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MASTER

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Sector: Food Sciences

Option: Food Safety and Quality Assurance

Phytochemical screening of aromatic plants used in the preparation of organic food packaging

Presented by:

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The jury:

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Résumé

La recherche a testé de nouveaux emballages alimentaires biologiques à base de gélatine, de glycérol et de sorbitol. La gélatine extraite à partir des pattes de poulet à l'aide d'acide acétique. Différentes combinaisons de biopolymères avec de l'huile essentielle ont été évaluées.

Le rendement en gélatine de 100 g de pattes de poulet était de 11,22 %, produisant une poudre cristalline jaune clair et inodore. Les films à base de sorbitol étaient plus transparents et flexibles, tandis que les films à base de glycérol étaient légèrement jaunes avec une flexibilité normale. Les deux types offraient une protection alimentaire raisonnable et un impact environnemental réduit.

L'huile essentielle semble avoir une activité antimicrobienne contre E. coli, *Kluyvera sp* et *Enterobacter cloacae* de Gram-. Le CMB de l'huile essentielle de girofle est compris entre 3 mg/ml chez *Kluyvera sp*, 5 mg/ml chez E. coli, et 4 mg/mL pour *Enterobacter cloacae*.

L'huile essentielle de Girofle d'*Eugenia caryophyllus* a un fort effet antimicrobien ; il inhibe toutes les bactéries (*E. coli, Kluyvera sp, Enterobacter cloacae*).

Mots clés : pattes de poulet, gélatine, huile essentielle de girofle, emballage biodégradable, activité antibactérienne.

ملخص:

ركز عملنا على الفحص الكيميائي النباتي للجزيئات الحيوية (الزيت العطري لقرنفل يوجينيا كاريوفيلوس) المستخدم لإنشاء عبوات أغذية عضوية جديدة تعتمد على الجيلاتين والجلسرين والسوربيتول. تم استخلاص الجيلاتين من أقدام الدجاج باستخدام حمض الأسيتيك. وقد تم اختبار مجموعات مختلفة من هذه البوليمرات الحيوية، بالإضافة إلى الزيوت العطرية.

قدرت نسبة إنتاج الجيلاتين المستخرج من 100 جرام من أفخاذ الدجاج بـ 11.22%. وكان الإيلاتين المستخرج من أقدام الدجاج عبارة عن مسحوق بلوري ممتاز، أصفر فاتح وبدون رائحة.تشمل الخصائص الحسية لزيت القرنفل مظهره السائل، ولونه الأصفر، ورائحته العطرية والتوابل.

كشف زيت يوجينيا كاريوفيلوس العطري عن تركيبة كيميائية غنية، حيث يشكل الفينول المكون الرئيسي، يليه الاسترات والسيسكويتيربين.

يبدو أن الزيت العطري له نشاط مضاد للميكروبات ضد البكتيريا الإشريكية القولونية و كلويفيرا إس بي و الأمعائية المذرقية من الجرام. يتراوح الحد الأدنى لتركيز مبيد الجراثيم للزيت العطري للجيروفل بين 3 ملغم/مل في كلويفيرا إس بي ، و5 ملغم/مل في الإشريكية القولونية ، و4 ملغم/مل في الأمعائية المذرقية.

الزيت العطري من القرنفل من يوجينيا كاريوفيلوس له تأثير قوي مضاد للميكروبات؛ فهو يثبط جميع البكتيريا الإشريكية القولونية، كلويفيرا إس بي، الأمعائية المذرقي .

الكلمات المفتاحية: أقدام الدجاج، الجيلاتين، التعبئة والتغليف القابلة للتحلل، زيت القرنفل الأساسي، نشاط مضاد للجراثيم.

Abstract:

Our work focused on the phytochemical screening of biomolecules (essential oil of glove *Eugenia caryophyllus)* used to create a new organic food packaging based on gelatin, glycerol, and sorbitol. The gelatin was extracted from chicken feet using acetic acid. Different combinations of these biopolymers, in addition to the essential oil, have been tested.

The yield of gelatin extracted from 100 g of chicken legs was estimated at 11.22%. The elatin extracted from the chicken feet was an excellent crystal powder, light yellow and without smell.

The organoleptic properties of clove oil include its appearance as liquid, color as yellow, and odor as aromatic and spicy.

Eugenia caryophyllus's essential oil revealed a rich chemical composition. Phenol was the major component, followed by esters and sesquiterpenes.

The essential oil appears to have antimicrobial activity against E. coli, Kluyvera sp, and Enterobacter cloacae of gram-. The BMC of the essential oil of girofle is between **3 mg/ml** in *Kluyvera sp*, **5mg/ml** in *E. coli*, and **4 mg/mL** for *Enterobacter cloacae*.

The results show that the clove oil positively affects the gram-negative. The essential oil of clove Girofle of *Eugenia caryophyllus* has a strong antimicrobial effect; it inhibits all bacteria (*E. coli, Kluyvera sp, Enterobacter cloacae*).

Keywords: chicken feet, gelatin, biodegradable packaging, essential oil of girofle, activity antimicrobial.

Knowledge

First, I would like to thank Allah for the will, ability, and patience he has given me to accomplish this work.

I want to thank Dr. FERHI Selma, our officer, for her confidence, patience, availability, sensible advice, and kindness and for the time and attention she has devoted to us.

We also thank the jury members Dr. MENSOUR Fadila and Pr. TALEB Salima for accepting to evaluate our work and who would like to honor us with their presence.

Also, I thank Dr.AZIZI Nasima and their students for her efforts and for participating in our work.

We thank all those who agreed to participate in this work.

اهداء

بدأنا بأكثر من يد و قاسينا أكثر من هم و عانينا الكثير من الصعوبات و ها نحن اليوم و الحمدلله نطوي سهر الليالي و تعب الأيام و خلاصة مشوارنا .

بعد الحمدلله رب العالمين

إلى امام الذاكرين و سيد الأولين و الآخرين و معلم المعلمين و رسول ربه العالمين محمد صلى الله عليه وسلم.

إلى من كان رضاها نادي في الحياة إلى من كان دعائها سببا في نجاحي إلى من انتظرت هذه اللحظة بفارغ الصبر إلى أمي الحبيبة

(نصرالله زينة) .

إلى الذي بذل كل غالي في سبيل اسعادي إلى النور الذي لا ينطفي بقلبي ابدا إلى من لا ينفصل اسمي عن اسمه و الحاضر بروح لا يغيب إلى أبي رحمه الله (شعبانة مقداد) .

إلى من اتاحت الفرصة لتحقيق هدفي بفارغ الصبر سندي و نور عيني الذي أبصر به صديقتي عواطف .♡

و لا أنسى أبدا عائلتي و صديقاتي و كل من أحاطوني بحبهم و كانوا عونا لي.

اخيرا لنفسي .

