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Field: Biological Sciences
Option: Applied Biochemistry

Title

Antimicrobial activity of natural extracts from spontaneousplants in comparison with some synthetic compounds

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DEDICATION

In memory of my beloved father, Mouhammed,

This work is the fruit of my years of study, I dedicate to you with all my love and respect. You have always beenmy role model, my inspiration and my greatest support. Unfortunately, life has decided otherwise and you areno longer here to see the outcome of this work.

Even though you are no longer physically present, your memory and your teachings remain engraved in my heart. You always believed in me and pushed me to give the best of myself. It's thanks to you if I am here today. Dad, this memoir is as much yours as it is mine. I hope you would be proud of the person I have become and what I have accomplished. I miss you every day. This work is a tribute to your memory and to the exceptional man that you were. I dedicate this success to you, hoping that you can share it with me up there.

To my dear mother, At this important moment in my life, I wish to dedicate this work to you in recognition of everything you have done for me. Your strength, your unconditional love and your unwavering support have been the pillars on which I have been able to build my academic career.

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DEDICATION

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**Thank you very much **

NAHOUI SIHAM

DEDICATION

الملخص

تهدف هذه المذكرة الى تقييم و مقارنة النشاط المضاد للميكروبات لبعض المركبات الاصطناعية مع الميثانول و مستخلصات الزيوت الطيارة من القرنفل ، القرفة و الخزامى .
تجمع المنهجية المعتمدة بين الاختبارات المعملية ضد السلالات البكتيرية المسببة للامراض المختلفة (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans)*
تم اختبار المستخلصات باستخدام طرق الانتشار القرصي و التخفيف الجزئي و ظهرت النتائج كالتالي :
بالنسبة للزيوت الطيارة فاظهرت نتائج الانتشار القرصي اقطار تثبيط مختلفة تتراوح بين 30mm و 20mm بالنسبة للزيت الطيار للقرنفل و بين 45mm و 28mm بالنسبة للزيت الطيار للقرفة مع مختلف السلالات المستعملة و سجلنا اعلى اقطار تثبيط تساوي 50mm للزيت الطيار الصافي للقرنفل مع *Candida albicans* و الزيت الطيار الصافي للقرفة مع *Escherichia coli* اما المضاد الحيوي الجنتاميسين فقطر تثبيطه يساوي 20 mm مع مختلف السلالات المستعملة.
اما بالنسبة للمستخلصات الطبيعية و الاصطناعية فاظهرت نتائج التخفيف الجزئي ان الحد الأدنى للتركيز لمستخلص القرفة مخفف 1/ 2 يساوي 0.475 mg /ml مع*Escherichia coli* و الحد الأدنى للتركيز لمستخلص الخزامى الصافي يساوي 1.49mg/ml مع *Candida albicans* اما الحد الأدنى للتركيز للمستخلص الاصطناعي حمض الاسكوربيك يساوي 1.59mg /ml مع *Staphylococcus aureus*.
ومنه نستنتج ان المستخلصات الطبيعية من القرفة و الخزامى و الزيوت الطيارة من القرنفل و القرفة لها نشاط كبير مضاد للميكروبات حتى اكبر من المستخلصات الاصطناعية و المضاد الحيوي الجنتاميسين .

في الختام تسلط هذه الدراسة الضوء على إمكانات المستخلصات الطبيعية و الزيوت الطيارة كبدائل واعدة للعوامل الاصطناعية المضادة للميكروبات .

الكلمات المفتاحية : النشاط المضاد للميكروبات ، المستخلصات الطبيعية ، الزيوت الطيارة ، قطر التثبيط ، الحد الأدنى للتركيز ، الحد الأدنى من تركيز مبيد الجراثيم .

ABSTRACT

This master thesis aimed to evaluate and compare the antimicrobial activity of some synthetic compounds with natural methanolic and essential oils extracts of cloves, cinnamon and lavender. The adopted methodology combines laboratory tests against different pathogenic bacterial strains (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*).

The extracts were tested using disk diffusion and microdilution methods, and the results appeared as follows:

As for the essential oils, the disc diffusion results showed different diameters of inhibition ranging between 30mm and 20mm for the essential oil of cloves and between 45mm and 28mm for the essential oil of cinnamon with the various strains used. We recorded the highest diameters of inhibition equal to 50mm for the pure essential oil of cloves with *Candida albicans* ,and the pure essential oil for cinnamon with *Escherichia coli*. As for the antibiotic gentamicin, its inhibition diameter is equal to 20 mm with the various strains used.

As for the natural and synthetic extracts, the results of microdilution showed that the MIC of the cinnamon extract diluted 1/2 is equal to 0.475 mg/ml with *Escherichia coli*, and the MIC of the pure lavender extract is equal to 1.49 mg/ml with *Candida albicans*. While the MIC of the synthetic extract Ascorbic acid is equal to 1.59mg/ml with *Staphylococcus aureus.*

From this, we conclude that natural extracts of cinnamon, lavender, and volatile oils of cloves and cinnamon have significant antimicrobial activity, even greater than synthetic extracts and the antibiotic gentamicin.

In conclusion, this study highlights the potential of natural extracts and essential oils as promising alternatives to synthetic antimicrobial agents.

Keywords: antimicrobial activity, natural extracts, essential oils, Inhibition diameter, MIC, MBC

RESUME

Ce mémoire vise à évaluer et comparer l'activité antimicrobienne de certains composés synthétiques avec des extraits méthanoliques naturels et d'huiles essentielles de clou de girofle, de cannelle et de lavande. La méthodologie adoptée combine des tests de laboratoire contre différentes souches bactériennes pathogènes (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*).
Les extraits ont été testés selon les méthodes de diffusion sur disque et la microdilution, et les résultats sont les suivants :
Quant aux huiles essentielle, les résultats de diffusion sur disque ont montré différents diamètres d'inhibition compris entre 30 mm et 20 mm pour l'huile essentielle de clou de girofle et entre 45 mm et 28 mm pour l'huile essentielle de cannelle avec les différentes souches utilisées. Nous avons obtenu les diamètres d'inhibition les plus élevés égal à 50mm pour l'huile essentielle pure de clou de girofle avec *Candida albicans* et l'huile essentielle pure de cannelle avec *Escherichia coli.* Et pour l'antibiotique gentamicine, son diamètre d'inhibition est égal à 20 mm avec les différentes souches utilisées.
Quant aux extraits naturels et synthétique, les résultats de la microdilution ont montré que la CMI pour l'extrait de cannelle dilué 1/2 est égale à 0,475 mg/ml avec *Escherichia coli*, et la CMI pour l'extrait de lavande pure est égale à 1,49. mg/ml avec *Candida albicans.* Tandis que la CMI pour l'extrait synthétique d'acide ascorbique est égale à 1,59 mg/ml avec *Staphylococcus aureus*.
Nous en concluons que les extraits naturels de cannelle, de lavande et les huiles essentielle de clou de girofle et de cannelle ont une activité antimicrobienne significative, encore plus grande que les extraits synthétiques et l'antibiotique gentamicine.

En conclusion, cette étude met en évidence le potentiel des extraits naturels et des huiles volatiles comme alternatives prometteuses aux agents antimicrobiens synthétiques.

Mots clés : activité antimicrobienne, extraits naturels, huiles essentielles, diamètres d'inhibition, CMI, MBC.

LIST OF ABBRIVIATION

MIC Minimum inhibitory concentration

MBC Minimal bactericidal concentration

E.O Essential Oil

NCTC National Collection of Type Culture

ATCC American Type Collection Culture

LAB Lactic Acid Bacteria

ICs Inhibitory Concentrations

ATP Adenosine Triphosphate

PC Phenolic compounds

DMSO Dimethyl sulfoxide

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INTRODUCTION

INTRODUCTION

Traditional medicine continues to play an important role in health care practices across the world, with a rich history rooted in the use of medicinal plants **(1)**.The use of phytochemicals and traditional remedies remains prevalent, with approximately 80% of the world's population using these traditional approaches for various health problems **(2)**. The value of traditional medicine in drug discovery is underscored by the wealth of information derived from ethnomedical systems, highlighting the potential for novel therapeutic interventions **(3)**.

Antibiotic resistance is a significant global concern, with bacteria evolving mechanisms to combat the effects of antibiotics. Studies have shown that bacteria can develop defenses against antibiotics through various means. For example, the production of hydrogen sulfide (H2S) has been identified as a universal defense mechanism in bacteria such as Bacillus anthracis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli, making them highly susceptible to antibiotics when producing H2S. is removed **(4)**. Additionally, exposure to antibiotics can induce pleiotropic effects in bacteria, leading to alterations in bacterial metabolism, cell wall structure, and antibiotic resistance**(5)**.

Bacterial infections caused by pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*, as well as fungal infections by *Candida albicans*, present significant challenges in healthcare settings. *Staphylococcus aureus*, a commensal bacteria, that can cause serious skin infections, abscesses, osteomyelitis, endocarditis, and other life- threatening conditions **(6)**. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections, contributing to significant morbidity and mortality worldwide **(7)**. On the other hand, *Pseudomonas aeruginosa* is known for its multidrug resistance, which requires new antibacterial strategies to combat infections caused by this pathogen. *Candida albicans*, a common fungal pathogen, can cause candidiasis, ranging from superficial mucosal diseases to deep mycoses**(8)**. Candida species, including *Candida albicans*, are opportunistic pathogens that can lead to infections, particularly in immunocompromised individuals**(9)**. the effectiveness of combinations of medicinal plant extracts against Candida albicans has been studied, highlighting the potential of traditional medicine in combating fungal infections**(10).**

The antimicrobial activity of natural extracts and essential oils, including cinnamon, clove and lavender, has been widely studied and recognized in the scientific literature. Essential oils and plant extracts have long been recognized for their antimicrobial properties**(11)**. Spices such as clove, oregano, thyme, cinnamon, and cumin have shown significant antibacterial and antifungal activities against various

pathogens and food-spoiling bacteria**(12)**. Studies have evaluated the antimicrobial activity of cinnamon, clove, basil, rosemary, dill and ginger essential oils, demonstrating their effectiveness against food-borne

bacterial and fungal strains**(13)**.Clove extract, known for its antimicrobial and antioxidant potential, has been highlighted as a natural preservative with excellent antimicrobial properties**(14)**. Additionally, the antimicrobial activity of clove and cinnamon extracts has been studied in the context of acute hematogenous pyelonephritis, showing promising results against pathogenic microorganisms**(15)**. Clove essential oil has been explored for its potential as an alternative biocide against biofilms, emphasizing the antimicrobial properties of essential oils extracted from medicinal plants**(16)**. Cinnamon and cloves, containing essential oils such as eugenol and cinnamic aldehyde, have been identified as potent antimicrobial agents with potential applications in food processing**(17)**. Additionally, cinnamon bark extract has demonstrated antimicrobial effects against various pathogens including *Escherichia coli* and Listeria innocua**(18)**. The use of natural oils such as garlic, clove and thyme has been proposed as a strategy to combat bacteria and reduce the risk of antibiotic resistance**(19)**. So this study aims to answer the following question What is the comparative effectiveness of synthetic extracts versus natural extracts from spontaneous plants in terms of antimicrobial activity?

The objective of this work is to estimate the antimicrobial performance of three synthetic and natural extracts and three essential oils of spontaneous plants and compare them with other studies of antimicrobial activity. evaluate the antibacterial and antifungal properties of the synthetic extracts and natural extracts from spontaneous plants, to determine their effectiveness in inhibiting the growth of bacteria, fungi and other pathogens.

In order to report on the scientific approach adopted, this manuscript will consist of three parts.

In a first part, a bibliographic review will be presented on medicinal plants, essential oils, secondary metabolites, antimicrobial activity and antibiotics as well as the extraction methods used in this study.

The second part of the manuscript will present the equipment and methods used, in particular the extraction of essential oils and phenolic compounds as well as the study of their antibacterial activity by the methods of diffusion of discs and Micro-dilution, which will conclude this second part.

The results obtained, followed by the discussion then the conclusion and the perspectives will be the subject of the third and fourth parts, respectively. The bibliographic references will constitute the last

INTRODUCTION

part of the manuscript

CHAPTER 1: Medicinal plants

1. General information about herbal medicine

Herbal plants, also known as medicinal plants or herbs, have been used for centuries across various cultures for their therapeutic properties**(20)**. Even with the advent of rational drug discovery in the early 19th century Herbal medicines continue to be widely used globally due to specific health beliefs and due to scientific information becoming more and more available on their benefits to patients as well as their safety**(21)**. These plants contain active compounds that can have medicinal benefits when used in different forms such as teas, extracts, tinctures, or topical applications. Herbal plants are valued for their natural healing properties and are often used as complementary or alternative treatments to conventional medicine**(20)**.

