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en « Biotechnologies et valorisation des plantes. »

THÈME

Ethnobotanical inquest and study of the therapeutic benefits of "*Eucalyptus globulus*", from the region of Raml Souk (El Tarf)

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Dedication

To those who sacrificed their lives for me, to those who gave me their love, to those who encouraged and supported me to realize my dream and always go forward, My dearest parents Dr.Rachid and Yassmina, may God protect them for me.

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To the whole HALIM family of which I am proud to be a member.

ملخص

في الوقت الحاضر، على الرغم من تطور الكيمياء الاصطناعية، احتفظ استخدام النباتات الطبية بمكانة كبيرة بسبب فعاليتها في مختلف الإجراءات العلاجية. يهدف هذا العمل إلى تحديد الاستخدامات التقليدية لنبات الأوكالبتوس جلوبولوس في منطقة «رمل السوق» - الطارف -. الخصائص الفيزيائية الكيميائية والبيولوجية المختلفة التي تميز الأنواع *Eucalyptus globulus* وتقييم التأثيرات المضادة للأكسدة والميكروبات. من ديسمبر إلى يناير 2024/2023، تم إجراء مسح عرقي على هذا المصنع بين 129 شخصًا في منطقة «رمل السوق» - الطرف من خلال مقابلات مباشرة باستخدام استبيان. تظهر هذه الدراسة الاستقصائية أن النساء أكثر اهتمامًا بالطب التقليدي من الرجال. وغالبية المعنيين ينتمون إلى الفئة العمرية 30-40 بمعدل 56 في المائة، وهم في أغلب الأحيان متعلمون. يستخدم السكان المحليون تفكيك الأجزاء المختلفة من النبات بما في ذلك الأوراق لعلاج عدد من الأمراض وخاصة أمراض الجهاز التنفسي على وجه التحديد الإنفلونزا.

يتراوح العائد الذي سجله هذا النوع بين 1.48٪ بالنسبة لغلوبولوس الأوكالبتوس الأقدم وبقيمة 3.26٪ بالنسبة لأنواع الأوكالبتوس جلوبولوس الصغيرة. تتوافق الخصائص الفيزيائية الكيميائية بشكل عام مع معايير AFNOR، مما يجعل زيت هذا النوع جيدًا.

تم تقدير النشاط المضاد للأكسدة باستخدام طرق DPPH. أظهرت النتائج تثبيطًا مهمًا للغاية للجذور الحرة ذات القيم المهمة القريبة من تلك الخاصة بالمعايير.

تم تقييم النشاط المضاد للميكروبات لهذا الزيت العطري على سلالات بكتيرية إيجابية الغرام: المكورات العنقودية الذهبية، المكورات العنقودية epidermidis (الأبيض)، الليستريا، وسلبية الغرام: الإشريكية القولونية، الزائفة الزنجارية، Efaumella، Salmonella تظهر النتائج أن الزيت أظهر نشاطًا مضادًا للبكتيريا ضد البكتيريا إيجابية الجرام بدرجات متفاوتة، بينما في البكتيريا سالبة الجرام هناك تأثير واضح صغير.

الكلمات المفتاحية.: الاوكالبتوس - الزيوت العطرية - النشاط المضاد للأكسدة - النشاطية ضد بكتيرية - ناحية طارف .

Résume

Nos jours, malgré le développement de la chimie de synthèse, l'utilisation des plantes médicinales a conservé une large place du fait de leur efficacité dans diverses procédures thérapeutiques. Ce travail a pour objectif de déterminer les usages traditionnels de la plante *d'Eucalyptus globulus* au niveau de la région de "Ramel El Souk " - El Tarf -. les différentes propriétés physicochimiques et biologiques qui caractérisent l'espèce *Eucalyptus globulus* et l'évaluation des effets antioxydants et antimicrobiens. De décembre au janvier 2023/2024, une enquête ethnobotanique a été réalisée sur cette plante auprès de 129 personnes dans la région de "Ramel ElSouk " - El Tarf par des interviews directes à l'aide d'un questionnaire. Ce sondage montre que les femmes sont plus préoccupées par la médecine traditionnelle que les hommes. Majoritairement les intéressés sont appartiens à la tranche d'âge 30-40 ans avec un taux de 56%,qui sont le plus souvent des scolarises. La population locale utilise decoction des différents parties de a plante notamment les feuilles pour traiter un certain nombre de pathologie particulièrement les maladies respiratoires spécifiquement l'influenza .

Le rendement enregistré par cette espèces varie entre 1.48% pour *l'Eucalyptus globulus* plus âgée et avec une valeur de 3.26% pour l'espèce d'*Eucalyptus globulus* jeune . Les propriétés physicochimiques sont en générale en accord avec les normes AFNOR, ce qui rend l'huile de cette espèce de bonne qualité .

L'activité antioxydante a été estimée par l'utilisation des méthodes DPPH. Les résultats ont montré une très importante inhibition des radicaux libres avec des valeurs significatives procEO à celles des standards.

L'activité antimicrobienne de cette huile essentielle a été évaluée sur des soucEO bactériennes pathogènes à Gram positif : Staphylococcus aureus, Staphylococcus epidermidis (blanc) , Listeria , et à Gram négatif : Escherichia coli, pseudomonas aeruginosa, E.faecalis, Salmonella, Proteus *mirabilis*, et Klebsiella pneumoniae. Les résultats mettent en évidence que l'huile à manifesté une activité antibactérienne contre les bactéries à Gram positif à des degrés variables, tandis que dans les bactéries à Gram négatif y'a un faible effet manifeste.

Mots clés : Eucalyptus globules - Huile essentielle - composition phytochimique

Jeune feuille - feuille âgée - Activité antioxydant - Activité antibactérienne

Abstract

Nowadays, despite the development of synthetic chemistry, the use of medicinal plants has retained a large place because of their effectiveness in various therapeutic procedures. This work aims to determine the traditional uses of the *Eucalyptus globulus* plant in the region of "Ramel ElSouk" - El Tarf -. the different physicochemical and biological properties that characterize the species *Eucalyptus globulus* and the evaluation of antioxidant and antimicrobial effects. From December to January 2023/2024, an ethnobotanical survey was carried out on this plant among 129 people in the region of "Ramel ElSouk " - El Tarf by direct interviews using a questionnaire. This survey shows that women are more concerned about traditional medicine than men. The majority of those concerned belong to the age group 30-40 with a rate of 56%, who are most often schoolgirls. The local population uses decoction of the different parts of a plant including leaves to treat a number of pathology especially respiratory diseases specifically influenza .

The yield recorded by this species varies between 1.48% for the older *Eucalyptus globulus* and with a value of 3.26% for the young *Eucalyptus globulus* species . The physicochemical properties are generally in accordance with AFNOR standards, which makes the oil of this species of good quality.

Antioxidant activity was estimated using DPPH methods. The results showed a very important inhibition of free radicals with significant values close to those of the standards.

The antimicrobial activity of this essential oil was evaluated on Gram-positive bacterial strains: Staphylococcus aureus, Staphylococcus epidermidis (white), Listeria , and Gram-negative: Escherichia coli, pseudomonas aeruginosa, E.faecalis, Salmonella, Proteus mirabilis, and Klebsiella pneumoniae. The results show that the oil showed an antibacterial activity against Gram-positive bacteria to varying degrees, while in Gram-negative bacteria there is a small obvious effect.

Key words: Eucalyptus globules - Essential oil - phytochemical composition

Young leaf - old leaf - Antioxidant activity - Antibacterial activity .

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Abbreviation list

AFNOR: French standardization association

CIM: minimal inhibitory concentration

d20: density at temperature 20.

DMASO: dimethylsyfoxide

DPPH: 2,2diphyenyl-pricylhydrazyl

EG: *Eucalyptus globulus*

E1: sample 1

E2: sample 2

E3: sample 3

E4: sample 4

E5: sample 5

FCC: food chemical codex

EO: essential oil

EO A: Essential oil of aged leaves

EO J: Essential oil of young leaves

EOs: essential oils

IA: acid index

IC50: 50% inhibition of the dpph radical

IE: ester index

Introduction

Since the beginning of time, humans have continued to draw inspiration from nature to provide for tEOe survival needs including its use of fruits and vegetables to feed, wood to warm up, and plants to heal. It is for this purpose that herbal medicine was born and continues to develop, or what is called phytotherapy, which refers to medicine based on plant extracts and natural active principles.
Today, the medicinal and aromatic plants sector mainly concerns markets such as: perfumery, cosmetics, agri-food and aromatherapy, branch of herbal medicine that takes advantage of the medical properties of aroma, essential oils or essences. It is home to natural medicines. (1)
In Algeria, herbal medicine is an integral part of the local culture. Its geographical location and climatic diversity have allowed the development of a very rich and diversified flora: Mediterranean, Saharan and a palaeotropical flora. Estimated to be more than 3000 subspecies of vascular plants belonging to several botanical families. (4)
Many Myrtaceae have been introduced in Algeria as ornamental trees or reforestation. This is particularly the case for Eucalyptus (2). The genus Eucalyptus includes at least 600 species scattered around the world. (3)
The genus Eucalyptus is vast, from the shrub to the very large tree about 55m for the globules (are among the largest trees in the world it is the case of the Eucalyptus régnas reaches 100 meters! It reaches records at 130 m in its original area).

Essential oils are attracting increasing interest because of their uses in the treatment of certain infectious diseases for which synthetic antibiotics are becoming less and less active or in the preservation of food against oxidation as alternatives to synthetic chemicals. (5)

As part of the valorization of the Algerian flora, we were interested in the species of the family Myrtaceae represented by the «Myrtus communus» and *«Eucalyptus globuls».*

This work is divided into three chapters:

•The first chapter is a bibliographic synthesis on medicinal plants, essential oils.

•The second part includes an ethnobotanical survey and an experimental part where we will present the methods and techniques used for the extraction and comparative study of essential oils of young and adult leaves of *Eucalyptus globulus*,

• Finally, the third part consists of the interpretation and discussion of the results, and we conclude with a general conclusion that will provide an overall synthesis of the various results obtained..

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Part 1:

Bibliographic

I. Medicinal plants

1 General information on medicinal plants

1.1 Spontaneous plants

They are difficult or impossible to grow. According to some importing firms, they still account for 60 to 70% of drugs on the European market. as for the medicinal value of spontaneous plants, it is very uneven and then varies according to origin, terrain and growing conditions. (6)

1.2 Cultivated plants

Cultivate is a plant production produced from the exploitation of the land. The term cultivated plant also refers to a cultivated plant, that is to say a cultivated plant species, for example wheat or potatoes. Cultivated plants have undergone a process of domestication, which has led them to develop characteristics different from their wild ancestors. Or A cultivated plant population is a collection of plants of a single species and variety (or population) grown to harvest a specific product desired by humans. (7)

1.3 Harvest period

Scientific studies have determined the optimal time of harvest. For example, harvesting is preferred:

- The roots at the time of vegetative rest (autumn, winter).

- Aerial parts, most often at the time of flowering.

- Leaves, just before flowering.

- Flowers in full bloom see in bud (hawthorn).

- Seeds when they have lost most of their natural moisture. (8)

1.4 Method of obtaining and harvesting

The conditions of cultivation, harvesting, drying and storage have a determining effect on the quality of plant drugs. Therefore, it is better to pick the plants in an infrequent place because the plants intended to be dried should in no case be washed. Therefore, they must be free of impurities such as earth, dust, dirt, as well as animal infection or contamination. They show no signs of rot or damage. (9)

1.5 Drying

Sun drying is the simplest and most economical method, used especially for roots, stems, seeds and fruits. Drying in the shade is indicated for leaves and flowers, because the sun-dried green leaves turn yellow, the flower petals lose their bright colors, which can alter the medicinal properties of tEOe products, Aromatic plants should not remain in the sun for too long to lose their scent. (10)

The maximum allowable temperature for proper desiccation of aromatic plants or plants containing essential oils is 30°C; in other cases, the desiccation temperature may vary from 15°C to 70°C. (11)

1.6 Conservation

During prolonged storage, storage methods and conditions must prevent any change in the nature of plants (vermin, mould, microorganisms) in order to preserve the integrity of their active properties. The quality of aromatic or medicinal plants depends on it. It is an important step in guaranteeing the properties of the plants studied or used to ensure a good conservation that is to promote the inhibition of any enzymatic activity after the avoid the degradation of certain components as well as bacterial proliferation. Indeed brightness can accelerate many chemical processes leading to a degradation or modification of the components present the temperature is another important parameter and it is accepted that a temperature rise of 10c doubles the rate of degradation. Therefore, it is best to store plants in a place with a constant temperature and relative humidity. (12)

2 Phytotherapy

2.1 Definition

Phytotherapy can be defined as an allopathic discipline intended to prevent and treat certain functional disorders using plants, plant parts or herbal preparations. It can be distinguished into three types of practices:

• A traditional practice, sometimes very old based on the use of plants according to the virtues empirically discovered.

• A practice based on advances and scientific evidence that seeks active plant extracts.

• A practice of prophylaxis, already used in antiquity. A balanced diet with certain active ingredients being prophylactic herbal medicine. (13).

2.2 Instructions for use of medicinal plants

To ensure the action of the drug, it is necessary to treat the plant, to transform it to extract the substance having a specific action. (14)

But the main types of preparation medicinal plants:

2.2.1 Decoction

For a decoction the mixture of herbs and solvent is placed in a container, this container is heated example .: using a steam jacket placement in a glycerin bath or similar (7)with reflux uncondensed connection, the contents of the container are brought to a boil and held in place for 30 minutes. (15)

2.2.2 Maceration

Maceration should be done in a container away from air and light, leaving a plant in a solvent (water, wine, alcohol or oil) cold for a fairly long time (from a few hours to several days, or even several weeks). After the time has elapsed, simply filter the mixture through a paper filter, and store the maceration obtained in a well-clogged. Cold maceration occurs when the active ingredients of a plant can be destroyed by heat. (16)

2.2.3 Infusion

Infusion is the most common and classic method of preparing herbal teas, it generally applies to the delicate organs of the plant: flowers, aromatic leaves and tops (12). The infusion is obtained by pouring boiling water over the plants in a covered container, to avoid any loss of volatile essence for a period of 5 to 15 minutes (depending on the plant), then filtration. (13)

2.2.4 Extraction of juices

This process requires plants to be absolutely fresh and rich in humidity. They contain the mineral salts, vitamins that the plant has developed, as well as the other substances obtained by pressure. By this method, not all the active ingredients are obtained, but the structure of heat-sensitive compounds will not be modified. For domestic use, one can extract the successive pressure sucs One can extract the successive pressure sucs

made with an appropriate apparatus, such a small press, or through a modern centrifuge that allows the recovery of all the juices contained in the plant. (14)

2.3 The benefits of phytotherapy

- Phytotherapy covers a very wide range of diseases and the pharmaceutical industry uses many plant active ingredients to treat all kinds of diseases. For example, taxol (a molecule used to treat cancer) extracted from the bark of If (19)
- Medicinal plants are much cheaper than drugs desyntEOis.
- Herbal medicine can be used as a means of prevention.
- The production of plants is very low pollution unlike chemical drugs. (20)

2.4 Disadvantages of phytotherapy

Herbal medicine is often a non-toxic therapy but requires a number of precautions, such as a good knowledge of plants because some may be toxic or manifest allergic reactions to certain subjects.

Make sure of the diagnosis and be attentive to the doses, especially for young children, pregnant or breastfeeding women and the elderly. Some plants may not be used at the same time as other medicines, or may have some toxicity if the dosage is increased or the treatment time is prolonged. (21)

II. Essential oils

1 Definition

The essential oil is a plant extract from so-called plants: aromatic which contain in their leaves, fruits, seeds, barks, or roots, a large number of aromatic molecules, which constitute the essential principle or principles of plants. (22)

Volatile oils can be considered residues of plant metabolism. Following photosyntEOis in chloroplasts, the energy produced (in the form of carbohydrates, NADPH and ATP) contributes to the development of the plant and indirectly to the biosyntEOis of multiple secondary compounds among them essential oils. (23)

2 Repair and rental of essential oils

Essential oils are widely distributed throughout the plant kingdom in a limited number of families that are particularly rich, for example in Myrtaceae, Lauraceae, Asteraceae, Apiaceae, Rutaceae and Lamiaceae. essential oils can be stored in all vegetal organs: flowers (bergamot), leaves (eucalyptus, laurel), roots (vetiver), bark (cinnamon), fruits (anise, citrus, star anise), seeds (nutmeg), rhizomes (ginger) and wood (rosewood).(24)

The amounts of essential oils produced by the plants are minimal, resulting in extremely low extraction yields, usually less than 2%.

