing membrane integrity and resistance against osmotic or thermal stress. Similar observations have been reported in previous studies demonstrating that plant extracts rich in polyphenols exhibit stronger membrane-protective properties due to their antioxidant and anti-inflammatory mechanisms (Anosike *et al.*, 2012).

6. Antibacterial activity:

The antibacterial activity of *Ephedra Alata* and *Aquilaria malaccinsis* is determined by measuring the diameter of the inhibition zone at different tablets on two extracts of medicinal plants in which the treatment is carried out on various bacterial strains (*E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *bacillus subtilis*).

The figure below presents the inhibition zone diameters produced by gentamicin at a concentration of 10 µg/mL, used as a positive control, as well as those obtained with the two plant extracts and their mixture at a concentration of 100 mg/mL against the four bacterial strains previously mentioned

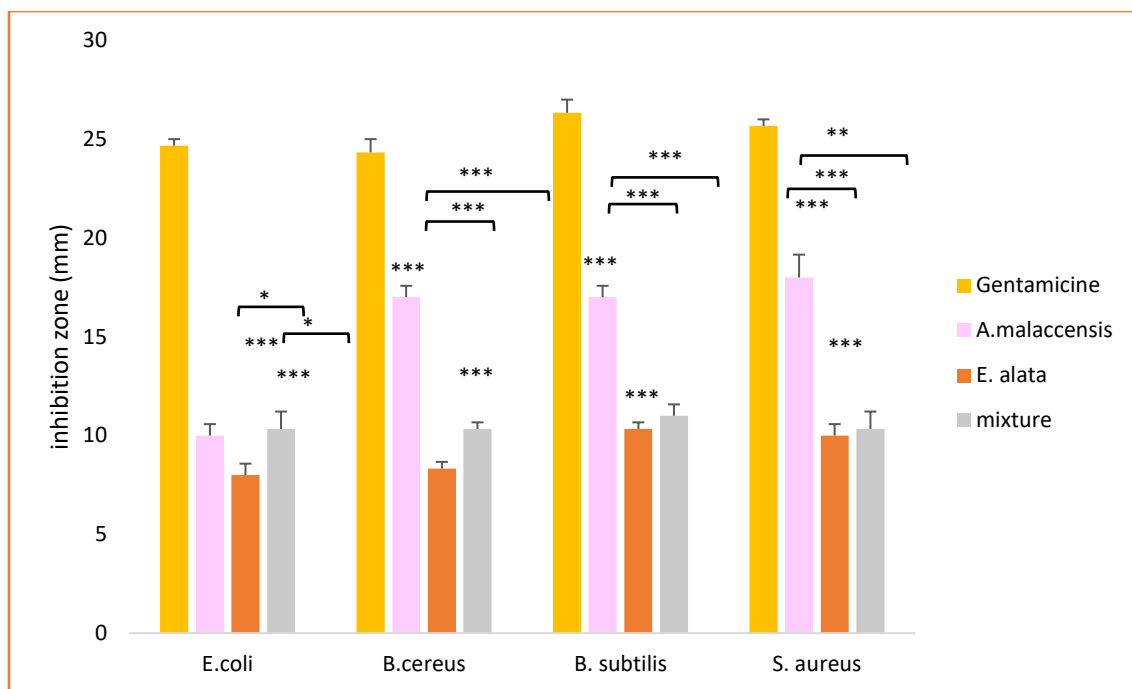


Figure 27: Inhibition zones induced by the plant extracts and their mixture. Values are expressed as mean $\pm$ SEM

Against *Escherichia coli*, gentamicin exhibited an inhibition zone diameter of 24.67 ± 0.33 mm, which was significantly higher ($p < 0.001$) than those produced by *A. malaccensis* and *E. alata*, with inhibition zones of 10.00 ± 0.58 mm and 8.00 ± 0.58 mm, respectively. *A. malaccensis* demonstrated significantly greater antibacterial activity than *E. alata* ($p = 0.037$). No significant difference was observed between *A. malaccensis* and the mixture, which produced an inhibition zone of 10.33 ± 0.88 mm. However, the mixture showed significantly greater activity than *E. alata* ($p = 0.04$), as reflected by its larger inhibition zone diameter.

Against *Bacillus cereus*, the inhibition zones of Gentamicin, *A. malaccensis*, *E. alata*, and the mixture were 24.33 ± 0.67 mm, 17.00 ± 0.58 mm, 8.33 ± 0.33 mm, and 10.33 ± 0.33 mm, respectively. Statistical analysis revealed that the inhibition zone produced by gentamicin was highly significantly greater than those of both plant extracts ($p < 0.001$). In addition, *A. malaccensis* exhibited a significantly ($p < 0.001$) stronger inhibition zone compared with *E. alata* and mixture. No significant difference was observed between *E. alata* and the mixture.

Against *Bacillus subtilis*, Gentamicin showed an inhibition zone of 26.33 ± 0.67 mm, which was highly significantly greater ($p < 0.001$) than those observed for *A. malaccensis* (17.00 ± 0.58 mm) and *E. alata* (10.33 ± 0.33 mm), *A. malaccensis* exhibited a highly significantly higher activity compared with *E. alata* ($p < 0.001$). The inhibition zone of the mixture was 11.00 ± 0.58 mm, showing no significant difference compared with *E. alata*. However, it exhibited a highly significant decrease compared with *A. malaccensis* ($p < 0.001$).

Regarding *Staphylococcus aureus*, Gentamicin exhibited an inhibition zone of 25.67 ± 0.33 mm, whereas the two plant extracts showed inhibition zones of 18.00 ± 1.54 mm for *A. malaccensis* and 10.00 ± 0.58 mm for *E. alata*. Statistical analysis revealed a highly significant difference between gentamicin and both plant extracts ($p < 0.001$), *A. malaccensis* exhibited a highly significantly higher activity compared with *E. alata* ($p < 0.001$). The inhibition zone of the mixture was 10.33 ± 0.88 mm, which showed a highly significant decrease compared with *A. malaccensis* ($p = 0.009$). However, no significant difference was observed compared with *E. alata* ($p \geq 0.05$).