Summary

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Abbreviation list

CO2 : Carbon dioxide

C° : Degree Celsius

% : Percentage

MH : Mueller Hinton

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Introduction

Introduction

Globally, the packaging industry is worth more than $650 billion and employs 5 million people in 100,000 companies (of which more than 65% are in the food sector). According to the Persistance Market Research report, the overall nano-packaging market in food and beverages was estimated to be $6.5 billion in 2013. Its annual growth rate would be 12.7%, which could reach approximately $15.0 billion in 2020 (Bumbudsanpharoke & Ko, 2015).

The packaging material defines an atmosphere favorable to preserving the food's taste, nutrition, and health qualities. As a result, food is stored in an atmosphere safe from contaminants (dust, microorganisms, chemicals) and external agents (oxygen), which can accelerate the degradation of its quality and safety (Guillard & Gontard, 2017).

Plastic is manufactured and used for packaging applications in various sectors. As food industries grow, the demand for packaging materials increases. However, conventional plastics are not biodegradable, creating serious environmental problems, threatening aquatic life and deteriorating air quality. Bio-degradable polymers have emerged as an alternative approach to many industrial applications to control the risk of non-biodegradable plastics. According to the type of raw material, it has been classified as polymers extracted from biomass (Shaikh, 2021).

Essential oils are obtained from medicinal and aromatic plants using different methods, such as steam distillation and hydro distillation (Barka & Berrich, 2021). They are widely recognized for their bioactive properties, making them valuable in various fields, including medicine, cosmetics, and agriculture. Due to their volatile compounds, such as terpenes, phenols, and esters, Essential oils possess significant antimicrobial, anti-inflammatory, and antioxidant properties (Bakkali & al., 2008).

The use of essential oil as natural preservatives in food products has been explored, offering a potential alternative to synthetic preservatives (Burt, 2004).

The objective is to extract gelatin from chicken feet and use it to create an active biodegradable packaging material and phytochemical screening of aromatic plants used to prepare organic food packaging.

This study comprises five parts: the first is a general introduction, the second is a bibliography devised into 3 chapters, essential oil, the second is a part of bio packaging, and the third is the use of bioactive molecules in bio-packaging. The fourth part presents the

material and methods, containing a presentation of place and raw material, gelatin extraction, film creation, antimicrobial activity , the fourth part results, and a discussion of our results.

I. Bibliography

Essential oil:

1. Definition of essential oil

An essential oil is an odorous and volatile substance, non-greasy, extracted from a vegetable in liquid form. It comes from a secretion developed by certain plants and contained in specialized structures (hairs, pockets, and secretory channels). Depending on the essential oil, the entire aromatic plant, or more specifically, some of its organs (root, bark, leaf, flower, fruit, seed, etc.) will be selected to extract the aromatic compounds. (Couic-Marinier & Lobstein, 2013).

2. Type of essential oïl extraction

2.1. Distillation with water vapor

It is the most common extraction process because it is suitable for most plants (wild or cultivated). In water vapor distillation, the plant is not in contact with water but with water vapor which, when passing through it, then leads to volatile compounds. Heat and steam burst the plant's cellular structure, releasing the essential oil. Vapor Sumer, volatile compounds are driven by water vapor to a cooling tank. Since oil and water are not miscible, it is easy to separate them by decanting (Couic-Marinier& Frély, 2019).

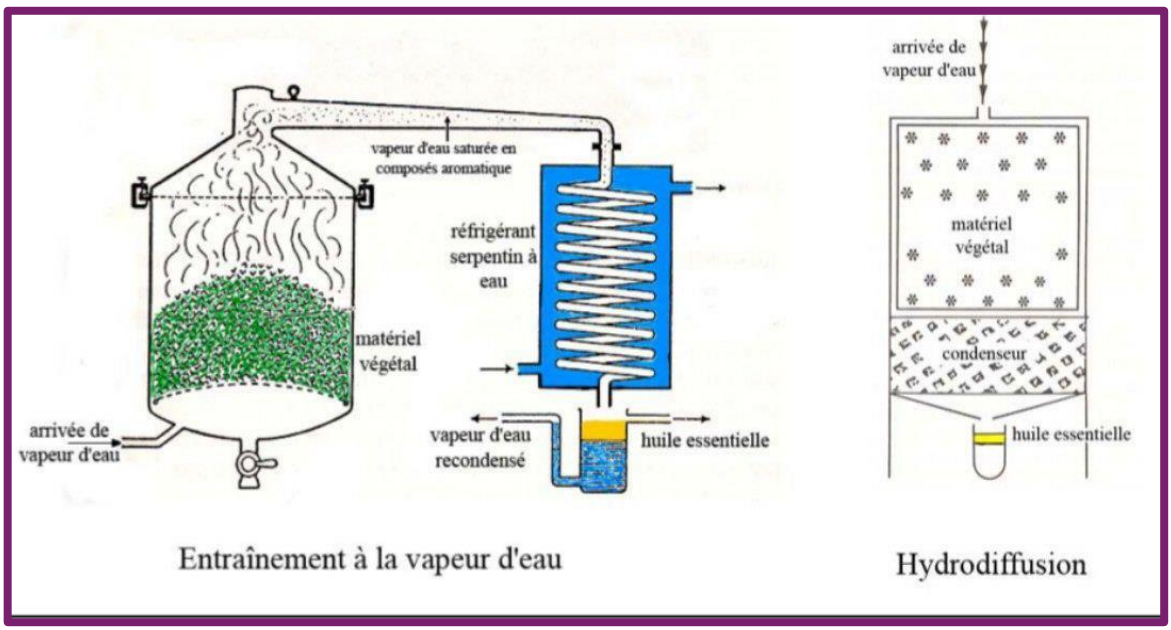


Figure 01: Upward and downward steam drive (Lucchesi, 2005).

2.2. L'hydrodistillation

This method, widely used in the perfumery industry, is rarer for the pharmacy. It consists of immersing a whole plant, fresh or crushed (turbo-distillation) in a still filled with water, brought to a boil. The vapors are cooled along a coil, and the essential oil separates from the water thanks to the difference in density (Couic-Marinier& Frély, 2019).