The specific anatomical structures specialized in the secretion of essential oils are very diverse: schizogenic secretory pockets (Myrtaceae) or schizolyzigenic secretory pockets (Aurantiaceae), they can also be secretory channels (Conifers and Apiaceae), secretory hairs (Lamiaceae and Asteraceae) and isolated secretory cells (Lauraceae, Magnoliaceae and Piperaceae) (25)

In the same plant, they may be present in different organs. The composition of EO may vary from one organ to another. (26)

3 Physical and chemical properties of essential oils

All EOs are volatile, odorous and flammable. Their density is most often less than 1. Only three medicinal EOs have a density greater than that of water, tEOe are the EOs of cinnamon, clove and sassafras. EOs are generally found colorless or pale yellow in liquid state at room temperature. Essential oils are soluble in alcohols, in fixed oils and in most organic solvents. (24-26-27)

Their boiling point is always above 100°C and depends on their molecular weights, this point varies from 160°C to 240°C. They have a high refractive index and most deflect polarized light. (28-29)

They sometimes have a greasy or oily feel but they are not fatty substances. By evaporation, they can return to the vapor state without leaving traces, which is not the case for fixed oils (olive, sunflower, etc.) which are not volatile and leave a persistent greasy mark on the paper. (30)

4 The toxicity of essential oils

Essential oils are not products that can be used without risk. Like all natural products: "just because it's natural doesn't mean it's safe for the body".

Some essential oils are dangerous when applied to the skin because of their irritating power (oils rich in thymol or carvacrol), allergenic (oils rich in cinnamaldehyde) (31) , or phototoxic (citrus oils containing furocoumarins ,Other essential oils have neurotoxic effect. (32), Ketones such as a-thujone are particularly toxic to nervous tissues, there are also some essential oils whose certain compounds are able to induce the formation of cancers. (33) Researchers have demonstrated the carcinogenic hepato activity of tEOe compounds in rodents (34)

However, tEOe results are controversial because there are differences in humans in the process of metabolizing tEOe compounds. Carole, for example, is metabolized in humans to non-carcinogenic dihydroxysafrole and trihydroxysafrole. (35)

5 Chemical composition of essential oils

The chemical composition of species is complex and can vary depending on the organ, climatic factors, the nature of the soil, cultural practices and the method of extraction,

EOs are a mixture of constituents that belong to three categories of terpene, aromatic and miscellaneous compounds. (31)

5.1 Terpenes

Terpenes are hydrocarbons formed by the assembly of two or more isoprene units, they are polymers of isoprene with the crude formula (C5H8). Essential oils particularly contain monoterpenes, sesquiterpenes and rarely diterpenes (Finar, 1994). Terpenes have very diverse structures (acyclic, monocyclic, bicyclic, etc.) and contain most of the chemical functions of organic materials. (37)

5.1.1 Monoterpenes

Monoterpenes have two isoprene units (C5H8). Volatile, enterable with water vapor, often with a pleasant odor including the majority of the constituents of EOs, sometimes 90%. They can be acyclic (myrcene, ocymene), monocyclic (terpene) or bicyclic (pinene, sabinene). TEOe terpenes include a certain number of natural products with special chemical functions: alcohol (geraniol, menthol), aldehydes (geranial, citronellal), ketones (carvone, menthone) and esters (geranyl acetate, linalyl acetate). (22)

5.1.2 Sesquiterpenes

This is the most diverse class of terpenes. They contain more than 3000 molecules such as: B-caryophylene, B-bisabolene, α-humilene, α-bisabolol, farnesol.(24)

5.2 Aromatic compounds

Aromatic compounds derive from phenylpropane (C6-C3). They are less common than terpenes. This class includes odorous compounds such as vanillin, eugenol, anethole, estragole. They are frequently encountered in Apiaceae EOs (cumin, fennel, parsley, etc.) and are characteristic of those of vanilla, tarragon, basil, clove. (38)

5.3 Compounds of various origins

TEOe are products resulting from the transformation of non-volatile molecules entrainable by water vapor. TEOe are compounds resulting from the degradation of fatty acids, terpenes, essential oils can contain various aliphatic compounds, generally of low molecular mass, entrainable during hydro distillation carbide, acid (C3 to C10), alcohols , aldehydes (octanal, decanal, etc.), esters, lactones, nitrogen or sulfur products. (39)

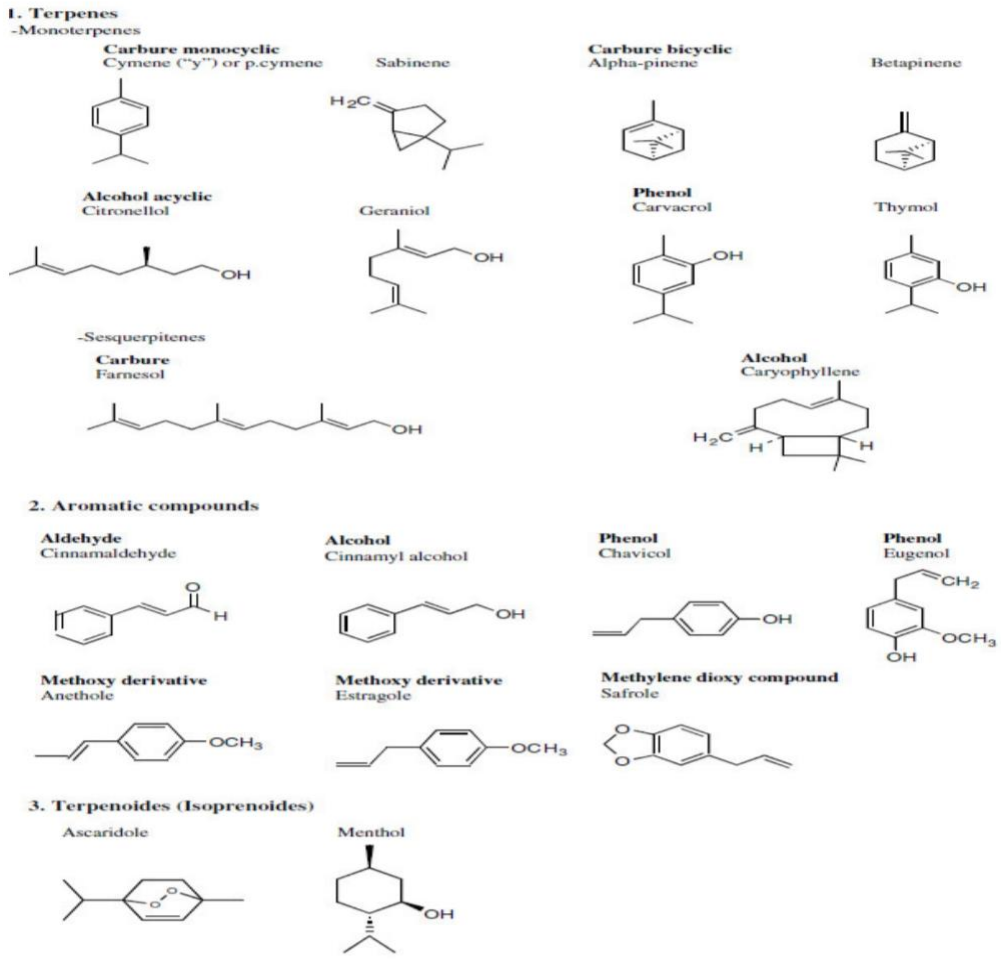

Figure 1: Classes of essential oil constituents **(Anonym 1).**

6 Obtaining essential oils

EOs and their components are generally extracted from a natural material or produced by chemical syntEOis after characterization and elucidation of their structure. There are three different strategies for obtaining EOs: extraction from a natural material, chemical syntEOis and bioconversion.

6.1 Extraction from natural material

6.1.1 The enfleurage

This method of extraction is used in the treatment of fragile parts of plants such as flowers, which are sensitive to the action of temperature. The petals and flowers of the plants are spread on fats to extract their aromas. Once saturated the fat is treated with alcohol. (40)

6.1.2 Pressing

EO is extracted under simple pressure from plant material. This is for example the case of lemon EO which is extracted by cold pressing from the peel of a fresh lemon.

6.1.3 Hydrodistillation

This is the simplest method and therefore the oldest used, it consists of distilling the plant material in water. The water vapor causes the volatile constituents to be condensed in a refrigerant. The distillate obtained will be decanted and then processed. Residual water may contain a small proportion of certain volatile compounds and may be used as floral water.

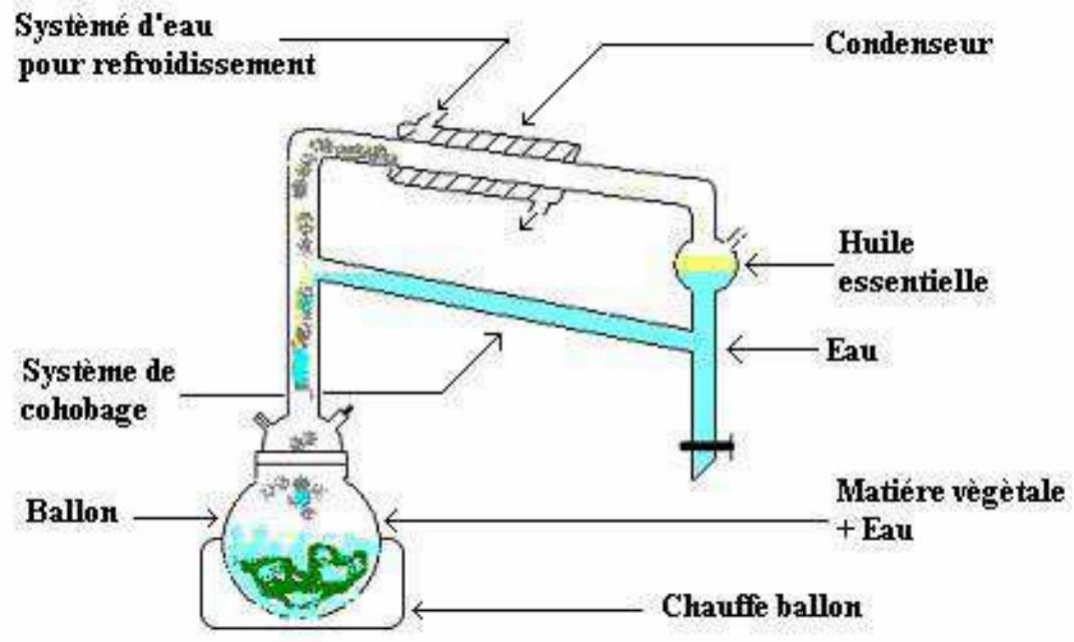

Figure 2: Equipment used for hydrodistillation of oil **(Anonym 2).**

6.1.4 Dry steam training

This process was developed to avoid certain degradation or hydrolysis reactions of the components of the EO. The vegetable mass rests on a grid towards which dry steam is pulsed. The cells distend and the odorous molecules are released and will be vaporized and condensed in a refrigerant.

6.1.5 Supercritical carbon dioxide (CO2) extraction

Devices on an analytical, pilot or industrial scale allow extraction using supercritical CO2. This has the advantage of being a non-flammable, non-explosive, non-toxic,

non-corrosive, odorless and inert fluid. Operating at a critical low CO2 temperature (31.1°C) makes it possible to extract and save the fragile components of EOs (lilac, lily of the valley, etc.). This process is widely used in the pharmaceutical industry allowing the extraction of active compounds.(41)

6.2 Chemical syntEOis

The composition of EO is extremely complex. It is therefore impossible to recreate the natural balance by a simple syntEOis or mixture of constituents. However, synthetic EO components are widely used because the world's needs for flavors and fragrances are increasing and the cost of extraction is high. Nowadays, there are two types of synthetic flavors: synthetic flavors identical to natural flavors and artificial flavors that do not exist in nature.

Synthetic flavorings obtained purely chemically have a structure and properties identical to those of natural flavorings; they can also be syntEOized from a wide variety of molecules. (42)

6.3 Bioconversion or biosyntEOis

This procedure is an alternative to classic chemical syntEOis for economic and environmental reasons mainly in a context of sustainable development. It consists of transforming a raw material using biological systems (microorganisms, plants, enzymes). (43).

Bioconversion/biosyntEOis requires that the precursor comes from a plant and obtained by natural processes and that the biological systems used are GRAS.

7 Function of essential oils

7.1 Anti-infective Properties

7.1.1 Antibacterial properties

The aromatic molecules with the most antibacterial activity are the phenols contained, for example, in clove essential oil. oregano or thyme.

7.1.2 Antiviral properties

Viruses are quite sensitive to phenol and monoterpenol essential oils. More than a dozen essential oils have antiviral properties. We can mention the essential oil of Ravintsara, the essential oil of Hô Wood, or the essential oil of Ceylon Cinnamon**.**

7.1.3 Antifungal properties

The essential oils used for their antifungal properties are the same as those mentioned above however the duration of treatment will be longer. For example, essential oils of cinnamon, clove or niaouli are antifungals.

7.1.4 Pest control properties

Aromatic molecules with phenols have a powerful action against parasites. Thyme with linalool, savory from the mountains are excellent antiparasitic essential oils.

7.1.5 Antiseptic properties

Antiseptic and disinfectant properties are often found in essential oils with aldehydes or terpenes functions such as Eucalyptus radiata essential oil.

7.1.6 Insecticidal property

Some essential oils are insect repellents or insecticides such as those with aldehydic functions such as citronella contained in lemon eucalyptus or lemongrass.

7.1.7 Antioxydant property

Essential oils containing flavonoids have antioxidant properties. Antioxidants act against free radicals by neutralizing them when they are in excess in the body. They act as a sort of “bulletproof” by preventing free radicals from developing harmful reactions against the organism.

7.2 Anti-inflammatory properties

Essential oils with aldehydes have active properties against internal inflammation such as ginger essential oil.

7.2.1 Digestive properties

Essential oils of cumin (with the molecule of cuminal), star anise or for example tarragon have a digestive and aperitif action. They stimulate the secretion of digestive juices. Peppermint essential oil reduces nausea.

7.3 Regulators of the nervous system

7.3.1 Calming, anxiolytic

Aldehydes such as citrals contained for example in the essential oil of lemon balm or lemon verbena promote relaxation and sleep.

7.3.2 Analgesics, analgesics

The essential oils best known for their analgesic action are the essential oils of lemon eucalyptus, ginger, true lavender.(44)

Part 2:

Methods part

Objective In this modest work our objective is a comparison between the young leaves of Eucalyptus and the oldest (adults) by the determination of the yield and the phyto and physico-chimc composition and also by the comparative study of some bioactivities of our oils including antioxidant and antibacterial activity.

I. Ethnobotanical survey

1 Definition

The ethnobotany survey, was carried out in the region of El Tarf , aims to know the richness of medicinal plants and to raise the maximum information on the traditional uses of plants *Eucalyptus globulus* among residents. This survey is based on a questionnaire; distributed in 129 copies in the study area "Raml El Souk".

2 Presentation of the study area «Oriental Numidia»

Numidia is an ancient appellation of the northern region of Algeria and Tunisia (Figure 3). It is the country of the Berbers of North Africa. It was broadly divided into two major regions: Western Numidia and Eastern Numidia, the latter extending from Jijel region in Algeria to the eastern regions of Tunisia.

