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SUBJECT OF THE MEMORY:

Preparation and Evaluation of a Therapeutic Ointment Using Beehive Derivatives

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In front of the jury:

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Dedication

To my beloved parents,

To my dear wife,

To my sister,

To all the members of my big family,

To all my teachers and instructors,

To all my friends and classmates,

To the pursuit of knowledge, the thrill of discovery and the hope of their use for the

betterment of humankind

ABDELHAK

Dedication

To my mother and father,

To those who were my home when the world felt tight, and my support when life

grew heavy.

You filled my life with a love like no other, and a giving spirit beyond measure.

Thank you for your patience during my illness, for embracing my pain, and for your

prayers that lit my way in every moment of weakness.

This graduation is the fruit of your love, your prayers, and your unwavering belief in

me. You deserve all the glory.

To my beloved husband,

You were the light in my return, the hand that held me when I was on the verge of

giving up.

I dedicate this achievement to you because you didn’t just complete our marriage —

you completed my heart and guided me back to my path when I had lost my way.

You supported me with your love, your patience, and your faith in me when I had

given up on my dreams.

You are the most beautiful chapter in my life, and the partner of a dream that has

come true.

To my dear siblings,

You are the support that never bends, the silent smile that lifted me through my

struggles.

Thank you for being the warm presence in my darkest moments, and the sincere joy

in my triumphs.

My success would not be complete without you, and my pride would not be whole

without your proud eyes.

And to the soul of my little child,

To you who came into my life like a beautiful dream and left before I could call your

name enough.

I didn’t hold you long in my arms, but you will forever hold my heart.

You were the light in my hardest moments, and the tear that never dries.

You left too soon, but taught me so much — patience, acceptance, and unconditional

love.

I dedicate this graduation to your pure soul, my little piece of heaven.

I will always remember you in my prayers, and smile through my tears whenever Ilook to the sky

ASMA

Abstract

Natural therapeutic alternatives have become a growing area of interest in recent years, yet they remain a subject of debate within the medical and scientific communities. This controversy is largely due to the lack of rigorous scientific studies confirming their efficacy and safety. Contributing factors include their potential competition with conventional pharmaceutical industries, the shortage of validated research that facilitates scientific advancement, and the ease of public access, which may lead to unsupervised use and unintended health consequences.

This study aims to formulate a 100% natural therapeutic ointment using beehive derivatives, primarily beeswax, blended with natural oils (*olive oil, clove oil*, *Artemisiaoil*, and *rosemary oil*). Beeswax was obtained from a modern apiary in Guemar, and olive oil from a local press. Essential oils were extracted through hydrodistillation using a Clevenger apparatus. Characterization of the three essential oils and the prepared ointment was performed using Fourier-transform infrared spectroscopy (FTIR) to observe the formation of new chemical bonds after blending.

In the experimental phase, 100 grams of beeswax were dissolved in 600 mL of olive oil. Three samples of 10 grams each were prepared by adding 0.05 grams of Indigofera tinctoria (natural blue dye) to each, followed by varying concentrations of the three essential oils (10 µL, 15 µL, and 20 µL, respectively). The biological activity was assessed against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and certain fungal strains using the agar well diffusion method. The results showed notable antibacterial activity against Gram-positive bacteria and antifungal activity, while activity against Gram-negative bacteria was limited. These findings open promising prospects for the development and use of this ointment as a natural remedy for skin allergies and infections of bacterial, fungal, or potentially viral origin.

Keywords: Rosemary essential oil, Artemisia essential oil, Clove essential oil, Beeswax, Olive oil, Indigofera tinctoria, FTIR, Bioactivity.

الملخص

تُعدّ البدائل العلاجية الطبيعية مجالًا متنامي الاهتمام في السنوات الأخيرة، إلا أنها لا تزال محل جدل في الأوساط الطبية والعلمية، ويرجع ذلك أساسًا إلى نقص الدراسات العلمية الدقيقة التي تثبت فعاليتها وسلامتها. ومن أبرز العوامل المؤدية إلى هذا الجدل منافستها المحتملة لصناعة الأدوية التقليدية، وغياب الأبحاث المعمّقة التي تسهم في تسهيل التقدم العلمي، بالإضافة إلى سهولة الحصول عليها من قِبل العامة دون إشراف طبي، مما قد يؤدي إلى استخدامها العشوائي وما يترتب على ذلك من مضاعفات صحية غير محسوبة.

تهدف هذه الدراسة إلى تحضير مرهم علاجي طبيعي 100% باستخدام مشتقات خلية النحل، وعلى رأسها شمع النحل، ممزوجًا بمجموعة من الزيوت الطبيعية (زيت الزيتون، وزيت القرنفل، وزيت الشيح، وزيت إكليل الجبل). تم الحصول على شمع النحل من منحلة حديثة بمنطقة قمار، أما زيت الزيتون فتم استخراجه من معصرة تقليدية محلية. كما تم استخلاص الزيوت الأساسية للنباتات الثلاثة عبر التقطير بالماء المقطر باستخدام جهاز كليفنجر. وتم تشخيص هذه الزيوت الأساسية والمرهم المحضر باستخدام تقنية التحليل الطيفي بالأشعة تحت الحمراء (FTIR) بهدف الكشف عن تكوّن روابط كيميائية جديدة بعد المزج.

في الجانب التطبيقي، تم إذابة 100 غرام من شمع النحل في 600 مل من زيت الزيتون، ثم قمنا بتحضير ثلاث عينات بوزن 10 غرام لكل منها، أضيف إليها 0.05 غرام من النيلة الزرقاء، بالإضافة إلى تراكيز مختلفة من الزيوت الأساسية (10 ميكرولتر، 15 ميكرولتر، و20 ميكرولتر على التوالي). وقد تم تقييم النشاط الحيوي لهذه العينات ضد بكتيريا موجبة الغرام(Staphylococcus aureus) ، وسالبة الغرام(Escherichia coli) ، وبعض السلالات الفطرية باستخدام طريقة الانتشار في آبار الآغار. أظهرت النتائج فعالية واضحة ضد البكتيريا موجبة الغرام والفطريات، في حين كانت الفعالية محدودة ضد البكتيريا سالبة الغرام.

تظهر هذه النتائج آفاقًا واعدة لاستخدام هذا المرهم كعلاج طبيعي محتمل لأمراض الجلد المرتبطة بالعدوى البكتيرية والفطرية، وربما الفيروسية، مع إمكانية تطويره وتحسين خصائصه العلاجية مستقبلاً.

الكلمات المفتاحية: زيت إكليل الجبل، زيت الشيح، زيت القرنفل، شمع النحل، زيت الزيتون، النيلة الزرقاء،النشاط الحيوي.

Résumé

Les alternatives thérapeutiques naturelles suscitent un intérêt croissant ces dernières années, mais elles demeurent controversées au sein des milieux médicaux et scientifiques. Cette controverse est principalement due à l'insuffisance d'études scientifiques rigoureuses prouvant leur efficacité et leur innocuité. Parmi les facteurs expliquant cette situation figurent la concurrence qu'elles représentent face à l'industrie pharmaceutique conventionnelle, le manque de recherches approfondies facilitant l'avancement scientifique, ainsi que l'accès facile du grand public à ces produits sans prescription médicale, ce qui peut entraîner une utilisation aléatoire et des effets indésirables potentiels.

Cette étude vise à formuler une pommade thérapeutique 100 % naturelle à base de dérivés de la ruche, principalement la cire d'abeille, mélangée à une sélection d'huiles naturelles (huile d'olive, huile essentielle de clou de girofle, d'armoise et de romarin). La cire d'abeille a été obtenue d'un rucher moderne situé à Guemar, et l'huile d'olive d'un moulin local. Les huiles essentielles ont été extraites par hydrodistillation à l'aide d'un appareil de Clevenger. La caractérisation des huiles et de la pommade a été effectuée par spectroscopie infrarouge à transformée de Fourier (FTIR) afin de détecter la formation de nouvelles liaisons chimiques après mélange.

Sur le plan expérimental, 100 grammes de cire d'abeille ont été dissous dans 600 mL d'huile d'olive. Trois échantillons de 10 grammes chacun ont été préparés, auxquels ont été ajoutés 0,05 gramme d'indigo naturel (Indigoferatinctoria), ainsi que différentes concentrations d'huiles essentielles (10, 15 et 20 µL respectivement). L'activité biologique a été évaluée contre des bactéries à Gram positif (Staphylococcus aureus), à Gram négatif (Escherichia coli), et certaines souches fongiques en utilisant la méthode de diffusion en puits sur gélose. Les résultats ont révélé une activité significative contre les bactéries à Gram positif et les champignons, tandis que l'effet sur les bactéries à Gram négatif s'est avéré limité.

Ces résultats ouvrent des perspectives prometteuses pour le développement et l'utilisation de cette pommade comme traitement naturel potentiel des affections cutanées d'origine bactérienne, fongique, voire virale.

Mots-clés : Huile essentielle de romarin, huile essentielle d'armoise, huile essentielle de clou de girofle, cire d'abeille, huile d'olive, indigo naturel, FTIR, activité biologique

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General Introduction

Among the growing global trend toward the use of natural products as safer alternatives to synthetic drugs, bee-derived substances have attracted increasing scientific and medical interest due to their diverse biological properties, including antimicrobial, anti-inflammatory, and wound-healing effects[1]. Among the most studied bee products are honey, propolis, and beeswax—natural substances that have long been used in traditional medicine, with modern research now confirming their broad therapeutic potential[2].

Despite this promise, there remains a significant research gap concerning the development of stable and effective pharmaceutical formulations based on these substances[3]. Specifically, challenges persist in optimizing the physicochemical properties of such formulations while ensuring their topical safety and therapeutic efficacy. Moreover, incorporating essential oils with known antiseptic properties—such as those derived from Artemisia, clove, and rosemary—may enhance the healing activity, provided their concentrations are optimized and their safety profiles are established[4].

Accordingly, the present study aims to formulate a therapeutic ointment using beeswax and olive oil as the lipid base, enriched with essential oils extracted from Artemisia herba-alba, Syzygium aromaticum (clove), and Rosmarinus officinalis through hydro distillation using a Clevenger apparatus[5]. The formulated ointment was subjected to laboratory evaluations, including antimicrobial and antifungal activity assays, as well as toxicity screening to assess its safety. Additionally, the ointment’s physical characteristics were studied and its performance compared to established criteria, offering insight into its potential as a safe and natural treatment for skin wounds and dermatological conditions[6].

References :

[1] D. J. Newman and G. M. Cragg, “Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019,” Journal of Natural Products, vol. 83, no. 3, pp. 770–803, 2020.

[2] V. R. Pasupuleti, L. Sammugam, N. Ramesh, and G. Q. Hua, “Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits,” Oxidative Medicine and Cellular Longevity, vol. 2017, Article ID 1259510, 2017.

[3] F. Fratini, G. Cilia, B. Turchi, and A. Felicioli, “Bee products and their potential use in modern medicine,” Antibiotics, vol. 5, no. 4, p. 19, 2016.

[4] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, “Biological effects of essential oils – A review,” Food and Chemical Toxicology, vol. 46, no. 2, pp. 446–475, 2008.

[5] R. Kowalski and M. Wójciak-Kosior, “Formulation and evaluation of herbal ointments containing propolis, calendula, and plantain extracts for wound healing,” Molecules, vol. 26, no. 5, p. 1273, 2021.

[6] European Pharmacopoeia, 7th ed., Council of Europe, 2010.

Chapter I

Overview on The Honest Derivatives

1.1 Introduction

The beehive is a rich source of natural products with diverse therapeutic benefits, such as honey, propolis, and beeswax. Beeswax, in particular, has gained special attention in pharmaceutical formulations—especially ointments—due to its soothing and antibacterial properties, as well as its role as a natural lipid base that helps protect the skin and promote wound healing.

1.2 Literature Background of Honey

1.2.1 Definition of Honey

Honey is a natural sweet substance produced by bees from the nectar of flowers or from plant secretions rich in sugars. Bees collect these substances, transform them using specific enzymes, and store the final product in wax combs within the hive. Honey primarily consists of simple sugars and is rich in compounds with significant nutritional and medicinal properties. It has been used since ancient times both as food and as a remedy [1].

Figure 1: Illustration of Bees Honey

1.2.2 Physical and Chemical Properties

- ✓ **Color:** Varies from transparent to dark amber, depending on the type of nectar and plant source [2].
- ✓ **Density:** Higher than water, ranging between 1.38 and 1.45 g/cm³ at 20°C [2].
- ✓ **Viscosity:** Very high and influenced by water content and temperature [3].
- ✓ **pH Level:** Mildly acidic (pH 3.4 to 6.1), which helps inhibit microbial growth [3].
- ✓ **Crystallization Tendency:** Depends on the glucose-to-fructose ratio; honey with higher glucose content crystallizes more rapidly [4].

1.2.3 Chemical Composition

- ✓ **Sugars:** Constitute approximately 95% of the dry matter, primarily fructose (38%) and glucose (31%) [5].
- ✓ **Water:** Comprises about 17–20% of fresh honey [5].
- ✓ **Organic Acids:** Such as gluconic acid, which contributes to honey's acidic nature [6].
- ✓ **Enzymes:** Including amylase, glucose oxidase, and catalase, which aid in breakdown processes and biological activity [6].
- ✓ **Vitamins:** Such as B1, B2, B6, and folic acid, present in trace amounts [6].
- ✓ **Minerals:** Including iron, calcium, magnesium, and potassium [6].
- ✓ **Phenolic Compounds:** Possess antioxidant properties [7].

