DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

Ministry of Higher Education and Scientific Research

Yahia Fares University of Medea

Faculty of technology

Department of chemical engineering

Graduation Project Report Submitted for Master of chemical engineering

And obtaining the Startup-Brevet diploma within the framework of Ministerial Decree 1275

Extraction and synergistic study of a mixture of some Algerian medicinal plants for therapeutic use

---

Prepared by :

. *HAROUN Maroua*

. *ATMANIA Ismahane*

Supervised by :

Dr. LETTREUCH Hichem

Academic Year : 2024/2025

Thanks

We thank Almighty ﷲ for giving us the health and the will to begin

and Complete this dissertation.

First of all, this work would not be as rich and could not have

happened without the help and supervision of Dr.

LETTREUCH Hichem we thank for the quality of his exceptional

supervision for his patience, his rigor and his availability during our

preparation of this project.

We would like to thank Dr. DRIOUCHE Asma for her constant assistance, support, and guidance, which was extremely helpful.

We thank all the university staff, especially the laboratory engineers, who helped us until the last minute.

Special thanks to Dr. HAROUN Omar, who supported us throughout this long journey.

Finally, we thank our parents, family, and friends for their support and trust, without them we would not have been able to complete this work.

إهداء

الحمد لله أولاً وآخرًا، ظاهرًا وباطنًا، الذي بنعمته تتمّ الصالحات، وبتوفيقه تذلل الصعوبات، وتتحقق الإنجازات

بفضل الله, بعد رحلة علمية حافلة امتدت على مدار سنوات من السعي والمثابرة، أصل اليوم إلى نهاية مشواري الدراسي، الذي كان مليئًا بالتحديات، لكنه أيضًا زاخرًا بالتجارب والدروس التي ستبقى محفورة في الذاكرة.

وقد كان لهذا المشوار أثر بالغ في بناء شخصيتي، وتنمية قدراتي، وفتح آفاق جديدة أمامي.

وفي هذه اللحظة، لا يسعني إلا أن أتوجه بأسمى عبارات الشكر والامتنان لكل من ساندني ووقف إلى جانبي خلال هذه المسيرة:

شكر خاص وعميق لوالديّ العزيزين،

إلى والدتي الحبيبة، رمز الحنان والصبر والدعاء، التي كانت دومًا النور الذي أنار طريقي.

وإلى والدي الغالي، الذي كان السند والداعم الأول، بتوجيهه وتشجيعه وثقته الدائمة بي.

كما أتقدم بخالص الشكر والامتنان لأخي العزيز **عمر**، الذي لم يدّخر جهدًا في دعمي، وكان لي سندًا في الأوقات الصعبة، ورفيقًا في اللحظات الحاسمة.

ولزوجته الطيبة **سلمى**، التي كانت دومًا قريبة من القلب، بحبها وطيبتها وتقديرها.

إلى أختيَّ الحبيبتين **فاطمة** و**مريم**، اللتين كانتا لي أكثر من مجرد أخوات؛ كانتا صديقتين ومصدرًا للدعم والتشجيع، فشكري لكما لا يكفيه كلام، ومحبتكما في قلبي لا توصف.

كما لا أنسى صغيرَي العائلة، **هبة** و**محمد**، من يزرعان البهجة في القلب، ويجددان الأمل بابتسامتهما البريئة.

وإلى أصدقائي الأوفياء، من شاركوني هذه الرحلة بكل تفاصيلها، ووقفوا بجانبي بمحبة وصدق.

وإلى أساتذتي الكرام، الذين كان لتوجيههم وعلمهم أثر بالغ ف ما وصلت إليه، كل الشكر والتقدير.

وإن كانت هذه نهاية لمسيرة دراسية، فإنها بداية لطريق جديد من العمل والطموح والعطاء.

إهداء

إلى من كان دعاؤها سندي الخفي، وصبرها زادي في كل مراحل الطريق،

إلى من علّمتني أن الحب تضحية، وأن العطاء بلا شرط هو أسمى معاني الأمومة،

.إلى أمي الغالية... يا نبع الحنان ،ويا حضن الطمأنينة، أهديكِ ثمرة هذا الإنجاز، عرفانًا لا يكفيه الكلام

وإلى أبي، الرجل الذي جعل من صمته حكمة، ومن تعبه أمانًا،

إليك يا من زرعت في داخلي معاني المسؤولية،

يا من كنت الحصن إذا مالت الأيام، والنور حين يعتم الدرب،

.أهديك هذا التخرّج، تقديرًا لتضحياتك، وامتنانًا لا يعرف حدًّا

إلى أختي الحبيبة، رفيقة قلبي، وصديقة روحي،

يا من كنتِ لي دفء اللحظة، وملاذ الحزن، وشريكة الفرح،

.شكرًا لحنانكِ الذي لا يُشبهه شيء، ولسندكِ الصامت الذي طالما خفّف عني كثيرًا

وإلى أخويَّ العزيزين،

يا من كنتما لي القوة حين ضعفت، والسند حين تراجعت،

لكما كل الشكر على وجودكما الثابت، وعلى دعمكما الذي لم يخذلني يومًا،

.بكما شعرت دومًا أن لي ظهرًا لا ينكسر

إلى كل من كان جزءًا من هذه الرحلة،

وإلى كل من ترك أثرًا جميلاً في قلبي،

.أهديكم هذه الصفحات التي خُطّت بالتعب، وغُذّيت بالأمل، ونضجت بالحب

بكم بدأ الحلم، وبكم اكتمل واقعه الجميل.

الملخص

هذا العمل هو جزء من البحث عن النباتات الطبية ويهدف إلى استخراج وتثمين الزيوت الأساسية التي تم الحصول عليها من ثلاثة نباتات: الزعتر وإكليل الجبل والشمر ، وكذلك دراسة تأثير مزج هذه الزيوت بهدف تطوير منتج شبه صيدلاني على شكل كريم مرطب مضاد للبكتيريا

تم تحديد الخصائص الفيزيائية والكيميائية بالإضافة إلى التحليل بواسطة كروماتوغرافيا الغاز الموصولة بطيف الكتلة جعل من الممكن تحديد التركيب الكيميائي لكل زيت ، على وجه الخصوص: استراغول) 28٪(في زيت الشمر ، الثيمول 86٪(في زيت الزعتر والكافور) 78٪(في زيت إكليل الجبل.

وكشفت نتائج النشاط المضاد للأكسدة من خلال طريقة الجذور الحرة باستعمال 2،2 ثنائي فنيل -1- بكريل هيدرازيل نتائج واعدة والتي تختلف وفقا لكل زيت ، تم الحصول على قيم نصفية للتراكيز تقدر بـ 5.2 مغ/مل لزيت الزعتر و 3.3 مغ/مل لزيت إكليل الجبل. أظهرت الاختبارات المضادة للميكروبات للزيوت الأساسية ضد السلالات البكتيرية والفطرية أن هذه الزيوت توفر قوة جيدة مضادة للميكروبات ، وقد تم تحسين هذا النشاط باستخدام مزيج من الزيوت الأساسية.

بعد التركيب التجريبي للكريم القائم على هذه الزيوت ، خضع المنتج لاختبارات بيولوجية ، بما في ذلك تقييم النشاط المضاد للميكروبات وكذلك قياس مستوى الحموضة ، من أجل ضمان توافقه مع المعايير الجلدية والصيدلانية

الكلمات المفتاحية: زيت عطري، زعتر، شمر، إكليل الجبل ،كروماتوغرافيا الغاز الموصولة بطيف الكتلة ;مضاد للأكسدة، نشاط مضاد للبكتيريا

Abstract

This work is part of the research on medicinal plants and aims at the extraction and valorization of essential oils obtained from three plants: thyme, rosemary and fennel, as well as the study of the synergistic effect of mixture obtained from them and subsequently the development of a Para pharmaceutical product in the form of antibacterial moisturizing cream.

The physico-chemical indices were determined and analysis by gas chromatography coupled with mass spectroscopy made it possible to identify the chemical composition of each oil, in particular: estragole (28%) in fennel oil, thymol (86%) in thyme oil and camphor (78%) in rosemary oil.

The results of the antioxidant activity by the method of free radical trap by DPPH revealed promising results which vary according to the HE, EC50 values were obtained of 2.5mg/ml for thyme oil and 3.3mg/ml for rosemary oil. Antimicrobial tests of essential oils against bacterial and fungal strains have shown that these oils provide a good antimicrobial power, this activity has been improved by using a mixture of essential oils.

After the experimental formulation of the cream based on these oils, the product was subjected to biological tests, including an evaluation of the antimicrobial activity as well as the measurement of the acidity level, in order to guarantee its compatibility with dermatological and pharmaceutical standards.

Keywords: Essential oil;Thyme;Fennel;Rosemary;CG-MS:Antioxidant, antibacterial activity

Résumé

Ce travail s'inscrit dans le cadre de la recherche sur les plantes médicinales et a pour objectif l'extraction et la valorisation des huiles essentielles obtenues à partir de trois plantes : le thym, le romarin et le fenouil, ainsi que l'étude de l'effet synergique de mélange obtenu à partir d'eux et par la suite le développement d'un produit parapharmaceutique sous forme de crème hydratante antibactérienne.

Les indices physico-chimiques ont été déterminé et l'analyse par chromatographie en phase gazeuse couplée à la spectroscopie de masse a permis d'identifier la composition chimique de chaque huile, notamment : l'estragole (28%) dans l'huile de fenouil, le thymol (86%) dans l'huile de thym et le camphre (78%) dans l'huile de romarin.

Les résultats de l'activité antioxydante par la méthode de piège de radicaux libre par DPPH ont révélé des résultats prometteuses qui varie selon l'HE, des valeurs de EC_{50} ont été obtenus de 2,5mg/ml pour l'huile de thym et de 3,3mg/ml pour celle de romarin. Les tests antimicrobiens des huiles essentielles contres des souches bactériennes et fongiques ont montré que ces huiles fournissent un bon pouvoir antimicrobien, cette activité a été améliorée on utilisant un mélange des huiles essentielles.

Après la formulation expérimentale de la crème à base de ces huiles, le produit a été soumis à des tests biologiques, incluant une évaluation de l'activité antimicrobienne ainsi que la mesure du niveau d'acidité, afin de garantir sa compatibilité avec les normes dermatologiques et pharmaceutiques.

Mots clés : Huile essentielle; Thym; Fenouil; Romarin; CG-MS : Activité antioxydante et antibactérienne

Table of Contents

Dedication

Summary

The keywords.

The table of contents.

List of abbreviations

List of figures

List of tables

General introduction …….[2]

Chapter I: introduction to medicinal plants and bioactive compounds

I.History and Use……...….[5]

I-1Historisal use ……...…[5]

I-2 Tradutional medicine ……...…..[5]

II- Definition of medicinal plants…….……...[5]

III -Types of Compounds …….……[5].

IV Challenges and Future Directions……..[5]

V - the plants we those for our study…….……[6]

VI- THYME…….……[8]

VI-1-1 . Role of thyme in Traditional and Modern Medicine……..[8]

VI- 1-2. The difference between traditional and modern medicine……...[9]

VI- 1-2-1. Traditional Medicine……..[9]

VI- 1-2-2. Modern Medicine……...…[9]

VI- 1-3. Why thyme?.....[9]

VII- [VI- 1- 4. The Journey Begins - Selecting thyme……[9]

VI-1-4-1 Historical Uses……..……..[9]

VI-1-4-2. Cultural Significance…….....[10]

VI-1-5. Medicinal Properties and Rationale for Selection…….....[10]

VI-1-5-1. Medicinal Properties……..…[10]

VI-1-5-2 Why Selected……...[10]

VI-1-6. Thyme bioactive compound…….[11]

VI-1-7. Potential Therapeutic Applications ……[11]

VII-2- FENNEL……...…….[11]

VII-2-1.Roleof fennel in Traditional and Modern Medicine……[11]

VI-2-1.The difference between traditional and modern medicine……...….[12]

VI-2-1-1Traditional Medicine……...…[12]

VII-2-1-2Modern Medicine……..….[12]

VI-2-1.Why fennel seeds……....[13]

VI-2-1.The Journey Begins - Selecting fennel……..……...…[13]

VII-2-1-1 Historical Uses and Cultural Significance……..……[13]

VII-2-2. Medicinal Properties and Rationale for Selection……..[13]

VII-2-2-1 Medicinal Properties……..……..[13]

VII-2-2-2 why selected……...…[14]

VII-2-3. Fennel bioactive compounds……[14]

VII-2-4. Potential Therapeutic Applications……..…[14]

VII-3- ROSEMARY……[14]..

VI-3 -1. Role of rosemary in Traditional and Modern Medicine……[15]

VI-3 -2. The difference between traditional and modern medicine……[15]

VI-3 -2-1. Traditional Medicine…….[15]

VI-3 -2-2. Modern Medicine…….…[15]

VI-3 -3. Why rosemary?.....[15]

IV-3-4. The Journey Begins - Selecting rosemary……[16]

IV-3-4-1. Historical Uses and Cultural Significance……...[16]

IV-3-5. Medicinal Properties and Rationale for Selection…….……[17].

IV-3-5-1. Medicinal Properties…….[17]

IV-3-5-2. Why Selected…….[17]

IV-3-6. Plants bioactive compounds……..[17]

IV-3-Potential Therapeutic Applications…….[17]

VII.. Comparison of Applications…….[19]

VIII. The Search for Bioactive Compound…….……[20]

VIII -1. Importance of discovering new compounds in the modern world of health and wellness…….…….[21]

VIII -2. Purpose of the research……[22]

VIII -3. Summary Table of Medicinal Properties and Study Selection Reasons……..[22]

VIII -4. Vernacular names of Foeniculum vulgare……..[23]

VIII -5. Results collected about Rosmarinus officinalis uses……..[23]

IX Plant Collection and Preparation (this is a bit mixed — adjust number if needed)[25]

IX -1. Gathering the Plants…….……[25]

IX -2. The Process of Extraction……..……[25]

IX -2-1 . Extraction by Clevenger Type Assembly……[25]

IX -2-2 Summary of the Key Stages of the Clevenger Assembly……[26]

IX -2-3 Conclusion……[26]

Chapter 2: Extraction Methods and Materials with Results and Discussion……[28]

I- Extraction Methods and Solvent……[28]

I.1 Detailed Explanation of Extraction Methods……..……[29]

I-1.1 Maceration Extraction……[29]

I-1-2. Soxhlet Extraction……..……[29]

I- 1-3. Ultrasonic-Assisted Extraction (UAE)……[29]

I-1-4. Microwave-Assisted Extraction (MAE)……[30]

I-1-5. Summary Table of Extraction Methods……[30]

I -2. Factors Influencing the Choice of Extraction Techniques……[31]

II-2-1. Nature of the Target Compounds……... [31]

II-2-2. Plant Material Characteristics…… [31]

II-2-3. Extraction Efficiency and Selectivity……..……[32]

II-2-4. Solvent Compatibility and Safety……[32]

II-2-5. Intended Use and Cost Considerations……..……[32]

II-2-6. Summary of Why Specific Techniques Are Chosen……..……[32]

1I -Materials and Methods…….[33]

1I-1 Materials and Equipment……..……[34]

1I-1.1 Plant Materials……[34]

1I-1-2. Chemicals and Reagents……..……[35]

1I-1-3. Apparatus……[35]

1I-1-3-1. Refractometer…… [36]

1I-1-3-2 UV-Vis Spectrophotometer……..……[37]

II-2. Biological Materials……[38]

II-2-1. Experimental Protocols……[39]

II-2-1-1. Essential Oil Extraction Protocol…….……[39]

II-2-1-2 Physicochemical Analysis of Essential Oils……..…[40]

II-2.2 Physical Analysis of Essential Oils…… [40]

II-2-2-1. Refractive Index Measurement……...…[40]

II-2-2.2 Density Measurement……[41]

II-2-2-3. Ethanol Solubility Test……[42]

II -3. Chemical Characteristics of Essential Oils……[42]

II 3-1. Acid Value (IA)……..……[42]

II -3-2. Saponification Value (IS)……[43]

II -3.3 Ester Value (IE)……[44]

II -4GC-MS Analysis……[44]

II-5 DPPH Antioxidant Activity Assay……[45]

II-5-1 Introduction…….[45]

II-5-2 Procedure…… [45]

II-5 -2-1. Preparation of DPPH Solution……..…[45]

II-5 - 2-2. Preparation of Working Solutions……[45]

II-5 -2-3. Reaction Conditions…… [46]

II-5 -2-4. Measurement and Calculation……...…….[46]

II-6 Antibacterial Activity Assay of Essential Oils…….……...[46]

II-6 -1. Introduction…….....[46]

. II-6 -2 Procedure……..…….[46]

II-7 Formulation……....[47]

II-7-1 Introduction…….[47]

II-7-2 Materials Used in the Formulation……[47]

III - Results and Discussion…….……...[49]

III-1- Yield of extraction…… [49]

III -2 Interpretation…… [49]

III-2-1 Organoleptic characteristics…… .[50]

III -2-2 Interpretation of Observed Physical and Sensory Properties of Essential Oils[51]

III-3 Physical Analysis of Essential Oils…….[52]

III-3-1 Refractive index……[52]

III-3-2 Comparative Analysis of Essential Oil Quality…….[52]

III-3-3 Miscibility test in ethanol……....[53]

III -4- Chemical Characteristics of Essential Oils…….[54]

III -5 Résultats des analyses GC/MS…….……..…... [55]

III -5 -1Chemical composition of thyme essential oil…….……[55]

III -5 -1-1 Interpretation of the Chemical Composition of the Essential Oil……...[56]

III -5 -2 Chemical composition of fennel essential oil…….……[57]

III -5 -2-1 Interpretation of the Chemical Composition of Fennel Essential Oil……[57]

III -5 -3 Chemical composition of Rosmarinus officinalis essential oil……[58]

III -5 -3-1 Interpretation of the Chemical Composition of Rosemary Essential Oil..[59]

III -6- Comparative Composition Analysis of Essential Oils: This Study vs. Literature…….....[60]

III -7- DPPH Antioxidant Activity Assay…….…… [60]

III-8-Antibacterial Activity Table…….[63]

III-9 Formulation properties…….……...[63]

III-10 Stability Study for the Cream…….……..[63]

Chapter : Guide startup……..…[64]

Information card……..…[65]

1- Management team ……..……..….[65]

2- Project Team…….……..[65]

Introduction……[66]

first axis : project presentation…….……...[66]

1. **The project idea (proposed solution)……..[66]**
2. **The proposed values:……[66]**
3. **Work team…….....[67]**
4. **Goals of the project :……...….[67]**
5. **Project implementation schedule……....[67]**

Second axis: Innovative aspects…….[68]

1. Nature of innovations……....[68]

Third axis :Strategic market analyse …….……[68]

1. **The market segment……[69]**
2. **Measuring the intensity of competition……[69]**
3. **The marketing strategy…….[69].**
4. **Fourth axis……..……[70]**

Production and organization plan……[70]

1.The Production Process……..……[70]

2. Supply……..….. [70]

3. Labor……[70]

4. The Main Partners……..[70].

Fifth axis:Financial plan…….[71]

1. **Costs and charges……[71]**
2. **Turnover…….…….[71]**
3. **Income Statements expected……..[72]**
4. **The Cash Flow**

Plan……[72]

Sixth axis: Experimental

prototype……...[72]

GENERAL CONCLUSION……...…….[73]

The bibliography…….…….