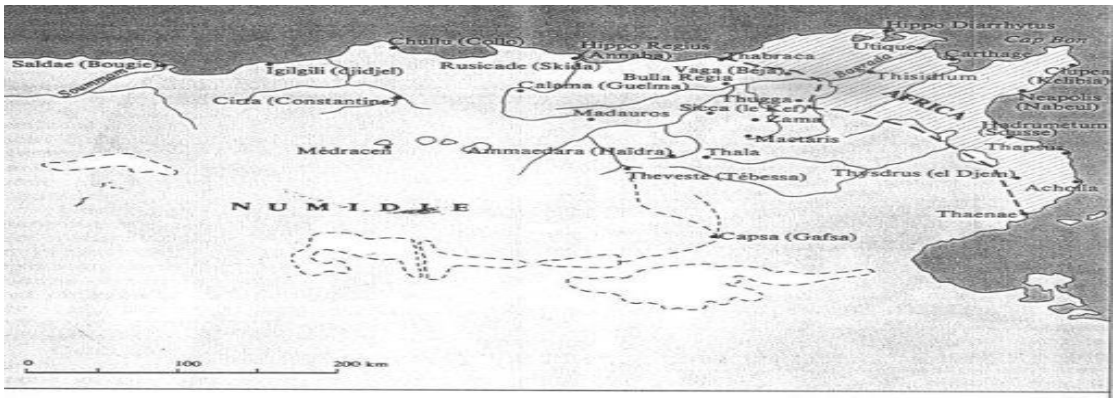

Figure 3: Ancient map of the nineteenth century delimiting Eastern Numidia and Western Numidia **(Anonym 3)**

2.1 Presentation of state of El Tarf

The region of El-Tarf (Far North-East Algeria) is a forest area encompassing a particular ecosystem mosaic. From its geographical location, the region benefits from a varied and characteristic floristic richness.

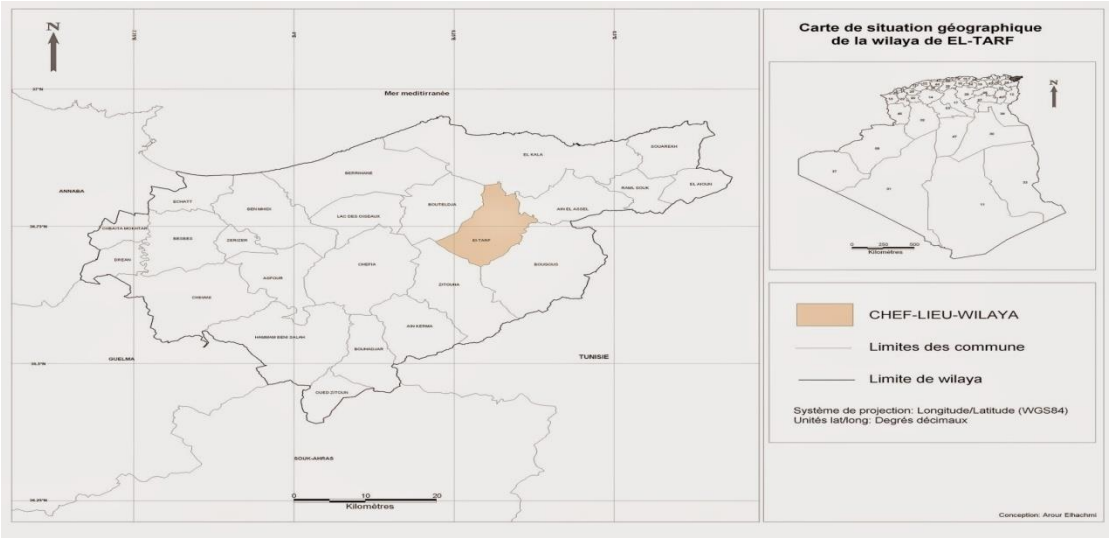

Figure 4: geographical map of the wilaya of El-Tarf **(Anonym 4).**

2.2 Population

The total population of the wilaya is estimated at 474,030 inhabitants, a density of 163 inhabitants per km². This population occupies the land in a heterogeneous way, describing a strong exploitation of agricultural areas northwest of the Wilaya and a significant implantation also in tourist areas and major roads northeast of the wilaya. Population growth rate: 1.38%.

3 Survey Methodology

3.1 Investigation Process: Direct Interview

The ethnobotanical survey is field work that allowed us to have direct contact with the population of the commune study, to allow the assessment of the knowledge of local populations and their relationship with the plant.

3.2 Survey Tool

The answers to the interviews were recorded using a form filled in by ourselves, while keeping the anonymity of the population questioned. We designed this questionnaire ourselves according to our research objectives (Figure 3).

Questions were directed as follows

Sociodemographic information

- Age
- Sex
- School level
- Living environment

Plant Information

- Parts used: stems, roots, leaves, grains, aerial part,
- Preparation mode ‘decoction, maceration, infusion, powder, c…)
- Mode of use (internal, external, etc.)
- Origin of plants
- Method of use
- Source of information
- Plant effectiveness

Enquête ethnobotanique et l'études thérapeutiques d'Eucalyptus Globulus
Dans le traitement traditionnel d'eucalyptus globulus à El taref

FICHE ENQUETE ETHNOBOTANIQUE

Profil de personne :

Age : A1 : (20-30) ☐ A2 : (30-40) ☐ A3 : (40-50) ☐ A4 : (50-60) ☐ A5 > 60 ans ☐

Sexe : Masculin ☐ Féminin ☐

Niveau scolaire : Non scolarisé ☐ scolarisé ☐ universitaire ☐

Milieu de vie : Rural ☐ Urbain ☐

La plante médicinale : d'eucalyptus globulus

plante	pathologie	Partie utilisées						Mode de préparation				Mode d'utilisation		Origine de plante	
		Entière	Feuilles	Fruit	Fleurs	Graines	Racines	décoction	infusion	macération	poudre	interne	externe	cultivé	sèche

Methode d'utilisation :

Origine de l'information : utilisée ☐ lecture ☐ expérience des autres ☐

Efficacité du plante : faible ☐ efficace ☐ meilleure que la médicaments ☐

Figure 5 : Example of the survey questionnaire used.

3.3 Data Processing

The data recorded on the survey sheets were processed and entered into the Excel software. Data analysis used simple methods of descriptive statistics.

I. Extraction of essential oil

1 Plant presentation studies: Ecalyptus globulus

Eucalyptus are mostly very large trees that are part of the Myrtaceae family. There are now more than 500 different species of Eucalyptus. (46)(47)

They originated in Australia but are also found in South America, Africa and Europe, where they learned to acclimatize, The term Eucalyptus was first used in 1777 by a French botanist, Charles-Louis L'héritier de Brutelle.(49)

A large number of Eucalyptus species were introduced in Algeria, notably by M Cordier from 1864 to 1876. (48)

The first planters were missionaries of European origin, they exploited it in order to clean up the marshlands where the anopheles and the mosquitoes which are vectors of an endemic parasitic disease: malaria. (50)

Eucalyptus are trees that grow very quickly. *Eucalyptus globulus* is 30 to 60 metres high and can reach up to 100 metres in some cases. (51)

Figure 6 : *Eucalyptus globulus* in region of Raml El Souk – el kala – El Tarf– **(Halim, 2024).**

1.2.1 Description *Eucalyptus globulus*

The *Eucalyptus globulus* tree, from the Myrtaceae family, is also called «blue gum», «koala tree» and «fever tree». Eucalyptus are large trees, some of which may exceed 100 m in height, (55)

- ✓ Leaves: *Eucalyptus globulus* leaves are an important component of the genus Eucalyptus(56), give off a pleasant and distinct fragrance (57), has endogenous secretory structures in the form of a cavity, they are close spherical schizogeny secretors that are numerous and active (58).
- ✓ flowers: Flowers are very varied. They have many stamens that can be white, cream, yellow, pink or red.(59) Visible in spring, are born in the axils of the leaves, the calyx in the shape of a bumpy top whose wide part is covered by an operculum that detacEO at the time of flowering revealing many stamens. The cream-colored flowers are solitary in the axils of the leaves and produce an abundant nectar that the bees transform into a honey with a pronounced flavor (60)
- ✓ Fruit: Fruit a woody capsule in the shape of 15 mm, (61) largely summit or rounded, black Term. (62)
- ✓ Pollen grains are Triangular flattened obtuse - convex in polar view. (63)
- ✓ The Stem smooth and perfectly straight, trimmed from the top to the bottom of branc EO (64).

Figure7 : *Eucalyptus globulus* flower and leaf and fruit **(Anonym 5).**

1.2.2 Systematic position of *Eucalyptus globulus*

The taxonomic classification of *Eucalyptus globulus* is as follows:

Reign: planted

Imprint: Spermaphyte

Under Mortgage: Angiosperm

Class: dicotylidones

Order: Myrtles.

Family: Myrtaceae.

Gender: Eucalyptus.

Species: *Eucalyptus globulus*. (54)

1.3 Origin and geographical distribution:

1.3.1 Species origin

The genus Eucalyptus is endemic to Australia and Tasmania. It is now grown in some subtropical regions of Africa, Asia (China, India, Indonesia) and South America as well as in southern Europe and the United States. (52)

1.3.2 Geographic Distribution:

Its introduction in Algeria was by the French in 1860. In this context no less than 130 species were planted on the national territory. During the 1960s to 1970s, Eucalyptus-based reforestation affected the East (El-Kala, Annaba, Skikda). (53)

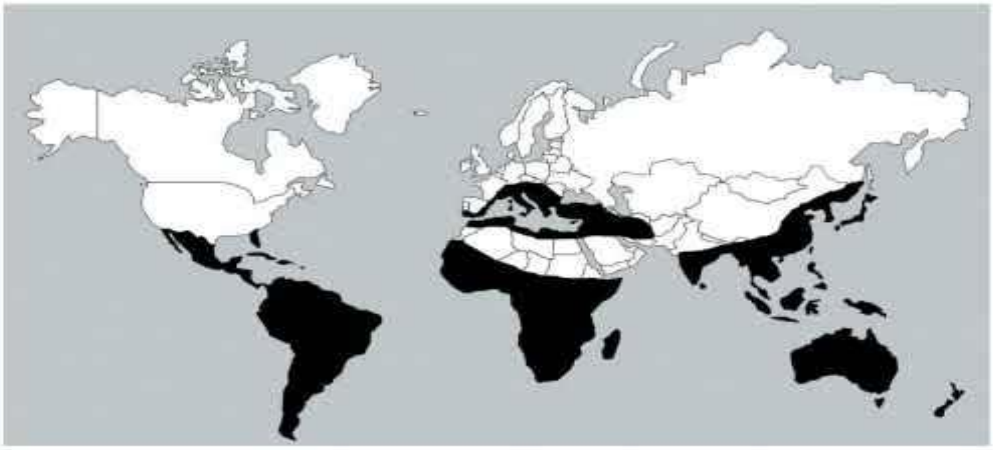

Figure 8 : Range of Myrtaceae worldwide **(Anonyme 6)**.

2 Study Area Description: Raml El Souk

1.1 Geographical location of the study area

It is a mountainous area, an important sector of the El-Kala National Park. Rich in aromatic and medicinal resources, sources of income for rural populations in the region. This area covers a total area of 6215 hectares, located on the north side of El Tarf.

1.2 Climatology

Climate is certainly a very important environmental factor. It has a direct influence on wildlife. A Mediterranean climate prevails in the region with abundant rainfall during the wet season and cold months and drought during the summer. (45)

The fragmentary data on the climatology of the region unfortunately do not provide a detailed picture of the climatic conditions prevailing there. If the mesoclimate remains known in its broad strokes, there remain many facts, such as the nature and distribution of vegetation for example, which can only be explained by the presence of a more localized climate of which we know no characteristics.(46)

1.3 Vegetation cover

The wilaya of El-Tarf is one of the ricEOt in terms of plant biodiversity and soil cover rate in Algeria. It is divided into two natural geographical areas: the mountainous areas located 500 m above the sea and the inland plains located 24m above the sea The wilaya of El-Tarf is a wilaya for agriculture (mixed farming, livestock) ; the agricultural area is 81,000 hectares.

1.4 Water resources

The wilaya is rich on hydrographic sources, Among most of the hydrographic network and the stream head of the wilaya of El-Tarf:

1.4.1 The lakes

The wilaya of El-Tarf has a wet complex formed by several lakes including

• Tonga Lake,

• Oubeira Lake,

• Bird Lake, Mellah Lake,

• Blue Lake. (Figure 5)

2.4.2 Waterways

The wilaya of El-Tarf is drained by a large water network consisting mainly of the following wadis:

• West: Oued Seybouse,

• East: Oued El Kebir,

• in the centre: oued Bounamoussa

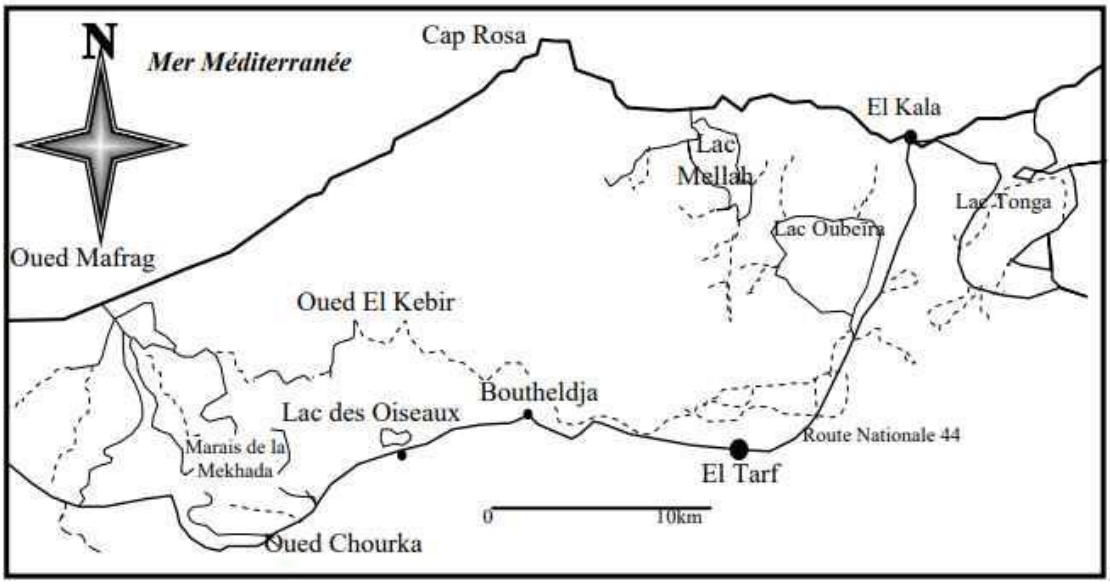

Figure 9 : The zone complex of Eastern Numidia **(Anonyme 7).**

2.4.3 The dams

The wilaya of El-Tarf has two important dams: Mexna dam on the Oued El Kebir and Cheffia or Bounamoussa dam.

2.5 Harvest

The harvest of our samples of the plant of Eaclyptus Globulus was carried out in November in the region of "Raml El Souk" El tarf .

2.6 Drying and conservatiom

There are several methods of drying or preserving plants. Some plants lend themselves to one or more drying methods that should be tested according to the means and varieties available.

The aerial parts must first be dried in the shade in a dry atmosphere for a few weeks.

Once dried, the leaves are ground with an electric mixer until a fine powder is obtained.

Figure 10 : Eucalyptus after drying **(Halim, 2024).**

Figure 11 : Storage of plant material **(Halim, 2024).**

3 Essential Oil Extraction

3.1 Principle

Hydrodistilation is the simplest and oldest method used. (66)

The principle is to bring to a boil in a flask a mixture of water and vegetable biomass from which the essential oil is extracted. Plant cells burst and release odorous molecules, which are then carried away by the water vapour created. They pass through a water refrigerant where they are condensed, then recovered in a container, and then separated by density difference. (24)

3.2 Procedure

The extraction of EO of Euclyptus globulus is carried out by hydro-distillation for 3 hours. The hydrodistillation was performed using a Clevenger type device.