The antibacterial activity of *A. malaccensis* observed in the present study showed similarities and differences compared with previous reports. According to **Bouhaous (2022)**, the ethanolic extract of *A. malaccensis* exhibited an inhibition zone of 20 mm against *Escherichia coli*, which is higher than the value obtained in our study. In contrast, **Hendra et al. (2016)** reported that methanolic extracts of *A. malaccensis* at a concentration of 150 mg/mL produced an inhibition zone of 6.83 mm against *E. coli*, which is lower than our findings.

For *Staphylococcus aureus*, **Bouhaous (2022)** reported an inhibition zone of 25 mm for the ethanolic extract of *A. malaccensis*, indicating stronger antibacterial activity than that observed in the present study. However, **Batubara et al. (2020)** found an inhibition zone of 9.23 ± 0.97 mm against *S. aureus*, which is lower than our results. Similarly, **Hendra et al. (2016)** reported an inhibition zone of 6.83 mm using methanolic extracts, also lower than the antibacterial activity observed in our study.

Regarding *Bacillus subtilis*, **Bouhaous (2022)** reported an inhibition zone of 11 mm for ethanolic extracts of *A. malaccensis*, which is lower than the value obtained in the present work. These variations in antibacterial activity may be attributed to differences in extraction solvents, extract concentrations, plant parts used, and experimental conditions.

Our results for *E. alata* are generally consistent with previous studies investigating its antibacterial activity. **Ouartal et al. (2024)** reported that the methanolic extract of the whole plant of *E. alata* collected from the Ouargla region exhibited an inhibition zone against *Escherichia coli* of 7.48 ± 1.05 mm, which is close to the value obtained in our study. The same authors also reported an inhibition zone of 10 mm against *Staphylococcus aureus*, which was nearly identical to our findings. Similarly, **Cherchab (2019)** observed an inhibition zone of 9 mm against *Bacillus cereus* using methanolic extracts of the whole plant, showing comparable antibacterial activity to that observed in the present work.

In contrast, **Bouhaous (2022)** reported that ethanolic extracts of *E. alata* showed no inhibitory effect against *Bacillus subtilis*, whereas our results demonstrated an inhibition zone of 10 mm. These differences may be explained by variations in plant origin, environmental conditions, phytochemical composition, and experimental protocols used in antibacterial evaluation.



Conclusion and perspective

Conclusion and perspective:

In recent years, the therapeutic use of medicinal plants has attracted increasing interest due to their richness in bioactive compounds and their potential effectiveness in preventing and treating various health disorders, including oxidative stress-related diseases, inflammatory conditions, and microbial infections.

Within this context, the present study focused on the phytochemical characterization and the evaluation of the antioxidant, anti-inflammatory, and antibacterial activities of ethanolic extracts obtained from *Ephedra alata*, *Aquilaria malaccensis*, and their mixture.

The extraction process using ethanol yielded extraction rates of 8.5% and 26% for *E. alata* and *A. malaccensis*, respectively, indicating differences in extractable phytochemical composition between the two species. Phytochemical screening revealed the presence of several classes of secondary metabolites, including alkaloids, anthocyanins, coumarins, tannins, flavonoids, sterols and terpenoids confirming the richness of these plants in bioactive constituents.

The antioxidant activity assessment demonstrated promising results. Regarding total antioxidant capacity, *A. malaccensis* exhibited the highest activity, reaching 112 mg AAE/g extract. In the DPPH radical scavenging assay, the mixture of both extracts showed the strongest activity among the tested samples, with an IC_{50} value of $93.09 \pm 2.59 \mu\text{g/mL}$, suggesting a possible synergistic interaction between the phytochemicals present in both plants.

Evaluation of anti-inflammatory activity confirmed the therapeutic potential of the studied extracts. The results showed that *A. malaccensis* exhibited the strongest anti-inflammatory activity, with an IC_{50} value of $115.53 \pm 3.78 \mu\text{g/mL}$ in the bovine serum albumin denaturation assay and an erythrocyte membrane protection percentage of $70.90 \pm 0.58\%$, indicating greater efficacy than *E. alata* and their mixture.

Furthermore, antibacterial activity evaluation using the agar well diffusion method against four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*,

and *Bacillus subtilis*) demonstrated that *A. malaccensis* generally exhibited stronger antibacterial effects compared with *E. alata* and the mixture, highlighting its potential as a source of antimicrobial compounds.

Overall, the findings of this study confirm that both *A. malaccensis* and *E. alata* possess important biological activities associated with their phytochemical composition. Among the tested samples, *A. malaccensis* demonstrated the most promising pharmacological potential, while the mixture showed interesting synergistic effects in certain assays. The results obtained open several perspectives for future investigations:

- Quantitative characterization and identification of the major bioactive compounds using advanced analytical techniques such as HPLC, GC–MS, or LC–MS/MS.
- Evaluation of additional biological activities, including antifungal, anticancer, and antiviral properties.
- Assessment of toxicity and safety profiles through in vivo studies before therapeutic application.
- Development of pharmaceutical formulations or natural products based on these extracts for potential medicinal applications.
- Exploration of synergistic interactions between plant extracts and conventional drugs to improve therapeutic efficacy.

These future studies would contribute to a better understanding and valorization of these medicinal plants as promising sources of natural bioactive compounds.

Reference bibliography

Reference bibliography:**A**

Abd Rashed, A., Jamilan, M. A., Abdul Rahman, S., Amin Nordin, F. D., & Mohd Nawi, M. N. (2024). The therapeutic potential of agarwood as an antimicrobial and anti-inflammatory agent: A scoping review. *Antibiotics*, 13(11), 1074.

Ahmaed, D. T., & Kulkarni, A. D. (2017). Sesquiterpenes and chromones of agarwood: a review. *Malaysian Journal of Chemistry*, 19(1), 33-58.

Ahmed, D., Khan, M. M., & Saeed, R. (2015). Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. *Antioxidants*, 4(2), 394-409.