LIST OF ABBREVIATIONS

BHT :	Butylhydroxytoluene
DPPH :	2,2-Diphenyl-1-picrylhydrazyl
DMSO :	Dimethyl sulfoxide
EO :	Essential oil
GC/MS :	Gas Chromatography/Mass Spectrometry
HCl :	Hydrochloric acid
IA :	Acid Index
IE:	Ester Index
IS:	Saponification Index
KOH :	Potassium hydroxide
MAE :	Microwave-Assisted Extraction
M :	Mass
Na_2SO_4:	Sodium sulfate
T:	Temperature
UAE:	Ultrasonic-Assisted Extraction
µL :	Microliter

List of images

image 01: Dioscorides's 1st century De materia medica, seen here in a c. 1334 copy in Arabic, describes some 1000 drug recipes based on over 600 plants…….[6]

Image 2: Plants part use and their percentage……...….[6]

Image 03: pictures of thyme plant……...……..[8]

Image 04: pictures of fennel speed……[12]

Image05: pictures of rose mary plant……[16]

Image 06: pictures of chemical structures of the main components of thyme, fennel seeds and rosemary oils respectively…….....……[19]

Image07: refractometer……..……...[37]

Image08: spectrophotometer…….[37]

Image09: extract oil in the laboratory……..……[39]

Image10: pycnometer with fennel oil……..…….[41]

Image11: The quantities of oil extracted in a single process from plants…….....[49]

Image12: Chromatogram of thyme essential oil……..…….[55]

Image13: Chromatogram of the fennel essential oil……..……..[57]

Image14 : Chromatogram of the rosemary essential oil……..…….[58]

Image15 :represent DPPH Antioxidant Activity Assay of BHT……..……[61]

Image16: represent DPPH Antioxidant Activity Assay of fennel oil…...……[61]

Image 17: represent DPPH Antioxidant Activity Assay of thyme oil……...……[62]

Image18: represent DPPH Antioxidant Activity Assay of rosemary oil……[62]

Image19 : Nature of innovations…….…...[68]

Image20: The Production Process of the cream…….[70]

Image 21: the prototype of the cream MI……...[72]

List of tables

Table 01: Medicinal plant species were used in treating different types of ailment……..[7]

Table 02 : Comparison of Applications……..…[19]

Table 03: Summary Table of Medicinal Properties and Study Selection Reasons……[22]

Table04 : Vernacular names of Foeniculum vulgare.……[23]

Table 05 : Results collected about Rosmarinus officinalis uses. ……[24]

Table 06: Table of Extraction Methods……[31]

Table 07: Summary of Why Specific Techniques Are Chosen……[33]

Table 08 : The medicinal plants used……[35]

Tabel 09 : chemical and Reagents Used……...[35]

Tabel 10: Apparatus Used……[36]

Table 11: Instruments and Equipment Used……[38]

Table 12: Biological materials and Equipment……[39]

Table 13:materials and equipment for GC-MS protocol……..[44]

Table 14: represent different volumes of the stock solution (EO, BHT)……..[46]

Table 15: represent different volumes of the stock solution (EO, ethanol, UI, DPPH)……....[46]

Table 16: Material and their Function in the Formulation……[48].

Table 17: yield of extraction……..[49]

Table 18: represent the Organoleptic characteristics for the three oils……..[51]

Table 19: represent the refractive index……[52]

Table 20: represent the results of oil density…….[53]

Table 21: represent the results of Miscibility test in ethanol…….[54]

Table 22: the results of Chemical Characteristics of Essential Oils……..[54]

Table23: Chemical composition of the thyme essential oil……...[56]

Table 24 : Chemical composition of the fennel essential oil……[57].

Table 25: Chemical composition of the rosemary essential oil…….……[59]

Table26 : Table of value EC……[62]

Table 27 : table of formulation properties……..……[63]

GENERAL INTRODUCTION

Medicinal Aromatic Plants play a valuable and important role in economic, social, cultural and ecological aspects of local communities the world over. They can be defined as botanicals that provide people with medicines to prevent disease, maintain health or cure ailments. In one form or another, they benefit virtually everyone on Earth through nutrition, toiletry, bodily care, incense and ritual healing.

Medicinal Aromatic Plants are an integral component of many local trade supply chains. They are part of traditional medicine systems found in numerous local communities around the world, and comprise a wide range of species which have different sources, characteristics and uses. Since time immemorial these products have made a significant contribution to human health and well-being as well as contributing to farm household income generation through trade. 'Traditional medicine' is defined by the World Health Organization as the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.[1]

In this research work, we choose three plants (thyme, rosemary and fennel), which are distinguished by their many pharmaceutical properties, including antioxidant, and antibacterial. To extract these essential oils from the three plants, we used a Clevenger device to ensure good quality of the obtained oil.

Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound [2], this is what we followed during our research.

Our main objective of this study is to formulate a natural cream based on these extracted oils The study aims to evaluate the physical and functional properties of the extracted oils, such as their aroma, consistency, and potential antimicrobial or antioxidant effects. Additionally, the cream will be assessed for its stability, texture, and suitability for topical use. This research seeks to explore the potential of using plant-based ingredients in skincare products, promoting natural alternatives to synthetic compounds and contributing to the development of eco-friendly and health-conscious cosmetic formulations.

In addition to the scientific study of our selected medicinal and aromatic plants and the development of a natural cosmetic product, this project also aims to establish the basis for a

sustainable and innovative startup. Our vision is to bridge the gap between academic research and real-world application by transforming our laboratory results into a practical, high-quality product that meets consumer needs and market demands. The startup will focus on the local valorization of natural resources, promoting the use of renewable plant materials and eco-friendly extraction methods, such as the Clevenger apparatus, to ensure both effectiveness and sustainability.

This entrepreneurial aspect includes careful planning for the production process, from raw material sourcing and quality control to packaging, marketing, and distribution. We also aim to create partnerships with local suppliers and distributors to support the regional economy and encourage responsible production practices. By combining scientific innovation, environmental responsibility, and a strong business model, our startup aspires to create new job opportunities, stimulate local industry, and contribute to the growing market for natural and organic cosmetic products

Finally, through this project, we hope to demonstrate how research can be turned into a successful business venture, inspiring other students and young entrepreneurs to invest in ideas that add value to our natural heritage and respond to modern consumer expectations.

Our memory is devided on three chapters:

chapter 01 :Medicinal plants have long been valued for their rich content of bioactive compounds, which play a vital role in traditional and modern healthcare. These natural substances, including essential oils, alkaloids, and phenolics, are known for their therapeutic properties such as antimicrobial, antioxidant, and anti-inflammatory effects. Understanding the nature and function of these bioactive compounds helps in developing safe, effective products for pharmaceutical, cosmetic, and wellness applications.

chapter 02 : This chapter describes the materials used and the methods applied for extracting essential oils from selected medicinal plants. It also presents the results obtained from different analyses, including yield, physical and chemical properties, and discusses their significance. By comparing the findings with previous studies, this chapter highlights the efficiency of the extraction methods and the quality of the oils produced.:

Chapter 03: This chapter provides a practical guide for launching a startup based on the production and commercialization of essential oils and related products. It outlines key steps

such as business planning, market analysis, legal requirements, production setup, and quality control. The aim is to help transform the research findings into a sustainable and profitable business venture.

CHAPTER 01

Introduction to medicinal plants and bioactive compounds

Introduction to medicinal plants:

From antiquity to the present day, various diseases that afflict and kill people have varied from so the man resorted to his surroundings, trying to extract the treatment from him necessary. And here is the role of the kingdom of plants because he was the only one who had everything for the human medicines and is still one of the most important sources, because the scientists have estimated that there are about 500,000 medicinal plants distributed in the world that the population depends on the treatment, due to their abundance, low cost and their effectiveness .Herbal medicine was known to the oldest civilizations on earth, or the therapeutic properties of certain plants are taken advantage of, either by taking some of its parts such as the roots, the leaves and the bark, and the methods varied use of them, such as putting them on a direct wound, and here the journey has began to look for innovative ways to extract effective compounds and try to make the most of these. Despite the many benefits that medicinal plants enjoy, many of them they are toxic and the degree of their danger reaches death, so scientists have to act with caution on this point and seek to separate the beneficial parts of the plants from the toxic and to divide the latter into families and species to facilitate their use and with the development of science and the development of various methods extraction methods allow humans to extract the basic materials of these plants by

using their basic parts which have been previously mentioned and employing them in the pharmaceutical aspect in order to manufacture various medicines. If the medicine modern tends today to use the active components of plants instead of using them in the natural state. Of the common plants among us that have been used in the past and still today, in order to find celery that is used as a diuretic. as for the chamomile, it has been used for thousands of years for several ailments, especially insomnia, anxiety and digestive disorders such as gastric disorders, gas, diarrhea and many others. So far, science and scientists are still in their continuous attempts to obtain the highest yield of the latter.

Medicinal plants, also known as medicinal herbs, have been used in traditional medicine for thousands of years, providing a rich source of pharmacologically active compounds. These plants synthesize a wide range of chemical compounds, including alkaloids, glycosides,

polyphenols, and terpenes, which serve various functions such as defense against insects and diseases. [3] [4] Here is an overview of medicinal plants:

I- History and Use:

I - 1 Historical Use: The earliest recorded use of medicinal plants dates back to ancient civilizations such as the Sumerians and Egyptians. The Greek physician Dioscorides documented over 600 medicinal plants in his work De Materia medica, which remained influential for centuries . [3] [6]

I **– 2 Traditional Medicine:** Medicinal plants have formed the basis of traditional medicine systems worldwide, including ancient Chinese medicine, which utilizes various plant parts for treating different ailments. [4] [5]

II **Definition of medicinal plants:**

Medicinal plants are plant drugs within the meaning of the Pharmacopoeia

European (1433), at least part of which has medicinal properties. It is uncommon for the plant to be used whole; most often it is one or more several parts - defined in the Glossary of anatomical terms useful for identification A or identification B (plant drugs) used in the Pharmacopoeia French– which can each have different uses. By extension, we call often "medicinal plant" or "plant" not only the botanical entity, but also the part used. Plants with medicinal properties can also have uses food or condiments, or even be used for the preparation of hygienic drinks. For these various uses, they are either the same parts of plants, or the parts different.

III **Types of Compounds:**

Alkaloids: These are bitter-tasting and often toxic compounds found in plants like nightshade (atropine, scopolamine), coffee (caffeine), and opium poppy (morphine). [3]

Terpenes: Known for their aromatic properties, terpenes are found in plants like thyme and lavender, and are used in essential oils for antiseptic and antifungal purposes. [3]

Glycosides: These compounds are found in various medicinal plants and have diverse pharmacological effects.

Polyphenols: Known for their antioxidant properties, polyphenols are abundant in many plants and contribute to their medicinal value.

IV **Modern Applications:**

Pharmaceuticals: Many modern drugs, such as aspirin and quinine, are derived from medicinal plants. Ethnobotany plays a crucial role in discovering new pharmacologically active substances . [3] [5]

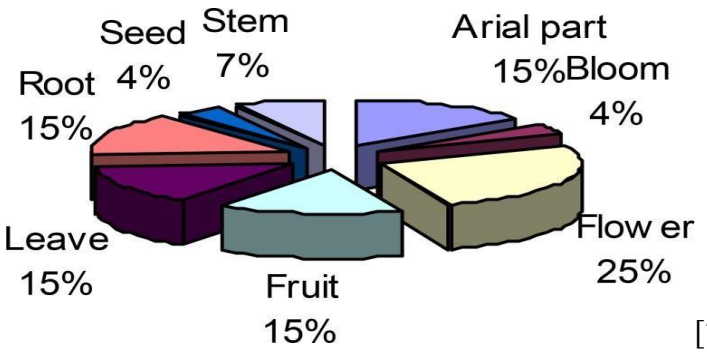
Antimicrobial Properties: Plants like oregano and thyme have essential oils that exhibit antimicrobial properties, helping to prevent bacterial and fungal infections . [4] V Challenges and Future Directions:

Safety and Efficacy: While medicinal plants offer numerous health benefits, some can be unsafe if not used properly. Scientific validation of their efficacy is essential for modern medical applications. [4]

Conservation: Over-harvesting of certain medicinal plants has led to their decline in the wild, emphasizing the need for sustainable cultivation practices [7]



image 01: Dioscorides's 1st century De materia medica, seen here in a c. 1334 copy in Arabic, describes some 1000 drug recipes based on over 600 plants [3]



[7] Image 2: Plants part use and their percentage

Medicinal plant species were used in treating different types of ailment……[7]

Table 01: Medicinal plant species were used in treating different types of ailment

No.	Ailment	Medicinal plants
1	Anemia	Berberis integerrima
2	Ascaris worms	Heracleum persicum
3	Asthma	Achilla millefolium
4	Backache	Achilla millefolium, Ferula persica, Juglans regia
5	Common cold	Anchusa italica, Echium amoenum, Ziziphora clinopodioides
6	Constipation	Malva neglecta, Viola odorata
7	Diabetes	Berberis integerrima, Juglans regia
8	Enteric fever	Berberis integerrima
9	Fever	Viola odorata
10	Gonalgia	Juglans regia
11	Gout	Ferula persica
12	Hand schism	Fumaria asepala
13	Headache	Capparis spinosa, Echium amoenum, Fumaria asepala, Heracleum persicum, Stachys lavandulifolia, Viola odorata, Ziziphora clinopodioides
14	Heart oxygenating	Ferula persica
15	Hyperlipidemia	Berberis integerrima, Grammosciadium platycarpum, Hippophae rhamnoides
16	Hypertension	Hippophae rhamnoides, Viola odorata
17	Increase nausea	Ziziphora clinopodioides
No.	Ailment	Medicinal plants
18	Infections	Achilla millefolium, Malva neglecta, Ziziphora clinopodioides
19	Mange	Fumaria asepala
20	Pectoralgia	Achilla millefolium
21	Pododynia	Capparis spinosa, Ferula persica, Juglans regia
22	Renal calculus	Capparis spinosa, Stachys lavandulifolia
23	Sedative	Echium amoenum, Viola odorata

24	Sinusitis	Ferula persica
25	Stomachache	Echium amoenum, Ziziphora clinopodioides
26	Tonic	Grammosciadium platycarpum, Smyrnium cordifolium
27	Tremor	Heracleum persicum

VI The plants we chose for our study

Medicinal plants have played a crucial role in the history of healthcare across various cultures, serving as a primary source of therapeutic agents for thousands of years. Their significance extends beyond their medicinal properties, often intertwining with cultural, spiritual, and traditional practices.

VI -1 THYME

VI -1 -1 Role of thyme in Traditional and Modern Medicine:

Thymus vulgaris L. (Thyme) is an aromatic plant belonging to the Lamiaceae family, used for medicinal and spice purposes almost everywhere in the world. Thymusvulgaris L. shows a polymorphic variation. in monoterpene production, the presence of intra specific chemotype variation being common in the genus Thymus. Each of the 6 chemotypes, Geraniol, alphaTerpineol, Thuyanol-4, Linalool, Carvacrol, and Thymol, is named after its dominant monoterpene. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma.. In addition,Thyme has been proven with pharmacological effects such as antifungal and antibacterial, spasmolytic and antitussive, expectorant and secretomotor. The aim of this study was to evaluate the botanical, microscopic characteristics, cultivation, biochemical Compounds, and pharmacological effects.

Thyme is very aromatic and generally reaches a height of 4 to 8 inches high. The aroma mostly comes from the leaves, which is the part of the herb most commonly used. The leaves are generally used fresh or dried. They can be added to a dish for flavoring as a sprig of Thyme, to later be removed before eating. The leaves are generally less than a half an inch long and are an oval shape.



Image 03: pictures of thyme plant

VI -1-2 The difference between traditional and modern medicine:

VI-1-2- 1 Traditional Medicine:

Thyme has been used historically for respiratory conditions such as bronchitis, asthma, and colds due to its expectorant, anti-inflammatory, and antibacterial properties [9] [10]

It is also employed as an antiseptic for wounds and skin conditions like acne [11] VI

-1 -2-2 Modern Medicine:

The active compound thymol in thyme essential oil exhibits antiviral, antifungal, antibacterial, and antioxidant effects. It has shown promise in treating infections, reducing inflammation, and even combating cancer cells [10]

Thyme is used in modern formulations like tinctures and nanocapsules to enhance bioavailability for therapeutic purposes. [10]

Studies suggest thyme may help manage blood pressure and cholesterol levels through its antihypertensive and antioxidant actions [9] [11] VI -1-3Why thyme?:

Many studies have been conducted on thyme species to identify their chemical composition. A wide variety of chemical compounds as well as essential oils constitute the main composition of thyme that varies with climate and geographical area. Investigations have reported that thyme contains 56.53% monoterpenes, 28.69% monoterpene hydrocarbons, 5.04% sesquiterpene hydrocarbons and 1.84% oxygenated sesquiterpenes . Thyme is rich in many flavonoids and phenolic antioxidants like zeaxanthin, lutein, pigenin, naringenin, luteolin and thymonin . Fresh thyme has one of the highest antioxidant levels being rich in minerals and vitamins that are

essential for optimum health. Potassium, iron, calcium, manganese, magnesium and selenium are concentrated in the leaves, and the main constituent of the oil extracted is thymol. Thymol is an important phenolic component mainly responsible for thyme's antioxidant activity . The thyme's flowered stem contains flavonoid derivatives such as apigenol and luteolol, phenolic acids such as cafeic and rosmarinic acids, and tannins .

VI -1-4The Journey Begins - Selecting thyme

VI -1 – 4-1 Historical Uses:

Thyme is a common household plant with many varieties with a panel of strong odours and flavours from lemon-like to camphorous. It is used for culinary, medicinal, cosmetic and ornamental purposes. Thyme was used in medicine and for embalmment by the Sumerians and Egyptians and is still used in ethnomedicine today. Traditionally associated with supporting the respiratory system and helping with pulmonary diseases. Research, primarily from Europe, the Far East and Middle East, has shown that thyme possesses, via its bioactive constituents, antioxidant, anti-inflammatory, antinociceptive/analgesic and chemopreventive/anti-cancer, hepato-protective and anti-microbial properties.[27] VI -1 – 4-2 Cultural Significance:

Thymus vulgaris commonly known as "thyme" has been used for many centuries for its flavoring, culinary, and medicinal properties . The name thyme derives from the Greek word 'thymos' which means courage or strength. In the first century AD, thyme was used mainly as a medicinal plant, which was mentioned in Dioscorides' work. However, in the Mediterranean region, it was used mainly as spice and then spread all over the world.[36]

VI -1 – 5 Medicinal Properties and Rationale for Selection

VI -1 -5-1 Medicinal Properties:

thymus vulgaris L. (T. vulgaris) a significant aromatic plant with around 100 species in the world is widely used for medicinal purposes as well as in culinary dishes. The genus Thymus are important medicinal plants, highly recommended due to a variety of therapeutic properties of their essential oils, normally known as Thyme oil. Thymus species are considered as medicinal plants due to their pharmacological and biological properties. Its properties are due to its main components, thymol and carvacrol. Fresh Thyme has the highest level of antioxidants among all herbs. Thymus vulgaris L. is widely used in folk medicine in the treatments of variety of diseases such as gastroenteric and bronchopulmonary disorders. The essential oil of thyme and the compound thymol have antimicrobial activity in vitro against E.

coli strains. The essential oil of thyme has been found to possess the strongest antimicrobial properties[32]

VI -1 -5-2 Why Selected:

Its broad spectrum of pharmacological activities, low toxicity, and traditional use in digestive, respiratory, and pain-related conditions make thyme a valuable plant for medicinal studies. Its antioxidant and anticancer potential further justify its inclusion.

VI -1 -6 thyme bioactive compound

Thyme, particularly Thymus vulgaris, is rich in bioactive compounds, including:

Phenolic Monoterpenes: Thymol and carvacrol are the primary phenolic compounds responsible for thyme's antioxidant and antimicrobial properties. These compounds are known for their high antioxidant activity. [39]

Flavonoids: Thyme contains flavonols like quercetin, which have anti-inflammatory and antioxidant effects. [39]

Essential Oils: Thyme's essential oils include limonene, linalool, and p-cymene, which exhibit antioxidant and antimicrobial activities. [39]

VI -1-7 Potential Therapeutic Applications

Antimicrobial and Antiviral Properties: Thymol, the primary active compound in thyme essential oil, has demonstrated antibacterial, antifungal, and antiviral effects. These properties make thyme effective against biofilms, infectious diseases, and fungal infections such as those caused by Candida species. [40]

Anti-inflammatory and Antioxidant Effects: Thyme essential oil exhibits strong antiinflammatory activity, which can be beneficial for respiratory conditions like asthma and bronchitis. Its antioxidant properties also contribute to its potential in preventing oxidative stress-related disorders. [40]

Anticancer Potential: Recent studies suggest that thymol has anticancer properties, making thyme a candidate for cancer therapies. [40]

Emerging Formulations: Novel pharmaceutical forms, such as nanocapsules containing thymol, are being explored to enhance bioavailability and therapeutic efficacy. [40]

VI -2 fennel:

VI -2-1Role of fennel in Traditional and Modern Medicine:

Foeniculum vulgare Mill with the English name of Fennel, Fenouil in French, belongs to the Apiaceae family. It is a 1 to 2-meter tall, herbaceous, and fragrant plant that is found throughout Asia, the Mediterranean, and many regions of Europe.[Citation3] A perennial herb known as fennel has grooved stems, intermittent leaves that are combined with dark green, fluffy leaves and sheathed petioles. Its flowers are typically bisexual, irregular, or regular, and have yellow umbrellas that resemble oval beads. Small seeds from fennel have a fragrant scent and pleasant flavor. They are around 8 mm in length and 3 mm in width. Fennel seeds have a long, thin, cylindrical shape, and their sizes fluctuate with plant growth.

