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أنا الممضي أدناه،

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أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات المهنية والنزاهة الأكاديمية المطلوبة في انجاز البحث المذكور أعلاه.

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وزارة التعليم العالي والبحث العلمي

بسكرة في: ....

جامعة محمد خيضر–بسكرة

كلية العلوم والتكنولوجيا

قسم الكيمياء الصناعية

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Oil extraction from rosemary plant : process and characterization

أرخص بإيداع المذكرة المذكورة.

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Oil Extraction from Rosemary Plant: process and characterization

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Dedication

First, I would like to thank God for giving me the strength and patience to complete this work successfully.

I dedicate this work to my dear family…

I am very grateful to my wonderful parents, Mom and Dad, for their love and support every day. I also want to thank my brothers, Zaki and Oussama, and my sister Hidaya, for their motivation.

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Abstract

This study explores the potential for establishing a sustainable rosemary (*Rosmarinus officinalis L.*) essential oil industry in Algeria, focusing on the Ain El Melh and Biskra regions. These areas offer suitable environmental conditions for rosemary cultivation, making them ideal for developing value-added botanical products. Various extraction methods were evaluated to optimize oil yield and quality, including hydrodistillation and steam distillation. Chemical and physical analyses of the oils were conducted using techniques such as gas chromatography-mass spectrometry (GC-MS), FTIR, and UV-Vis spectroscopy. Results show that Algerian rosemary oil contains significant levels of 1,8-cineole, camphor, and α-pinene, compounds known for their therapeutic and antioxidant properties. This composition highlights the oil's suitability for cosmetic and medicinal applications.

Keywords: *Rosmarinus officinalis L;* essential oil; hydrodistillation; steam distillation.

الملخص

تتناول هذه الدراسة إمكانات إنشاء صناعة مستدامة لإكليل الجبل (.Rosmarinus officinalis L) لصناعة الزيوت العطرية في الجزائر، مع التركيز على منطقتي عين الملح وبسكرة. توفر هذه المناطق ظروفًا بيئية مناسبة لزراعة إكليل الجبل، مما يجعلها مثالية لتطوير وتحسين منتجات نباتية ذات قيمة عالية. وقد تم استخدام طرق استخلاص مختلفة، بما في ذلك التقطير المائي والتقطير بالبخار، لتحسين إنتاج الزيت وجودته. تم إجراء التحاليل الكيميائية والفيزيائية للزيوت باستخدام تقنيات مثل التحليل اللوني الغازي - مطياف الكتلة(GC-MS) ، والتحليل الطيفي بالأشعة تحت الحمراء والفوق البنفسجية والمرئية. أظهرت النتائج أن زيت إكليل الجبل الجزائري يحتوي على مستويات كبيرة من 8،1-سينول والكافور والبينين ألفا، وهي مركبات معروفة بخصائصها العلاجية والمضادة للأكسدة. وتسلط هذه التركيبة الضوء على ملاءمة الزيت للتطبيقات التجميلية والطبية .

الكلمات الرئيسية :اكليل الجبل؛ زيت عطري؛ التقطير المائي: التقطير بالبخار.

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EU: European Union

GC-MS: Gas Chromatography-Mass Spectroscopy

IPM: integrated pest management

MAE: Microwave-Assisted Extraction

USM: ultrasonic machining

UV: ultraviolet

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General introduction

Through this study, we explored the vast potential of *Salvia rosmarinus L*, commonly known as rosemary, a plant of high value in many fields.

Rosemary (*Rosmarinus officinalis* L.), a perennial aromatic herb belonging to the Lamiaceae family, is widely recognized for its essential oil, which contains a rich array of bioactive compounds with antioxidant, antimicrobial, and anti-inflammatory properties. The composition and yield of rosemary essential oil are influenced by several factors, including environmental conditions, geographical origin, plant chemotype, and the method and solvent used for extraction [1,2].

With its diverse agro-climatic zones, Algeria offers an ideal setting to investigate regional influences on essential oil composition. This study collected rosemary samples from two distinct regions: Biskra, characterized by a hot and arid Saharan climate, and Ain El Melh (M'Sila), which experiences a more semi-arid, steppe-like environment. These contrasting climates are expected to influence the biosynthesis and accumulation of secondary metabolites in rosemary.

Moreover, the extraction method plays a critical role in determining the yield and chemical integrity of the essential oils. Traditional techniques such as hydro-distillation and Clevenger apparatus are commonly used for essential oil recovery, while Soxhlet extraction, particularly when coupled with organic solvents, may offer higher yields but can alter the oil's composition due to thermal and solvent effects [3,4].

The present study aims to evaluate the effect of regional origin (Biskra and Ain El Melh) and extraction technique (hydro-distillation, Soxhlet, and Clevenger methods), as well as the choice of solvent (ether and hexane), on the yield and quality of rosemary essential oil. By identifying the optimal conditions for extraction, this work contributes to enhancing the valorization of rosemary as a natural resource for the pharmaceutical, cosmetic, and food industries.

In the first chapter, we provided a comprehensive overview of this plant, focusing on its botanical characteristics, richness in active chemical compounds, and diverse industrial applications, emphasising its importance in Algeria. We also addressed the main challenges facing its cultivation, stressing the need for sustainable agricultural practices to improve quality and productivity. In the second chapter, we examined the various techniques for extracting essential oils from rosemary. By comparing traditional and modern methods, such as steam distillation, solvent extraction, Soxhlet method, hydrodistillation, ultrasound-assisted

extraction, and microwave-assisted extraction, we highlighted the impact of each technique on the yield and quality of the extracted oil. We also emphasized the importance of controlling extraction conditions to ensure an effective oil with high therapeutic and industrial properties. In the third chapter, we presented the practical aspect of our work. We applied selected extraction methods to obtain rosemary oil, and the characterization was carried out using advanced analytical techniques. The comparative analysis between the different methods gave us a deeper understanding of the efficiency and practicality of each technique in real applications.

Chapter I

A Review on Salvia Rosmarinus

Rosemary (Salvia rosmarinus) is an aromatic evergreen shrub from the Lamiaceae family, primarily native to the Mediterranean region. It is known for its diverse applications in culinary, medicinal, and industrial fields due to its rich phytochemical composition. Traditionally, rosemary has been used to treat digestive issues, respiratory disorders, and menstrual pain [5]. Its bioactive compounds have shown anticancer effects and the ability to inhibit enzymes associated with Type 2 Diabetes Mellitus [6]. Moreover, its antioxidant properties help reduce oxidative stress, which is linked to many chronic diseases. In culinary contexts, rosemary enhances flavour in various dishes and acts as a natural preservative, preventing oxidation and microbial growth [7]. Rosemary emerges not only as a palatable herb but also as a botanical entity with considerable health advantages and cultural significance [5].

Figure 1: Salvia Rosmarinus [8].

1. Salvia Rosmarinus in Algeria:

Salvia Rosmarinus, commonly known as rosemary, is a significant medicinal plant in Algeria, particularly within the diverse ecological zones of the Sahara. Its geographical distribution reflects the unique adaptations of flora in arid environments, where it is utilized in traditional medicine across various regions [9].

2. Geographical Distribution

- **Northern Sahara**: In the northern Sahara, Salvia rosmarinus is found among 130 species identified in five geomorphological zones, highlighting its adaptability to desert conditions [10].
- **Southern Regions**: Ethnobotanical surveys in southern Algeria (Ouargla, El-Oued, Adrar, Illizi) reveal that the Lamiaceae family, which includes rosemary, is prevalent and treats various ailments [11].
- **Semi-arid Areas**: In semi-arid regions like El Bayadh, Salvia rosmarinus contributes to the local medicinal flora, and it has been reported to be used significantly to treat gastrointestinal issues [9].

Rosemary is cultivated in several northern regions of Algeria, notably Blida, Djelfa, and Tebessa. It is valued for its essential oils extracted from its leaves and flowers. A study on rosemary grown in Blida found that the essential oil yield reached 1.6%, with two dominant compounds being 2-bornanone (19.47%) and eucalyptol (18.58%) [12].
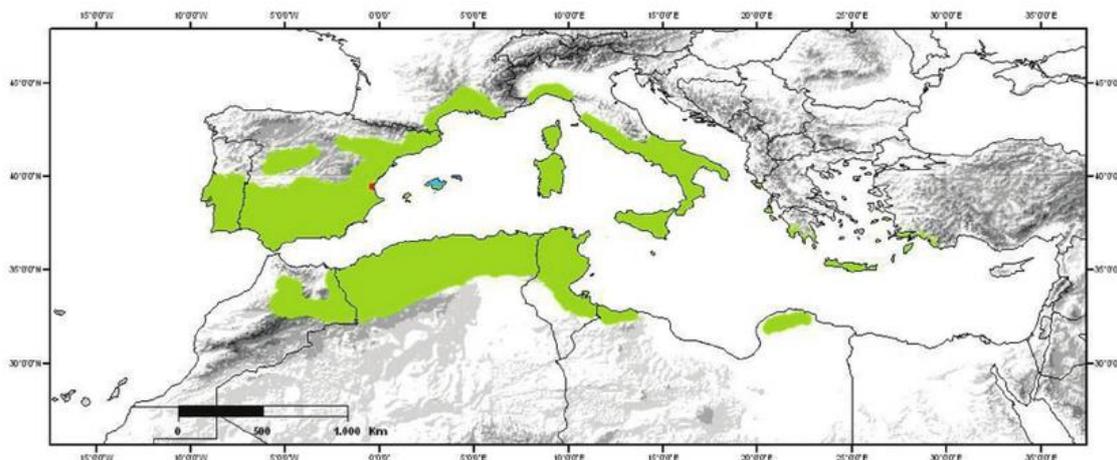
Figure 2: Distribution Map to the subspecies of the Rosmarinus officinalis complex [13].
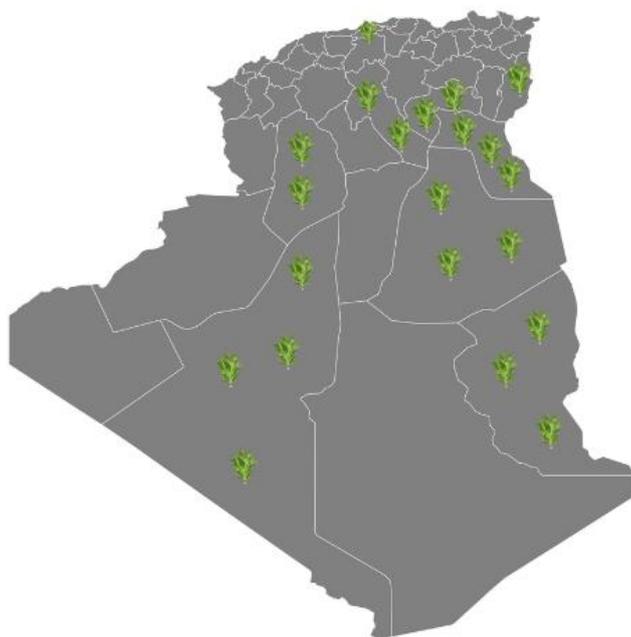
Figure 3: Rosemary in Algeria.