Ais M, S & Boughazzel, M. (2024). *Étude phytochimique, activité antiradicalaire et pouvoir hémolytique de deux plantes toxiques à effets thérapeutiques (Echinops spinosus et Aquilaria malaccensis)*. Mémoire de Master en Sciences Écologiques, option Toxicologie Industrielle et Environnementale, Université Aboubakr Belkaïd, Tlemcen, Algérie.

Aiyegoro, O. A., & Okoh, A. I. (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC complementary and Alternative Medicine*, 10, 1-8.

Ali, N. A. M., Jin, C. B., & Jamil, M. (2016). Agarwood (*Aquilaria malaccensis*) oils. In *Essential Oils in Food Preservation, Flavor and Safety* (pp. 173-180). Academic Press.

Al-Qarawi, A. A., Abd Allah, E. F., & Abeer, H. (2013). Effect of *Ephedra alata* Decne. on lipids metabolism of *Aspergillus flavus* Link. *Bangladesh Journal of Botany*, 42(1), 45-50.

Al-Snafi, A. E. (2017). Therapeutic importance of *Ephedra alata* and *Ephedra foliata*-A review. *Indo American Journal of Pharmaceutical Sciences*, 4(2), 399-406.

Andraws, R., Chawla, P., & Brown, D. L. (2005). Cardiovascular effects of ephedra alkaloids: a comprehensive review. *Progress in cardiovascular diseases*, 47(4), 217-225.

Anosike, C.A., Obidoa, O., & Ezeanyika, L.U.S. (2012). *Membrane stabilization as a mechanism of anti-inflammatory activity of methanol extract of garden egg*. DARU Journal of Pharmaceutical Sciences, 20, 76.

Ansel, F. (2019). Chemical and biological study of agarwood from the genus *Aquilaria* (Thymelaeaceae). PhD Thesis, University of Yaoundé I, Cameroon.

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Applied Sciences*, 12(4), 2107.

Apak, R., Ozyurek, M., Guclu, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *Journal of agricultural and food chemistry*, 64(5), 997-1027.

Arpita, R and Navneeta, B., (2017). *Plant Biotechnology*.

Azzini, E., & Maiani, G. (2015). Mediterranean diet: antioxidant nutritional status. In *The Mediterranean Diet* (pp. 249-257). Academic Press.

B

Bahri, F., Bouyahyaoui, A., Kouidri, M., & Meziane, M. (2022). Screening of phytochemical, evaluation of phenolic content, antibacterial and antioxydant activities of *Ephedra alata* from the Algerian Sahara. *Journal of Applied Biological Sciences*, 16(2), 220-229.

Bailey-Shaw, Y. A., Williams, L. A. D., Junor, G. A., et al. (2017). *The anti-inflammatory activity of natural products in heat-denatured protein assays*. *Pharmaceutical Biology*, 55(1), 59–66.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.
<https://doi.org/10.1016/j.jpha.2015.11.005>

Baslam, A., Aitbaba, A., Aboufatima, R., Agouram, F., Boussaa, S., Chait, A., & Baslam, M. (2023). Phytochemistry, Antioxidant Potential, and Antibacterial Activities of *Anacyclus pyrethrum*: Promising Bioactive Compounds. *Horticulturae*, 9(11), 1196.

Batubara, R., Hanum, T. I., Handika, A. R. B. I., & Affandi, O. D. I. N. G. (2020). The screening of phytochemical and antioxidant activity of agarwood leaves (*Aquilaria malaccensis*) from two sites in North Sumatra, Indonesia. *Biodiversitas*, 21(4), 1588-1596.

Bellahcene, Ahlem. (2020). *Étude phytochimique et évaluation de l'activité antioxydante des extraits méthanoliques d'Ephedra alata*. Mémoire de Master en Biologie, option Biochimie, Université Abou Bekr Belkaïd, Tlemcen, Algérie.

Ben Guedouad N, Taleb A (2021). Enquête ethnobotanique des plantes médicinales utilisées pour le traitement du cancer dans la région de Bordj Bou Arreridj. Mémoire de Master. Université Mohamed El Bachir El Ibrahimi- B.B.A – Algérie.

Bensam, M., Rechreche, H., Abdelwahab, A. E., Abu-Serie, M. M., & Ali, S. M. (2023). The role of Algerian *Ephedra alata* ethanolic extract in inhibiting the growth of breast cancer cells by inducing apoptosis in a p53-dependent pathway. *Saudi Journal of Biological Sciences*, 30(6), 103650.

Bouhaous, L., (2022). *Étude ethnobotanique, phytochimique et activités biologiques de quelques plantes utilisées contre le cancer dans la phytothérapie traditionnelle en Algérie*. Thèse de doctorat en sciences, Université Ibn Khaldoun de Tiaret, Algérie.

Bruneton, J. (1999). Pharmacognosie, phytochimie, plantes médicinales. (3ème éd) Tec&Doc, Paris.

Bruneton, J. (2001). *Plantes toxiques: végétaux dangereux pour l'homme et les animaux*. Tec & Doc Lavoisier.

C

Cantau, A. (2011). La thérapeutique par les plantes à travers les âges (I).

Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and chemical toxicology*, 51, 15-25.

Castañeda-Corral, G., Cedillo-Cortezano, M., & Petricevich, V. L. (2025). Parallel in vitro and in silico studies of the anti-inflammatory activity of bioactive compounds found in different ethanolic extracts of bracts from *b. x buttiana* (var. rose): A comparative analysis. *Pharmaceuticals*, 18(6), 821.

Cesbron M., (2020) Vers l'élaboration de surfaces modifiées par des monocouches organiques mixtes issues de la réduction de précurseurs diazonium. HAL ((open science), p.169.

Chaves, J. O., De Souza, M. C., Da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. D. F., ... & Rostagno, M. A. (2020). Extraction of flavonoids from natural sources using modern techniques. *Frontiers in chemistry*, 8, 507887.

Cherchab, M. (2019). Étude phytochimique et évaluation de l'activité biologique de deux plantes (*Ephedra major* et *Ephedra alata*) dans la région de Naâma (Mémoire de master, Centre Universitaire Salhi Ahmed de Naâma, Algérie).