1.2.4 Medicinal Uses

- ✓ **Wound and ulcer treatment:** Due to its antibacterial properties and ability to accelerate tissue healing [8].
- ✓ **Skin hydration and treatment of dryness:** Attributed to its moisturizing sugars and soothing compounds [9].
- ✓ **Acne treatment:** Thanks to its anti-inflammatory and antimicrobial characteristics [10].
- ✓ **Facial masks:** Used to nourish the skin and enhance its radiance [11].
- ✓ **Burn relief:** When combined with other ingredients, honey can help treat minor burns and reduce skin inflammation [8].

1.3 Literature Background of Beeswax

1.3.1 Definition of Beeswax

Beeswax is a natural waxy substance secreted by worker bees from specialized wax glands located on the sides of their abdomen. It is used in constructing honeycombs, where honey and pollen are stored, and where brood is raised. Initially colorless, beeswax gradually turns yellow or brown due to the absorption of pigments from pollen, propolis, and aromatic oils [1].

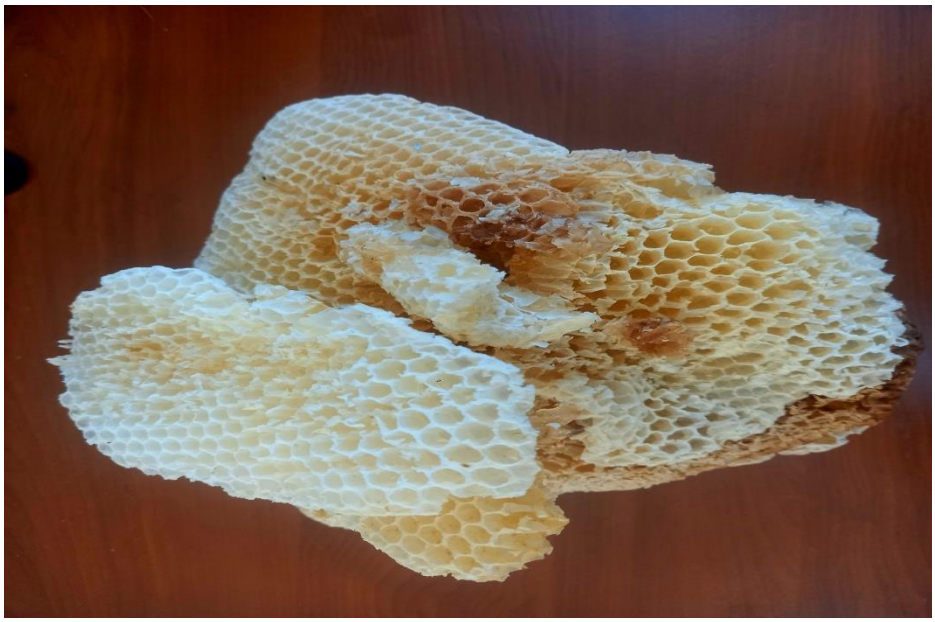

Figure 2: Photograph of the beeswax employed in the formulation process of the therapeutic ointment developed in this study

1.3.2 Physical and Chemical Properties

- ✓ **Color:** Ranges from white to dark yellow or brown, depending on the nectar and pollen source [12].
- ✓ **Odor:** Characteristic aromatic scent derived from honey and volatile oils.
- ✓ **Specific Gravity:** Ranges between 0.95 and 0.98 at 15°C [13].
- ✓ **Melting Point:** Between 61°C and 65°C, considered one of its key physical properties [13].
- ✓ **Solubility:** Insoluble in water but soluble in hot alcohol, ether, chloroform, and certain volatile oils [14].

- ✓ **Chemical Stability:** Exhibits relative stability and is resistant to oxidation, making it suitable for long-term storage [13].

1.3.3 Chemical Composition

- ✓ **Esters:** Comprise approximately 70–80% of beeswax, primarily esters of long-chain fatty acids and alcohols, such as myricyl palmitate [15].
- ✓ **Free Fatty Acids:** Make up about 12–15%, notably palmitic acid [15].
- ✓ **Saturated Hydrocarbons:** Represent around 14–15%, including hexadecane and heptadecane [16].
- ✓ **Alcohols:** Account for 1–2%, mainly long-chain fatty alcohols [16].

1.3.4 Medicinal Uses

- ✓ **Wound and burn treatment:** Owing to its antibacterial and anti-inflammatory properties, beeswax acts as a protective barrier and promotes skin healing [17].
- ✓ **Topical treatment for eczema and psoriasis:** Due to its moisturizing and anti-inflammatory effects [18].
- ✓ **Ingredient in skin ointments:** Especially effective in treating skin fissures, including nipple inflammation in breastfeeding women [19].
- ✓ **Treatment of fungal and bacterial skin infections:** Contains bioactive compounds with antimicrobial activity [17].
- ✓ **Used with honey in preparations for chronic wound and ulcer treatment:** Enhances healing and provides microbial protection [20].

1.4 Literature Background of Propolis

1.4.1 Definition of Propolis

Propolis, commonly referred to as “bee glue,” is a resinous, sticky substance collected by honeybees from plant resins, such as those found in tree buds or sap flows. Bees mix these substances with enzymes and wax to form propolis, which is used to seal hive cracks, inhibit microbial growth, and preserve hive sterility [21].

Figure 3: Illustration of Propolis in a Beehive

1.4.2 Physical Properties of Propolis

- ✓ **Color:** Ranges from yellow-green to dark brown depending on botanical origin and storage conditions [22].
- ✓ **Texture:** Soft and pliable when warm, hard and brittle when cooled [22].
- ✓ **Melting Point:**Typically between 60°C and 70°C [22].
- ✓ **Solubility:** Insoluble in water, but soluble in ethanol, ether, and alkaline solutions [23].
- ✓ **Odor and Taste:** Characteristic aromatic scent and a bitter, slightly pungent flavor due to essential oils and plant compounds [21].

1.4.3 Chemical Composition of Propolis

The chemical composition of propolis is influenced by factors such as geographic region, the botanical sources available to bees, and the time of year. Typically, propolis is composed primarily of resins and balsamic substances—particularly flavonoids and phenolic acids—which make up about half to over half of its content. Waxes account for roughly one-third, while essential oils are present in smaller amounts, generally ranging from 5% to 10%. Additionally, pollen contributes around 5%, and minor quantities of various other organic compounds may also be present [24].

1.4.4 Key bioactive compounds include

- ✓ Flavonoids: such as pinocembrin, galangin, and chrysin
- ✓ Phenolic acids and esters: e.g., caffeic acid, ferulic acid, and caffeic acid phenethyl ester (CAPE)
- ✓ Aromatic aldehydes and alcohols
- ✓ Triterpenes and steroids
- ✓ Vitamins: B1, B2, B6, C, and E in minor concentrations
- ✓ Minerals: such as calcium, magnesium, zinc, and iron [25].

1.4.5 Chemical Formulas of Key Compounds

- ✓ Caffeic Acid Phenethyl Ester (CAPE): $C_{17}H_{16}O_4$
- ✓ Chrysin: $C_{15}H_{10}O_4$
- ✓ Galangin: $C_{15}H_{10}O_5$
- ✓ Pinocembrin: $C_{15}H_{12}O_4$ [25]

1.4.6 Biological and Pharmacological Properties

Propolis exhibits a wide range of pharmacological activities, making it a valuable natural product in therapeutic applications. It demonstrates antibacterial properties, showing efficacy against both Gram-positive and Gram-negative bacterial strains [21]. Its antiviral action has been observed against pathogens such as the herpes simplex virus and the influenza virus [24]. In terms of antifungal activity, propolis is capable of inhibiting species like *Candida albicans* and various dermatophytes [25]. Additionally, it possesses anti-inflammatory effects by modulating cytokine production and interfering with inflammatory pathways [22]. As an antioxidant, propolis helps protect cells by neutralizing reactive oxygen species [23]. It also plays an immunomodulatory role, either enhancing or regulating immune function [25]. Furthermore, its contribution to wound healing is significant, as it supports tissue regeneration while reducing the risk of infection [21,24]

References

[1] J. W. White, "Composition of honey," in *Honey: A Comprehensive Survey*, E. Crane, Ed., London: Heinemann, 1975, pp. 157–206.

[2] S. Bogdanov, *Physical properties and chemical composition of honey*, Bee Product Science, 2009.

[3] P. M. da Silva, C. Gauche, L. V. Gonzaga, A. C. O. Costa, and R. Fett, "Honey: Chemical composition, stability and authenticity," *Food Chem.*, vol. 196, pp. 309–323, 2016.

[4] A. S. Alqarni, A. A. Owayss, and A. A. Mahmoud, "Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia," *Arab. J. Chem.*, vol. 7, no. 6, pp. 1019–1026, 2014.

[5] R. A. Gleiter, H. Horn, and H. D. Isengard, "Influence of type and state of crystallisation on the water content of honey," *Food Chem.*, vol. 96, no. 3, pp. 441–445, 2006.

[6] S. Ahmed *et al.*, "Honey as a potential natural antioxidant medicine: An insight into its molecular mechanisms of action," *Oxid. Med. Cell. Longev.*, vol. 2018, Art. no. 8367846, 2018.

[7] I. C. Ferreira, E. Aires, J. C. Barreira, and L. M. Estevinho, "Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract," *Food Chem.*, vol. 114, no. 4, pp. 1438–1443, 2009.

[8] P. C. Molan, "Potential of honey in the treatment of wounds and burns," *Am. J. Clin. Dermatol.*, vol. 2, no. 1, pp. 13–19, 2001.

[9] N. S. Al-Waili, "Topical application of natural honey for wound healing: A review," *Complement. Ther. Clin. Pract.*, vol. 9, no. 1, pp. 38–43, 2003.

[10] A. A. Alangari*et al.*, "Topical honey application in the management of seborrheic dermatitis: A randomized controlled pilot study," *Clin. Cosmet. Investig. Dermatol.*, vol. 10, pp. 23–27, 2017.

[11] B. Burlando and L. Cornara, "Honey in dermatology and skin care: A review," *J. Cosmet. Dermatol.*, vol. 12, no. 4, pp. 306–313, 2013.

[12] H. R. Hepburn and S. P. Kurstjens, "The combs of honeybees," *Apidologie*, vol. 19, no. 4, pp. 388–407, 1988.

[13] E. A. Adebayo and T. L. Akindele, "Beeswax: Properties, uses, and future directions," *Int. J. Res. Innov. Appl. Sci.*, vol. 5, no. 10, pp. 118–123, 2020.

[14] J. W. White, "Composition of honey," in *Honey: A Comprehensive Survey*, E. Crane, Ed., London: Heinemann, 1975, pp. 157–206.

[15] C. Turek and F. C. Stintzing, "Stability of essential oils: A review," *Compr. Rev. Food Sci. Food Saf.*, vol. 12, no. 1, pp. 40–53, 2013.

[16] A. P. Tulloch, "Composition of beeswax and other waxes," *Lipids*, vol. 15, no. 12, pp. 969–975, 1980.

[17] N. S. Al-Waili, "Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: Partially controlled, single-blinded study," *Complement. Ther. Med.*, vol. 11, no. 4, pp. 226–234, 2003.

[18] A. Peterfalvi*et al.*, "Immunomodulatory effects of propolis: A review," *Nutrients*, vol. 12, no. 3, pp. 1–24, 2020.

[19] N. H. Abdel-Azim *et al.*, "Formulation of topical beeswax-based ointment: Evaluation of its wound healing activity," *J. Drug Deliv. Sci. Technol.*, vol. 61, Art. no. 102085, 2021.

[20] P. C. Molan, "Potential of honey in the treatment of wounds and burns," *Am. J. Clin. Dermatol.*, vol. 2, no. 1, pp. 13–19, 2001.

[21] V. Bankova, S. L. de Castro, and M. C. Marcucci, "Propolis: Recent advances in chemistry and plant origin," *Apidologie*, vol. 31, no. 1, pp. 3–15, 2000.

[22] A. Kujumgiev, I. Tsvetkova, Y. Serkedjieva, V. Bankova, R. Christov, and S. Popov, "Antibacterial, antifungal and antiviral activity of propolis of different geographic origin," *J. Ethnopharmacol.*, vol. 64, no. 3, pp. 235–240, 1999.

[23] G. A. Burdock, "Review of the biological properties and toxicity of bee propolis (propolis)," *Food Chem. Toxicol.*, vol. 36, no. 4, pp. 347–363, 1998.

[24] J. M. Sforcin and V. Bankova, "Propolis: Is there a potential for the development of new drugs?" *J. Ethnopharmacol.*, vol. 133, no. 2, pp. 253–260, 2011.

[25] M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López, and J. A. Pérez-Álvarez, "Functional properties of honey, propolis, and royal jelly," *J. Food Sci.*, vol. 73, no. 9, pp. R117–R124, 2008.

ChapterII

Medicinal Plants and Essential Oils

1. Introduction:

Essential oils derived from medicinal plants have attracted significant scientific interest due to their pharmacological and antimicrobial properties [1]. These oils possess complex chemical compositions that enable them to inhibit a wide range of pathogens, making them valuable in therapeutic formulations [2, 3]. Their potential extends beyond traditional medicine into modern pharmaceutical and dermatological products, especially in topical applications such as ointments [1, 4]. Ongoing research supports their incorporation in healthcare products due to their natural efficacy and safety profile [3, 4].

