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Evaluation of the Antibacterial Properties of *Eucalyptus globulus* Essential Oil

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Dedication

*I dedicate this work to my lovely parents, who have been and continue to be
my source of encouragement.*

To my beloved sister and brother.

*To all my friends and family members,
For their constant love and unwavering support.*

To all those who love HOUDA and who I love.



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List of abbreviations

µl: micro liter

AMK: Amikacin

ATCC : American Type Culture Collection

D: diameter

DMSO: dimethyl sulfoxide

DMSO-EO: dimethyl sulfoxide-essential oil

E. coli : *eschirechia coli*

E. globulus : *eucalyptus globulus*

EO: essential oil

G: gram

Gent: gentamycin

Gram-: négatif Gram. .

Gram+: positif Gram.

Ha : Hechter

K+: potassium ion

MH: Muller-Hinton

Pen: penicillin

S. aureus : staphylococcus aureus

TM: traditional medicine

Van: vancomycin

Y%: yield in percentage

Abstract:

Essential oils extracted from *Eucalyptus globulus* by hydrodistillation could be a plausible alternative for people with antibiotic resistance, and also for elderly individuals who can no longer tolerate the conventional administration of these drugs. The yield of essential oil varies significantly depending on the extraction method, the season during which the leaves are harvested, and other biotic and abiotic factors, culminating in a low yield value of 0.1%. The efficacy of the essential oil from this aromatic plant at different concentrations (pure, 50%, 25%) is estimated by determining the zones of inhibition using the aromatogram and antibiogram methods on the growth of the tested microorganisms (clinical *Escherichia coli* and ATCC strain, as well as clinical and ATCC *Staphylococcus aureus*). Indeed, the essential oil extracted from the leaves of this plant shows a good antibacterial effect compared with the antibiotics tested (Penicillin and Vancomycin for the clinical and ATCC *Staphylococcus aureus* strains, and Amikacin and Gentamicin for the clinical and reference strains of *Escherichia coli*). Our experiments show that the inhibitory power of *Eucalyptus globulus* essential oil is superior to that of the antibiotics against all the bacterial strains tested. In conclusion, this study underlines the effectiveness of this aromatic and medicinal plant in combating bacterial diseases and highlights its potential to replace antibiotics with a natural remedy.

Key words: *Eucalyptus Globulus*, Essential oil, Hydrodistillation, Antibacterial activity.

Résumé :

L'huile essentielle extraite d'*Eucalyptus Globulus* par la méthode de l'hydrodistillation pourrait être une alternative plausible pour les personnes présentant une résistance à l'usage des antibiotiques, et aussi pour les personnes âgées ne supportant plus l'administration normale de ces médicaments. Le rendement d'huile essentielle varie significativement en fonction de la méthode d'extraction, la saison de cueillette des feuilles associée à d'autres facteurs biotiques et abiotiques, pour atteindre à cette faible valeur de rendement qui est égale à 0,1%, tel est le résultat de ce mémoire. L'efficacité de l'huile essentielle de cette plante aromatique pour différentes concentrations (pure, 50%, 25%), est estimée par la détermination des zones d'inhibition en utilisant les méthodes d'aromatogramme, et d'antibiogramme sur la croissance des micro-organismes testés (*Escherichia Coli* ATCC et clinique, le *Staphylococcus aureus* ATCC et clinique). En effet, l'huile essentielle extraite des feuilles de cette plante manifeste un bon effet antibactérien par rapport aux antibiotiques testés (Pénicilline, vancomycine, pour *Staphylococcus aureus* et *Staphylococcus aureus* ATCC) et (Amikacine et Gentamicine pour les souches clinique et ATCC de *Escherichia coli*). Notre expérimentation a montré que le pouvoir inhibiteur de l'huile essentielle d'*Eucalyptus globulus* a une activité antibactérienne contre toutes les bactéries testées supérieur à celui des antibiotiques. En conclusion, cette étude souligne l'efficacité de cette plante aromatique et médicinale dans la lutte contre les maladies d'origine bactérienne et met en évidence son potentiel en tant que remède naturel pouvant remplacer les antibiotiques.

Mots clés : *Eucalyptus Globulus*, Huile essentielle, Hydrodistillation, Activité antibactérienne.

ملخص:

يمكن أن يكون الزيت العطري المستخرج من نبات *الأوكالبتوس غلوبولوس* بطريقة التقطير المائي بديلاً معقولاً للأشخاص الذين يعانون من مقاومة المضادات الحيوية، وكذلك لكبار السن الذين لم يعودوا قادرين على تحمل التناول العادي لهذه الأدوية. يختلف محصول الزيت العطري اختلافاً كبيراً حسب طريقة الاستخلاص، والموسم الذي يتم فيه حصاد الأوراق وعوامل حيوية وغير حيوية أخرى، حيث بلغت قيمة المردود 0.1% تم تقدير فاعلية الزيت العطري لهذا النبات العطري بتركيزات مختلفة (نقي، 50%، 25%) عن طريق تحديد مناطق التثبيط باستخدام اختبار الحساسية للزيوت العطرية واختبار الحساسية للمضادات الحيوية على نمو الكائنات الدقيقة المختبرة (*الإشريكية القولونية* المرجعية و*السريرية* و*المكورات العنقودية الذهبية* المرجعية و*السريرية*). الزيت العطري المستخلص من أوراق هذا النبات له تأثير جيد مضاد للجراثيم مقارنة بالمضادات الحيوية التي تم اختبارها (*البنسلين* و*الفانكوميسين* و*المكورات العنقودية الذهبية* المرجعية و*السريرية*) و (*الأميكاسين* و*الجنتاميسين* للسلالات *السريرية* و*المرجعية* من *الإشريكية القولونية*). أظهرت تجاربنا أن القوة التثبيطية لزيت *الأوكالبتوس غلوبولوس* العطري كانت أكبر من قوة المضادات الحيوية ضد جميع البكتيريا التي تم اختبارها. في الختام، تؤكد هذه الدراسة على فعالية هذا النبات العطري والطبي المدروس في مكافحة الأمراض البكتيرية واستبدال المضادات الحيوية بهذا العلاج الطبيعي.

الكلمات المفتاحية: *أوكالبتوس غلوبولوس*، زيت عطري، تقطير مائي، نشاط مضاد للبكتيريا.

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I. Introduction

Every culture has used medicinal plants to treat a variety of ailments throughout history. The study of plants and their derivatives has seen a notable resurgence in interest in recent years, especially in light of possible uses in public health, medicine, and food preservation. Since many of these plants are known to be abundant sources of natural antioxidants with potential applications in the food industry, researchers are now carefully analyzing plants that have historically been used in folk medicine to determine their viability as safer, more sustainable substitutes for artificial chemical preservatives (Voon et al., 2012; Tiwari et al., 2009).

Aromatic plants provide fragrant essences, aromas, cosmetics, health drinks and chemical terpenes. These plants are highly valued and sold in large quantities in many developing nations. By minimizing the emergence of antibiotic-resistant foodborne pathogens and improving the management of cross-contamination risks, the use of natural antibiotics derived from plants may prove to be very effective in reducing an excessive dependence on synthetic antibiotics (Voon et al., 2012).

The World Health Organization (WHO) claims that traditional medicine is founded on a wealth of information, customs, and beliefs that have been cultivated over many generations. Whether or not these practices are formalized, their goal is to treat and maintain physical and mental health. Bioactive compounds found in medicinal plants are essential for the treatment of a variety of illnesses. In fact, according to the WHO, about 75% of people in developing nations get their primary medical care from herbal remedies (WHO, 2002).

Among these plants is *Eucalyptus*, one of the major genera of the Myrtaceae family. It originated in Australia and Tasmania, but has become acclimatized in other countries such as Brazil (Ghedira et al., 2008), as well as in the Mediterranean basin (Aït youssef, 2006). According to Jacovelli (2002), in 1995, *Eucalyptus* covered an area of 39,000 ha in Algeria, while *Eucalyptus Globulus* is one of the most widespread species, according to Aït youssef (2006).

According to popular tradition, its leaves are used as a tonic remedy in herbal tea form to treat seasonal flu, respiratory infections, diarrhea, fevers, toothache, frostbite and other problems (Aït youssef, 2006; Kim et al., 2011).

According to Vankar et al., (2006), the pharmacological properties of *Eucalyptus* are mainly attributed to its variety of secondary metabolites, including essential oils, tannins and

flavonoids. Beyond conventional therapeutic benefits, leaf extracts from this plant are listed as a food additive in Japan (Ministry of Health Notice No. 120, April 16, 1996) (**Amakura et al., 2002**).

Eucalyptus Globulus extracts and essential oils are also used in the composition of a number of medicines to treat various pathologies, such as respiratory disorders, as well as in certain cosmetic products.

Furthermore, the extraction of essential oils from medicinal plants could represent a major economic lever for our country.

The use of essential oils to treat various ailments (digestive, infectious, among others) is based on their attributes: anti-infectious, analgesic, anti-inflammatory, soothing, antimicrobial, antispasmodic and antioxidant properties, they are also used clinically to treat inflammatory diseases.