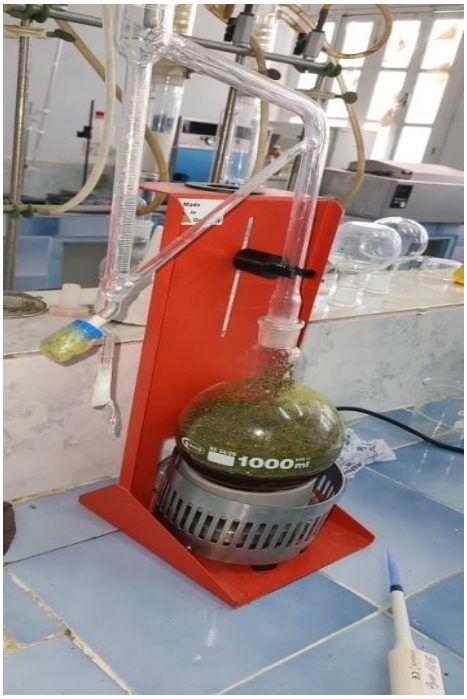

Figure 12 : Hydro distillation device.

A 1 L flask, a quantity of plant material (70 g) is put in direct contact with 700 ml of distilled water. The mixture is brought to a boil using a heating tank. The heterogeneous vapours are condensed on a cold surface and the essential oil separates by density difference The essential oil is separated from the aromatic water by decantation as shown in the following figure:

Figure 13 : Oil and water separation by density.

The resulting essential oil is kept in a well sealed opaque vial away from light and kept at 4°C until analyzed.

3.3 Determination of Essential Oil Yield

Essential oil yield (Re) is defined as the ratio of the mass of essential oil obtained from extraction (m HE) to the mass of plant material used (m0 M). It is given by the following formula:

$$Re(\%) = \frac{\text{m HE}}{m0\ M} \times 100$$

Re : Essential Oil Yield.

m HE: Essential Oil Mass.

m0 M: Mass of plant matter.

4 Essential Oil Testing

After the extraction of eucalyptus essential oils the following analyses were made:

4.1 Physical Analysis

4.1.1 Measurement of ph

Ph stands for Hydrogen potential, it measures the chemical activity of hydrogen ions (H+) (also commonly called protons) in solution. More commonly, pH measures the acidity or basicity of a solution. It is a coefficient of whether a solution is acidic, basic

or neutral: it is acidic if its pH is less than 7, neutral if it is equal to 7, basic if it is greater than 7. (67).

4.1.2 Density Measurement

The relative density at a temperature of 20°C of an oil or grease is the quotient of the mass in the atmosphere of a certain volume of that oil or at a given temperature t by the mass of the equal volume of water at the same temperature essential substance or oil is the ratio of the mass of a given volume of that substance to the mass of an equal volume of distilled water at a temperature of 20°C. (68).

The relative density is calculated using the following formula:

$$d20 = m2-m0 / m1- m0$$

m0: the mass of the empty pycnometer (in grams).

m1: the mass of the water-filled pycnometer (in grams).

m2: pycnometer mass filled with EO (in grams).

4.2 Chemical analysis

4.2.1 Acid index

The acid index of a fatty substance is the amount of potash in mg necessary to neutralize its free acidity. The acid index is used to verify the quality of an HE, particularly with respect to its degradation over time during storage. (69)

Protocol

-Introduce 0.2 g of the *Eucalyptus globulus* essential oil sample into the clean and dry Erlenmeyer.

-Add 5 ml of ethanol with the pipette and put 3 drops of phenolphthalein used as a colored indicator.

-Using the burette, neutralize the solution obtained with a KOH solution with a concentration (molarity) of 0.1 mol/l.

-The titration is stopped when the color turns.

-Note the volume KOH consumed (V KOH).

for acid index calculation using the following relation:

$$IA = \frac{56.1 \times V \times N}{m}$$

V = volume of KOH.

56.1 = mass of KOH.

m=mass of oil.

N = molarity of KOH.

4.2.2 Saponification index

This is the amount in milligrams of KOH (potash) needed to transform free fatty acids and glycerides into soap contained in one gram of fat. (70)

Protocol

One gram of oil to be analyzed are introduced into a round-bottomed balloon then 12, 5ml of KOH solution (0.5M) are added with pumice stone fragments. The mixture is brought to a boil in a balloon heater topped with a reflux refrigerant for one hour. Then a few drops of the colored indicator (phenolphthalein) are added to the mixture to titrate the solution with hydrochloric acid HCl at 0.5 N until the disappearance of the pink color and reappearance of the initial color of the mixture (transparent). Note the HCl volume drop.

Saponification index is given by the following formula:

$$I = \frac{V - v}{m} \times 56.11$$

V: The 0.5 N HCl volume required to titrate white.

v: The volume of 0.5 N HCl required to titrate the sample.

m: Test intake in grams.

4.2.3 Ester index

Recall that the ester index of a fat is the amount of potash expressed in milligrams, necessary to saponify the combined fatty acids present in 1g of fat.

$$IE = IS - IA$$

IE: The ester index.

IS: The saponification index.

IA: The acid index.

4.2.4 Refractive Index

The refractive index of a matter is a magnitude that characterizes the power of that matter to slow down and deflect light. The refractive index of a material is measured by a refractometer.

• Refractometer is an optical instrument used to determine the refractive index of a substance, that is, the extent to which light is deflected through the substance.

Protocol

- Open the secondary prism and place 2 or 3 drops of HE on the central part of the main prism;
- Gently close the secondary prism. The sample spreads between the main prism and the secondary prism into a thin film;
- Wait until the temperature is stable to make the measurement. The measurement value for a liquid sample being changed according to the temperature change;
- Read the temperature indicator to know the actual measurement degree, and attach it without fail to the measured value.

5 Photochemical Screening

Photochemical screening does not provide information on the structure of a well-defined molecule. It only highlights the presence of a particular chemical family that may contain in a sample. (71)

5.1 Alkaloids

Protocol

- 1g of the dried and crushed plant powder are mixed with 10ml of HCl at 5c/o in a container. After half an hour of maceration. The mixture is filtered by adding or filtering a few drops of Mayer reagent, the appearance of a yellowish white precipitate indicates the presence of alkaloids. (73)

5.2 Tannins

Protoco

- 10g of dry powder, are placed in 100ml of MeOH at 80c/ o. After 15 minutes of agitation the extract is filtered and put in tubes. The addition of drops of a solution of FeCl3 at 1c/o makes it possible to detect the presence of tannins. The colour blue or green indicates the presence of tannins. (74).

5.3 Saponinsides

Protocol

- 1g of the dry powder is weighed in a vial in which 10ml of distilled water is added and boiled for 5mn, the mixture is filtered, 2.5ml of the filtrate are added to 10ml of distilled water in a test tube. The tube is shaken vigorously for 30s and then left to rest for half an hour. A cellular foam reveals the presence of saponins. (74).

5.4 Quinones

Protocol

- 1g of crushed powder is placed in a tube with 15 to 30ml of petroleum ether, After stirring and a rest of 24h, the extract is filtered and then concentrated with rotasteam, the presence of quinones is confirmed by the addition of some drops of NaOH1/10 when the aqueous phase turns to red or purple yellow. (72).

6 Antioxidant activity

6.1 Trapping the free radical DPPH

This method is described by several authors among others, (Iwashima et al., 2005; Rigane et al., 2011; Medjeldi et al., 2018). It is an advantageous technique because it is independent, simple and fast.

DPPH • (2,2 diphenyl-1 picrylhydrazyl) is a stable radical that absorbs in the visible between 515 and 520 nm. The test involves reacting the purple DPPH with so-called "antioxidant" molecules to measure their ability to reduce it to yellow DPPH-H (Figure 14). This color change reflects the power of the plant extract to trap this radical. The phenomenon can therefore be followed by visible spectrophotometry.

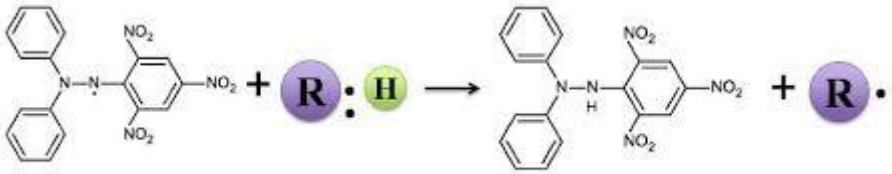

Figure 14 : DPPH reaction with a proton-donating molecule.

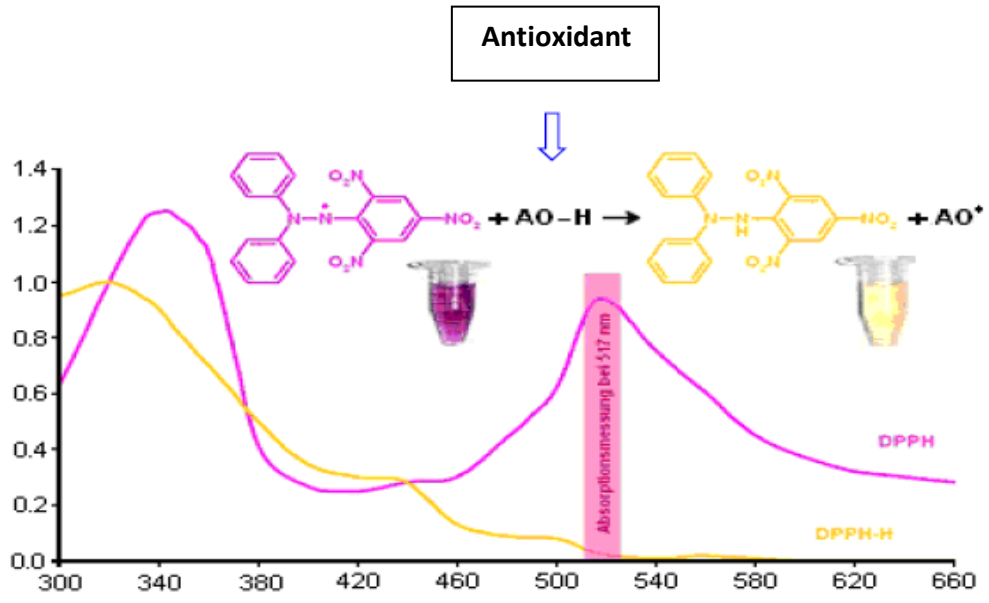

Figure 15 : The structure of the DPPH radical and its reduction by an antioxidant (AO-H).

Protocol

The estimation of the antiradical activity is determined according to the method described by El-Haci et al., (2011) with some modifications. At 975 µl of a methanolic solution of DPPH at (4%) are added 25 µl of the extract at different concentrations. After vigorous stirring, the mixture is kept in the dark for 1 hour. Absorbance is measured at 517 nm referring to a control without extract. Methanol is used as white. Expression of antiradical activity.

It is expressed as a percentage of inhibition which is calculated according to the following formula:

PI% = [(DO witness – DO extract) / DO witness] x 100

PI%: percentage of inhibition

Control OD: Absorbance of control solution (DPPH).

DO extract: Absorbance of antioxidant solution (extract).

The linear regression of the curve: PI% = f [C extracted] allows to determine the IC50 which corresponds to the extract concentration responsible for the inhibition of 50% of radical DPPH.

• IC50 is expressed in units of extract concentration. (75)

7 Antibacterial activity

7.1 Introduction

Microbiology is the science that deals with microscopic living beings, protozoa, algae, viruses, yeasts, fungi, bacteria.

Bacteria were first observed under optical microscope by Leeuwenhoek in 1675. (76)

The bacterial world includes a very large number of species. Some are typical pathogens. Others are commensal, they can cause human disease (infection) under favorable conditions. (77)

7.2 Evaluation of biological activity

The antimicrobial activity test of *Eucalyptus globulus* essential oils was performed at the university microbiology laboratory. We adopted the method of diffusion of discs on a agar medium (aromatogram). Then, the determination of antimicrobial parameters: the MIC in solid medium.

7.3 Bacterial strains uses

This study was conducted on germs, including 06 Gram-negative (-), 03 Gram-positive (+) bacterial strains (Table 1). The bacterial strains collected were stored at 5°C.

Tableau 1: Presentation of the microbial strains tested.

	Gram	Famille	Bactérium
01	Negative	*Entérobactéries*	*Klebsiella pneumonia*
02	Negative	*Entérobactéries*	*Escherichia coli*
03	Negative	*Pseudomonadaceae*	*pseudomonas aeruginosa*
04	Negative	*Entérobactéries*	*E.fuadis*
05	Negative	*Entérobactéries*	*Salmonella*
06	Negative	*Entérobactéries*	*Proteus mirabilis*
07	Positive	*Listeriaceae*	*Listeria*
08	Positive	*Staphylococcaceae*	*Staphylococcus aureus*
09	Positive	*Staphylococcaceae*	*Staphylococcus blanc*

a) Enterobacteriaceae

Enterobacteriaceae are a family of gram-negative bacilli of the order Enterobacterales. tEOe bacteria are usually normal or pathological hosts, depending on microbial species, of the digestive tract of humans and animals. (81)

b) Pseudomonadaceae

TEOe are strict aerobic gram-negative sticks. living in soils and wetlands (taps, plugs, sinks, surfaces of oral thermometers, etc.). This species can live commensal in humans in the skin, upper respiratory tract and digestive tract. (82)

c) Listeriaceae

of which Listeria monocytogenes, the only pathogen for humans where it causes listeriosis, are small, mobile bacilli

d) Staphylococcaceae

Staphylococcus aureus is the most pathogenic species of the genus Staphylococcus, they are Gram positive bacteria aerobic-anaerobic optional, tEOe bacterial cells have the form of shells grouped in clusters, having the shape of a bunch of grapes, they are immobile and not sporulated. (83).

7.4 Growing media

The culture medium used is the agar Mueller Hinton (MH) is the appropriate medium for testing the sensitivity of bacteria to plant extracts, it is a medium that is suitable for bacterial strains.

7.5 Dilutions of essential oils

The strains were tested with concentrated and diluted essential oil.

The essential oil being not miscible, the different dilutions were prepared in an organic solvent used in industry in this case dimethyl sulfoxide (DMSO) with a chemical structure C2H6SO. DMSO is inactive on microorganisms, so it cannot interfere with essential oil activity

The following crude and three dilutions are used: 1/2, 1/8, 1/32.

The aromatogram was tested on two samples of EO:

E1:EO young leaves known in the study area called (leaf female(Entaa , kafour).

E2: EO of the older leaves known in the study area called male leaf (Edkar).

From our two samples we chose to combine the two EO according to the following proportion:

E3: Antibiogram disc containing an equal dose between (E1 + E2)

E4: Antibiogram disc containing a dose of (E1 + 1/2 E2)

E5: Antibiogram disc containing a dose of (1/2 E1 + E2)

7.6 Evaluation Procedure

7.6.1 Transplanting and Reactivating Bacterial Strains

Well-isolated colonies of pure crops were transplanted to the surface of the pre-cast nutrient agar in a petri dish and incubated at 37°C for 24.

7.6.2 Bacterial suspensions (inoculum)

From the young bacterial cultures in the exponential growth phase on GN, 3 to 5 well isolated and identical colonies are taken from 4 ml of sterile physiological water, all will be stirred in the vortex for a few seconds. According to Mc Ferland, an Optical Density between 0.08 and 0.1 corresponds to a concentration of 107 to 108 germs/ml.(78)

7.7 Method of diffusion on agar

In Petri dishes, the Mueller Hinton agar culture medium was poured aseptically at a rate of 15ml per box. After solidification, a sterile swab was soaked into the bacterial suspension and spread on the surface of the agar three times, turning the can to about 60° after each application for equal inoculum distribution.

7.8 Reading

Once the diameter of the inhibitory zone was determined, the qualifying study of the essential oils category was done according to Ponce et al. (2003) as shown in Table 2.

Tableau 2: Categories of bacterial susceptibility to EO.

Catégories	Diameter of inhibiting zone (mm)	Presentation
Resistant	≤ 6	-
Sensitive	7 – 9	+
very sensitive	10 – 15	++

7.9 Determination of Minimum Inhibitory Concentration (MIC)

In general the Minimum Inhibitory Concentration (MIC) is the lowest concentration capable of inhibiting any visible growth after an incubation time of 18 to 24 h. The technique of micro dilution in 96-well microplates is the method chosen to determine the MIC of the tested extracts. (80)

Part 3:

Results and discussion

I. Ethnobotanical survey

1 Age distribution of the population surveyed

In this survey, 28% of the respondents have the age between 30 and 40 years, 23% have the age between 40 and 50 years, 16% of the people are over 60 years and 10% are in the age group under 30 years.