Fennel is utilized in all of its components, notably the seeds, roots, leaves, and fruit,[Citation3] 6.3% of fennel seed is water, 9.5% is protein, 10% is fat, 13.4% is mineral, 18.5% is fiber, and 42.3% is carbs. Its leaves include calcium, potassium, salt, iron, phosphorus, thiamine, riboflavin, niacin, and vitamin C, among other vitamins and minerals.[Citation7] Fruits contain between 10 and 12% oil, which is kept in the cotyledons of seeds. The fennel fruit oil contains 6% petrocylic acid, 22% oleic acid, 14% linoleic acid, and 4% palmitic acid. The fruit's essence content ranges from 4 to 6%, and the combination of elements depends on where the plant was grown.[Citation8] The essence of fennel is what gives it its fragrant quality. The essential oil of fennel contains more than 30 different types of terpene chemicals; the most significant of them are 50 to 80% trans-anethole and 5% limonene. Additionally, this plant includes phenolic substances: tannin, coumarin, hydroxycinnamic acids, flavonoids, and phenolic acids.



Image 04: pictures of fennel speed

VI -2-2 The difference between traditional and modern medicine:

VI -2-2-1 Traditional Medicine:

Fennel has been used historically for digestive issues such as bloating and indigestion. It is also known for its carminative properties that reduce gas formation. [14]

In traditional practices, fennel seeds are used to promote lactation in nursing mothers and to alleviate menstrual discomfort [14]

VI -2-2-2Modern Medicine:

Fennel contains bioactive compounds like anethole that exhibit antimicrobial, antiinflammatory, and antioxidant properties. These make fennel effective in supporting gastrointestinal health.

It has been studied for its potential in managing blood sugar levels, promoting cardiovascular health, and reducing symptoms of menopause due to its phytoestrogen content.

VI -2-3Why fennel seeds :

Water and ethanol extracts of fennel seeds show strong antioxidant activity in vitroIndications are that the fennel seed is a potential source of natural antioxidant.Antimicrobial property:A combination of oils of fennel, anise or basil with either benzoic acid or methylparaben was tested against Listeria monocytogenes and Salmonella enteriditis. S. enteriditis was more sensitive to inhibition by a combination of oil of anise, fennel or basil with methyl-paraben

VI -2-4 The Journey Begins - Selecting fennel

VI -2-4-1Fennel (Foeniculum vulgare): Historical Uses and Cultural Significance

Historical Uses:

Fennel's use goes back to Mycenaean Greece (13th century BC), where it was listed among important spices. The very name "marathon" comes from a region abundant with fennel. Hippocrates, the "Father of Medicine," included fennel in at least 30 medicinal formulas, especially for gynecological conditions such as promoting menstruation and lactation, and treating female infertility. It was also used for respiratory ailments and as a diuretic. Dioscorides, in his De materia medica, included fennel as a well-known medicinal plant. [24]

Cultural Significance:

Fennel is tied to Greek mythology and the famous Battle of Marathon. The “fennel field” gave its name to the historic site. Ancient tales, such as snakes using fennel to improve their eyesight, contributed to its reputation for enhancing vision. Fennel was a symbol of strength and longevity in ancient Mediterranean cultures and was believed to impart courage and vitality [24]

VI -2-5Medicinal Properties and Rationale for Selection:

VI -2-5-1Medicinal Properties:

Fennel is traditionally used for digestive, endocrine, reproductive, and respiratory ailments. It acts as a galactagogue, relieves menstrual cramps by modulating hormones like oxytocin and prostaglandin, and reduces inflammation and gas in the bowels. Fennel is rich in antioxidants (rosmarinic acid, chlorogenic acid, quercetin, apigenin) and exhibits antimicrobial, antiviral, antispasmodic, antitumor, hepatoprotective, hypoglycemic, and memory-enhancing effects. It also shows potential in reducing menopausal symptoms and hypertension. [29,30,31,35] VI -2-**5-2Why Selected:**

The diverse pharmacological profile of fennel, especially its hormone-modulating, digestive, and antioxidant activities, alongside its traditional use in women’s health and respiratory conditions, supports its selection for comprehensive medicinal research.

VI -2-6fennel bioactive compounds:

There is limited specific information available on fennel in the context of bioactive compounds. However, if referring to a plant like Artemisia, it would typically contain sesquiterpene lactones and other phenolic compounds with potential bioactivity.

In summary, both rosemary and thyme are valuable sources of bioactive compounds with significant health benefits, including antioxidant, anti-inflammatory, and anticancer properties.

VI -2-7Potential Therapeutic Applications :

Digestive Health: Fennel is traditionally used to treat gastrointestinal issues such as bloating, flatulence, and colic. Its antispasmodic and carminative properties make it effective for these conditions. [43]

Hormonal Regulation: Fennel has shown efficacy in reducing menstrual pain by modulating hormones like oxytocin and prostaglandins. [41]

Antioxidant Benefits: Rich in compounds like rosmarinic acid and quercetin, fennel helps combat free radicals, reducing risks of chronic diseases such as cancer and cardiovascular disorders. [41,43]

Broad Pharmacological Effects: Fennel exhibits antimicrobial, anti-inflammatory, hypoglycemic, hypolipidemic, antitumor, and hepatoprotective activities. These properties make it a versatile herb with applications across multiple health domains. [43]

VI -3 Rosrmary:

VI -3 – 1 Role of rosemary in Traditional and Modern Medicine:

R. officinalis L., popularly known as rosemary, is a plant belonging to the family Lamiaceae and originated from the Mediterranean region. However, it could be found all over the world. It is a perennial and aromatic plant, shrub-shaped with branches full of leaves, having a height of up to two meters and green leaves that exude a characteristic fragrance. R. officinalis may be used as a spice in cooking, as a natural preservative in the food industry, and as ornamental and medicinal plant.

Several phytocompounds presenting pharmacological activities may be isolated from essential oils and extracts of R. officinalis L , varying the concentration of these molecules in each specimen of the plant. The phytocompounds most reported include caffeic acid, carnosic acid, chlorogenic acid, monomeric acid, oleanolic acid, rosmarinic acid, ursolic acid, alpha-pinene, camphor, carnosol, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin. Pharmacological effects of phytocompounds from R. officinalis L.

VI -3 -2 The difference between traditional and modern medicine:

VI -3 -2-1 Traditional Medicine:

Rosemary has been traditionally used as an antispasmodic for conditions like renal colic and dysmenorrhea. It is also valued for relieving respiratory disorders and stimulating hair growth [13]

In folk medicine, it has been used to treat digestive issues and inflammatory diseases. [12] [13]

VI -3 -2-2 Modern Medicine:

Rosemary contains rosmarinic acid and other caffeic acid derivatives with potent antioxidant properties. These compounds are being explored for their therapeutic potential in preventing or treating asthma, inflammatory diseases, peptic ulcers, cancer, and cardiovascular disorders. [13]

Its antimicrobial properties have been studied against bacteria, fungi, and viruses. Rosemary oil also shows promise in lipid regulation and improving metabolic health [12] [13]

Recent clinical studies highlight rosemary's neuroprotective effects, particularly in improving memory speed at lower doses [12]

VI -3 -3 Why rosemary?:

Rosemary contains a number of powerful substances, including carnosic acid, carnosol, and rosmarinic acid, which offer anti-inflammatory, antioxidant, and antimicrobial effects

Rosemary oil differs from rosemary extract, as they contain different compounds in varying concentrations. Rosemary extract supplements can be taken orally, while rosemary oil is applied topically or inhaled as aromatherapy.

Applying rosemary oil to your skin and hair and inhaling it as aromatherapy may offer a few health benefits.

Molecules in rosemary essential oil are absorbed through the skin and impact pain pathways in several ways, including targeting pleasure memory sites in the brain and interacting with receptor sites for neurotransmitters involved in pain pathways. [15,16,17]



Image05: pictures of rose mary plant

<u>Part two:</u> bioactive compounds

VI -3 -4 The Journey Begins - Selecting rose mary

VI -3 -4-1 Rosemary (Rosmarinus officinalis): Historical Uses and Cultural Significance

Historical Uses:

Rosemary is native to the Mediterranean and has been used since ancient Egypt, where it was offered to deities and used in burial rituals. Ancient cuneiform tablets from 5000 BC mention rosemary. It spread to China by 220 CE and reached the UK by the 9th century. Traditionally, it was used for memory enhancement, as a symbol of fidelity, and in funerals to mask odors. Rosemary was also believed to have medicinal properties, including antioxidant, antibacterial, and skin-conditioning effects. [23,26]

Cultural Significance:

Rosemary is deeply woven into European folklore and Christian symbolism. In Spain, it is called "romero" or "pilgrim plant," with legends connecting it to the Virgin Mary. It is associated with remembrance, loyalty, and love, often used in weddings and funerals. Rosemary is mentioned in Shakespeare's plays as a symbol of memory and mourning. In various cultures, it was believed to ward off evil and was used in protective rituals. [23]

VI -3 -5Medicinal Properties and Rationale for Selection

VI -3-5-1Medicinal Properties:

Rosemary contains potent antioxidants such as carnosic acid and rosmarinic acid, which exhibit antimicrobial, antiviral, antifungal, anti-inflammatory, neuroprotective, antinociceptive, and antitumor properties. It supports immune function, improves eye health, regulates liver function, and may reduce the risk of asthma and cancer. Rosemary has been used traditionally for headaches, neuralgia, insomnia, depression, and memory enhancement. [28,34] VI -3 -5-

2Why Selected:

Rosemary's strong antioxidant and neuroprotective effects, combined with its traditional use for cognitive and mood disorders, as well as its antimicrobial and anti-inflammatory properties, make it an important candidate for studies targeting chronic diseases and nervous system health.

VI -3 -6Plants bioactive compounds:

Rosemary, Rosmarinus officinalis, is renowned for its diverse bioactive compounds, including:

Diterpenoids: Carnosic acid and carnosol are prominent diterpenoids known for their antioxidant and anticancer activities. These compounds have shown antiproliferative effects against various cancer cell lines, including colorectal and prostate cancer. [37, 38]

Flavonoids: Rosemary contains flavones, flavanones, and flavonols, which contribute to its antioxidant properties. Flavonoids like apigenin and luteolin are also present. [38]

Phenolic Acids: Rosmarinic acid is a significant phenolic acid found in rosemary, contributing to its high antioxidant potential. [36,38]

Triterpenes: Ursolic acid and betulinic acid are triterpenes with reported bioactivity, though not all extracts contain them. [37,38]

VI -3 -7Potential Therapeutic Applications :

Anti-inflammatory and Neuroprotective Effects: Rosemary contains compounds like carnosol and rosmarinic acid that inhibit inflammatory pathways. It also shows promise in improving memory speed and protecting against neurodegenerative conditions. [42,44]

Dermatological Applications: Rosemary extract is widely used in topical formulations for wound healing, skin cancer prevention, anti-aging treatments, and protection against UV damage. [44]

Metabolic Health: Rosemary has been studied for its potential to manage obesity, diabetes, dyslipidemia, and metabolic syndrome through its antioxidant and anti-inflammatory actions. [42,44]

Antimicrobial Activity: The herb demonstrates antibacterial effects against pathogens and is being explored for its chemopreventive properties. [44]

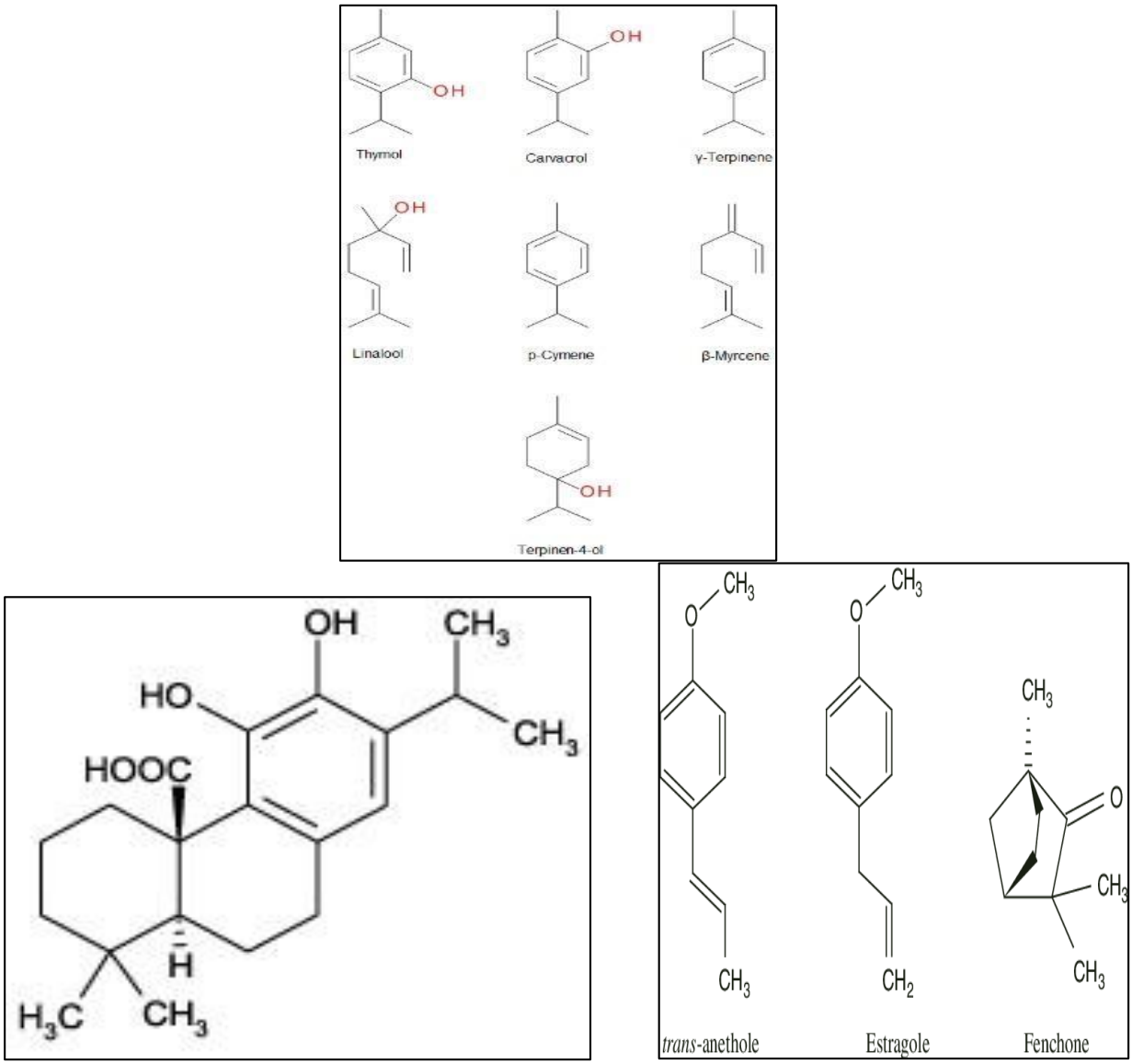


Image 06: pictures of chemical structures of the main components of thyme, fennel seeds and rosemary oils respectively.

VII **Comparison of Applications:**

Table 02 : Comparison of Applications

Herb	Traditional Uses	Modern Applications
Thyme	Respiratory issues, antiseptic	Antiviral, antihypertensive, anticancer

Rosemary	Antispasmodic, respiratory relief	Neuroprotective effects, metabolic health
Fennel	Digestive aid, lactation support	Blood sugar regulation, cardiovascular benefits

These herbs demonstrate the enduring importance of plants in medicine. Their traditional uses often align with modern scientific findings, underscoring their therapeutic potential across diverse health domains.

VIII -**The Search for Bioactive Compound :**

The potency of medicinal plants for healing can be traced back to prehistorical times. Man right in the past relied on nature for food and medicines . Nature served as the repository of diverse biodiversity and a unique laboratory for natural active ingredients of medicinal plants synthesis which man looks to for rescue in times of ailment.

Medicinal plants as major components of the biodiversity have been used in African traditional medicines to provide herbal remedies to different kinds of ailments among the various African cultures.

The application of medicinal plants as the active ingredients of African herbal medicines was rooted in African history, culture belief and religion accepted among the various subcultures before the advent of colonization which introduced Western medicines and Western religion that tended to erode the African healing culture . [15,16,17]

The African healing culture involves the application of the knowledge of medicinal plant healing potentials gained through trial and error experiments, experience and many years of careful observation passed across from one generation to another. This knowledge exists among cultures, tribes and families in the form of local folklore . In preparing the herbal medicines, it was noted that depending upon the individual recipe, all parts of the plant can be utilized, such as the whole plants, seeds, bark, leaves, roots, flowers, stems and fruits .

Conditions like diabetes mellitus (DM), cancer, infections, inflammation, cardiovascular diseases (CVDs), and gastrointestinal (GI) disorders, continue to have a major impact on mortality and morbidity worldwide, and in the case of chronic illnesses, often associated with multiple complications, can severely impact the quality of life . Many modern synthetic medicines that are used to manage the aforementioned diseases present limitations that restrict their use. This includes being associated with adverse side effects, triggering drug interactions, and/or hypersensitivity reactions . Additionally, a significant proportion of the world's population can neither afford nor easily access synthetic medicines .

Medicinal plants, which are generally considered safer, more affordable, and more accessible than synthetic medicines, have historically served as useful therapeutic agents in ethnomedicine. According to the World Health Organization (WHO), more than 80% of the world's population still relies on traditional medicines obtained from plants to meet their basic medical needs. Over the past few decades, there has been a surge in global interest in medicinal plants as alternatives to synthetic medicines. Unlike the latter, which is based on a single chemical entity, medicines based on plant extracts contain various phytoconstituents (e.g., flavonoids, alkaloids, polyphenols, and terpenoids). Interestingly, they have been demonstrated to exert their pharmacological activities by interacting simultaneously with numerous biological targets, thereby increasing their therapeutic potential . Moreover, the discovery that some phytoconstituents are able to enhance the bioactivity of others within a plant extract, an effect called "synergism", is another great incentive for the use of medicinal plants[15,16,17]

VIII -1-Importance of discovering new compounds in the modern world of health and wellness:

Large population of people are turning to herbal medicines for its therapeutic value from both developed and developing countries. This development is not necessarily because of poverty or lack of access to Western medicines but due to the realization that several advantages are presented by medicinal plants bioactive compounds such as the ability to cross blood-brain barriers , being of natural origin, little or no side effect compared to the Western medications, multiple target effects. Their ability for cellular process modulation, including immune responses, metabolic pathways, oxidative stress and inflammation all enhanced their therapeutic potentials .

However, the application of various advancements in medicinal plant research is essential to ensure that the full potential of medicinal plants bioactive compounds is fully realized.

Interdisciplinary collaborations combining traditional knowledge with modern scientific process is required for the future of utilizing medicinal plants bioactive substances. Novel therapies and personalized medicine approaches can be developed from the collaboration which can help in bridging the gap between traditional medicine and evidence-based medicine .

Additionally, exciting prospects for enhancing the therapeutic potential of these compounds are provided by advancements in nanotechnology, pharmacogenomics and phytopharmaceutical formulations. The therapeutic outcomes of these compounds can be optimized by improving their bioavailability, targeting specific tissues or cells and tailoring treatments based on individual genetic variations

VIII -2Purpose of the research :

We always need personal motivation to work or start any project to ensure continuity and dedication to get the best possible results. The first motivation we had when starting this project was our love for all kinds of plant sciences, especially medicinal ones, which contain many medical benefits, and therefore we chose this field to discover new things that are of course useful.