Chevalier A. (2017). Larousse des plantes médicinales : Identification, préparation, soins. Hong Kong, Larousse, 336.

CITES (Convention on International Trade in Endangered Species). (2025). Assessment of the trade of specimen with source code "w" for the species *Aquilaria malaccensis*. <https://cites.org/sites/default/files/documents/E-SC78-34-03-A2.pdf>

Cristina P., Ilonka S., Bartek T., (2009), Evaluation of the antioxidant activity of phenolic compounds by reactivity with the free radical DPPH, *Industrial Engineering Review*, 4, 25-39

Cui, Q., Du, R., Liu, M., & Rong, L. (2020). Lignans and their derivatives from plants as antivirals. *Molecules*, 25(1), 183.

D

Dabbs, D. M., Mulders, N., & Aksay, I. A. (2006). Solvothermal removal of the organic template from L 3 (“sponge”) templated silica monoliths. *Journal of Nanoparticle Research*, 8(5), 603-614.

Dalir, M., Choobchian, S., & Abbasi, E. (2025). Identifying the relative importance of cultivating medicinal plants in improving rural livelihoods. *Environmental Development*, 54, 101159.

Damhuri, Sitti W.A., Lili D., Deki Z., La Ode M., Munadi. (2023), Morphological Characteristics of Wild Plants as Medicines Traditional Muna Tribe *Universal Journal of Plant Science* 11(2):

Danciu, C., et al. (2018). Phytochemical characterization and biological effects of Ephedra alata Decne. extracts. (Une étude souvent citée par les chercheurs maghrébins pour l'activité anti-inflammatoire).

Dbeibia, A., Taheur, F. B., Altammar, K. A., Haddaji, N., Mahdhi, A., Amri, Z., ... & Jabeur, C. (2022). Control of Staphylococcus aureus methicillin resistant isolated from auricular infections using aqueous and methanolic extracts of Ephedra alata. *Saudi Journal of Biological Sciences*, 29(2), 1021-1028.

De Melo, L. F. M., de Queiroz Aquino-Martins, V. G., da Silva, A. P., Rocha, H. A. O., & Scortecci, K. C. (2023). Biological and pharmacological aspects of tannins and potential biotechnological applications. *Food Chemistry*, 414, 135645.

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H., Whitman, W.B., 2009. Bergey's manual of Systematic Bacteriology - second edition. Bergey's manual trust 3.

Desmier, T. (2016). *Les antioxydants de nos jours* (Thèse d'exercice en Pharmacie, Université de Limoges, France). Université de Limoges. <https://cdn.unilim.fr/files/theses-exercice/P20163309.pdf>

Devi, S.S. & Mehendale, H.M. (2014). Quinone, *Encyclopedia of Toxicology, Third Edition* 26-28

Dias, M. C., Pinto, D. C., & Silva, A. M. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, 26(17), 5377.

Dilafruz, M. (2023). Medicinal plants –sources of biologically active substances. *Journal of management and economics*, 4, 2751-1707

Dohou, R., Yamni, K., Tahrouch, S., Hassani, L. I., Badoc, A., & Gmira, N. (2003). Screening phytochimique d'une endémique iberomarocaine, Thymelaea lythroides. *Bulletin-Société de Pharmacie de Bordeaux*, 142(1/4), 61-78.

Dworkin MM and Falkow S. Proteobacteria: Gamma subclass. Ed. Springer, New York, NY, 2006, p. 1248.

E

Elazzouzi, H., Zekri, N., Zair, T., & Alaoui El Belghiti, M. (2019). Total phenolic and flavonoid contents of Anacyclus pyrethrum Link plant extracts and their Antioxidant activity. *Karbala International Journal of Modern Science*, 5(4), 10.

Elhadeif, K., Smaoui, S., Fourati, M., Ben Hlima, H., Chakchouk Mtibaa, A., Sellem, I., ... & Mellouli, L. (2020). A review on worldwide Ephedra history and story: from fossils to natural products mass spectroscopy characterization and biopharmacotherapy potential. *Evidence-Based Complementary and Alternative Medicine*, 2020(1), 1540638.

Elhadeif, K., Smaoui, S., Hlima, H. B., Ennouri, K., Fourati, M., Mtibaa, A. C., ... & Mellouli, L. (2020). Effects of Ephedra alata extract on the quality of minced beef meat during refrigerated storage: A chemometric approach. *Meat Science*, 170, 108246.

El-Najjar, N., Gali-Muhtasib, H., Ketola, R. A., Vuorela, P., Urtti, A., & Vuorela, H. (2011). The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochemistry Reviews*, 10(3), 353-370.

Elshibani, F., Gehawe, H. A., Fallah, G., & Alamami, A. (2020). Screening of in vitro cytotoxic activity of Ephedra alata used traditionally to treat cancer in Libya. *Int J Herb Med*, 8(5), 23-25.

Errington, J., & Aart, L. T. V. D. (2020). Microbe Profile: Bacillus subtilis: model organism for cellular development, and industrial workhorse. *Microbiology*, 166(5), 425-427.

F

Faried, A., El-Banhawy, A., & Elqahtani, M. (2018). Taxonomic, DNA barcoding and phylogenetic reassessment of the Egyptian Ephedra L.(Ephedraceae). *Catrina: The International Journal of Environmental Sciences*, 17(1), 1-13.

Firdiyani, F., Agustini, T. W., & Ma'ruf, W. F. (2015). Extraction of bioactive compounds as natural antioxidants from fresh spirulina platensis using different solvents. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 18(1).

G

Gao, M., Han, X., Sun, Y., Chen, H., Yang, Y., Liu, Y., ... & Han, J. (2019). Overview of sesquiterpenes and chromones of agarwood originating from four main species of the genus *Aquilaria*. *RSC advances*, 9(8), 4113-4130.

Głowacka, K., & Wiela-Hojeńska, A. (2021). Pseudoephedrine—benefits and risks. *International Journal of Molecular Sciences*, 22(10), 5146.