Our results are confirmed by Ait Ouakrouch (2015), while Lazli *and al.*, (2018) and Hamel *and al,*. (2018) used different ages in their study, the results are similar where individuals aged 40 to 60 are

included.

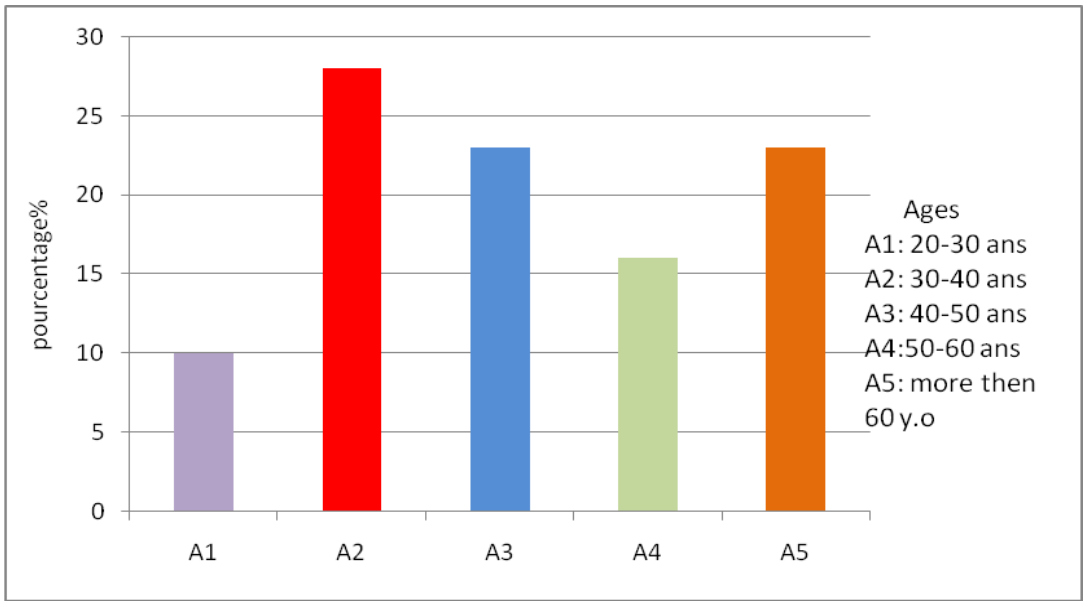

Figure 16 : **Age-band frequencies of the population surveyed.**

2 Distribution by gender of the population surveyed

The results indicate an interesting female dominance with a percentage of 56%, for the use of the plant studied by contribution to men with a percentage of 44%.

Same results inventory conducted by Hamel *and al.*, (2018), the result can be justified by the nature of the role of the woman in her family responsible for much of the health of her children.

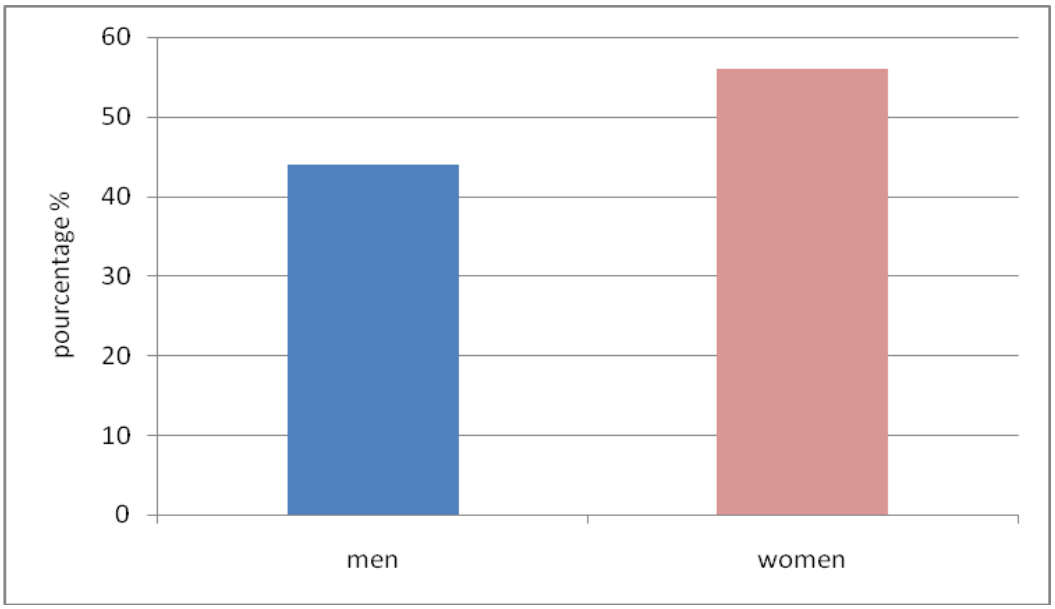

Figure 17 : **Gender groups frequencies.**

3 Distribution by school level

According to our results, the majority of the population surveyed is educated, with a percentage of 38.75%, but they have not followed higher education. Next, there is a similar percentage of academics and people out of school, with 31% and 30.25% respectively.

Our results are confirmed by Koto-Te-Nyiwa *and al.*, (2023), while other results from Ait Ouakrouch (2015) do not coincide with our results

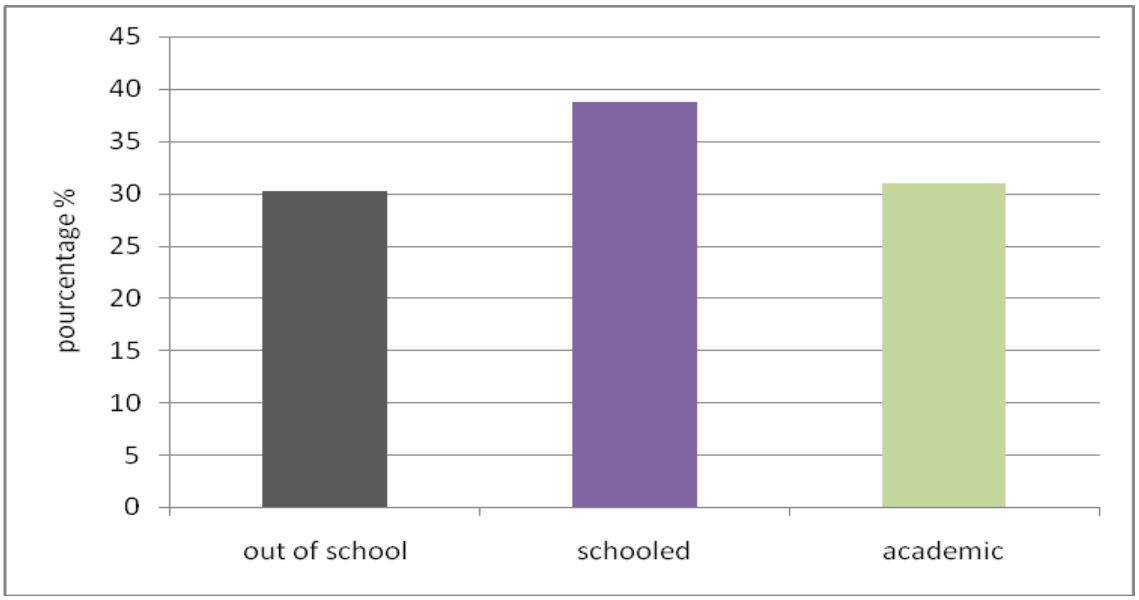

Figure 18 : Frequencies according to school level.

4 Distribution by living environment

All respondents have a rural environment. (Figure 19).

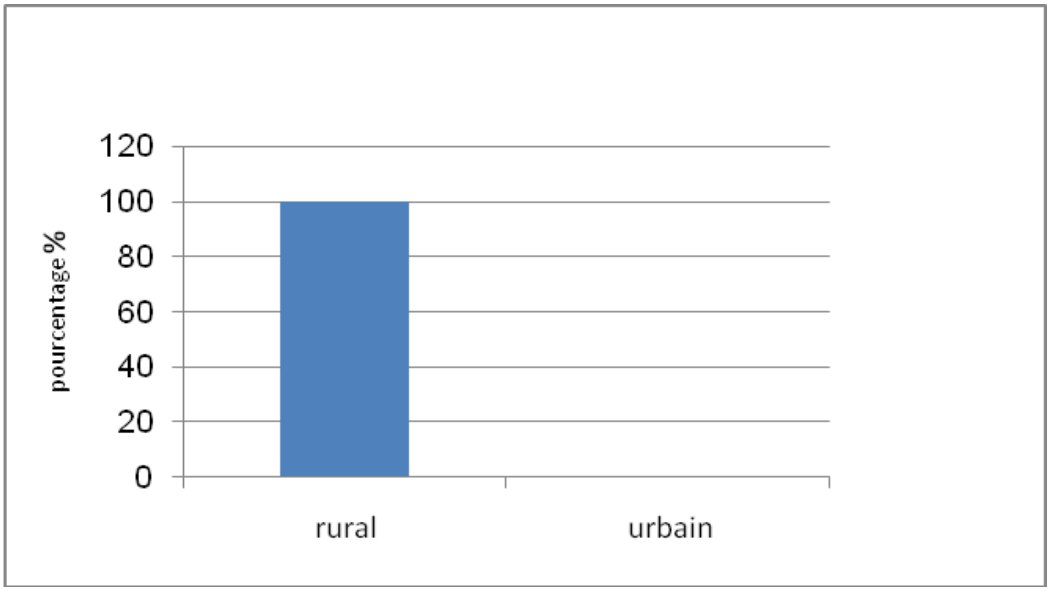

Figure 19 : Frequencies by living environment**.**

5 Distribution by source of information

According to our results presented in Figure 6, 74% of respondents have knowledge about their uses, while 23% have knowledge from the experiences of others. The lowest percentage is that of people who have knowledge through reading, with about 3%.

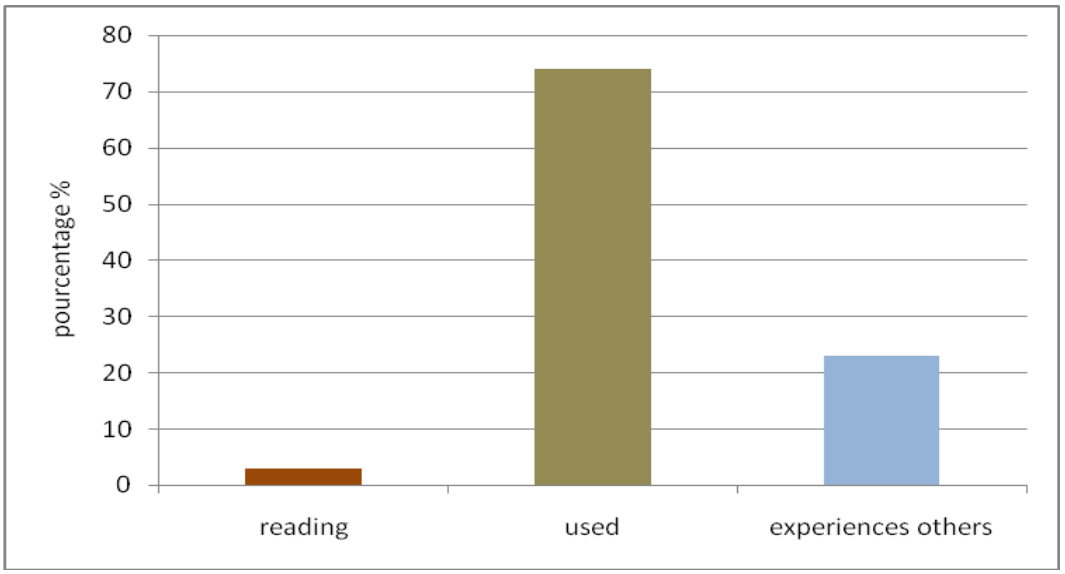

Figure 20 : Frequencies of the origin of information according to the population inquested**.**

6 Distribution by plant effectiveness

Regarding the effectiveness of the plant, 61.25% of the population says it is effective, while 27.13% say it is weak and 11.62% say it is better than drugs.

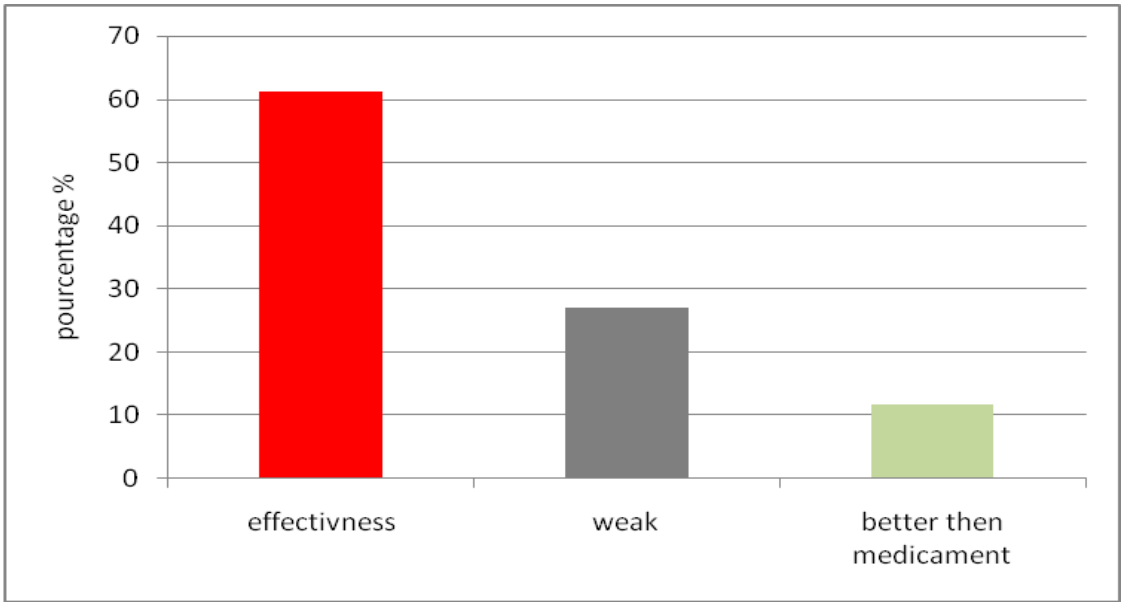

Figure 21 : Frequencies according to plant efficiency.

7 Distribution by used plant part Euclyptus globulus

According to our study, the utilization rate of the different parts reveals that the leaf is the most frequently used (82%), followed by whole plant with (18%), and no other element is used.

From our survey, the part of the plant most used as a remedy is the leaves, which is confirmed by the inventory of Boutabia *and al.*, (2020).

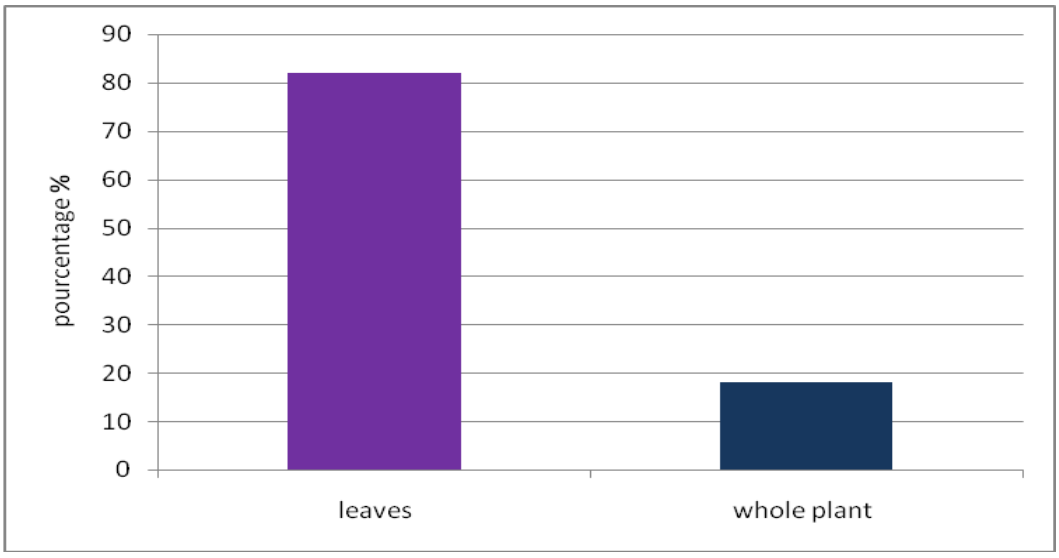

Figure 22 : Frequencies according to the used part of plant of *Eucalyptus globulus*.