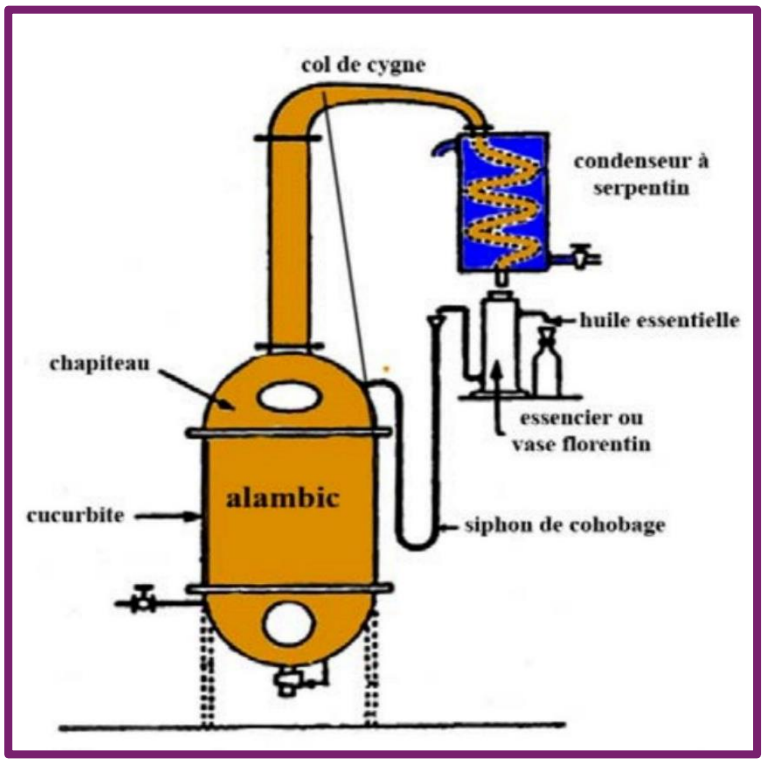


Figure 02: Traditional hydrodistillation (Lucchesi, 2005).

2.3. Cold expression extraction

This method is reserved for citrus fruits (lemon, grapefruit, tangerine, orange, bergamot). The zests are torn with mechanical presses, and the secretory pockets release the essential oils called ‘’Essences’. Formerly (and still today in Italy), they were obtained by scraping the zests with a tool. At the industrial level, citrus fruits are cold pressed by a press, allowing the release of essential oils in the fruit's peel. They swim on the juice obtained and are then separated by centrifugation. The citrus essences are kept cool (Couic-Marinier& Frély, 2019).

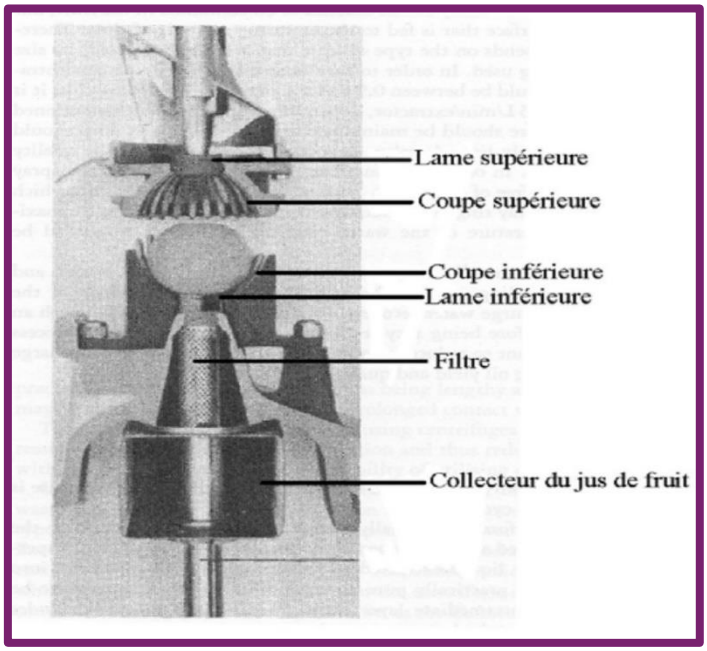


Figure 03: In-line extraction (Lucchesi, 2005).

2.4. Gas extraction

It is an extraction that uses a common gas (carbon dioxide, the same gas that we exhale from our lungs) put under high pressure and low temperature conditions so that it is no longer gaseous but liquid. This supercritical fluid "is at the limit of a liquid phase and a gas phase and thus presents the properties of both. This process is used in industrial perfumery for perfume plants to extract aromas. It is an effective method and sometimes less expensive than the previous ones but does not allow us to obtain products that can be qualified "essential oils" in the "medical" sense of the term, nor the "organic" label (the method is not authorized in the specifications of organic products) (Couic-Marinier& Frély, 2019).

2.6. Extraction with solvents

This process involves dissolving the essences in a volatile solvent (not in water). We obtain an absolute and not an essential oil used only in perfumery. Indeed, in a method of care such as aromatherapy, organic solvents - even residual ones are not allowed while in perfumery. This process is used for certain flowers, gums, or resins (incense, myrrh, musk, benzoin, etc.) (Couic-Marinier& Frély, 2019).

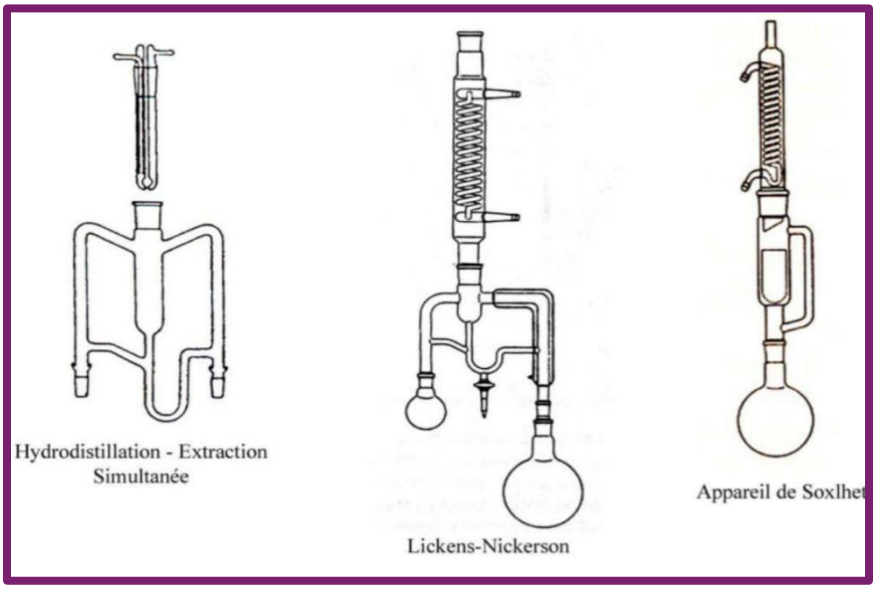


Figure 04: The different types of extraction by volatile solvents (Lucchesi,2005).

3. chemical composition of essential oils

3.1. Composes terpéniques:

Only mono terpenes in C10 and sesquiterpenes in C15 can be extracted by distillation, the other terpenes (di terpenes in C20 and tri terpenes in C30) are not driven by water vapor. They are classified according to (Couic, 2013)**:**

• their functions: alcohols (geraniol, linalool), esters (linalyl acetate), aldehydes (citral, citronellal), ketones (menthone, camphor, thuyone), ether-oxides (cineole);

• their structure: linear (farnesene, farnesol), monocyclic (humulene, zingiberene), bicyclic (cadinene, caryophyllene, chamazulene) or tricyclic (cubebol, patchoulol, viridiflorol) (Couic, 2013).