People use herbal plants for a wide range of health purposes, including managing common ailments like colds, digestive issues, insomnia, and skin conditions. Some herbs are also believed to support overall well-being and promote vitality. Examples of commonly used herbal plants include chamomile for relaxation, ginger for digestion, echinacea for immune support, and lavender for stress relief**(20)**. Indeed, many plants that have been used traditionally for therapeutic and culinary applications are generally safe**(22)**, but it is also important to note that while herbal plants can offer health benefits, they should be used with caution as they can interact with medications and may not be suitable for everyone. Consulting with a healthcare provider or a qualified herbalist before using herbal plants is recommended, especially if you have underlying health conditions or are pregnant or nursing**(20)**.

2. Medicinal plants

2.1. Definition

Medicinal plants are plants that have been traditionally used for their therapeutic properties in treating various ailments and promoting health and well-being. These plants contain bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, and others that have pharmacological effects on the human body. Medicinal plants have been an essential part of traditional medicine systems worldwide and continue to be studied for their potential in modern pharmacology and drug discovery**(23)**.

2.2. The origins of medicinal plants

The origins of medicinal plants can be classified into two categories:

2.2.1. **spontaneous plants:** also known as wild plants or native plants, In the context of medicinal plants, spontaneous plants are those that grow naturally in their native habitats without human intervention and have medicinal properties They are not intentionally planted but occur naturally in specific ecosystems.

2.2.2. **cultivated plants:** cultivated plants are intentionally grown by humans for various purposes, including medicinal use. they are not native to the region, they are actively cultivated and utilized for their medicinal properties. These plants are usually cultivated in gardens, farms, or other managed environments. Some of these species may have been imported from other countries, such as Asia, India, or Egypt**(24)**.

2.3. Plant Selection Criteria in Traditional Medicine

2.3.1. **Geographical Origin:**

Plants native to a specific region or ecosystem may be preferred for their adaptation to local environmental conditions and unique medicinal
properties. **(25)**

2.3.2. **Botanical and Chemotaxonomic Classification:**

Botanical and chemotaxonomic classification techniques are frequently used in traditional medicine as plant selection criteria. The process of botanical classification involves classifying and identifying various plant species based on their morphological traits, which include their structure, appearance, and reproductive properties. Traditional medicine practitioners can identify and distinguish between different plants using this classification system based on physical characteristics.

Conversely, chemotaxonomic categorization is the process of identifying and classifying chemical substances found in plants according to their biological makeup. This method aids in comprehending the therapeutic potential and medicinal qualities of plants, which makes it especially significant in traditional medicine. Traditional medicine practitioners can identify plants with specific bioactive

chemicals that are known to have therapeutic effects by researching the chemical composition of various plants. In general, a thorough method for choosing plants for traditional medicine is offered by the integration of chemotaxonomic and botanical classification techniques. Practitioners can choose**(26)**

2.3.3. **Harvest time:**

When using herbal treatments, the time of year that plants are harvested is a crucial consideration in traditional medicine. Many variables often influence the harvest timing, such as:

2.3.3.1. Plant Phenology: The amount of bioactive substances in a plant can vary depending on its stage of growth and development.

2.3.3.2. Seasonality: Many medicinal plants are collected at certain times of the year when their potency is peak.

2.3.3.3. Circadian Rhythms: To guarantee the best possible medicinal effects, several ancient medical systems advise harvesting herbs at particular times of the day.

2.3.3.4. Lunar Cycles: In certain customs, the ideal time to harvest plants is determined by taking into account the lunar calendar.

2.3.3.5. Traditional Knowledge: The best time to gather plants is determined by particular instructions that indigenous communities have. Using this traditional knowledge, practitioners can choose the strongest plants.

2.3.3.6. Environmental Factors: The timing of plant harvest can be influenced by climatic conditions, soil composition, and geographic location.

Traditional medicine practitioners can guarantee that herbal treatments are as effective as possible in treating a range of health disorders by taking these aspects into account and following customary norms**(27)**.

3. Botanical classification of the three selected plants

3.1. Cinnamon plant

Scientific Classification of *Cinnamomum cassia* (28)	
Kingdom	**Plantae**
Class	**Magnoliopsida**
Family	**lauraceae**
Genus	***Cinnamomum***
Species	***cassia***

chemical composition of cinnamon

Cinnamomum cassia, also known as Chinese cinnamon**(28)**, is Chemically composed of : resinous substances such as cinnamaldehyde, cinnamate, cinnamic acid, and various essential oils like eugenol, linalool, and caryophyllene oxide**(29)**. Possesses a variety of benefits, such as anticancer, anti-inflammatory, analgesic, anti-diabetic, anti-obesity, cardiovascular protective, cytoprotective, neuroprotective, immunoregulatory, antimicrobial, and other properties**(28)**.

3.2. Cloves plant

Scientific Classification of *Syzygium aromaticum* (30)	
Kingdom	**Plantae**
Class	**Angiosperms**
Family	**Myrtaceae**
Genus	***Syzygium***
Species	***S. aromaticum***

- ✓ Chemical composition of clove plant:

Principal Substance	**Additional Substances**
Eugenol: This compound is the primary component in the oil extracted from cloves, constituting 72 to 90% of the oil. Eugenol is responsible for the characteristic aroma of cloves and has various properties such as analgesic, antimicrobial, and mouthwash uses.	Phenol: Mainly Eugenol (80 to 88%) Acyl group eugenol: (10 to 15%) α and β-Caryophyllene Methyl radicalaldehyde and dimethyl aldehyde Essential oils: Including acetyl eugenol, beta-caryophyllene, and vanillin Flavonoids: Such as eugenin, kaempferol, rhamnetin, and eugenitin Triterpenoids: Oleanolic acid, stigmasterol, and campesterol Others: Crategolic acid, tannins, gallotannic acid, methyl salicylate, and several sesquiterpenes**(30)**

The properties of clove plant *(Syzygium aromaticum*)
1. Antibacterial Activity
2. Antinociceptive Activity
3. Anti-inflammatory Activity
4. Antifungal Activity
5. Anticancerous Qualities
6. Medicinal Potential
7. Phytochemical Importance
8. Antioxidant Properties Insecticidal Activity
9. Antiviral Activity
10. Antidiabetic Activity
11. Food Processing Applications .**(31)**

3.3. Lavender plant

Scientific Classification of *Lavandula angustifolia* (32)	
Kingdom	**Plantae**
Class	**magnoliopsida**
Family	**Lamiaceae**
Genus	***Lavandula***
Species	***Lavandula angustifolia***

chemical composition of lavender plant	chemical composition of lavender EO
Lavender plantare composed of a variety of chemicals, including: • Tannins • Anthocyanins • Saponins • Flavonoids • Polyphenols • Triterpenes • Sugars • Minerals (zinc, calcium, magnesium, manganese) • Phytosterols • Phenolic acids (glycolic acid, coumaric acid, ursolic acid, valeric acid) • Coumarin • Herniarin	Over 100 components are also present in the lavender essential oil that is produced from the plant; some of the more significant ones are: • Linalool • Linalyl acetate • 1,8-cineole • Camphor • Limonene • Lavandulol **(33)**

✓ Medical Properties:

Lavender has long been utilized for its therapeutic effects and is well known for its medical qualities. It is thought to possess anti-inflammatory, antifungal, and antibacterial qualities.

Aromatic Properties: Due to its pleasant fragrance, lavender is widely employed in the aromatherapy, cosmetic, and pharmaceutical industries. It is a highly aromatic plant.

Antioxidant Compounds: Compounds with antioxidant qualities found in lavender assist shield cells from harm brought on by free radicals.

Skin Healing Properties: Due to its ability to regenerate skin, lavender is used in skincare products. It is thought to aid in the healing of skin irritations, sunburns, wounds, and acne.

Stress-Relieving Properties: Aromatherapy uses lavender to promote relaxation and lessen stress. It is renowned for its ability to relax the body and mind.

Lavender has the ability to naturally repel insects, which makes it a valuable tool for keeping pests at bay.

Sleep-Inducing Properties: Lavender is used to encourage relaxation and enhance the quality of sleep, and it is said to have sedative properties.

Lavender plants are beneficial in a variety of industries, such as healthcare, cosmetics, and agriculture, due to their vast range of qualities**(33)**.

CHAPTER 2: secondary metabolites and Biological activities of plants

1. Secondary metabolites

Plants create substances known as secondary metabolites, which are not directly related to growth, development, or reproduction. They play a variety of functions in a plant's existence, including luring pollinators, facilitating plant-to-plant communication, and supporting defensesystems against external threats**(34).**

1.1. Phenolic compounds

Plants contain a class of secondary metabolites known as phenolic chemicals, which come in a wide range of structural variations. In addition to their immunomodulatory and anti-inflammatory qualities, they are recognized for their possible efficacy against a variety of human viruses.

Phenolic monoterpenes, diterpenes, hydroxybenzoic acids, and phenylpropanoic acids are a few types of phenolic chemicals. They have been researched for their potential uses in medicine andpharmaceuticals and are regarded as significant dietary supplements**(35).**

1.1.2. Flavonoids:

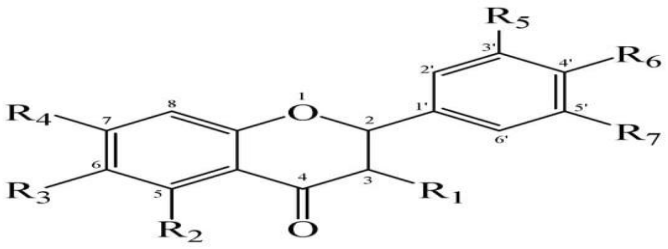


Figure 01 Structure of a typical flavonoid**(36)**

Flavonoid compounds, one of the main classes of plant secondary metabolites, are commonly present in plants. Some plant flavonoids may be used as dietary compounds, which can improve human health and prevent many diseases .Based on the epidemiological, clinical and animal studies, flavonoids may exert beneficial effects on human health when an individual is suffering from various diseases, such as cardiovascular disease and cancer**(37)**.

1.1.3. Tannins:

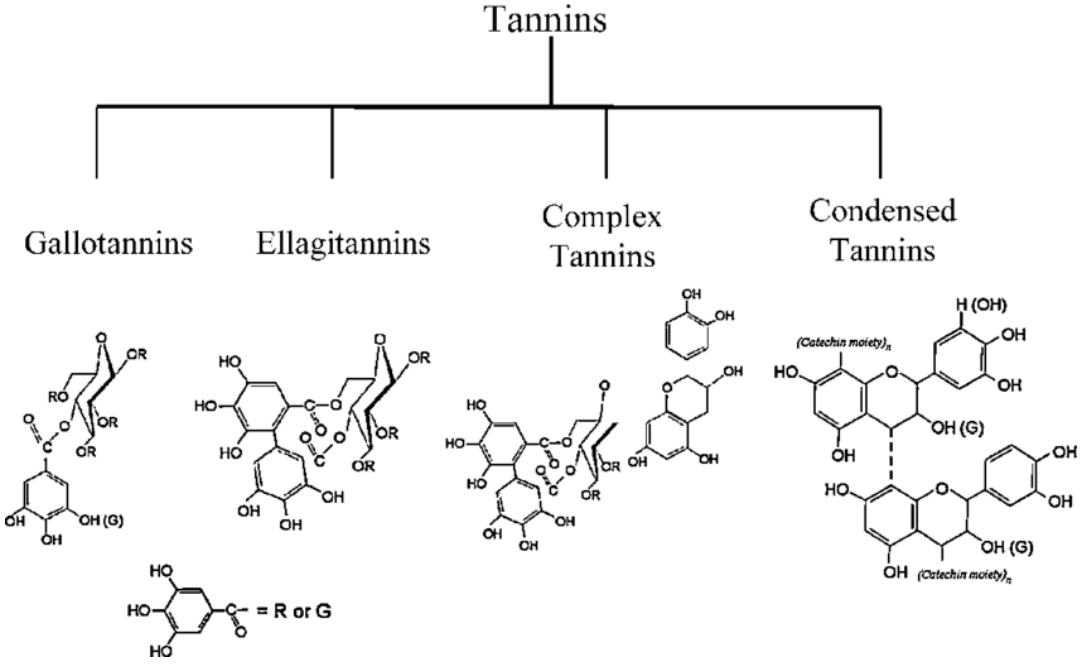


Figure 02 Main chemical structures of the tannins**(38)**

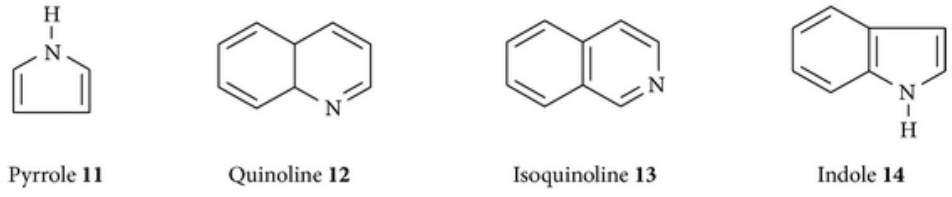
Tannins are polyphenols containing aromatic rings. These compounds are classified into two

main groups: hydrolysable and non-hydrolysable tannins. Different biological and pharmacological activities have been associated with the composition and function of tannins. Tannins and the multiple functional groups, such as hydroxyls, of their chemical structure enable them to develop links to establish a stable reticulate association within different molecules, such as proteins or carbohydrates. This unique feature allows their differentiation into polyphenolic groups**(39).**

1.2. Terpenoids

Terpenoids, also known as isoprenoids, are the most numerous and structurally diverse natural products found in many plants. Several studies, in vitro, preclinical, and clinical, have confirmed that this class of compounds displays a wide array of very important pharmacological properties. The diverse collection of terpenoid structures and functions has evoked increased interest in their commercial use resulting in some with established medical applications being registered as drugson the market**(40)**.