8 Distribution by method of preparation

The results in Figure 23, The results, tell us that decoction is the dominant mode of preparation with 66.5%, followed by equal percentage of powder and infusion preparation modes about 15%, the lowest mode of preparation is maceration.

Our results agree with those of Boumediou and Addoun (2017) region of Tlemcen on the use of toxic plants while Rekioua (2023), find that the vapour bath is the most frequent mode of use and Tahri *and al.,(*2012), find that decoction the most useful mode for medicinal plants in morocco .

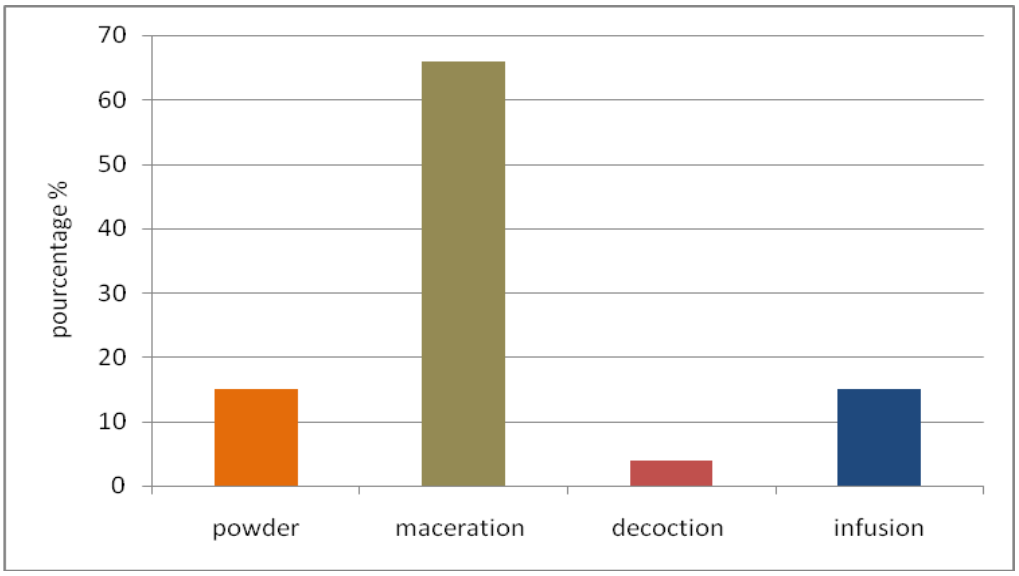

Figure 23 : Frequencies by method of preparation.

9 Depending on mode of use

It is noted that there is a strong dominance of internal use (90%) over external use (10%). Figure 24.

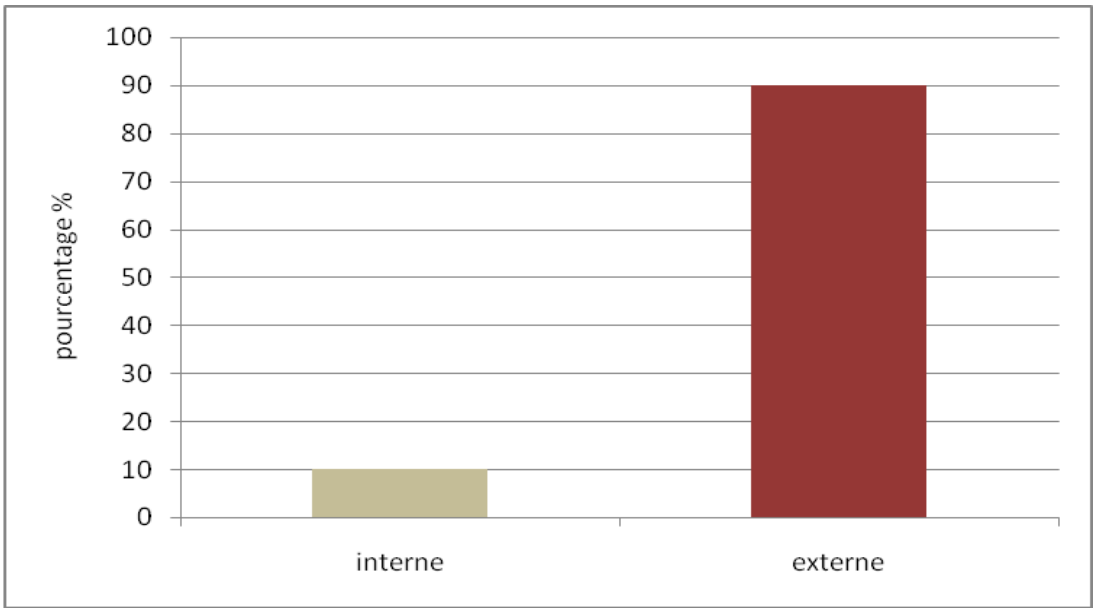

Figure 24: Mode of use frequencies .

10 Distribution by plant condition

According to the results, residents of El-Tarf mainly use *Eucalyptus globulus* in the fresh state, with a percentage of 72% than in the dry state 28%.

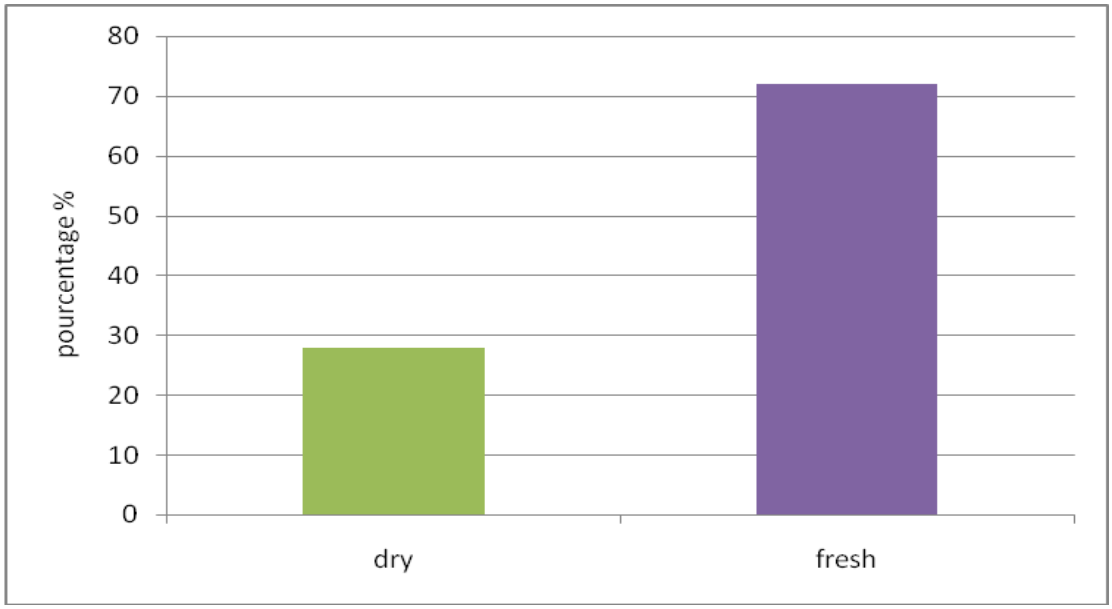

Figure25: Frequencies according to origin of the plant used.

11 Distribution by Treated Diseases

This study, which aims to deepen our understanding of traditional care used in eastern Algerian cities, gave us the opportunity to identify several diseases treated with

oregano. Analysis of the results reveals that the plant plays a role in the treatment of influenza syndrome (83%), stress (5%) and desinfector (12%).

Our results match the results found rekioua 2023.

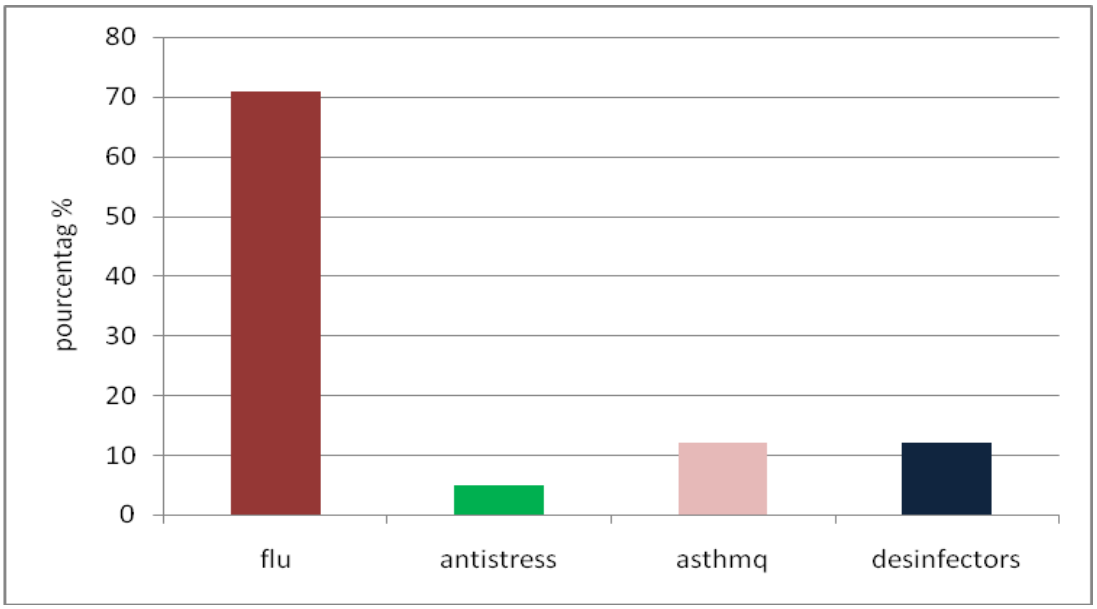

Figure 26: Frequencies according to diseases treated by the *Eucalyptus globulus* plant.

II. Essential Oils

1 Yield

The yield recorded for the essential oil species E. globulus in the Ramel El Souk region is shown in Figure (27).

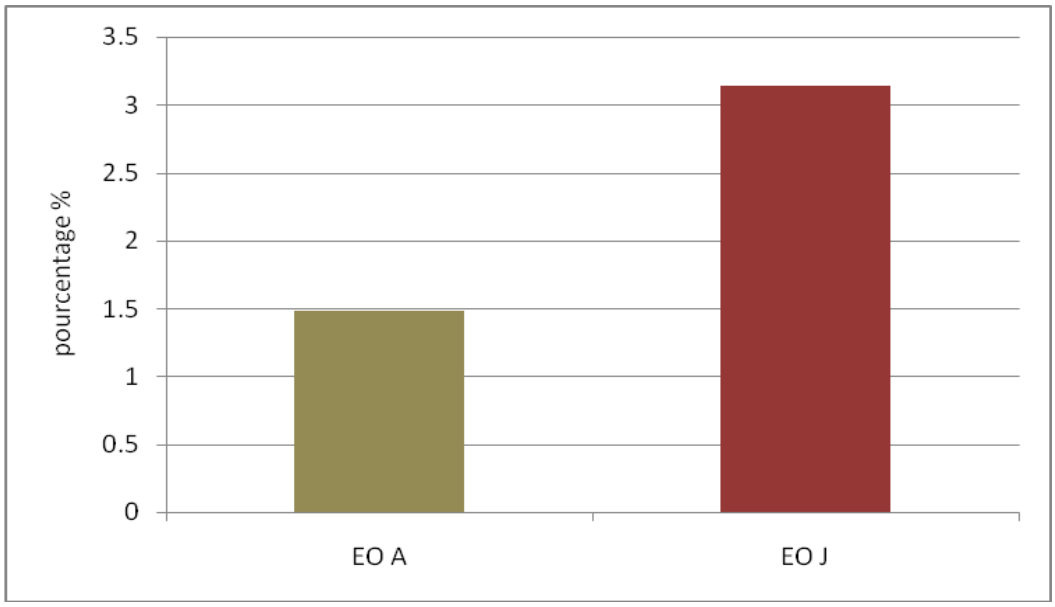

Figure 27 : Evolution of the essential oil yield of old and young leaves.

Regarding the extraction yield of essential oil studied, we note a significant variation in the amount of oil from study plants, whose leaves of aged *Eucalyptus globulus* provided us with a yield of 1,48% and leaves of young *Eucalyptus globulus* gave us a yield of 3.26%. In our study, it is noted that the yield of the essential oil extracted from the leaves of young *Eucalyptus globulus* is higher than that of the essential oil extracted from the leaves of older *Eucalyptus globulus*.

Eucalyptus globulus studied provided a yield of (3.26%), higher than the results of Harkat-Madouri et al., (2015) which is (2.53%) of the Tizi Ouzou region in Algeria and also higher than that obtained by Pino et al., in 2020 by a percentage of (0.17%).

The difference in yield between the same species can be attributed to many biotic factors: plant cycle, plant age, stage of growth and the part subjected to distillation

2 Physico-chemical characteristics of EO

Physico-chemical properties such as: ph, density, acid index, ester index, etc. etc, represent a means of verification and quality control of essential oils. Several tests have been done on the essential oils of our studied species.

2.1 The pH

In our work we use the pH meter to measure the pH of Eucalyptus oil. And we find the pH recorded by our species is 6 for both young and older EO in the French AFNOR standards.

2.2 Density

The relative density of essential oils can be considered as a criterion of purity (62). Density is generally lower than water

Table 3: Results of the relative density of the essential oil of the essential oil.

Plant	*EO A*	*EO J*
Density	0.908	0.910

The relative density at 20°C of tEOe essential oils varies between 0.9243 and 0.9043. The results obtained are in accordance with the AFNOR standard. .

2.3 Acid index

Allows to assess the degree of alteration by the hydrolysis of the oil.

Table 4: presents the results of the acid index of Eucalyptus oils in the Ramel El Soug region

Plant	*EO A*	*EO J*
Acide index	1.68	1.40

The acid index of the essential oil extracted from the leaves of young *Eucalyptus globulus* is 1.68 and for the leaves of aged *Eucalyptus globulus* and 1.40

Our results comply with the French standard AFNOR (0.84 – 3.74).

Acidity represents the percentage of free fatty acids expressed as oleic acid in an essential oil, this parameter can help to know the quality of the product. When the acid index is low means that the HE is stable and does not undergo worrying oxidation, because by oxidizing, it degrades quickly and causes an increase in the acid index (73), so we can say that our essential oils are stable.

2.4 Saponification index

The saponification index of the essential oil is the number of mg of potash necessary to neutralize the free acids and saponify the esters present in 1 g of essential oil. The saponification index has no unity (63). The SI values found are 38.027 for older *Eucalyptus globulus* and 42.082 for older *Eucalyptus globulus*.

2.5 Ester Index

The ester index is an indicator of oil quality, essential oils of very high quality contain a significant amount of esters. This index may be influenced by other factors such as the conditions under which hydrolysis occurs (i.e., in which type of still, the water constituents used for heating, etc.)(69),

The ester index found for aged *Eucalyptus globulus* leaves is 36.627 and for young *Eucalyptus globulus* leaves is 40.402.

From the results we find that the young *E.golobulus* essential HE has the higEOt ester index, so it is considered of better quality than the aged E. globulus essential oil . This is already confirmed by the value of its acid index.

2.6 The refractive index

The refractive index was measured using a refractometer at a temperature T = 20°C:

-Refractive index of the leaves of aged *Eucalyptus globulus* equals 1.469

- Refractive index of *Eucalyptus globulus* young leaves equals 1,472.