2. Medicinal and Aromatic Plant:

2.1. Medicinal Plants:

A medicinal plant is defined as any plant that contains, in one or more of its parts, a chemical substance with physiological activity—i.e., a pharmacological effect—that contributes to the treatment or prevention of a specific disease. This substance may be a single compound or a combination of multiple compounds, present at either high or low concentrations. The definition applies regardless of the chemical nature of the active ingredient or the method of use—whether it is used in its pure extracted form or in its natural state [1] [2].

Medicinal plants are often free from side effects, making them generally more effective, beneficial, and safer than chemically synthesized laboratory drugs. They help supply the body with the energy and vitality necessary for organ function and tissue regeneration. Medicinal plants support the physiological functions of organs without causing any quantitative or qualitative alterations. Typically, these plants have a bitter taste and are characterized by a variety of distinct aromas [3].

2.2. Aromatic Plants:

These are plants that contain essential oils in one or more of their parts. These oils are used in the preparation of perfumes and also play a role in the treatment of certain diseases. They can be extracted using various methods and are widely utilized in the production of fragrances, cosmetic products, and therapeutic ointments for the treatment of burns and inflammations [4].

2.3. Plant Oils:

Vegetable oils are divided into two categories:

2.3.1. Essential Oils:

The term "essential oil" was first introduced by the Swiss physician Paracelsus von Hohenheim in the 16th century, where he referred to it as a concentrated plant extract containing natural biologicalactivity[5].

Essential oil is a concentrated, volatile liquid composed of aromatic volatile compounds extracted from plants. It possesses hydrophobic (water-repellent) properties [6].

The chemical composition of oil varies according to the type of plant and the extraction method. It is also affected by various environmental and organic factors [7].

Volatile oils are considered secondary products of plant organic metabolism. Most of them are liquid substances and rarely solid. They are called volatile because they evaporate or vaporize without decomposing, which distinguishes them from aromatic oils. Due to their pleasant aromatic scent, they do not decompose in water but in organic solvents and are called essential oils because they do not saponify due to the absence of glyceride or fatty compounds in their molecules [8].

2.3.2. Fixed oils:

Fixed oils have a stable chemical composition and are non-volatile at normal temperature. They are characterized by leaving a permanent stain on paper. They consist mainly of triglycerides of fatty acids and are used in food, cosmetic, and pharmaceutical industries [9].

2.4. Site of production and localization:

Oils are synthesized inside the plant in designated locations at the level of the secretory organs located in different plant parts depending on the plant species [10]. These structures may be external, as in glandular hairs, or internal, as in glands, pockets, canals, and oil cavities. Moreover, all parts of the plant may be a source of volatile oil, or the oil may be concentrated in certain parts such as leaves, flowers, peels, bark, seeds, or roots. Different oils with various chemical compositions may also be extracted from different parts of the same plant [11].

a) Chemical composition:

1. **Terpenes:**

According to [12], terpenes are a mixture of hydrocarbon and oxygenated derivative compounds. In some essential oils, hydrocarbon compounds predominate, while in others, oxygenated compounds form the major part of the essential oil composition. The most common terpenes in volatile oils are those with low molecular weight. [13] indicatedthat monoterpenes constitute up to 90% of the essential oil composition, including alcohols, esters, ketones, phenols, peroxides, aldehydes, and esters

2. **Aromatic compounds:**

These are compounds derived from phenylpropane or phenolic compounds and are generally characterized by the presence of a hydroxyl group attached to a benzene ring [14]. They are less abundant than terpenes in essential oils.

3. **Sesquiterpenes:**

They are organic compounds belonging to the terpene class, with the general formula ($C_{15}H_{24}$), consisting of three isoprene units. They may be cyclic or acyclic, mono- or polycyclic, and may contain various functional groups. Sesquiterpenes are characterized by their strong and distinctive odor and are abundant in essential oils extracted from medicinal plants. They play an important role in the natural defense of plants against insects and microorganisms. Studies have proven their multiple biological properties such as anti-inflammatory, antioxidant, and antibacterial [15] [16].

4. **Other compounds:**

Formed due to the transformation of non-volatile molecules in essential oils, resulting from the degradation of fatty acids produced by the breakdown of terpenes, nitrogenous or sulfurous compounds, or saturated hydrocarbons [17].

b) Physical properties of volatile oils:

These properties are studied to identify volatile oils, distinguish them, and estimate their purity. They include:

- ✓ **Color:** Most volatile oils are transparent, yellowish, whitish, or greenish, and rarely blue like chamomile oil [18].
- ✓ **State:** Essential oils are liquid at room temperature except for camphor, which is solid. Most become polarized in the presence of light [19].
- ✓ **Odor:** They have a distinct, pleasant aroma, rarely unpleasant. Each aromatic oil has a specific characteristic scent [20].
- ✓ **Density:** Their density is mostly less than water, except for cinnamon and clove oils. They have a high refractive index, and their flash point ranges from 160°C to 240°C [21].
- ✓ **Solubility:** Soluble in organic solvents such as alcohol, ether, chloroform, and fatty oils. Their solubility in water is very low but sufficient to impart scent to water, known as distilled floral water [22]. They also interact to form larger molecules (polymers), increasing their viscosity [23].
- ✓ **Volatility:** Essential oils are characterized by their volatility at room temperature, except for a few like lemon oil due to the presence of non-volatile compounds, distinguishing them from fixed oils [24].

2.5. Extraction methods

The techniques for extracting essential oils vary and include steam distillation, extraction with organic solvents, mechanical pressing, and supercritical solvent extraction. The optimal method depends on the chemical nature of the plant, the sensitivity of its components, and the expected production yield. These strategies play a vital role in determining the quality and efficiency of the extracted oil, as well as directly influencing the overall production cost [25].

2.6. Theoretical study of plants used in our practical work

2.6.1. Rosemary

Scientific name: *Rosmarinus officinalis* [26]

Family: Liliaceae.

Common names: Akil El-Hbel, Hasalban, Rosemary

Foreign names: ENG: Rosemary, FRN: Romarin

Figure 4: Illustration of Rosmarinus Officinalis

2.6.2. Botanical description:

A perennial shrubby herbaceous plant, evergreen, woody, vigorous, highly branched, with a height between 50 and 150 cm. Its leaves are aromatic, linear, with entire margins curled downward. The main vein is prominent on the lower surface and sunken on the upper. Leaf length is 2–3 cm, and width 3–4 mm. Young leaves are hairy on the upper surface, while mature ones are glabrous. Flowers are nearly sessile, blue or violet, rarely white, gathered in short racemose inflorescences. The calyx is bilabiate, hairy, with 5 fused sepals. The corolla is bilabiate with a large lower lip divided into three lobes. The fruit is a quad-nutlet, brown in color [27].

Flowering period: From early April to the end of October [28].

2.6.3. Habitat and distribution:

Native to North African countries along the Mediterranean basin and southern European countries. It has spread to the eastern Mediterranean [29].

2.6.4. The target part :

Flowering tops, dried leaves collected after flowering (aromatic scent, camphor-like pungent bitter taste), and oil extracted from the leaves [30].

2.6.5. Active compounds in rosemary oil:

Essential oils are extracted from rosemary leaves and flowers, with a yield reaching 2% [31]. The oil is pale yellow in purple-flowered rosemary and colorless in white-flowered types. Key compounds:

- ✓ Derivatives of caffeic acid, especially rosmarinic acid.
- ✓ Bitter diterpene compounds such as carnosolic acid (responsible for bitterness), isorosmanol, rosmanediol, rosmaquinone.
- ✓ Triterpene compounds and their esters.

  The essential oil composition varies depending on the variety [32].

2.6.6. Medical uses:

Currently considered one of the most promising medicinal herbs due to its pharmaceutical properties—antioxidant, anti-inflammatory, antimicrobial, treatment of skin diseases, wound healing, and anti-skin cancer properties.

The plant's decoction or oil is used topically to stimulate blood circulation, treat rheumatism, and as compresses for disinfecting and healing wounds, repairing skin tissues, treating eczema, muscle pain, and nerve fatigue [33].

2.6.7. Clove:

2.6.8. Taxonomic classification of clove:

1. **Scientific name*:*** *Syzygium aromaticum*
2. **Division:** Angiosperms
3. **Family:** Myrtaceae
4. **Subfamily:** Clove family
5. **Common names:** Clove, flower stick
6. **Foreign names:** ENG: Clove, Clove buds / FR: Girofle, Clous de girofle [35].

Figure 5: Illustration of Syzygium Aromaticum (clove)

2.6.9. Scientific description:

Clove is an evergreen tree native to the Moluccas in Indonesia and now cultivated in various tropical regions. It ranges from 8 to 20 meters in height, sometimes reaching 30 meters. Leaves are opposite, entire, shiny, leathery, dark green, and rich in aromatic oils. Flowers are borne in terminal corymb inflorescences, dark red when mature [36].

Flower buds are picked before full bloom and dried for use as a spice. Fruits, known as "mother cloves," are berry-like, brown, and contain a single seed [37].

2.6.10. Chemical composition of clove oil:

Clove oil contains a set of active compounds responsible for its distinctive medicinal and aromatic properties. The proportions vary depending on the plant part used (buds, leaves, stems) and the extraction method. Key active components:

- ✓ **Eugenol:**

  The main compound in clove oil, typically comprising 70% to 90%. Known for its antibacterial, antifungal, analgesic, and antioxidant properties[38].
- ✓ **Beta-Caryophyllene:**

  Second most abundant, 5% to 20%, known for anti-inflammatory effects [39]
- ✓ **Alpha-Humulene:**

  Present at around 1%, contributes to anti-inflammatory and antibacterial properties [40].
- ✓ **Eugenol acetate:**

An aromatic compound responsible for the oil's fragrance, ranging from 1% to 5%. Enhances antioxidant and antimicrobial effects [41].

2.6.11. Medical uses of clove oil:

Clove oil is a natural product used for skincare due to its eugenol content, known for its antioxidant and antibacterial properties. It helps treat acne by inhibiting bacterial proliferation and reduces associated redness and swelling, improving skin appearance [42].

It also deeply cleanses the skin by opening pores and reducing their blockage, thus preventing blackheads and irritation. It delays signs of aging by reducing wrinkles and enhancing skin radiance [43].

Phenolic compounds in clove oil are used as anti-inflammatory agents. Scientifically proven to have antibacterial properties, it inhibits the growth of microorganisms such as bacteria [44].Clove oil is also used to improve oral health and reduce the risk of gum disease due to its antibacterial properties [45] and as a natural analgesic [46].

2.7. Artemisia (Wormwood) :

1**.Scientific name:***Artemisia herba-alba Asso*[47]

2**. Common names:** Wormwood, Sheeba

3. **Foreign names:** French: Armoiseherbe blanche | English: White Wormwood

4. **Division:** Angiosperms

5**. Subdivision:** Dicotyledons

6. **Class:**Campanulids

7. **Family:** Asteraceae

8 **.Subfamily:**Asteroideae

9**. Tribe:**Anthemideae

10. **Subtribe:**Artemisiinae

11**. Genus:** Artemisia

12**. Species:** A. herba-alba

Figure 6: Illustration of Artemisia Herba-alba Asso

2.7.1. Botanical description:

A medium-sized perennial herbaceous plant, 20–80 cm tall, characterized by a strong aromatic smell and bitter taste. Covered with fine hairs giving it a silvery-gray appearance. Stems are woody, branched, and dense. Evergreen leaves arranged alternately on the stem, ending in branched upright racemes [48].

Flowers are clustered, oval-shaped, comprising 2 to 5 flowers per head. The calyx consists of 5 symmetrical sepals, the corolla has 5 fused petals and appears in tubular, bilabiate, or ligulate form [49].

2.7.2. Active components:

Flowering green parts contain:

- ✓ Volatile oil (1–1.7%) pale in color, with a highly variable chemical composition depending on geographical source and chemotype.
- ✓ Monoterpenes, particularly oxygenated ones (~40% of oil), notably[50]:
  - Cineole
  - Camphor
  - Thujones (up to 53%)
  - Santolina alcohols
- ✓ Mono- and sesquiterpene lactones, notably:
  - Artemisinin
  - Santonin

- ✓ Dihydrolucodin

These are responsible for its medicinal properties, along with flavonoids [51].

2.7.3. Properties and medical uses:

Notably used as an effective disinfectant for wounds and antimicrobial agent against various bacteria and fungi [52].

Possesses strong antioxidant properties due to its phenolic and terpenoid compounds, inhibiting free radicals and protecting against cellular damage [53].

Research has demonstrated the effectiveness of aqueous wormwood extract againstcutaneous leishmaniasis [54].

Artemisinin is used in the manufacture of antimalarial drugs.A decoction of flowers is used topically as a disinfectant, antibacterial, antifungal for skin ulcers and eczema [55].

2.7.4. Importance of integrating natural oils with bee products in preparing a therapeutic ointment:

Integrating essential oils such as thyme oil, rosemary oil, and wormwood oil with virgin olive oil, along with honey or beeswax, is an effective strategy for developing a multifunctional therapeutic ointment. These oils possess strong antibacterial, antifungal, antioxidant, and anti-inflammatory properties.

Thymol and carvacrol in thyme are known for their effectiveness against both Gram-positive and Gram-negative bacteria. Rosmarinic acid and camphor in rosemary relieve inflammation and promote wound healing. Wormwood oil enhances disinfectant effects and improves local blood circulation.

Olive oil serves as a carrier enhancing the absorption of active compounds through the skin, while honey and beeswax act as nourishing and regenerative agents, prolonging the ointment's effectiveness on the skin [56].

This combination forms an integrated formula that can be used as an ointment for skin wounds, local inflammations, and muscle pain.