The love of providing assistance through our work and benefiting from all the information that we can collect during our research and work in the laboratory, and therefore we have applied the purpose of the scientific thesis and scientific work. [15,16,17]

VIII -3Summary Table of Medicinal Properties and Study Selection Reasons:

Table 03: Summary Table of Medicinal Properties and Study Selection Reasons

Plant	Key Medicinal Properties	Reason for Study Selection
thyme	Antioxidqnt, strongest antimicrobial propertiesantimicrobial, antiinflammatory.	considered as medicinal plants due to their pharmacological and biological properties.
Rosemary	Antioxidant, antimicrobial, neuroprotective, antitumor, anti-inflammatory, immune support, cognitive enhancement	Strong antioxidant and neuroprotective effects, traditional use in mood, memory, and inflammatory disorders
Fennel	Antioxidant, antiinflammatory, antispasmodic, hormone modulating, digestive aid, antimicrobial, antitumor	Diverse pharmacological effects, especially in women's health, digestive and respiratory systems

VIII - 4 Vernacular names of Foeniculum vulgare.:

Table04 :Vernacular names of Foeniculum vulgare.…[43]

Region/language/system of medicine	Local name
Arabic	Bisbas, razianaj
English	Bitter fennel, common fennel, sweet fennel, wild fennel
France	Fenouille
French	Fenouil
Latin	Foeniculum, maratrum

VIII-5 Results collected about Rosmarinus officinalis uses.

Table 05 : Results collected about Rosmarinus officinalis uses. [44]

topic	Results

Anti-inflammatory activity	• Carnosic acid inhibit NO. • Carnosic acid platelet was inhibited. • Carnosol reduces atopic dermatitis. • Rosemary extract showed anti-inflammatory activity similar to indomethacin.
Skin cancer	• Rosemary extract reduces number, diameter, weight, and incidence of tumors and increases the latency period. • Rosmarinic acid showed chameoprotective activity. • Carnosic acid showed protective effect against melanoma.
Wound healing	• Rosemary oil showed healing, angiogenesis, and improvements in granulation tissue. • Rosemary oil accelerated healing wounds in diabetic and nondiabetic animals. • Rosemary cream accelerated wound healing.
Skin flaps survival	• Rosemary oil showed improvement in tissue survival and viability, and tissue necrosis was lower.
Transdermal effects	• Monoterpertenes, presented in rosemary oil, promoted cutaneous absorption
Antifungal activity	• Rosemary oil was capable of inhibiting C. albicans growth. • Rosemary extract was responsible for inhibiting fungal growth.
Ginoid lipodystrophy (GLD, cellulite)	• A cream with carnosic acid was responsible for an improvement of cellulitis appearance
Alopecia	• Rosemary extract showed a hair growth.
Antiaging	• Roms1 has a strong antioxidant capacity. • Rosemary essential oil nanoparticles showed greater capacity of hydration and improves the elasticity

Ultraviolet protection	• Rosemary and citrus extracts were able to improve cell protection against UV. • Rosemary extract reduced skin damage caused by the sun
Other studies	• Secondary metabolites present in rosemary extract showed stabilizer emulsion properties.

IX -Plant Collection and Preparation:

IX -1 Gathering the Plants :

To extract the active substances from medicinal plants, one of the most important steps is required to ensure a good quality of the final product. In this search, we wanted to collect oils for the three plants thyme, fennel seeds and rosemary. we started looking for guaranteed places to acquire plants, so we chose to take dried thyme and fennel seeds from a medicinal herbs store after we confirmed that the product was the same from the period of January to April, as for Rosemary, we chose " Ben chikaou" area to get this plant, which is characterized by its abundance in that area.

There are some important steps that must be followed during the oil extraction process, depending on the plant to be worked on, each plant has a certain way to be able to get the best and most amount of oil

We had to crush the fennel seeds partially crushed to facilitate the extraction of oil, as well as for thyme, because they were originally dried, but the rosemary had other stages

When the rosemary is brought, it is purified from any impurities and distributed sparsely to be left to dry in a moderately hot and well-ventilated place for a period of 5 to 10 days. After making sure that it is completely dry, the dry leaves are removed from the branches, which originally contain oil

After taking the dry leaves, they are broken by hand to help the oils come out of theme during the extraction process.

IX -2 The Process of Extraction:

IX -2-1 Extraction by Clevenger Type Assembly:

Preparation of the plant material: The plant is cut into small pieces or crushed.

Loading the flask: The plant material is immersed in a glass flask containing a sufficient amount of distilled water (usually filled to a maximum of two thirds to avoid overflows).

Heating: The flask is heated to bring the water to a boil.
Steam rise: The water vapor loaded with volatile compounds rises in the glass assembly to the condenser.

Condensation: The steam is cooled in a refrigerant, forming a liquid condensate.

Separation and recirculation: The condensate falls back into a graduated burette (essentier). The essential oil, less dense, supernatates on the surface of the water. The condensed water (hydrolat) is returned to the heated flask via a return tube (cohobation principle).

Duration: The extraction lasts about 2 to 3 hours Oil

recovery :

The essential oil is recovered directly in the graduated burette and can be dried with a dehydrating agent (e.g. sodium sulfate) to remove traces of water.

Calculation of the yield: The volume or weight of oil is measured and related to the mass of dry plant used.

The Clevenger assembly, invented in 1928, is considered more exhaustive because the cohobation makes it possible to redistill the volatile compounds dissolved in water, thus increasing the yield of essential oil. However, it is limited to smaller quantities of vegetable matter and does not allow the recovery of the pure hydrolate.[63,64,65,66] IX -2-2 Summary of the Key Stages of the Clevenger Assembly :

- Place the crushed plant in a flask with distilled water (max 2/3 of the volume).
- Heat until boiling to generate steam.
- The steam passes through the plant, carrying away the essential oils.
- Condensing the steam into a liquid in a refrigerant.
- Separate the floating essential oil in the graduated burette.
- Recirculating the condensed water in the flask (cohobation).
- Extract the oil after 2-3 hours and dry if necessary.
- Calculate the yield of essential oil.

IX -2-3 Conclusion:

The extraction of essential oils in the laboratory is mainly based on hydrodistillation methods using either hydrodiffusion, adapted to large quantities and to the recovery of hydrolates, or Clevenger assembly, more exhaustive and precise for the yield of volatile compounds. The choice depends on the quantities to be extracted, the nature of the plant, and the analytical or industrial objectives. [63,64,65,66]

chapter 2 :

extraction methods and Materials with Results and discussion

Parte one: extraction methods and solvent :

I-Detailed Explanation of Extraction Methods:

I-1 Maceration Extraction:

Maceration is a traditional solid-liquid extraction technique where plant material is soaked in a solvent (such as methanol, ethanol, water, or their mixtures) at room temperature or slightly elevated temperatures for an extended period, allowing bioactive compounds to diffuse into the solvent. It relies on the solvent penetrating the plant matrix and dissolving the desired compounds without agitation or with minimal stirring. Maceration is simple and widely used but has limitations such as long extraction times and the need for large solvent volumes. The efficiency can be improved by optimizing factors like homogenization, temperature, solvent type, and extraction time. Maceration without stirring has been shown to yield higher total phenolic content in some cases. [52,53]

I-2 Soxhlet Extraction

Soxhlet extraction is a continuous extraction technique that repeatedly washes the solid sample with fresh solvent vapors condensed back into the extraction chamber. The process involves heating the solvent in a flask, vaporizing it, condensing the vapor, and allowing it to percolate through the solid sample repeatedly. This cycle continues until the extraction is complete, which is indicated by the solvent in the siphon becoming clear. Soxhlet extraction is more efficient than simple maceration because it uses hot solvent and continuous solvent recycling, leading to higher yields. It is suitable for compounds soluble in the solvent at elevated temperatures. However, it requires longer extraction times and uses relatively large solvent volumes. Modifications exist to improve efficiency and overcome limitations such as thermal degradation of sensitive compounds. [53,55,57]

I-3 Ultrasonic-Assisted Extraction (UAE)

Ultrasonic-assisted extraction uses ultrasound waves to enhance the extraction process by inducing acoustic cavitation-the formation, growth, and implosive collapse of bubbles in the solvent. This phenomenon generates localized high temperature and pressure

microenvironments, causing physical effects such as cell wall disruption, increased solvent penetration, and improved mass transfer. Ultrasound also causes acoustic streaming, which increases fluid turbulence and convection, further enhancing extraction efficiency. UAE can reduce extraction time and solvent consumption compared to conventional methods while improving yield and preserving thermolabile compounds. It is especially effective for extracting pigments, phenolics, and other intracellular compounds by promoting fragmentation, erosion, and pore formation in the plant matrix. [54,55]

I-4 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction uses microwave energy to heat the solvent and plant matrix rapidly and uniformly, accelerating the extraction process. The microwaves cause polar molecules and ions in the sample and solvent to oscillate, generating heat internally, which disrupts plant cell walls and enhances solvent penetration and solubilization of target compounds. MAE offers advantages such as shorter extraction times, reduced solvent use, and improved extraction yields compared to traditional methods. However, it may cause oxidation or thermal degradation of sensitive compounds if not properly controlled. MAE is often combined with modified conditions (e.g., nitrogen atmosphere, vacuum) to mitigate these drawbacks and improve extraction efficiency. [56,57]

I-5 Summary Table of Extraction Methods

Table 06: Table of Extraction Methods

Extraction Method	Principle	Advantages	Limitations	Typical Applications
Maceration	Soaking solid in solvent at room/low temp	Simple, low cost, minimal equipment	Long extraction time, large solvent use	Phenolics, alkaloids, flavonoids
Soxhlet Extraction	Continuous hot solvent reflux and siphoning	High yield, continuous extraction	Long time, high solvent use, thermal risk	Thermally stable compounds, oils

Ultrasonic-Assisted (UAE)	Acoustic cavitation and streaming enhance extraction	Faster, less solvent, better yield	Equipment cost, limited scale	Pigments, phenolics, heatsensitive compounds
Microwave-Assisted (MAE)	Microwave heating disrupts cells and enhances solubility	Very fast, efficient solvent use, high yield	Possible thermal degradation, equipment cost	Wide range of phytochemicals, thermolabile compounds

These extraction methods are chosen based on the nature of the target compounds, thermal sensitivity, solvent compatibility, and desired efficiency. Maceration and Soxhlet are traditional but often slower and solvent-intensive, while UAE and MAE represent modern, greener alternatives offering faster and more efficient extraction with less solvent and energy consumption [52,53,54,55,56,57]

The choice of specific extraction techniques for each plant and its compounds is fundamentally guided by the nature of the phytochemicals targeted, the physical and chemical properties of the plant material, and the desired quality and yield of the extract. Several critical factors influence this decision:

II- **Nature of the Target Compounds**

Polarity: Polar compounds such as glycosides, phenolics, and flavonoids are best extracted with polar solvents (e.g., aqueous methanol, ethanol), often using maceration or percolation for thermolabile compounds, while non-polar compounds like lipids or lupeol require nonpolar solvents (e.g., hexane) and sometimes Soxhlet extraction for thermostable constituents. .

[59,61,62]

Thermal Stability: Heat-sensitive compounds (thermolabile) necessitate mild extraction methods such as cold maceration, percolation, or ultrasonic-assisted extraction to avoid degradation, whereas thermostable compounds can withstand Soxhlet or microwave-assisted extraction that use elevated temperatures for better yield. . [59,61]

II-1 **Plant Material Characteristics**

Part of Plant and Condition: Different plant parts (leaves, roots, bark) contain different compounds and require tailored methods. The drying method and particle size after grinding affect extraction efficiency and must be carefully controlled to avoid heat damage or loss of volatile compounds. . [59,62]

Moisture Content: High moisture content in plant material may require weight corrections and influence the choice of extraction method and solvent. . [59]

II-2 **Extraction Efficiency and Selectivity**

Extraction Time and Solvent Volume: Maceration is suitable for long-duration extraction and large-volume products (e.g., tinctures), while ultrasonic- or microwave-assisted extraction significantly reduces time and solvent use, producing more concentrated extracts. . [61]

Yield and Purity: Soxhlet extraction offers continuous solvent recycling and higher yields but requires longer times and more solvent. Ultrasonic-assisted extraction enhances mass transfer and cell disruption, improving yield and preserving thermolabile compounds. . [58,59]

II-3 **Solvent Compatibility and Safety**

Solvent choice impacts extraction temperature (due to boiling point), selectivity, and safety. Lower boiling point solvents allow extraction at lower temperatures, protecting sensitive compounds, whereas higher boiling solvents suit thermostable phytochemicals. . [61]

Solvents must also be compatible with the extraction method and not form artifacts or degrade the phytochemicals. . [61]

II-4 **Intended Use and Cost Considerations**

For inexpensive drugs or large-scale production, simpler and cost-effective methods like maceration are preferred. For costly or high-value compounds, more refined methods such as percolation or Soxhlet extraction are used to maximize purity and yield. . [60]

II-5 **Summary of Why Specific Techniques Are Chosen**

Table 07: Summary of Why Specific Techniques Are Chosen

Factor	Choice of Extraction Technique and Reason.
Thermolabile compounds	Cold maceration, percolation, ultrasonicassisted extraction to avoid heat degradation. [59,60]
Thermostable compounds	Soxhlet extraction or microwave-assisted extraction for higher yield and efficiency. [59,60]
Polar compounds (e.g., glycosides, phenolics)	Polar solvents with maceration or ultrasonic extraction to maximize solubility and preserve activity. [59,62]
Non-polar compounds (e.g., lipids, lupeol)	Non-polar solvents like hexane with Soxhlet or maceration for effective extraction. [59,62]
Large volume, low-cost extracts	Maceration due to simplicity and low cost despite longer extraction time. [60]
Concentrated, high-purity extracts	Percolation or Soxhlet extraction for continuous and efficient extraction cycles. [60]
Heat-sensitive volatile compounds	Low-temperature methods with suitable solvents to prevent loss or degradation. [59,61]
Need for rapid extraction	Ultrasonic-assisted or microwave-assisted extraction to reduce time and solvent use. [58,60]

In conclusion, the selection of extraction techniques is a strategic decision balancing the chemical nature of the target compounds, plant material characteristics, extraction efficiency, solvent compatibility, and final product requirements. This ensures optimal recovery of bioactive compounds with preserved therapeutic properties and minimal degradation or contamination. [58,59,60,61,62]

Part two : Materials and Methods

Medicinal and aromatic plants represent a rich source of bioactive compounds and have been traditionally used for centuries in treating a wide range of diseases. With the advancement of biological and chemical sciences, these plants have gained significant attention in various sectors, including the pharmaceutical, cosmetic, and food industries, owing to their essential oils that possess therapeutic and biologically active properties.

Among these plants, Thymus vulgaris (thyme), Rosmarinus officinalis (rosemary), and Foeniculum vulgare (fennel) are particularly distinguished for their essential oils, which are known for their antioxidant activity, antibacterial potential, and favorable physicochemical characteristics, making them suitable candidates for natural product formulation.

The objective of this study is to extract the essential oils from these three plants and conduct a comprehensive set of analyses, including:

Physicochemical characterization (density, refractive index, ethanol solubility, acid value, saponification value, and ester value),

Gas Chromatography-Mass Spectrometry (GC-MS) to determine the chemical composition and identify major active constituents,

Evaluation of antibacterial activity to assess the effectiveness of each oil against selected bacterial strains.

As part of the practical application of these oils, a topical cream formulation was developed using a combination of the extracted oils. The cream was subsequently subjected to additional analyses, including: Antimicrobial activity testing to evaluate the efficacy of the final formulation, pH measurement to ensure skin compatibility.

This research aims to provide a thorough evaluation of the extracted essential oils in terms of their chemical composition, biological activities, and formulation potential. The findings may contribute to the development of natural, plant-based products for therapeutic and cosmetic use, supporting the growing interest in organic and alternative medicine-based applications.

I-Materials and Equipments:

I-1 Plants materials:

Table 08:the medicinal plants use

Plant Materials	Image

Dried thyme leaves (Thymus vulgaris) Ouamri -medea- (Spring)	
Fennel seeds (Foeniculum vulgare) (Winter, Spring)	
Fresh rosemary leaves (Rosmarinus officinalis) Ben chicao -medea- (Winter, Spring)	

I-2 Chemicals :

Tabel 09 : chemical and Reagents Used

chemical
Anhydrous sodium sulfate (Na_2SO_4)
70% Ethanol
Ethanol 95%
Potassium hydroxide (KOH) solution (0.1 mol/L)
Hydrochloric acid solution (HCl 0.5 mol/L)
DPPH (2,2-diphenyl-1-picrylhydrazyl)
BHT (reference antioxidant)
Reagents
Phenolphthalein indicator

I-3 Apparatus:

Tabel 10: Apparatus Used

Apparatus	society
Clevenger-type apparatus	Joanlab
Round-bottom distillation flask	Joanlab
Water bath or electric heating mantle	Joanlab
Electric grinder	Joanlab
Oil separator tube	Joanlab
Dark glass storage bottles	Joanlab
Refractometer (digital or optical)	infitek
Micropipette or dropper	/
Digital thermometer (if not integrated in the device)	/
Watch glass (for temporary sample placement)	Joanlab
Pycnometer	Glassco
Analytical balance	Joanlab
Small funnel (to avoid bubbles)	Joanlab
Test tubes	Joanlab
Test tube rack	Joanlab
Erlenmeyer flask	Joanlab
Burette	Joanlab
Stirring rod	Joanlab
Round-bottom flask (100 mL)	Joanlab
Reflux condense	Joanlab
Graduated burette (50 mL)	Joanlab
Magnetic stirrer with heating plate	Joanlab
Beakers	Joanlab
UV-Vis spectrophotometer	UVmini-1240

I-3-1Refractometer:

A refractometer is an instrument used to measure the refractive index of liquids and transparent materials. It is widely used in chemical and food applications to determine the refractive index

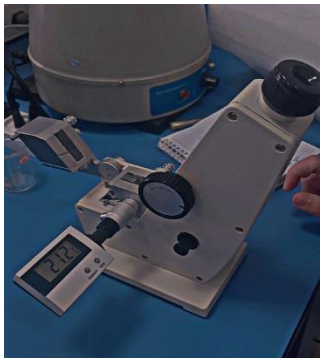


Image07: refractometer

I-3-2 UV-Vis spectrophotometer for absorbance measurement:

UV-Vis spectrophotometry is a powerful analytical technique used in various scientific fields to measure the absorbance of light in the ultraviolet (UV) and visible (Vis) domains of the electromagnetic spectrum. By measuring the intensity of the light passing through a sample solution and comparing it with the intensity of the incident light, a UV-Vis spectrophotometer provides valuable information on the properties of materials and their interactions with light. This blog post examines the components, operating principles, applications and advantages of UV-Vis spectrophotometry.



Image08: spectrophotometer

Table 11: Instruments and Equipment Used

Instrument / Device	Function

Electronic balance	Accurate measurement of material quantities
Magnetic stirrer	Stirring liquid components with a magnetic bar
Magnetic bar	Rotates inside the liquid to enable mixing
Mechanical stirrer	High-speed or prolonged stirring of mixtures
Glass beakers	Containing and preparing liquid mixtures
Glass rod	Manual stirring of components
Dropper	Adding precise drops (e.g., ethanolamine)
Graduated cylinder	Measuring exact volumes of liquids
Distilled water bottle	Source of pure water for aqueous phases
Storage containers	Holding the final product after preparation

II-Biological materials

The antibacterial effects of thyme, rosemary, and fennel essential oils were evaluated using the disc diffusion method. This assay helps assess the ability of oils to inhibit bacterial growth around impregnated discs.