1.2.1. Alkaloids:



Pyrrole **11** Quinoline **12** Isoquinoline **13** Indole **14**

Figure 03 Typical basic structure of alkaloids.**(41)**

Alkaloids are nitrogen-containing compounds derived from amino acids. These compounds areclassified into the following three types:

- ✓ alkaloids with heterocyclic nitrogen.
- ✓ protoalkaloids without cyclic nitrogen.

- ✓ pseudoalkaloids.

These nitrogenous compounds may act on lipid metabolism because enhanced de novo fatty acid synthesis in cancer cells is mainly used for membrane formation, energy storage, and signalling molecule production. In summary, lipid metabolism disorders can cause severe diseases and health complications**(39)**

1.2.2. Saponosids :

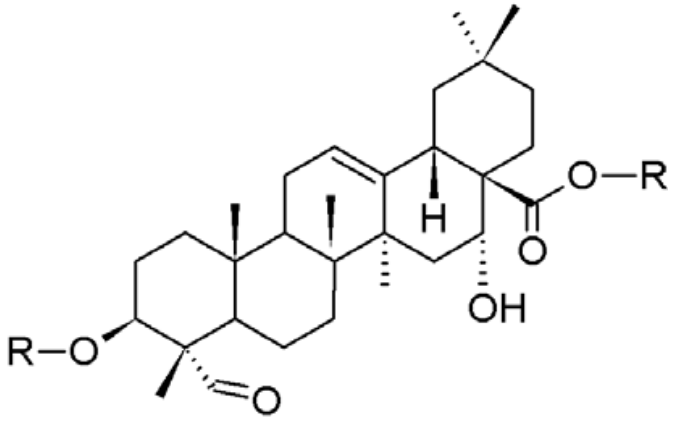


Figure 04 Generic structures of saponins. R= sugar moiety.**(42)**

Saponosides, also known as saponins, are secondary metabolites that are commonly found in medicinal plants. These compounds are characterized by their ability to produce soapy foam when shaken in water due to their amphipathic nature (having both hydrophilic and hydrophobic properties). Saponosides have been widely studied for their various biological activities and potential health benefits. In the context of the document snippets provided, saponosides were mentioned in relation to the phytochemical screening of several plants.

the presence of saponosides was observed in the following plants:

- Inula viscosa: Saponosides were found in the leaves of Inula viscosa in different percentages depending on the extraction solvent used, with the highest percentage observed in the ethanolic extract.
- Salvia officinalis: Salvia officinalis also contained saponosides, with the highest percentage present in the ethanolic extract.

- Rosmarinus officinalis: Saponosides were present in Rosmarinus officinalis as well, withsignificant levels found in the ethanolic extract.

The presence of saponosides in these plants supports their traditional therapeutic use and suggeststheir potential in treating various ailments. However, it is important to note that the specific therapeutic effects and mechanisms of saponosides may vary depending on the plant species and the specific compounds present. Further research is needed to explore the bioactive properties and potential applications of saponosides in modern medicine**(43)**.

Saponosides are a type of secondary metabolite found in medicinal plants. These compounds have been identified in several plant species used in traditional medicine for the treatment of various conditions, including hemorrhoids. The presence of saponosides in plants like Syzygium guineense, Balanite aegyptiaca, and Detarium microcarpum suggests their potential therapeutic properties against hemorrhoids. Other plants, such as Acacia nilotica and Ximenia americana, also contain saponosides. Saponosides are known for their diverse biological activities, including anesthetic, antitumor, cicatrizing (wound healing), and anti-inflammatory effects. They are also considered vasculoprotective and veinotonic, which may contribute to their potential effectiveness in treating hemorrhoids. These findings are based on the phytochemical screening of the selected plants in the study**(44)**.

2. Essential oil

2.1. Definition:

Essential oils (EOs) can be defined as complex natural mixtures of volatile, lipophilic, and odoriferous substances commonly found in aromatic plants. According to the "Association Française de NOR malisation" and the European Pharmacopoeia, essential oil is a product obtained from a natural raw material of plant origin. It can be obtained through distillation with water or steam, from the epicarp of Citrus sp. fruits by a mechanical process, or by dry distillation. Essential oils are separated from the aqueous phase by physical means. These oils arecharacterized by their low molecular weight, colorless or pale yellow appearance, and liquid stateat room temperature. They are less dense than water, except for a few exceptions. Essential oils consist of various compounds, typically 20-60 components found in different concentrations.

They are primarily composed of terpenoids and non-terpenoids, which include aldehydes, ketones, alcohols, oxides, esters, phenols, and other classes of compounds**(45)**.

2.2. General:

Essential oils are complex natural mixtures of volatile, lipophilic, and odoriferous substances commonly found in aromatic plants. They are typically colorless or pale yellow, liquid at room temperature, and less dense than water. The chemical constituents of essential oils have a low molecular weight and can be optically active. Essential oils have been extensively studied for their therapeutic potential in various pathologies. They exhibit a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antitumor, and antioxidant effects. These oils are obtained from natural plant sources through processes such as distillation with water or steam, mechanical extraction from citrus fruit epicarps, or dry distillation. Essential oils are separated from the aqueous phase by physical means. The diversity of chemical compounds found in essential oils contributes to their biological and pharmacological activities. Their lipophilic nature allows them to penetrate cells and tissues to interact with biological targets. Dueto their ability to restore balance in oxidative stress-related disorders and their antimicrobial activity, essential oils are considered a promising source for developing new drugs**(45)**.

3. Extraction methods used

3.1. solvent extraction : The process of solvent extraction, also called liquid-liquid extraction, involves transferring a solute from one solvent (the filler) to another solvent (the extractant) where the solute is more soluble. Methanol is often employed as a solvent in the extraction of bioactive compounds from plant materials due to its ability to efficiently dissolve a variety of compounds**(46)**.

3.2. Hydrodistillation: is a technique for obtaining essential oils from plant materials like flowers, herbs, and spices. Using heat to cause vaporization and water as a solvent**(47)**.

4. Biological activities of plants

Plants exhibit a wide range of biological activities due to the presence of various bioactive compounds, including toxic proteins. These activities play crucial roles in plant defense mechanisms, interactions with other organisms, and adaptation to environmental stresses. Somecommon biological activities of plants include:

- **Defense Mechanisms:** To protect themselves from infections, parasites, pests, and insects, plants create toxic proteins such lectins, ribosome-inactivating proteins, protease inhibitors, and antimicrobial peptides. These proteins have the ability to interfere with vital biological functions in invasive species, causing inhibition or even death.
- **Antimicrobial Properties:** A variety of pathogens, such as bacteria, fungi, and viruses, can be inhibited by plant-derived chemicals, such as antimicrobial peptides. These peptides are essential for shielding plants against microbial diseases.
- **Enzyme Inhibition:** Certain plant proteins, such as α-amylase and protease inhibitors,might prevent pests or diseases from using certain enzymes, which will interfere with their ability to survive and carry out their physiological functions.
- **Plant chemicals with Anticancer Potential:** By causing cancer cells to undergo apoptosis, or programmed cell death, or by preventing tumor growth, several plant chemicals have demonstrated encouraging results in cancer therapy.
- **Phytotoxicity:** Toxins originating from plants can also impede the growth and development of other plants. Plants benefit from this competitive advantage in terms ofacquiring resources and surviving.
- **Nutritional Benefits:** Not all plant components are harmful to humans; a

number of phytochemicals found in fruits, vegetables, and herbs have anti-inflammatory, antioxidant, and other health-promoting qualities.**(48)**

4.1. Allelopathy phenomenon

Allelopathy is a fascinating process in which plants send biochemical into the surrounding environment that affect nearby microbes, other plants, and organisms' growth, development, and behavior. Allelochemicals are these substances that can affect species around them in both stimulatory and inhibitory ways. Some salient features of allelopathy are as follows:

- ✓ **Inhibition of Competitors:** Plants can produce allochemicals that prevent other plant species from germinating, growing, or developing in their immediate environment. Allelopathic plants benefit from this competitive advantage in terms of resource acquisition and domination.
- ✓ **Defense strategy:** By generating allelochemicals that repel or discourage herbivores, pests, and infections, certain plants employ allelopathy as a defensestrategy.
- ✓ **Effect on Soil Microorganisms:** Allelochemicals have the ability to modify the makeup and activity of soil microorganisms, which in turn affects plant-microbe interactions, soil health, and the cycling of nutrients.
- ✓ **Use in Agriculture:** Creating natural pesticides from allelochemicals to suppress weeds and increase crop yields is one way that an understanding of allelopathic interactions can be put to use in agriculture.
- ✓ **Environmental Significance:** By affecting the species composition and distribution, allelopathy shapes plant communities and biodiversity in naturalecosystems.
- ✓ **Research and Studies**: To understand the mechanisms underlying these interactions, discover allelochemicals, and investigate their possible uses in pharmacological, ecological, and agricultural fields, scientists investigate allelopathy.**(48)**

4.2. Antimicrobial activity

The ability of some compounds to stop the growth of microorganisms, such as bacteria,

fungus, viruses, and parasites, or to eradicate them is known as antimicrobial activity. Many substances having antimicrobial qualities, including lectins, antimicrobial peptides, and

other bioactive molecules, are produced by plants. The following are important details about plant antibacterialactivity:

- ✓ **AMPs, or antimicrobial peptides**: Plants create tiny peptides known as AMPs as a defensive mechanism. These peptides are excellent candidates for the development of novel antimicrobial medicines because they have strong antibacterial action against a range of diseases.
- ✓ **Lectins:** Present in plants, lectins are proteins that bind carbohydrates and possess antibacterial and agglutinating abilities. They have demonstrated promise in the management and prevention of infections, such as HIV and other coinfections.
- ✓ **Secondary Metabolites**: Alkaloids, terpenoids, tannins, and glycosides are just a few of the many secondary metabolites that plants make that have antibacterial qualities. These substances function as natural defense mechanisms by preventing the growth of germs.
- ✓ **Uses in the Medical Field**: The possibility of plant-derived antimicrobial chemicals as atherapeutic intervention for a range of infectious disorders has been investigated. They provide an alternative to traditional antibiotics and might be useful in the fight against antibiotic resistance.
- ✓ **Food Preservation:** By preventing the growth of diseases and bacteria that cause spoiling, antimicrobial substances derived from plants are also utilized in food preservation to increase the shelf life of food products.

Research and Development: Current investigations try to discover new antimicrobial chemicals derived from plants, comprehend their modes of action, and investigate their potential usage inhealthcare, agriculture, and other sectors**(48)**.

CHAPTER 3: Antimicrobial activity and antibiotics

1. Overview of bacteria

Bacteria are metabolically active, single-celled microorganisms that divide by binary fission and lack a nuclear membrane. In terms of medicine, they are a major cause of disease. On the surface,bacteria seem like very basic life forms, but they are actually very intelligent and very adaptive. Numerous types of bacteria may use a wide range of hydrocarbon substrates, such as phenol, rubber, and petroleum, and many of them reproduce quickly. These creatures are extensively distributed in both free-living and parasitic forms. due to their widespread distribution and extraordinary ability to select for spontaneous mutations that adapt to changing settings **(49)**.

There are various ways that scientists categorize and define bacteria.

- ✓ **Scientific name:** A scientific name is one method used by scientists to categorize germs. The scientific name contains the species of the bacteria as well as their genus, which is determined by the traits of the bacterium. For instance, the bacteria that causes botulism is known scientifically as "Clostridium botulinum."
- ✓ **forms of bacteria :**Scientists may find various bacterial strains, or kinds, with in a species forms of bacteria, the forms of bacteria are another method that scientists categorize them. There are three basic bacterial shapes:
    - Spheres or ball-shaped (cocci bacteria).
    - Rod-shaped bacteria (bacilli)
    - Spirals or helixes (spirochetes).
- ✓ **Need for oxygen:** Bacteria are also categorized by scientists

according to their requirement for oxygen in order to survive and proliferate. Aerobes are bacteria that require oxygen to survive. Anaerobes are bacteria that are incapable of growing or surviving in the presence of

oxygen. Oxygen is not necessary for the survival and growthof certain bacteria. We refer to these as facultative bacteria.