The use of plant-based extracts for therapeutic purposes has a long history. According to **Raphael (2011) and Sawadogo et al. (2012)**, between 66 and 85 percent of people worldwide, particularly those in developing nations, particularly those in sub-Saharan Africa, rely on medicinal plants as a substitute for or addition to traditional medicine.

The botanical diversity of the area must be taken into account in order to guarantee that plant-based extracts are effective in curing illnesses or reducing their symptoms. Due to its advantageous geographic location, Algeria boasts a rich and varied natural flora, including a large number of aromatic and therapeutic plants that grow naturally throughout its land. Because of its established therapeutic potential, *Eucalyptus globulus* stands out among these.

The purpose of this study is to assess the antibacterial properties of essential oil extracted from *Eucalyptus globulus*. It aims to evaluate its effectiveness against selected bacterial strains through laboratory-based testing. This leads us to an important research question:

- What extent does the essential oil of *Eucalyptus globulus* exhibit antibacterial activity against specific strains of both Gram-positive and Gram-negative bacteria?

Our dissertation is divided into two parts:

The first part deals with theories. It is composed of 3 main AXES. The first deals with phytotherapy, medicinal plants in general, and the second with *Eucalyptus globulus* in particular, highlighting essential oils in the third. The second part is experimental, presenting

the materials and methods used in our experiments. We then analyse the results obtained, with discussion and interpretation.



**PART 01 : LITERATURE
REVUE**

1. Phytotherapy

1.1. History of phytotherapy

The Egyptians used several hundred plants for their culinary and medicinal properties. For centuries, these two uses remained closely linked. A major milestone occurred in the 18th century when the botanist Jussieu identified the cinchona tree. In 1820, two French researchers, Pelletier and Caventou, succeeded in isolating its active ingredient, quinine. However, by the late 19th century, the use of medicinal plants rapidly declined in the West with the rise of scientific medicine and the development of modern pharmaceuticals (**Sebai et Boudali, 2012**).

Throughout the 20th century, phytotherapy was largely neglected in favor of compounds derived from synthetic chemistry. Groundbreaking scientific discoveries—such as Fleming's identification of antibiotics in 1928 and Pasteur's development of vaccines—transformed the field of medicine (**Cazau-Beyret, 2013**).

In the early 1990s, research began to highlight the effectiveness of traditional Chinese medicine in treating eczema by addressing its root causes. The addition of a single herb to an existing formula even led to significant improvement in a patient who had previously not responded to conventional treatments. This personalized approach stands in stark contrast to modern medicine, which typically favors standardized therapies for each disease (**Iserin, 2001**).

1.2. Definition of phytotherapy

According to etymology, the word phytotherapy comes from two Greek roots: *therapeia* (meaning "treatment") and *phuton* (meaning "plant"), which together mean "healing through plants" (**Nogaret, 2011**).

Using whole plants, plant parts, or plant-based extracts, phytotherapy is now recognized as an allopathic field that helps prevent and treat a variety of functional disorders and/or medical conditions (**Wichtl et al., 2003**).

1.3. Phytotherapy Interest

Phytotherapy has been popular since ancient times. The potential for advancement in this broad field has grown dramatically with continuous scientific and technological advancements.

Because of its many benefits, phytotherapy now faces competition from traditional medicine (**Prigent, 2018**). These benefits include:

- ✓ There are typically few adverse effects due to the natural makeup of medicinal plants.
- ✓ Medicinal plants are less expensive and easier to use than synthetic substances and traditional medications.
- ✓ They are employed in the creation of numerous medicinal products and, occasionally, even narcotics.
- ✓ The protective qualities of medicinal plants aid in the fight against progressive and chronic illnesses.

1.4. Types of phytotherapy

There are two categories :

A. Traditional herbal medicine is based on a set of concepts, beliefs, philosophies and knowledge that lack experimental scientific foundation due to a lack of scientific instruments at the time (**Jorite, 2015**).

B. Contemporary phytotherapy came into being following the advent of modern chemistry, which greatly contributed to its evolution. It is explained and justified by the study of medicinal plants, which has facilitated its understanding. It also explores the mechanisms of action of the therapeutic components of these plants, while encouraging the use of products derived from extraction or synthesis. (**Merad et Mahiout, 2019**).

2. Medicinal plants

2.1. History of medicinal plants

More than four out of five people in Africa use traditional medicine (TM).

In Algeria, the first references to medicinal plants date back to the 9th century, by Ishâ-Ben-Amran and Abdallah-BenLounès, the latter born in Oran. They documented the use of numerous plants for medicinal purposes.

During the French colonial period from 1830 to 1962, botanists were able to record a large number of medicinal species. In **1942, Fourment et Roques** published a book on the medicinal and aromatic plants of Algeria, in which they documented, described and analyzed 200 species. Beloued's book contains the most recent published work on medicinal plants in Algeria.

2.2. Definition of medicinal plants

A plant is considered medicinal when at least one of its parts possesses curative or preventive properties against disease, with or without identified active compounds (**Claisse-Dauchy, 1996; Bruneton, 1999**).

It is estimated that there are approximately 500,000 plant species on Earth, of which around 10,000 exhibit therapeutic properties. These plants function as natural chemical factories, producing biochemical compounds such as alkaloids, essential oils, flavonoids, and tannins. They offer these substances to humanity, which can harness them to improve health and fulfill essential needs. (**Iserin et al., 2001**)

2.3. Components of medicinal plants

A wide variety of bioactive substances, sometimes known as secondary metabolites, are responsible for the therapeutic qualities of medicinal plants. These substances have significant ecological roles, such as protecting the plant from diseases and herbivores, even though they are not directly involved in the growth or reproduction of the plant (**Roy et al., 2022**). These compounds fall into the following primary categories:

- **Alkaloids**

Substances that contain nitrogen and are well-known for their strong biological effects. Examples include atropine (anticholinergic), quinine (antimalarial), and morphine (analgesic) **(Bruneton, 2009)**.

- **Flavonoids**

Polyphenolic substances that have anti-inflammatory, anti-cancer, and antioxidant qualities. found in a variety of medicinal plants, fruits, and vegetables. **(Ghedira, 2005)**

- **Tannins**

Astringent polyphenols with antibacterial and anti-diarrheal qualities. prevalent in unripe fruits, leaves, and bark **(Ali Delille, 2013)**.

- **Essential Oils**

Volatile aromatic compounds that have antiseptic, antifungal, and antimicrobial properties. prevalent in fragrant plants such as thyme, lavender, and eucalyptus **(Thormar, 2011)**.

- **Glycosides**

Substances called aglycones that are made up of sugar molecules bound to other molecules. renowned for their laxative, anti-inflammatory, and cardiac properties **(Iserin et al., 2001)**.

- **Saponins**

Substances with antimicrobial, anti-inflammatory, and cholesterol-lowering properties that create soapy lathers in water **(Iserin et al., 2001)**

- **Terpenoids and Terpenes**

A broad class of hydrocarbons that contribute to flavor, aroma, and therapeutic qualities (such as anti-inflammatory and anti-cancer effects) **(CoxGeorgian et al., 2019)**.

- **Coumarins**

Natural phenolic compounds that have antimicrobial, anticoagulant, and anti-inflammatory properties. **(Vanier, 2022)**

2.4. Interest and importance of medicinal plants

Medicinal plants play a crucial role in pharmaceutical research and drug production, not only as treatment agents, but also as a source of raw material for drug synthesis or as a reference for effective pharmaceutical compounds. Tubocurarine, the most powerful muscle relaxant, is derived from curane (*Chondroendron tomentosum*). The most powerful analgesic used in major surgery and cancer treatment is morphine, a specific alkaloid derived from papaver (*Papaver somniferum*). (Verdrage, 1978; Herbel, 1999).

It has been established that medicinal plants have the capacity to treat minor ailments such as the common cold, or to prevent more serious problems such as ulcers, migraines, heart attacks and certain allergies (Khemies, 2013).

One of the major advantages of frequently-used medicinal plants is that they usually have few, if any, side effects (Decaux, 2002).

Some plants pose no danger at all, while others, such as certain species (*foxglove, belladonna, colchicum, etc.*), are toxic and should only be used in strictly controlled forms, sold exclusively in pharmacies. Careless use of wild-gathered plants can lead to severe and fatal poisoning (Williamson, 2001).

II.1. Essential oils

A mixture of terpene compounds (essential oils are derived from secondary metabolism; they have various functions: insect control, herbivore deterrence; derived from flowers, they are used to make perfumes (**Larousse, 2006**).

According to **Ben Kaab (2020)**, essential oils are produced by aromatic plants as secondary metabolites.

Essential oils are aromatic substances, often of complex composition, extracted from a specifically identified plant material, either by steam distillation, dry distillation, or through a suitable mechanical method without the application of heat (**Lecomte, 2022**).

Essential oils can be extracted from roots (such as iris and vetiver), bulbs (such as garlic) or rhizomes (such as *ginger*). It can also come from the stem (*fine grains*), wood (*rose, cedar, sandalwood*) or even bark (*cinnamon*). Leaves (*eucalyptus* and *citronella*), buds (*pine*), sap (*myrrh*), flowers (*ylang-ylang, rose*), fruit (*orange*) and seeds (*nutmeg, anise*) are all sources of essential oils. For essential oils such as lavender or sage, the whole plant is used (**Fillatre, 2011**).