The refractive index indicates the ability of HE to reflect light. This ratio is generally high, it is higher than those of water at 20ºC = 1.3356. This index depends on the chemical composition that increases with the lengths of the acid chains, their degree of unsaturation and temperature, it varies essentially with the content of monoterpenes and oxygenated derivatives. A high content of monoterpenes gives a higher index.

As an indication, the acceptance interval identified in F.C.C. III (Food Chemical Codex) for eucalyptus essential oil is: 1.458 to 1.470.

Based on the results in Table 14, all refractive indices measured are FCC compliant.

3 Photochemical Screening

The phytochemical screening of the two oils of *E.globulus* allowed, qualitatively, the highlighting of the different classes of the secondary metabolites of the species *E.globulus* whose results are gathered in table 5.

Table 5: Chemical screening results.

Tests	Resultat	
	EO A	EO J
Alkaloides	+ + +	+ +
Saponosides	+ + +	+ + +
Tannins	+ + +	+ + +
Quinones	− − −	− − −

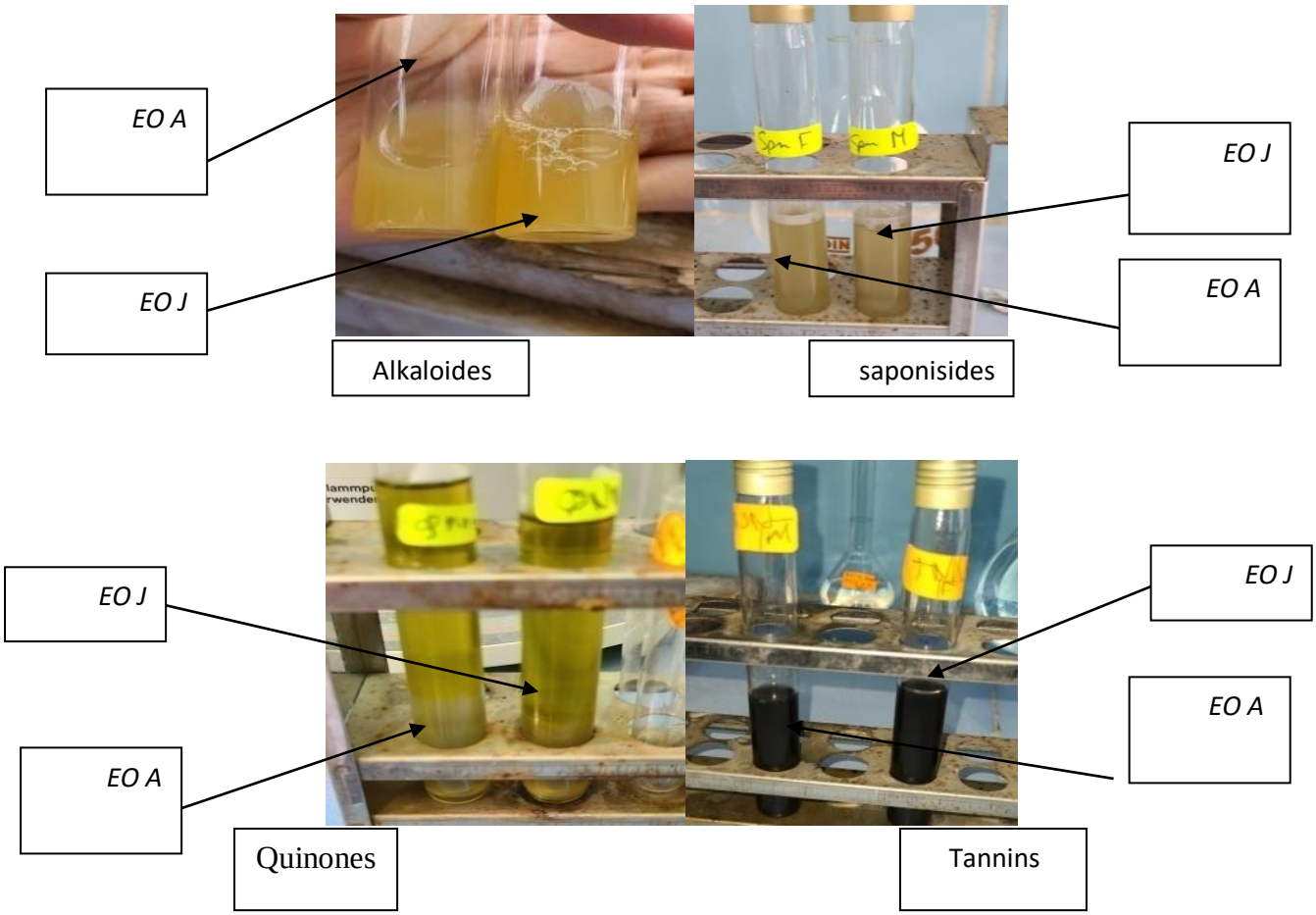

Figure 28 : phytocimical Scrrening .

Our preliminary phytochemical tests presented in (Tab.04) indicate that the leaves of young and old *Eucalyptus globulus* are rich in Alkaloids, Saponosides, Tannins, and show an absence of quinones.

This phytochemical study carried out on the species *E.globulus* showed results namely the presence of certain chemical families. However, there are no other chemical families. This variability in the results can be explained by a difference in several biological, geographical or physicochemical parameters such as: the influence of the vegetative stage, the organ of the plant studied the difference in the harvest site including the plant environment, light, precipitation, soil conditions, harvest period, genetic heritage and extraction procedure used. (69-54)

4 Antioxidant activity

The antioxidant activity of *Eucalyptus globulus* oil was evaluated in vitro by the DPPH free radical reduction method. The presence of free radical scavengers reduces purple DPPH to yellow DPPHH.

Determination of IC50: The oil IC50 is determined from the linear portion of the inhibition percent curve as a function of concentration.

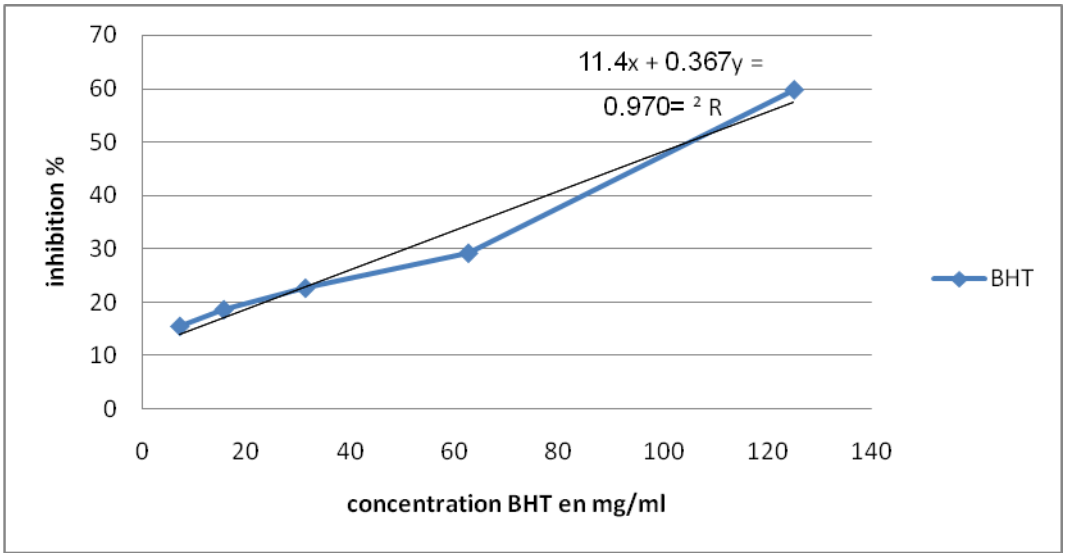

Figure 29 : Percentages of DPPH inhibition as a function of different BH concentrations.

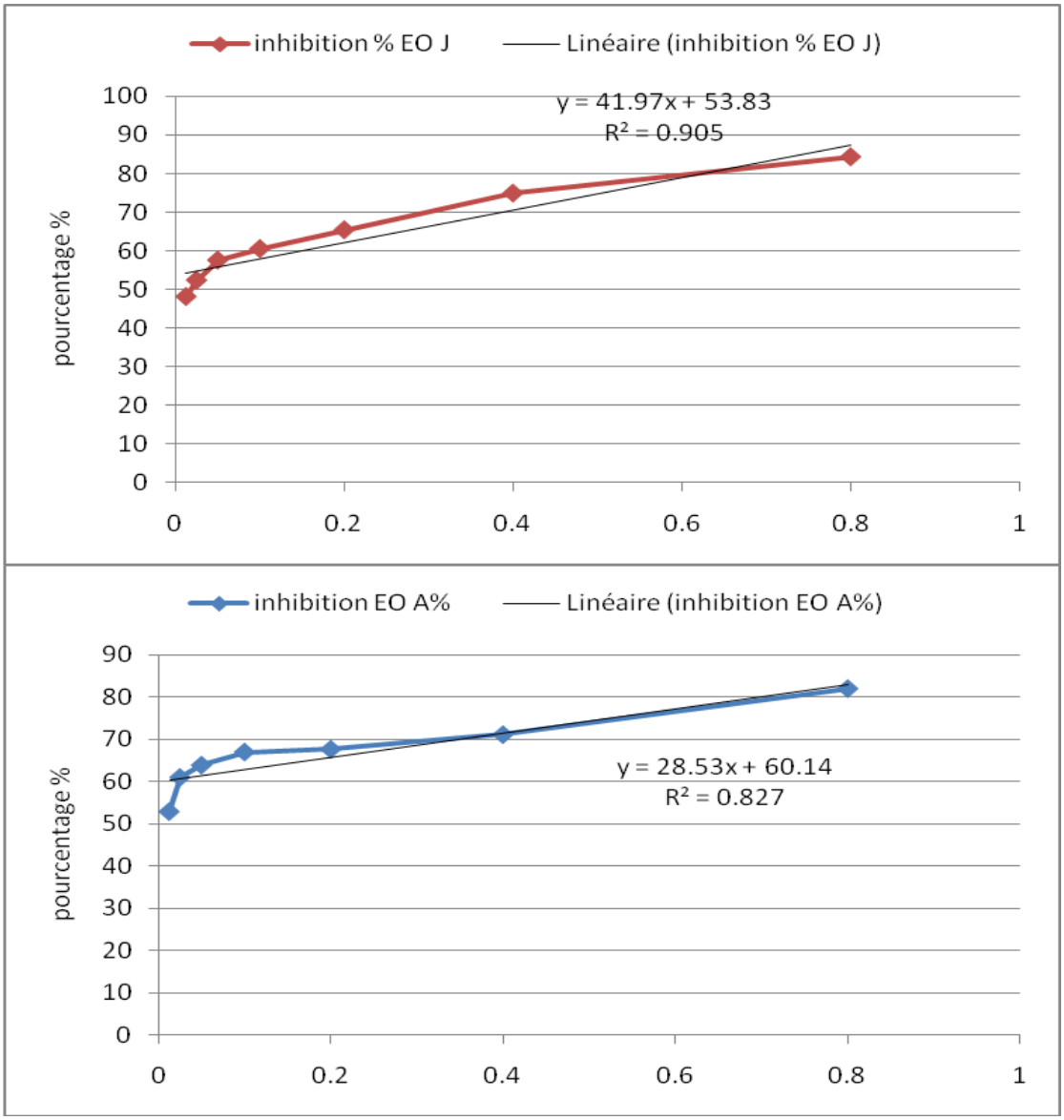

Figure 30 : DPPH inhibition percentages according to different oil concentrations of young and old Eucalyptus gobulus leaves.

The antiradical activity of the extract has been evaluated by the DPPH test, which is often used for rapid results as it is used for screening molecules with antioxidant activities in plant extracts. (70)

IC50 is inversely related to the antioxidant capacity of a compound, as it expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The lower the IC50 value, the greater the antioxidant activity of a compound.(71)

The results of the antiradical action of young *Eucalyptus globulus* oil leaves show an IC50 (leaves) of the order of (23 mg/ml), also the aged *Eucalyptus globulus* oil leaves show an IC50 of the order of (11mg/ml) while BHT show an IC50 (105.19 mg/ml).

TEOe results suggest that both young and old *Eucalyptus globulus* oil contains free radical scavenging agents acting as primary antioxidants.

the results of antioxidant potency may be influenced not only by chemical composition but also by test conditions (reaction temperature, antioxidant ratio/DPPH). (72)

5 Antibacterial activity

The qualitative evaluation of the antimicrobial activity of our essential oils was made on nine bacteria, by the method of aromatograms. The antimicrobial power is obtained by measuring the diameters of the inhibition zones (mm).

Note

E1: EO of young leaves known in the study area called leaf female

E2: EO of the older leaves known in the study area called male leaf

E3: Antibiogram disc containing an equal dose between (E1 + E2)

E4: Antibiogram disc containing a dose of (E1 + 1/2 E2)

E5: Antibiogram disc containing a dose of (1/2 E1 + E2)

The diameters of the inhibition zones (mm) obtained are shown in the following tables:

5.1 Klebsiella pneumonia

Tableau 6: Bacterial sensitivity of bacterium Klebsiella pneumonia of different concentration of essential oils studied.

Dilution / Samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	-(0)	-(0)	-(0)	-(0)
E2	++(11)	+(8)	-(0)	-(0)
E3	-(0)	-(0)	-(0)	-(0)
E4	++(11)	+(8)	-(0)	-(0)
E5	-(0)	-(0)	-(0)	-(0)

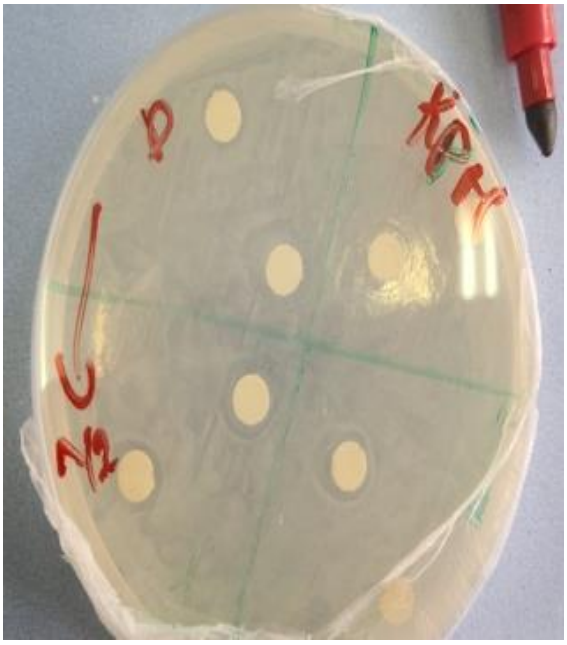

Figure 31: Photograph showing the action of *E.globulus* EO on the bacterial strain .

5.2 Escherichia coli

Tableau 7: Bacterial sensitivity of bacterium E.Coli deferens concentration of essential oils studied.

Dilution / Samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	-(0)	-(0)	-(0)	-(0)
E2	-(0)	-(0)	-(0)	-(0)
E3	-(0)	-(0)	-(0)	-(0)
E4	-(0)	-(0)	-(0)	-(0)
E5	-(0)	-(0)	-(0)	-(0)

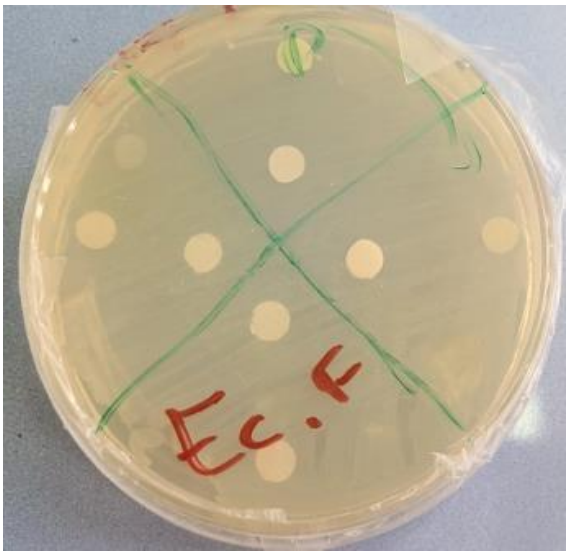

Figure 32: Photograph showing the action of *E.globulus* EO on the bacterial strain E.Coli.