- ✓ **Genetic makeup:** The genetic makeup of bacteria is another method used by scientists to categorize them. Every bacterium has a unique genetic composition. We refer to this as their genotype. The variations in the genotype of each bacteria can be ascertained throughspecialized testing.
- ✓ **Staining:** After applying particular chemicals, or stains, to bacteria, scientists can categorize the bacteria based on the color changes. Gram staining is a popular staining method. There are two types of bacteria: gram-positive and gram-negative. Because gram-positive and gram-negative bacteria react differently to specific antibiotics, gramtaining also aids in treatment guidance**(50).**

2. Antibacterial activity

The World Health Organization states that the greatest place to find a wide range of more recent herbal medications is from medicinal plants. Approximately 80% of people in developed nations use traditional medicine, which contains substances derived from therapeutic plants. To learn more about these plants' characteristics, safety, and effectiveness, further research should be done on them. With their established antibacterial qualities, the application of phytochemicals and plant extracts can be very important to medical interventions. Numerous research demonstrating this efficiency have been carried out in various nations in the past few years**(51)**.

The antibacterial properties of many plants have been exploited; these properties are a

result ofchemicals that are produced during the secondary metabolism of the plant. These products are identified by their active ingredients, such as the essential oils' phenolic components**(52)**.

Currently, there are a number of methods available to detect their antimicrobial activity.

However, not all of these methods are based on the same principles, so the method chosen, themicroorganisms used, the extraction technique, and the degree of solubility of each test compound all affect the results. To handle a variety of extracts and fractions, antimicrobial test

systems should ideally be easy to use, quick, repeatable, affordable, and able to handle a highvolume of samples**(53)**.

3. The selection criteria applied to the bacteria

Certain bacterial pathogens are more common in health-related contexts, the plant's traditionaluses, and the availability of standardized testing procedures all play a role in the selection of bacteria used to test the antibacterial activity of medicinal plants like lavender, cinnamon, and clove. Regular selection criteria for microorganisms could consist of:

- Pathogenicity: Staphylococcus aureus, Escherichia coli, Salmonella spp., and Pseudomonas aeruginosa are among the bacteria that are frequently selected for testing because of their reputation for causing human infections or foodborne diseases.
- Clinical relevance: Priority is given to bacterial strains that are linked to common infections, such as those that are acquired in the community or identified in hospitals.
- Standardization: To guarantee uniformity and comparability among investigations, standard bacterial strains suggested by institutions such as NCTC or ATCC may beutilized.

- Diversity: To evaluate the broad-spectrum activity of the therapeutic plant extract or molecule under evaluation, researchers may choose a variety of bacterial species.
- Prior studies: To make comparisons with the body of literature easier, bacterial strains that have been previously investigated in relation to the antibacterial activity of the medicinal plant are frequently selected. Most people agree that the strain needs to be

well- characterized, of host origin, capable of colonizing and surviving the harsh conditions of the digestive tract, physiologically active against the target, stable, and adaptable to commercial production and distribution. It is also necessary to gather data on doses and proof of efficacy. To investigate the various aspects, in vitro and in vivo investigations areoften combined, and ultimately, clinical trials are needed. While lactic acid bacteria are widely acknowledged to be harmless, the safety of both LAB and non-LAB probiotic

strains is questioned in terms of the strains' potential pathogenicity and potential risks tothe community and individual.

Finally, despite the availability of methods for genetically modifying numerous probiotic strains, it is unlikely that this matter will be tackled anytime soon because of potential regulatory ramifications. It is suggested that probiotic strains with observable activity can be obtained by using this kind of selection criteria**(54)**.

4. mechanism of action of phenolic compounds

The discovery of antibiotics is among the most significant medical advancements of the 20th century. Unfortunately, the selection and spread of resistant bacterial strains as well as a sharp rise in the treatment failure ratio have resulted from their excessive, irrational, and inappropriateusage. There are numerous resistance mechanisms that bacteria have evolved, including:

- modification of the antibiotic binding site.

- production of enzymes which can degrade or change the antibiotic structure.
- mutations in genes encoding transport proteins resulting in cell wall permeabilitydisruptions.
- active pumping out of the antibiotics molecules**(55)**.

Several mechanisms, including multi-target action, in which each compound acts on a differentsite in the bacterial cell, pharmacokinetic or physicochemical properties, such as an increase inthe solubility or bioavailability of the antibiotics, or targeted at a specific bacterial resistance mechanism, can explain why natural compounds enhance the antibacterial action of antibiotics**(56)**

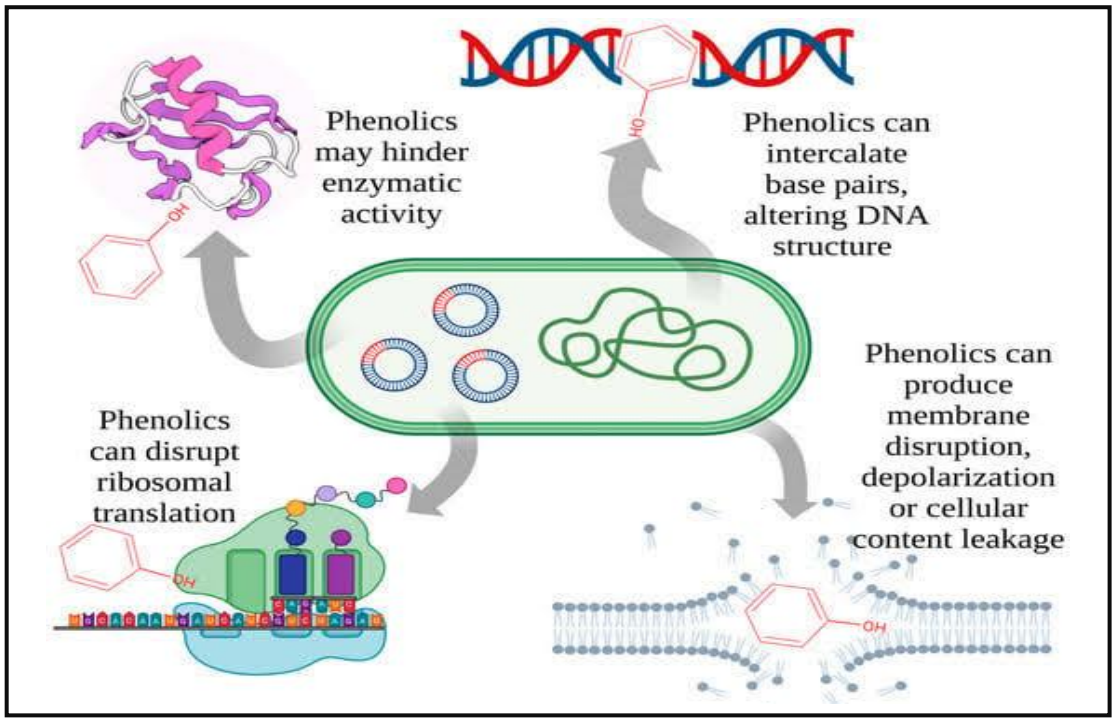


Figure 05 : Schema mechanism of action of phenolic compounds on Pathogenic Bacteria(56).

Each bacteria has a different response to these latter compounds, we can determine multiplemodes of action:

4.1. Interaction in the Bacterial membrane

Antimicrobial action of phenolic acids is demonstrated against both Gram-positive and Gram- negative microorganisms. Their antibacterial potential is dependent on species, strains, and the chemical structure of the compounds, specifically the number and location of substituents in the benzene ring and the length of the saturated chain, and their activity is less than that of other phenolic compounds **(57)**. As with gallic and chlorogenic acids, one theory about the mechanism of action of phenolic acids is a reduction in extracellular pH**(58)**.

The antibacterial activity is dependent on the interaction location with target molecules, resulting in distinct action mechanisms, in addition to its basic chemical features. When bacteria are present, phenolic acids can operate as a barrier to stop the leaking of various components of cells, such as proteins, nucleic acids, and inorganic ions like phosphate or potassium. They function at the cytoplasmic and membrane levels. Hyperacidification, which modifies cell membrane potential, is another mode of action at the membrane level. The dissociation of phenolic acids results in an increase in membrane permeability, which impacts the sodium-potassium pump.

Because they lack an outer membrane, gram-positive bacteria are more vulnerable to this antibiotic action. A high quantity of phenolic acids (1000 µg/mL) was discovered to haveantibacterial activity against lactobacilli such as L. paraplantarum LCH7, L.
plantarum LCH17, L. fermentum LPH1, L. fermentum CECT 5716, L. brevis LCH23 and L.coryniformis CECT 5711**(59)**.

Phenolic acids, like gallic acid, can also alter the membrane's charge, hydrophobicity, and permeability. In the case of Gram-negative bacteria, they can even cause the membrane todissolve by the chelation of divalent cations**(60)**.

In the meantime, the dissociation capacity influences the antibacterial activity, which is dependent on the strains and the number and location of substituents in the benzene ring. For instance, phenolic acids inhibit the growth of bacteria like S. aureus ATCC 29213 and EP167, with respective ICs of 1000 µg/mL and 667 µg/mL, and dissociate at the cell membrane level ofGram-positive pathogens **(59)**.

Although bacteria have intricate, multilayered structures that help shield them from the environment, polyphenols can directly attach to and harm bacterial cell membranes. The intricacyof the cell membrane facilitates the flow of nutrients or waste products in addition to aiding in the survival of the bacterium. Because they lack an outer membrane and have peptidoglycans on their surface, gram-positive bacteria are the most susceptible to phenolic chemicals. This amount of response is caused by the presence of functional groups from the membrane structure.

Polyphenols, for example, have the ability to harm the bacterial cell wall by binding peptidoglycans from the membrane structure through their hydroxylic groups. Both the kind of bacteria and the structure of the polyphenol affect how resistant they are to this kind of antibacterial action. The outer membrane, the peptidoglycan layer, and the inner membrane make up the cell membrane of Gram-negative bacteria, as opposed to Gram-positive bacteria. These three layers are more resistant to the antibacterial activity of phenolic compounds. The high concentrations of phospholipids on the lipophilic outer membrane are linked to this resistance **(61)**.

In this instance, the accumulation of hydroxylic groups in lipid bilayers damages lipoprotein association and increases cell membrane permeability as part of the antibacterial mechanism. Moreover, polyphenols have the ability to alter cell architecture, disrupt membrane integrity, impact metabolism, and release cellular contents. The phospholipid bilayer's destruction alters physiological processes and cell division, ultimately leading to cell death **(57)**.

4.2. Penetration of phenolic compounds into the cytoplasm

Depending on their physicochemical characteristics, some phenolic compounds have the abilityto pass through membrane borders and enter the cytoplasm. The cytoplasmic level is where phenolic acids function in the case of Gram-negative bacteria. The concentration of phenolic acids in their undissociated state determines the antibacterial action. In this state, phenolic acids can passively diffuse across the cell membrane due to their somewhat lipophilic nature **(62)**.

Polyphenols have the ability to affect the permeability of cell membranes, which can result in the leakage of cellular contents, including DNA. Additionally, phenolic chemicals have the ability to bind to genomic DNA, changing the shape and secondary structure of DNA **(63)**.

4.3. Acidification of the cytoplasm

Intracellular phenolic acidification lowers the pH of the cytoplasm. They induce protein denaturation by lowering pH and rupturing the structure of the cell membrane. Potassium effluxes rise as a result of increased permeability brought on by the membrane degradation. On the cell membranes of E. Coli, P. aeruginosa, S. aureus, and L. monocytogenes, the antibacterial activity of hydroxycinnamic acids (ferulic acid) and hydroxybenzoic acids (gallic acid) was evaluated. It was discovered that ferulic acid acts against bacteria with MICs between 100 and 1250 µg/mL, while gallic acid acts with MICs between 500 and 2000 µg/mL **(62)**.

Moreover, by inhibiting enzymatic activity, phenolic substances have antimicrobial properties. Protein-phenolic interactions, which can be covalent or noncovalent and rely on the structural characteristics of proteins such as hydrophobicity, molecular weight, conformational configuration, amino acid composition, and sequence, are the mechanism regulating such expression. These substances are capable of complexing with metal ions, forming ligands with iron, copper, and zinc that influence the activity of bacterial enzymes **(64)**

By inhibiting bacterial enzymes like hydrolase, oxidoreductase, lyase, and transfer enzymes,

polyphenols alter the metabolism of bacteria. For instance, because epigallocatechin gallate inhibits S. maltophilia's dihydrofolate reductase, it has antibacterial activity against the bacterium**(65)**.

The oxidation of phenolic compounds is catalyzed by polyphenoloxidase, which results in oxidized chemicals that inactivate the enzyme glucan synthase, which is attached to the plasma membrane. Oxidized polyphenols undergo covalent changes that permanently alter the structure of glucan synthase. Furthermore, even in the absence of polyphenoloxidase, several polyphenols, including epicatechin, pyrogallol, tannic acid, and catechin, inactivate glucan synthase activity **(66)**.

4.4. Disturbance of metabolism

the antibacterial mechanism's objective is the suppression of gene expression, which is occasionally connected to virulence factors generated by pathogenic bacteria. For example, the virulence factors staphyloxantin, generated by S. aureus, and listeriolysin O, produced by L. monocytogenes, which causes gastroenteritis and meningitis, are inhibited by flavone and flavonol, respectively, in gene expression **(67)**. Methyl gallate, a phenolic molecule, regulates autoinducer synthase, lasl and rhll, and cognate receptors, lasR and rhIR, in addition to controlling the gene expression of C violaceum and P. aeruginosa **(68)**. Gallic, protocatechuic, and vanillic acids are examples of phenolic acids that can downregulate the expression of genes involved in the synthesis and assembly of the Type III Secretion System (T3SS), which is responsible for invading host cells. Other virulence genes that can be downregulated include flic, which increases motility, and sipA, which is responsible for intracellular survival **(69)**.