González-Juárez, D. E., Escobedo-Moratilla, A., Flores, J., Hidalgo-Figueroa, S., Martínez-Tagüena, N., Morales-Jiménez, J., ... & Bautista, E. (2020). A review of the *Ephedra* genus: distribution, ecology, ethnobotany, phytochemistry and pharmacological properties. *Molecules*, 25(14), 3283.

Guitton, Y. (2010). Diversité des composés terpéniques volatils au sein du genre *Lavandula*: aspects évolutifs et physiologiques, Université Jean Monnet-Saint-Etienne p :100.

H

Hadjadj, K., Daoudi, B. B., & Guerine, L. (2020). Importance thérapeutique de la plante *Ephedra alata* subsp. *alenda* dans la médecine traditionnelle pour la population de la région de Guettara (Djelfa, Algérie). *Lejeunia, Revue de Botanique*.

Halim, A. P., Wijayanti, N., Hidayati, L., & Nuringtyas, T. R. (2022, May). Antioxidant activity evaluation of agarwood *Aquilaria malaccensis* Lamk. leaves extract using DPPH, FRAP and ABTS assays. In 7th International Conference on Biological Science (ICBS 2021) (pp. 18-25). Atlantis Press.

Hamia C., Guergab A., Rennane N., Birache M. (2014). Influence des solvants sur le contenu en composé phénolique et l'activité antioxydante des extraits du *Rhanterium adpreddim*, *Annales des Sciences et technologie*, 6(1). 33-39

Harbone J.B. 1998. *Phytochemical Methods: A guide to moderne techniques of plant analysis*. 3e ed. chapman and hill. 303p

Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules*, 26(7), 1836.

Hendra, H., Moeljopawiro, S., & Nuringtyas, T. R. (2016, July). Antioxidant and antibacterial activities of agarwood (*Aquilaria malaccensis* Lamk.) leaves. In AIP Conference Proceedings (Vol. 1755, No. 1, p. 140004). AIP Publishing LLC.

Herlina, T., Mardianingrum, R., Gaffar, S., & Supratman, U. (2017, February). Isoquinoline alkaloids from *Erythrinapoeppigiana* (Leguminosae) and cytotoxic activity

against breast cancer cells line MCF-7 in silico. In Journal of Physics: Conference Series (Vol. 812, No. 1, p. 012091). IOP Publishing.

Huda, A. W. N., Munira, M. A. S., Fitrya, S. D., & Salmah, M. (2009). Antioxidant activity of *Aquilaria malaccensis* (Thymelaeaceae) leaves. *Pharmacognosy research*, 1(5), 270.

I

Ibn sina, K. et Bounab triki, N. (2017). Etude ethnobotanique de la flore médicinale des milieux Steppiques de M'sila. [Mémoire de master, Université Mohamed Boudiaf - m'sila]

J

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., & Ishii, S. (2017). Environmental *Escherichia coli*: ecology and public health implications—a review. *Journal of applied microbiology*, 123(3), 570-581.

Jaradat, N., Hussen, F., & Al Ali, A. (2015). *Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne.* *Journal of Materials and Environmental Science*, 6(6), 1771–1778.

Jdaidi N, Selmi H, Aloui F, Jedidi S, Chaabane A, (2023), Évaluation des facteurs de menace et de vulnérabilité potentielles des plantes médicinales et aromatiques au nord-ouest tunisien, vol 11, No 1.

Jerbi, A., Zehri, S., Abdnnabi, R., Gharsallah, N., & Kammoun, M. (2016). Essential oil composition, free-radical-scavenging and antibacterial effect from stems of *Ephedra alata* alenda in Tunisia. *Journal of Essential Oil-Bearing Plants*, 19(6), 1503-1509.

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, 119, 579-591.

Juang, Y. P., & Liang, P. H. (2020). Biological and pharmacological effects of synthetic saponins. *Molecules*, 25(21), 4974.

K

Kebili, Z. (2016). *Contribution à l'étude de quelques activités biologiques des extraits d'Ephedra alata de la région de Ouargla* (Mémoire de master, Université Kasdi Merbah Ouargla, Algérie).

Khalaf, M. H., Amer, W. M., Shaye, N. A. A., Hassan, M. O., & Gomaa, N. H. (2024). Taxonomic Revision of Genus *Ephedra* Tourn. ex L. in Egypt with Intra-Gender Diversity in Morphometric Traits and Fatty Acid Composition. *Plants*, 13(17), 2442.

Khattabi, L., Harchaoui, L., Hacini, S., Derguine, R., & Sobhi, W. (2025). Multifaceted methodology to study the safety aspects of *Ephedra alata* Decne and its ability to prevent chemical toxicity. *Journal of Ethnopharmacology*, 120324.

Kholkhal, F. (2014). Etude Phytochimique et Activité Antioxydante des extraits des composés phénoliques de *Thymus ciliatus* ssp *coloratus* et ssp *euciliatus*. *Université Abou Bekr Belkaid, Tlemcen*.

Khongthaw, B., Chauhan, P. K., Dulta, K., Kumar, V., & Ighalo, J. O. (2023). A comparison of conventional and novel phytonutrient extraction techniques from various sources and their potential applications. *Journal of Food Measurement and Characterization*, 17(2), 1317-1342.

Kisiriko, M., Anastasiadi, M., Terry, L. A., Yasri, A., Beale, M. H., & Ward, J. L. (2021). Phenolics from medicinal and aromatic plants: Characterisation and potential as biostimulants and bioprotectants. *Molecules*, 26(21), 6343.

Kittana, N., Abu-Rass, H., Sabra, R., Manasra, L., Hanany, H., Jaradat, N. Zaid, A. N. (2017). Topical aqueous extract of *Ephedra alata* can improve wound healing in an animal model. *Chinese Journal of Traumatology*, 20(02), 108-113.

Kurin, E., Mučaji, P., & Nagy, M. (2012). In vitro antioxidant activities of three red wine polyphenols and their mixtures: An interaction study. *Molecules*, 17(12), 14336-14348.