2. The chemical composition of essential oils

From a chemical point of view, essential oils are extremely complex structural combinations, likely to contain over 300 distinct compounds. These compounds are highly volatile molecules mainly classified as terpenes, including monoterpenes and sesquiterpenes (**Kurkin, 2003**).

- **Terpenes**

Terpenes are naturally occurring hydrocarbons that can have a cyclic or linear structure. Their most significant structural feature is the inclusion of a 5-carbon isoprene unit (C₅H₈) in their skeleton. They are divided into different categories according to the number of isoprene units: monoterpenes, made up of two isoprenes (C₁₀H₁₆), sesquiterpenes, which are made up of three isoprenes (C₁₅H₂₄) and diterpenes, composed of four isoprenes (C₂₀H₃₂) (**Vila et al., 2002**).

The eight tetraterpene isoprenes lead to the carotenoids. Polyterpenes (C₅H₈) may or may not range from 9 to 30 (**Vila et al., 2002**).

Terpenoids are terpenes with one or more chemical functions, such as alcohols, aldehydes, ketones, acids and so on (Vila et al., 2002).

- **Monoterpenes**

Monoterpenes are volatile substances that can be transported by water vapor, generally have a pleasant odor and often make up the bulk of essential oils, sometimes exceeding 90%. They can be acyclic (myrcene, oxymene), monocyclic (terpinene, p-cymene) or bicyclic (pinene, sabinene). Several substances with chemical functions are associated with these terpenes: alcohols (geraniol, citronellal, sinensal), ketones (carvone, menthone, 3-vetivone) and esters (geranyl acetate, geranyl acetate, linalyl acetate, cedryl acetate, α -terpinyl acetate) (Triaux, 2019).

- **Sesquiterpenes**

This is the most varied class of terpenes. It contains over 3,000 molecules, including β -caryophyllene, β -bisabolene, α -humulene and α -bisabolol (Triaux, 2019).

3. Aromatic compounds present in essential oils

There are fewer phenylpropane derivatives than terpenoids. This category includes aromatic compounds such as vanillin, eugenol, anethole, estragole and many others. They are most commonly found in apiaceae essential oils (*anise, fennel, cinnamon, basil*) (Hurtel, 2006).

4. Compounds of various origins

A considerable set of products is found to originate from the conversion of non-volatile molecules, either through the decomposition of non-volatile terpenes or through the auto-oxidation of compounds such as carotenes or fatty acids like linoleic acid and α -linolenic acid into (3-cis-hexanol, decanal, and β -ionone) (Bruneton, 1999).

5. Concept of chemotype

The chemotype of an essential oil refers to a specificity indicating the predominant or characteristic biochemical constituent present in the essential oil. It is the element that helps

identify EOs from the same botanical species, but with a distinct biochemical composition **(Thompson et al., 2003)**.

This categorization facilitates the choice of EOs for a specific, safer and more efficient application. This chemical polymorphism is found in certain species:

Thymus vulgaris, *monarda spicata*, *origanum vulgare*. It should be emphasized that EOs with different chemotypes not only exhibit distinct activities, but also very different toxicities **(Pibiri, 2005)**.

6. Storage of essential oils

- The use of opaque glass bottles is recommended, as products can deteriorate on contact with light and plastic.

- Essential oils should be stored in a cool place, protected from light.

-It is important to note that some essential oils contain active agents that give them a natural preservative capacity.

-However, be careful when using them in the sun, as they also have a photosensitizing effect. **(Sarrouy, 2013)**

7. Toxicity of essential oils

Essential oil (EO) poisoning, whether voluntary or involuntary, is one of the most frequent forms of intoxication linked to overdose **(Baudoux, 2001)**. Young children are the most exposed to this danger. In adults, poisoning can be intentional, as in the case of suicide attempts. For example, an 18-year-old woman died of liver failure six days after ingesting 30 ml of menthol essential oil. What's more, the multitude of molecules present in EO can have a sensitizing effect, indicating that the danger of allergies should not be minimized **(Festy, 2018; Uter et al., 2010)**. Most allergic reactions to essential oils occur on the skin. Keep this in mind. As a safety precaution, pregnant women are advised not to use essential oils during the first trimester of pregnancy. It is during embryogenesis and organogenesis, two critical phases of pregnancy, that it is essential to avoid any interference **(Staub et Bayer, 2013; Faucon et Lobstein, 2015)**.

III.1. History of *Eucalyptus globulus*

The discovery of *Eucalyptus globulus* was made by French botanist La Billardière, a naturalist who was part of the expedition led by French explorer Bruni d'Entrecasteaux in 1791. In 1792, he named this plant *Eucalyptus globulus Labill*, combining the Greek “*eu*”, meaning “well”, and “*calyptos*”, meaning “covered”, in reference to the operculum present on the *Eucalyptus* fruit, known as capsules (Orme, 2000).

Eucalyptus trees, mainly of the *globulus* species, have been introduced to many parts of the world: Portugal, California, North America, Chile (1823), South Africa (1828), India (1843), Argentina (1857), as well as Egypt and Andalusia (Orme, 2000).

Eucalyptus globulus originated in Tasmania and south-western Australia. Due to its accelerated growth and high quality, it is now being established in cultivation in Mediterranean areas. Ramel introduced the concept to Algeria in 1854 (Makhlouf et al, 2016).

2. Common name of *Eucalyptus globulus*

- **Scientific name:** *Eucalyptus globulus Labill*.
- **French names:** *Eucalyptus globuleux*, gommier bleu, *eucalyptus* bleu, arbre à fièvre, *eucalyptus* commun, *eucalyptus* officinal.
- **In Australia** the *eucalyptus* are commonly known as gum trees or stringybark trees.
- **Vernacular names:** Calitous “the most recognized name in Algeria”, Calibtus, Kafor. According to Aït Youssef (2006), these names are very common in Algeria and reflect great regional diversity.

3. The Myrtaceae family

3.1. Botanical description

Eucalyptus trees belong to the vast Myrtaceae family. According to Warot (2006), they comprise some 600 to 700 species and varieties. Members of this botanical family are mainly trees or shrubs, with leaves that are generally opposite and entire, of considerable size, large and translucent, with stipules that are diminished or completely absent (Judd et al., 2002; Ozenda, 1982). These plants are distinguished by their olfactory-cycle, actinomorphic and hermaphrodite flowers (Deysson, 1979; Spichiger et al., 2002). Their fruits are generally berries or capsules, and sometimes drupes (Ozenda, 1982; Spichiger et al., 2002).

4. Definition of *Eucalyptus*

Eucalyptus is a tree with mottled bark and fragrant grey-green foliage that has become acclimatized in European and American gardens (**Larousse, 2006**), also known as the “blue gum tree” because of its abundant aromatic essence, which makes its young branches sticky, and the slightly blue color of its leaves.

Eucalyptus globulus is a tree that can sometimes exceed 25 to 30 meters in height. It is distinguished by its rapid growth and is used in reforestation projects and Arboretum centers particularly in the Mitidja region and the coastal plains of northern Algeria. There are two types of leaves, depending on their origin: those from young plants and those from older twigs. Young, opposite leaves are arranged horizontally at the base of branches. These have a lanceolate, slightly curved petiole, 16 to 25 cm long and 2 to 5 cm wide. When crumpled, the leaves give off a strong balsamic, camphorated scent (**Beloued, 2009**).



Figure 01: *Eucalyptus Globulus* tree (Original)

4.1. Botanical aspects

➤ Leaves

Most *eucalyptus* trees retain their leaves, although some tropical species lose them at the end of the dry season.

Like other members of the Myrtaceae family, *eucalyptus* leaves are covered with oil glands. Generous oil production is a notable characteristic of this species (Serventy, 1968).

The bluish-tinted leaves have an interesting peculiarity: on young trees, they grow in opposite directions, stalkless, oval and glaucous. However, as the tree develops, they begin to grow in an alternating pattern, with petioles, elongated and sometimes slightly curved like harvesting scythes, sporting a brilliant green color. In the same forest, two types of foliage coexist, giving the illusion that they are composed of distinct tree species (Serventy, 1968).



Figure 02: *Eucalyptus Globulus* leaves (Original)

➤ Flowers

They are very diverse. These plants have a multitude of stamens, which can appear in shades of white, cream, yellow, pink or red.

Initially, the stamens are contained in an envelope sealed by an operculum formed by the fusion of the petals and sepals (Serventy, 1968).

The opercules can take a variety of forms, even on the same plant. When the stamens develop, they lift the operculum and extend to form the flower. Flowers are mainly pollinated by insects, attracted by nectar (Serventy, 1968).



Figure 03: Flowers of *Eucalyptus Globulus* (GCLN, 2025)

➤ **Fruit**

Ripe fruits are conical in shape, dry and brown in color. They also have valves that open to release the seeds when they fall to the ground (Serventy, 1968).