5. The essential oil’s mechanisms of action

The chemical composition of essential oils determines how they work, and research indicates thatthe antimicrobial activity of these oils is not attributed to a single mechanism, but rather to a series of reactions involving the entire bacterial cell **(70)**. These characteristics collectively are known as the "essential oils versatility." EOs generally have

the effect of preventing bacterial cellproliferation as well as the generation of harmful bacterial metabolites. Due to variations in the makeup of their cell membranes, Gram-positive bacteria are more susceptible to the effects of most EOs than Gram-negative species **(71).**

Many different methods have been proposed to explain how an EO acts on bacterial cells. An EO's activity can impact a cell's cytoplasm as well as its external envelope. Due to the inability to separate the EOs from the bacterial cell membrane, the hydrophobicity characteristic of EOs causes disruption of bacterial structures and increased permeability. The permeability barrier that cell membranes provide is essential for a variety of cellular operations, such as solute transport, metabolic control, membrane-coupled energy transduction, and preserving the energy status of the cell. Turgor pressure regulation also depends on the cell membrane **(72).**

Damaging the membrane proteins, increased permeability that causes cell contents to leak out**(73)**, a decrease in the proton motive force**(74)**, a reduction in the intracellular ATP pool due to a decrease in ATP synthesis, increased hydrolysis that is distinct from the increased membrane permeability, and a decrease in the membrane potential due to increased membrane permeability **(70)**.

The double lipid layer of the membrane is permeable to the EOs and their constituents due to their hydrophobicity. Membrane protein function and permeability can both be changed by the EOS. Certain essential oils, especially those high in phenolics, have the ability to penetrate thephospholipid bilayer of bacterial cell walls, attach to proteins, and stop those proteins from carrying out their intended task **(75)**.

This phenomenon indicates that the membrane is the first target of EOs . As previously reported, the EO's mode of action is not solitary; rather, it involves a number of processes that occur both inside the cytoplasm and on the cell surface. A "disbalance" develops within the microbial cell as a result of changes in membrane permeability and flaws in the movement of molecules and ions. This ultimately results in the loss of metabolites and ions, cytoplasm coagulation, denaturation ofmany enzymes, and cellular proteins **(76)**.

A single mechanism cannot be responsible for the antibacterial action of the EOS due to the

large range of chemicals found in the natural extracts. Rather, various biochemical and structural processes are engaged **(77)** at various locations on the cell surface as well as within the cell.

These methods, which have the ability to entirely alter the shape of the microbial cell, involve chemical alterations of the cytoplasm, enzymes, proteins, and cell membrane. Moreover, exposure to an EO can compromise microbial metabolism and cause cell death through the prolonged loss of ions or minerals **(76).**

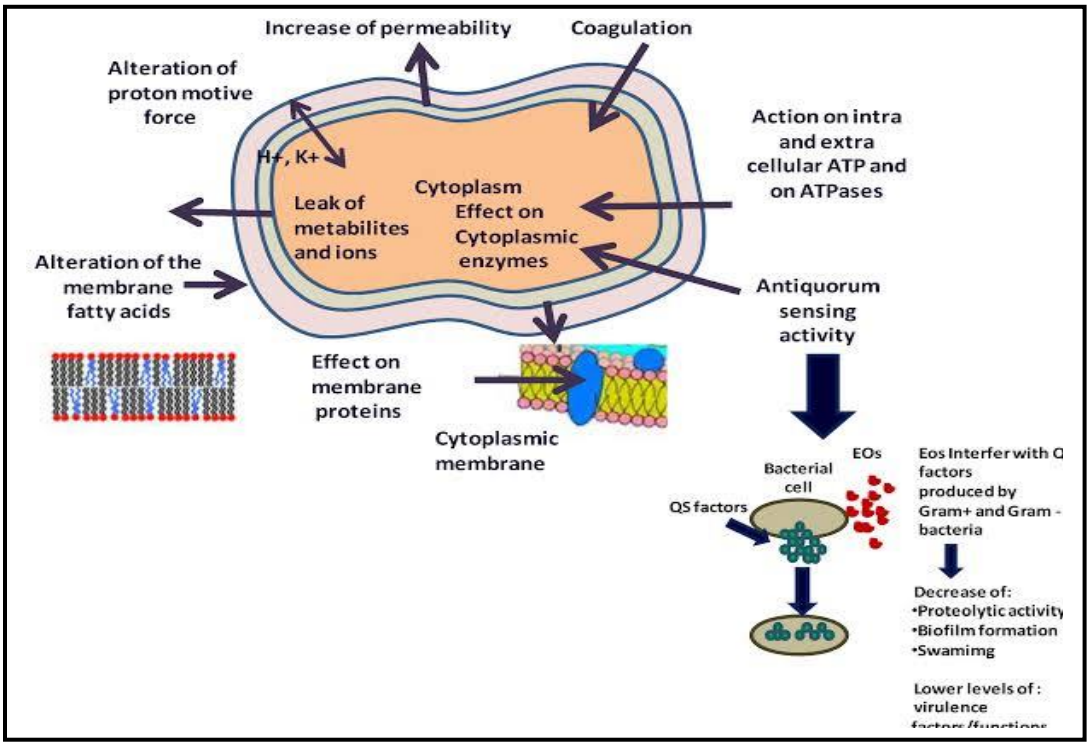


Figure 06 : Schema mechanism of action of Essential Oils on Pathogenic Bacteria(72)

6. Antifungal Activity

As fungal infections are caused by eukaryotic organisms, as opposed to bacterial infections, it is more challenging to identify them and administer the proper therapeutic intervention. The chitin structure found in the cell walls of fungi, which is lacking in human cells, makes them an ideal target for highly toxic antifungal medicines. Although chemical treatments are often effective, it is possible to generate resistant strains and intrinsically resistant species. The inoculum charge, the

host's immune system, and resistance all affect the fungal infection's development and severity. One of the most promising natural items for inhibiting fungus is essential oils (EOs) **(78).**

Through certain mechanisms, EOs and other phytochemicals may inhibit the growth of microorganisms and the formation of biofilms (79).This is a particularly valuable aspect: Microorganisms are known to initiate a certain mechanism that results in the synthesis and manufacturing of chemicals, microbial communication signals, and the development of specific pathogenicity parameters, like the formation of biofilms, in addition to a specific growth threshold value. Many essential oils have broad antibacterial capabilities that make them useful for preventing microbiological deterioration, preserving food safety and quality, and extending the shelf life of food **(80).**

The Food and Drug Administration (FDA) classifies essential oils as "Generally Recognised as Safe" (GRAS), meaning that they are safe and, because of their natural nature, are more commonly accepted by consumers than "synthetic" substances **(81)**. The properties of terpenes/terpenoids, which are highly lipophilic and have a low molecular weight, may be responsible for the antimicrobial or antifungal activity of essential oils. These properties can disrupt the cell membrane, resulting in cell death, or prevent food spoilage fungi from sporulating and germination. Therefore, when terpenes/terpenoids are employed as single compounds as opposed to the entire EO, many in vitro experiments reveal inadequate antibacterial efficacy **(82).**

Flavonols make up 83% of the primary phenolic chemicals found in leaves, while flavan-3-ols make up 62% in stems and canes.Stilbenes accumulate primarily in the canes and stems.

Pterostilbene (PTB), an inducible antifungal molecule found in grapevine leaves, is one of the most potent antifungal substances even more potent than viniferins and resveratrol **(83).**

The genus of fungi Candida is the predominant fungal infection that affects humans. The primary systemic fungal disease and the primary cause of morbidity and mortality globally is candidiasis.

Numerous disease categories, including invasive or systemic illnesses as well as mucocutaneous infections like vaginitis and intertrigo, are included in the category of candidiasis. C. albicans is the most frequent species of Candida that infects people and causes illnesses. Biofilm development is often linked to C. albicans infections. Compared to planktonic cells, the cells within biofilms are far more resistant to the standard antifungal

therapy **(84)**

7. Natural antibiotics

One of the greatest medical breakthroughs ever made is the discovery of antibiotics. Since nothing was known about infections, how to prevent them, about antibiotics or vaccines, people were defenseless against massive waves of epidemics, including cholera, smallpox, plague, typhoid fever, malaria, tuberculosis, leprosy, and syphilis, for thousands of years. The discovery of the antibiotic-producing bacteria' therapeutic properties brought about a gradual improvement in the condition. The development of contemporary medicine was made possible by the discovery of antibiotics. Antibiotics, in addition to a thorough understanding of pathogens, upholding hygienic practices, and controlling infectious diseases, have improved life expectancy and qualityof life today **(85)**.

Antibacterial agents can be categorized into multiple primary categories based on data found inthe literature. These groups can be identified by factors such as source, chemical structure, method of action, type of action, and spectrum of activity **(86).**

Antibiotics are specifically made to affect microorganisms, and the way bacteria react to them depends on their concentration**(87)** .Antibiotics have the ability to act both bacteriostatically and bactericidally on bacteria at high doses, albeit deadly amounts are rarely achieved outside of therapeutic applications **(88)**. While bactericidal treatments kill bacteria, bacteriostatic drugs just inhibit the growth of bacterial cells. Since certain antimicrobials are bacteriostatic for some bacterial pathogens but bactericidal for others, the words "bacteriostatic" and "bactericidal" are general classifications that may not apply to a particular agent against all organisms. Because the lethal impact of each medicine varies depending on the test technique and species studied, these categories are therefore not absolute**(88)** .

Antibiotics have a negative impact on natural microbialcommunities because they can have bactericidal and bacteriostatic effects, which can lead to the extinction or suppression of some microbial groups that are essential to the functioning of ecosystems. Antibiotics, however, have the potential to selectively affect certain microbial

populations, leading to the development of resistance, generating genetic and phenotypic variability, and affecting a range of physiological activities; in certain instances, bacteria may even acquire the ability to degrade antibiotics (an indirect effect) as a homeostatic reaction to astressful situation**(89)** .

8. Concept of Minimum Inhibitory Concentration and Minimal bactericidalconcentration (MIC) (MBC)

MIC is the minimum inhibitory concentration (measured in mg/L or μg/mL) of an antibacterial drug that, when applied under carefully regulated in vitro conditions, totally stops the test strainof an organism from growing **(90)**.

-This is the lowest concentration of extracts for which growth bacterial is inhibited by (99.9%) after 18 to 24 hours of contact at 37 degrees, and allows determine whether a strain is sensitive orresistant to the antibiotic or extract tested**(91).**

Minimal bactericidal concentration (MBC) : (MBC) become recorded as a lowest extract attention killing 99.9 % of the bacterial inocula after 24 h incubation at 37 °C. Each test becomerepeated as a minimum 3 times. MBC values have been decided with the aid of using getting ridof one hundred μl of bacterial suspension from tradition demonstrating no seen boom and inoculating nutrient agar plates. Plates have been incubated at 37 °C for a complete length of 24

h. The MBC is decided with the wells whose the concentrations are ≥ MIC

- ✓ Evaluation of bactericidal and bacteriostatic capacity:

The movement of an antibacterial at the bacterial traces may be characterised with parameters which include Minimum inhibitory attention (MIC) and Minimum bactericidal attention (MBC). According to the ratio MBC/MIC, we preferred antibacterial activity. If the ratio

MBC/MIC = 1 or 2, the impact become taken into consideration as bactericidal however if the ratio MBC/MIC =four or 16, the impact become described as bacteriostatic.**(92)**

MATERIAL AND METHODS

WORK PLACE :

-This work was carried out at the level of the microbiological engineering laboratory and Applications of Amar Telidji university of laghouat . The aim of this work is to extract and identify the roles of the antimicrobial activity of the extracts obtained via the three chosen plants.

1. Plant material

The Bark of *Cinnamon cassia*, cloves of *Syzygium aromaticum* and the aerial part of *Lavandulaangustifolia* used in this study was purchased from royal mill store

We used approximately 100 grams of crushed cloves, lavender and cinnamon for the extractionof their essential oil and for the extraction of phenolic compounds we used 10 grams of each crushed plant.

2. Extraction of natural extracts for the three chosen plants

2.1. Extraction of essential oils

2.1.1. The hydrodistillation for extracting essential oils was carried out in the research laboratory of Mr. Chaibi according to the following process :

- ✓ Loading the still: 50 g of cloves powder is placed into a distillation apparatus, such as astill or distillation flask.
- ✓ Adding water: Water is added to the still containing the cloves. The quantity of wateradded is 500 ml, covers the plant material but without excess in order to dilute the essential oil.
- ✓ Heating: The still is heated, generally using a balloon heater. The heat boils the water andproduces steam.
- ✓ Distillation: When water boils, the steam carries away the volatile compounds present inthe clove, including the essential oil. The steam passes through the plant material, extracting essential oil and other aromatic compounds.