L

Laccourreye, O., Werner, A., Giroud, J. P., Couloigner, V., Bonfils, P., & Bondon-Guitton, E. (2015). Apport, dangers et limites de l'éphédrine et de la pseudoéphédrine en tant que décongestionnant nasal. *Annales françaises d'Oto-rhino-laryngologie et de Pathologie Cervico-faciale*, 132(1), 28-31.

Lee, M. R. (2011). The history of *Ephedra* (ma-huang). *Journal of the Royal College of Physicians of Edinburgh*, 41(1), 78-84.

Lee, S. Y., Faridah-Hanum, I., & Mohamed, R. (2013). Vegetative description of three *Aquilaria* (Thymelaeaceae) saplings in Malaysia. *Pertanika J Trop Agric Sci*, 36(S), 287-94.

Levasseur-Garcia, C., Kleiber, D., and Surel, O (2013) "Utilisation de la spectroscopie infrarouge comme élément d'aide à la décision pour la gestion du risque fongique et mycotoxique." *Cahiers Agricultures*, 22(3) (1) :216-227.

Liang, J. J., Lv, T. M., Xu, Z. Y., Huang, X. X., & Song, S. J. (2022). *Aquilaria sinensis* (Lour.) Spreng: phytochemical review and chemotaxonomic values. *Biochemical Systematics and Ecology*, 102, 104413.

M

Mahmod, N. H., Johar, A. J., Hamid, M. H. A., & Ali, A. M. (2017). Comparative assessment of antioxidant activities in *Aquilaria malaccensis* leaf extracts. *Journal of Agrobiotechnology*, 8(2), 77-85.

Makhloufi, A. (2013). Etude des activités antimicrobienne et antioxydante de deux plantes médicinales poussant à l'état spontané dans la région de bechar (*Matricaria pubescens* (Desf.) et *Rosmarinus officinalis* L) et leur impact sur la conservation des dattes et du beurre cru p :50 -90.

Mansour, D. H. (2014). Evaluation chimique et activite antidermatophyte de quelques plantes medicinales d'algerie.

Martínez-Moreno, A., Toledo-Gil, R., Bautista-Ortin, A. B., Gómez-Plaza, E., Yuste, J. E., & Vallejo, F. (2024). Exploring the impact of extended maceration on the volatile compounds and sensory profile of Monastrell red wine. *Fermentation*, 10(7), 343.

Masyita, A., Sari, R. M., Astuti, A. D., Yasir, B., Rumata, N. R., Emran, T. B., ... & Simal-Gandara, J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food chemistry: X*, 13, 100217.

Merzougui, I., and Tadj, H. (2015). Etude de l'effet antibactérien et antioxydant d'*Ammoides verticillata* De la région de Tlemcen p :120-200

Mighri, H., Akrouit, A., Bennour, N., Eljeni, H., Zammouri, T., & Neffati, M. (2019). LC/MS method development for the determination of the phenolic compounds of Tunisian *Ephedra alata* hydro-methanolic extract and its fractions and evaluation of their antioxidant activities. *South African Journal of Botany*, 124, 102-110.

Miranda, J. J. M. (2021). Medicinal plants and their traditional uses in different locations. In *Phytomedicine* (pp. 207-223). Academic Press.

Mohamed R, Jong PL, Zali MS (2010) Fungal diversity in wounded stems of *Aquilaria malaccensis*. *Fungal Diversity* 43: 67-74

Mohammed, H. A., Said, R., Abbas, M. M., Al-Najjar, B. O., Abd-Elmoniem, E., Khan, R. A., ... & Ismail, A. (2024). Phytochemical, biological, and computational investigations of *Ephedra alata* Decne. growing in salinity conditions of Arabian Peninsula. *Scientific Reports*, 14(1), 21987.

Mustaqim, W. A. (2021). *Aquilaria malaccensis* Lam. Thymelaeaceae. In *Ethnobotany of the mountain regions of Southeast Asia* (pp. 109-126). Cham: Springer International Publishing.

N

Naef R (2011) The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: a review. *Flavour and Fragrance Journal* 26(2): 73–87, doi: 10.1002/ffj.2034

Naziz PS, Das R, Sen S (2019) The scent of stress: evidence from the unique fragrance of agarwood. *Frontiers in Plant Science* 10:840, doi: 10.3389/fpls.2019.00840

Nurtamin, T., Rahim, A. H., & Yusuf, M. (2018). *Evaluation of anti-inflammatory activity by human red blood cell membrane stabilization method*. *Asian Journal of Pharmaceutical and Clinical Research*, 11(5), 176–179.

O

Ouartal, I., Ferhati, N., & Chebah, A. (2024). *Analyse physico-chimique et évaluation des activités biologiques des deux plantes médicinales (Ephedra alata et Rubus fruticosus)*. Mémoire de Master, Université Abbès Laghrour-Khenchela, Faculté des Sciences de la Nature et de la Vie

Ouasti, M., Bussmann, R. W., & Elachouri, M. (2023). *Ephedra alata* Decne, *Ephedra altissima* Desf., *Ephedra fragilis* Desf., *Ephedra major* Host, *Ephedra alata* subsp. *alenda* (Stapf) Trab, *Ephedra transitoria* Riedl, *Ephedra foliata* Boiss. ex CA Mey., *Ephedra foeminea* Forssk., and *Ephedra aphylla* Forssk.: *Ephedraceae*. In *Ethnobotany of Northern Africa and Levant* (pp. 1-11). Cham: Springer International Publishing.

P

Palazon, J., & Alcalde, M. A. (2025). Secondary Metabolites in Plants. *Plants*, 14(14), 2146.

Pandiselvam, R., Dinesha, B., & Kothakota, A. (2023). Novel Processing Methods for Plant-Based Health Foods.

Percival SL. Microbiology of waterborne diseases. Ed. Elsevier Academic Press, Amsterdam; Boston, 2004, p. 480.

Perez, C., Pauli, M., & Bazerque, P. (1990). *An antibiotic assay by the agar well diffusion method*. *Acta Biologica et Medicinæ Experimentalis*, 15, 113–115.