3. Botanical Characteristics

This evergreen plant typically grows upright, reaching up to 2 meters in height and width. It has a woody stem and narrow, needle-like leaves about 2 to 3 centimetres long. The leaves are dark green on the upper surface and pale on the underside, often covered with fine hairs, and they emit a strong aromatic scent when crushed due to their high concentration of essential oil glands. Rosemary produces small, pale blue to violet flowers, mainly during the spring and summer seasons, although blooming can occur throughout the year in mild climates. Native to the Mediterranean region, rosemary thrives in dry, rocky, and calcareous soils, particularly in areas with full sunlight exposure. The plant is well adapted to drought conditions and is known for its resistance to pests and diseases, which makes it relatively easy to cultivate and maintain in suitable environments [14].

4. Phytochemical Composition

Rosemary is valued for its rich content of bioactive compounds, especially essential oils and antioxidants, which give it notable aromatic and therapeutic qualities [15].

4.1. Essential Oils

Essential oils are among the most prominent constituents of rosemary and are typically extracted from the plant's leaves and flowers using steam distillation, a method widely recognized for its ability to preserve the volatile and bioactive components of aromatic plants [16]. These oils are composed of several key compounds, including α-pinene, 1,8-cineole (commonly known as eucalyptol), camphor, borneol, and camphene, which are collectively responsible for the plant's distinctive aroma and therapeutic properties such as antimicrobial and anti-inflammatory effects [15]. However, it is important to note that the chemical composition of rosemary essential oils is not fixed; it varies significantly depending on a variety of factors such as the plant's geographic location, the season during which it is harvested, and the stage of its growth cycle [17].

4.2. Polyphenols and Flavonoids

In addition to its essential oils, rosemary contains a diverse range of powerful phenolic compounds, with rosmarinic acid being one of the most prominent. This compound is well-regarded for its strong antioxidant activity, which allows it to neutralize free radicals and protect cells from oxidative damage [18]. Furthermore, rosemary includes carnosic acid and carnosol, two diterpenes known for their potent antioxidant effects as well as their promising anticancer properties, including the ability to inhibit tumour growth and induce apoptosis in cancer cells [19]. The plant also contains important flavonoids such as luteolin and apigenin, which have demonstrated anti-inflammatory properties and immune-boosting effects, thereby enhancing the plant's therapeutic applications in both traditional and modern medicine [14].

4.3. Antioxidant Properties

Due to its high concentration of antioxidant compounds, rosemary is extensively used in the food industry as a natural preservative. These antioxidants are capable of delaying the oxidation of lipids, thereby preventing rancidity and extending the shelf life of food products [15]. Moreover, rosemary extracts are commonly found in cosmetic formulations aimed at skincare and anti-ageing, where their antioxidant activity helps to combat premature ageing, protect the skin from environmental stressors, and promote overall skin health [18].

4.4. Environmental Influence on Chemical Composition

The chemical profile of rosemary is greatly influenced by environmental factors, which play a critical role in determining both the quality and quantity of its bioactive compounds. Elements such as soil composition, climate, sunlight exposure, and water availability can significantly alter the concentration and diversity of essential oils and other phytochemicals

present in the plant. As a result, the variability in environmental conditions directly affects the efficacy and application of rosemary in industrial and medicinal contexts, highlighting the importance of cultivation practices tailored to optimize its phytochemical yield [16].

5. Benefits and Industrial Applications

Rosemary has been known since ancient times. It has been widely used in traditional medicine due to its unique combination of active compounds. The therapeutic properties of rosemary, particularly its antioxidant and anti-inflammatory effects, are utilized in treating inflammatory diseases, wound healing, and skin conditions such as mycoses and skin cancer [19].

Rosemary's bioactive compounds are also explored for their potential in drug formulations, offering benefits without systemic absorption and adverse side effects [19].

Rosemary plays a significant role in the cosmetic and perfumery industry due to its remarkable therapeutic and antioxidant properties. Its extract and essential oil are widely used in skincare and haircare formulations, primarily for their anti-ageing, anti-inflammatory, and anti-cellulite effects. The high content of natural antioxidants, including rosmarinic acid and carnosic acid, helps combat free radicals, which are responsible for premature skin ageing and loss of elasticity [20]. As a result, rosemary is commonly incorporated into anti-ageing creams, serums, and body lotions to promote youthful and firm skin [19].

Moreover, rosemary is known to stimulate blood circulation in the scalp, thereby supporting hair follicle health and promoting hair growth. This makes it a popular ingredient in shampoos, hair oils, and scalp treatments aimed at reducing hair loss and enhancing hair density [7]. In addition to its skin and hair benefits, recent research has emphasized rosemary's potential to protect the skin from ultraviolet (UV) radiation and various environmental stressors such as pollution and oxidative damage, making it a valuable component in sun protection products and cosmeceutical formulations [19].

Its strong, fresh, and herbal aroma also contributes to its wide usage in the perfumery sector, where it is utilized as a natural fragrance in perfumes, deodorants, and personal care products, offering both olfactory appeal and therapeutic benefits [19].

In the food industry, rosemary is used as a natural ingredient to add a distinctive flavour to meat, fish, and bread, in addition to being used in the preparation of flavoured oils and some beverages. In addition to its unique taste, its antioxidant properties make it a natural alternative to synthetic preservatives in food preservation, especially meat and dairy products, as it extends their shelf life and prevents fat rancidity. Therefore, rosemary is considered an ingredient that plays an integrated role in health, beauty, and nutrition, which enhances its position in global industrial markets as an effective and multifunctional ingredient [21].

6.   Challenges in Rosemary Cultivation

Rosemary cultivation presents several challenges that can impact both yield and quality. These challenges stem from environmental factors, cultivation methods, and pest management, which require careful consideration to optimize production.

6.1. Environmental Factors:

Rosemary is notably sensitive to environmental fluctuations and seasonal variations, which often impose constraints on its vegetative and reproductive growth. Fluctuations in temperature, light intensity, and photoperiod can disturb its physiological processes. However, controlled growing environments—such as artificial light plant factories—have demonstrated a capacity to alleviate these stresses. By offering consistent and optimized light conditions throughout the various phenological stages of growth, these systems can improve biomass accumulation and overall plant health [22].

The effectiveness of rosemary cultivation is heavily dependent on soil conditions. The traditional cultivation approach highlights the importance of proper soil preparation, including nutrient enrichment through fertilization, as well as early pest and weed control interventions. These steps are crucial for promoting healthy root development and nutrient uptake. In contrast, inadequate or degraded soils—whether due to compaction, poor drainage, or nutrient depletion—can drastically reduce the yield and compromise the concentration of valuable essential oils [22].

6.2. Cultivation Techniques:

The introduction of modern cultivation methods such as clonal micropropagation and hydroponic systems offers notable improvements in the concentration of bioactive compounds and the extraction yield of essential oils. These methods provide controlled growth environments and sterile conditions that enhance plant uniformity and phytochemical stability. Nevertheless, such techniques require access to advanced infrastructure, as well as specialized technical knowledge, which may not be readily available to all producers [23].

Fertilizer selection plays a critical role in determining the quality and quantity of rosemary's essential oil production. Studies have shown that organic seaweed-based fertilizers are more effective than conventional inorganic fertilizers, leading to higher essential oil content and improved crop health. The organic components in seaweed fertilizers contribute to soil microbiome diversity and enhance the plant's nutrient uptake efficiency [24].

6.3. Pest and Disease Management:

Rosemary, despite its aromatic defences, remains vulnerable to several pests and pathogens that can undermine plant vitality and significantly lower productivity. Therefore, effective integrated pest management (IPM) strategies are essential. These should ideally include the development of organic and sustainable approaches, which ensure the continued

production of high-quality rosemary without compromising environmental or consumer health [25].

Chapter II

Rosemary Oil Extraction

Essential oils are volatile aromatic compounds extracted from various parts of plants using methods such as steam distillation, solvent extraction, and cold pressing [26]. The choice of extraction method significantly influences the yield and chemical composition of the oil, as some methods better preserve the active compounds [27]. Quality control during extraction is essential, as factors like plant origin, harvesting time, and processing conditions directly affect the purity and efficacy of the final product [26]. Rosemary is a widely studied medicinal and aromatic plant, and its essential oil is commonly obtained by steam distillation due to its efficiency and high-quality yield [28]. Rosemary oil is rich in bioactive compounds such as 1,8-cineole, camphor, and α-pinene, making it valuable in cosmetics, food preservation, and traditional medicine [28].

1. Oil extraction methods

1.1. Steam Distillation Method

Steam distillation is considered the primary technique for extracting authentic essential oils, although several distillation variants are available. Generally, this process involves heating water to generate steam, which carries the volatile aromatic constituents from the plant material. These vapours are then condensed and gathered as part of the final distillate [29]. As the hot water vapour infiltrates plant structures, it dissolves portions of the essential oil stored within, and this aqueous solution passes through cellular membranes, allowing the oil to evaporate immediately upon reaching the surface. The distillation continues until all volatile compounds are fully extracted from the plant cells [30].
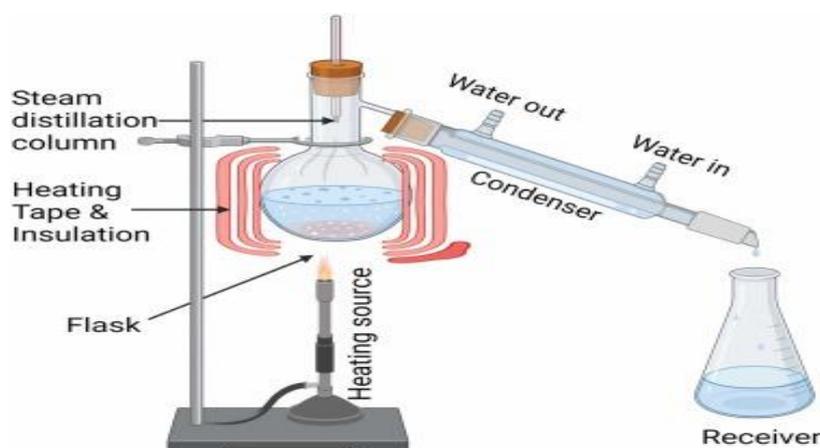
Figure 1: Steam distillation method [31].

One of its key benefits is the relatively low cost of the equipment required for the process. Moreover, steam distillation is highly efficient and well-suited for large-scale, industrial production of essential oils. It does not require any further separation steps after extraction and reliably produces oils that are free from organic solvents. Due to its efficiency, cost-effectiveness, and ability to maintain the purity of the final product, steam distillation remains one of the most trusted and widely used methods in the essential oil industry [32].

1.2. Solvent-Extraction

The solvent-extraction method is widely employed for the recovery of essential oils from plant materials, particularly when delicate aromatic compounds are involved. In this technique, an extraction unit is carefully loaded with perforated trays containing the essential oil-bearing plant matter. A suitable solvent—most commonly hexane—is repeatedly passed through the plant material to extract its volatile and non-volatile constituents. The solvent dissolves not only the highly volatile aromatic molecules responsible for the plant's fragrance but also non-aromatic substances such as waxes, pigments, and other lipid-soluble compounds [29].