- ✓ Condensation: The vapor containing the essential oil is then cooled and condensed into a liquid. The resulting mixture of water and essential oil is collected in a separate containercalled a receiver.
- ✓ Separation: Since essential oils are immiscible with water, they float on the surface of

thecollected distillate. The essential oil is separated from the water and collected for further processing and storage.

- ✓ Storage: The extracted clove essential oil is stored in dark, airtight containers away fromlight and humidity in the refrigerator to preserve its quality and potency**(93)**.
- ✓ The experiment was repeated two times.
    - ❖ we followed the same extraction process for cinnamon (repeat twice with 50 g of plant material and lavender once with 50 g of plant material.

Figure 07: Extraction of E.O from cloves. (personal prise)

2.2. Extraction of phenolic compounds

Solvent extraction using methanol proceeds in the following steps:

- Solvent Selection: Methanol is selected as the extraction solvent due to its polarity, itsability to dissolve different compounds **(46)**.
- Contact and separation phase: we dissolved each plant material in a dark bottle (10 g crushed cloves, 10 g crushed cinnamon and 10g lavender flowers) with 50 ml of methanolin each bottle then we subjected the 3 bottles to stirring for 24 hours.**(4)**

after 24 hours of contact we filtered the three solutions and kept the filtra in dark vials, and thefiltered plant material in other dark vials with 50 ml of methanol in each vial on the shaker foranother 24 hours then we filtered the plant material again to obtain the new filtra and mixed it with the other filtra from the first filtration

This step facilitates the transfer of target compounds from the plant material to methanol**(46)**.

- Concentration: in this step we concerted the methanolic extracts obtained using a rotaryevaporator

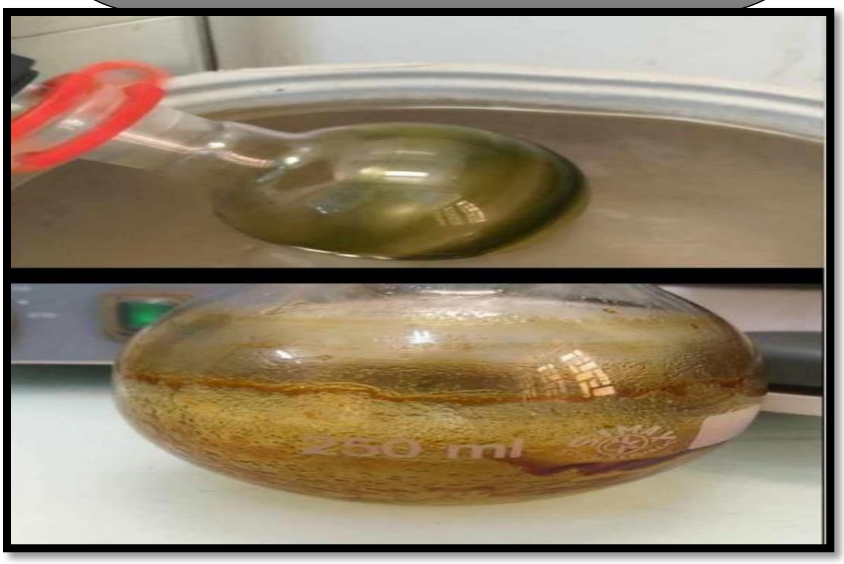


Figure08: natural extracts of lavender and cloves in the rotary evaporator. (personal prise)

3. Determination of antibacterial activity

3.1. Sterilization of equipment

In the microbiological part we need not only cleanliness, but also to respect the conditions of asepsis and sterilization. It is for this reason that we wrapped the following material used in aluminum foil and sterilized it in autoclave at 121°C: distilled water, ivory petri dishes, clamps,

tips for micro pipettes, test tubes used for the preparation of bacterial solutions and Wattman N°1paper disks (6 mm of diameter).

3.2. Preparation of extract solutions

3.1.1. synthetic extract :

We used three synthetic extracts: gallic acid, ascorbic acid and

quercetin.In three tubes containing 10 ml of distilled water we put:

- ✓ 1.59 g of ascorbic acid
- ✓ 0.39 g of quercetin

- ✓ 0.91 g of gallic acid

And we mix the three tubes well until we obtain solutions of synthetic extracts.

3.1.2. natural extracts:

In the preparation of natural extract solutions we mixed

- ✓ 0.95 g Cinnamon phenolic compounds with 10 ml of 5% DMSO in a tube.
- ✓ 3.03 g phenolic compounds of Clove with 10 ml of DMSO 5% in a tube.
- ✓ 1,49 g lavender phenolic compounds with 10 ml of 5% DMSO in a tube.

And we mix the three tubes well until we obtain solutions of natural extracts.

3.3. Bacterial strains tested

The antimicrobial activity of the extracts was evaluated on three bacteria and one fungus (ATCC). The bacterial and fungal strains come from the Medical Analysis Laboratory ofMicrobiology, el Akid lotfi laghouat hospital.

- ✓ Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923
- ✓ Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853
- ✓ *Escherichia coli* ATCC 25922
- ✓ The fungus: *Candida albicans* ATCC 10231

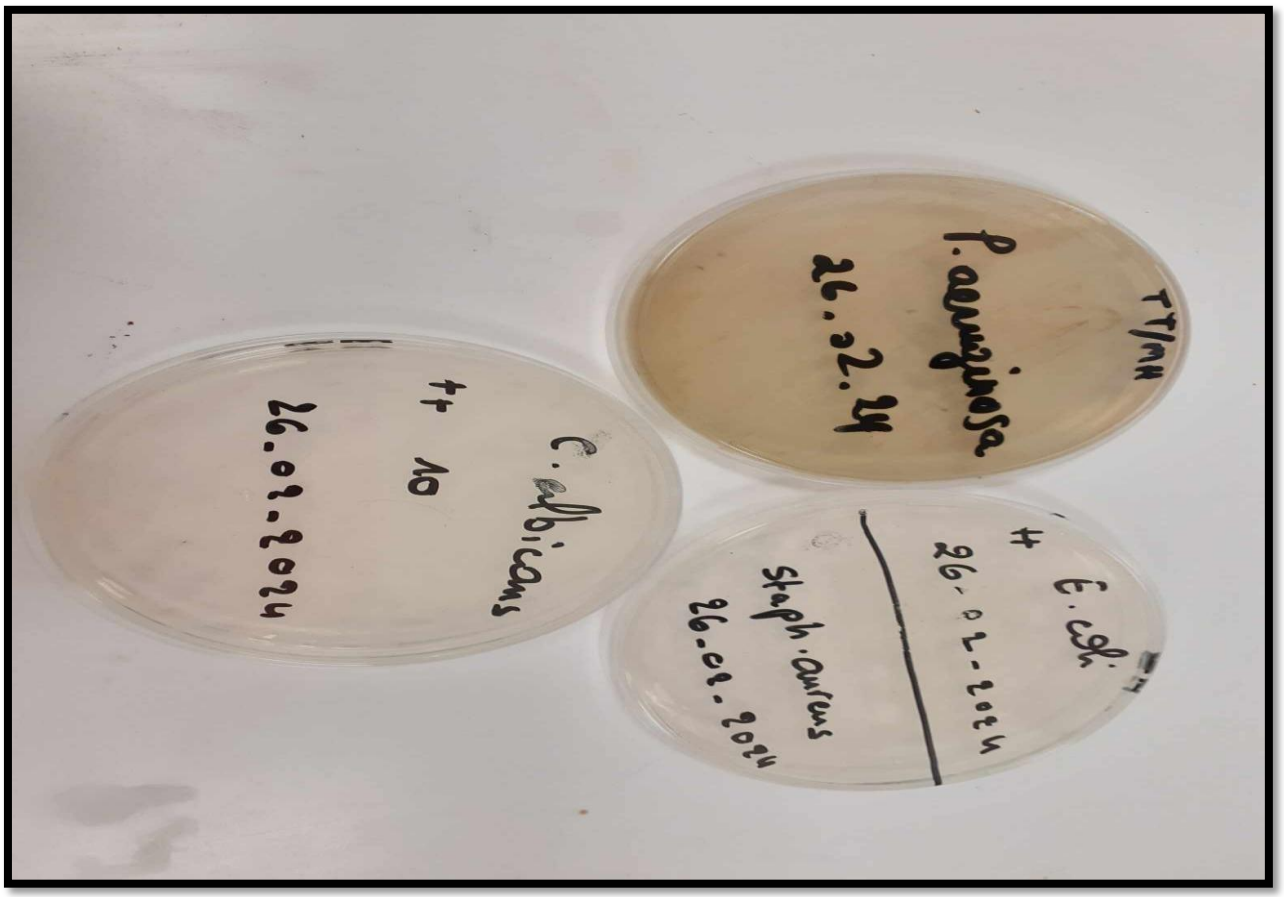


Figure09: The tested strains. (personal prise)

3.3.1. Sub-cultivation of bacterial strains:

In sterile conditions, from storage boxes, we took isolated colonies identical to the tested strain, and collected sterile tools and culture media. This includes sterile swabs, Petri dishes, and any necessary nutritional supplements or antibiotics.

First we used a Bunsen burner to sterilize the work area. This helps prevent contamination duringthe process. Then using a sterile swab, we transferred a small amount of bacteria from the original culture to the new culture medium.

We distribute the inoculated bacteria evenly by spreading in a narrow stripe manner on the surface of the transfer media present in Petri dishes that are suitable for the microbial strains

used(PDA medium for fungi and nutrient agar for bacteria) and which were incubated at 37 °C for 24

hours. to obtain small, well-isolated colonies. This allows bacteria to grow and reproduce. It ispossible to continue cultivating it and cultivating strains of it.

3.4. Preparation of the bacterial inoculum

After 24 hours of incubation of the strains tested, we took 1 to 2 colonies in exponential growth phase of each strain, well isolated and perfectly identical, at using a platinum loop, then the latter is placed in a tube containing water sterile physiological 0.9% Na Cl, and finally we homogenized the tubes with a vortex in such a way as to obtain an optical density of between 0.1to 0.2 for a length waveform of 620 nm (approximately 10^(6) CFU/m).

3.5. Preparation of culture media

The next step is to prepare the culture media to test the activity, antibacterial properties of the extracts used on the strains obtained. We sank the middle Mueller Hinton in petri dishes (4 mmthick) for the bacterial strains, and allowed them to dry .

3.6. Inoculation

Using sterile swabs, penetrate each of them into the different suspensions bacterial (the inoculum), we inoculated Mueller Hinton dishes using the four-quadrant method and thenincubated them at 37°C.

4. Antibacterial tests

Two different methods are used for the evaluation of the activity antimicrobial properties of thethree different plant extracts (cinnamon/clove/lavender):

1- The most famous method is the disc diffusion method from a Whatman paper

disk.2- The micro dilution (96-well microplate)

4.1. The disc diffusion method

4.1.1. Antibiogram sensitivity test:

One of the strategies to use antibiotics wisely is performed by generating and using anantibiogram **(94).**

This test was carried out to study the standard antibiogram of the germs used and the comparewith the effect of our extract obtained –positive control‖.

We took a seeded box for each strain and placed it on its antibiotic surface to be tested(Gentamicin 15µg).

4.1.1. The method of diffusion of extract discs –antibioaromatogram‖

The disc diffusion method (DDM) is classified as an agar diffusion method (ADM) because theplant extracted to be tested diffuses from its reservoir through the agar medium seeded with themicroorganism test. Generally, the reservoir is a filter paper disk, which is placed on top of an agar surface. If tested plant extracts or isolated compounds are

Microbiologically active, an inhibition zone develops around the filter paper disk after incubation. The diameter of the inhibition zone properly describes the antimicrobial potency of plant extracts or individual compounds **(95)** .A Mueller-Hinton medium is necessary to carry outthis technique.

The discs of Whatman N°1 papers were cut with a diameter equal to 6 mm, then sterilized in aglass box, they were then impregnated by a micropipette filled with 25µl of different concentrations by dilution (pure, (½), (1/10)) of the three extracts (cinnamon/clove/lavender:

3 essential oil extracts and other 3 phenolic compounds extracts‖). We also have prepared a negative control disk of DMSO. The prepared discs are placed using sterile forceps on the surface of the media which are already dawn. Each disc indicates a concentration of the extractused. The Control disk (Gentamicin) is placed at the top of the petri dish. The boxes are then kept for 12 hours at 4°C, then incubated at 37°C for 18h-24h for bacteria.

We obtained three disks of each extract (for the essential oils and for the extracted phenolic compounds) of course With a DMSO negative control disk and control disk

(Gentamicin) foreach Bacterial strain studied.

4.2. Microdilution method (96-well microplate)

A microplate assay was modified for the detection of antimicrobial activity in plant extracts. Theaim was to develop an in vitro assay that could rapidly screen plant extracts to provide quantitative data on inhibition of microbial growth. A spectrophotometric assay using a microplate with serial dilutions of the plant extract and the bacteria was developed**(96)**.

In order to study the antibacterial activity of phenolic compounds, we brought 3 sterile microplates and 6 solutions of the studied extracts, including 3 natural (cinnamon/cloves/lavender) and 3 synthetic (ascorbic acid/gallic acid/quercetin), and we used 4tubes for the sterile swab.