Figure 04: The fruit of *Eucalyptus Globulus* (Original)

➤ **Trunk**

The trunk is smooth and ashy, wrapped in bark that peels off in long strips (**Ghedira et al., 2008**). It also has crevices containing a gum called “Kito”, which can range in hue from bright red to deep red (**Aït Youssef, 2006**).



Figure 05: The trunk of *Eucalyptus Globulus* (Original)

➤ **Roots**

The majority of eucalyptus trees also have underground reserve organs known as lignotubes. These lignotubes appear as protuberances at the base of the root collar; they are made up of undifferentiated cell masses containing carbohydrate reserves such as starch (**Serventy, 1968**).



Figure06: The roots of *Eucalyptus globulus* (Original)

5. Main chemical components of the *eucalyptus* genus

- Essential oil (terpene oxides: 1,8-cineole; monoterpenes: alpha-pinene, limonene, gamma-terpinene, para-cymene; sesquiterpenes: aromadendrene; sesquiterpenols: globulol, ledol).
- Flavonoids (flavone heterosides containing the following aglycones: quercetin, myricetin, kaempferol and rutin).
- Tannins (**Daroui-Mokaddem, 2012**).

6. Properties and composition of *Eucalyptus globulus* essential oil

Eucalyptus globulus essential oil is pale yellow in color, extremely fluid, with an invigorating, pleasant, balsamic fragrance and a refreshing, aromatic taste.

The main active and aromatic ingredient, dominant in its composition, is cineol or eucalyptol (70-80%), which is associated with terpenes, ketones, sesquiterpenes and various aldehydes. Rectification of the crude essence is necessary to remove aldehydes, notably isovalerian aldehyde, which is irritating and gives off an unpleasant odor (**Bardeau, 2009**).

7. Geographical distribution of the plant in Algeria:

Eucalyptus occupied a surface area of 5,855 hectares, more than half of which in the Oranaise region (**Boudy, 1955**). Today, plantations line the coast at El-Kala and Azzefoun. The species is also found in the Mitidja and Hadjout regions. The geographical distribution

of the *E.globulus* species is expressed as follows: **(Foudil-Cherif, 1993)**.

BLIDA : 41 HA

BOUMERDES : 93HA

RELIZANE: 70 HA

SKIKDA: 2250 HA

SIDI BELABAS: 342 HA

SETIF: 10 HA

EL TAREF: 1000 HA

8. Using *Eucalyptus globulus* essential oil

Eucalyptus globulus is best known for its ability to promote respiratory tract health, making it a popular treatment for colds, coughs, bronchitis and nasal congestion **(Danvers, 2025)**. It is also very useful for flu-like symptoms **(Sarrouy, 2013)**.

In addition, *eucalyptus* essential oil has antibacterial, antiviral and anti-inflammatory properties, making it an effective remedy for healing wounds, easing pain and fighting infections. *Eucalyptus globulus*, used to relieve toothache and reduce fever, is also employed in cases of superficial burns and earwax accumulation **(Sarrouy, 2013)**.

9. The benefits of *eucalyptus* essential oil

Eucalyptus essential oil has antimicrobial, antifungal and decongestant attributes that can help maintain respiratory tract health. It can be used in a diffuser or applied locally to the chest and throat to help relieve congestion and facilitate easier breathing.

✓ Anti-inflammatory:

It has anti-inflammatory and analgesic properties that can bring relief to muscle and joint pain. It can be used locally on the affected area, mixed with a carrier oil, to soothe and relieve pain.

✓ Protection against insects:

It has a natural repellent action on insects such as mosquitoes and ticks. It can be used in a diffuser or applied directly to the skin to keep insects away.

- ✓ **Soothing:** Provides a feeling of calm for both body and mind. It can be used in a diffuser or incorporated into bath water to promote relaxation and relieve stress (**Danvers, 2025**).

10. The side effects of *eucalyptus* oil

- ✓ **Allergic reactions:** *Eucalyptus* oil may cause allergic reactions or skin irritation in some individuals, particularly if applied undiluted or in large quantities. Before applying *eucalyptus* oil topically, it is crucial to perform a patch test on a limited area of the skin. It is recommended to perform a patch test on a small area of skin before applying *eucalyptus* oil topically.
- ✓ **Absorption is not safe:** *Eucalyptus* oil is risky to consume and should only be used externally or inhaled as part of aromatherapy. *Eucalyptus* oil, when ingested, may cause nausea, diarrhea and various other side effects.
- ✓ **Harmful to young children:** *Eucalyptus* oil may be toxic to young children, especially those under the age of six. Before applying *eucalyptus* oil to children, it is essential to seek professional medical advice (**Danvers, 2025**).

11. Antimicrobial activity

From the moment of birth, an individual is exposed to micro-organisms which gradually begin to invade the mucous membrane of the skin. Various means are employed to combat these micro-organisms, and three categories can be schematically identified: anatomical barriers, natural resistance processes and acquired immunity (**García-Ruiz et al., 2008**).

The treatment of bacterial infections relies mainly on the use of antibiotics. However, over-prescription and sometimes inappropriate use of these substances can lead to the selection of multi-resistant strains. It is therefore crucial to direct research towards the discovery of new avenues for the development of innovative plant-based drugs (**Jürgen et al., 2009**).

When it comes to antimicrobial activity, two types of effect can be distinguished: a lethal or bactericidal effect, and growth inhibition, known as the bacteriostatic effect. Essential oils are generally associated with a bacteriostatic effect. Nevertheless, it appears that some of their chemical components possess antibacterial properties (**Ayachi, 2014**).

12. Antimicrobial action of essential oils

- ✚ The effect of essential oils is mainly determined by the nature and characteristics of the active ingredients, particularly their hydrophobic capacity, which enables them to cross the phospholipid bilayer of the bacterial cell membrane. This may result in a modification of the membrane structure, disruption by chemiosmosis and loss of ions (K⁺) : this mechanism was observed with *Melaleuca alternifolia* oil on Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria and yeast (*Candida albicans*) in vitro (Cox et al., 2000; Carson et al., 2002).
- ✚ The effect of essential oils is also linked to the nature of the microorganisms, The type of microorganisms targeted is another key factor influencing the antimicrobial activity of essential oils. Usually, different microorganisms react differently to essential oils. Bacteria can be distinguished using a staining technique called Gram. It is thanks to variations in the size and characteristics of the peptidoglycan that we can distinguish these two types of bacteria (Gram-/Gram+) through staining (Burt, 2004).



Part 02 : Experimental part

1. Materials

1.1. Biological Material (Plant)

The aerial part of the plant, specifically the leaves of *Eucalyptus globulus*, was selected. It should be noted that the leaves are dimorphic meaning that the older and younger leaves exhibit different morphologies (see figures 07 and 08).

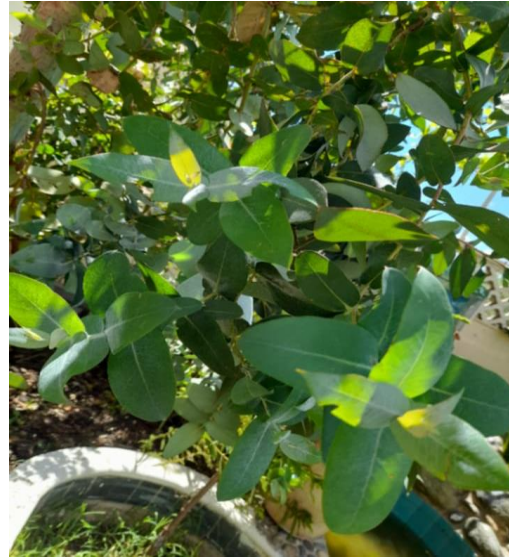


Figure 07: Older leaves of *EG*(Original) **Figure 08:** Younger leaves of *EG*(Original)

The leaves used in this study were collected in December from the Chéraiaa forests near the commune of Tamalous. This site is located away from sources of pollution. The collected leaves, originating from mature branches of large trees, were relatively old.

1.2. Classification of *Eucalyptus globulus*

The taxonomic classification provided by **Guignard on 2001** has enabled us to determine the following systematics:

Table 01: Classification of *Eucalyptus globulus*

Kingdom	Plants
Phylum	Spermatophytes
Sub-branch	Angiosperms
Class	Eudicots
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	<i>Eucalyptus</i>
Species	<i>Eucalyptus Globulus</i>

1.3. Essential Oil Extraction Equipment

Still: Holds the mixture of plant material and water to be distilled.

Heat source: Heats the flask containing the mixture.

Condenser/Refrigerant: Cools and condenses the steam.

Receiving vessel: Collects the distillate.

Water.

Plant material: Leaves of *Eucalyptus globulus*.

1.4. Bacteriological Material

Antibacterial tests were performed on both standard reference strains (from the Institut Pasteur) and clinical isolates from Abed Rezak Bouharra Hospital, Skikda.

Gram-positive Bacteria: *Staphylococcus aureus* (ATCC 25923)

A member of the Micrococcaceae family, *S. aureus* is a Gram-positive coccus arranged in grape-like clusters. It is non-motile, non-sporulating, and catalase-positive. *S. aureus* is a major pathogen in both community-acquired and hospital-acquired infections (**Pibiri, 2005**).