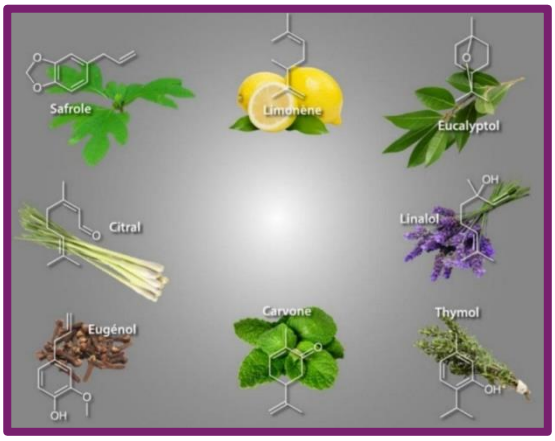


Figure 05: Example of essential oils and their majority constituent (Fourmentin & Kfoury, 2024).

3.2. Aromatic compounds derived from phenylpropane:

Aromatic compounds derived from phenylpropane are much less common in essential oils than monoterpenes and sesquiterpenes. These include cinnamic acid and cinnamic aldehyde (cinnamon essential oil), eugenol (clove essential oil), anethol and anisic aldehyde (anise, anise, fennel) and safrole (sassafras essential oil). Lactones derived from cinnamic acids, such as coumarins, are mostly entrainable by water vapour and thus present in certain essential oils (celery essential oil) (Couic, 2013).

4. The properties of essential oil

Essential oils possess various properties, including relaxation, toning, energizing, regenerative, deodorizing, anti-inflammatory, and antioxidant effects. Their biological activity is closely linked to their chemical composition and the potential interactions between their various constituents. Some essential oils and their components have demonstrated powerful anticancer activities in both laboratory and real-world conditions. Additionally, their potential use in the pharmaceutical industry requires absolute specificity and pharmacodynamic selectivity to ensure both efficacy and safety (Bouyahya & al, 2018).

Les huiles essentielles sont solubles dans les alcools, dans les huiles fixes et dans la plupart des solvants organiques (Paris & al, 2001).

5. field of use of essential oils

- **food sector**

In the food sector, essential oils and plant extracts, known as photogenic food additives, are increasingly considered viable alternatives to synthetic additives. These natural products are pivotal for food security due to their antimicrobial properties, effectively combating a wide range of pathogenic microorganisms in food. A recent review consolidates the literature on using essential oils to extend the shelf life of various foodstuffs, including fruits, dairy products, meat, fish, and seafood. In deli meats, natural extracts offer similar benefits to synthetic additives but without the associated cancer risks from nitrites and nitrates (Fourmentin & Kfoury, 2024).

Essential oils have different chemical composition profiles and are used as natural food preservatives. Its use as an antiseptic is due to the presence of compounds with antibacterial and antioxidant properties. In the crop protection and agro-food sectors, essential oils or their active ingredients can also be used as protective agents against mushrooms and phytopathogenic microorganisms that enter foodstuffs (Menaceur, 2015)

- **cosmetics sector**

In the cosmetics sector, since antiquity, essential oils have been key ingredients not only for perfumery, but also for the whole field of beauty. Nowadays, essential oils have regained their place among the essential ingredients for cosmetic products because they make it possible to offer certified organic cosmetic formulations (Fourmentin & Kfoury,2024).

In perfumery and beauty products: Essential oils are used in the cosmetics sector, especially for the manufacture of perfumes, fragrance compositions of detergents, and functional perfume products. The use of essential oils for perfume preparation is obvious (Besombes, 2008).

- **pharmaceutical sector**

In the pharmaceutical sector, research is particularly interested in exporting traditional knowledge of herbal medicine, so-called alternative medicine, to propose alternative molecules to synthetic products to deal mainly with antibiotic resistance (Fourmentin & Kfoury,2024).

Bio packaging

1. Food packaging

The International Packaging Institute defines packaging as the process of enclosing products, articles, or items within various containers such as pouches, bags, boxes, cups, trays, cans, bottles, or other receptacles. This fulfils one or more functions: containment, safeguarding, preservation, communication of information, facilitation of use, and enhancement of performance (Robertson, 2013).

Today, food packaging materials are often combined with several other materials to exploit each material's functional or aesthetic properties. As research continues to improve the properties of food packaging, the industry will undoubtedly reduce its impact on the environment and improve its degradation properties (Marsh & Bugusu, 2007).

2. Definition of a biodegradable film

Biodegradable films are produced using sustainable resources and are specifically engineered to decompose into organic components, such as carbon dioxide and water. Within a few months instead of centuries, it a far more ecologically sustainable choice for the packaging sector. They provide comparable resistance to moisture and contamination as plastic but exert a lesser ecological footprint. Food goods can be packaged using biodegradable films (Han, 2000).

3. Biodegradation

Biodegradation is a primary process through which most chemicals are released into the environment. Biodegradation is the process of deterioration caused by biological activity, primarily by microorganisms or enzymatic action. The final byproducts consist of carbon dioxide (CO_2), fresh biomass, and water (in aerobic conditions, i.e., in the presence of oxygen) or methane (in anaerobic situations, where oxygen is absent). Hence, the process of biodegradation can be categorized into two distinct components: (1) Aerobic and (2) anaerobic (Ayhllon-Meixueiro, 2000).

4. Features of Active Biodegradable Packaging:

Bio packaging films are extensively utilized across various industries. Biodegradable films were initially developed as a substitute for polyethylene films. Biodegradable plastics possess superior qualities compared to non-biodegradable plastics. Crucial characteristics of a high-quality packaging film encompass:

- Exhibit effective protective characteristics;
- Sustain the structural soundness;
- Inhibit or minimize microbial decay;
- Facilitate regulated respiration; (Shaikh & al, 2021)
- They exhibit resistance to moisture, heat, and organic matter for an extended duration of many weeks or even several months while maintaining their physical qualities unchanged;
- These products are free of polyethylene, do not leave any residue after composting, and are manufactured with recyclable biomaterials (Ivonkovic & al,2017).

Use of bioactive molecules in bio-packaging

1.Emballages actifs antioxydants

In addition to causing rancidity, the oxidation of lipids in food might potentially result in the creation of toxic aldehydes and the loss of nutritional value (Appendini & Hotchkiss, 2002).

Antioxidants are typically included in the first dietary formulation to prevent lipid oxidation. However, their inability to selectively target the food surface, where most oxidation processes take place, and their involvement in intricate reactions that can occasionally turn them into prooxidants limit their direct inclusion in food (Ribeiro-Santos et al., 2017).