Q

Qadir, S., Bashir, S., & John, R. (2020). Saffron—immunity system. In *Saffron* (pp. 177-192). Academic Press.

R

- Rahman, H., Eswaraiah, M. C., & Dutta, A. M. (2016). *Anti-arthritic activity of leaves and oil of Aquilaria agallocha*. *Haya: The Saudi Journal of Life Sciences*, 1(1), 34–43
- Rasheed, I., & Gruber, R. (2025). Crude Plant Extracts and Their Anti-Inflammatory Potential in Oral Inflammatory Cell Models: A Systematic Review of In Vitro Studies. *International Journal of Molecular Sciences*, 26(23), 11253.
- Remya V., Alex M.J., Thomas A. *Ayurveda in The New Millennium*. CRC Press; Boca Raton, FL, USA: 2020. *Quality Control of Ayurvedic Medicines*; pp. 71–100.
- Ridwanto, Trizaldi, A., Rani, Z., Daulay, A. S., Nasution, H. M., & Miswanda, D. (2022). Antioxidant activity test of methanol extract of Gaharu (*Aquilaria malaccensis* Lam.) bark with DPPH (1,1 diphenyl-2-picrylhydrazyl) method. *International Journal of Health and Pharmaceutical*, 232.

S

- Sahi, L. (2016). La dynamique des plantes aromatiques et médicinales en Algérie [Troisième partie]. *Le marché des plantes aromatiques et médicinales : analyse des tendances du marché mondial et des stratégies économiques en Albanie et en Algérie*, (73), 101-140.
- Salah, B. A., Aid, S., & Djenidi, D. (2022). Évaluation de l'activité antioxydante des extraits de la plante *Ephedra alata* alenda par le test de DPPH (Mémoire de master). Université Mohamed Boudiaf de M'sila, Faculté des sciences.
- Sarma, D. R., Sarmah, J., Gupta, A., & Mishra, R. K. (2015). *Aquilaria malaccensis*, an ayurvedic medicinal herb found in Assam—its therapeutical and pharmacological aspect. *Indian J Trop Biodiv*, 23(2), 218-222.
- Sarveswaran, R., Jayasuriya, W. J. A. B. N., Suresh, T. S., & Thayumanavan, S. (2017). *In vitro assays to evaluate anti-inflammatory activity of plant extracts*. *Journal of Ethnopharmacology*, 196, 150–159.
- Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757–781. <https://doi.org/10.1016/j.jff.2015.01.047>
- Shahidi, F., Zhong, Y. (2011). Revisiting the Polar Paradox Theory: A Critical Overview. *Journal of Agricultural and Food Chemistry*, 59, 3499–3504.
- Shivanand, P., Arbie, N. F., Krishnamoorthy, S., & Ahmad, N. (2022). Agarwood—the fragrant molecules of a wounded tree. *Molecules*, 27(11), 3386.

- Siddiqui, A. A., & Ali, M. (1997). *Practical pharmaceutical chemistry*. CBS Publishers & Distributors.
- Siddiqui, S., Verma, A., Rather, A. A., Jabeen, F., & Meghvansi, M. K. (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. *Advances in biological research*, 3(5-6), 188-195.
- Sioud, F., Mangelinckx, S., Lahmer, A., Bonneure, E., & Chaabene, F. (2021). Alkaloids isolated from ephedra alata: characterization and protective effects against cisplatin-induced liver and kidney injuries in mice. *Biomedical Journal of Scientific & Technical Research*, 36(3), 28591-28602.
- Song Y T, Ren J, Ling K, Guangli Y, Chang L, Ying H, Soleil H, Xi W, (2023). Ephedrae Herba : Un examen de sa phytochimie, de sa pharmacologie, de son application clinique et de sa toxicité des alcaloïdes, *Molécules* 2023, 28 (2), 663.
- Soua, L., Koubaa, M., Barba, F. J., Fakhfakh, J., Ghamgui, H. K., & Chaabouni, S. E. (2020). Water-soluble polysaccharides from Ephedra alata stems: structural characterization, functional properties, and antioxidant activity. *Molecules*, 25(9), 2210.
- Soumaya, B., Yosra, E., Rim, B. M., Sarra, D., Sawsen, S., Sarra, B., ... & Wided, M. K. (2020). Preliminary phytochemical analysis, antioxidant, anti-inflammatory and anticancer activities of two Tunisian Ephedra species: Ephedra alata and Ephedra fragilis. *South African Journal of Botany*, 135, 421-428.
- Sumantri, I. B., Saragih, A., Batubara, R., Wahyuni, H. S., Laila, L., Yuliasmi, S., ... & Mustanti, L. F. (2025). The Effect of Solvent Polarity and Extraction Time on the Antioxidant of Aquilaria malaccensis Leaves. *Majalah Obat Tradisional*, 31(1), 140-150.
- Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, 28(4), 1845.

T

- Thilakarathna, R. C. N., Siow, L. F., Tang, T. K., Chan, E. S., & Lee, Y. Y. (2023). Physicochemical and antioxidative properties of ultrasound-assisted extraction of mahua (Madhuca longifolia) seed oil in comparison with conventional Soxhlet and mechanical extractions. *Ultrasonics sonochemistry*, 92, 106280.
- Tiss, M., Souiy, Z., Boujbiha, M., Achour, L., & Hamden, K. (2020). Ephedra alata extracts exhibit anti-obesity, anti-hyperlipidaemic, anti-hyperglycemia, anti-antipyretic and analgesic effects through the inhibition of lipase, α -amylase and inflammation.
- Trabelsi N., Megdiche W., Ksouri R., Falleh H., Oueslati S., Bourgou S., Hajlaoui H., Abdelly C. (2010). Solvent effects on phenolic contents and biological activities of the halophyte Limoniastrum monopetalum leaves. *Food Science and Technology*, 43(4): 632-639.