Gram-negative Bacteria: *Escherichia coli* (ATCC 25922)

This bacterium, belonging to the Enterobacteriaceae family, is a Gram-negative, peritrichously flagellated bacillus commonly found in the intestinal flora. In food safety contexts, *E. coli* is used as an indicator organism for fecal contamination during processing. While generally commensal, it can become pathogenic via virulence factors (**Cuq, 2007**).

2. Methods

2.1. Drying and Preservation of Plant Material

The drying process removes moisture from the plant to prevent enzymatic degradation and bacterial growth, thereby preserving the chemical integrity (**Wichtel et Anton, 1999**).

Approximately 10 kg of leaves were cleaned and dried in a well-ventilated, shaded area for three days.



Figure 09: Drying of *Eucalyptus* leaves (Original)

2.2. Extraction of *Eucalyptus globulus* essential oil

The hydrodistillation method were used in the lab of BIO-VERMA which is an institution for extracting essential and vegetable oils and natural cosmetics located in Collo-Chraiaa west of Skikda, The hydrodistillation method was used, as it is one of the simplest and oldest extraction techniques. Ten kilograms of plant material were directly immersed in a still containing 60 liters of water, which was then placed on a heating device. The mixture was brought to a boil under atmospheric pressure. Heat was applied to rupture the plant cells and release the odorant molecules contained within.

The resulting mixture of water and essential oil was subsequently cooled and condensed in an essencier. After condensation, due to differences in density, the components separated into two distinct phases: an aqueous phase and an organic phase containing the essential oil. This protocol is illustrated in the following figure.



Figure 10: The hydrodistillation technique (Original)

2.2.1. Yield calculation

EO yield was determined on the basis of plant material. It is the ratio between the mass of oil extracted and that of the plant used. The yield is expressed as a percentage and is calculated using the following formula:

$$Y = \frac{\text{the mass of the essential oil (g)}}{\text{the mass of the plant material used (g)}} \times 100$$

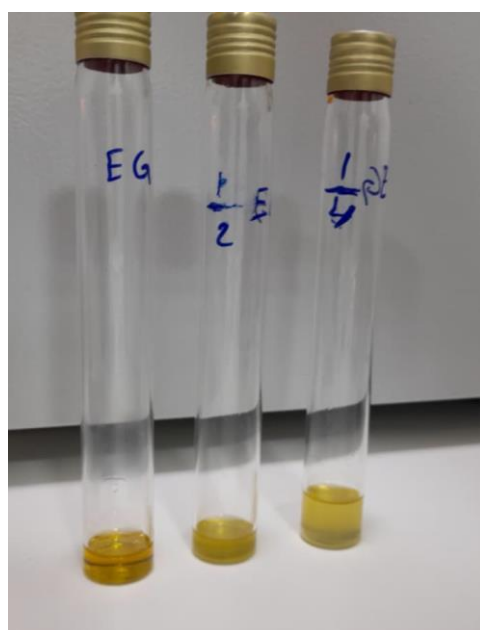
2.3. Preparation of dilutions

Two dilutions were prepared using three test tubes:

- The first tube contained pure essential oil (EO).
- The second tube contained a 1/2 mixture of dimethyl sulfoxide (DMSO) and EO.
- The third tube contained a 1/4 dilution, in which one part of the DMSO-EO mixture was added to three parts of DMSO, as detailed in the table and illustrated in the figure below.

Table 02: Dilution of essential oil in each tube

Test tube	Essential oil (μl)	DMSO (μl)
Pure	1000	000
Test tube $\frac{1}{2}$	1000	1000
Test tube $\frac{1}{4}$	1000 from $\frac{1}{2}$ tube	1000

**Figure 11:** Test tubes containing dilutions (Original)

2.4. Preparation of MULLER-HINTON culture medium

According to **Mayachiew et Devahastin (2008)**, Muller-Hinton Agar (MH) is the culture medium of choice for investigating antibacterial activity, as it is the most widely used medium for antibiotic susceptibility testing.

To prepare the medium, the following steps were carried out: 500 grams of Muller-Hinton agar were mixed with 500 milliliters of distilled water in a beaker. The mixture was boiled and stirred until fully dissolved. It was then poured into glass vials and autoclaved for 20 minutes at 121°C.

After sterilization, the medium was ready to be poured into Petri dishes for use in antibacterial testing.



Figure 12: Bottle of MULLER-HINTON agar (Original)

2.5. Preparation of petri dishes

After the culture medium was melted in a water bath at 96°C, it was poured into 12 Petri dishes. The dishes were then placed in an oven and left to dry for nearly 24 hours to ensure that the medium was properly solidified and free of air bubbles, allowing for optimal use in subsequent experiments.



Figure 13: Petri dishes after preparation (Original)

3. Antimicrobial activity tests

To assess the inhibition of bacterial proliferation upon contact with the test sample, a qualitative analysis of the antibacterial activity of essential oils extracted from *Eucalyptus globulus* leaves is carried out. An aromagram is used in conjunction with the agar diffusion method to accomplish this. The diameters (in mm) of the transparent, bacterial-free zones that develop around the discs are used to measure the antibacterial activity.

The diameters of the zones of microbial growth inhibition (D) are divided into four groups, per (Ponce *et al.*, 2003).

Table 03: Reference of inhibition diameters of impregnated discs

Diameters(D) Of Inhibition Zone(mm)	Transcription	Germ Sensitivity
Less than 8 mm	-	Resistant
Between 9 mm and 14 mm	+	Sensitive
Between 15 mm and 19 mm	++	Very sensitive
More than 20 mm	+++	Extremely sensitive

3.1. Inoculation

About 10 ml of distilled water is introduced into the swab, then the surface of the bacterial strains is simply rubbed to remove them. The swab is then mixed to homogenize the bacterial suspension, and culture dishes are inoculated with MH. This operation is divided into four experimentations:

- The first for the *S. aureus* clinic strain and its two repeats.

- The 2nd for the *S. aureus* reference strain and its repeats.
- The 3rd for the *Escherichia coli* reference strain and its repeats.
- The last for the clinical *E. coli* strain and its repeats.

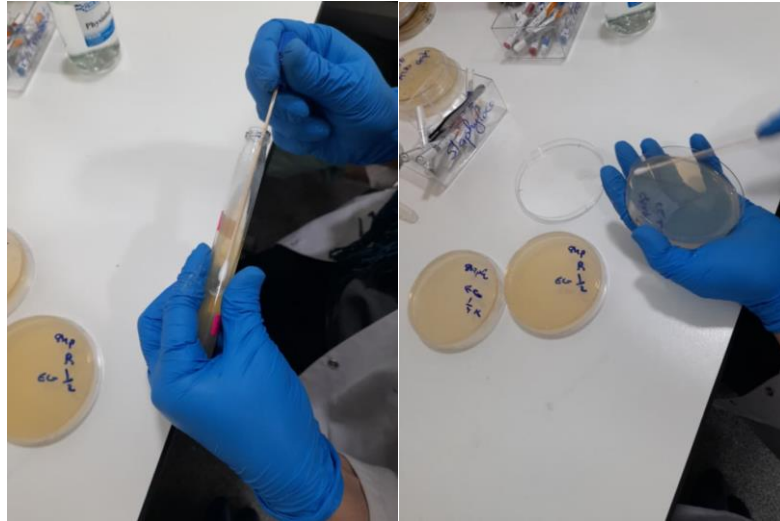


Figure 14: Inoculation of bacterial strains (Original)

3.2. Preparation of Aromatogram wells

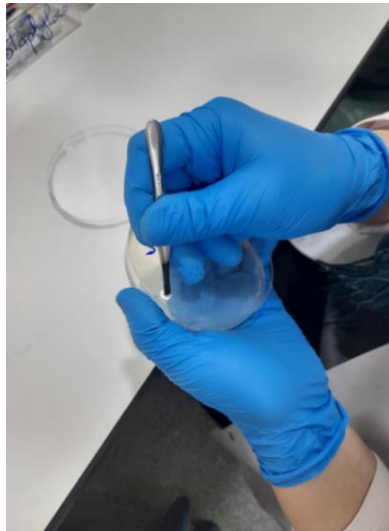
Wells were prepared in the agar of 12 petri dishes containing bacterial strains using a sterile cork borer (lombouts). Each dish contained:

- One well filled with DMSO, serving as the negative control,
- Three wells filled with the essential oil (EO).
- For *Escherichia coli*, two antibiotic discs Gentamicin and Amikacin were placed using sterile forceps,
- For *Staphylococcus aureus*, two antibiotic discs Vancomycin and Penicillin were also applied using sterile forceps.

The dishes were then incubated at 37°C for 24 hours. Details are illustrated in the table and figure provided below.

Table 04: Present the experimentation protocol of aromatogram.

Bacterial strains	essential oil	Antibiotics	DMSO
Clinical strain <i>E. coli</i>	3wells each 40 μ l	One disk AK and CN in each petri dish	One well in each petri dish
Reference strain <i>E. coli</i>	3wells each 40 μ l		
Clinical strain <i>S. aureus</i>	3wells each 40 μ l	One disk P and VA in each petri dish	
Reference strain <i>S. aureus</i>	3wells each 40 μ l		

**Figure 15:** Fill wells with EO(Original)**Figure 16:** Placement of an antibiotic disc(Original)**Figure 17:** Incubation in oven (Original)

3.3. Reading the results

Following incubation, the precise measurements will be taken of the zones of inhibition using a caliper or ruler (Figure 18).