Table 12: Biological materials and Equipment

Materials
Essential oils (thyme, rosemary, fennel)
Active bacterial cultures (e.g., E. coli, S. aureus)
Nutrient agar or Muller-Hinton agar
Sterile paper discs (6 mm)
DMSO or ethanol (as solvents)
Reference antibiotic (e.g., amoxicillin)
Distilled water
Equipment
Sterile Petri dishes

Test tubes
Micropipettes
Disc dispenser or sterile tweezers
Bunsen burner
Autoclave
Refrigerator
Incubator (37°C)
Sterile cotton swabs

II-1 Experimental protocols:

II-1-2-Essential Oil Extraction Protocol

Opening Statement:

Essential oils were extracted from the selected plant materials (Thymus vulgaris, Rosmarinus officinalis, and Foeniculum vulgare) using the hydrodistillation method, in order to carry out various physicochemical, biological, and formulation-related analyses. The extracted oils were later used in the preparation of a topical cream prototype.

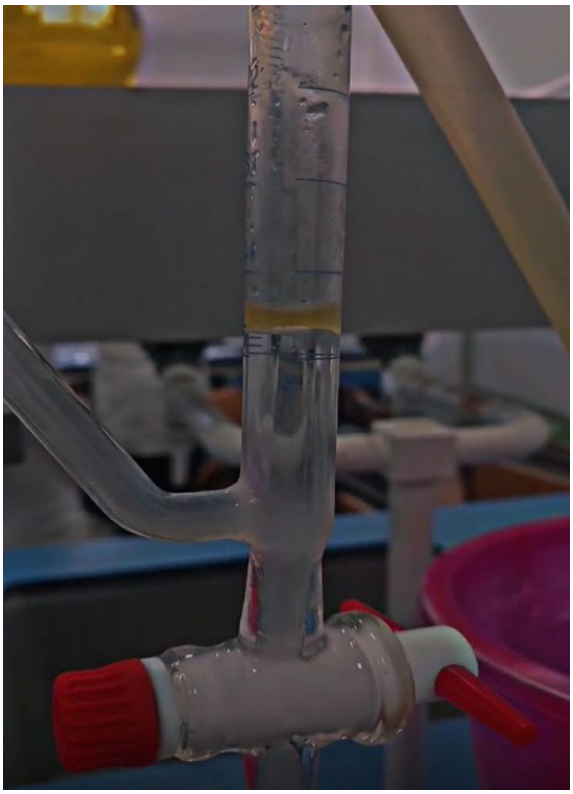


Image09: extract oil in the laboratory

Procedure:

- Each plant material (thyme, rosemary, fennel) was coarsely ground using a clean electric grinder, avoiding heat generation during the process.
- The ground plant material was placed in an appropriately sized round-bottom flask and completely submerged in distilled water.
- The Clevenger apparatus was assembled and securely connected to the condenser.
- The mixture was gradually heated using a water bath until boiling, and hydrodistillation was maintained for approximately 3 hours per sample, until oil collection ceased.
- The essential oil was separated from the aqueous phase and carefully collected from the Clevenger oil separator tube.
- The extracted oil was dried by placing it in contact with anhydrous sodium sulfate for 24 hours inside a sealed container.
- After drying, the oil was filtered and stored in dark, airtight glass bottles at a low temperature (approximately 4°C) until further use in analytical procedures or formulation testing.

II-2 Physical Analysis of Essential Oils

II-2-1-Refractive Index Measurement

Purpose:

To assess the purity and homogeneity of the essential oil by comparing its optical behavior to standard references.

- Allow the oil sample to reach room temperature (approx. 20°C).
- Place a drop of oil on the surface of the refractometer.
- Close the lid and wait 30 seconds for thermal equilibrium.
- Record the refractive index value.
- Repeat the measurement three times and calculate the average.

If the measurement was not performed at 20°C, apply the correction formula:

$$\eta_D^{20} = \eta_D^{t'} + \frac{0,0004}{\frac{t'}{20}} \qquad (1)$$

Where:

η_D^{20} : Corrected refractive index at 20°C

$\eta t'_D$: Measured refractive index at the current temperature

t': Temperature during measurement Notes:

Clean the refractometer lens thoroughly after each sample.

Avoid direct light during measurement.

II-2-2 Density Measurement

Purpose:

To determine the physical integrity and identity of the oil sample, supporting its standard classification.



Image10: pycnometer with fennel oil

Protocol Steps:

- Weigh the empty pycnometer.
- Fill it carefully with oil (no air bubbles), seal it.
- Weigh the filled pycnometer.
- Calculate density using:

$$\rho = \frac{m}{v} \qquad (2)$$

Where:

ρ: density (g/cm³)

m : oil mass = (filled – empty weight)

V: pycnometer volume

- Repeat three times and take the average.
- Record values at ambient temperature.

Notes:

- The pycnometer must be dry and clean.
- Results should be compared to standard reference values for each oil.

II-2-3 Ethanol Solubility Test

Purpose:

To evaluate the oil's compatibility with ethanol, which is important in pharmaceutical and cosmetic formulations.

Protocol Steps:

- Add 1 mL of essential oil to a clean test tube.
- Add 5 mL of 70% ethanol gradually, shaking gently.
- Observe the mixture (full solubility, partial, or phase separation).
- Repeat using 95% ethanol.
- Record results for each oil sample.

Notes:

- Solubility may vary between ethanol concentrations.
- Avoid strong agitation to reduce foam formation. III- Chemical Characteristics of

Essential Oils

III-1 Acid Value(IE):

Purpose:

To assess the amount of free fatty acids present in the oil, which indicates its quality and oxidative state.

Procedure:

- Sample Preparation:

  Weigh 0.5 g of essential oil accurately and transfer it to an Erlenmeyer flask.
- Adding the Solvent:

  Add 25 ml of ethanol to the sample and mix gently to ensure homogeneity.
- Adding the Indicator:

  Add 5 drops of phenolphthalein solution as a color indicator.
- Titration Process:

  Fill the burette with potassium hydroxide (KOH) solution (0.1 mol/L).

  Gradually add the KOH solution while continuously stirring until the color changes to a stable light pink, indicating the neutralization point ➢ Recording the Volume:

  Record the volume of KOH used to reach the endpoint.

  We applied to 3 types of oil and the results were as follows

III-2 Saponification Value (IS):

Purpose:

To determine the average molecular weight of fatty acids in the oil by measuring the amount of alkali required to saponify them.

Procedure:

- Introduce 0.5 g of the essential oil into a round-bottom flask.
- Add 25 mL of alcoholic KOH solution (0.5 mol/L).
- Heat the mixture under reflux for 1 hour.
- Allow the mixture to cool to room temperature.
- Add 20 mL of distilled water and 5 drops of phenolphthalein.
- Titrate the excess KOH with HCl solution until the pink color disappears.
- Record the HCl volume used (V) and compare with blank (V_0).

Calculate:

III-3 Saponification index (IS):

$$IS = \frac{28,05 * \frac{v_0}{v}}{m} \qquad (3)$$

V0= 35ml (blank test)

V = volume of HCl used with the essential oil m=

0.5g (sample mass)

III-3 Ester Value(IE) Purpose:

Indicates the amount of esterified fatty acids, calculated as the difference between saponification and acid values.

Calculate:

$$IE = IS - IA \qquad (4)$$

Ester value=Saponification value−Acid value

IV- GC-MS Protocol for Essential Oils Analysis

GC-MS analysis was performed to identify the volatile chemical compounds present in the essential oils of thyme, rosemary, and fennel. This method allows for detailed profiling of bioactive constituents.

Table 13:materials and equipment for GC-MS protocol

Materials
Essential oils (Thymus vulgaris, Rosmarinus officinalis, Foeniculum vulgare)
n-Hexane or high-purity ethanol (as solvent)
Internal standard (e.g., toluene, if applicable)
Equipment
GC-MS system (Gas Chromatograph coupled with Mass Spectrometer) SHIMADZU GCMS
GC sample vials with caps
Micropipettes
Data analysis software (e.g., NIST or Wiley Library)

Procedure:

- Sample Preparation:

Dilute each oil at 1:100 ratio using hexane or ethanol (e.g., 10 µL oil + 990 µL solvent).

Transfer to GC vials and seal properly.

- GC-MS Conditions:

Column: capillary (e.g., HP-5MS)

Carrier gas: helium, 1 mL/min

Initial temperature: 60°C → ramped to 250°C at 3°C/min

Injector temperature: 250°C

Injection volume: 1 µL

Injection mode: split or splitless

Total runtime: ~40–60 minutes MS

Detection Settings:

Ionization mode: EI (Electron Impact)

Scan range: 50–550 m/z

Acquisition time: per method setup ➢

Data Analysis:

Identify compounds by comparing mass spectra against a reference library (e.g., NIST).

Report retention times, molecular formulas, and relative peak areas.

V-DPPH Antioxidant Activity Assay V-1

Introduction:

The antioxidant potential of thyme, rosemary, and fennel essential oils was evaluated using the DPPH method due to its sensitivity and simplicity in assessing free radical scavenging activity. BHT was used as a positive reference compound.

V-2 Procedure:

V-2-1 Preparation of DPPH Solution:

A quantity of 4 mg of DPPH was dissolved in 100 ml of ethanol. The solution was stirred using a magnetic stirrer for 30 minutes in the dark to ensure complete dissolution.Sample Preparation:

BHT: 5.5 mg of BHT was dissolved in 5 ml of ethanol to prepare a solution with a concentration of 5×10^{-3} mol/L.

Essential oils: 0.5 g of each essential oil was diluted in 5 ml of ethanol, resulting in a concentration of 100 mg/ml.

V-2-2 Preparation of Working Solutions

Different volumes of the stock solutions were taken as follows:

0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml for both BHT and the essential oils.

Table 14: represent different volumes of the stock solution (EO, BHT)

V(EO)	0.05	0.1	0.12	0.2	0.4	0.6	0.8	1
V(BHT)	0.05	0.1	0.12	0.2	0.4	0.6	0.8	1

Each volume was adjusted to a final volume of 1 ml using ethanol. Subsequently, 1 ml of the DPPH solution was added to each tube.

Table 15: represent different volumes of the stock solution (EO, ethanol, UI, DPPH)

V(EO) ul	0.05	0.1	0.12	0.2	0.4	0.6
V(ethanol)	0.95	0.9	0.88	0.8	0.6	0.4
Ul	1	1	1	1	1	1
V(DPPh)	2	2	2	2	2	2

V-2-2Reaction Conditions:

One milliliter of each working solution was mixed with 1 ml of DPPH solution. The tubes were then kept in the dark for 30 minutes to allow the reaction to proceed under light-protected conditions. The absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer

V-2-3 Measurement:

Measure absorbance at 517 nm using a spectrophotometer.

Use a blank sample (DPPH + ethanol only) as control.

V-2-4 Calculation of Radical Scavenging Activity:

$$\text{Inhibition (\%)} = [\,[A(\text{blank}) - A(\text{sample})\,] \div A(\text{blank})\,] * 100 \quad \textbf{(5)}$$

VI-Antibacterial Activity Assay of Essential Oils

VI-1 Introduction:

The antibacterial effects of thyme, rosemary, and fennel essential oils were evaluated using the disc diffusion method. This assay helps assess the ability of oils to inhibit bacterial growth around impregnated discs.

VI-2 Procedure:

Oil Preparation:

Dilute oils in DMSO or ethanol to a fixed concentration (e.g., 10%).

Plate Inoculation:

Inoculate agar plates uniformly using sterile cotton swabs dipped in bacterial suspension.

Disc Preparation:

Soak sterile paper discs in the oil solution for 1–2 minutes and allow slight drying under aseptic conditions.

Disc Placement:

Place oil-loaded discs onto the inoculated agar using sterile tweezers. Add negative control discs (solvent only) and positive control discs (reference antibiotic).

Incubation:

Incubate plates at 37°C for 24 hours.

Results Reading:

Measure the diameter of inhibition zones around each disc in millimeters.

Evaluation:

- A larger inhibition zone indicates stronger antibacterial activity.
- Results are compared to control discs to confirm significance.

VII-Formulation:

VII-1 Introduction

In the pursuit of safer and more effective skin care formulations, the use of natural plant-based oils has gained significant attention due to their therapeutic properties and biocompatibility with the skin. This experiment aims to formulate a topical emulgel using three medicinal essential oils: fennel seed oil (Foeniculum vulgare), rosemary oil (Rosmarinus officinalis), and thyme oil (Thymus vulgaris).

These oils were selected for their well-documented antibacterial, anti-inflammatory, and antioxidant properties, which make them promising candidates for treating various skin conditions.

The main objective is to develop a stable emulgel with desirable cosmetic and therapeutic properties, using a carefully optimized formulation protocol

Table 16: Material and their Function in the Formulation

Material	Function in the Formulation
Fennel oil	Anti-inflammatory, soothing, smoothens skin and reduces irritation

Rosemary oil	Antioxidant, stimulates circulation, promotes skin regeneration
Thyme oil	Antimicrobial, antifungal, beneficial for acne-prone skin
Liquid Vaseline	Emollient and occlusive, prevents transepidermal water loss
Tween 80	Non-ionic surfactant, helps emulsify oil and water phases
Span 80	Co-surfactant, stabilizes emulsion
Xanthan gum	Gelling agent, provides viscosity and texture
Aloe vera gel	Soothing agent, moisturizes and accelerates wound healing
Vitamin E	Antioxidant, protects skin and prevents oxidation of oils
Distilled water	Solvent and medium for the aqueous phase
Ethanolamine	pH adjuster to ensure skin compatibility

Part three :Results and discussion:

In this part of the research, we will talk about the results we obtained from all the analyses we conducted for the three oils (rosemary, fennel seeds, and thyme), and we will discuss them and compare them with previous studies to confirm the effectiveness of the results obtained.

I-Yield of extraction

Tabel 17 :yield of extraction

Plant name	Plant quantity used (g)	Oil remaining (ml)	N of extractions	Actual yield (%)
Fennel	2400	16.1	28	0.67
Thyme	940	17.00	24	1.81
rosemary	3690	5	56	0.13

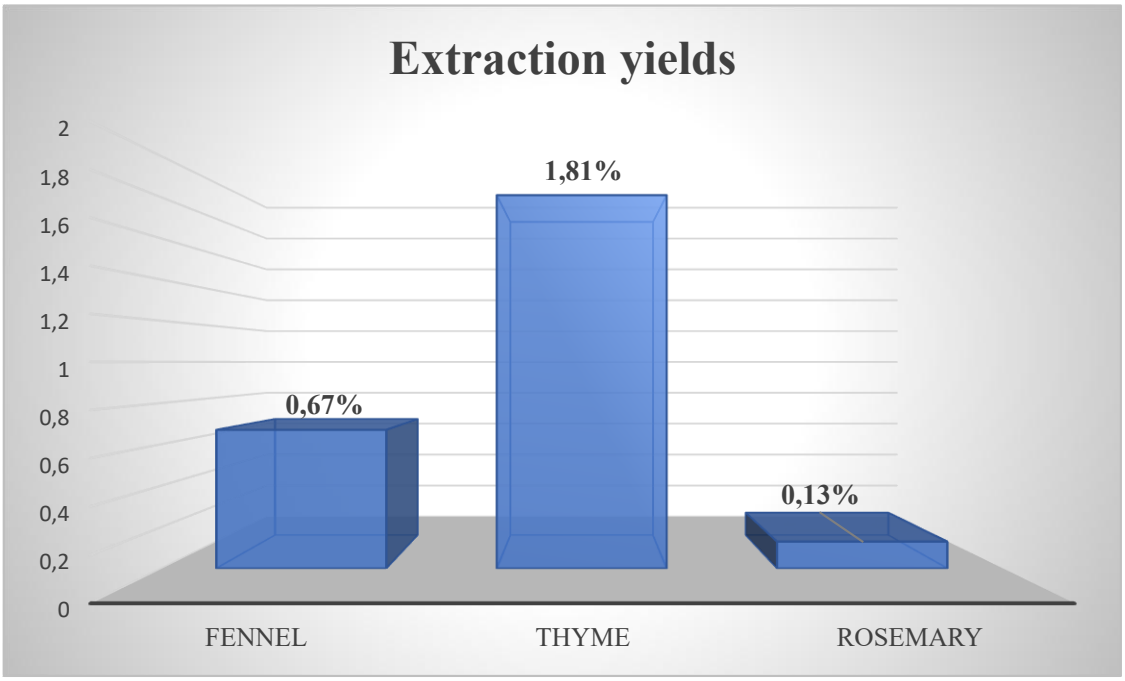


Image11: The quantities of oil extracted in a single process from plants

II-Interpretation:

The table above summarizes the quantitative data related to the extraction of essential oils from three medicinal plants: fennel (Foeniculum vulgare), thyme (Thymus vulgaris), and rosemary (Rosmarinus officinalis). The results reveal a significant variation in both the total and actual yields of essential oils, as well as in the rate of oil loss during the extraction process.

The highest gross yield was obtained from thyme, reaching 3.14%, followed by fennel at 0.99%, while rosemary exhibited the lowest yield with only 0.203%, despite being used in the largest quantity (3690 g). This low yield may be attributed to the intrinsic characteristics of the rosemary plant, including its lower essential oil content or reduced responsiveness to the extraction method applied.

Regarding the actual yield (the amount of oil effectively collected and retained), thyme again showed the highest value (1.81%), compared to 0.67% for fennel and 0.13% for rosemary. These values reflect the real efficiency of oil recovery from each plant material.

In terms of loss rate, thyme recorded the highest loss at 42.4%, followed by rosemary (33.3%) and fennel (32.6%). Such losses could be due to evaporation of volatile components, adherence of oil to the apparatus, or inefficiencies in the condensation and collection stages.

When considering the number of extractions performed, thyme also showed the highest average oil output per extraction (1.23 ml/extraction), compared to 0.85 ml for fennel and 0.13 ml for rosemary. This further confirms thyme's superior performance in essential oil production under the experimental conditions used.

These results highlight the critical role of plant selection in essential oil extraction processes. The considerable differences in oil yield and loss across the tested species underscore the need for optimizing both the choice of botanical material and the extraction parameters to enhance efficiency and ensure cost-effectiveness.

II-1Organoleptic characteristics:

Table 18: represent the Organoleptic characteristics for the three oils

oils	Appearance	color	aroma

Thyme	Clear appearance, a yellow to golden color, a light texture, and the absence of any visible residues.	Yellowishgolden	Strong and herbaceous
Fennel	Clear appearance, a yellowish-green color, a light non-greasy texture, and is free from impurities or residues.	Yellowishgreen	Sweet and mild
rosemary	Clear appearance, a golden yellow color, and a light texture, free from any residues	Golden yellow	Refreshing and camphoraceous

II-2 Interpretation of Observed Physical and Sensory Properties of Essential Oils:

The essential oils extracted from Thymus vulgaris (thyme), Foeniculum vulgare (fennel), and Rosmarinus officinalis (rosemary) exhibited distinct physical characteristics and aromatic profiles, reflecting both their botanical origins and the efficiency of the extraction process.

All three oils presented a clear appearance and absence of visible residues, indicating successful separation from aqueous phases and plant debris during distillation. This clarity suggests that the oils were free from emulsified water, suspended particles, or post-extraction contamination, which is crucial for maintaining stability and efficacy.

In terms of color, thyme oil showed a yellow to golden hue, fennel oil displayed a yellowishgreen tint, while rosemary oil exhibited a golden yellow coloration. These color differences may be attributed to variations in the chemical composition of each oil, including the presence of pigments, phenolic compounds, and oxygenated monoterpenes. The yellowish-green tone observed in fennel oil, for instance, may result from its higher content of anethole or other green-tinted aromatic compounds.

Regarding texture, all oils were described as light and non-greasy, which is characteristic of high-quality essential oils. This property facilitates their application in aromatherapy, topical formulations, and pharmaceutical preparations without leaving heavy or sticky residues on the skin.

The aromatic profile of each oil was distinctive and consistent with known characteristics of the plant species:

Thyme oil was noted for its strong and herbaceous scent, typically attributed to compounds such as thymol and carvacrol, which are also responsible for its antimicrobial and antioxidant activities.

Fennel oil exhibited a sweet and mild aroma, often associated with anethole, which provides a pleasant licorice-like scent and is known for its soothing properties.

Rosemary oil had a refreshing and camphoraceous fragrance, linked to the presence of camphor, 1,8-cineole, and borneol — compounds recognized for their stimulant and respiratory benefits.