By regulating the amount of oxygen that the product is exposed to, antioxidant-active packaging can be utilized to enhance product quality and prolong the shelf life of food, particularly meat and meat products (Majid & al ,2016).

2.Antimicrobial active packaging

Antimicrobial packaging is a novel solution derived from issues linked to food. This option prevents the growth of microorganisms, hence maintaining the food's quality, freshness, and safety. Antimicrobial food packaging functions to diminish, impede, or postpone the proliferation of germs that might exist in packed food or the packaging materials themselves (Quintavalla & Vicini, 2002).

The most effective method of packaging can be using packaging films that incorporate antimicrobial agents. These films gradually release the agents from the packaging material onto the surface of the product, ensuring that high concentrations are maintained in the areas where they are required. If an antimicrobial can be continuously released from the packaging over an extended duration, its effectiveness can also be prolonged during food storage, transportation, and distribution (Quintavalla & Vicini, 2002).

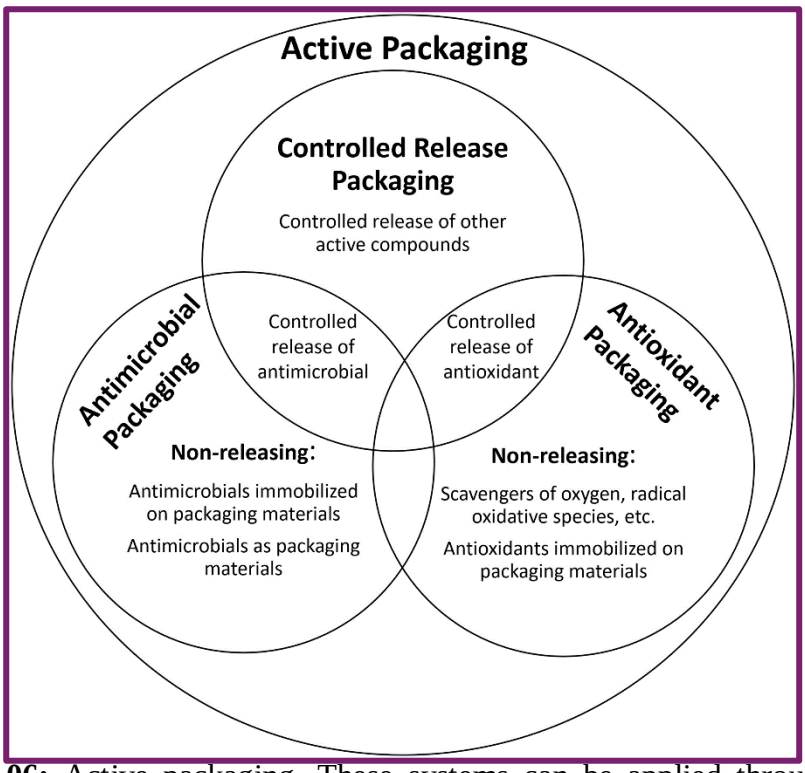


Figure 06: Active packaging. These systems can be applied through different techniques such as controlled release packaging, antimicrobial packaging, and antioxidant packaging (Casalini & Giacinti,2023).

II. Material and Methods

1. Objectives:

The most important thing is to provide biodegradable packaging materials comparable in characteristics and advantages to the conditioning materials because we intend to use poultry feet instead of destroying them.

The objectives of this study are to extract gelatin from chicken feet and use it to create an active biodegradable packaging material and phytochemical screening of aromatic plants used in the preparation of an organic food packaging.

2. Study Location:

Our study was conducted at laboratory No. 01, Microbiology, in teaching block B, Department of Applied Biology, Faculty of Natural Sciences and Life Sciences, Echahide Larbi University Tebessa –Tebessa.

3. Animal Material:

The chicken feet used in this study were brought from the poultry slaughterhouse Boutarfa -Tébessa-, more precisely, in the Industrial area. The feet have four fingers and are covered with scales. They end up with big claws and an unpleasant smell. They are transported to the Lab at one °C. We chose Fresh feet, not bleeding and not moldy yellow.

Figure 07: Chicken feet after cutting nails and removing crust external (personal photo).

For the extraction of gelatin, the products and materials are listed in the following table:

Table 01: Material and products used in gelatin extraction.

Materiel and Glassware	Products
• cooler. • Refrigerator. • ADE Scout Pro analytical scale. • Heated agitator by LAB TECH. • Continued at 45C° • Benzene beak • Beakers (500ml). • Erlenmeyer (1l). • Graduated pipette. • Petri dish. • Micropipettes. • Aluminum foil. • Parafilm. • Muslin cloth • Spatulas. • Scratch limes. • Knife.	• Chicken feet. • NaOH SIGMA ALDRICH. • acetic acid Ridel-de Haen. • Distilled water. • Tween 20. • Glycerol. • Ethanol. • Sorbitol. • Agar PCA CONDALAB. • GN • MacConkey. • Aluminum sulfate.

4. Sample preparation:

- Wash chicken feet with tap water at least twice to remove dust and all pollutants and impurities;

Figure 08: Washing chicken feet (personal photo).

-Remove nails by nail clipping and pliers, remove crust from the skin of feet of chicken with a knife, and wash for another time with tap water;

- Cut chicken feet into small pieces (5cm), then ground them with a meat grinder.

Figure 09: Cutting the feet (personal photo).

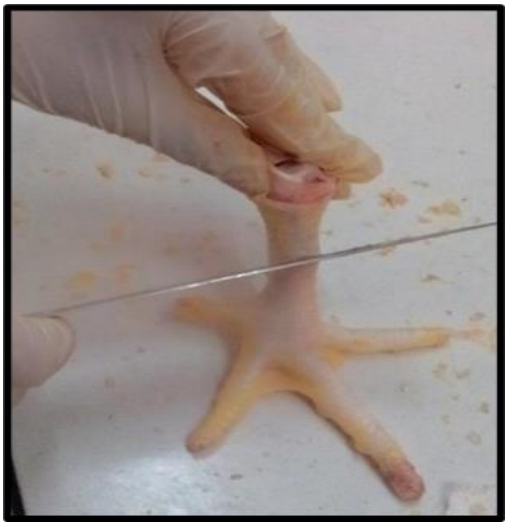


Figure 10: Cut the nails and decoule the skin (personal photo).

-Weigh each 100g in a plastic bag and store them in the freezer at 4°C until the chicken feet are useful.

Figure 11: Grinding of chicken feet by meat grinder (Personal photo).

Figure 12: Weigh each 100g in a plastique bags (personal photo).

5. Preparation of solutions

5.1. Preparation of sodium hydroxide (NaOH) solution 0.5 M:

-Introduce a mass of 20 g NaOH into a 1L volumetric flask, half filled with distilled water, using a funnel;

-Homogenize the solution by turning and shaking the blocked vial, then complete

to the gauge line.