Once the extraction process is complete, the mixture undergoes distillation to remove and recover the solvent for potential reuse. The residue left behind is a waxy, fragrant substance known as the "concrete." This semi-solid material is rich in aromatic compounds but still contains waxes and other components that dilute the purity of the essential oil. To obtain the more refined "absolute," the concrete is gently warmed and stirred with ethanol (ethyl alcohol). During this process, the concrete breaks into fine globules, and since aromatic molecules are significantly more soluble in alcohol than waxes, this facilitates efficient separation between the fragrance compounds and non-aromatic waxes [29].

One of the major advantages of solvent extraction over traditional distillation is its use of low boiling-point solvents, which allows for the extraction to occur at relatively low temperatures typically below 50°C. This lower-temperature environment is beneficial because it minimizes the risk of thermal degradation or transformation of the sensitive aromatic molecules, which can often happen during high-heat distillation processes. Consequently, the oils produced by solvent extraction tend to have a composition that more closely resembles the natural aroma profile of the plant material [29].

However, the use of solvents is not without drawbacks. There is the potential for the formation of artefacts and undesirable chemical byproducts that can alter the fragrance and quality of the extracted oil. Schnelle and Horster identified several such artefacts in volatile oils extracted from a mint species when a mixture of petroleum ether and acetone was used as the solvent. They discovered that these byproducts resulted from chemical reactions between acetone and non-terpenoid compounds naturally present in the plant material [31].
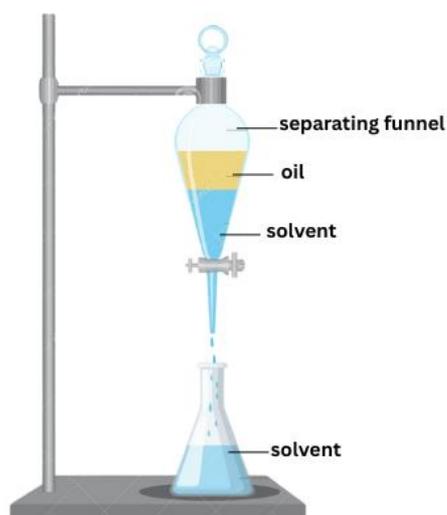
Figure 2: Separation funnel of oil-solvent [33].

1.3. Cold Press Method

The cold press method is more energy-efficient and environmentally friendly compared to other oil extraction techniques. It preserves bioactive components by avoiding thermal, chemical, and refining processes during oil extraction and processing [34]. This technique is considered safe and non-hazardous. It is specifically developed for oil production from oilseeds and is used to extract oil from a wide range of raw materials [35].

By operating at low temperatures, the cold press method enables the production of high-quality and high-purity oils with no solvent residues making it suitable for food and cosmetic applications. This technology is applied in extracting oils from soybeans, sunflower seeds, rapeseed, corn, grape seeds, hemp, flaxseeds, rice bran, olives, and pumpkin seeds [35].

Moreover, the natural and non-toxic nature of these oils appeals to consumers, as they contribute to disease prevention and overall human health. Compared to refined oils, cold-pressed oils retain higher levels of antioxidants and essential nutrients, offering superior health benefits [35].

1.4. Soxhlet extraction method.

Soxhlet extraction is a commonly employed technique for isolating target analytes from solid matrices, recognized for its high efficiency and reliability. The process is based on the repeated circulation of a solvent through the solid sample, which facilitates the exhaustive extraction of desired compounds. In recent years, technological improvements have led to enhanced versions of the traditional Soxhlet apparatus, aiming to optimize extraction time, minimize solvent consumption, and improve overall process sustainability [36]. Commonly used for extracting essential oils, such as from neem seeds and Moringa oleifera leaves, with varying yields depending on solvent choice and extraction conditions [37,38].

New devices allow for multiple samples to be extracted simultaneously, significantly increasing efficiency [39]. Innovations like microwave-assisted and ultrasound-assisted Soxhlet extraction have been developed to further enhance extraction performance and reduce time [40].

The choice of solvent (e.g., hexane, ethanol) greatly influences the yield and quality of the extracted compounds. For instance, a mixture of ethanol and hexane yielded the highest oil extraction from neem seeds [40].
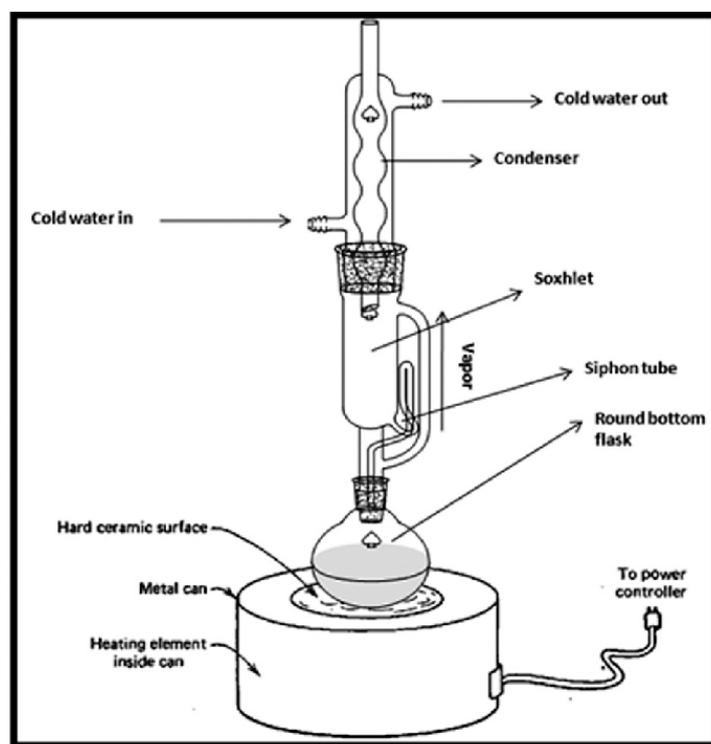
Figure 3: Soxhlet extraction method [41].

1.5. Hydrodistillation method

Hydrodistillation remains a commonly used and time-honoured technique for extracting essential oils from plant-based materials. This method utilizes water to isolate volatile compounds, eliminating the necessity for solvent recovery, which contributes to improved efficiency and a lower environmental footprint. Recent research underscores the method's effectiveness in obtaining essential oils from diverse botanical sources, with extraction yields being affected by variables such as the amount of raw material, the volume of water applied, and the physical characteristics of the plant material [42,43].

Hydrodistillation is particularly effective for spices like cloves and lemongrass, with yields varying significantly based on plant type and extraction conditions [44,45]. The method is noted for its lower energy consumption and reduced loss of volatile compounds, making it a cleaner extraction technology compared to others. While hydrodistillation is effective, alternative methods such as steam distillation may yield better results for certain plants, indicating that the choice of extraction technique should be tailored to the specific plant material and desired outcomes [45].
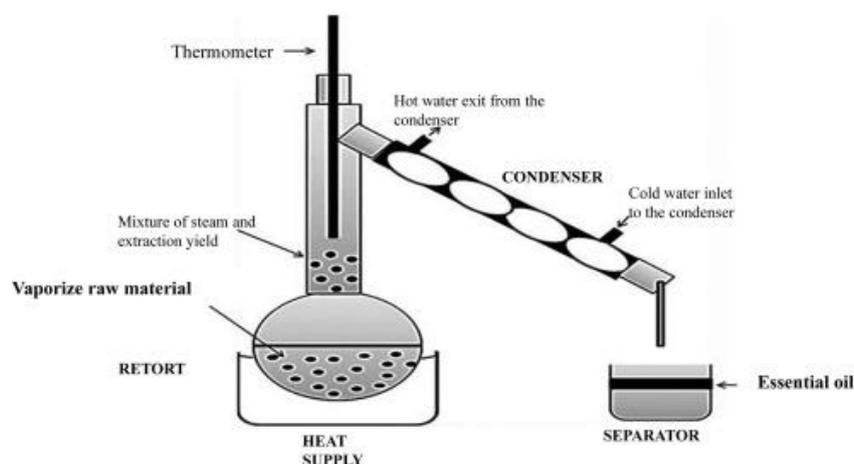
Figure 4: Hydrodistillation method [46].

1.6. Ultrasound processing:

Ultrasound processing encompasses a range of applications across various industries, particularly in food processing and machining. This technology utilizes high-frequency sound waves to induce physical effects that enhance efficiency and effectiveness in various processes [47].

Ultrasound is also employed in food decontamination and drying operations, accelerating the removal of moisture and microorganisms from raw materials. This leads to faster drying cycles, improved hygiene, and higher product yields. Additionally, its role in enhancing mixing and emulsification processes ensures better uniformity and consistency in products such as sauces, dressings, and beverages [48].

Despite its clear advantages, ultrasound processing is not without challenges. The adoption of this technology may be hindered by high initial costs and the requirement for specialized equipment and trained personnel. These factors can limit its accessibility, especially for small and medium enterprises. Nonetheless, the growing demand for efficient, sustainable, and high-quality processing continues to drive interest in ultrasonic technologies across various sectors [49].
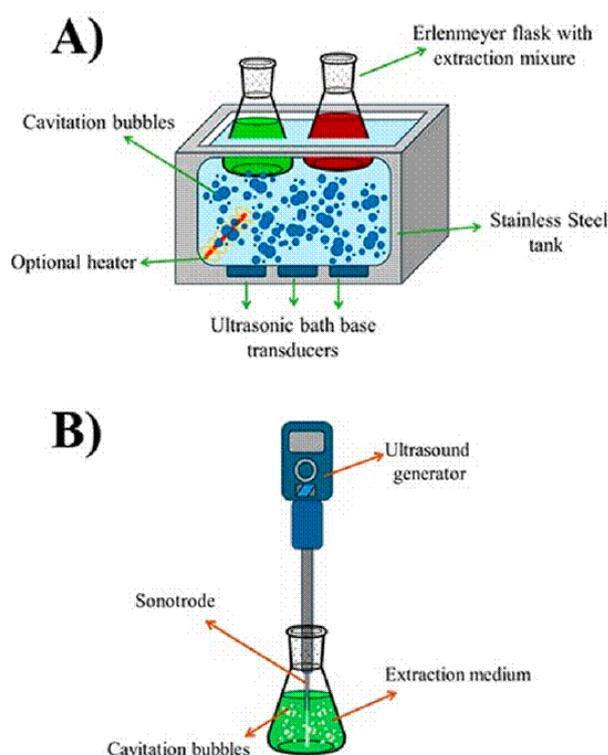
Figure 5: Ultrasonic bath (A) and ultrasonic probe (B) [50].

1.7. Microwave-Assisted Extraction (MAE):

Microwave-assisted extraction (MAE) is a modern and increasingly adopted technique that utilizes microwave energy to facilitate the transfer of bioactive compounds from solid plant materials into suitable solvents. Distinguished by its rapid and uniform heating mechanism, this method significantly improves extraction efficiency while reducing both time and solvent

usage, positioning it as a more sustainable and eco-friendly alternative to conventional extraction methods [51,52].

At the core of MAE lies the application of electromagnetic radiation in the microwave frequency range, which induces rapid heating within the sample matrix. This localized and volumetric heating disrupts plant cell walls, thereby accelerating the release of target phytochemicals into the solvent medium. The process is governed by a complex interplay of electromagnetic energy, thermal dynamics, and mass transfer mechanisms, all of which must be carefully balanced to maximize extraction efficiency and product quality [53].