A comparison will be made between the measured inhibition diameters and the critical diameters to determine the classification of bacteria as: Resistant, Susceptible or Extremely Susceptible according to the table 04.



Figure 18: The method of reading the results (Original)

1. Characteristics of *Eucalyptus Globulus* essential oil

The organoleptic characteristics of the extracted essential oil are compared with the **AFNOR (2000)** standards, as presented in the following table:

Table 05: Organoleptic characteristics of *Eucalyptus globulus* essential oil

characteristics	Aspect	Color	Odor
Norme AFNOR (2000)	Clear liquid and mobile	Fluid Very pale yellow to transparent	Fresh and spicy
Our oil	Clear liquid	Yellow to pale yellow	Fresh and spicy

2. Extraction Yield

The yield (in %) from *Eucalyptus Globulus* leaves is presented in the following table.

Table 06: Yield value of the extracted *Eucalyptus globulus* essential oil

Yield (%)	Quantity of oil extracted (g)	Quantity of <i>EG</i> leaves (g)
0.1	9.1	10000

$$Y(\%) = \frac{9.1}{10000} \times 100 = 0.091 \approx 0.1$$

Looking at the table above, the yield of essential oil from extracted *Eucalyptus Globulus* leaves (10000g) is equal to $\approx 0.1\%$.

2.1 Literature-Based Yield Comparison

In Algeria, yields of *E. globulus* essential oil vary from one region to another. According to studies published in various regions of Algeria, yields of *Eucalyptus globulus* EO vary from 0.13% to 2.83%, Wilaya as the following table shows:

Table 07: Yields of essential oil from *Eucalyptus globulus* in various Wilayas of Algeria

The wilaya	The yield (%)	Reference
Ghardaïa	0.94	(Nasri et Lebouabi, 2024)
Ouargla	0.315	(Nasri et Lebouabi, 2024)
El-Menia	0.43	(Nasri et Lebouabi, 2024)
Boumerdes	1.90	(Oulebsir-Mohandkaci et al., 2015)
Tebessa	1.40	(Ghenaiet et Aouidet, 2016)
Hajout	2.83	(Faudil Chérif et al., 1993)
Média	0.2	(Benabdallah et Drici, 2018)
Bainem	0.69	(Faudil Chérif et al, 1993)
Azzefoune	1.20	(Faudil Chérif et al., 1993)
Constantine	0.13	(Abed et Morsli, 2021)
Ain-defla	0.33	(Benabdallah et Drici, 2018)

It is to be pointed out that **Nasri and Lebouabi's (2024)** values, which had calculated the yield of *Eucalyptus globulus* essential oil on 100 g of dry matter, provided a yield of 0.315% in the area of Ouargla. If we compare their values with ours, we observe that the yield in Ouargla (0.315%) is quite close to our value (0.1%). But in Ghardaïa and El-Menia wilayas, their yields were higher than ours.

The same pattern is indicated in the study by **Oulebsir-Mohandkaci et al. (2015)**, who had a yield of 1.90% in the Boumerdes region. Likewise, **Ghenaiet and Aouidet (2016)** had a yield of 1.40% in the Tebessa region. In the study by **Faudil-Chérif et al. (1993)** on yields in *Eucalyptus* leaves in three areas in Algiers, the yields were 2.83% in Hajout, 1.20% in Azzefoune, and 0.69% in Bainem. Apart from that, **Benabdallah and Drici (2018)** reported 0.33% in Ain Defla and 0.20% in Médéa. Moreover, **(Abed et Morsli, 2021)** reported the yield in Constantine as 0.13%.

A comparison between the yields obtained in this study and those reported in the literature indicates that the values for Ouargla (0.315%), Médéa (0.2%), Ain Defla (0.33%), and Constantine (0.13%) are very similar to the yield obtained in this study. In contrast, higher yields were reported in Ghardaïa (0.94%), El-Menia (0.43%), and Bainem (0.69%). Even

greater yields were observed in Boumerdes (1.90%), Tebessa (1.40%), Azzefoune (1.20%), and Hajout (2.83%).

In short, extraction efficiency has been discovered to be affected by a variety of inherent factors such as the plant's genetic composition and external factors such as geographical origin. The method of extraction (e.g., hot or cold) also affects the yield of secondary metabolites in the overall content and hence the resultant biological activities concerned with such compounds (**Lee et al., 2003**). This explains the varying essential oil yields obtained from various regions.

Furthermore, other studies have determined that the variations in the yield of essential oils are not merely a function of origin but also influenced by several biotic and abiotic parameters. These include temperature, relative humidity, sun exposure time, wind regime, and use of organic or mineral fertilizers (**Mehalaine et Chenchouni 2021**).

3. Antibacterial activity

The aromatogram aims to investigate the sensitivity of bacterial strains to *Eucalyptus globulus* essential oil and its dilutions. Accordingly, the antibiogram was performed for two antibiotics against each bacterial strain. The zones of inhibition are presented in the following tables and figures.

3.1. Antimicrobial Activity Against Clinical and Reference Strains of *Staphylococcus aureus*:

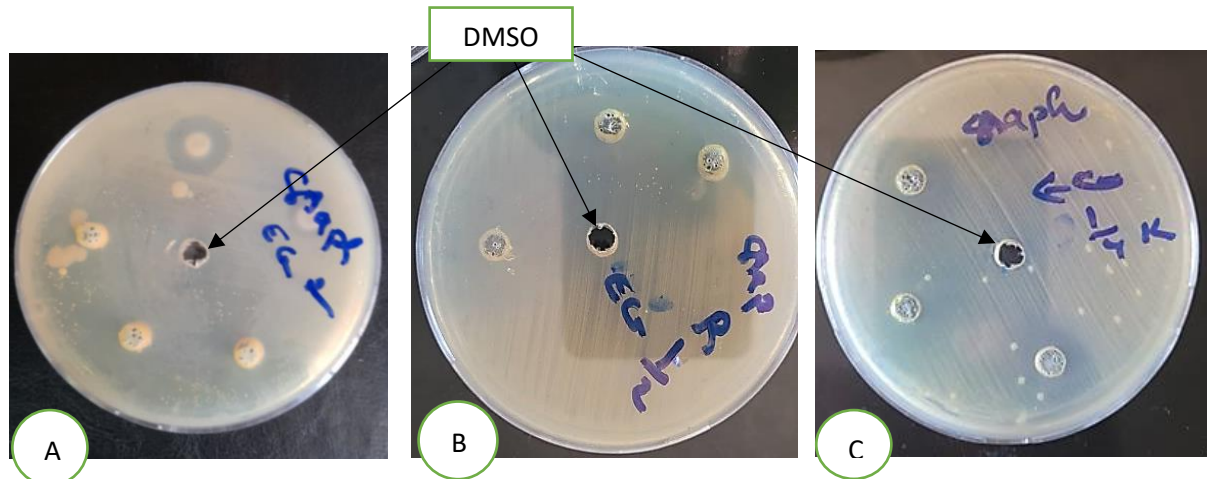


Figure 19: Photograph showing the action of EO from *E. globulus* on bacterial strains of *S. aureus* ATCC 25923 (Original).

A: the pure EO + antibiotics (P, VA), **B:** The $\frac{1}{2}$ EO dilution, **C:** The $\frac{1}{4}$ EO,

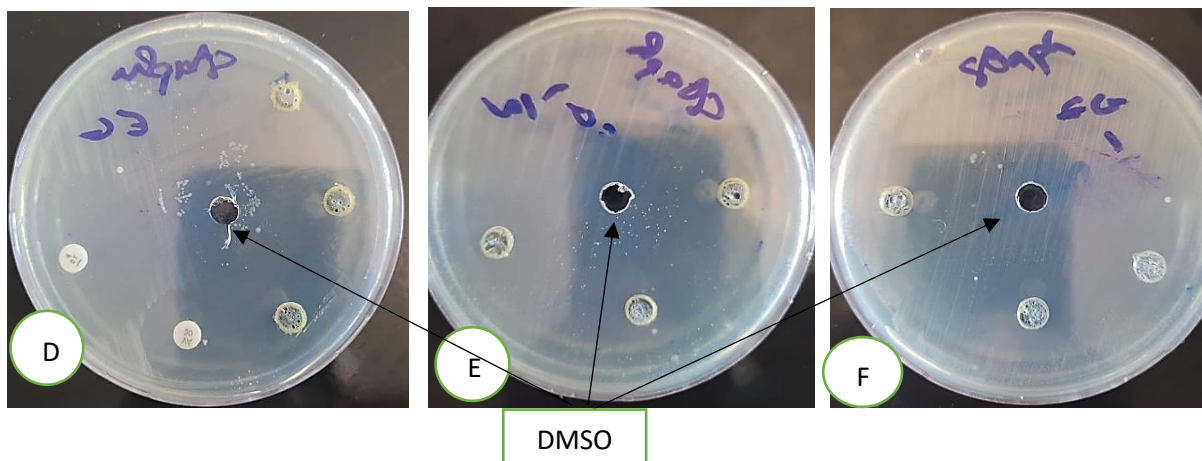


Figure 19: Photograph showing the action of EO from *E. globulus* on bacterial strains of clinical *S. aureus* (Original).