These organoleptic observations, when combined with quantitative and chromatographic analysis, serve as valuable indicators of oil quality, chemical integrity, and potential therapeutic applications.

III- **Physical Analysis of Essential Oils:**

III-1 **refractive index:**

Table 19: represent the refractive index

oil	**Refractive index**
Thyme oil	1.506
Fennel oil	1.520
Rose mary oil	1.477

III-2 **Comparative Analysis of Essential Oil Quality:**

The refractive index is a key parameter in assessing the quality and purity of essential oils. In this analysis, we compared three types of essential oils: thyme,rosemary and fennel seed oils.

Thyme Oil :

The measured refractive index values for thyme oil were consistent, indicating high purity and uniformity. These values align well with standard reference values, suggesting that the oil has been distilled and preserved correctly.

Fennel Seed Oil :

Similarly, the refractive index values for fennel seed oil showed stability and consistency. This reflects good quality and compliance with typical quality standards for this type of essential oil.

Rosemary oil :

Rosemary refractive index results are within the standards set for rosemary oil at 24 degrees ,meaning the oil is fairly good with a good purity.

Determination of Oil Density Using a Pycnometer:

Table 20: represent the results of oil density

Oil	m (water)	m (pycno)	m (oil)	ρ (water) g/ml	ρ (oil) g/ml
Thyme	9.71	13.344	9.19	0.998	0.9447
Fennel	9.71	13.344	9.419	0.998	0.9682
Rosemary	9.71	13.344	7.380	0.998	0.7586

Notes:

-We must accurately record the temperature

-Water density at 20°C is 0.9982 g/ml

-We compare the result to standard oil values

III-3 Miscibility test in ethanol:

Results**:**

Table 21: represent the results of Miscibility test in ethanol

oil	**Time duration (min)**	**Volume of ethanol (ml)**	**Notes**
Thyme	4.43	20.6	mixes quickly with ethanol, indicating the presence of polar or easily soluble compounds. This suggests a composition that allows for rapid interaction with organic solvents.

Fennel	5	29	takes longer to mix and requires a larger volume of ethanol to achieve full miscibility. This may indicate a higher viscosity or the presence of non-polar compounds, making it less reactive with ethanol compared to thyme oil.
	7.40	28	Rosemary essential oil shows good solubility in ethanol, which suggests a
Rosemary			balance in its components between polar and non-polar compounds. This indicates that the oil is relatively pure and of high quality.

Conclusion:

- ✓ Through the experiment we conducted with thyme, rosemary and fennel oils using ethanol as a solvent, we observed notable differences in the rate of miscibility and the volume of ethanol required for each oil.
- ✓ Thyme Oil mixed quickly with ethanol, indicating that it contains more polar or lightweight compounds that easily dissolve in ethanol. This suggests the purity of the oil and its composition with reactive components towards organic solvents.
- ✓ Fennel Oil took longer and required a larger volume of ethanol to achieve full miscibility, indicating that it contains more viscous or non-polar compounds, making the miscibility more difficult.
- ✓ Rosemary oil took longer than other oils to dissolve with ethanol, but the amount of ethanol was acceptable, indicating a balance in the components of the oil and a high degree of purity.
- ✓ Through this experiment, we can conclude that each oil has specific chemical properties that influence its interaction with organic solvents like ethanol. These results enhance our understanding of the compositions of essential oils and how their components affect their reactivity with solvents, which is crucial for various pharmaceutical and industrial applications.

IV-Chemical Characteristics of Essential Oils

Table 22: the resuits of chemical characteristics of essential oils

Essential Oil	Acid index	Saponification index	Ester index
Thyme	12.34	561	548.66
Fennel	4.49	897.6	893.11
rosemary	13.464	785.4	771.936

- Thyme and rosemary oils show a high acid index (12.34), (13.46) which may indicate the presence of a significant amount of free acids, possibly due to oxidation or degradation. Fennel oil has a low IA (4.49) compared to the other two oils, suggesting better chemical stability.
- Fennel and rosemary oils show a higher IS than thyme oil, suggesting a greater proportion of esterified compounds (fatty acid esters or aromatic esters).
- The high IE in fennel oil (893.11) confirms its richness in esters, which likely contribute to its aromatic profile.
- Rosemary oil also has a high IE (771.936) which indicates that it is also rich in esters ,as it belongs to the aromatic family.
- On the other hand, thyme oil, while rich in active compounds, contains fewer esters (IE = 548.66), which may affect its sensory or medicinal properties.

Conclusion:

Fennel and rosemary oils are chemically more stable and richer in esters, making it more suitable for cosmetic or aromatic uses. Thyme oil, despite its higher acidity, may still be valuable for medicinal purposes due to its bioactive components.

V-Résultats des analyses GC/MS

V-1 Chemical composition of thyme essential oil

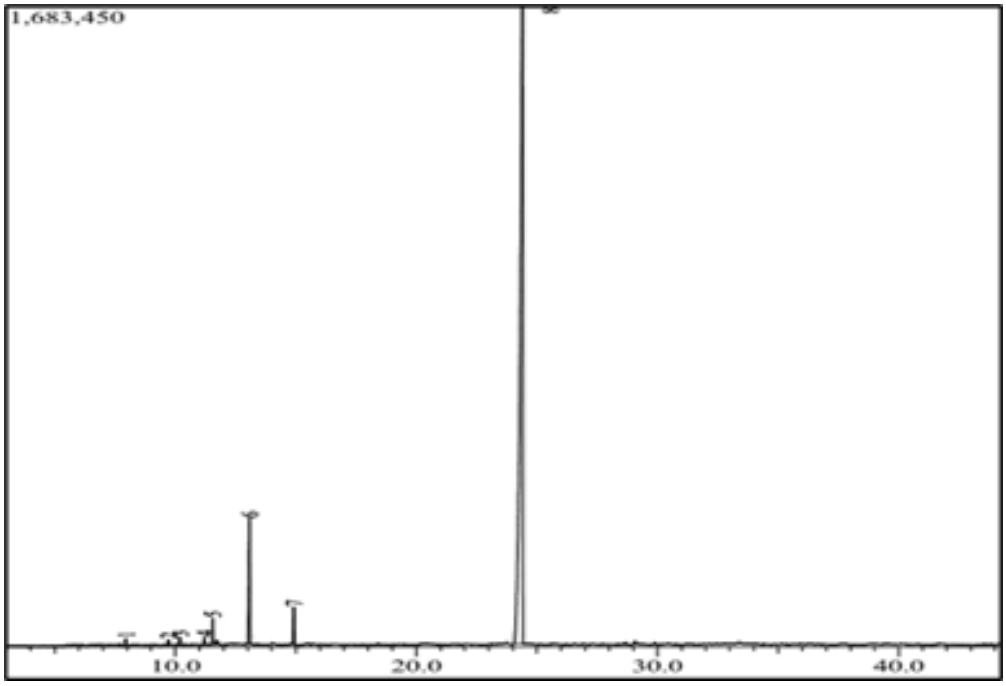


Image12: Chromatogram of thyme essential oil

Table23: Chemical composition of the thyme essential oil

N°	Compounds	Retention time (min)	% Content
1	α-pinene	7,95	0,34
2	Octene-3-ol	9,71	0,33
3	β-myrcene	10,19	0,40
4	γ-terpinene	11,21	0,48
5	p-cymene	11,55	1,46
6	β-ocimene	13,08	7,08
7	Linalool	14,92	2,39
8	**Thymol**	**24,39**	**87,52**

V-1-2 Interpretation of the Chemical Composition of the Essential Oil:

The table above presents the chemical composition of the essential oil as determined by gas chromatography analysis. Eight volatile compounds were identified, showing significant variation in their relative concentrations within the oil sample.

The most abundant compound was thymol, accounting for 87.52% of the total oil content. Thymol is well known for its potent biological properties, particularly its strong antimicrobial and antifungal activities, as well as its antioxidant potential. Its dominance in the composition confers powerful therapeutic characteristics to the oil and enhances its applicability in both pharmaceutical and cosmetic formulations.

The second most prominent compound was β-ocimene, present at 7.08%, a fragrant monoterpene associated with floral and herbal aromas. β-ocimene also contributes to the oil's biological profile through its reported anti-inflammatory and antiviral properties.

Other compounds such as linalool (2.39%) and p-cymene (1.46%) were identified as minor constituents, yet they significantly influence the overall aroma profile and may support the biological activity of the oil through synergistic interactions with major components.

Additional compounds were present in trace amounts below 1%, including γ-terpinene, βmyrcene, α-pinene, and Octene-3-ol. Despite their low concentrations, these molecules may play subtle roles in enhancing the fragrance and overall functional complexity of the oil.

Overall, the results indicate that the essential oil is chemically characterized by a dominant bioactive constituent—thymol—supported by several minor compounds that contribute to its sensory qualities and potential therapeutic effects. This composition suggests a high-value essential oil suitable for natural medicinal and therapeutic applications. V-2 Chemical composition of fennel essential oil:

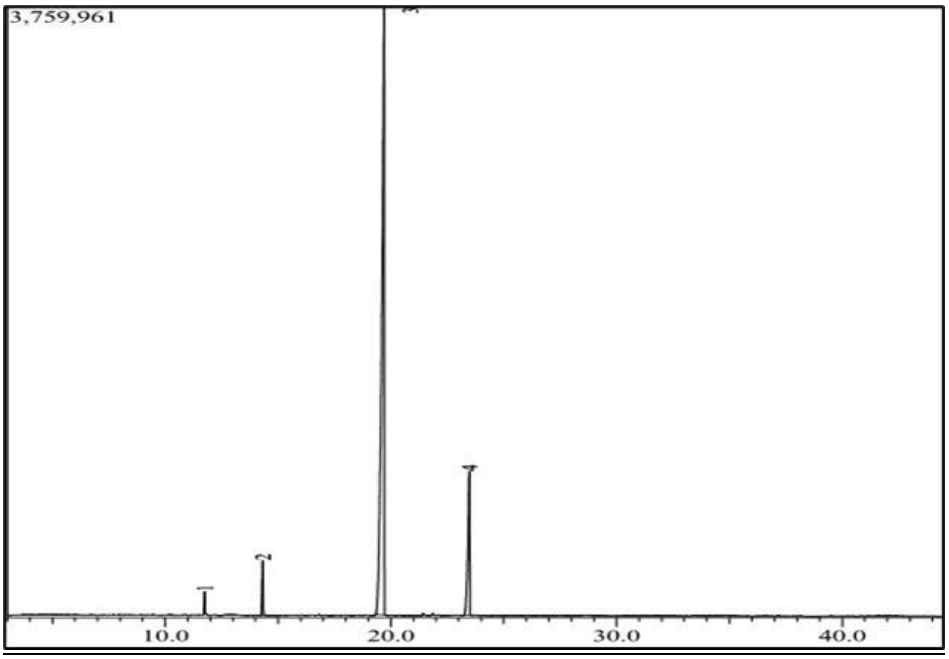


Image13: Chromatogram of the fennel essential oil

Table 24 : Chemical composition of the fennel essential oil

N°	Compounds	Retention time (min)	% Content
1	Limonene	11,75	1,12
2	Fenchone	14,32	3,24
3	**Estragole**	**19,33**	**81,04**
4	Anethole	19,7	14,6

V-2-1 Interpretation of the Chemical Composition of Fennel Essential Oil:

The table above presents the chemical profile of fennel essential oil, as determined through gas chromatography analysis. Four major volatile compounds were identified, showing distinct variations in their concentrations.

The dominant compound is estragole, which accounted for 81.04% of the total oil content. Estragole is a phenolic compound known for its characteristic sweet and aromatic scent, widely used in flavoring and perfumery industries. It has been reported to possess sedative and antispasmodic properties.

The second most abundant compound is anethole (14.6%), another key aromatic component typical of fennel essential oil. It significantly contributes to the oil's sweet fragrance and is recognized for its antioxidant and anti-inflammatory activities.

Other minor constituents include fenchone (3.24%) and limonene (1.12%). These compounds enhance the overall aromatic complexity of the oil. Limonene, in particular, is associated with a refreshing citrus scent and is known for its antioxidant and stimulating effects.

Overall, the results indicate that fennel essential oil is chemically characterized by a dual dominance of estragole and anethole, supported by secondary components that contribute to both its sensory appeal and therapeutic potential. This chemical balance makes the oil suitable for use in natural medicinal and aromatic formulations.

V-3 Chemical composition of Rosmarinus officinalis essential oil

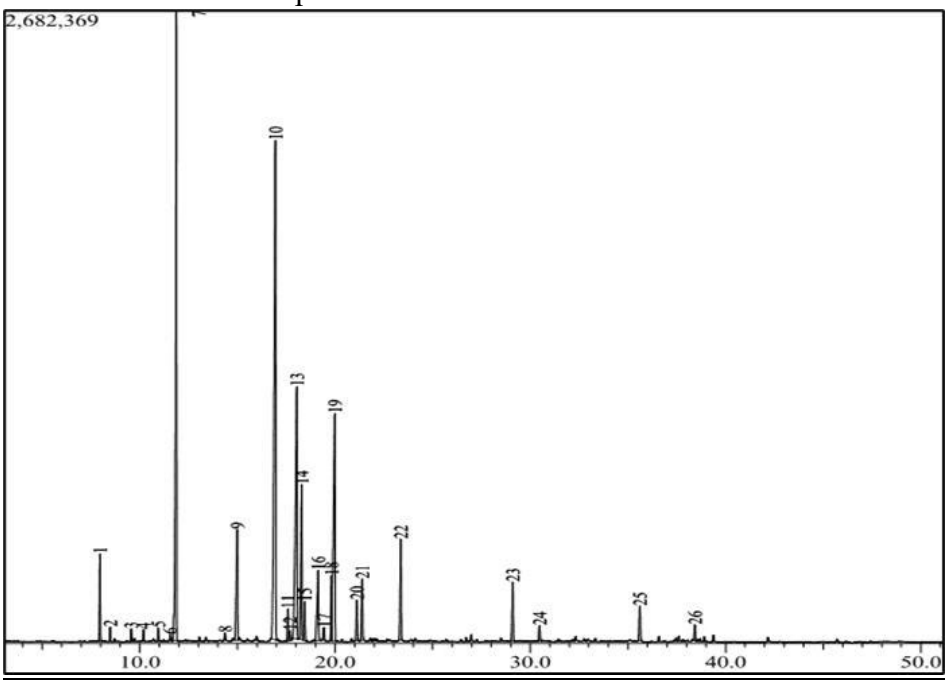


Image14 : Chromatogram of the rosemary essential oil

Table 25: Chemical composition of the rosemary essential oil

N°	Compounds	Retention time (min)	% Content
1	α-pinene	7,96	1,94
2	Camphene	8,48	0,35
3	β-pinene	9,55	0,34
4	β-myrcene	10,19	0,28
5	Phellandrene	10,94	0,35
6	p-cymene	11,55	0,07

7	1,8-cineol	11,87	17,84
8	Terpinolene	14,36	0,22
9	Linalool	14,99	4,55
10	**Camphor**	**16,94**	**23,26**
11	Iso-pinocamphone	17,59	1,04
12	Pinocarvone	17,68	0,30
13	Borneol	18,04	13,40
14	Verbenol	18,29	5,63
15	Terpinen-4-ol	18,44	1,14
16	α-terpineol	19,13	3,41
17	Fenchol	19,42	0,51
18	4-(1,2-dimethyl-2-cyclopenten-yl) -2butanone	19,82	2,26
19	D-verbenone	19,98	12,00
20	Carvone	21,11	1,41
21	Geraniol	21,39	2,21
22	Bornyl acetate	23,36	3,20
23	β-Farnesene	29,1	1,98
24	NI	30,47	0,51
25	Caryophyllene oxide	35,60	1,24
26	NI	38,43	0,54

V-3-1 Interpretation of the Chemical Composition of roemary Essential Oil:

The chemical analysis of rosemary essential oil revealed a total of 26 volatile compounds, with a composition dominated by camphor (23.26%), 1,8-cineole (17.84%), borneol (13.40%), and D-verbenone (12.00%), which together account for over 66% of the oil. These major constituents belong mainly to the ketone, oxide, and alcohol families, known for their antiseptic, anti-inflammatory, and cell-regenerating properties. In addition to these, the oil contains notable secondary compounds such as verbenol, linalool, α-terpineol, and bornyl acetate, which contribute to its aromatic profile and enhance its biological activity. The presence of a rare ketone, 4-(1,2-dimethyl-2-cyclopenten-yl)-2-butanone (2.26%), adds further complexity to the oil's composition. Overall, the richness and balance of oxygenated monoterpenes, alcohols, and

esters in rosemary essential oil support its traditional use in respiratory, dermatological, and aromatherapeutic applications.

VI-Comparative Composition Analysis of Essential Oils: This Study vs. Literature:

Thyme essential oil extracted in this project showed an exceptionally high concentration of thymol (87.52%), making it a nearly monocomponent oil. In contrast, previous studies on Thymus vulgaris typically report thymol levels ranging between 40–60%, accompanied by notable amounts of p-cymene, carvacrol, and linalool. The absence or minimal presence of these common secondary compounds in our extract suggests a distinct chemotype or a possible influence of local environmental conditions. This high thymol dominance may enhance the oil's antimicrobial potential, but also reflects a deviation from the more balanced compositions reported in literature.

Fennel essential oil obtained here is primarily composed of estragole (81.04%) and anethole (14.6%), which together constitute over 95% of the oil. This is largely in agreement with common fennel oil profiles described in the literature, though some studies report slightly lower estragole and higher anethole content, along with the presence of fenchone or other minor constituents. The near absence of fenchone in our sample suggests chemotypic or processing differences. Nevertheless, the overall composition maintains the characteristic sweet aromatic profile and potential digestive effects attributed to fennel oil.

Rosemary essential oil from this study exhibited a diverse chemical profile dominated by camphor (23.26%), 1,8-cineole (17.84%), borneol (13.40%), and D-verbenone (12.00%). While camphor and 1,8-cineole are commonly found as major components in rosemary oil according to previous reports, the elevated levels of D-verbenone and verbenol (5.63%) represent a distinctive feature of our extract. These compounds are typically present in trace amounts or even absent in standard chemotypes. This unique composition may be linked to specific environmental or botanical factors, and could enhance the oil's utility in respiratory or dermatological applications due to the synergy between oxygenated monoterpenes and ketones.