One of the standout benefits of microwave-assisted extraction is its remarkable efficiency. Extraction times are drastically reduced—often requiring just 20 to 40 seconds for compounds such as polyphenols—compared to the lengthy durations associated with traditional methods. Additionally, MAE promotes higher extraction yields and better reproducibility [54].

From an economic standpoint, the technique offers clear advantages by lowering energy demands and minimizing solvent consumption. These factors contribute to reduced operational costs and enhance the consistency and quality of the final product [55].

Despite its many benefits, MAE is not without limitations. The requirement for specialized microwave extraction systems and the need to tailor operational parameters for each plant matrix can pose challenges. Optimizing variables such as microwave power, extraction time, solvent type, and sample-to-solvent ratio remains essential for ensuring optimal outcomes. As such, ongoing research is needed to refine the method and expand its applicability across a broader range of plant materials and industrial contexts [54].
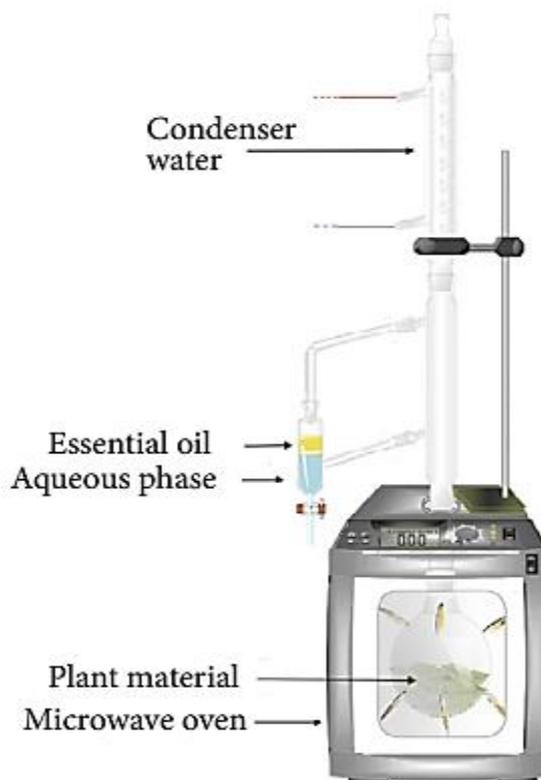
Figure 6: Schematic representation of the microwave-Clevenger [56].

2. Factors Affecting Oil Quality and Yield

Several factors, including soil amendments, environmental conditions, and post-harvest processing methods influence the quality and yield of rosemary oil. Understanding these factors is crucial for optimizing oil production and ensuring high-quality extracts.

2.1. Soil Amendments

Soil quality and amendments play a significant role in the growth and oil yield of rosemary. Studies have shown that the application of organic fertilizers, such as cow manure, can enhance plant growth parameters like height and the number of branches, which in turn can lead to higher oil yields. For instance, rosemary plants grown with cow manure exhibited better growth compared to those treated with synthetic fertilizers alone [57]. The use of organic amendments not only improves soil fertility but also positively affects the chemical composition of the essential oils produced. In particular, rosemary oil extracted from plants grown with cow manure contained a higher number of oil compounds compared to those grown with synthetic fertilizers [58].

2.2. Environmental Conditions

Climate and environmental factors such as temperature, humidity, and light exposure significantly impact rosemary oil quality. Rosemary thrives in Mediterranean climates, characterized by warm, dry summers and mild, wet winters. Optimal growing conditions can enhance the concentration of key oil constituents like alpha-pinene and eucalyptol, which are crucial for the oil's aromatic and therapeutic properties [49]. Additionally, variations in rainfall and temperature during the growing season can lead to fluctuations in oil yield and composition [51].

2.3. Harvesting and Drying Techniques

The method of harvesting and subsequent drying of rosemary also affects oil yield and quality. Freshly harvested rosemary typically yields more oil than dried herbs, but the drying process is essential for preserving the oil's volatile compounds. Research indicates that drying rosemary for about one week optimizes oil yield, while extended drying periods can lead to a decrease in oil quality due to the degradation of sensitive compounds [59]. The drying method (natural vs. artificial) can also influence the chemical profile of the oil, with natural drying often preserving more of the desirable constituents [52].

2.4. Extraction Methods

The method used to extract rosemary oil is another critical factor. Common extraction techniques include steam distillation and solvent extraction. Each method can yield different oil compositions and qualities. For example, steam distillation is generally preferred for its ability to produce high-quality oils with a more favourable aromatic profile [60]. The choice of extraction method can also affect the concentration of specific compounds, such as camphor and 1,8-cineole, which are important for the oil's therapeutic properties [52].

2.5. Genetic and Chemotypic Variability

Genetic factors also play a role in the quality and yield of rosemary oil. Different chemotypes of rosemary can produce varying oil compositions, influenced by their genetic makeup and environmental adaptations. For instance, some varieties may have higher concentrations of certain terpenes, which can affect both the aroma and therapeutic efficacy of

the oil [53]. Understanding these genetic differences can help in selecting the best cultivars for specific applications.

3. Applications of Rosemary Oil

Rosemary oil, derived from the leaves of the rosemary plant, has a wide range of applications across various fields, including cosmetics, medicine, and the food industry.

3.1. Ccosmetics

Rosemary oil is highly valued in the cosmetics industry for its aromatic properties and potential benefits for skin and hair. It is often included in formulations for shampoos and conditioners due to its ability to stimulate hair growth and improve scalp circulation, which may help combat hair loss [47,48]. Additionally, rosemary oil is recognized for its antioxidant and anti-inflammatory properties, making it a popular ingredient in skincare products aimed at reducing signs of ageing and promoting healthy skin [61,62].

3.2. Medicine

In the realm of medicine, rosemary oil has been traditionally used for its therapeutic effects. It is known for its analgesic, anti-inflammatory, and anti-anxiety properties. Research suggests that rosemary oil may enhance cognitive function and memory, making it beneficial for brain health. Furthermore, it has been used as a mild pain reliever and to alleviate symptoms of conditions like alopecia areata [63].

3.3. Food Industry

In the food industry, rosemary oil serves as a natural preservative due to its antibacterial properties. It is commonly used in meat and poultry products to extend shelf life and enhance flavour [64]. However, it is important to note that while rosemary oil can be beneficial in food preservation, it is generally advised against direct ingestion in large amounts [57].

Chapter III

Experimental section (Rosemary Oil Extraction and Characterizations)

1. Materials and Equipment

1.1. Raw materials

1.1.1. Rosemary plant and origin

The rosemary plants used in this study were harvested and collected in February 2025 from two distinct regions in Algeria: Aïn Melah (in the Wilaya of M'Sila, central Algeria) and Biskra (southeastern Algeria). These regions were selected to assess potential variations in the rosemary plant's chemical composition and biological properties based on differences in climatic and geographical conditions.

Aïn Melah is in a semi-arid region with relatively cold winters and hot, dry summers. The area ranges between 700 and 800 meters above sea level. The soil is generally clay-loamy, with moderate organic matter content, offering suitable conditions for the growth of aromatic and medicinal plants, including rosemary.

In contrast, Biskra, known as the "Gateway to the Sahara," is situated in a desert-like environment with a hot arid climate. It experiences high temperatures during the summer, mild winters, and very low annual rainfall, averaging less than 100 mm per year. The soil is predominantly sandy and poor in organic matter, but its well-drained nature allows rosemary to thrive, particularly in rocky or stony terrains.

Both regions are known for wild and cultivated rosemary, which is traditionally used for culinary, medicinal, and aromatic purposes. The plants harvested were healthy and at full vegetative growth, ensuring optimal levels of essential oils and phenolic compounds for analysis. The comparison between these two distinct ecological zones allows for a better understanding of the environmental influence on the phytochemical profile of Rosmarinus officinalis L. As shown in Figure 1,1)**,** rosemary bushes are seen growing in the rocky terrain of Aïn Melah in February 2025, and Figure 1,2) shows more mature shrubs from Biskra in the same month.

Figure 1: Rosemary of 1) Aïn Melah and 2) Rosemary of Biskra.

1.2. Products and materials:

Table 1: Products and materials used in this study.

Item	Description
n-Hexane	(C_6H_{14}) is a colourless, volatile organic solvent belonging to the alkane family. It is commonly used in the extraction of essential oils and lipids due to its non-polar nature and high solvent efficiency. n-Hexane has a low boiling point, making it suitable for use in Soxhlet extraction, and it is often removed afterwards using a rotary evaporator. It is flammable and must be handled with proper safety precautions in a well-ventilated area.
petroleum ether	Petroleum Ether is a clear, highly volatile hydrocarbon mixture derived from petroleum distillation. Despite its name, it contains no ether functional group. It is widely used as a non-polar solvent in chemical laboratories, particularly for lipid extraction and purification processes. Petroleum ether evaporates quickly and has a low boiling range, making it ideal for gentle extraction in procedures such as Soxhlet. It should be

	handled carefully due to its flammability and potential health hazards when inhaled.
Dried Rosemary	Main plant sample collected from Aïn M'lila and Biskra regions
Distilled Water	Used as a solvent in Clevenger and hydrodistillation processes
Condenser, Round-bottom Flask (Ballon), Extraction Thimble (Cartridge), balance, beakers, heat plate, Clevenger, Filter Paper, Rotary Evaporator (Rotavapor), Hydrodistillation Setup, Soxhlet Apparatus.	

2. Rosemary oil extraction methods:

In this study, rosemary essential oil was extracted from dried leaves of Rosmarinus officinalis L. collected from two distinct Algerian regions: Aïn Melah and Biskra, during February 2025. The plant material was subjected to three different extraction techniques: hydrodistillation, Clevenger apparatus extraction, and Soxhlet extraction. Each method was carried out separately for samples from each region, to compare the yield and quality of essential oils obtained under identical experimental conditions.

2.1. Soxhlet Method

The Soxhlet method was used to extract lipid compounds from rosemary plant using two different organic solvents: n-hexane and petroleum either, four complete experiments were carried out, two for each region 15 grams of dried and ground rosemary leaves were placed in a filter paper cartridge inside the Soxhlet apparatus and 100 mL of each solvent in the experiment for 2h.

Figure 2: Soxhlet extraction.

Figure 3: Extractors Rosemary oil samples.

2.2. Hydrodistillation:

For each trial, 15 grams of dried plant material were placed in a round-bottom flask containing 250 mL of distilled water. Each experiment lasted approximately two hours. At the end of the distillation, the essential oil floating on the water surface was carefully collected.

Figure 4: Hydrodistillation extraction oil.

2.3. Clevenger method:

The Clavenger method was performed on the same two rosemary samples, using 15 grams of powdered plant material and 250 mL of distilled water for each. The apparatus was assembled with a specialized oil separator and a condenser equipped with water circulation tubes to ensure the condensation of the steam. As the oil condensed, it gradually rose within the sidearm tube and was collected without the need for manual separation. The experiment lasted two hours.
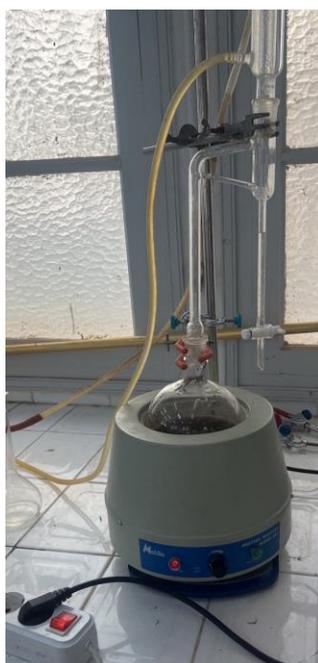
Figure 5: Clavenger extraction oil.