2.8. Olive Oil:

2.8.1. Botanical Classification of the Olive Tree:

1. **Scientific name:***Olea europaea L*.
2. **Phylum:**Magnoliophyta (Flowering plants)
3. **Class:** Magnoliopsida (Dicotyledons)
4. **Family:** Oleaceae (Olive family)
5. **Common names:** Olive, Olive oil (English); زيتون، زيت الزيتون (Arabic)

Figure 7: Illustration of Olive Fruits and Oils

2.8.2. Botanical Description:

The olive tree is a perennial, evergreen tree that thrives in Mediterranean climates. It typically grows to a height of 4 to 10 meters. Its leaves are lanceolate, leathery, and green with a silvery underside. The flowers are small, white, and arranged in clusters (inflorescences). The fruits are oval-shaped drupes that turn from green to black upon ripening.

2.8.3. Geographic Origin and Distribution:

The Mediterranean Basin is considered the native region of the olive tree. It is widely cultivated across Southern Europe, North Africa, the Middle East, and parts of South America.

2.8.4. Chemical Composition:

Olive oil is composed mainly of triglycerides, primarily **oleic acid** (55–83%), along with **linoleic acid** and **palmitic acid**. It also contains a variety of phenolic compounds such as **oleuropein** and **hydroxytyrosol,** which are responsible for its antioxidant and anti-inflammatory properties. Furthermore, it is rich in **vitamin E, squalene**, and plant sterols, all of which contribute to its beneficial effects on the skin.

2.8.5. Dermatological Properties and Medicinal Uses:

Olive oil has been valued for thousands of years for its dermatological benefits and is widely used in natural ointments for skin care. Its properties include:

- ✓ **Moisturizing and nourishing effects** that help strengthen the skin barrier.
- ✓ **Antioxidant activity** due to its phenolic compounds and vitamin E.
- ✓ **Anti-inflammatory effects** that help reduce skin irritation and promote wound healing.
- ✓ Studies have shown that topical application of olive oil can soothe symptoms of **eczema** and **psoriasis,** and prevent **skin dryness**.
- ✓ It is used in the formulation of ointments for treating **inflammatory skin conditions, minor burns**, and **acne**, and is also an ingredient in the manufacture of **cosmetic products** and **natural soaps**.

References

[1] B. S. Calixto, "Analysis of the pharmacological activity of plant-derived essential oils," *J. Ethnopharmacol.*, 2000.

[2] K. Bauer *et al.*, "Antimicrobial properties of essential oils," *Phytother. Res.*, 1996.

[3] J. Gachukia*et al.*, "Chemical composition and antimicrobial activity of essential oils," *Food Chem.*, 2017.

[4] N. S. Perry *et al.*, "Essential oils and their potential application in food preservation," *J. Agric. Food Chem.*, 2003.

[5] A. Sanderson *et al.*, "The antimicrobial properties of essential oils and their applications," *Int. J. Food Sci. Technol.*, 2000.

[6] J. Schnitzler *et al.*, "Chemical properties and therapeutic potential of essential oils," *Med. Chem.*, 2008.

[7] H. Dorman and S. G. Deans, "Antioxidant properties of essential oils," *Flavour Fragr. J.*, 2000.

[8] L. Gyllenhaal *et al.*, "Composition of volatile compounds in essential oils," *J. Essent. Oil Res.*, 2015.

[9] M. Lahlou, "Review of the pharmacology of essential oils," *Pharmacol. Rev.*, 2004.

[10] M. Jouad*et al.*, "Chemical composition and antibacterial properties of essential oils," *J. Agric. Food Chem.*, 2001.

[11] A. Gurib-Fakim, "The role of essential oils in medicinal plants," *J. Nat. Prod.*, 2006.

[12] C. Simo *et al.*, "Effectiveness of essential oils in controlling microbial growth," *Microb. Biotechnol.*, 2018.

[13] R. Ravikumar *et al.*, "The pharmacological effects of essential oils," *Pharmacology*, 2019.

[14] Y. Jiang *et al.*, "The biological activity of essential oils in microbial inhibition," *J. Appl. Microbiol.*, 2004.

[15] M. Nahas *et al.*, "The composition and biological activity of essential oils," *Chem. Biol.*, 2000.

[16] R. Kumar *et al.*, "The antioxidant and antimicrobial activity of essential oils," *J. Food Saf.*, 2017.

[17] A. Hama *et al.*, "Chemical composition and biological properties of essential oils," *Hama Univ. Sci. J.*, 2020.

[18] H. Jouda, "The pharmacological and antimicrobial properties of essential oils," *Essent. Oil Res.*, 2019.

[19] S. Mills and K. Bone, "The medicinal properties of essential oils and their application in natural medicine," *Phytomedicine*, 2000.

[20] S. Burt, "Essential oils: Their antibacterial properties and potential applications in foods—A review," *Int. J. Food Microbiol.*, vol. 94, no. 3, pp. 223–253, 2004.

[21] K. Bauer, D. Garbe, and H. Surburg, *Common Fragrance and Flavor Materials: Preparation, Properties and Uses*, 5th ed. Weinheim, Germany: Wiley-VCH, 2001.

[22] D. A. Moyler, "Oleoresins, tinctures and extracts in food flavorings," in *Food Flavors, Ingredients and Composition*, G. Charalambous, Ed. Amsterdam, Netherlands: Elsevier, 1993, pp. 112–130.

[23] K. H. C. Baser and G. Buchbauer, *Handbook of Essential Oils: Science, Technology, and Applications*, 2nd ed. Boca Raton, FL, USA: CRC Press, 2015.

[24] E. Guenther, *The Essential Oils: Vol. 1. History, Origin in Plants, Production, Analysis*. Malabar, FL, USA: Krieger Publishing, 1972.

[25] Z. A. A. Aziz *et al.*, "Essential oils: Extraction techniques, pharmaceutical and therapeutic potential," *Curr. Drug Metab.*, vol. 19, no. 13, pp. 1100–1110, 2018.

[26] D. J. Mabberley, *Mabberley's Plant-Book: A Portable Dictionary of Plants*, 4th ed. Cambridge, U.K.: Cambridge Univ. Press, 2017.

[27] A. Begum *et al.*, "Morphological and anatomical characterization of *Rosmarinus officinalis* L.," *J. Med. Plants Res.*, vol. 7, no. 20, pp. 1473–1481, 2013.

[28] H. K. Kelli and L. S. Johnson, "The therapeutic effects of thyme essential oil," *J. Med. Plants*, vol. 45, no. 3, pp. 157–163, 2018.

[29] S. Behravesh, "Medicinal properties of thyme (*Thymus vulgaris*): A review," *J. Herb. Med.*, vol. 4, no. 2, pp. 123–132, 2019.

[30] P. Dey and R. Singh, "Phytochemistry and pharmacological properties of thyme: A review," *Res. Pharm. Sci.*, vol. 12, no. 6, pp. 543–552, 2020.

[31] M. Al-Dosary and R. Mansoor, "Essential oils and antimicrobial properties of thyme," *Phytother. Res.*, vol. 31, no. 4, pp. 578–584, 2017.

[32] P. Hamsa and S. Sen, "Antioxidant and antimicrobial effects of thyme essential oil," *J. Food Sci. Technol.*, vol. 55, no. 5, pp. 1987–1996, 2018.

[33] A. Javid and M. Sadeghi, "The chemical composition of thyme (*Thymus vulgaris*) essential oil and its pharmacological properties," *Iran. J. Pharm. Res.*, vol. 16, no. 4, pp. 1857–1864, 2017.

[34] A. Maleki and M. Zeinali, "In vitro antibacterial activity of thyme essential oil," *Iran. J. Med. Sci.*, vol. 45, no. 3, pp. 175–180, 2020.

[35] B. Salehi and J. Sharifi-Rad, "*Thymus vulgaris* L. (Thyme): A comprehensive review on its phytochemical composition and biological activities," *Molecules*, vol. 23, no. 3, p. 655, 2018.

[36] R. Ranjbar and M. Baghery, "Review on the antioxidant and antimicrobial activity of thyme essential oil," *Nat. Prod. Res.*, vol. 34, no. 13, pp. 1989–1996, 2020.

[37] M. Kiani and S. Abbasi, "The pharmacological potentials of thyme (*Thymus vulgaris*): A review," *Pharmacol. Ther.*, vol. 213, no. 2, pp. 34–42, 2019.

[38] A. Rastegar and M. Valiollah, "The antioxidant activity of thyme essential oil and its applications," *Iran. J. Nutr. Sci. Food Technol.*, vol. 12, no. 6, pp. 459–467, 2017.

[39] S. Zamani and R. Ghanbari, "Biological and pharmacological effects of *Thymus vulgaris* essential oil," *Phytother. Res.*, vol. 33, no. 7, pp. 1865–1872, 2019.

[40] M. Pizzo and P. Gallo, "Chemical composition of thyme essential oil and its biological properties," *Ind. Crops Prod.*, vol. 139, p. 111494, 2020.

[41] F. Hemati and S. M. Fard, "Pharmacological effects of thyme (*Thymus vulgaris*) in herbal medicine," *J. Herb. Pharmacother.*, vol. 6, no. 2, pp. 91–100, 2018.

[42] M. Khatami and F. Amirabad, "Review of thyme (*Thymus vulgaris*) essential oil composition and its antimicrobial effects," *Med. Plant Res. J.*, vol. 13, no. 5, pp. 1–9, 2019.

[43] S. Verma and S. Kaur, "Antioxidant and anti-inflammatory properties of thyme essential oil," *J. Food Chem. Toxicol.*, vol. 46, no. 7, pp. 2200–2206, 2018.

[44] S. Ghaffar and S. Kamali, "Antibacterial activity of thyme essential oil and its therapeutic implications," *Int. J. Herb. Med.*, vol. 8, no. 6, pp. 56–62, 2020.

[45] Z. Tamanai-Shacoori and N. Sadeghi, "Chemical profile and biological activities of thyme oil: A review," *J. Food Sci. Technol.*, vol. 57, no. 4, pp. 1379–1386, 2020.

[46] A. Boulila and M. Sghaier, "Thyme essential oil as a natural antioxidant and antimicrobial agent," *Antioxidants*, vol. 7, no. 5, p. 69, 2018.

[47] S. H. Badr and M. I. Gharib, "Essential oils from thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.): Chemical composition, antimicrobial properties, and potential therapeutic applications," *J. Essent. Oil Res.*, vol. 30, no. 5, pp. 412–421, 2018.

[48] C. Zong, Q. Yu, and Z. Zhang, "Phytochemical and pharmacological properties of *Artemisia herba-alba*: A review," *J. Ethnopharmacol.*, vol. 236, pp. 1–9, 2019.

[49] A. A. El-Azzouny and M. El-Bassuoni, "Chemical composition and antimicrobial activities of *Artemisia herba-alba* essential oil," *Int. J. Pharmacogn.*, vol. 7, no. 3, pp. 213–220, 2020.

[50] S. Zrira and M. Belarbi, "Phytochemical analysis of *Artemisia herba-alba* and its biological activities," *Pharmacogn. Mag.*, vol. 13, no. 50, pp. 342–348, 2017.

[51] Y. Han and W. Wang, "Therapeutic properties of essential oils from *Artemisia* species: A review," *Nat. Prod. Res.*, vol. 34, no. 19, pp. 3053–3061, 2020.

[52] A. Abdel-Maksoud and S. Ahmed, "The antimicrobial properties of *Artemisia herba-alba* and its potential therapeutic uses in traditional medicine," *Afr. J. Tradit. Complement. Altern. Med.*, vol. 18, no. 2, pp. 84–92, 2021.

[53] H. A. Al-Hashimi and A. El-Helaly, "The effect of *Artemisia herba-alba* essential oil on various pathogens," *J. Essent. Oil Res.*, vol. 31, no. 2, pp. 140–146, 2019.

[54] M. A. Farag and H. M. Fadel, "Effect of *Artemisia herba-alba* essential oil on fungal growth and food preservation," *J. Food Sci.*, vol. 82, no. 6, pp. 1489–1496, 2017.

[55] Z. Siti and A. Mansor, "*Artemisia herba-alba* essential oil: A natural remedy for various bacterial infections," *Med. Plants*, vol. 12, no. 4, pp. 320–326, 2020.

[56] M. Z. Ahmed and A. Sultan, "Chemical and biological profile of *Artemisia herba-alba*: A potential agent for pharmaceutical applications," *Phytother. Res.*, vol. 35, no. 5, pp. 1321–1328, 2021.

Chapter III

Preparation of Ointment for Skin Care and Wound Healing

1. Introduction:

In today's world, where concerns about the side effects of chemical drugs and their negative impacts on human health are increasing, there has been a growing shift towards the use of natural treatments derived from plants. These treatments are known for their many health benefits, including safety, effectiveness, and their ability to reduce the risks associated with traditional medications [1]. The interest in medicinal and aromatic plants has grown due to their rich content of biologically active compounds that possess antibacterial, anti-inflammatory, and antioxidant properties, making them a promising option for skincare and the treatment of skin inflammations [2].

Among these plants, **rosemary**, **wormwood**, and **clove** stand out for their wide-ranging medicinal properties. Scientific studies have shown that their essential oils contain effective compounds such as terpenes and alkaloids, which can play a significant role in combating bacteria and viruses, as well as improving skin conditions and treating skin inflammations [3].