Prepare a solution of bacteria in advance, bring DMSO and Mueller-Hinton (MH) liquid, andhave it all near the Bunsen burner to sterilize and ensure the validity of the experiment.

- ✓ **First step:** we used a micropipette holding 70µl of MH liquid and filled it into each 96wells of the microplate.
- ✓ **In the second step**: we divided each microplate into two parts (4 × 12) for each extractedsolution. We now have 4 rows (A/B/C/D). In each row, we placed one type of bacteria in the following order (*Escherichia coli* / *Staphylococcus aureus* / *Pseudomonas aeruginosa*/*Candida albicans*), and using a fine pipette we filled 5µl of bacteria into their designated places. We then rearranged the same bacterial species and filled them into the second part(E/F/G/H) of the microplate.
- ✓ **Third step**: we set the first line as positive control and the second line we filled with 25µL of DMSO, which is considered as negative control during the final readout. As forthe remaining ten lines, we filled them using micropipette, 25µL, for different concentrations of the extracted solutions, with dilution according to

the following order (1/2, 1/4, 1/6, 1/8, 1/10, 1/12, 1/14, 1/16, 1/18, 1/20) In the same way, we completed thesix extracts.

- ✓ **The final step:** is to store the three paintings at a temperature of 37 degrees Celsius for 18 to 24 hours. The next day, we read the results on an ELISA analyzer, Mindray MR-96A.

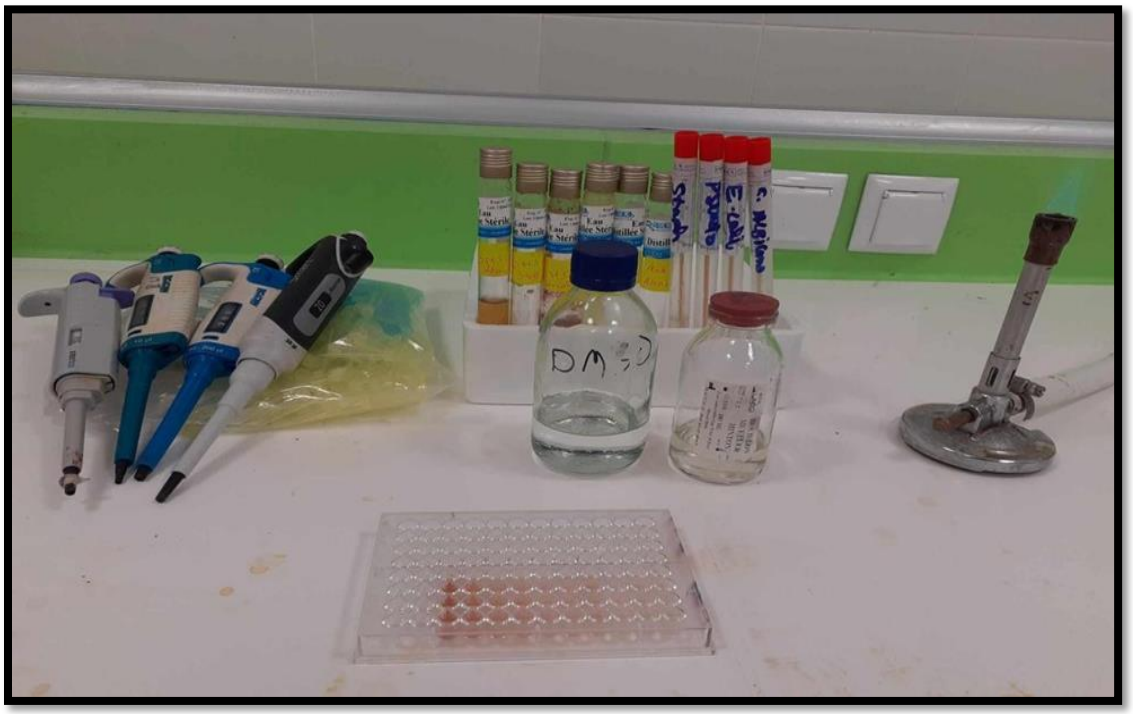


Figure10: Work tools (personal prise).

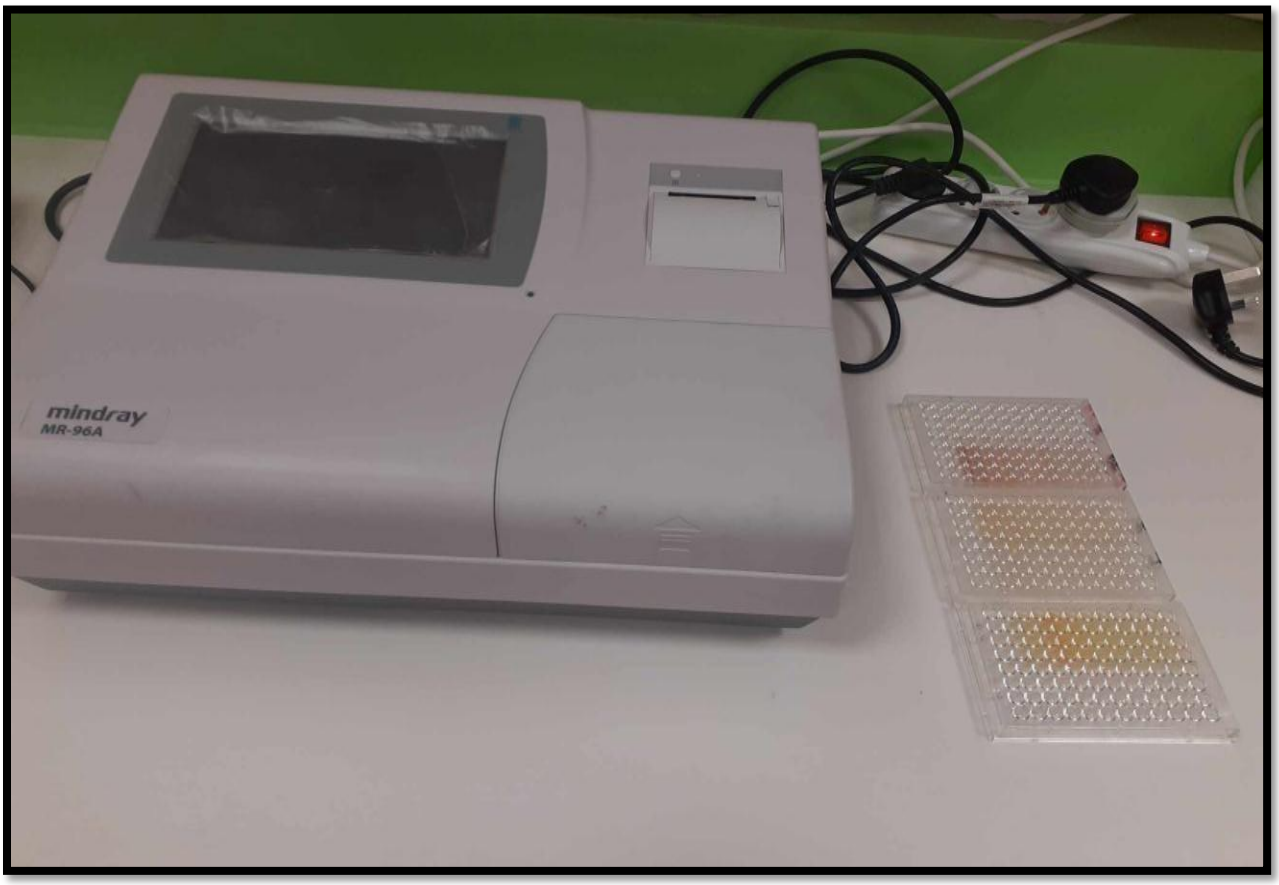


Figure 11: prepared microplates and elisa analyzer (personal prise)

RESULTS AND DISCUSSION

1. Resuts

1.1. Determination of the yield of the three raw extracts

1.1.1. Essential oil:

$$\text{Yield EO} = \frac{EOM}{MFP} \times 100$$

Yiled EO: Essential oil yield (%)

- EOM:Mass Essential oil (g)
- MFP: Mass of fresh plant material (g)

Table 01: the yield of the three tested essential oils

extracts	The Yield
cinnamon	2.969%
Cloves	6.118%
lavender	4.095%

1.1.2. Natural extracts

Table 02: the yield of the three PC extracts

extracts	The results
Cinnamon	9.5%
cloves	30.3%
Lavender	14.9%

1.2. Antimicrobial activity by the disk method

1.2.1. Essential oils and gentamicin antibiotic:

Table03: the areas of inhibition of the antibiotic and the three essential oils.

Strains	*Staphylococcus aureus* ATCC 25923	*Candida albicans* ATCC 10231	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922
DMSO	0	0	0	0
Gentamicin	20mm	30mm	20mm	20mm
pure cloves E.O	32mm	50mm	30mm	40mm
cloves E.O diluted ½	30mm	30mm	18mm	26mm
cloves E.O diluted 1/10	20mm	28mm	15mm	20mm
pure cinnamon E.O	40mm	35mm	35mm	50mm
cinnamon E.O diluted ½	35mm	30mm	30mm	45mm
cinnamon E.O diluted 1/10	28mm	25mm	25mm	30mm
lavender E.O pure and diluted ½ and1/10	0	0	0	0

We note that the diameter of the inhibition zones of pure and diluted (1/2) clove essential oil is larger than the diameter of the inhibition zone of the antibiotic, and for the (1/10) dilution the diameter converges to the diameter of the antibiotic. Therefore, we can conclude that clove essential oil has strong antimicrobial activity against the strains tested ,and for cinnamon E.O we notice that the diameters of the inhibition zones of pure and diluted cinnamon essential oil (1/2) (1/10) are larger than the diameters of the inhibition zones of the antibiotic. Therefore, we can conclude that clove essential oil has strong antimicrobial activity against the strains tested.

Lavender essential oil gave zero results in the diks diffusion method which indicates that it has low or no antimicrobial activity.

Figure 12: Results of disc diffusion method of cinnamon and cloves E.O against some tested strains.

1.2.1. Antimicrobial activity of lavender CP:

Table 04: the inhibition zones of the natural lavender extract and the three syntheticextracts.

Strains	***Escherichia coli ATCC 25922***	***Staphylococcus aureus ATCC 25923***	***Candida albicans ATCC 10231***
DMSO	0	0	0
S.E Quercitin	10mm	10mm	10mm
S.E gallic acid	19mm	14mm	15mm
S.E ascorbic acid	20mm	8mm	15mm
N.E lavender	30mm	10mm	20mm
N.E lavender diluted ½	18mm	9mm	12mm
N.E lavender diluted 1/10	13mm	8mm	0

The obtained results indicating that the natural extract of pure and diluted (1/2; 1/10) lavender have inhibition diameters converging to the inhibition zones of the three synthetic extracts used. therefore natural lavender extract has antimicrobial activity.

1.3. Antimicrobial activity by the microdilution method

Table 05: Results of the antimicrobial activity of natural and syntheticextracts on the strains studied by microdilution method.

CP extract / Strain	inhibition zone					
	natural extracts			synthetic extracts		
	cinnamon	cloves	lavender	Quercetin	A. ascorbic	A. gallic
Escherichia coli ATCC 25922	+ +	– –	– –	– –	– –	– –
Staphylococcus aureus ATCC25923	– – – –	– – – –	– – – –	– – – –	+ + + +	– – – –
Pseudomonas aeruginosa ATCC 27853	– –	– – – –	– –	– – – –	– –	– – – –
Candida albicans ATCC10231	– – – –	– – – –	+ + + +	– – – –	– – – –	– – – –

- (+) : inhibition zone observed
- (-) : No inhibition zone observed

✓ MICRODILUTION METHOD

The antibacterial activity by the microdilution method was expressed as determining theminimum inhibitory concentration (MIC).

Results of the microdilution test are grouped in the table bellow:

Table 06: MIC of Active extracts on the strains studied.

Active area	**cinnamon x ½ *Escherichia coli* ATCC 25922**	**lavender pure *Candida albicans* ATCC 10231**	**A.ascorbic pure *Staphylococcus aureus* ATCC 25923**
MIC (mg/ml)	0.475	1.49	1.59

1.3.1. Antimicrobial activity of natural extracts:

1.3.1.1. phenolic compounds extracts:

we noticed an antimicrobial activity for cinnamon on *Escherichia coli* ATCC 25922 by dilution(1/2) and an MIC=0.475 , and no activity with the 3 remaining germs.

we noticed an antimicrobial activity for lavender on *Candida albicans* ATCC 10231 with aMIC=1.49 , and no activity with the 3 remaining germs.

No antimicrobial activity for Clove with all the 4 germs studied.

1.3.1.2. Synthetic extracts :

we noticed an antimicrobial activity for Ascorbic Acid on *Staphylococcus aureus* ATCC 25923with a

MIC=1.59 (mg/ml). and no activity with the remaining 3 germs.