Afterwards, all the extracted samples were placed in the rotary evaporator (Rotavapor) under reduced pressure. This step was essential to remove any remaining solvents or moisture, ensuring a higher purity of the final essential oils.

3. Rosemary oil analysis and characterizations:

3.1. Yield:

Yield refers to the amount of essential oil extracted from a given weight of plant material. It is typically expressed as a percentage and is used to evaluate the efficiency of the extraction process.

$$Yield = \frac{Mass\ of\ extracted\ oil\ (g)}{Mass\ of\ Rosemary\ used\ (g)}\ 100$$

Where:

- Mass of extracted oil (g): The amount of rosemary oil you obtained after the extraction process.

- Mass of rosemary used (g): The initial weight of the dried rosemary plant material used in the extraction.

3.2. UV-Visible spectroscopy:

An analytical tool called a UV-Vis spectrophotometer calculates how much ultraviolet (UV) and visible light a sample absorbs. It is a commonly used method for identifying and quantifying compounds in essential oils and other substances.

3.3. FTIR analysis:

FTIR spectroscopy is a technique used to obtain the infrared spectrum of absorption of a substance. It helps identify functional groups and molecular structures by measuring how the sample absorbs infrared radiation at various wavelengths.

3.4. Gaz chromatography:

Gas chromatography is an analytical technique used to separate and analyze volatile compounds in a mixture. It is widely used to identify and quantify the individual chemical components in essential oils by comparing their retention times. The GC analysis was performed using a GCMS-QP2020 NX chromatography by split injection, with a high split ratio (80:1), likely to reduce overloading from volatile compounds.

4.   Results and discussion

4.1. Yield and physical properties

The rosemary oil obtained from the two regions (Biskra and Ain El Melh from M'Sila) is a clear yellow viscous liquid (figure 6) with a Specific odour of rosemary attributed to the presence of α-pinene or camphor [65].
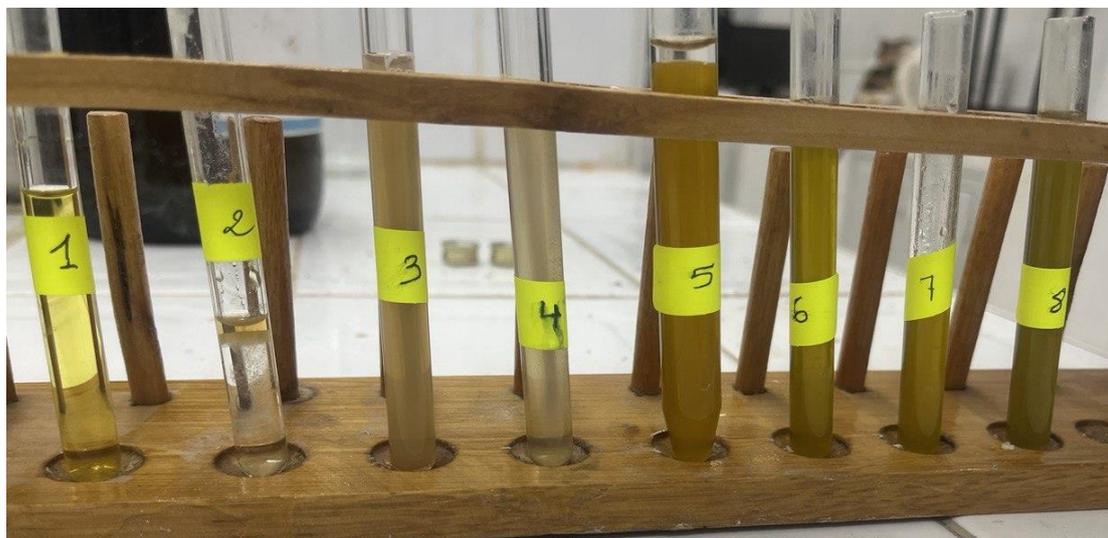
Figure 6: The rosemary oil obtained from the two regions (Biskra and Ain El Melh) was extracted using three methods.

The table shows the yield (%) and oil weight of rosemary essential oil extracted from plant material collected in two different regions **(**Biskra and Ain El Melh) using three extraction methods**.**

The extraction yield of rosemary essential oil varies significantly depending on the plant material's geographical origin and the extraction method employed, as evidenced by the comparative data from Biskra and Ain El Melh. Samples from Biskra generally produced higher yields than Ain El Melh, regardless of the method employed.

Among the tested techniques, Soxhlet extraction using hexane produced the highest yields, with Biskra samples reaching 74.13% and Ain El Melh samples 66.2%, indicating the high efficiency of non-polar solvents in extracting lipophilic components. In contrast, hydrodistillation yielded the lowest oil quantities, particularly from Ain El Melh (8.6%), highlighting the limitations of this traditional method in terms of volatile oil recovery. The Clavenger apparatus, which also employs a water distillation principle but allows better condensation and separation, showed moderate effectiveness, particularly for samples from Ain El Melh (33.8%).

Interestingly, Soxhlet extraction using ether resulted in a relatively low yield for Ain El Melh (15.7%) but a significantly higher yield for Biskra (59.8%), suggesting that the solvent's polarity and the regional phytochemical composition influence extraction efficiency. Overall, the results demonstrate that rosemary plants from Biskra generally contain higher oil content, potentially due to environmental factors such as climate and soil composition. Moreover, solvent-based methods, especially Soxhlet with hexane, appear more suitable for maximizing essential oil recovery than hydrodistillation.

Table 2: The yield (%) and oil weight of rosemary essential oil extracted from plants collected in Biskra and Ain El Melh using three extraction methods.

Methode	Region	Oil weight	Yield %
Hydro distillation	Ain El Melh	1,29	8,6
	Biskra	2,01	13,4
Clevenger	Ain El Melh	5,08	33,8
	Biskra	3,66	24,4
Soxhlet/ Hexane	Ain El Melh	9,94	66,2
	Biskra	11,12	74,13
Soxhlet/ Ether	Ain El Melh	2,36	15,7
	Biskra	8,98	59,8

In comparison with another study, the yield of essential oil of *Rosmarinus officinalis L.* plants from Biskra and Ain El Melh (M'Sila) is higher than that obtained by T. T. K. Ngan et al. [66], achieving 1 %, using the hydro-distillation method. The authors compare the physical characteristics of *Rosmarinus officinalis L.* from Vietnam with those from Kabylia (Bouira, Algeria) and conclude that the composition of the oils is significantly sensitive to climatic conditions.

To assess the quality of rosemary essential oils, we identify some of their characteristic physicochemical properties using UV-Vis, IR spectroscopy, and GC-MS analysis.

4.2. UV-Visible spectroscopy

Figure 2 presents the UV-Vis spectra of rosemary essential oil extracted using three methods from plants collected in Biskra and Ain El Melh.

The UV-Vis spectral analysis of rosemary essential oil extracted using three different methods: hydrodistillation, Soxhlet extraction, and clavenger extraction from two regions, Biskra and Ain El Melh, reveals notable differences in the absorbance profiles that reflect variations in the chemical composition and concentration of chromophoric compounds. The oils extracted via Soxhlet methods, particularly with hexane, exhibited higher absorbance intensities in the ultraviolet region (typically between 200–300 nm), suggesting a greater presence of conjugated double bonds and aromatic compounds, such as phenolic diterpenes and flavonoids, which are known for their antioxidant properties.

In contrast, oils obtained through hydro-distillation displayed lower absorbance values, indicating a reduced concentration of UV-absorbing constituents, likely due to the loss or degradation of heat-sensitive compounds during thermal processing. Additionally, the spectral differences between oils from Biskra and Ain El Melh further underscore the influence of geographical origin on phytochemical composition. Oils from Biskra consistently showed higher absorbance across all extraction methods, suggesting a richer content of UV-active secondary metabolites. These results highlight both the efficiency of solvent extraction in preserving bioactive compounds and the impact of regional variation on the quality of rosemary essential oil.
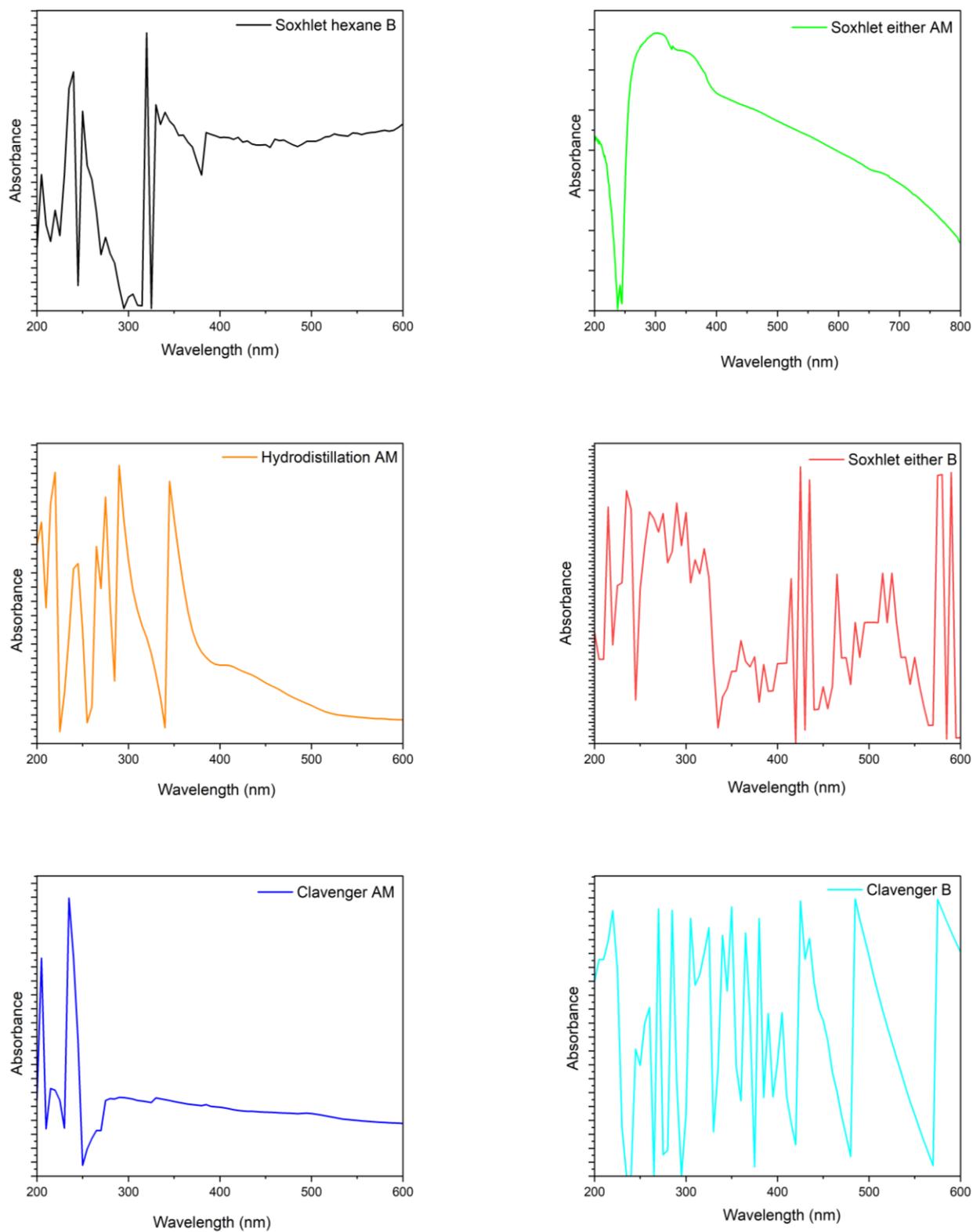
Figure 2. The UV-Vis spectra of rosemary oil were obtained from the two regions (Biskra and Ain El Melh) using three extraction methods.