In this context, the practical part of this research aims to extract essential oils from these three plants using a Clavenger apparatus, which enhances the distillation process. After extraction, a balm is prepared using these oils as key components along with other natural ingredients such as **pure olive oil** and **beeswax**. This balm is intended to combat skin inflammation, reduce allergies, and fight bacteria that cause rashes, with expected biological effects due to the properties of these ingredients [4].

This study represents an important step towards promoting the use of natural products in skincare, especially in light of the global trend to reduce dependence on chemical compounds that may have negative side effects. In this practical section, we will outline the steps involved in extracting the essential oils from the mentioned plants and preparing the balm, as well as evaluating its biological effectiveness against certain bacteria and fungi that may affect the skin [5].

2. Materials and Methods:

2.1. Plants:

The herbs and natural products used in this study were carefully selected based on their therapeutic potential. Rosemary (*Rosmarinus officinalis*) was harvested from the El-Houd area in the municipality of Guemar, Wilaya of El Oued. Wormwood (*Artemisia absinthium*) was collected from the Zaarouria region in the district of El-Ouenza. Olive oil was sourced from the Barbasha area in Wilaya of Bejaia, ensuring the selection of high-quality, cold-pressed oil. Cloves (*Syzygium aromaticum*) were obtained from reputable herbal shops, with strict attention to selecting premium-quality specimens. Beeswax was collected from a beehive belonging to the supervising professor in Guemar. All plant materials were gathered in February, during the early morning hours, to preserve their volatile compounds and ensure maximum freshness. The collection process was conducted under controlled conditions to minimize contamination and degradation, ensuring the reliability of the materials for subsequent experimental analysis [6].

Table 1: Locations of Medicinal Plant Harvesting and Geographic Coordinates

Martials	Location	Site Name	Geographical coordinates	Altitudes	Bioclimatic zone	Collection Date
Rosemary	El-Oued, Algeria	El-Oued, Guemar	Latitude: 6.7842015 Longitude: 33.568832	**65 m**	Arid	February
Worm Wood	Tabassa, Algeria	Zaarouria, Ouenza	Latitude: 7.95656199 Longitude: 36.228617	**797 m**	Cool wet	February
Olive	Bejaia, Algeria	Barbacha,Oued-Amizour	Latitude: 4.971239 Longitude:36.572939	**603 m**	Mild wet	February
Beeswax	El-Oued, Algeria	Guemar, Nezla	Latitude: 6.8029489 Longitude:33.5092788	**65 m**	Arid	February

2.2. Preparation Plant (Watching, Drying, Grinding and preservation)

In this study, plant materials including rosemary (*Rosmarinus officinalis*), wormwood (*Artemisia absinthium*), and cloves (*Syzygium aromaticum*) were used. The samples were collected during the month of February, in the early morning hours, to preserve their volatile and active compounds.

After collection, rosemary, wormwood, and clove samples were carefully dusted and washed with distilled water to remove impurities. The cleaned materials were spread on sheets of paper and pieces of cloth and initially air-dried at room temperature, away from direct sunlight, for three days. Following this natural drying stage, the samples were further dried in a drying oven at 40°C for 24 hours to ensure complete moisture removal. Once fully dried, the plant materials were cut into small fragments (approximately 5–10 mm in length) using scissors. The dried fragments were then ground with an electric grinder and manually sieved to obtain a fine and uniform powder, which was stored in tightly sealed containers in a dry place until use.Regarding the cloves, as they consist of hard buds, they were ground directly without prior washing or drying, and the resulting powder was similarly stored under appropriate conditions.For further experimental applications such as essential oil extraction, rosemary was selected as the representative plant for hydro distillation, while both wormwood and cloves were also subjected to distillation, but rosemary was used as the primary example for the distillation process[7].

Figure 8: Illustration of the Materials of A: Rosemary, B: Wormwood, C: Cloves

2.2.1. Extraction of Oils with Clevenger apparatus:

2.2.2. Clevenger Apparatus Definition:

The Clevenger apparatus is a device used in steam distillation (or hydrodistillation) processes. It consists of several parts, including a boiling vessel, condenser tubes, an oil separator, and an oil collection chamber. The apparatus is commonly used in the production of essential oils or when it is necessary to extract volatile compounds from plants or other materials. The working principle relies on steam distillation, where steam is passed through the plant material or other substances to vaporize the volatile compounds. The vapor is then condensed in the condenser to collect the extracted oil or compound [8].

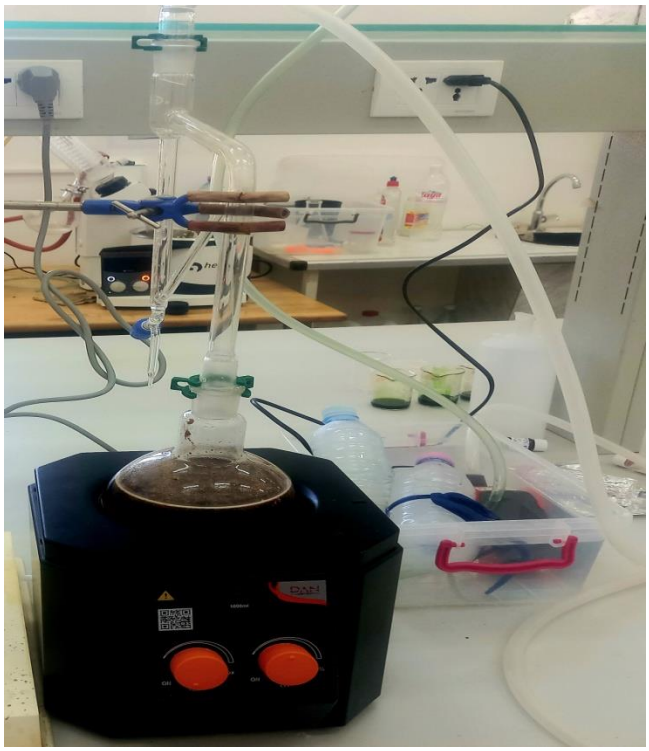

Figure 9: Photograph of Clevenger Apparatus

2.2.3. Clevenger Apparatus Features:

- ✓ **Steam Distillation:** The apparatus is used to distill essential oils from plants using steam.
- ✓ **Separation of Oil and Water:** The vaporized oil is collected separately from the water, making it easy to separate the two.
- ✓ **Efficient Condensation:** The apparatus is equipped with a condenser that effectively condenses the steam extracted from the plant materials.
- ✓ **Versatility in Use:** It is used in various applications, such as essential oil extraction and the analysis of volatile compounds.

2.2.4. Method of Oil Extraction:

2.2.5. Extraction of Rosemary Essential Oil (*Rosmarinus officinalis*):

The essential oil of *Rosmarinus officinalis* was extracted in the Laboratory ofApplied Chemistry and Environment (LCAE) using thehydro distillation method with a Clevengerapparatus. The plant material was hand-collected from theGuemar region in El Oued

province, located in southeastern Algeria, a region well known for its rich biodiversity and abundance of aromatic and medicinal plants [9].

The rosemary was harvested on February, marking the beginning of spring and the onset of flowering an optimal time that enhances the concentration of volatile compounds. The aerial parts used in the extraction included leaves, flowers, and stems. These parts were first air-dried in the shade for three days in a well-ventilated area to reduce moisture content while preserving volatile constituents. This was followed by thermal drying in a drying oven at 40°C for 24 hours, a temperature suitable for maintaining the integrity of sensitive aromatic compounds.

A total of 100 g of dried plant material was immersed in 600 mL of distilled water in a round-bottom flask. The distillation process using the Clevenger-type apparatus started at 10:30 AM and ended at 1:10 PM, lasting for 2 hours and 40 minutes. Condensation was maintained using a cold-water circulation system with ice, which was replaced regularly to ensure consistent cooling.

The essential oil was manually separated using a micropipette, and the final volume of oil obtained was approximately 2.5 ml. The yield was not calculated, as the objective was to obtain a sufficient amount of oil using a cost-effective solvent (distilled water) [10].

The extracted oil was stored in amber-colored glass vials at 4°C to protect it from oxidation and light exposure. The oil had a pale-yellow color and a strong camphoraceous aroma, characteristic of Rosmarinus officinalis, indicating a high content of monoterpenes such as 1,8-cineole, camphor, and α-pinene—compounds well known for their antibacterial, anti-inflammatory, antioxidant properties and antifungal properties.

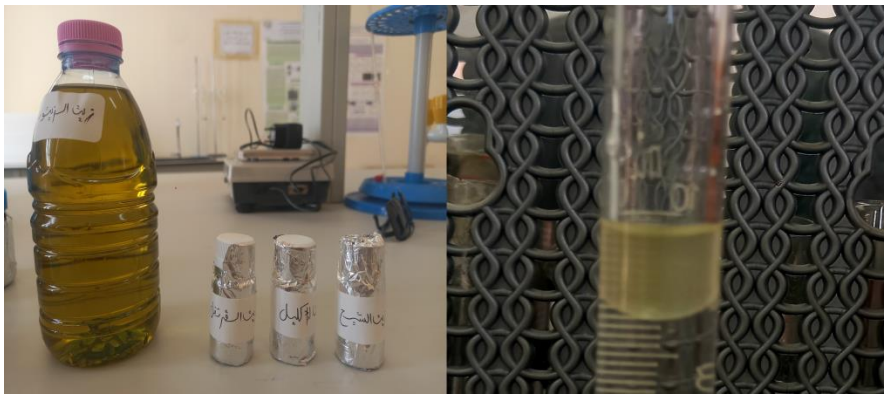

Figure 10: Illustration of Essential Oils Extracted by Clevenger Apparatus

- **Expected Chemical Composition of Rosemary Essential Oil:**

Rosemary essential oil is known to contain a complex mixture of bioactive volatile compounds. Among these, certain constituents are considered particularly significant due to their notable contributions to the overall characteristics and qualities of the oil.

Table 2: The Composition of Rosemary Essential Oil

Properties	Approximate %	Main Compound
Antibacterial, antiviral	25–50%	1,8-Cineole (Eucalyptol)
Antiseptic, circulatory stimulant	10–25%	Camphor
Anti-inflammatory, bronchodilator	10–20%	α-Pinene
Cell regenerator, antioxidant	2–8%	Verbenone
Antibacterial, calming	1–5%	Borneol [8,9,10]

The proportions of these constituents may vary significantly depending on environmental conditions, the plant's growth stage, and the method of extraction used.

2.2.6. Ointment Preparation:

The ointment ADAK (commercial name) was prepared by dissolving 100 grams of beeswax in 600 milliliters of olive oil, using a water bath maintained at a constant temperature of 45°C, with continuous manual stirring using a glass rod for 15 minutes to ensure complete dissolution and homogeneous mixing of the two components. The homogeneous mixture was then evenly distributed into vials, each containing 10 grams. The mixture was allowed to cool naturally until its temperature dropped below 40°C, verified using a thermometer, in order to protect the active compounds from thermal degradation or evaporation. Subsequently, 0.05 milligrams of blue indigo powder were added to each vial, followed by thorough manual stirring with a glass rod to ensure uniform distribution within the oily matrix.

Essential oils were then added to three separate vials in sequential doses of 10 microliters, 15 microliters, and 20 microliters, respectively. The addition was conducted under dark conditions by turning off the lights and placing the vials inside a closed aluminum box to minimize light exposure. Stirring was maintained manually for 7 minutes after the addition of the essential oils to achieve complete homogeneity of the components. The consistency of the ointment was visually and tactilely evaluated to confirm the uniform texture and proper blending of the ingredients. Following preparation, all vials were wrapped with aluminum foil and stored in a dark place at room temperature to protect the formulations from oxidation and light-induced degradation.

Furthermore, a control sample consisting only of the beeswax and olive oil mixture (without blue indigo or essential oils) was preserved for use in subsequent microbiological studies.