5.2. Preparation of 5% acetic acid solution from 100% concentration stock solution:

-Pour 50 ml of acetic acid into a 1L volumetric flask containing little distilled water, plug the vial, and shake the solution;

-Complete with distilled water to gauge line, shake to make homogeneous;

-Acetic acid solution should be prepared when used.

Note! During the preparation of acetic acid solution, wear glasses, protective gloves, and lab coats. Work under a hood or in a ventilated area.

6. Gelatin extraction

Extraction was carried out according to (Suparno & Prasetyo, 2019),) with modification, and after several tests, we followed the present protocol with (Dr. Ferhi Selma)

The first step is the pretreatment process with a NaOH solution to eliminate non-collagen proteins and other impurities such as fats, minerals, pigments, and odors (Suparno & Prasetyo, 2019). 100g of ground chicken feet were mineralized with sodium hydroxide for 20 h at refrigeration, then filtered by muslin cloth three times (Chakka & al, 2017). The residue was washed repeatedly with distilled water until pH became neutral.

Excess water was removed by filtration using a muslin cloth (Fatima & al,2022).

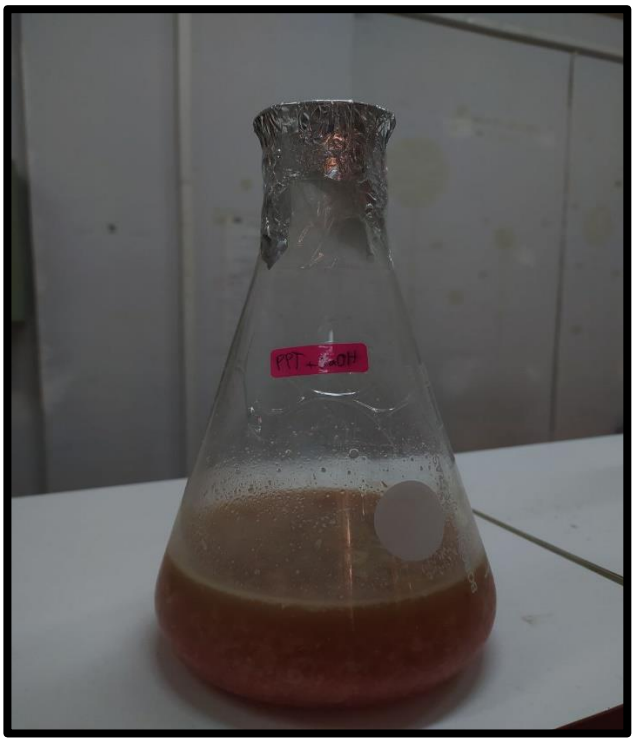


Figure 13: feet after treatment By NaOh (personal photo).

Figure 14: Muslin filtration (personal photo).

7. Hydrolysis with a solution of acetic acid

The second step was hydrolysis with an acetic acid solution (CH_3COOH) to modify the structure of collagen fibres to facilitate the extraction process (Suparno & Prasetyo, 2019). Chicken feet are soluble in acetic acid for at least 3.5 hours under heating agitation at 66 °C until acetic acid is evaporated and filtered by muslin cloth.

After putting the gelatin in Petri, small holes appeared on the surface. For one night, keep the boxes in the freezer at -4 °C.

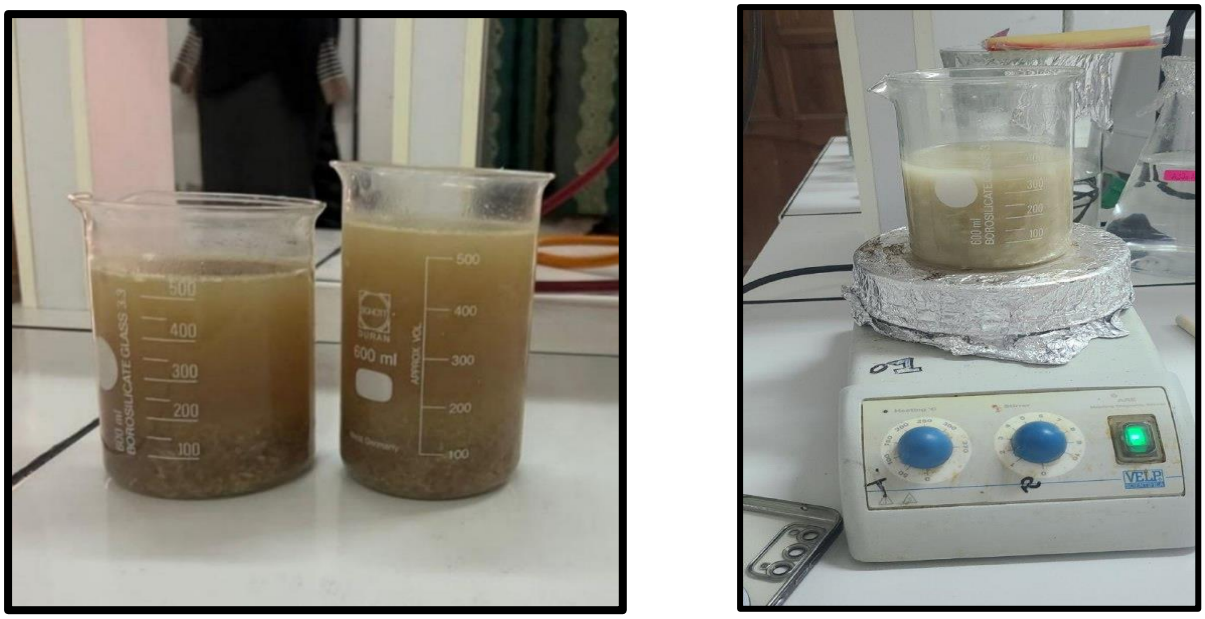


Figure 15\16: Acetic acid hydrolyzation (personal photo).

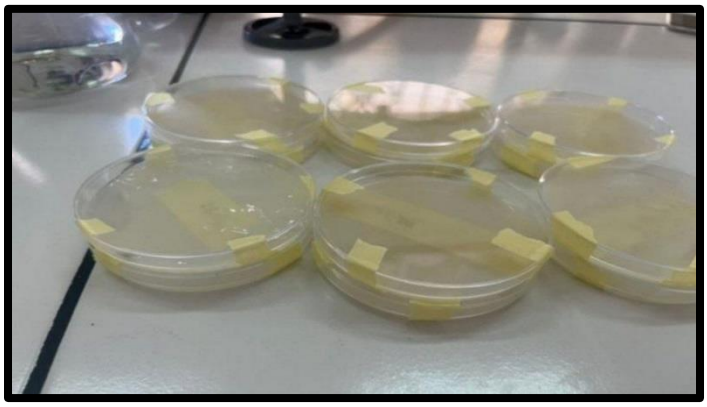


Figure 17: Put the gelatin in the petris boxes (personal photo).