D: The pure EO + antibiotics (P, VA), **E:** The $\frac{1}{2}$ dilution, **F:** The $\frac{1}{4}$ dilution

Table 08: Aromatogram and antibiogram of *Eucalyptus globulus* essential oil against *Staphylococcus aureus*

Bacterial strains	Dilutions	Average inhibition zone (mm±standard deviation)	Average zone of P (mm±standard deviation)	Average zone of VA (mm±standard deviation)	DMSO
<i>S. aureus</i> Clinical	EG Pure	39.66 ± 0.57	33.5± 3.53	18.5 ± 3.53	00
	EG ½	33.66 ± 3.21			00
	EG ¼	23.33 ± 0.57			00
<i>S. aureus</i> Reference	EG Pure	23.66 ± 0.57	11.5 ± 0.70	15.5 ± 0.70	00
	EG ½	20 ± 0.60			00
	EG ¼	17.33 ± 1.52			00

These results indicate that *Eucalyptus globulus* essential oil exhibits antimicrobial activity against both clinical and reference strains of *Staphylococcus aureus*.

When compared to its dilutions, the pure essential oil consistently produces a larger zone of inhibition. For instance, the average inhibition zone in the reference strain with pure essential oil measures 23.66 mm, which is greater than that observed with the half dilution (20 mm) and the quarter dilution (17.33 mm).

For the clinical strain, pure essential oil invariably generates a wider zone of inhibition. Hence, the average zone of inhibition with pure essential oil is 39.66 mm, which exceeds that measured with half dilution (33.66 mm) and quarter dilution (23.33 mm).

A significant zone of inhibition is observed around the penicillin 33.5 mm disc in the clinical *Staphylococcus aureus* strain, while a smaller zone of 11.5 mm is recorded in the reference strain.

In the case of vancomycin, the inhibition zone measures 18.5 mm in the clinical strain and 15.5 mm in the reference strain.

The most remarkable inhibition diameter recorded for the clinical strain of *S. aureus* is 39.66 mm, whereas the reference strain exhibits a maximum inhibition diameter of 23.66 mm.

Table 09: Transcript of inhibition diameters of *Eucalyptus* plant EO against *staphylococcus aureus*

Germes	Pure EO	½ EO	¼ EO
<i>Staphylococcus aureus</i> clinical	+++	+++	+++
<i>Staphylococcus aureus</i> ATCC	+++	+++	++

The transcription of inhibition diameters of *Eucalyptus* plants can be seen from the table above, in the case of reference *S. aureus* strain it shows that in the matter of pure essential oil and ½ EO, the strain is extremely sensitive. However, in the case of 1/4 EO the reference strain is very sensible.

For the clinical *S. aureus* strain, the zone of inhibition transcription table shows that at all dilutions the strain is highly sensitive to *Eucalyptus globulus* essential oil.

3.2. Analysis and Interpretation of Data:

The Gram-positive clinical bacterium *Staphylococcus aureus* appears to be highly sensitive to *Eucalyptus globulus* essential oil, with an average inhibition zone of 23.66 mm. This result is consistent with the findings of **Messabhia and Bouacha (2024)**, who reported that the pure essential oil produced the largest inhibition zone, measuring 26 mm. For the half dilution, a zone of 19.33 mm was recorded for the same strain. These findings closely align with the current data.

Aidet and Filali (2015) find that pure essential oil exhibits an inhibition zone of 22 mm, which is slightly lower compared to our result of 23.66 mm. In their study, the inhibition zone for the $\frac{1}{2}$ dilution is 19 mm, and for the $\frac{1}{4}$ dilution, it is 16 mm. By comparison, our corresponding values are 20 mm and 17.33 mm, respectively. These results are nearly identical, confirming the reproducibility of antimicrobial activity across similar studies.

Additionally in a study conducted by **Demars (2021)** on the antibacterial activity of *Eucalyptus globulus* essential oil and its dilutions against *S. aureus* and *E. coli*, the reported inhibition zones for *S. aureus* are 25 mm (100%), 13 mm (50%), and 11 mm (25%). When compared to our results—23.66 mm (100%), 20 mm (50%), and 17.33 mm (25%)—we find a consistent trend in inhibition relative to oil concentration. The ranking of inhibitory effects from pure to more diluted forms corresponds well with the pattern described by Demars.

According to **Damjanović-Vratnica (2011)**, the highest inhibition zones are observed for *S. aureus* strains, with 33 mm for clinical isolates and approximately 34 mm for the ATCC reference strain. In contrast, our results show a larger inhibition zone for the clinical strain (39.66 mm) than for the ATCC 25923 reference strain (23.66 mm), which is the opposite trend. This significant difference likely stems from environmental adaptations of clinical isolates.

Exposure to frequent and high doses of antibiotics may lead to either heightened resistance or, in some cases, increased susceptibility due to evolutionary trade-offs, while reference strains remain less affected by external factors.

The antibiogram performed on the *Staphylococcus aureus* strain revealed an inhibition zone of 11.5 mm against penicillin. In comparison, **Messabhia and Bouacha (2024)** reported a larger zone of 18 mm for the same antibiotic, indicating a relatively lower sensitivity in the present strain.

In **Bachheti's (2015)** study, a 28 mm inhibition zone is observed for *S. aureus*, which is slightly higher than our result of 23.66 mm with pure essential oil. Likewise, **Mekonnen et al. (2016)** report a zone of 32 mm, a value that diverges more significantly from our data, likely due to differences in the bacterial strain's resistance profile or in the chemical composition of the essential oil tested.

The biological activity of essential oils is closely related to their chemical composition, where functional groups of the main constituents often act synergistically (**Rather et al., 2012**).

Variability in antimicrobial activity may also result from structural differences between bacterial strains, including membrane composition and resistance mechanisms.

As described by **Poole (2001)**, Gram-negative bacteria possess a double membrane, with an outer membrane that serves as an effective barrier against hydrophobic molecules such as essential oils. In contrast, Gram-positive bacteria like *Staphylococcus aureus* lack this outer membrane, making them more permeable to essential oil components and, consequently, more susceptible to their antimicrobial action.

3.3 Results of the both clinical and reference strains of *E. coli* :

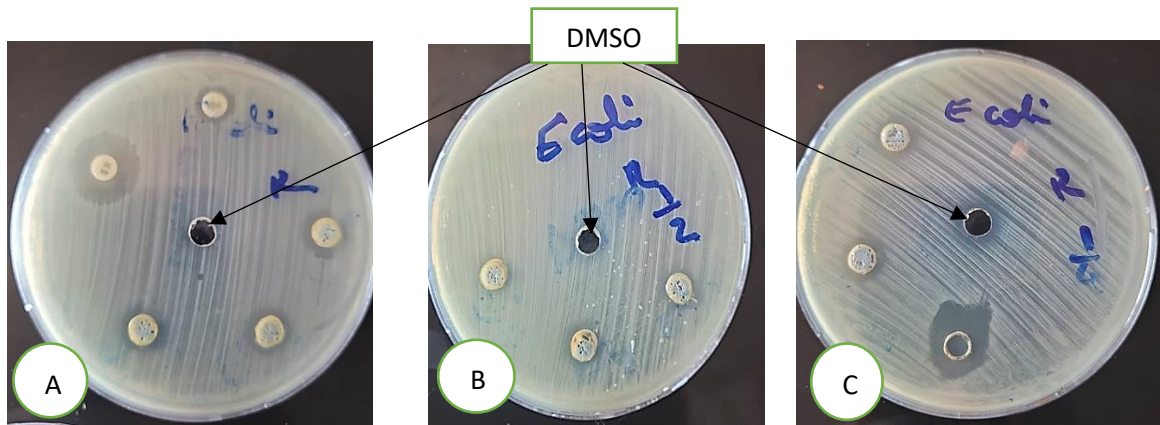


Figure 20: Photograph showing the action of EO from *E. globulus* on bacterial strains of reference *E. coli* ATCC 25922 (Original)

A: the pure EO + antibiotics (AK, CN), **B:** The $\frac{1}{2}$ EO dilution, **C:** The $\frac{1}{4}$ dilution

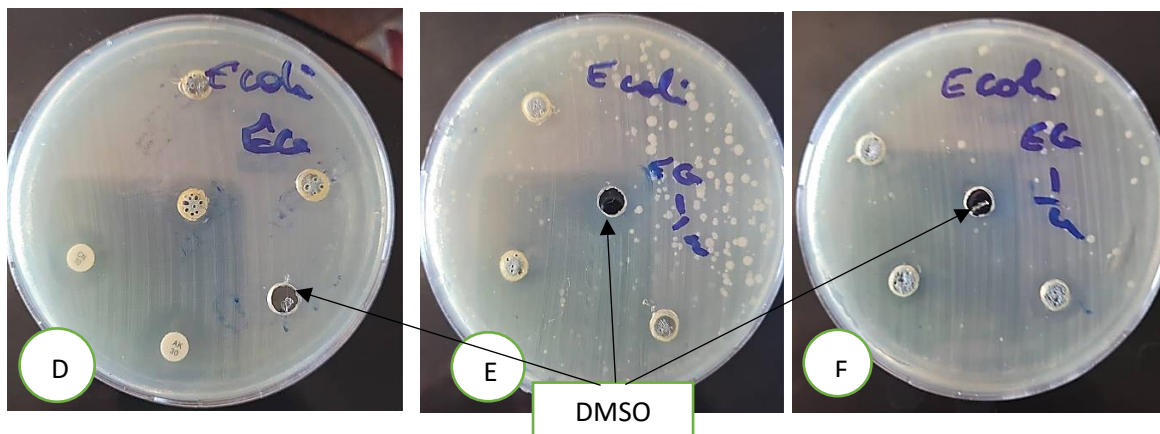


Figure 20: Photograph showing the action of EO from *E. globulus* on bacterial strains of clinic *E. coli*. (Original).