VII- **DPPH Antioxidant Activity Assay**

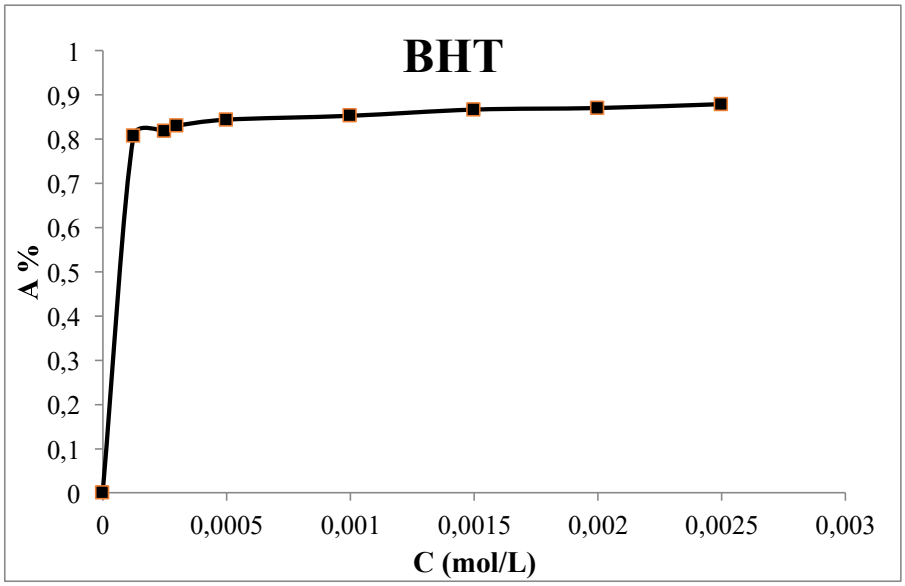


Image15 :represent DPPH Antioxidant Activity Assay of BHT

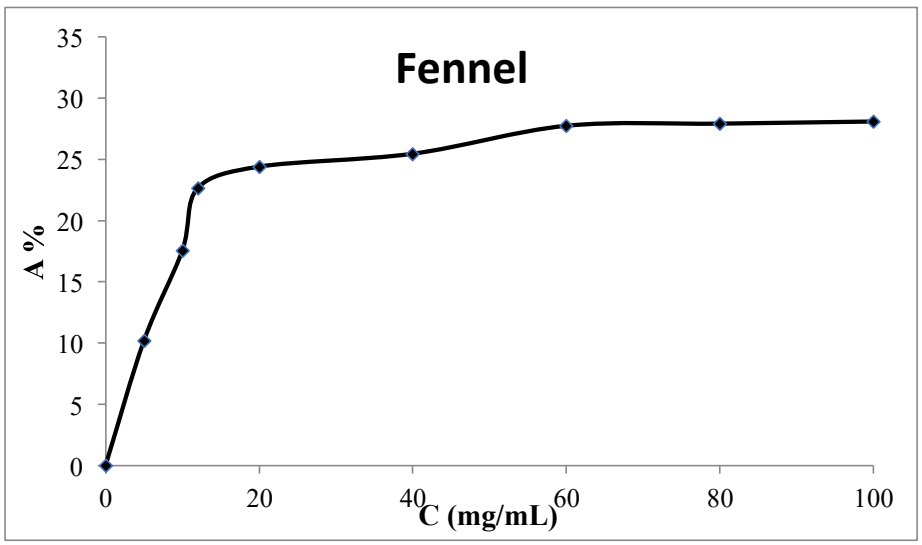


Image16: represent DPPH Antioxidant Activity Assay of fennel oil

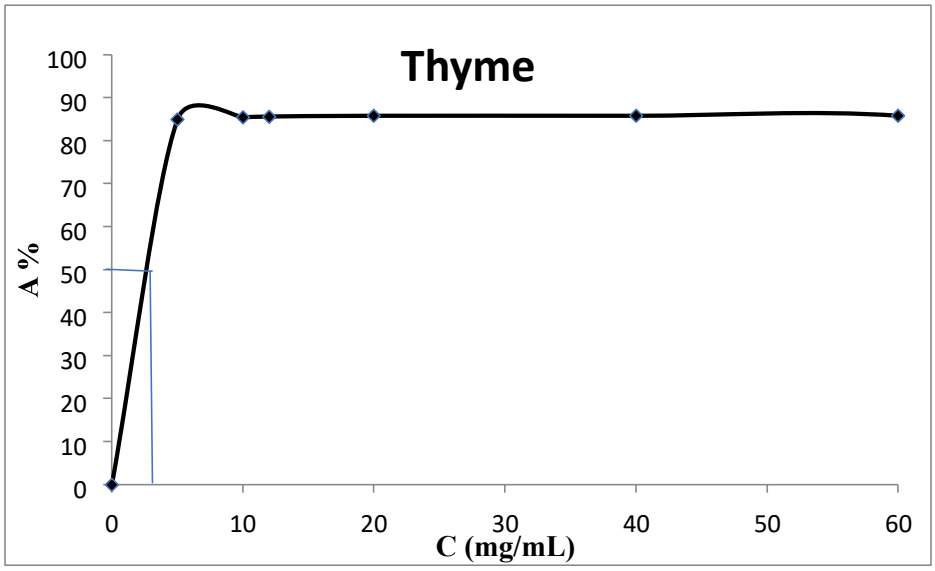


Image 17: represent DPPH Antioxidant Activity Assay of thyme oil

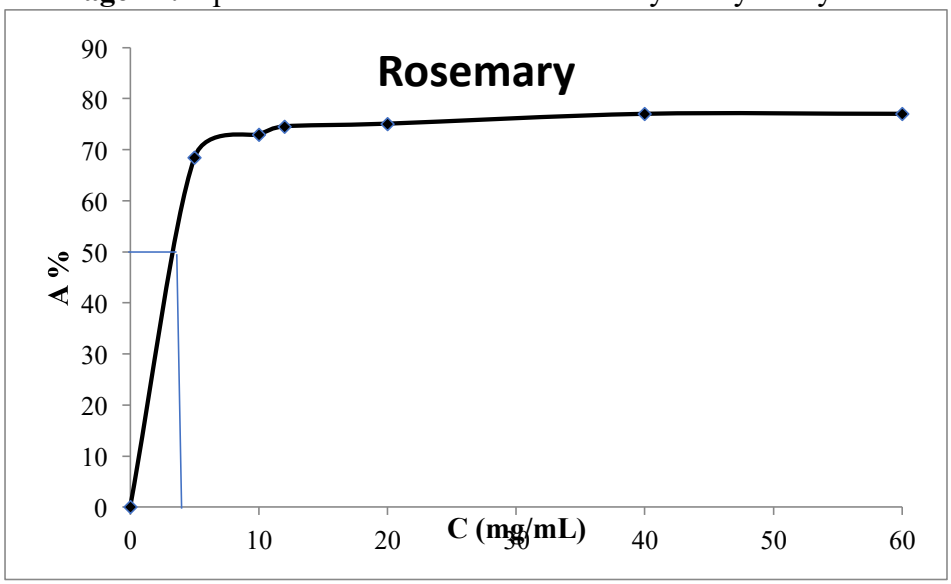


Image18: represent DPPH Antioxidant Activity Assay of rosemary oil

Table26 :Table of value EC

EO	Thyme	Rosemary	fennel
EC_{50} (mg/mL)	2,5	3,3	>100

VIII-Antibacterial Activity Table :

				Essential Oils			
				Rosemary	**Fennel**	**Thyme**	**Mixture(1:1:1)**
Bacteria	**Gram(+)**	**Staphylococcus aureus *ATCC 25923***	MIC(mg/ml)	1	2	0.25	0.125
			MBC(mg/ml)	2	4	0.5	0.25
			ID(mm)	15.22	12	22.01	25.90
		Sulfite-reducing Clostridium	MCI(mg/ml)	2	4	0.75	0.375
			MBC(mg/ml)	4	8	1.5	0.75
			ID(mm)	12.04	10	18.52	22
	Gram(-)	**Escherichia coli *ATCC 25922***	MCI(mg/ml)	1.5	3	0.5	0.25
			MBC(mg/ml)	3	6	1	0.5
			ID(mm)	13.1	10.0	10.05	23.45
		Pseudomonas aeruginosa *ATCC 27853*	MCI(mg/ml)	2	4	1	0.5
			MBC(mg/ml)	4	8	2	1
			ID(mm)	11	9.5	17.14	21.73
Fungi		**Aspergillus**	MCI(mg/ml)	1	2	0.5	0.25
			MBC(mg/ml)	2	4	1	0.5
			ID(mm)	14	11.2	20	23.95

Analysis of Results

The table presents a comprehensive assessment of the antimicrobial activity of four essential oils—rosemary, fennel, thyme, and an equal-parts blend (1:1:1)—tested against standard strains of Gram-positive bacteria (Staphylococcus aureus, Clostridium sulfito reducteur), Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and a fungal species (Aspergillus).

Thyme essential oil exhibited the most pronounced antimicrobial effect, characterized by the lowest values for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) across nearly all tested microorganisms, along with the largest Inhibition Zone Diameters (DI). This strong performance is attributed to its high content of bioactive compounds such as thymol and carvacrol, which are known to disrupt microbial membranes and interfere with essential enzymatic functions.

The mixture of the three essential oils outperformed each individual oil, suggesting a synergistic interaction that enhances antimicrobial efficacy. This synergy was especially evident against Staphylococcus aureus and Aspergillus, with inhibition zones reaching 25.90 mm and 23.95 mm respectively, accompanied by significant reductions in MIC and MBC values.

It is also notable that the oils were generally more effective against Gram-positive bacteria compared to Gram-negative ones. This difference can be explained by structural variations in the bacterial cell wall: the presence of an outer lipopolysaccharide membrane in Gram-negative bacteria acts as a barrier to the penetration of active compounds.

In conclusion, these findings highlight the promising potential of essential oils—particularly thyme and its combination with other oils—as natural antimicrobial agents suitable for use in pharmaceutical or cosmetic formulations for both preventive and therapeutic purposes.

IX-formulation properties

Table 27 : table of formulation properties

Property	Description
texture	Smooth, easy to spread, non-greasy, and quickly absorbed by the skin
color	Light creamy color, natural appearance without artificial dyes
PH	Balanced, around 6.2, skin-friendly and suitable for all skin types

IX-1 Stability Study for the Cream :

The stability study involves testing the cream's ability to maintain its physical, chemical, and microbiological properties during storage. This is done by keeping samples of the cream under different conditions of temperature, humidity, and light, and observing any changes in color, texture, smell, and pH level. It also ensures that there is no separation of ingredients or unwanted microbial growth. The results show that our cream remains stable and keeps its original quality when stored at cold and ambient temperatures, and even after one month it maintains the same texture, appearance, and effectiveness. The purpose of this study is to guarantee the quality, safety, and effectiveness of the cream throughout its shelf life, which is an essential step for approving and marketing the product with confidence.

To ensure the safety and tolerance of the formulated cream, a sensibility test was carried out on a small group of volunteers. A small amount of the cream was applied to a limited area of the skin, typically on the forearm, and observed for any signs of irritation, redness, or allergic reaction over a period of 24 to 48 hours. The results indicated that the cream was well tolerated by the skin, with no adverse reactions reported, confirming its suitability for topical application.

Chapter 03:

Guide startup

Information card

About the Working Group Leadership Team

1-Management team :

Management team (for information purposes only)	
Main superviser: LETTREUCH Hichem	Speciality : Process engineering

2-Project Team:

Project Team (for information purposes only)	Faculty	Speciality
Student 01 **:** Haroun maroua	Faculty of technology	Chemical engineering
Student 02: Atmania ismahane	Faculty of technology	Chemical engineering

Introduction:

In order to enable students with innovative projects to develop their work, and to facilitate the design and drafting of their patents and enable them to move from a simple idea to a complete

invention complete with details and information that clarify the innovative aspects of the project, its attractiveness and the integrity of the logical sequence of ideas, the national commission for coordination, monitoring of innovation and university incubators is developing this document which serves as a guide for the student to write their dissertation in accordance with the provisions of ministerial decree no. 1275 Diploma – Startup.

The necessary presentations and explanations in the guide are illustrated through a very simple example to facilitate understanding of the different steps. This example is related to a project for the production of a cream based on extraction of a mixture of some Algerian medicinal plants for therapeutic use.

First axis: Presentation of the project 1-The

project idea:

The project idea stemmed from a previous study we conducted in our third year of bachelor's degree, which focused on methods for extracting essential oils from medicinal plants. Therefore, we decided to continue with the idea by selecting three medicinal plants (rosemary, fennel seeds, and thyme) and developing the oil extraction component and using it to produce a cream that includes all the bioactive components found in the oils.

The main problem that prompted us to develop our own product[MI] is that all similar products on the market contain chemical ingredients. Our product has antibacterial and antioxidant properties that soothe the skin with natural, environmentally friendly ingredients.

2-The proposed values: the proposed

values can be the following :

- Rosemary essential oil provides antioxidant, , and antimicrobial benefits, helping to improve circulation and soothe acne or muscle soreness.
- Thyme essential oil is a powerful antimicrobial and antifungal agent that supports wound healing and helps control skin infections and acne.
- Fennel seed essential oil has detoxifying and anti-aging properties; it reduces puffiness, tones the skin, and may minimize the appearance of fine lines

• The cream has a smooth, creamy texture that is easily absorbed into the skin **3-Work team:**

The project team is composed of two members: Maroua and Ismahane. Together, we are responsible for the formulation, testing, and improvement of the cream made from natural oils. Maroua also leads the project and focuses on market research and marketing strategies, while Ismahane contributes to quality control and production planning to ensure the final product meets high standards.

Our work was an integrated team, from idea to completion, and this is what guarantees the success of any project.

4**. Goals of the project :**

Our goal is to create a natural, high-quality cream using selected oils, offering effective skincare while promoting eco-friendly and sustainable practices. We aim to meet market demand and add value to local resources.

We aspire to move from the prototype to the MVP(minimum viable product) stage, meaning that our product will be ready to be presented to investors. Therefore, we are seeking a recommendation from the incubator to continue developing the product and move to a semiindustrial scale.

5-Project implementation schedule:

			Mois ou semaines						
			1	2	3	4	5	6	7
1		Preliminary studies: choice of location of the production unit, preparation of the necessary cream documents						✓	
2		Equipment order		✓	✓				
3		Construction of a production headquarters (factory)		✓	✓	✓			

…		Installation of equipment			✓	✓	✓		
n		Purchase of raw materials						✓	
…		Production of the prototype							✓

Second axis: Innovative aspects:

1-Nature of innovations:

The nature of the innovations adopted in the project must be specified according to the proposals given in the following diagram :

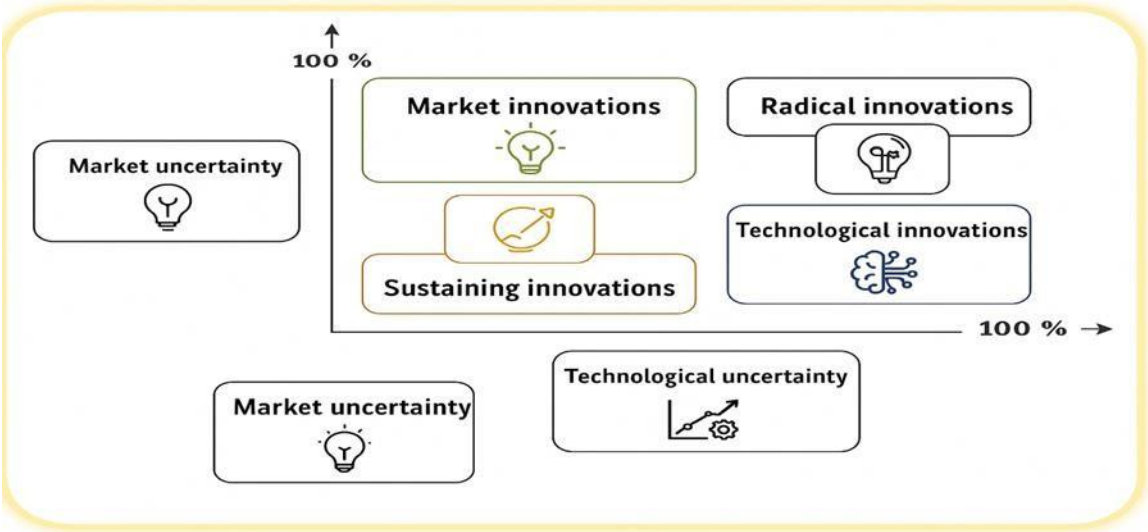


Image19 : Nature of innovations

2.Areas of innovation:

3.Overall, innovation can include the following areas:

- **New processes (increasing profitability by increasing operational efficiency).**
- **New features (offers improved products or services).**
- **New customers (offer the usual range of products or services for new customer segments).**
- **New offers (offering innovative products).**
- **New models (change of business model, adoption of a new "system" of value creation).**

- **Customizable cream bases where users can adjust essential oil blends based on skin type (e.g., oily, dry, sensitive).**
- **- Target a new category of consumers (who choose a cream that preserve the state of health)**
- **Our product is made from natural materials and is free of chemicals.**

Third axis: Strategic analysis of the market:

1-The market segment:

Potential Market: This is anyone who wants to consume natural products like our cream (they have no reason not to express the need to consume it).

Target Market: We are looking to provide natural cream to people who follow a specific skin car routine .

Most people now go for products with a natural background to lighten the skin for a long time.

2-Measuring the intensity of competition:

The most important competitors on the Algerian market, produce, for the most part cream with preservatives, and they are classified according to their market share as follows: Arvea, Santé Tech……..

Among their key assets, we can highlight their long-standing presence in the Algerian market and the strength of their brand, which has earned trust and recognition over the years..

. Among their weaknesses, we can mention concerns about product quality and the frequent use of chemical ingredients, which may not align with the growing consumer demand for natural and safer alternatives

3.The marketing strategy:

The market for our natural healing cream, made from essential oils like thyme, rosemary, and fennel, presents strong opportunities in both B2B (Business to Business.) and B2G (Business to Government) partnerships with our university. For the B2B market, the cream can be supplied to organic shops, beauty salons, wellness resorts, and distributors looking for high-quality, locally produced natural skincare products. This sector values eco-friendly

solutions and unique local brands, creating chances for bulk sales and long-term partnerships. Through our university, the cream can be developed, tested, and promoted as a safe, natural option for health centers, research projects, and student wellness initiatives, while also supporting local production and innovation. By meeting quality standards and working closely with the university's labs and programs,our cream can gain credibility, attract institutional support, and create opportunities for future collaborations. Together, these strategies strengthen our cream's competitive position and expand its reach to institutions, businesses, and end consumers alike

Fourth axis: Production plan and organization

1-The Production Process:

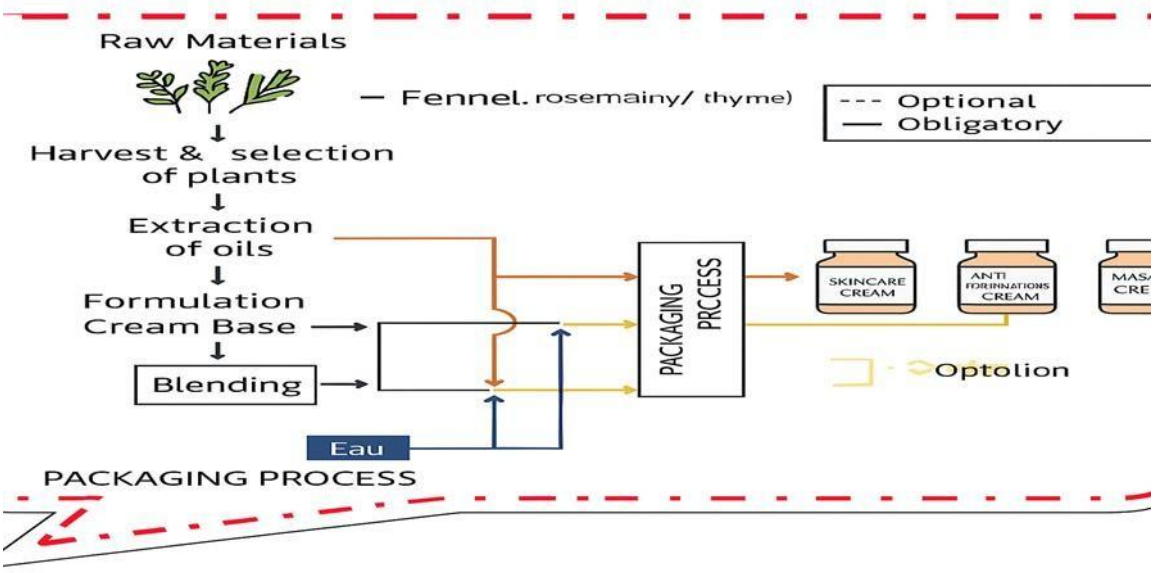


Image20: The Production Process of the cream

2-Supply:

In the purchase process, we deal directly with the farm owners (suppliers), which is a competitive advantage for our project **3- Labor:**

The project can create between 9 and 13 job positions, depending on the production scale. These positions will cover plant harvesting, oil extraction, cream formulation, packaging, quality control, administration, and sales. The project will require manual laborers ,Technicians and skilled workers, Packaging operators and Administrative and sales staff. The use of handling equipment is possible and recommended to improve efficiency.

4. The Main Partners:

The most important partnerships in our case are the suppliers, given the importance of their services for the success of the project. University incubators also provide valuable assistance to projects. Not to mention the weight of the financing structures.