8. Drying

The third step is drying the gelatin solution using chamber environmental (streamer) at 45°C for at least 48 hours until total water removal; then, the gelatin sheet was ground manually using mortar to obtain gelatin powder.

Figure 18: put the boxes in the streamer (personal photo).

Figure 19: Gelatin (personal photo)

8. Determination of extraction yield:

The yield of gelatin extraction was expressed as a percentage and calculated as follows:

$$R = (PG/PP) \times 100$$

R: extraction yield in %.

P.G.: Weight of gelatin powder.

PP.: Weight of chicken feet.

9. Preparation of gelatin film

9.1. Materials used in film preparation.

9.1.1. Gelatin: Extracted from poultry feet:

Gelatin's gelling ability is undoubtedly one of its most essential properties. When placed in cold water, gelatin swells, absorbing 5 to 10 times its volume of water. When heated to temperatures above its melting point, swollen gelatin dissolves, forming a gel on cooling. This sol-gel conversion is reversible and can be repeated, which is advantageous in many food applications.

Also, gelatin gels begin to melt between 27 and 34°C and tend to melt in the mouth. This is a desirable property in many foods (Imeson, 1992).

9.1.2. Essential oil of organic CLOVE:

Essential oil of organic CLOVE of Girofle (*Eugenia caryophyllus*) extracted by steam distillation is one of the official and most used methods for manufacturing essential oils (European Pharmacopoeia, 2007). It is the most used in French Pharmacopoeia (Roux, 2008). In this method, there is no direct contact between water and plant matter and then between the water and aromatic molecules; this avoids certain phenomena of hydrolysis (especially esters) or degradation that may harm the quality of the oil (Raaman, 2006). This operation is carried out in an alembic. This method is used for fresh plants such as mint, myrtle, and plants that carry their essential oils in the leaves that are collected, partially cut, and then brought to the distillation device. The principle of water vapor distillation consists of:

A boiler produces steam that destroys plant cell structure, which is then sent into the alambic, containing vegetable material for distillation. The steam ruptures aromatic glands, releasing aromatic and volatile molecules. These molecules are dragged to the top of the alambic, reaching the swan's neck. The steam, loaded with aromatic molecules, condenses through a

refrigerant serpentine, cooling and condensing to become liquid again. The liquid is collected in a decantation vase, called Florentin or essencier, where the essential oil separates from the distillation water. The hydrolate, or distillation water, is recovered or returned to the boiler for a new cycle (Rullière & Porraz, 2015).

Table 03: Identity of GIROFLE oil (*Eugenia caryophyllus*) .

Identity	Girofle oil
Process of obtaining	Distillation by steam distillation
Part of the plant extracted	Nails
Botanical name	Aromatic syzygium
Botanical family	Myrtaceae
Country of origin	Madagascar
Culture	Organic, certified by Ecocert FR-BIO-01
Presentation	Amber glass bottles with Codi goutte
Obtaining process	Cold expression
Quality	Food quality
Conservation	Essential oils are sensitive to UV radiation and the gradual evaporation of their constituents. Therefore, they must be stored in a colored bottle at 5°C.

10. Gas chromatography essential oil of *Eugenia caryophyllus*:

Gas chromatography is a separation technique used to analyze volatile compounds in a sample. In gas chromatography, the sample is injected into a column containing a stationary phase, which interacts with the compounds in the sample as they pass through (Skoog & al, 2017). The sample components are separated based on their affinity for the stationary phase and their volatility, allowing for their identification and quantification (Straka & al, 1992).

For the creation of films, the products and materials are listed in the following table:

Table 04: Material and products used in the creation of the film.

Materiel and Glassware	Products
• Refrigerator. • Scout Pro analytical scale. • Precision scale KERN ALS220-4N. • LAB TECH heating agitator. • Streamer in 45C°. • Benzene beak - Beakers (500ml). - Beakers (50 and 20 ml) • Erlenmeyer (1l). • Graduated pipette. • Petri. • Micropipettes. • Aluminum foil. • Parafilm. • A muslin cloth. • Spatulas.	• Gelatin extracted from chicken feet. • Tween 20. • Glycerol. • Essential oil of incense. • Distilled water. • Sorbitol.

11. film Creation

Gelatin-based film preparation was performed according to (Lee & al, 2015) and (Nazim & Sarbon, 2019) with modification, and after several tests, we followed this protocol with (Dr. Ferhi Selma).

To prepare a gelatin powder film-forming solution, we mixed gelatin with 100 ml of bi-distilled cold water (Lee & al., 2015), ensuring that all particles were moistened evenly and allowed to swell sufficiently for at least 5 min; the mixture was stirred continuously for 10 min at 60 °C. Then, glycerol (plasticizer), tween 20 (emulsifier), and sorbitol (Lee & al, 2015) were added, stirring for 10 min at 40°C.

Add essential oil, stirring for 2 min after lowering the solution's temperature to preserve the oil's volatile compounds. After filtration of the film-forming solution a muslin cloth was poured onto the Petri dish, left at room temperature for 18h, and oven-dried to 45°C (Nazmi & Sarbon, 2019).

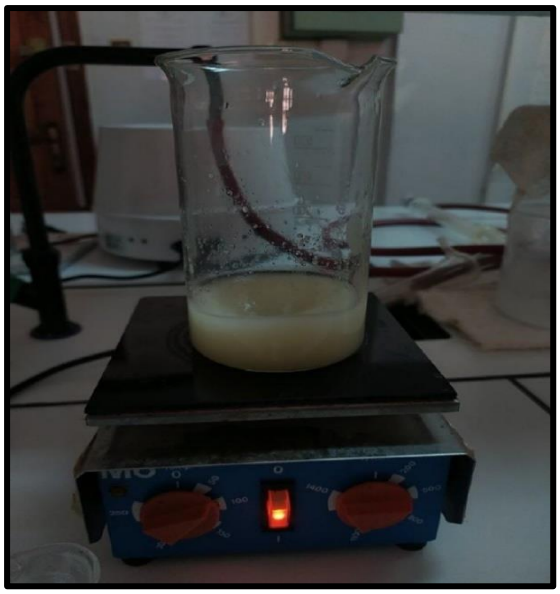


Figure 20\21: Preparation of the filmogenic solution (personal photo).

Figure 22\23: Filtration of the filmogenic solution (personal photo).