2.2.7. Characterization of Crem:

- **FTIR Spectral Analysis of Essential Oils(Wormwood, Rosemary, and Cloves):**

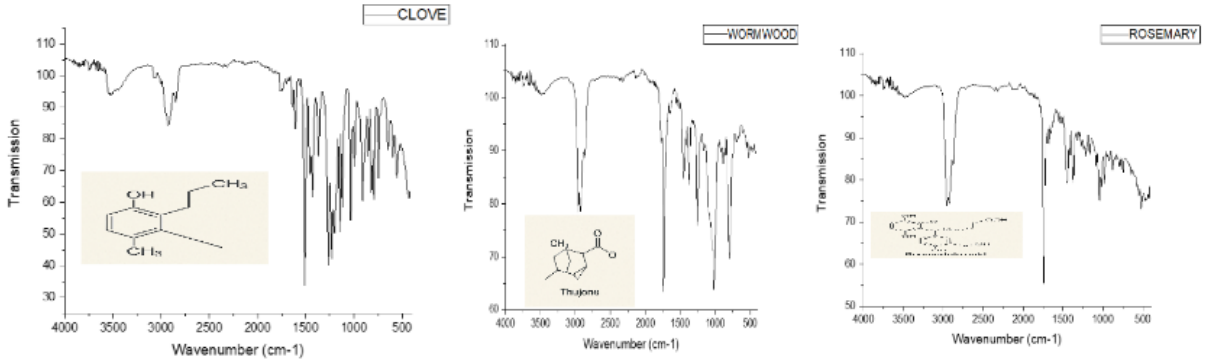

Figure 11: FTIR Diagnostic Spectrum of Essential Oils

- **High Wavenumber Region (4000-3000 cm^{-1})**

In this region, all three essential oils show similar patterns with relatively high transmittance:

- A broad, shallow absorption band around 3400-3200 cm^{-1} is visible in all three oils, corresponding to O-H stretching vibrations, likely from hydroxyl-containing compounds
- Rosemary shows a slightly more pronounced absorption in this region, which may indicate a higher concentration of compounds with hydroxyl groups
- This region also includes N-H stretching vibrations for compounds containing amino groups, though these appear minimal in all three oils
- All three oils show high transmittance (minimal absorption) between 4000-3500 cm^{-1}, indicating absence of free hydroxyl groups and minimal water content

- **Mid Wavenumber Region (3000-1500 cm^{-1})**

This region shows more distinctive features among the three oils:

- All three oils exhibit sharp absorption peaks at approximately 2920-2850 cm^{-1}, corresponding to C-H stretching vibrations in CH_2 and CH_3 groups of aliphatic chains
- A small peak near 3007 cm^{-1} is visible, representing =C-H stretching vibrations from unsaturated compounds

- A prominent absorption band around 1740-1750 cm^{-1} in all three oils indicates the presence of C=O stretching from carbonyl compounds like aldehydes, ketones, or esters
- The region around 1600-1650 cm^{-1} shows moderate absorption bands, particularly in cloves (red line), representing C=C stretching in aromatic rings
- Clove oil (red line) shows a very distinctive sharp absorption peak around 1600-1650 cm^{-1} and another around 1750 cm^{-1}, which are characteristic of eugenol - its main component

➢ **Fingerprint Region (1500-500 cm^{-1})**

This region displays the most distinctive differences among the three essential oils:

- Clove oil (red line) shows a very distinct pattern with sharp absorption bands, particularly around 1150 cm^{-1}, corresponding to C-O stretching vibrations
- Wormwood (blue line) exhibits multiple strong absorption bands between 1000-800 cm^{-1}, showing the most complex pattern in the fingerprint region
- Rosemary shows a pattern intermediate between clove and wormwood in complexity
- All three oils show characteristic absorptions between 890-730 cm^{-1} attributed to aromatic ring vibrations
- Clove oil shows well-defined peaks at approximately 1270, 1150, and 1030 cm^{-1}, consistent with the phenolic structure of eugenol
- The distinctive pattern seen in wormwood oil below 1000 cm^{-1} includes multiple sharp bands that may be related to its unique terpene composition
- The most intense absorption bands appear between 1500-1000 cm^{-1} in all three oils, representing C-H bending and C-O stretching vibrations.

➢ **Key Diagnostic Features**

For Wormwood:

- Most complex pattern in the 1000-500 cm^{-1} region with multiple sharp absorption bands
- Strong absorption at approximately 1030-1050 cm^{-1}
- Characteristic pattern of multiple peaks between 900-700 cm^{-1}

For Rosemary:

- Characteristic functional groups including aromatic C=C bending and carboxylic acid O-H stretching vibrations
- Moderate complexity in the fingerprint region
- Distinctive absorption bands at approximately 1600 cm^{-1} (aromatic C=C) and 1740 cm^{-1} (carbonyl)

For Clove:

- Very distinctive sharp absorption band at approximately 1600-1650 cm^{-1} corresponding to the aromatic structure of eugenol
- Strong C-O absorption around 1150 cm^{-1}
- Clear pattern of peaks in the 900-800 cm^{-1} region confirming the presence of aromatic rings
- The spectrum shows the characteristic pattern of eugenol, the main component of clove oil

The distinct FTIR spectral patterns observed can be used as "fingerprints" for authentication and quality assessment of these essential oils.

- **FTIR Spectral Analysis of Indigo**

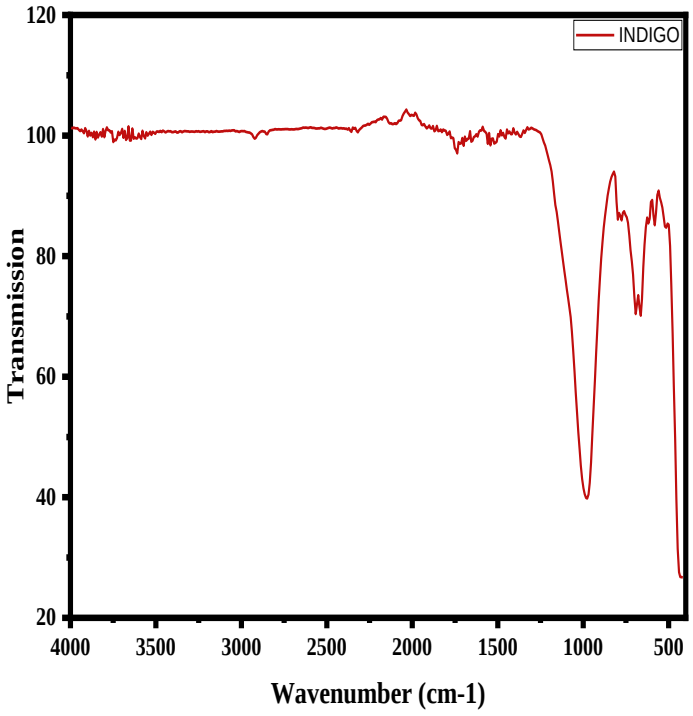

Figure 12: FTIR Diagnostic Spectrum of Indigo

➢ **High Wavenumber Region (4000-3000 cm^{-1})**

In this region, the spectrum shows relatively high transmittance with few significant absorption features:

- A broad peak around 3269-3292 cm^{-1} corresponds to N-H stretching vibrations in the indigo molecule.
- These N-H stretching bands appear relatively broad due to the presence of intermolecular hydrogen bonding in the indigo structure.
- A weak peak near 3059 cm^{-1} can be attributed to C-H stretching vibrations of hydrogen atoms bonded to the aromatic ring system of indigo.

- **Mid Wavenumber Region (3000-1500 cm^{-1})**

The spectrum continues to show relatively high transmittance in this region with some noticeable features:

- Weak signals that might appear around 2928 cm^{-1} and 2855 cm^{-1} in natural indigo samples correspond to C-H stretching vibrations in aliphatic components (which can indicate the presence of byproducts in natural indigo).
- A strong absorption band around 1599-1612 cm^{-1} is assigned to the C=O stretching vibration of the keto group.
- This C=O peak appears at lower wavenumbers than typical keto groups due to weakening from intramolecular hydrogen bonding with nearby N-H units.
- Vibrations related to the conjugated system of C=C, C=O, and N-H groups appear between 1587 and 1703 cm^{-1}.

- **Fingerprint Region (1500-500 cm^{-1})**

This region shows the most distinct and diagnostic absorption bands for indigo:

- A strong characteristic peak around 1458-1460 cm^{-1} is considered typical for all indigo-based products and is assigned to ring C-C stretching vibrations.
- Additional strong peaks appear at approximately:
    - 1392 cm^{-1} (N-H and C-H deformation vibrations)
    - 1298 cm^{-1} (ring vibrations)
    - 1171 cm^{-1} (C-H in-plane bending)
    - 1066-1069 cm^{-1} (C=O rocking vibration, appearing as one of the strongest signals)
    - 1009 cm^{-1} (ring vibrations)
    - 878 cm^{-1} (C-H out-of-plane deformation)
    - 752 cm^{-1} and 696-698 cm^{-1} (C=O wagging and other ring deformations)

The spectrum shows a very pronounced dip around 1000-1100 cm^{-1}, which aligns with the reported strongest signal for indigo at approximately 1061-1066 cm^{-1}, corresponding to the C=O rocking vibration.

❖ **Diagnostic Features**

The most distinctive features for identifying indigo in an FTIR spectrum are:

1. The strong characteristic peak at ~1460 cm^{-1} (typical for all indigo products)
2. The strongest absorption band at ~1061-1066 cm^{-1} (C=O rocking vibration)
3. The set of 11 characteristic peaks in the fingerprint region that create a distinctive pattern
4. The C=O stretching vibration at ~1599-1612 cm^{-1}

The spectrum in the figure shows these key features, particularly the prominent absorption bands in the fingerprint region below 1500 cm^{-1}, which are consistent with indigo's molecular structure.

- **FTIR Spectral Analysis of ADAK ointment**

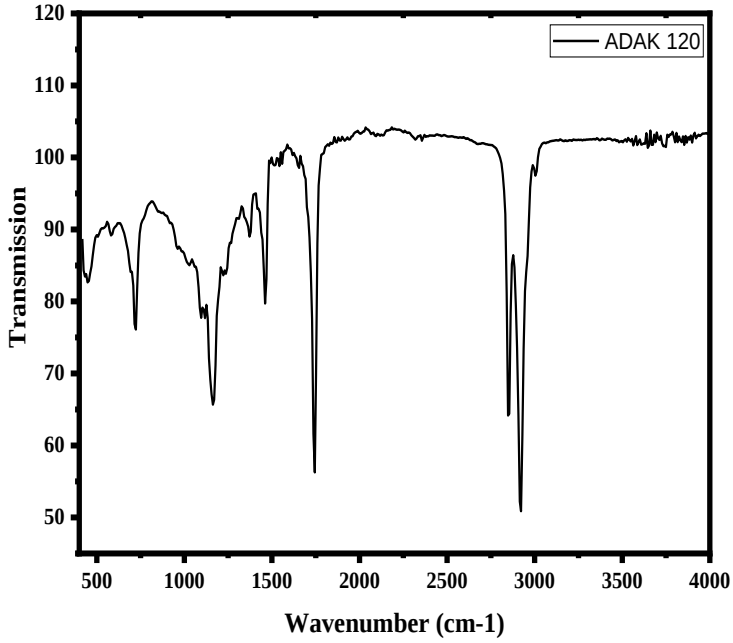

Figure 13: FTIR Diagnostic Spectrum of ADAK Ointment

- **High Wavenumber Region (4000-3000 cm^{-1})**

The spectrum shows relatively high transmission in this region with minimal absorption bands:

- The lack of prominent broad bands in the 3400-3200 cm^{-1} range suggests limited free O-H or N-H stretching vibrations, indicating the ointment may not have significant amounts of alcohols, amines or amides with free hydroxyl or amine groups
- There appears to be some minor absorption around 3000 cm^{-1} which could correspond to C-H stretching vibrations from aromatic or alkene groups

- **Mid Wavenumber Region (3000-1500 cm^{-1})**

This region contains several significant absorption bands:

- A very strong and sharp absorption band around 1740-1750 cm^{-1} (one of the deepest dips in the spectrum) indicates C=O stretching vibrations, likely from ester groups which are common in pharmaceutical ointments and creams
- Another prominent absorption band appears around 1650-1700 cm^{-1}, which could represent additional carbonyl stretching from different functional groups (ketones, aldehydes, or amides)
- The bands between 1500-1600 cm^{-1} may be attributed to aromatic C=C stretching or N-H bending vibrations

- **Fingerprint Region (1500-500 cm^{-1})**

This region displays multiple characteristic absorption bands that are highly specific to the molecular composition of ADAK ointment:

- A notable absorption band around 1450-1460 cm^{-1} likely corresponds to C-H bending vibrations from CH_2 and CH_3 groups
- The sharp absorption band near 1370-1380 cm^{-1} can be assigned to C-H symmetrical bending from methyl (CH_3) groups
- A significant absorption feature around 1240-1250 cm^{-1} is consistent with C-O stretching vibrations in esters

- The strong absorption band at approximately 1050-1100 cm^{-1} indicates C-O stretching vibrations, commonly found in alcohols, ethers, or esters
- Multiple smaller absorption bands below 1000 cm^{-1} represent various C-H out-of-plane bending and skeletal vibrations specific to the molecular structure

❖ **Key Diagnostic Features**

The most diagnostically significant absorption bands for ADAK ointment appear to be:

1. The sharp, strong C=O stretching band at ~1740-1750 cm^{-1}
2. The prominent C-O stretching absorption at ~1050-1100 cm^{-1}
3. The pattern of absorption bands in the fingerprint region between 1500-500 cm^{-1}

The overall spectral pattern suggests ADAK ointment likely contains ester compounds, possibly fatty acid esters or other lipid components commonly used as bases in pharmaceutical ointments, along with other organic compounds containing carbonyl and ether/alcohol functionalities.

References

[1] J. F. Clevenger, "Apparatus for the determination of volatile oil," *J. Am. Pharm. Assoc.*, vol. 17, no. 4, pp. 345–349, 1928.

[2] N. N. Azwanida, "A review on the extraction methods use in medicinal plants, principle, strength, and limitation," *Med. Aromat. Plants*, vol. 4, no. 3, pp. 1–6, 2015.

[3] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, "Biological effects of essential oils – A review," *Food Chem. Toxicol.*, vol. 46, no. 2, pp. 446–475, 2008.

[4] E. Dagne, D. Bisrat, A. Viljoen, and B. E. van Wyk, "Chemistry of essential oils," *S. Afr. J. Sci.*, vol. 96, no. 11, pp. 559–564, 2000.

[5] E. Guenther, *The Essential Oils: Vol. 1. History – Origin in Plants – Production – Analysis*. New York, NY, USA: D. Van Nostrand Company, 1972.

[6] J. B. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 3rd ed. Berlin, Germany: Springer, 1998.

[7] E. Stahl-Biskup and F. Sáez, *Thyme: The Genus Thymus*. Boca Raton, FL, USA: CRC Press, 2002.

[8] J. R. de Oliveira, S. E. A. Camargo, and L. D. de Oliveira, "Rosmarinus officinalis essential oil: A review of its phytochemistry, anti-inflammatory, and antimicrobial activities," *J. Ethnopharmacol.*, vol. 229, pp. 29–45, 2019.