4.3. FTIR spectroscopy analysis

Figure 2 presents the IR spectra of rosemary essential oil extracted using three methods from plants collected in Biskra and Ain El Melh.

The infrared (IR) spectral analysis provides insights into the functional groups and chemical composition of the oils. Across all samples, characteristic absorption bands were observed around 3400 cm^{-1}, corresponding to O–H stretching vibrations of phenolic or alcoholic compounds, and near 2920–2850 cm^{-1}, indicating C–H stretching of aliphatic hydrocarbons. Prominent peaks around 1740 cm^1 and 1640 cm^1 suggest the presence of carbonyl (C=O) and C=C stretching vibrations, respectively, consistent with ester, ketone, and terpene constituents commonly found in rosemary oil. Oils extracted via Soxhlet methods exhibited more intense and well-defined peaks, particularly in the fingerprint region (1500–600 cm^{-1}), reflecting a higher concentration and better preservation of bioactive compounds. Comparatively, oils from Biskra showed stronger absorption intensities than those from Ain El Melh, suggesting a higher phytochemical richness influenced by regional factors such as soil composition and climate. These findings demonstrate that the extraction technique and geographic origin significantly affect the molecular profile of rosemary essential oil, with Soxhlet/hexane extraction from Biskra yielding the most chemically rich samples as evidenced by IR spectral features.

Table 2. Characteristic absorption bands of rosemary essential oil on the IR spectra.

| Wavenumber (cm^{-1})	Functional Group	Vibration Type	Associated Compounds
~3400	O–H	Stretching (broad)	α-Terpineol, borneol, chrysanthenol
2920–2850	C–H (aliphatic)	Stretching	Alkanes, methyl and methylene groups
~1740	C=O (carbonyl)	Stretching	camphor, Eucalyptol, Verbenone
~1640	C=C (alkenes)	Stretching	limonene, α-Pinene, Camphene, β-Pinene, p-Cymene, γ-Terpinene
1450–1370	C–H bending	Bending (scissoring and wagging)	Alkanes, methyl groups
1260–1000	C–O	Stretching	α-Terpineol, borneol, chrysanthenol, Eucalyptol
900–650	=C–H (aromatic or alkene)	Out-of-plane bending	Aromatic and unsaturated compounds
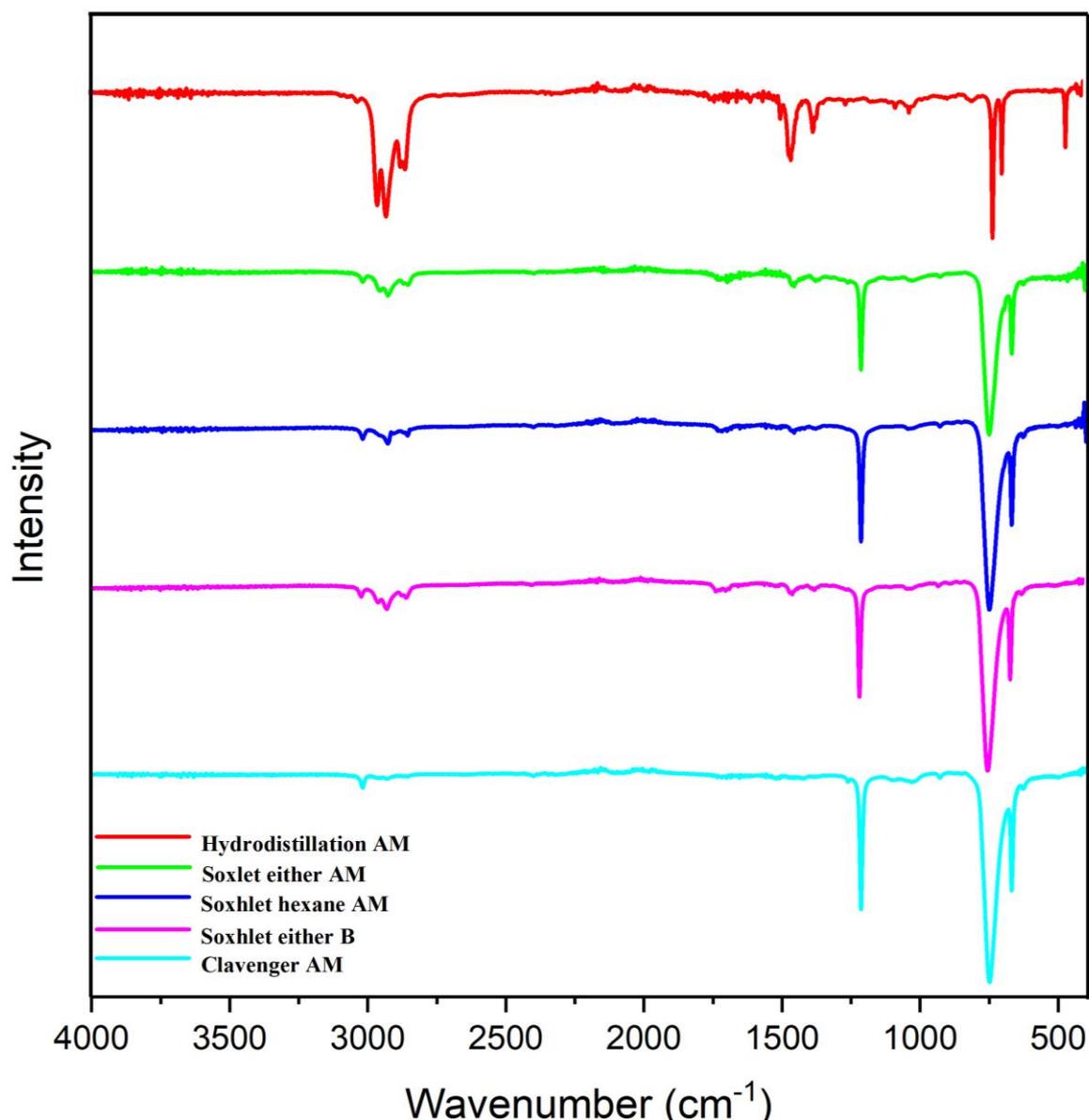
Figure 7: The FTIR spectra of rosemary oil were obtained from the two regions (Biskra and Ain El Melh) using three extraction methods.

4.4. Gas chromatography- Mass Spectrometry (GC-MS analysis)

Rosemary essential oil is typically rich in oxygenated monoterpenes like eucalyptol (1,8-cineole), camphor, α-pinene, and borneol. The GC-MS (Gas Chromatography-Mass Spectrometry) analysis of rosemary essential oil gives detailed information about its chemical composition and indicates the presence of these products.

4.4.1. GC-MS Analysis of Rosemary Essential Oil -Soxhlet either Biskra

The GC-MS analysis of rosemary oil revealed a complex phytochemical composition rich in terpenoids, esters, and hydrocarbons, reflecting its biological and aromatic potential (**Table 3**). A total of 42 compounds were identified in the sample, with Tetrapentacontane (15.59%),

α-Amyrin (8.05%), and 1,4-Bis(trimethylsilyl)benzene (8.44%) being the most abundant constituents. Other significant components included β-Amyrin, Betulin, and Lanosterol, indicating a strong presence of triterpenoids known for anti-inflammatory and antimicrobial properties. Minor components such as Camphene, **(+)-**2-Bornanone (Camphor), and endo-borneol contribute to the typical rosemary scent and are associated with therapeutic effects. The oil's rich composition of oxygenated monoterpenes and long-chain hydrocarbons suggests potential uses in pharmaceuticals, cosmetics, and food preservation. The presence of biologically active compounds such as α-Tocopherol-β-D-mannoside further supports its antioxidant potential.

Table 3: Major Constituents Identified in Rosemary Oil (Soxhlet either Biskra).

No.	Compound Name	Retention Time (RT min)	Area (%)	Class
1	Tetrapentacontane	50.91, 54.33, 55.63	15.59 + 5.03 + 4.99 ≈ 25.61	Long-chain hydrocarbon
2	α-Amyrin	53.96, 54.98	8.05 + 4.79 = 12.84	Triterpenoid
3	1,4-Bis(trimethylsilyl)benzene	61.92	8.44	Aromatic derivative
4	β-Amyrin	51.90	3.51	Triterpenoid
5	Betulin	52.55	3.43	Triterpenoid
6	α-Tocopherol-β-D-mannoside	57.99	3.32	Vitamin E derivative
7	Methyl commate C	52.93	2.67	Triterpenoid derivative
8	Lanosterol	53.25	2.53	Sterol
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	41.99	0.09	Fatty acid ester
10	9-Octadecenoic acid, methyl ester, (E)-	42.09	0.06	Fatty acid ester
11	(+)-2-Bornanone (Camphor)	19.34	0.05	Monoterpene ketone
12	endo-Borneol	20.07	0.02	Monoterpene alcohol

4.4.2. GC-MS Analysis of Rosemary Oil -Soxhlet hexane Ain El Melh

The GC-MS analysis of rosemary oil Soxhlet hexane Ain El Melh sample revealed a distinct phytochemical profile dominated by oxygenated monoterpenes and sesquiterpenes, with 39 compounds identified (Table 4). The most abundant compound was (+)-2-Bornanone (camphor), accounting for 28.27% of the total area, followed by 2-Pinene (12.19%) and Camphene (11.57%). Other significant constituents included Dotriacontane (11.68%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (4.28%), and D-Limonene (3.74%). This chemical profile is characteristic of traditional rosemary chemotypes with high monoterpene content, especially those with camphor-type signatures. Minor but relevant compounds such as Eucalyptol, α-Bisabolol, and Caryophyllene contribute to both the aroma and potential therapeutic effects of the oil. The high content of camphor and related compounds indicates strong antimicrobial and analgesic properties, making this sample suitable for pharmaceutical and aromatherapeutic applications.

Table 4: Major Constituents Identified in Rosemary Oil (Soxhlet hexane Ain El Melh).