D: The pure EO+ antibiotics (AK, CN), **E:** The $\frac{1}{2}$ dilution, **F:** The $\frac{1}{4}$ dilution.

Table 10: Presentation of aromatogram and antibiogram of *eucalyptus globulus* essential oil against *Escherichia Coli*

Bacterial strains	Dilutions	Average inhibition zone (mm±standard deviation)	Average zone of CN (mm±standard deviation)	Average zone of AK (mm±standard deviation)	DMSO
<i>E.coli</i> Clinical	<i>EG</i> Pure	14 ± 1	10.5 ± 0.70	18.5 ± 0.70	00
	<i>EG</i> ½	13.66 ± 1,15			00
	<i>EG</i> ¼	12.33 ± 0,57			00
<i>E.coli</i> Reference	<i>EG</i> Pure	11.66 ± 0,57	11.5 ± 0,70	16.5 ± 0.70	00
	<i>EG</i> ½	12 ± 2			00
	<i>EG</i> ¼	11.66 ± 0,57			00

Table 11: Transcript of inhibition diameters of *Eucalyptus* plant EO against *Escherichia Coli*

Germes	Pure EO	EO 1/2	EO1/4
<i>Escherichia Coli</i> clinical	+	+	+
<i>Escherichia Coli</i> ATCC	+	+	+

It is clear that the transcription of the inhibition diameters of *eucalyptus* essential oil, for both the clinical and reference strains of *E. coli*, reveals that in all dilutions, both strains show sensitivity to this essential oil.

Eucalyptus globulus essential oil has an antibacterial effect on both clinical and reference types of *Eschirechia coli*. Aromatogram results show that clinical *E. coli* shows a large zone of inhibition of 14 mm for pure essential oil, followed by 13.66 mm for 1/2 EO dilution and 12.33 mm for 1/4 EO dilution. As for reference *E. coli*, the largest zone of inhibition observed is 12 mm for the 1/2 dilution. Noting the impressive zone of inhibition for pure essential oil and 1/4 dilution, we note that they have the same diameter (11.66).

3.4. Interpretation and Discussion of Results of *Escherichia coli* strain

In the study by **Djendli and Bouali (2022)**, *Escherichia coli* shows sensitivity to gentamycin, a result that is consistent with our findings. In fact, both Gram-negative and Gram-positive bacteria demonstrate susceptibility to gentamycin, as noted by **Marquet (2004)**. This broad-spectrum activity may be attributed to the shared mode of action of gentamycin on bacterial cells, particularly among Gram-negative strains.

The results of **Damjanović-Vratnica in 2011** shows that the same inhibition zone values were observed against the medically important pathogens, *Escherichia Coli* and the *E. coli* ATCC the zone is about 32, our results are with the comparison of his results the zone of inhibition is for the reference *E. coli* (12) than the *E. coli* clinical (14), This marked variation in the zone of inhibition in *E. coli* strains, whether reference or pathogenic, is indicative of the nature of these bacteria. Indeed, the divergence observed in this zone is the result of differences inherent to the

environment in which these bacteria live, particularly in the clinical setting. The latter have been exposed to higher doses of antibiotics, which have increased their resistance compared with reference strains that have not been influenced by external factors.

In the study of **Damjanović-Vratnica in 2011** the results of the antimicrobial assay with the Amikacin antibiotic the inhibition zone is 16.5 for ATCC *E. Coli*, and 18.5 for pathogenic strain, in our results the inhibition zone of clinical *E. Coli* and ATCC 25922 *E. Coli* are 14 and 12 respectfully.

Demars (2021) conducted a study on the antibacterial activity of *Eucalyptus globulus* essential oil and its dilutions against *Staphylococcus aureus* and *Escherichia coli*. The inhibition zones reported for *E. coli* were 24 mm (100%), 15 mm (50%), and 9 mm (25%). In comparison, the current findings show inhibition zones of 11.66 mm for the pure essential oil, 12 mm for the 50% dilution, and 11.66 mm for the 25% dilution. These results indicate a significantly lower sensitivity of *E. coli* to the tested oil samples.

In **2022, Ben Bekkou et al.** examine the antibacterial effect of pure *Eucalyptus globulus* essential oil on the *Escherichia coli* ATCC strain and report an inhibition zone of 18.33 mm. Our result is slightly lower, which may be attributed to several influencing factors.

Most of the antimicrobial activity of essential oils has been attributed to the presence of oxygenated monoterpenes, which constitute a series of volatile organic compounds with strong biological activity (**Knobloch et al., 2011; Aggarwal et al., 2002**). Oxygenated monoterpenes are lipophilic and thus able to interact and disrupt microbial cell membranes, leading to increased permeability, leakage of cell material, and eventually cell death.

In the case of the *Eucalyptus globulus* essential oil, its antimicrobial potential is largely due to major constituents such as 1,8-cineole, linalool, and pinocarveol. Of these, the most prevalent compound is 1,8-cineole or eucalyptol, and it has been extensively studied for its bactericidal activity as well as its bacteriostatic activity. It disrupts membrane integrity and inhibits enzymatic action within the bacterial cell, making it extremely effective against a broad range of Gram-positive and Gram-negative bacteria. Similarly, linalool, a terpene alcohol found in other medicinal plants, also possesses strong antimicrobial activities due to the disruption of structural components of the bacterial envelope as well as interfering with metabolic processes (**Tzakou et al., 2001; Mourey et**

Canillac, 2002; Viljoen et al., 2003).

Pinocarveol, although less common, is also synergistic to the overall activity of the oil in enhancing the activity of the principal components. The synergism between these monoterpenes is speculated to result in a multifaceted mode of antibacterial action including interference with cell wall biogenesis, inhibition of quorum sensing, and production of reactive oxygen species (ROS) resulting in oxidative stress (**Moldoch et al., 2025**).

Therefore, the antimicrobial activity of *Eucalyptus globulus* essential oil is not attributed to a single compound but synergistic action of a mixture of bioactive constituents that can interact in such a way that they create a synergistic total inhibitory effect. The variability in chemical composition among various samples of the oil depending on geographical area, season of cultivation, and method of processing can contribute to its antibacterial activity to a significant extent.



CONCLUSION AND PERSPECTIVES

VI. Conclusion and perspectives

This study reveals the strong antimicrobial activity of *Eucalyptus globulus* essential oil, which has variable but efficient inhibitory activity against reference and clinical strains of *Staphylococcus aureus* and *Escherichia coli* at different concentrations. Particularly, *S. aureus* ATCC 25923 shows its strong sensitivity toward pure essential oil with the largest inhibition zone and *E. coli* minimal inhibition at half concentration. These findings corroborate previous studies confirming the bactericidal activity of *Eucalyptus globulus* essential oil against different types of bacterial strains.

The well-documented efficacy of this natural substance against major pathogenic bacteria justifies its assessment as an effective alternative or adjuvant to conventional antibiotics, especially in the context of growing antibiotic resistance. The results support the idea of reconsidering phytotherapy, which leads to the use of *Eucalyptus globulus* essential oil in treating bacterial infections caused by *S. aureus* and *E. coli* strains.

In general, resistance profiling of *Escherichia coli* indicates that this bacterium exhibits low sensitivity to essential oils and their dilutions, which can be attributed to its high intrinsic resistance potential. This reduced susceptibility is common among Gram-negative bacteria and is primarily due to the structural complexity of their cell envelope.

While our study presents valuable information regarding the antimicrobial activity of this essential oil, it is not exhaustive. We therefore encourage researchers to expand on this work by testing its activities against a wider range of bacterial pathogens and under varying experimental conditions. Overall, this work points to the possibility of plant-based antimicrobials as effective, environmental-friendly agents in the management of bacterial diseases.

Perspectives

Many perspectives can be considered:

- ✚ It could be testing the anti-inflammatory, antifungal effects or antioxidant of *Eucalyptus globulus* essential oil.
- ✚ Evaluate the antibacterial activity with other strains or with other extracts from the same plant.
- ✚ In light of the results of this study, it would be interesting to produce medications or syrups with this essential oil rich in active compounds.

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