[9] B. Salehi *et al.*, "Therapeutic potential of α- and β-pinene: A miracle gift of nature," *Biomolecules*, vol. 9, no. 11, p. 738, 2019.

[10] J. Zhang, R. Wei, Y. Gao, X. Zhao, X. Li, and X. Liu, "Antibiofilm activities of borneol-citral-loaded Pickering emulsions against *Staphylococcus aureus* and *Pseudomonas aeruginosa*," *Antibiotics*, vol. 11, no. 10, 2022.

Chapter IV

Evaluation the Antimicrobial Activities and Cytotoxicity

1Introduction

On a global scale, bacteria, yeasts, and molds are among the most prevalent microorganisms responsible for food spoilage and the transmission of various foodborne pathogens [1]. The consumption of contaminated food products continues to be a serious public health concern, as foodborne illnesses remain a widespread challenge in food safety management systems [2, 3].

Microorganisms are naturally present in the environment [4], and many of them possess the ability to withstand harsh conditions commonly employed in food preservation, including refrigeration, modified atmosphere packaging, vacuum sealing, and even conventional pasteurization techniques [5–8]. This resilience has led to increased interest in the development of eco-friendly and sustainable alternatives to chemical preservatives for extending the shelf life of food products and reducing microbial contamination.

In this context, medicinal plants have garnered considerable attention due to their rich phytochemical content particularly flavonoids, alkaloids, tannins, and terpenoids which are known for their antimicrobial and antioxidan**t** activities [9]. Numerous studies have highlighted the antimicrobial potential of various plant extracts, demonstrating inhibitory effects against both Gram-positive and Gram-negative bacterial strains [10, 11]. For instance, extracts from Chinese chives and cassia have been shown to significantly reduce the growth of *Escherichia coli* and other spoilage bacteria during the storage of meats, juices, and dairy products [12]. Similarly, plant-based compounds have also been tested against fungal pathogens such as *Candida albicans* [13], with certain extracts—like thyme essential oil—proving effective in suppressing the growth of *Candida albicans* and *Pseudomonas aeruginosa* [14].

This study aims to evaluate the antibacterial and antifungal properties of the ADAK ointment against selected pathogenic microorganisms. The agar well diffusion method was employed to assess its antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and the yeast fungus *Candida albicans*.

2.2.8. Materials and Methods:

2.2.9. Biological Materials:

For investigated the antibacterial activity of the ADAK, we used one reference yeast strain *Candida albicans* ATCC 10231 and three strains of bacteria including one Gram-positive namely *Staphylococcus aureus* ATCC 25923, and two Gram-negative strains, namely Escherichia coli ATCC 25922 and *Klebsiella pneumonia* ATCC 70603. The bacterial strains obtained from ElMedjed Laboratory, El-Oued, Algeria**..**

2.2.10. Preparation of Microbial Inocula:

Prior to conducting the antimicrobial assays, two successive subcultures were performed for each microbial strain to ensure optimal viability. For the bacterial isolates, the initial revival was carried out in nutrient broth (NB)**,** followed by incubation at 37°C for 24 hours**.** A second subculture was then performed by streaking the revived cultures onto nutrient agar (NA)plates to ensure colony purity and vigor. These plates were also incubated at 37°C for 24 hours. To maintain the strains in their exponential growth phase, they were subsequently stored at 4°Con nutrient agar slants until use.In the case of *Candida albicans*, the strain was reactivated in nutrient broth and incubated at30°C for 24 hours, after which it was subcultured onto Sabouraud dextrose agar (SDA**)** plates. These fungal cultures were incubated at 30°C for 72 hoursand stored at 4°Cfor further use.

According to [16], several well-isolated and morphologically consistent colonies were selected from both bacterial and fungal cultures. These were suspended in 10 mL of sterile physiological saline solution (0.9% NaCl**)** and thoroughly mixed to obtain a 10^{-1} dilution, ensuring a homogeneous microbial suspension suitable for antimicrobial testing.

2.3. Agar well Diffusion Method:

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [17, 18]. This experiment was carried out according to the method described by [19-22] with a slight modification.

2.3.1. Seeding:

The agar well diffusion method was employed as a standard technique to assess the antimicrobial activity of the ADAK ointment. This method is widely recognized for its reliability and precision in evaluating the efficacy of natural formulations against microbial strains [16].

The experiment began with the preparation of Mueller-Hinton agar (MHA), which was heated to approximately 55°C, then aseptically poured into Petri dishes at a volume of 15–20 mL per plate, ensuring a uniform thickness of about 4 mm. The plates were then left to dry at room temperature.Afterward, the agar surface was inoculated with a bacterial or fungal suspension using a sterile cotton swab to ensure even distribution of the microorganisms. Circular wells with a diameter of 6 mm were then aseptically punched into the agar, maintaining a minimum distance of 20 mm between each well, using a sterile cork borer or pipette tip.

Each well was filled with 50 µL of the previously prepared ADAK ointment The plates were sealed and incubated under appropriate conditions depending on the tested strain: Bacterial strains were incubated at 37°C for 24 hours, while *Candida albicans* was incubated at the same temperature for 48 hours.To ensure the reliability of the results, the experiment was repeated three times for each microbial strain.The agar well diffusion method was employed as a standard technique to assess the antimicrobial activity of the ADAK ointment. This method is widely recognized for its reliability and precision in evaluating the efficacy of natural formulations against microbial strains [16]. The experiment began with the preparation of Mueller-Hinton agar (MHA), which was heated to approximately 55°C, then aseptically poured into Petri dishes at a volume of 15–20 mL per plate, ensuring a uniform thickness of about 4 mm. The plates were then left to dry at room temperature.Afterward, the agar surface was inoculated with a bacterial or fungal suspension using a sterile cotton swab to ensure even distribution of the microorganisms. Circular wells with a diameter of 6 mm were then aseptically punched into the agar, maintaining a minimum distance of 20 mm between each well, using a sterile cork borer or pipette tip.Each well was filled with 50 µL of the previously prepared ADAK ointment, The plates were sealed and incubated under appropriate conditions depending on the tested strain: Bacterial strains were incubated at 37°C for 24 hours, while *Candida albicans* was incubated at

the same temperature for 48 hours.To ensure the reliability of the results, the experiment was repeated three times for each microbial strain.

2.3.2. Reading:

The efficacy of ADAK ointment against the tested microbial strains was determined by measuring the diameter of the inhibition zone resulting from the diffusion of the active compound in the agar medium, which inhibits the growth of microorganisms surrounding the well (*Figure IV 2*). Readings were taken after 24 hours of incubation at 37°C using a caliper or a precise ruler, with the well diameter included in the total inhibition zone measurement [19].

The results were expressed either as the absolute values of inhibition zone diameters (in millimeters) or as the mean ± standard deviation from three independent experiments. Values less than 6 mm were considered ineffective and indicative of no significant antimicrobial activity against the tested microorganisms.

1. Result and discussion:

Pathogenic bacteria and fungi cause a wide spectrum of diseases in humans and animals. Intensive research and advanced scientific approaches have become imperative to find new therapeutic values to overcome microbial resistance and the non-selective and irregular use of antibiotics. In this study, the well diffusion (WD) method on agar was used to study the antimicrobial activities of the prepared Adak therapeutic ointment against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and one fungus (*Candida albicans*).Furthermore, the effect of different concentrations on the aforementioned bacterial strains was investigated.In general, Gram-positive bacterial strains and fungal species exhibited concentration-dependent inhibition when treated with ADAK ointment. *Staphylococcus aureus*, *Bacillus subtilis*, and fungal strains such as *Candida spp.* showed the highest susceptibility, particularly at higher concentrations.

In contrast, Gram-negative bacterial strains, including *E. coli* and *Pseudomonas aeruginosa*, did not display any significant inhibitory response, as illustrated in the (**Table 1**).

Table 3: Antimicrobial and Anti-Candida Activity

Microbial inhibition (mm) P × (100%)	
Strains Used	**Microbial Inhibition (mm)**
Escherichia coli ATCC 25922	NI
Pseudomonas aeruginosa ATCC 27853	NI
Staphylococcus aureus ATCC 25932	17
Bacillus subtilis ATCC 25973	15
Anti-Candida activity	
Candida albicans ATCC 10231	19

- Inhibition Zone in mm
- NI = No Inhibition

Table 4: The inhibition Zones for the ADAK and Mentha Piperita

Microbial inhibition (mm)					
Strains Used	**P1**	**P2**	**P3**	**P4**	**Co. Neg.**
Escherichia coli ATCC 25922	NI	NI	NI	NI	NI
Pseudomonas aeruginosa ATCC 27853	9	11	13	NI	NI
Staphylococcus aureus ATCC 25932	8	NI	8	NI	NI
Bacillus subtilis ATCC 25973	14	NI	NI	NI	NI
Anti-Candida activity					
Candida albicans ATCC 10231	20	15	13	NI	NI

2.3.3. Test Cytotoxicity:

Following the promising biological activity of all three ointment formulations, a comprehensive toxicity assessment was undertaken to ensure their safety for topical application. The cytotoxicity test was performed using the yeast model Saccharomyces cerevisiae to establish an initial biocompatibility profile, determining LC_{50} values across serial dilutions. These in vitro findings will guide subsequent evaluation on mammalian skin cell lines (e.g., HaCaT) and in vivo irritation models, thereby confirming the formulations' suitability for further development.

Table 5: Estimated LC_{50} values for the tested cosmetic ointments based on Saccharomyces cerevisiae cytotoxicity assay

Sample Code	Estimated LC_{50} (dilution)	Toxicological Interpretation
P1	$>> 2^{-2}$	No cytotoxic effect observed at any tested dilution; the ointment is likely biocompatible under the tested conditions.
P2	$>> 2^{-2}$	Maintained high yeast cell viability across all dilutions; considered non-toxic and biocompatible.
P3	$>> 2^{-2}$	No significant growth inhibition detected; the product is non-cytotoxic at experimental concentrations.

All three formulations (P1, P2, P3) showed no cytotoxicity up to a 1/4 dilution, indicating good biocompatibility at the tested concentrations. These preliminary results support their safety profile.

The results obtained in this study demonstrated that the natural ointment "ADAK," composed of essential oils extracted from *Artemisia herba-alba, Rosmarinus officinalis,* and *Syzygium aromaticum,* and prepared in a lipid base of beeswax and olive oil, exhibits broad-spectrum and effective antimicrobial activity. The formulations prepared at different concentrations (P1, P2, P3) showed notable efficacy against Gram-positive bacterial strains, including *Bacillus subtilis* (ATCC 25973), with a maximum inhibition zone of 14 mm, and *Staphylococcus aureus* (ATCC 25932), with inhibition zones of 8 mm. Remarkably, the

ointment also displayed activity against the Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 27853), with inhibition zones of 9 mm, 11 mm, and 13 mm for samples P1, P2, and P3 respectively, suggesting that the mixture of essential oils is partially capable of penetrating the outer membrane barrier of Gram-negative bacteria.

In contrast, the formulation exhibited no inhibitory effect against *Escherichia coli* (ATCC 25922), indicating that this strain may possess more efficient resistance mechanisms such as efflux pumps or membrane compositions that limit the uptake of active compounds. Regarding antifungal activity, all three formulations demonstrated strong inhibition against *Candida albicans* (ATCC 10231), with inhibition zones of 20 mm for P1, 15 mm for P2, and 13 mm for P3, reflecting potent antifungal capacity, particularly at higher concentrations.

To assess the potential for broadening the bioactive spectrum of the formulation, a modified version (P4) was developed by incorporating peppermint essential oil (Mentha piperita). However, this formulation exhibited no antimicrobial or antifungal activity, confirming that peppermint oil did not contribute the desired bioactivity in this context. In contrast, a commercial product (PX) showed inhibition zones of 15 mm against *B. subtilis*, 17 mm against *S. aureus*, and 19 mm against *C. albicans*, while showing no activity against Gram-negative bacteria. Comparatively, the ADAK formulation demonstrated similar or superior efficacy against fungi and Gram-positive bacteria, with the added advantage of partial activity against *P. aeruginosa*, which was not observed in the commercial product.

Moreover, cytotoxicity assays revealed no toxic effects for formulations (P1–P3) even at a 1:4 dilution in vitro, indicating good biocompatibility and supporting their safety for topical use. This result is of significant importance, as it confirms that the active plant-based compounds are effective without compromising cellular safety. The synergistic interaction between the essential oils—particularly eugenol in clove oil and oxidized monoterpenes in Artemisia and rosemary—is likely responsible for the enhanced antimicrobial effects observed in this study.

Overall, the ADAK ointment, as a promising natural formulation, presents an effective alternative to conventional chemical-based topical treatments. It combines broad-spectrum efficacy against Gram-positive bacteria, moderate activity against certain Gram-negative strains,

and strong antifungal performance, all while demonstrating a favorable safety profile. Future improvements to the formulation may include the incorporation of natural permeation enhancers or other phytochemicals to boost efficacy against resistant bacteria. These findings highlight the potential of ADAK ointment as a natural, safe, and eco-friendly therapeutic solution for treating microbial skin infections.

2.4. Conclusion:

The antimicrobial activity of the laboratory prepared ointment "ADAK," composed of essential oils from *Artemisia herba-alba* (wormwood), *Rosmarinus officinalis* (rosemary), and *Syzygium aromaticum* (clove), formulated in a lipid base of beeswax and olive oil, with natural indigo, was evaluated using the well diffusion method. The results demonstrated significant inhibitory effects against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and the fungus *Candida albicans*, with a direct correlation observed between the oil concentration and the antimicrobial potency. The largest inhibition zones were observed at the highest tested concentration (20 µL), indicating a strong antimicrobial activity.