Fifth axis: Financial plan 1-Costs

and Charges:

The costs for our natural cream project include several essential components. First, there are raw material costs for high-quality essential oils like thyme, rosemary, and fennel, as well as other ingredients needed for the formulation. Next, I must cover production expenses such as equipment, packaging materials, and laboratory supplies for small-scale testing and prototype development. Additional costs include certification fees, quality control tests, and any necessary safety or dermatological analyses.we will also account for marketing and branding expenses, including packaging design, labels, and promotional materials. If I plan to sell through partners , we must include transportation, storage, and distribution costs. Finally, the total charges must reflect a fair profit margin to ensure the project is sustainable and can cover unexpected costs or future improvements. Careful cost planning will help price the cream competitively while maintaining product quality and business growth.

2-Breakdown of revenue:

Item	**Estimated Cost (DA)**
Essential Oils	**~1,650 DA**
Other Ingredients	**~1,000 DA**
Packaging	**~400–500 DA**
TOTAL	**~3,000 – 3,200 DA**

Sixth axis: Experimental prototype

To create a prototype of our natural healing cream, we start by researching and selecting the best quality of plants , such as thyme, rosemary, and fennel, make the extraction and ensuring that the oils are pure and suitable for skin application. Next,we will define the right formulation by testing different ingredient ratios to achieve the desired texture, absorption, and effectiveness. Once we have a basic formula, we prepare small test batches in a clean lab environment, following good manufacturing practices. After that,we conduct basic stability and safety tests to check for any unwanted reactions, .we will then adjust the formula if needed, choose suitable packaging that preserves the product's quality, and design a simple label. Finally, we will prepare the final prototype samples, ready to be presented to potential partners, my university, or health experts for further testing, certification, or approval, .and this is our final result **.**



Image21 : the prototype of the cream MI

GENERAL CONCLUSION

In conclusion,this research was conducted to investigate and evaluate the therapeutic and medicinal properties of three well-known aromatic plants: thyme (Thymus vulgaris), rosemary (Rosmarinus officinalis), and fennel (Foeniculum vulgare). The primary objective was to understand the active compounds present in each plant and how their properties can be utilized, particularly in natural formulations such as healing creams.

The extraction of essential oils by hydrodistillation gave an average yield of 0.67 % for fennel 1.81.% for thyme and 0.13% for the plant of rosemary The physico-chemical characteristics were studied.

The study of the chemical composition by GC/MS revealed that thymol, accounting for is the majority compound of thyme .EO (more than 87.52 %) estragole, is the majority for fennel (.81.04%.), as well as camphor for the plant of rosemary with a percentage of (23.26%),

The study of antimicrobial activity on bacterial and fungal strains showed that the EOs provided strong antimicrobial power while the results of the antioxidant activity by free radical scavenging by DPPH show an EC50 of (2,5 and 3,3) mg/ml for the EO of. Fennel and of rosemary .

An antimicrobial moisturizer was prepared based on its three essential oils, which were studied and showed good effectiveness.

This project allowed us to explore the rich potential of medicinal and aromatic plants—specifically thyme, rosemary, and fennel—through scientific analysis, essential oil extraction, and cosmetic formulation. Beyond the laboratory work, the entrepreneurial dimension of our project represents a vital step toward real-world application. By designing a startup around our cosmetic cream, we have transformed academic knowledge into a viable business idea that supports local agriculture, promotes sustainable production, and meets the growing consumer demand for natural products.

This initiative not only highlights the economic value of plant-based ingredients but also encourages innovation and self-employment among young researchers. We believe that our project demonstrates how a small-scale idea, when supported by research and thoughtful

planning, can evolve into a larger vision for industrial and economic development. We hope that this startup will serve as both a model and a motivation for future projects that combine science, entrepreneurship, and environmental awareness.

Looking ahead, it is recommended to:

-Study the anti-inflammatory activity of essential oils

-Conduct more advanced in vivo studies on the efficacy of emulgel

-Study the toxicity of emulgel

The bibliography

1. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011, octobre). Extraction, isolation and characterization of bioactive compounds from plants' extracts.
African Journal of Traditional, Complementary and Alternative Medicines, 8(1), 1–10. https://doi.org/10.4314/ajtcam.v8i1.65226

2. Marshall, E. (2011). Health and wealth from medicinal aromatic plants. Rome: Rural Infrastructure and Agro-Industries Division, Food and Agriculture Organization of the United Nations.

3 Duke, J. A. (2002). Handbook of Medicinal Herbs (2nd ed.). CRC Press

4 Marshall, E. (2011). Health and wealth from medicinal aromatic plants. Rome: Rural Infrastructure and Agro-Industries Division, Food and Agriculture Organization of the United Nations.Study.com. (n.d.). Medicinal plants: Uses & definition. Retrieved June 28, 2025, from https://study.com/academy/lesson/medicinal-plants-uses-definition.html

5 University of Pretoria. (n.d.). Chapter 1: Introduction [Master's thesis, University of Pretoria]. University of Pretoria Repository. Retrieved June 28, 2025, from https://repository.up.ac.za/bitstream/handle/2263/24934/01chapter1.pdf?sequence=2

6Historic review of medicinal plants' usage. (2012, January–June). [Title of Journal if known]. Retrieved June 28, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC3358962/

7 Introduction of medicinal plants species with the most traditional usage in Alamut region. (n.d.). Retrieved June 28, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC3813099/

8 Khan, H. (2014, July). Medicinal plants in light of history: Recognized therapeutic modality. Journal of Evidence-Based Complementary & Alternative Medicine, 19(3), 216–219. https://doi.org/10.1177/2156587214533346

9 Dr. Sruthi M., MBBS, & Uttekar, P. S., MD. (n.d.). What is thyme good for? MedicineNet. Retrieved June 28, 2025, from https://www.medicinenet.com/what_is_thyme_good_for/article.htm

10 Kowalczyk, A., & Przychodna, M. (2020, September 9). Thymol and thyme essential oil— New insights into selected therapeutic applications. Molecules, 25(18), Article 4125. https://doi.org/10.3390/molecules25184125

11 Leal, K. S. (2023, December 20). Top 9 thyme benefits (plus how to take & make thyme tea). Tua Saúde. Retrieved June 28, 2025, from https://www.tuasaude.com/en/thyme-benefits/

12 Drugs.com. (2024, June 20). Rosemary. Retrieved June 28, 2025, from https://www.drugs.com/npp/rosemary.html

13 Al-Sereiti, M. R., Abu-Amer, K. M., & Sen, P. (1999, February). Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. Indian Journal of

Experimental Biology, 37(2), 124–130. Retrieved June 28, 2025, from https://pubmed.ncbi.nlm.nih.gov/10641130/

14Ruggeri, C. (2022, April 1). Top 11 fennel benefits (plus nutrition facts, recipes & more). Dr. Axe. Retrieved June 28, 2025, from https://draxe.com/nutrition/fennel-benefits/

15 Azeez, S. (2025). 12 Fennel [En ligne], pp. 7–9. Retrieved June 28, 2025, from https://cabidigitallibrary.org/terms-and-condition

16 Martins Emeje, E., Ogu, E. O., Chidebe, I., Roko, G., Abdullahi, M., & Bibinu, D. S. (2024, July 25). Therapeutic potential of medicinal plants: Current situation and outlook [En ligne]. IntechOpen. https://doi.org/10.5772/intechopen.1005828

17 Adam, F. (2024, October 24). What are the benefits of thyme [En ligne]. Medical News Today. Retrieved June 28, 2025, from https://www.medicalnewstoday.com/articles/266016

18 Unsplash. (n.d.). Photos de plants de romarin [Photographies]. Retrieved June 28, 2025, from https://unsplash.com/fr/s/photos/rosemary-plant

19 Noreena, S., Tufaila, T., Badar Ul Aina, H., & Awuchi, C. G. (2023). Pharmacological, nutraceutical, functional and therapeutic properties of fennel (Foeniculum vulgare). International Journal of Food Properties, 26(1), 915–927. https://doi.org/10.1080/10942912.2023.2192436

20 Khosravipour, B., & Direkvand-Moghadam, F. (2016). The development of thyme plant as a medicinal herb: A review article. Advances in Herbal Medicine, 2(2), 47–53.

21 Oliveira, J. R., Camargo, S. E. A., & de Oliveira, L. D. (2019). Rosmarinus officinalis L. (rosemary) as therapeutic and prophylactic agent. Journal of Biomedical Science, 26, Article 5. https://doi.org/10.1186/s12929-019-0499-

22 Silva, H. (2020, December 21). A descriptive overview of the medical uses given to Mentha aromatic herbs throughout history. Biology, 9(12), Article 484. https://doi.org/10.3390/biology9120484

23 Letlow, O. (2023). The history and usage of rosemary [Research paper]. HSABR Garden Intern. Retrieved June 28, 2025, from https://www.hsabr.org/uploads/1/2/5/5/12552641/history_and_usage_of_rosemary_report_hsa_research_paper.pdf

24 Touwaide, A., & Appetiti, E. (2022, December). Herbs in history: Fennel. American Herbal Products Association. Retrieved June 28, 2025, from https://www.ahpa.org/herbs_in_history_fennel

25 Silva, H. (2020, December). A descriptive overview of the medical uses given to Mentha aromatic herbs throughout history. Biology, 9(12), Article 484. https://doi.org/10.3390/biology9120484

26 Thiab, S., Nassar, R. I., Alamleh, S. A., Aboqubo, A., & Aljebori, A. (2024, August 26). Investigation of the knowledge, attitudes, and perceptions regarding the utilization of rosemary among the population in Jordan. PLoS One, 19(8), e0307575.

https://doi.org/10.1371/journal.pone.0307575

27 Opara, E. I., & Chohan, M. (2021). Chapter 30: Thyme (Thymus vulgaris). In Culinary Herbs and Spices: A Global Guide (pp. 533–548). Royal Society of Chemistry. https://doi.org/10.1039/9781839164446-00533

28 Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014, August 3). Foeniculum vulgare Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Research International, 2014, Article 842674. https://doi.org/10.1155/2014/842674

29 Zelman, K. M. (2024, October 14). Health benefits of rosemary. WebMD. Retrieved June 28, 2025, from https://www.webmd.com/diet/health-benefits-rosemary

30 Sheikh, Z. (2024, April 16). Fennel: Health benefits, nutrients, and recipes. WebMD. Retrieved June 28, 2025, from https://www.webmd.com/food-recipes/health-benefits-fennel

31 Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014, August 3). Foeniculum vulgare Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Research International, 2014, Article 842674. https://doi.org/10.1155/2014/842674

32 Zelman, K. M. (2024, October 14). Health benefits of rosemary. WebMD. Retrieved June 28, 2025, from https://www.webmd.com/diet/health-benefits-rosemary

33 Sheikh, Z. (2024, April 16). Fennel: Health benefits, nutrients, and recipes. WebMD. Retrieved June 28, 2025, from https://www.webmd.com/food-recipes/health-benefits-fennel

34.Ghasemzadeh Rahbardar, M., & Hosseinzadeh, H. (2020, September). Therapeutic effects of rosemary (Rosmarinus officinalis L.) and its active constituents on nervous system disorders. Iranian Journal of Basic Medical Sciences, 23(9), 1100–1112. https://doi.org/10.22038/ijbms.2020.45269.10541

35.ScienceDirect. (n.d.). Fennel – An overview. Retrieved June 28, 2025, from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fennel

36.Sharma, Y., Velamuri, R., Fagan, J., & Schaefer, J. (2020). Full-spectrum analysis of bioactive compounds in rosemary (Rosmarinus officinalis L.) as influenced by different extraction methods. Molecules, 25(20), Article 4599. https://doi.org/10.3390/molecules25204599

37.Untitled preprint (n.d.). [Unpublished preprint]. Universidad de Granada Institutional Repository. Retrieved June 28, 2025, from https://digibug.ugr.es/bitstream/handle/10481/102683/preprint.pdf?sequence=1&isAllowed=y

38 .Mena, P., Cirlini, M., Tassotti, M., Herrlinger, K. A., Dall'Asta, C., & Del Rio, D. (2016). Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (Rosmarinus officinalis L.) extract. Molecules, 21(11), 1576. https://doi.org/10.3390/molecules21111576

39.Stojanović-Radić, Z., Đurđević, P., Belović, M., Lakic, N., & Cvetković, D. (2020, September). A review on applications and uses of Thymus in the food industry. Food Chemistry, 334, Article 127513. https://doi.org/10.1016/j.foodchem.2020.127513

40.Kowalczyk, A., Przychodna, M., & Sopata, S. (2020, September). Thymol and thyme essential oil—New insights into selected therapeutic applications. Molecules, 25(18), Article 4125. https://doi.org/10.3390/molecules25184125

41.Sheikh, Z. (2024, April 16). Fennel: Health benefits, nutrients, and recipes. WebMD. Retrieved June 28, 2025, from https://www.webmd.com/food-recipes/health-benefits-fennel

42.Drugs.com. (2024, June 20). Rosemary. Retrieved June 28, 2025, from https://www.drugs.com/npp/rosemary.html

43. Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014, August 3). Foeniculum vulgare Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Research International, 2014, Article 842674. https://doi.org/10.1155/2014/842674

44. Macedo, L. M. de, Santos, É. M. dos, & Custódio, D. L. (2020, June). Rosemary (Rosmarinus officinalis L., syn Salvia rosmarinus Spenn.) and its topical applications: A review. Plants, 9(6), Article 651. https://doi.org/10.3390/plants9060651

45 .Academia Stack Exchange. (n.d.). Practical strategies for increasing one's passion and excitement about research. Retrieved June 28, 2025, from https://academia.stackexchange.com/questions/27762/practical-strategies-for-increasing-onespassion-and-excitement-about-research

46. University of Montana. (n.d.). Undergraduate research and creative scholarship. Retrieved June 28, 2025, from https://www.umt.edu/undergraduate-research/research/why-research.php

47 .STEMCELL Technologies. (n.d.). How to stay motivated in research. Retrieved June 28, 2025, from https://www.stemcell.com/efficient-research/

48 .Soomro, M. M. (2023, October 17). Research challenges and how to overcome them. LinkedIn. Retrieved June 28, 2025, from https://www.linkedin.com/pulse/researchchallenges-how-overcome-them-moomal-soomro-hcndf

49. Al Dalbhi, S., Alodhayani, A., Alghamdi, Y., Alrasheed, S., Alshehri, A., & Alotaibi, N. (2019). Difficulties in conducting clinical research among healthcare practitioners in Saudi Arabia: A cross-sectional survey. Journal of Family Medicine and Primary Care, 8(7), 2281–2285. https://doi.org/10.4103/jfmpc.jfmpc_288_19

50. Valentino, A. L., & Juanico, J. F. (2020, December). Overcoming barriers to applied research: A guide for practitioners. Behavior Analysis in Practice, 13, 767–777. https://doi.org/10.1007/s40617-020-00458-4

51. Fu, N., Hou, Y., Li, K., Li, M., Tang, S., & Yu, C. (2021, April). A time of transition: Stories from starting a new research program in 2020. Biophysical Reviews, 13, 311–317. https://doi.org/10.1007/s12551-021-00801-z

52. da Silva Ferreira, V., de Lima Silva, M. F., da Silva Gadelha, T., Cavalcante da Silva, M. R., & Pereira, G. A. (2020, October 8). Maceration extraction conditions for determining the phenolic compounds and the antioxidant activity of Catharanthus roseus (L.) G. Don. Ciencia e Agrotecnologia, 44, e012220. https://doi.org/10.1590/1413-7054202044012220

53. BCL UAE. (2022, November 28). Soxhlet extraction 101: Understanding basics of the technique. Retrieved June 28, 2025, from https://www.bcluae.com/know-about-soxhletextraction-system

54. Meliza, G. L., & Rojas, L. (2022, May 24). Ultrasound-assisted extraction of natural pigments from food processing by-products: A review. Frontiers in Nutrition, 9, Article 891462. https://doi.org/10.3389/fnut.2022.891462

55. Sherley, N. P. E. H., & Agustin, M. (2023, May 8). The effect of extraction methods on total phenolics and antioxidant activities in Caulerpa racemosa (Forsskal) J. Agardh extracts. [PDF]. Retrieved from https://docs.bvsalud.org/biblioref/2024/04/1538037/the-effect-ofextraction-methods-on-total-phenolics-and-antiox_9jv4Pmj.pdf

56. Cao, S., Liang, J., & Chen, M. (2025, March 3). Comparative analysis of extraction technologies for plant extracts and absolutes. Frontiers in Chemistry, 13, Article 1536590. https://doi.org/10.3389/fchem.2025.1536590

57 .Okoduwa, S. I. R., Umar, I. A., James, D. B., Inuwa, H. M., & Habila, J. D. (2016, December 15). Evaluation of extraction protocols for anti-diabetic phytochemical substances from medicinal plants. World Journal of Diabetes, 7(20), 605–614. https://doi.org/10.4239/wjd.v7.i20.605

58. Alenezi, S. (2025, May 1). Innovative extraction technologies for the development of herbal-based functional products: A green chemistry perspective. Trends in Food Science & Technology, 145, 182–193. https://doi.org/10.1016/j.tifs.2024.12.005

59. Tamil Nadu Agricultural University. (n.d.). Extraction techniques of medicinal plants. Retrieved June 28, 2025, from http://www.agritech.tnau.ac.in/horticulture/extraction_techniques%20_medicinal_plants.pdf

60. Abubakar, A. R., & Haque, M. (2020, June 29). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bioallied Sciences, 12(3), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_20

61 .Ramesh, M. M., Shankar, N. S., & Venkatappa, A. H. (2024). Driving/critical factors considered during extraction to obtain bioactive enriched extracts. Pharmacognosy Reviews, 18(35), 68–76. https://doi.org/10.5530/phrev.2024.18.11

62. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011, October). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines, 8(1), 1–

10. https://doi.org/10.4314/ajtcam.v8i1.65226

63. Phytochemia. (2014, août 4). L'extraction d'huiles essentielles en laboratoire. Consulté le 28 juin 2025, à l'adresse https://phytochemia.com/fr/2014/08/04/lextraction-dhuilesessentielles-en-laboratoire/

64. Université de M'Sila. (n.d.). Extraction des huiles essentielles d'eucalyptus par Clevenger. [Fichier PDF]. Consulté le 28 juin 2025, depuis https://elearning.univ-msila.dz/moodle/pluginfile.php/271187/mod_resource/content/1/TP%2004%20EXTRACTION%20DES%20HUILES%20ESSENTIELLES%20D%E2%80%99EUCALYPTUS.pdf

65. Laiche, C., & Mecheri, M. (2023). Extraction des huiles essentielles et hydrolats. Mémoire de fin d'études, Université des Frères Mentouri Constantine. Année universitaire 2022–2023. https://fac.umc.edu.dz/snv/bibliotheque/biblio/mmf/2023/Extraction%20des%20huiles%20essentielles%20et%20hydrolats.pdf

66. Jeulin. (n.d.). Appareil de Clevenger. Consulté le 28 juin 2025, à l'adresse https://jeulin.com/lelaborantin_fr/714260.html

67. Hielscher Ultrasonics. (n.d.). Hydrodistillation ultrasonique des huiles essentielles. Consulté le 28 juin 2025, à l'adresse https://www.hielscher.com/fr/ultrasonichydrodistillation-of-essential-oils.htm

68. Université de Blida 1. (2020). Extraction et caractérisation des composés secondaires de la plante (Mentha longifolia) de la région de Tamanrasset. Mémoire de Master. Année universitaire 2019–2020. https://di.univ-blida.dz/jspui/bitstream/123456789/7308/1/30%20M.SP.pdf

67. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011, octobre). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines, 8(1), 1–10. https://doi.org/10.4314/ajtcam.v8i1.65226

68. Marshall, E. (2011). Health and wealth from medicinal aromatic plants. Rome: Rural Infrastructure and Agro -Industries Division, Food and Agriculture Organization of the United Nations.

69. Jannavi R. Srinivasan, Ph. D., and reviewed by Yoko Kawamura, Ph. D. ROSEMARY EXTRACT[en lign], 82nd JECFA - Chemical and Technical Assessment (CTA), 2016

70. Fennel Seeds https://www.viralspices.com/products/fennel-seeds

71. thyme ,https://wandernana.com/blogs/blog-conseils-astuces/tisane-de-thym-bienfaits

72. Ingestion and Therapeutic Grade Essential Oils, By sal battaglia Apr 6, 2019 https://www.perfectpotion.com.au/blogs/blog/ingestion-and-therapeutic-grade-essential-oils