5.3 Pseudomonas aeruginosa

Tableau 8: Bacterial sensitivity of bacterium Pseudomonas aeruginosa deferens concentration of essential oils studied.

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	++(10)	-(6)	-(0)	-(0)
E2	++(11)	+(8)	-(0)	-(0)
E3	-(0)	-(0)	-(0)	-(0)
E4	-(0)	-(0)	-(0)	-(0)
E5	-(6)	-(0)	-(0)	-(0)

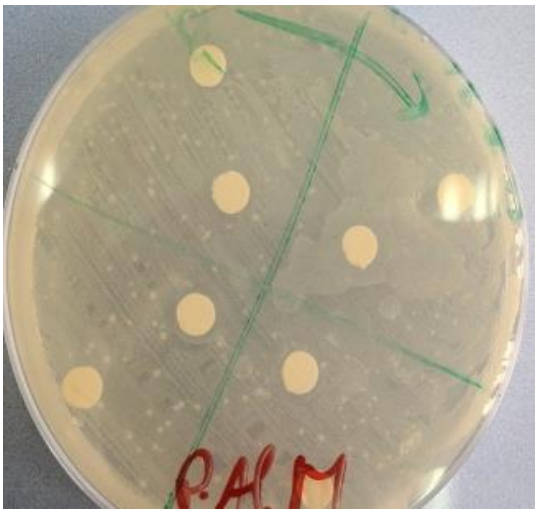

Figure 33: Photograph showing the action of *E.globulus* EO on the bacterial strain Pseudomonas aeruginosa.

5.4 E.faecalis

Tableau 9: Bacterial sensitivity of deferential bacterium E.faecalis concentration of essential oils studied.

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	++(11)	+(8)	-(0)	-(0)
E2	-(0)	-(0)	-(0)	-(0)
E3	-(0)	-(0)	-(0)	-(0)
E4	++(11)	+(8)	-(0)	-(0)
E5	-(0)	-(0)	-(0)	-(0)

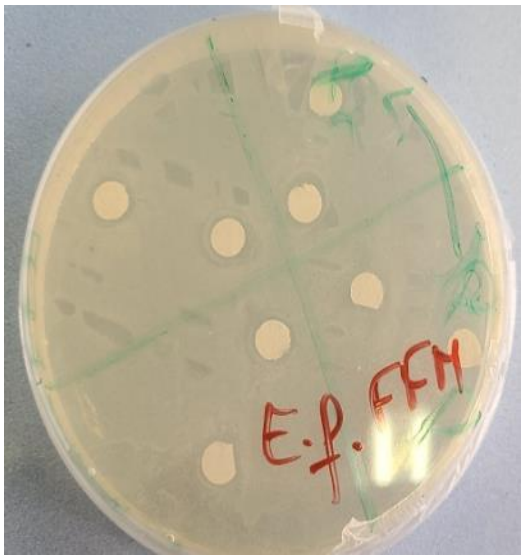

Figure 34: Photograph showing the action of *E.globulus* EOs on the bacterial strain E.faecalis.

5.5 Salmonella

Tableau 10: Bacterial sensitivity of Salmonella bacteria of deferential concentration of essential oils studied

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	++(12)	++(11)	-(0)	-(0)
E2	++(12)	+(8)	-(6)	-(0)
E3	++(12)	-(6)	-(0)	-(0)
E4	-(0)	-(0)	-(0)	-(0)
E5	-(0)	-(0)	-(0)	-(0)

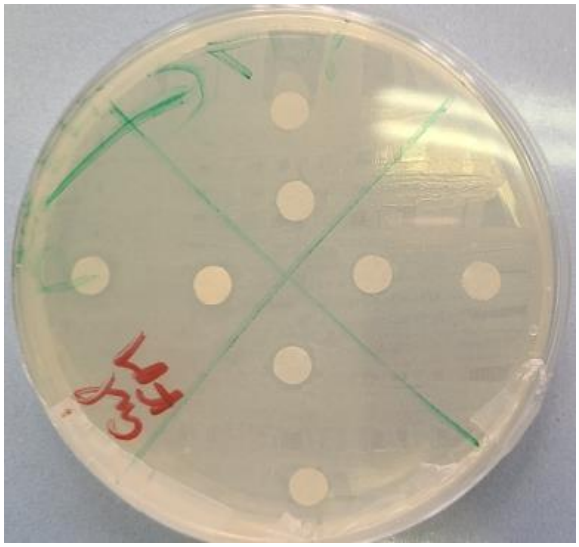

Figure 35: Photograph showing the action of *E.globulus* EO on the bacterial strain Salmonella.

5.6 Proteus mirabilis

Tableau 11: Bacterial sensitivity of Proteus mirabilis a bacteria of deferent concentration of essential oils studied

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	+(9)	-(6)	-(0)	-(0)
E2	++(12)	+(10)	+(8)	-(7)
E3	++(10)	+(8)	-(0)	-(0)
E4	+(8)	-(6)	-(0)	-(0)
E5	+(8)	-(0)	-(0)	-(0)

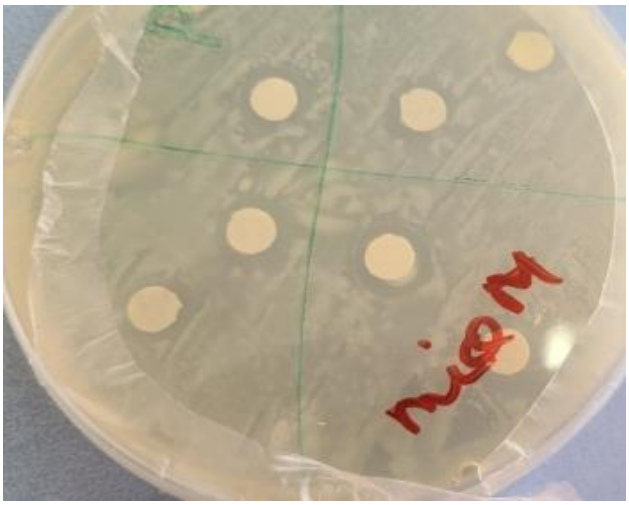

Figure 36: Photograph showing the action of *E.globulus* EO on the bacterial strain Proteus mirabilis.

5.7 Listeria

Tableau 12: Bacterial sensitivity of listeria bacteria of deferent concentration of essential oils studied

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/16
E1	++(12)	+(9)	-(0)	-(0)
E2	+(9)	-(0)	-(0)	-(0)
E3	++(10)	+(8)	-(6)	-(0)
E4	++(10)	+(8)	-(0)	-(0)
E5	+(9)	-(0)	-(0)	-(0)

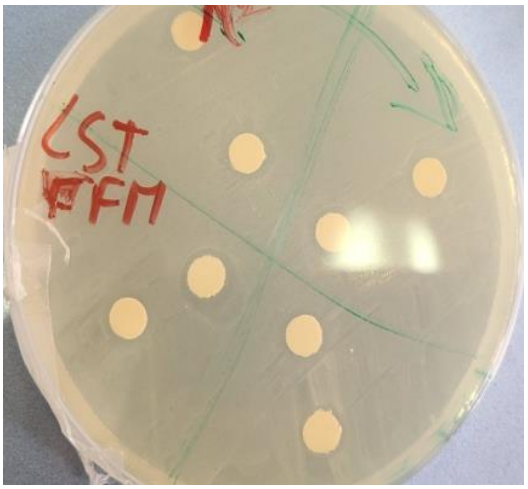

Figure 37: Photograph showing the action of *E.globulus* EO on the bacterial strain Listeria.

5.8 Staphylococcus aureus

Tableau13: Bacterial sensitivity of staphylococcus aureus bacteria of deferent concentration of essential oils studied.

Dilution / Samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	++(10)	+(8)	-(0)	-(0)
E2	+(8)	-(0)	-(0)	-(0)
E3	-(0)	-(0)	-(0)	-(0)
E4	++(10)	-(0)	-(0)	-(0)
E5	++(10)	-(0)	-(0)	-(0)

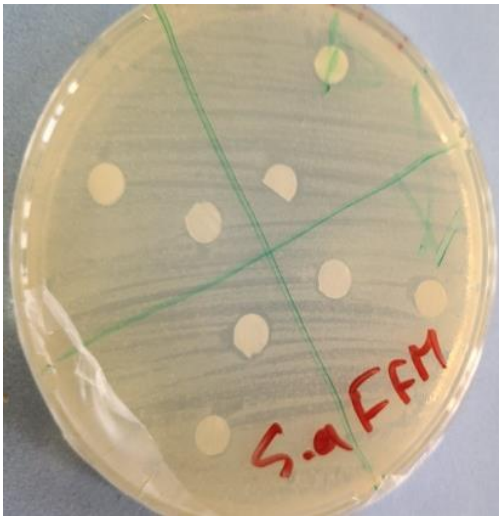

Figure 38: Photograph showing the action of *E.globulus* EO on the bacterial strain Staphylococcus aureus.

5.9 *Staphylococcus epidermidis (blanc)*

Tableau 14: Bacterial sensitivity of *Staphylococcus epidermidis (blanc)* bacteria of deferent concentration of essential oils studied.

Dilution / samples	EO pure	EO 1/2	EO 1/8	EO 1/32
E1	-(7)	-(0)	-(0)	-(0)
E2	++(10)	-(7)	-(0)	-(0)
E3	++(15)	+(8)	-(0)	-(0)
E4	++(10)	+(8)	-(0)	-(0)
E5	++(10)	+(8)	-(0)	-(0)

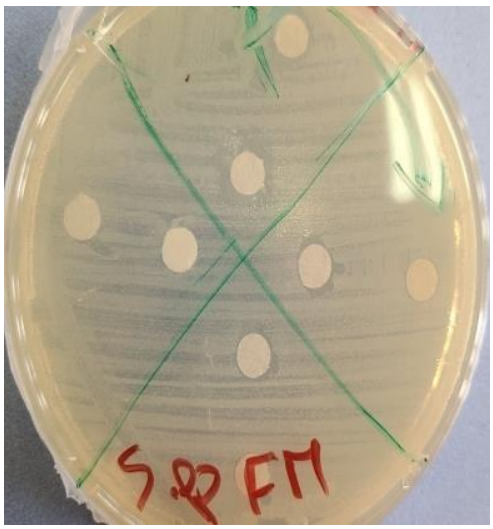

Figure39: Photograph showing the action of *E.globulus* EO on the bacterial strain *Staphylococcus epidermidis* ***(blanc)*.**

From our results we found that the sample (E1) of *Eucalyptus globulus* of young leaves, has a weak antibacterial activity against the strains of Pseudomonas

aeruginosa, E.faecalis, Salmonella, Proteus mirabilis, Listeria, Staphylococcus aureus, White staphylococcus and that sample (E2) of *Eucalyptus globulus* of old leaves, has antibacterial activity against Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella, Proteus mirabilis, Listeria, Staphylococcus aureus, Staphylococcus epidermidis (blanc).

The investigations established for the mixture of old and young Eucalyptus essential oils present the following results:

• (E3): Salmonella, *Staphylococcus epidermidis (blanc)*.

• (E4): Pseudomonas aeruginosa, Proteus mirabilis, E.faecalis, Staphylococcus aureus, *Staphylococcus epidermidis (blanc)*.

• (E5): Proteus mirabilis, E.faecalis, Staphylococcus aureus, *Staphylococcus epidermidis (blanc)*.

The even mixing of the two EO of *Eucalyptus globulus* seems more effective against *Staphylococcus epidermidis (blanc)* with an inhibition diameter interval of 15 mm and a MIC of 0.04 mg/ml.

• The sensitivity of Klebsiella pneumoniae to *Eucalyptus globulus* essential oil appears to be moderate, which does not corroborate the results of Bouras (2019) which show that the strain was sensitive.

• Results with Escherichia coli do not support Results from rekioua (2023).

•The results agree with those of Raho and benali,(2008) on the sensitivity of S. aureus and do not have the same sensitivity to P. aerugenosa strain.

• Salmonella strain results with *Eucalyptus globulus* EO were lower than those calculated by Belhaji et al.,(2016) noted resistance of the bacterium to natural honey of Moroccan origin and similar with Kouamé *and al.*, (2004) with EO Of Ocimum gratissimum.

• The sensitivity of Proteus mirabilus to the essential oil of *Eucalyptus globulus* has the same sensitivity to Belgaid, Chikhoun.(2013) with Noah's Phlomis Bovei extract.

• The listeria strain does not have a sensitivity according to R. Ben Abdallah ,*and al.,(*2019) sensitivity. which does not agree with our results which show that even the strain has a sensitivity .

• *Eucalyptus globulus* EO has been shown to be active against *Staphylococcus epidermidis (blanc)*, corroborating the work of Abdelli et al. (2016).

Conclusion

Conclusion

Medicinal plants remain an important source of active ingredients known by their various properties, moreover essential oils hold an important place in recent research for their multiple biological activities (Bactericide, insecticide, herbicide, antiseptic, fungicide, etc.) etc. This work enabled us to carry out the ethnobotanical survey and the study of the therapeutic interests of *Eucalyptus globulus* in the Ramel El Souk region – El Taref – It can be concluded that:

The ethnobotanical survey revealed a multitude of results on traditional population use in the Ramel El Souk – El Tarf region:

• The 30-40 age group predominates in plant use (28%) and (56%) are women and the majority are educated (38.75) with a rural living environment (100%).

• The majority of people use the plant (74%) and it is effective (61.25).

• the population of the study area uses the cultivated plant (72) with predominance of use of the leaves of the plant (82%) by contribution of the other parts, the dominant mode of preparation is decoction (68%) with an internal mode of use (90%).

• the majority of uses are for treating influenza (71%).

- ✓ The extraction of essential oil by hydro distillation showed a yield of 3.26% of the EO of the young *E.globulus* leaves and 1.48% for the EO of the old *E.globulus* leaves.
- ✓ Physico-chemical characteristics (acid index, refractive index, saponification index, ester index, density and pH measurement) for both and phytochemical screening.
- ✓ DPPH test results showed an antiradical activity equal to 84% and 82% with an IC50= (23 and 11 mg/ml). It can be noted that this activity was lower than that of the BHT.
- ✓ The evaluation of the antibacterial activity of EO at different concentrations against the strains tested. . The results obtained throughout this test showed that the essential oils of *Eucalyptus globulus* have bacterial activity against a

number of bacteria, especially against Staphylococcus aureus, Staphylococcus epidermidis (white).

- ✓ It is interesting to combine between the two EO since they demonstrate a diameter of inhibition more interested against Staphylococcus epidermidis (white) with a MIC of 0.04mg/ml .

The result obtained for antibacterial activity confirms the choice of remedies used by the indigenous population of the region.

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Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
جامعة الشادلي بن جديد - الطارف
Université Chadli Bendjedid - El Tarf

CERTIFICATE OF PARTICIPATION

THIS IS TO CERTIFY THAT

MS. HALIM N-I.

DELIMI A., MEDJELDI S., GUENADIL F.

has participated in to "SNBSE - 24"

National Seminar on Bioindicators and Environmental Health

organized by SNV Faculty, CHEDLI -BENDJEDID El-TARF UNIVERSITY , En ligne on 3 et 4 Mai 2024 . with

Poster Communication

Titled

ETHNOBOTANICAL INQUEST AND STUDY OF THE THERAPEUTIC BENEFITS OF *EUCALYPTUS GLOBULUS*, FROM THE REGION OF RAML SOUK (EL TARF).

Session Chair

Université Chadli Ben Djedid el Tarf
Faculté Des Sciences De la Nature et De la Vie
Présidente Dr BECIR FARIDA
National Seminar on Bioindicators and Environmental Health

UCBET 3 & 4 MAY 2024

The Dean of the faculty

جامعة الشادلي بن جديد - الطارف
عميد كلية علوم الطبيعة والحياة
الأستاذ الدكتور: هشام ساعد