Interestingly, the formulation showed moderate activity against the Gram-negative bacterium *Pseudomonas aeruginosa*, with inhibition zones ranging from 9 mm to 13 mm. However, no activity was observed against *Escherichia coli*, suggesting a difference in permeability resistance between the Gram-negative strains. A modified version (P4) was developed by incorporating peppermint oil as an active ingredient, but this formulation did not show any improvement in biological activity, indicating that the synergistic effect observed in previous samples may have been altered due to this substitution.

When compared to a commercial product, the latter exhibited strong activity against Gram-positive bacteria and fungi but showed no effect against Gram-negative bacteria. This comparison highlights the advantage of "ADAK" in targeting a broader microbial spectrum, especially with its partial efficacy against *Pseudomonas aeruginosa*, which was not observed in the commercial product.

Furthermore, cytotoxicity tests confirmed the safety of formulations P1–P3, as no toxic effects were observed even at a 1:4 dilution, supporting the safety of topical use. These findings

reinforce the promising therapeutic potential of "ADAK" as a natural antimicrobial agent, combining efficacy against bacteria and fungi with a favorable safety profile.

In conclusion, the strategic combination of active essential oils in a natural lipid base offers a viable approach for developing alternative topical treatments. These formulations provide safe, environmentally friendly, and effective options for managing skin infections and promoting skin healing, especially in the context of increasing microbial resistance to synthetic antimicrobial agents.

2.5. The Annex:

Figure 14: The preparation of Mueller-Hinton agar of the agar well diffusion Method

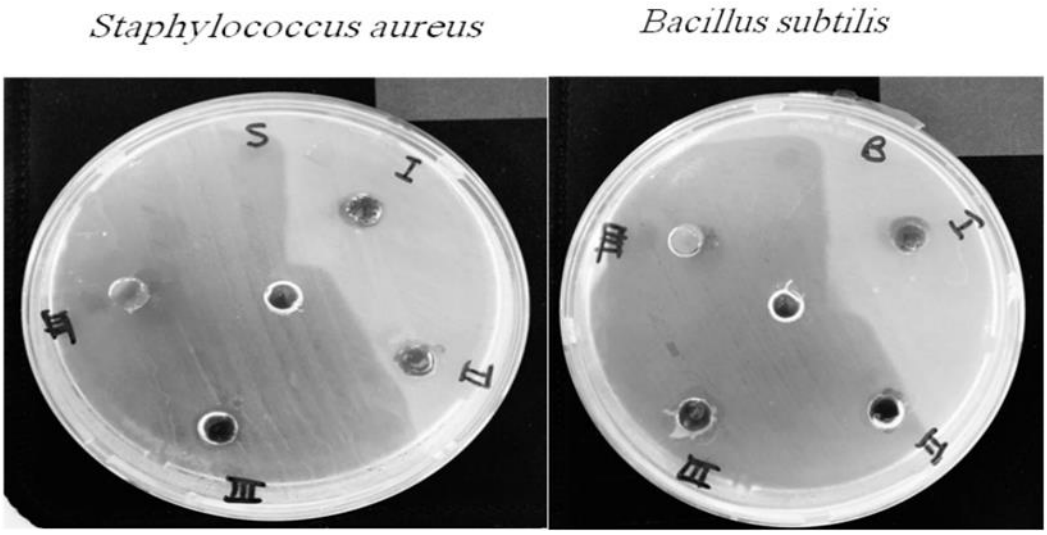

Figure 15: Antibacterial Activity of ADAK ointment against Staphylococcus aureus and Bacillus subtilis evaluated by agar diffusion assay

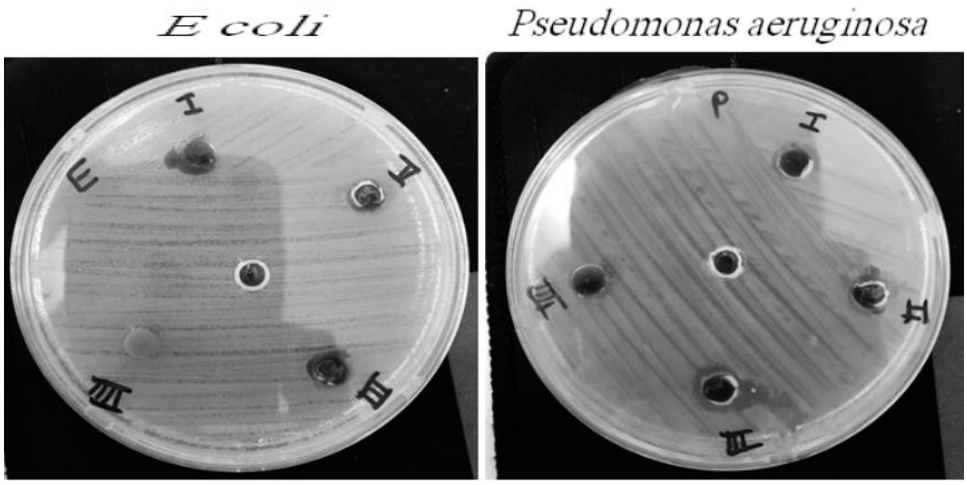

Figure 16: Antibacterial Activity of ADAK ointment against E. coli and Pseudomonas aeruginosa evaluated by agar diffusion assay

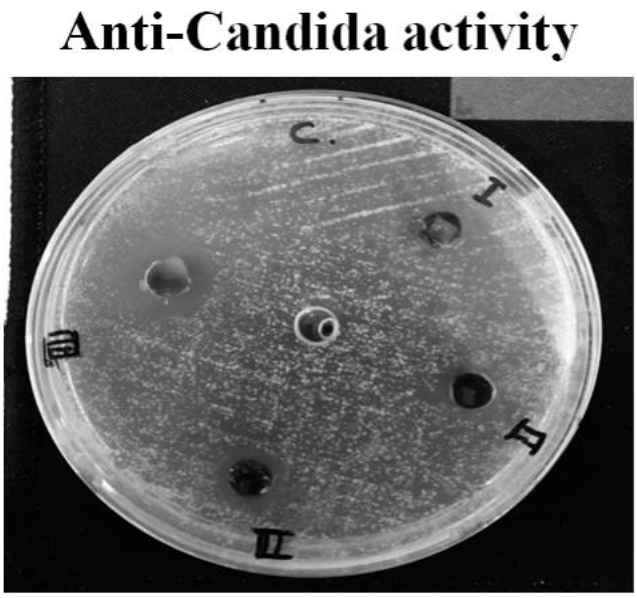

Figure 17: Illustration of Anti-Candida effect of ADAK ointment assessed by agar diffusion Method

References:

[1] A. Lianou, E. Panagou, and G.-J. Nychas, "Microbiological spoilage of foods and beverages," in *The Stability and Shelf Life of Food*, Elsevier, 2016, pp. 3–42.

[2] L. Lewis *et al.*, "Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya," *Environ. Health Perspect.*, vol. 113, no. 12, pp. 1763–1767, 2005.

[3] F. J. Angulo *et al.*, "Diarrhoeal disease in children due to contaminated food," 2017.

[4] E. Abatenh*et al.*, "The role of microorganisms in bioremediation – A review," *Open J. Environ. Biol.*, vol. 2, no. 1, pp. 038–046, 2017.

[5] S. I. Dimitrijević *et al.*, "A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L.," *Food Chem.*, vol. 104, no. 2, pp. 774–782, 2007.

[6] L. Provincial *et al.*, "Survival of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in sea bream (*Sparus aurata*) fillets packaged under enriched CO_2 modified atmospheres," *Int. J. Food Microbiol.*, vol. 166, no. 1, pp. 141–147, 2013.

[7] C. Saraiva *et al.*, "Modelling the fate of *Listeria monocytogenes* in beef meat stored at refrigeration temperatures under different packaging conditions," *Procedia Food Sci.*, vol. 7, pp. 177–180, 2016.

[8] E. Säde *et al.*, "Exploring lot-to-lot variation in spoilage bacterial communities on commercial modified atmosphere packaged beef," *Food Microbiol.*, vol. 62, pp. 147–152, 2017.

[9] W. H. Talib and A. M. Mahasneh, "Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine," *Molecules*, vol. 15, no. 3, pp. 1811–1824, 2010.

[10] N. Alzoreky and K. Nakahara, "Antibacterial activity of extracts from some edible plants commonly consumed in Asia," *Int. J. Food Microbiol.*, vol. 80, no. 3, pp. 223–230, 2003.

[11] S. Castro *et al.*, "Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria," *Braz. J. Microbiol.*, vol. 39, pp. 756–760, 2008.

[12] J.-L. Mau, C.-P. Chen, and P.-C. Hsieh, "Antimicrobial effect of extracts from Chinese chive, cinnamon, and corni fructus," *J. Agric. Food Chem.*, vol. 49, no. 1, pp. 183–188, 2001.

[13] S. J. Doddanna*et al.*, "Antimicrobial activity of plant extracts on *Candida albicans*: An in vitro study," *Indian J. Dent. Res.*, vol. 24, no. 4, pp. 401, 2013.

[14] N. B. C., A. K. Zahra S. N., and A. M. Zahra, "Antimicrobial activities of clove and thyme extracts," 2006.

[15] R. P. Samy and S. Ignacimuthu, "Antibacterial effects of the bark of *Terminalia arjuna*: Justification of folklore beliefs," *Pharm. Biol.*, vol. 39, no. 6, pp. 417–420, 2001.

[16] P. Bazerque, C. Perez, and M. Pauli, "Antibiotic assay by the Agar-well Diffusion Method," *Acta Biol. Med. Exp.*, vol. 15, pp. 113–115, 1990.

[17] S. Magaldi *et al.*, "Well diffusion for antifungal susceptibility testing," *Int. J. Infect. Dis.*, vol. 8, no. 1, pp. 39–45, 2004.

[18] C. Valgas*et al.*, "Screening methods to determine antibacterial activity of natural products," *Braz. J. Microbiol.*, vol. 38, pp. 369–380, 2007.

[19] Y. Choi *et al.*, "Antioxidant and antimicrobial activities of propolis from several regions of Korea," *LWT-Food Sci. Technol.*, vol. 39, no. 7, pp. 756–761, 2006.

[20] I. Wiegand, K. Hilpert, and R. E. Hancock, "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances," *Nat. Protoc.*, vol. 3, no. 2, pp. 163–175, 2008.

General Conclusion

The results obtained in this study demonstrated that the natural ointment "ADAK," composed of essential oils extracted from *Artemisia herba-alba* (wormwood), *Rosmarinus officinalis* (rosemary), and *Syzygium aromaticum* (clove), and formulated in a lipid base of beeswax and olive oil, exhibits broad-spectrum and effective antimicrobial activity. The formulations prepared at varying concentrations (P1, P2, P3) showed notable efficacy against Gram-positive bacterial strains, including *Bacillus subtilis* (ATCC 25973), with a maximum inhibition zone of 14 mm, and *Staphylococcus aureus* (ATCC 25932), with inhibition zones of 8 mm. Remarkably, the ointment also demonstrated activity against the Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 27853), with inhibition zones of 9 mm, 11 mm, and 13 mm for P1, P2, and P3, respectively. This suggests that the combination of essential oils is partially capable of overcoming the outer membrane barrier of Gram-negative bacteria.

In contrast, the formulation exhibited no inhibitory effect against *Escherichia coli* (ATCC 25922), indicating that this strain may possess more efficient resistance mechanisms, such as efflux pumps or membrane compositions that restrict the uptake of active compounds. Regarding antifungal activity, all three formulations demonstrated strong inhibitory effects against *Candida albicans* (ATCC 10231), with inhibition zones measuring 20 mm for P1, 15 mm for P2, and 13 mm for P3, indicating potent antifungal capacity, particularly at higher concentrations.

To explore the possibility of broadening the antimicrobial spectrum, a modified version of the ointment (P4) was developed by incorporating peppermint essential oil (*Mentha piperita*). However, this modified formulation did not exhibit any antimicrobial or antifungal activity, confirming that peppermint oil did not contribute the desired bioactivity in this context. By contrast, a commercial product (PX) showed inhibition zones of 15 mm against *B. subtilis*, 17 mm against *S. aureus*, and 19 mm against *C. albicans*, but displayed no activity against Gram-negative bacteria. Comparatively, the ADAK formulation demonstrated similar or superior efficacy against fungi and Gram-positive bacteria, with the additional advantage of partial activity against *P. aeruginosa*, a property not observed in the commercial product.

Furthermore, cytotoxicity assays revealed no toxic effects for the ADAK formulations (P1–P3) even at a 1:4 dilution in vitro, indicating good biocompatibility and supporting their safety for topical application. This finding is of particular significance as it confirms that the

active plant-based components are effective without compromising cellular viability. The synergistic interaction among the essential oils—particularly eugenol from clove oil and oxidized monoterpenes from wormwood and rosemary—is likely responsible for the enhanced antimicrobial effects observed in this study.

Overall, the ADAK ointment represents a promising natural alternative to conventional chemical-based topical treatments. It offers broad-spectrum efficacy against Gram-positive bacteria, moderate activity against selected Gram-negative strains, and strong antifungal performance, alongside a favorable safety profile. Future improvements to the formulation may include the incorporation of natural permeation enhancers or additional phytochemicals to improve efficacy against resistant bacteria. These findings underscore the potential of ADAK ointment as a safe, natural, and environmentally friendly therapeutic option for treating microbial skin infections.