No antimicrobial activity for Quercitine and Gallic Acid with all the 4 germs studied.

note: we did not carry out a CMB manipulation because the results gave a pure MIC of ascorbic acid and lavender therefore CMB=0, and a low MIC of cinnamon so it is impossible to find a CMB of it.

2. Discussion

- The analysis indicates that the natural extracts of the three studied plants showed varying yieldsof essential oils and phenolic compounds. We found that cloves have a particularly high yield of essential oils and phenolic compounds. Followed by lavender and then cinnamon, which provided the lowest returns. Natural extracts showed significant antimicrobial activities in both methods, with clove and cinnamon leading the way. Among the synthetic compounds, ascorbic acid showed the best antimicrobial activity, in contrast to quercetin and gallic acid. The disk diffusion method allowed direct visualization of microbial inhibition which gave different diameters of the inhibition zones. The result of the largest diameter indicating the strength of the extract against strains, while the microdilution method provided accurate quantitative measurements of MICs(the smallest MIC= the strongest extract) , confirming the relative potency of the extracts and compounds tested.

- The comparison results between our extracts and the synthetic antibiotic tablet (Gen) confirm the antibacterial power of the different extracts. However, the difference in the diameter of the inhibition zones between the two may be due to the nature of the pure and natural extracted materials as well as the amount taken for each. The absence of inhibition zones in certain strains indicates a difference in the chemical composition of each extract, as well as the type of bacteria and their sensitivity to the extracts.

- The results confirm that cinnamon extract is the strongest, because cinnamon essential oil (CEO) showed a larger zone of inhibition of 80 mm at pure concentration against Escherichia coli ATCC 25922, and there was also other zones of inhibition with variable values against the rest of the types of bacteria studied. And cinnamon phenolic compound has the lowest MIC value MIC=0.47 mg/ml at 1/2 dilution against the same bacteria Escherichia coli ATCC 25922, confirming its antibacterial activity. Confirming the hypothesis according to **(Upadhyaya, (97))** also reported ethanolic extract of cinnamon. showed 14 mm and 11 mm inhibitory zone against Staphylococcus aureus and Escherichia coli, respectively. **(Similarly, (98))** noted the 10 mm inhibitory zone with cinnamon extracts against Staphylococcus aureus and **(M El-Shreef, (99))**E. coli ATCC 25922 was found to be

partially sensitive to the cinnamon extract with an inhibition zone diameter (IZD) of 14.85 mm. But contrary to the hypothesis association **(Keloth,(100))** observed no inhibitory effect of cinnamon aqueous extract against E. Coli and S. aureus. **(Abdul Rasheed, (101))** also Observe no zone of inhibition against E. coli, S. aureus and P .aeruginosa at 20% concentration of cinnamon powder aqueous extract. **(Kavitha, (102))** also found no inhibitory zone with aqueous extract of cinnamon bark powder against Staphylococcus aureus and Escherichia coli. The results with respect to MIC of ethanolic and aqueous extracts of cinnamon powder were in accordance with **(Liang, (103))** found 20 mg/ml MIC of cinnamon powder ethanolic extract for E. coli.

-The results of clove natural extract prove that clove essential oil has antimicrobial activity due to the appearance of diameters inhibitions zones with varying values against all strains, and we recorded its largest inhibition zone 70 mm at a pure concentration against the yeast Candida albicans ATCC 10231. In contrast to that, the second method shows that the cinnamon phenolic compounds are not It is capable of inhibiting bacterial and yeast species and not resisting them, as we did not record any CIM value for the different concentrations studie . of confirming the hypothesis , The other reports showed that Clove Essential oil similar activity to our results. According to **(Haomin Sun , (104))** The inhibition zones of the clove essential oil against Escherichia coli and Staphylococcus aureus were 9.32 and 13.47 mm, respectively.**(Maggini,(105))** Concerning the Minimal Inhibitory Concentration (MIC), clove EO was more effective against S. aureus MIC = 0.05 mg/mL . Gram-negative species E. coli andP. aeruginosa were less inhibited by clove EO, probably because of the presence of the external membrane that represents an additional barrier to the introgression of EO components, if compared to Gram-positive bacteria . **(Gucwa, (106))** the MIC of Clove EO was 1.25 mg/mL against C.albicans ATCC 10231 **.(Hakalova, (107))** Some studies have observed a difference in antibacterial activity depending on the extraction method, bacterial strain, and also experimental conditions. For example, with certain strains the phenolic compounds in cloves may not exhibit strong antibacterial activity or may require higher concentrations to achieve effect .

But contrary to the hypothesized association According to **(Jingwen Bai, (108))**Notably,

phenolic compound of clove extract (eugenol) resulted in obvious inhibition zones of S.aureus 24mm and E.coli 21.9 mm. The MIC=0.26 mg/mL against S. aureus were lower than those againstE. coli MIC = 0.32 mg/mL . **(Lee Rosarior, (109))** clove ethanolic extract (CEE) was evaluated for their antimicrobial properties. Results are the presence of a zone of inhibition with Escherichia coli ATCC 25922 (12.7 mm ± 0.6) and Staphylococcus aureus ATCC 25923 (14.7 mm ± 0.6). and no zone of inhibition was observed on Pseudomonas aeruginosa disc diffusion plate. and theoretically the MIC for (CEO) be 0.15 mg/mL.**(Biernasiuk, (110))** the result of activity of CEO and EUG was shown at MIC = 1–2 mg/mL with C. albicans ATCC 10231.

- The results of lavender natural extract show that lavender essential oil is not effective against all strains, as the diameters of the inhibition zones (0 mm) were recorded at different concentrations. As for the phenolic compound of lavender, it was active against the strains studied, and the largest zone of inhibition recorded was 30 mm at a pure concentration against the bacteria E.coli ATCC 25922. We also recorded a CIM = 1.49 mg/ml at a pure concentration against the yeast C.albicans ATCC 10231. of confirming the hypothesis There are studies that indicate little or no antibacterial activity of lavender essential oil against various bacterial strains. Among them **(Moon, (111))** In this study, the antibacterial activity of lavender essential oil was tested against arange of bacteria, including Escherichia coli and Staphylococcus aureus. The results showed that the effectiveness of the oil is limited, especially against gram-negative bacteria. the findings of this study contradict those **(Predoi, (112))** results antimicrobial activity of lavender essential oilagainst E.coli ATCC 25922, the inhibition zone was (20 ± 1 mm) .and MIC=0.15 mg/ml . **(Todorova, (113))** Lavender oil inhibit the growth of the yeast Candida albicans ATCC 10231

- the result of inhibition zone was (18.6 ± 0.3 mm). **(Stoyanova, (114))** found out that Bulgarian lavender oils have an antimicrobial effect on Gram-positive bacteria . In other studies **(Predoi, (113))** lavender oil from southern Romania showed stronger antimicrobial activity against the Gram-negative bacteria E. coli. Lavender oil from Montenegro also exhibits antibacterial activity. **(S Perovic, (115))** These differences are due to differences in the composition of lavender oil depending on its origin.

- The results of industrial extracts showed antimicrobial activity of ascorbic acid extract

against the studied strains, as the largest diameter of the inhibition zone reached 20 mm against Escherichia coli bacteria.Its effect is considered weak compared to the strength of the effectiveness of natural extracts. This was proven by the second method, where we recorded MIC = 1.59 mg/ml in pure concentration against the Gram-positive bacteria Staphylococcus aureus ATCC 25923. We did not record any activity for both quercetin and gallic acid against the studied strains.To confirm the hypothesis, according to studies **(Gyawali, (116))**, it has been demonstrated that ascorbic acid (or vitamin C) can either inhibit or increase the latency of pathogenic bacteria such as S. aureus and E. coli .

It is interesting to note that synthetic extracts of gallic acid and quercetin, although they contain antioxidants and other potential health benefits, may not necessarily show antibacterial activity. But contrary to the supposed association, some other studies have shown that gallic acid is effective. Against a variety of bacterial strains, including both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. Its effectiveness varies depending on theconcentration and specific bacterial strain, and quercetin has shown broad antibacterial activity, being effective against many Gram-positive and Gram-negative bacteria. Like gallic acid, its effectiveness depends on the strain and concentration of bacteria.

.the obstacles in collecting data results: We know that The MIC value confirms the presence or absence of antimicrobial activity of natural extracts essential oils. However, unfortunatelydue to the amount of essential oils extracted in work of the diffusion disk method running out when we faced contamination problems with the bacterial strains used, we repeated the experiment more than once to ensure the validity of the final results. Due to time constraints, we were unable to finish our work in the second method

CONCLUSION

3. Conclusion

This final thesis aims to answer the questions asked above in the introduction where we described the context in which this work takes place.

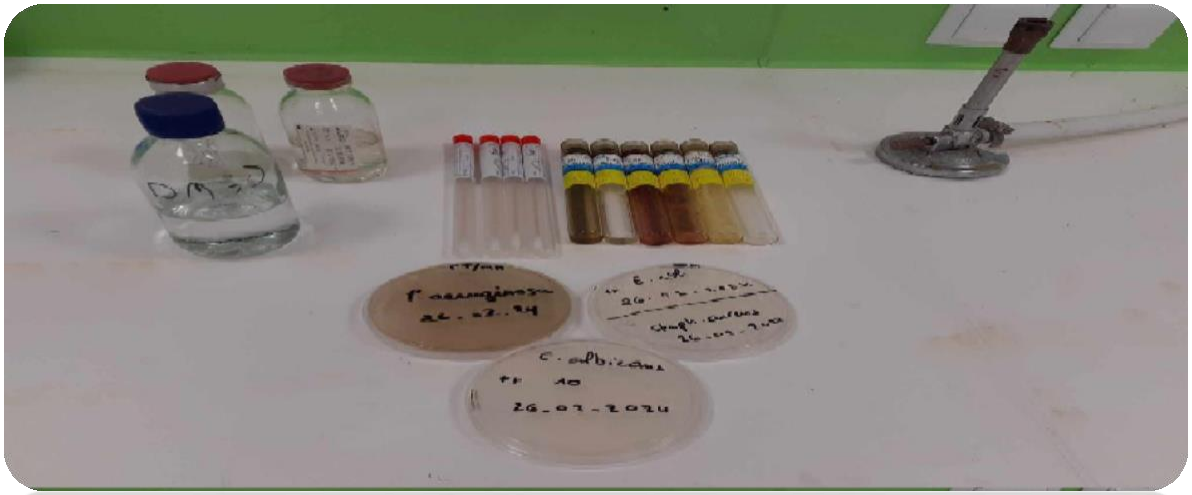
We have had the opportunity to study in our memory the properties of some medicinal plants that are indispensable in our daily lives, to demonstrate their virtues and multiple benefits. He explained the role of these plant extracts in traditional medicine, including their effect on human health.

Some of the objectives of this study were achieved by the results obtained, including antimicrobial activity. It should also be noted that the interpretations presented are in addition to findings arising from other work carried out against bacterial strains on the same topic, but in a different geographical context. Referring to the results obtained, we clearly confirmed that the types of natural extracts of cinnamon, cloves and lavender, rich in their essential oils and phenolic compounds, as well as the synthetic compounds tested, represent promising alternatives to traditional antimicrobials. Their use may be considered in the development of effective antimicrobial agents. Emphasizing the importance ofcontinuing research in this field to better understand its working mechanisms and improve its practical application.

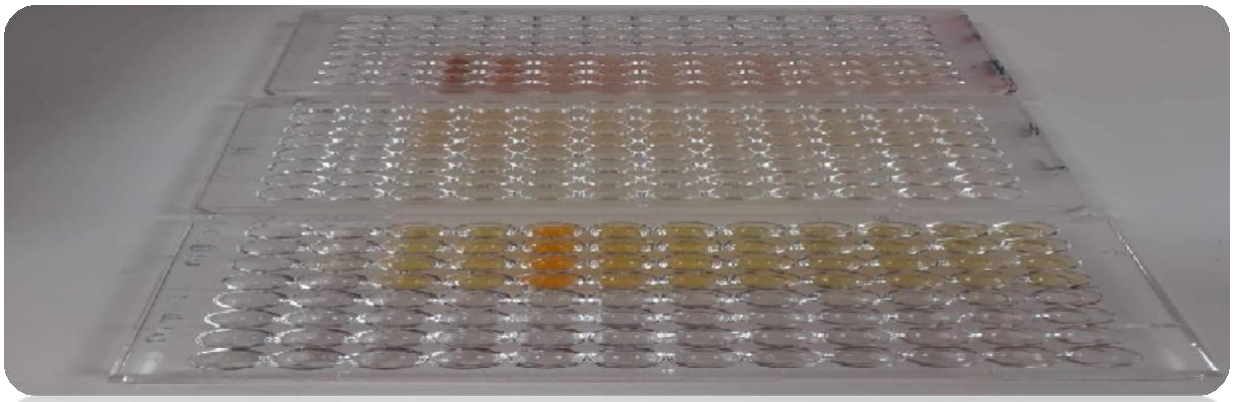
Ultimately, this detailed work is just the beginning step in our future careers.

4. Annexes

The strains tested and the synthetic and natural extract solutions



Prepared microplates



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