No.	Compound Name	Retention Time (min)	Area (%)	Class
1	(+)-2-Bornanone (Camphor)	19.32	28.27	Monoterpene ketone
2	2-Pinene	10.72	12.19	Monoterpene hydrocarbon
3	Camphene	11.41	11.57	Monoterpene hydrocarbon
4	Dotriacontane	59.44	11.68	Long-chain hydrocarbon
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	41.96	4.28	Fatty acid ester
6	D-Limonene	14.86	3.74	Monoterpene hydrocarbon
7	Eucalyptol	15.05	3.64	Oxygenated monoterpene
8	9-Octadecenoic acid, methyl ester, (E)-	42.06	2.71	Fatty acid ester
9	Caryophyllene	27.58	1.73	Sesquiterpene
10	α-Bisabolol	33.87	1.21	Oxygenated sesquiterpene

4.4.3. GC-MS Interpretation of Rosemary Oil – Soxhlet either Ain El Melh

The GC-MS analysis of Soxhlet either Ain El Melh sample (**Table 5**). indicates a highly diverse phytochemical composition with a total of 34 identified compounds. The oil is remarkably rich in Tetrapentacontane (40.12%) and Dotriacontane (sum of three peaks ≈ 14.55%), indicating a substantial presence of long-chain alkanes, which are typically associated with emollient, protective, or film-forming properties. Among the biologically active terpenes, significant quantities of (+)-2-Bornanone (5.15%), 2-Pinene (1.13%), and Camphene (1.25%) are observed, confirming the identity of typical rosemary chemotypes. Other therapeutic constituents include D-Limonene, Eucalyptol, endo-borneol, and α-Bisabolol, each contributing to the aroma and pharmacological potential (e.g., anti-inflammatory, antimicrobial). Notably, the presence of Caryophyllene oxide and Thunbergol suggests anti-oxidative and neuroprotective relevance.

Table 5: Major Constituents Identified in Rosemary Oil (Soxhlet either Ain El Melh)

No.	Compound Name	Retention Time (min)	Area (%)	Class
1	Tetrapentacontane	59.56	40.12	Long-chain alkane
2	Dotriacontane (x3 peaks)	51.71, 53.21, 54.96	1.53 + 0.69 + 11.82 ≈ 14.04	Long-chain alkane
3	Toluene	4.56	16.27	Aromatic compound
4	(+)-2-Bornanone (Camphor)	19.33	5.15	Monoterpene ketone
5	Thunbergol	41.55	2.90	Diterpene alcohol
6	2-Pinene	10.74	1.13	Monoterpene hydrocarbon
7	Camphene	11.42	1.25	Monoterpene hydrocarbon
8	D-Limonene	14.88	0.82	Monoterpene hydrocarbon
9	Eucalyptol	15.06	0.29	Oxygenated monoterpene
10	endo-Borneol	20.07	0.41	Monoterpene alcohol
11	α-Bisabolol	33.88	0.40	Oxygenated sesquiterpene
12	Caryophyllene oxide	31.75	0.19	Oxygenated sesquiterpene
13	9,12-Octadecadienoic acid, methyl ester	41.98	0.34	Fatty acid ester

4.4.4. GC-MS Interpretation of Rosemary Oil – Soxhlet Hexane Biskra

Soxhlet Hexane Biskra sample (table 6) shows a complex and rich composition typical of rosemary chemotypes, featuring volatile monoterpenes and non-volatile long-chain alkanes and esters. The dominant compound is Dotriacontane (major peaks totalling ~38.56%), followed by (+)-2-Bornanone (Camphor) at 17.62%, and Tetrapentacontane (~3.45% across peaks). These major constituents highlight the dual nature of this oil, which is rich in aromatic monoterpenes and heavy alkanes.

Other notable compounds include Eucalyptol (2.12%), Linalool (0.64%), D-Limonene (0.75%), and α-Terpineol (0.91%), which contribute significantly to the oil's anti-inflammatory, antimicrobial, and antioxidant potential. Long-chain alkanes like Hexatriacontane, Squalene, and Octacosane, along with tocopherol derivatives (e.g., α-Tocopherol-β-D-mannoside), increase the oil's cosmetic and skin-healing value.

The diversity of volatile and non-volatile fractions makes the Soxhlet Hexane Biskra sample a balanced chemotype, suitable for aromatherapeutic, dermatological, and nutraceutical applications.

Table 6: Major Constituents of Rosemary Oil –Soxhlet Hexane Biskra sample.

No.	Compound Name	Retention Time (min)	Area (%)	Chemical Class	Biological/Functional Notes
1	**Dotriacontane** (multiple peaks)	54.90, 56.90, 59.40	~38.56	Long-chain alkane	Emollient, barrier function
2	**(+)-2-Bornanone (Camphor)**	19.29	17.62	Oxygenated monoterpene	Antimicrobial, analgesic
3	**Tetrapentacontane**	58.38, 61.56	3.45	Long-chain hydrocarbon	Occlusive, cosmetic use
4	**Eucalyptol**	14.99	2.12	Monoterpene oxide	Antibacterial, decongestant
5	**Linalool**	17.62	0.64	Oxygenated monoterpene	Calming, anti-inflammatory
6	**D-Limonene**	14.82	0.75	Monoterpene hydrocarbon	Antioxidant, solvent
7	**α-Terpineol**	20.81	0.91	Monoterpene alcohol	Antiseptic, fragrance component
8	**Squalene**	52.86, 53.76	1.66	Triterpene hydrocarbon	Skin barrier repair, antioxidant
9	**Hexatriacontane**	56.35	1.12	Long-chain alkane	Lubricant, skin protectant

No.	Compound Name	Retention Time (min)	Area (%)	Chemical Class	Biological/Functional Notes
10	α-Tocopherol-β-D-mannoside	60.78	0.52	Vitamin E derivative	Antioxidant, anti-aging
11	Isocarnosol	47.61	0.99	Diterpene phenol	Antioxidant, neuroprotective
12	Octacosyl acetate	50.39, 53.38, 57.24	~2.95	Alkyl ester	Skin conditioning agent
13	Ferruginol	46.34	0.37	Diterpene phenol	Anti-inflammatory, antimicrobial
14	Caryophyllene oxide	31.71	0.13	Oxygenated sesquiterpene	Anti-inflammatory, antifungal

4.4.5. GC-MS Interpretation of Rosemary Oil – Clevenger Ain El Melh

The GC-MS analysis of Clevenger Ain El Melh sample identified 18 peaks, showing a balanced composition between fatty acid esters, siloxanes, and terpenoid-derived molecules, indicating a complex essential oil profile with bioactive potential **(Table 7)**. The most dominant compound was 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (30.21%), followed by 9-Octadecenoic acid, methyl ester (E)- (18.47%), both of which are unsaturated fatty acid esters known for their anti-inflammatory, emollient, and skin-conditioning properties.

Other significant constituents include Hexadecanoic acid, methyl ester (7.69%), Andrographolide (4.08%), and Phytol (2.52%), which are bioactive terpenoid derivatives contributing to the oil's antioxidant, antimicrobial, and neuroprotective potential. A range of cyclosiloxanes (Cycloocta-, Cyclonona-, Cyclododecasiloxane) make up smaller but relevant proportions, possibly linked to the formulation or extraction method. Minor aromatic and hydrocarbon impurities such as Toluene (3.32%) and Cyclohexane (14.29%) may suggest slight contamination or solvent residue.

Table 7: Major Constituents of Rosemary Oil – Clevenger Ain El Melh

No.	Compound Name	Retention Time (min)	Area (%)	Chemical Class
1	9,12-Octadecadienoic acid, methyl ester	41.99	30.21	Unsaturated fatty acid ester
2	9-Octadecenoic acid, methyl ester (E)-	42.09	18.47	Unsaturated fatty acid ester
3	Cyclohexane	3.13	14.29	Hydrocarbon (contaminant)
4	Hexadecanoic acid, methyl ester	38.74	7.69	Saturated fatty acid ester
5	n-Hexadecanoic acid (Palmitic acid)	39.45	3.53	Saturated fatty acid
6	Toluene	4.57	3.32	Aromatic hydrocarbon

No.	Compound Name	Retention Time (min)	Area (%)	Chemical Class
7	**Andrographolide**	42.84	4.08	Diterpenoid lactone
8	**Phytol**	42.33	2.52	Diterpene alcohol
9	**Methyl stearate**	42.53	2.76	Fatty acid ester
10	**Cycloocta-/Cyclonona-/Cyclododecasiloxanes**	33.27–42.59	~4.84	Siloxanes
11	**7-epi-cis-sesquisabinene hydrate**	41.27	2.18	Sesquiterpene alcohol
12	**6,6-Diethylhoctadecane**	54.95	1.78	Alkane
13	**1-t-Butyl-4-(adamantyl-1)benzene**	42.71	3.04	Aromatic derivative

4.4.6. GC-MS Interpretation of Rosemary Oil – Hydrodistillation Ain El Melh

The GC-MS analysis of the Hydrodistillation Ain El Melh sample (**table 8**) revealed a complex composition rich in light hydrocarbons, monoterpenes, and fatty acid esters. The oil is dominated by Heptane, 4,4-dimethyl- (27.38%), Methylbenzene (Toluene, 26.59%), and Hexane, 3,3,4-trimethyl- (20.02%)—which collectively form over 70% of the total peak area. These compounds are typically not dominant natural constituents of rosemary oil, suggesting potential contamination with solvents or hydrocarbon residues, or a non-traditional extraction process.

Despite this, the sample still retains typical rosemary compounds: (+)-2-Bornanone (Camphor) at 0.33%, Eucalyptol at 0.32%, D-Limonene and Linalool, each under 0.2%, This indicates that rosemary's essential profile is present but in very low proportions.

On the other hand, fatty acid esters such as 9,12-Octadecadienoic acid, methyl ester and Hexadecanoic acid, methyl ester contribute bioactivity (anti-inflammatory, emollient), though they remain minor components. Hydrodistillation Ain El Melh sample appears chemically imbalanced, with likely contamination or adulteration, reducing its value for therapeutic or aromatic applications.

Table 8: Major Constituents of Rosemary Oil – Hydrodistillation Ain El Melh.

No.	Compound Name	Retention Time (min)	Area (%)	Notes
1	**Heptane, 4,4-dimethyl-**	3.12	27.38	Likely a contaminant, non-typical for rosemary
2	**Hexane, 3,3,4-trimethyl-**	3.18	20.02	Common solvent-like compound

No.	Compound Name	Retention Time (min)	Area (%)	Notes
3	Methylbenzene (Toluene)	4.57	26.59	May indicate contamination
4	Propanoic acid, 2-methyl-, 3-methylbutyl ester	3.31	6.40	Flavor additive, not typical
5	Heptane	3.43	12.24	Light hydrocarbon
6	Cyclohexane, methyl-	3.80	2.20	Often found in synthetic blends
7	(+)-2-Bornanone (Camphor)	19.33	0.33	Signature rosemary compound
8	Eucalyptol	15.06	0.32	Therapeutic agent
9	D-Limonene	14.87	0.10	Citrus scent, antimicrobial
10	Linalool	17.68	0.10	Fragrance and calming agent
11	9,12-Octadecadienoic acid, methyl ester	41.98	0.79	Anti-inflammatory
12	Hexadecanoic acid, methyl ester	38.73	0.24	Emollient property

4.5. Comparative Synthesis of Rosemary Essential Oil Samples

   Table 9 compares the chemical composition of the oil obtained using two types of Rosemary: one from the Biskra region and the second from the Aïn Melah region (Wilaya of M'Sila). Three extraction methods were used to study the effect of the region and the type of solvent on oil yield and quality. Overall, the GC-MS profile of the rosemary oil sample demonstrates a diverse and bioactive phytochemical makeup.