U

USDA, U.S. Department of Agriculture. (2025). Animal and Plant Health Inspection Service. <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomydetail?id=410926>

USFS, United State Forest Service. (2025), Plant Parts Used for Medicinal Purposes <https://www.fs.usda.gov/wildflowers/ethnobotany/medicinal/parts.shtml>

W

Wang, Y., Hussain, M., Jiang, Z., Wang, Z., Gao, J., Ye, F., ... & Li, H. (2021). Aquilaria species (Thymelaeaceae) distribution, volatile and non-volatile phytochemicals, pharmacological uses, agarwood grading system, and induction methods. *Molecules*, 26(24), 7708.

Widad, T., Nadjwa, S., Fatna. G. (2023). Étude phytochimique et activités biologiques de la plante Ephedra alata alenda dela région Messaad – Djelfa. Memoire master en Sciences Alimentaire

Y

Yang, Q., Zhang, Q., Zhang, W., Liu, K., Wang, Z., Yang, C., ... & Fan, Z. (2026). Sesquiterpenoids in Agarwood: Composition, biosynthesis, and metabolic regulation. *Industrial Crops and Products*, 240, 122628.

Yinyang, J., Mpondo, E. M., Tchatat, M., Ndjib, R. C., Ottou, P. M., & Dibong, S. D. (2014). Les plantes à alcaloïdes utilisées par les populations de la ville de Douala (Cameroun). *Journal of Applied Biosciences*, 78, 6600-6619.

Z

Zhao, Y., Wu, Y., & Wang, M. (2015). Bioactive substances of plant origin 30. *Handbook of food chemistry*, 967, 967-1008.

Ziani, B. E., Heleno, S. A., Bachari, K., Dias, M. I., Alves, M. J., Barros, L., & Ferreira, I. C. (2019). Phenolic compounds characterization by LC-DAD-ESI/MSn and bioactive properties of Thymus algeriensis Boiss. & Reut. and Ephedra alata Decne. *Food Research International*, 116, 312-319.

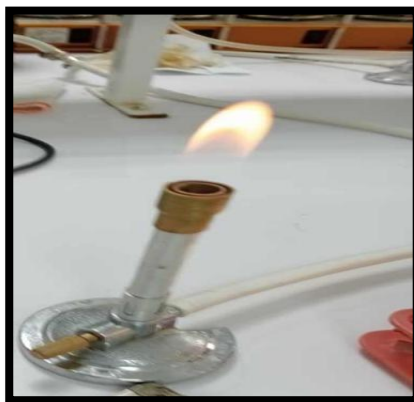
Appendices

Appendices:

Appendices 01: Equipment



Steamer



Bunsen burner



Water bath



Magnetic stirrer

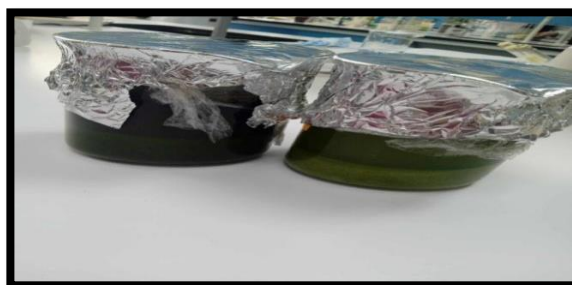
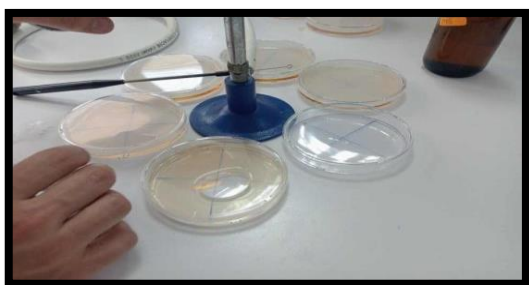
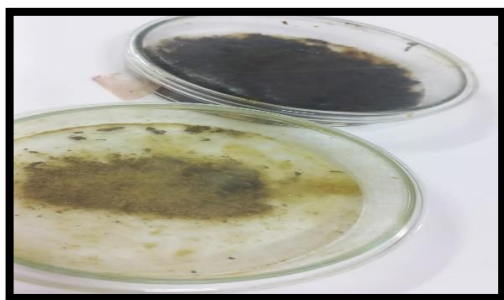


Smart scale

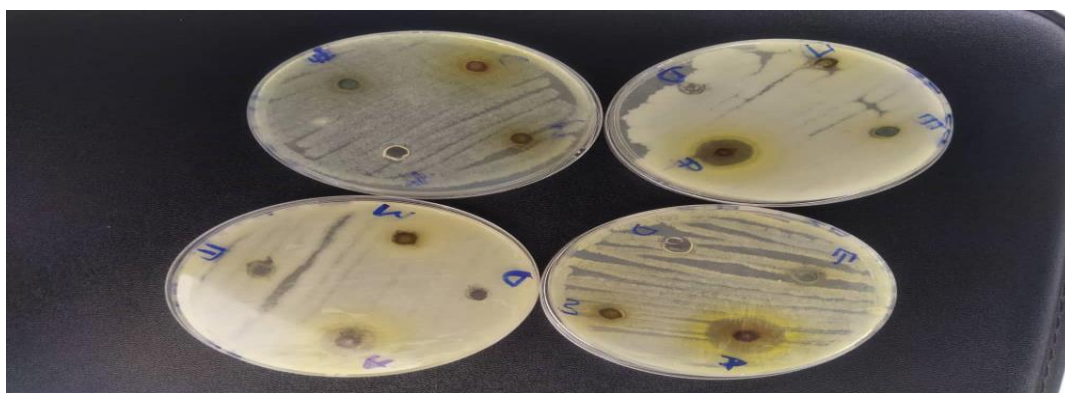
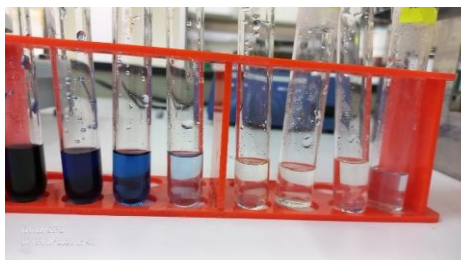


Spectrophotometer

Appendices 02: Preparations



Appendices 03: the different results



Results of antibacterial activity