   The GC-MS, UV-Vis, and IR analyses of rosemary essential oil samples revealed significant variation in chemical profiles, reflecting differences in chemotype, extraction conditions, and potential contamination. Soxhlet hexane Ain El Melh sample exhibited a classic camphor-type rosemary chemotype, dominated by (+)-2-Bornanone (28.27%), 2-Pinene, and Eucalyptol, indicative of high volatile monoterpene content suited for aromatherapy and topical medicinal use. In contrast, Soxhlet either Biskra sample displayed a unique triterpenoid-rich profile with major constituents like α-/β-Amyrin, Betulin, and Tetrapentacontane, aligning it more closely with therapeutic or cosmetic applications due to its anti-inflammatory potential.

   Soxhlet Hexane Biskra sample offered a balanced profile, combining moderate camphor content (17.62%) with a high proportion of long-chain alkanes (Dotriacontane ~38%) and fatty acid esters, making it suitable for skin care formulations. Meanwhile, Soxhlet either Ain El
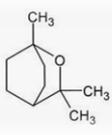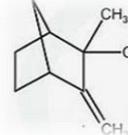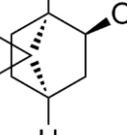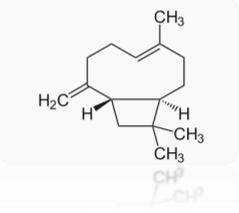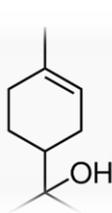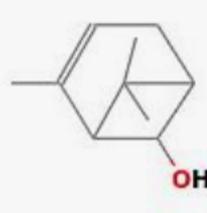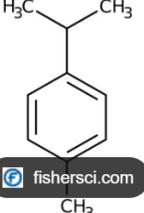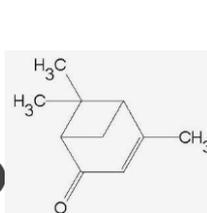
Melh sample showed an alkane-dominant composition (Tetrapentacontane 40.12%, Dotriacontane), but was marred by the presence of Toluene (16.27%), which points to possible solvent contamination, reducing its suitability for medicinal use.

Clevenger Ain El Melh sample exhibited a unique profile dominated by unsaturated fatty acid esters like 9,12-Octadecadienoic acid, methyl ester (30.21%), and Cyclohexane (14.29%), again suggesting non-standard distillation or external contamination, although therapeutic compounds like Phytol and Andrographolide were present. Hydrodistillation Ain El Melh sample stood out with over 70% composed of hydrocarbon solvents and alkanes such as Heptane, Hexane isomers, and Toluene, with only trace amounts of rosemary terpenes, indicating it is chemically atypical and likely contaminated, unsuitable for therapeutic use.

The compositional differences may arise from geographical variation, harvest timing, or distillation methods. In contrast to more volatile rosemary oils, Soxhlet either Ain El Melh sample also features Toluene (16.27%), a potential contaminant or solvent marker rather than a natural constituent. However, the unusual abundance of hydrocarbons and the solvent-like Toluene peak raises questions about extraction or storage conditions.

These results highlight the chemical diversity among rosemary essential oils and underscore the need for strict quality control to ensure proper usage in pharmaceutical, cosmetic, or aromatic products.

Table 9: Chemical composition of Rosmarinus officinalis Essential Oil.

Eucalyptol (1,8-cineole)	α-Pinene	Camphor (2-Bornanone)	Camphene	β-Pinene	Borneol

β-Caryophyllene	α-Terpineol	Cis-chrysanthenol	p-Cymene	Verbenone

D-Limonene   Isopulegol   γ-Terpinene   Linalool

Chemical Structure of Compounds	Biskra percentage (%)				Ain El Melh percentage (%)		Ref Jiangsu, China
	Soxhlet method		Clevenger method	Hydro distillation method (1)	Soxhlet method	Clevenger method	hydro-distillation method
	Hexane (5)	Ether (6)			Hexane (7)	Ether (8)	
Eucalyptol (1,8-cineole) (Major in natural rosemary)	2.12	3.64		3.2	0.29		26.54%
0α-Pinene		12.19			1.13		20.14%
Camphor (2-Bornanone) (Key rosemary constituent)	17.62	28.27		3.3	5.15	0.05	12.88%
(Typical rosemary terpene)	0.55	11.57		0.9	1.25	0.02	11.38%
β-Pinene							6.95%
Borneol (common in rosemary)		1.87		3.7	0.41	0.02	3.06%
β-Caryophyllene	0.13	1.73			0.19		2.37%
α-Terpineol	0.91				0.18		1.95%
Cis-chrysanthenol							1.93%
p-Cymene	0.28	1.91			0.28		1.59%
Verbenone							1.36%

Chemical Structure of Compounds	Biskra percentage (%)				Ain El Melh percentage (%)		Ref Jiangsu, China
	Soxhlet method		Clevenger method	Hydro distillation method (1)	Soxhlet method	Clevenger method	hydro-distillation method
	Hexane (5)	Ether (6)			Hexane (7)	Ether (8)	
D-Limonene (Citrus note)	0.75	3.74		1.0	0.82		1.32%
Isopulegol		0.42					1.03%
γ-Terpinene		0.38					1.02%
Linalool (Floral aroma)	0.64			1.0			

Table 10: Comparative Table: Key Constituents of Rosemary Oil Samples.

Feature / Compound	Hydrodistillation Ain El Melh	Clevenger Ain El Melh	Soxhlet hexane Biskra	Soxhlet hexane Ain El Melh	Soxhlet either Ain El Melh	Soxhlet either Biskra
Main Compound	Heptane, 4,4-dimethyl (27%)	9,12-Octadecadienoic acid (30%)	Dotriacontane (~38.56%)	(+) -2-Bornanone (28.27%)	Tetrapentacontane (40.12%)	Tetrapentacontane (25.61%)
Camphor [(+)-2-Bornanone]	0.33%	–	17.62%	**28.27%**	5.15%	0.05%
Eucalyptol	0.32%	2.12%	2.12%	3.64%	0.29%	–
Toluene (contaminant)	26.59%	3.32%	–	–	**16.27%**	–
Fatty acid esters	~1.5%	**High (~50%)**	Moderate	Moderate	Low	Low
Terpenes (Total)	Trace	Low	Medium	**High**	Medium	Low
Triterpenoids (Amyrins, Betulin)	–	–	–	–	–	**High (~12%)**
Long-chain alkanes	Present	Present	**Very High**	Medium	**Very High**	High
Oxygenated monoterpenes	Trace	Moderate	Moderate	**High**	Low	Trace

Feature / Compound	Hydrodistillation Ain El Melh	Clevenger Ain El Melh	Soxhlet hexane Biskra	Soxhlet hexane Ain El Melh	Soxhlet either Ain El Melh	Soxhlet either Biskra
Overall Profile	Contaminated, solvent-rich	Fatty ester-rich, non-typical	Balanced (terpenes + alkanes)	Aromatic, monoterpene-dominant	Alkane-heavy, possibly altered	Triterpenoid-heavy, therapeutic
Suitability	Unsuitable	Cosmetic/ industrial use only	Cosmetic/ pharma blend	Therapeutic/aromatherapy	Industrial/ caution advised	Pharma/ cosmetic (anti-aging)

Table 11: Chemical Class and Biological Function of the Main Constituents of Rosemary Oil.

No.	Compound Name	Chemical Class	Biological/Functional Notes
1	Dotriacontane (multiple peaks)	Long-chain alkane	Emollient, barrier function
2	(+) -2-Bornanone (Camphor)	Oxygenated monoterpene	Antimicrobial, analgesic
3	Tetrapentacontane	Long-chain hydrocarbon	Occlusive, cosmetic use
4	Eucalyptol	Monoterpene oxide	Antibacterial, decongestant
5	Linalool	Oxygenated monoterpene	Calming, anti-inflammatory
6	D-Limonene	Monoterpene hydrocarbon	Antioxidant, solvent
7	α-Terpineol	Monoterpene alcohol	Antiseptic, fragrance component
8	Squalene	Triterpene hydrocarbon	Skin barrier repair, antioxidant
9	Hexatriacontane	Long-chain alkane	Lubricant, skin protectant
10	α-Tocopherol-β-D-mannoside	Vitamin E derivative	Antioxidant, anti-aging
11	Isocarnosol	Diterpene phenol	Antioxidant, neuroprotective
12	Octacosyl acetate	Alkyl ester	Skin conditioning agent
13	Ferruginol	Diterpene phenol	Anti-inflammatory, antimicrobial
14	Caryophyllene oxide	Oxygenated sesquiterpene	Anti-inflammatory, antifungal
1	9,12-Octadecadienoic acid, methyl ester	Unsaturated fatty acid ester	Anti-inflammatory, skin conditioning
2	9-Octadecenoic acid, methyl ester (E)-	Unsaturated fatty acid ester	Emollient, wound healing
4	Hexadecanoic acid, methyl ester	Saturated fatty acid ester	Emollient, lipid metabolism
5	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	Antioxidant, antimicrobial
6	Toluene	Aromatic hydrocarbon	Possible contaminant
7	Andrographolide	Diterpenoid lactone	Anti-inflammatory, hepatoprotective

No.	Compound Name	Chemical Class	Biological/Functional Notes
8	**Phytol**	Diterpene alcohol	Antioxidant, precursor of Vitamin K/E
9	**Methyl stearate**	Fatty acid ester	Emollient, surfactant
11	**7-epi-cis-sesquisabinene hydrate**	Sesquiterpene alcohol	Anti-inflammatory, neuroprotective

Conclusion

Through this study, we explored the vast potential of Salvia rosmarinus, commonly known as rosemary, a plant of high value in many fields. In the first chapter, we provided a comprehensive overview of this plant, focusing on its botanical characteristics, richness in active chemical compounds, and its diverse industrial applications, with particular emphasis on its importance in Algeria. We also addressed the main challenges facing its cultivation, stressing the need for sustainable agricultural practices to improve both quality and productivity. In the second chapter, we examined the various techniques for extracting essential oils from rosemary. By comparing traditional and modern methods—such as steam distillation, solvent extraction, Soxhlet method, hydrodistillation, ultrasound-assisted extraction, and microwave-assisted extraction—we highlighted the impact of each technique on the yield and quality of the extracted oil. We also emphasized the importance of controlling extraction conditions to ensure an effective oil with high therapeutic and industrial properties. In the third chapter, we presented the practical aspect of our work. We applied selected extraction methods to obtain rosemary oil, and the characterization was carried out using advanced analytical techniques. We also studied the biological activity of the oil, particularly from a medical perspective, which supported the theoretical findings. The comparative analysis between the different methods gave us a deeper understanding of the efficiency and practicality of each technique in real applications. In conclusion, this study not only contributes to a deeper understanding of Salvia rosmarinus but also highlights its importance as a promising natural resource with wide-ranging applications. The results of this work can serve as a solid scientific foundation for future research aimed at optimizing extraction methods and promoting the sustainable exploitation of this valuable plant, especially within the Algerian context.

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