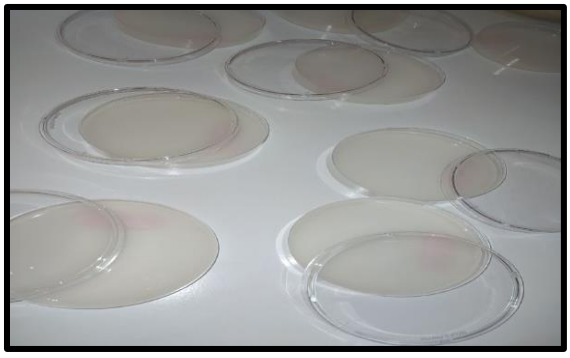


Figure 24: Injection of the filmogenic solution in the boxes Petries (personal photo).

12. Evaluation of the antibacterial activity of the essential oil of *Eugenia caryophyllus* :

For the microbial activity, we used the material and product in this table:

Table 05: Materials and products used in antibacterial activity.

Material	Product
• Micropipette • Bec Bensen • petrie • Emboulle • Streamer	• Gelose MH (Mueller Hinton) • NaCl • Wattman paper • Essential oil

12.1. Aromatogram:

This is an *in vitro* method of measuring the antibacterial power of essential oils. The antibacterial activity of the studied oil was evaluated using the gelose diffusion method (disc method) (Hammer & al,1999).

For the test of aromatogram, the bacterial are listed in the following table:

Table 06: Name of bacterial.

Family	Gam	Espece
Enterobacteriaceae	**Gram -**	*Kluyvera sp 153.* *Escherichia coli 214.* *Enterobacter cloacea 9.*

- ***Kluyvera sp. 153 :***

  Researchers are studying the genetic makeup and virulence factors of *Kluyvera sp. 153*, a lesser-known Gram-negative bacteria in the Enterobacteriaceae family with emerging pathogenic potential. This bacterium has been linked to nosocomial infections and resistance to multiple antibiotics, making it a significant challenge in clinical settings (Smith & al, 2021).

- ***Escherichia coli 214*** *:*

  Escherichia coli is a familiar name in microbiology. It is a Gram-negative *Enterobacteriaceae* family bacterium known for its diverse strains with varying pathogenic potentials. E. coli 214, a particular strain, has been implicated in various infections, including urinary tract infections and food-borne illnesses. The evolution of antibiotic resistance in this strain adds complexity to infection management (Gyles & Prescott, 2016).

- ***Enterobacter cloacae 9*** :

  Enterobacter cloacae is another noteworthy pathogen. It is a Gram-negative bacterium in the *Enterobacteriaceae* family. Strain 9 of Enterobacter cloacae is of concern due to its association with hospital-acquired infections and its ability to develop resistance to multiple antibiotics. Antimicrobial stewardship and infection control practices are crucial in combating its spread (Palma & al, 2020).

13. Evaluation of antibacterial activity

In order to evaluate the antibacterial activity of essential oil, we used the Muller-Hinton disc diffusion method described by (BARRY & al, 1985). The antibacterial activity is determined by the diameter of the area of inhibition of bacterial growth produced around the discs after incubation (BUZID & al, 2011).

13.1. The bacterial strains used

13.1.1. Preparation of the inoculum

- **Pre-culture preparation**

  The bacterial strains to be tested are enriched in nutritious broth for 30 min at 37 °C and then grown in dried boxes containing nutrient Gelose incubated 24h at 37 °C to obtain a young culture of isolated bacteria and colonies.

- **Preparation of bacterial suspension**

  We took well-isolated and identical colonies through a pastoral pipette and placed them in 10 ml of sterile salt water at 0.9% (NaCl). The bacterial suspension is well homogenized. Its opacity must equal 0.5 Mc Farland (10 CFU/ml).

13.2. Aromatogram test by solid medium diffusion technique (Disc method)

Aromatogram is a test used in a laboratory to determine a microorganism's sensitivity to different antibiotics. The antibacterial activity of the studied oil was evaluated using the gelose diffusion method (disc method) (Hammer & al,1999).

a. Preparation of discs:

Cut the Wattman paper into 6 mm plates. We sterilized the discs by autoclave; the white discs of Wattman paper were impregnated with essential oil in a sterile petri box near the beak. Then, they are deposited using a sterile pince on the Gelose, previously sown, on which they were dried. To determine the antimicrobial activity of oil.

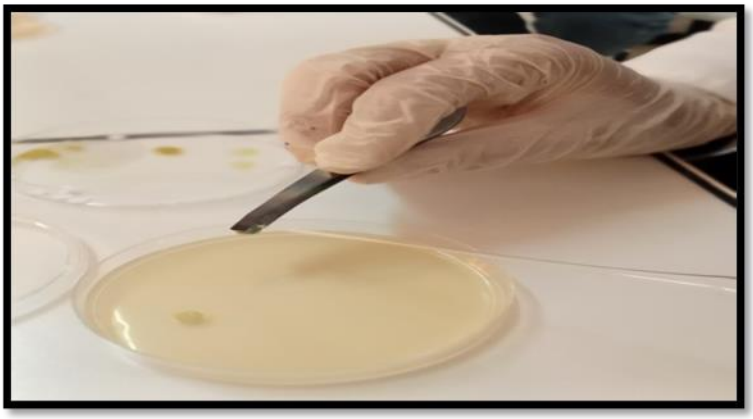


Figure 25: Disk repository (personal photo).

b. Technical

After 15 minutes, use the bacterial suspension. It is used to sow Mueller Hinton (MH) Gelose poured into Petri boxes with a thickness of approximately 4 mm.

c. Reading

The antibacterial activity is evaluated by measuring the diameter of the inhibition area around each disc using a rule in mm.

-Less than 6: The strain is **resistant.**

- Between 6-8: the strain is **sensitive +.**

-Between 8-14: the sensitive **strain**++

- greater than 14: the strain has a **high sensitivity** +++

14. Determination of minimum inhibitory concentrations (MIC) for essential oils:

14.1.Minimum Inhibitory Concentration (MIC):

a. Principle:

The principle of MIC is to determine the minimum concentration of a plant extract needed to inhibit the growth or multiplication of a target microorganism. Therefore, a measure of the antimicrobial activity of this Clove Girofle oil.

b. Technical:

CMI were determined using the liquid dilution technique, using a concentration of 0.3 and 0.5 mg of each extract.

First, disinfect the straw and the instruments used to avoid contamination. We prepared the extract from each plant using the same method used in the aromatogram test. The technique is made on a plate with 96 wells or a dome.

• The first cupola for each line serves as the cupola of the mother solution for each antibacterial test; 170uL of liquid Mueller-Hinton broth is added and mixed with an adequate solvent for the test with a well-adjusted concentration.

• Add 20uL of extract in the first cupola to obtain a volume of 190 Ul.

• Distribution of 95uL Mueller-Hinton broth to all other cups.

• Semi-logarithmic dilutions of reason 2 (transversing 95uL of homogenized from one dome to another in the horizontal direction in the same line) are made from the mother solution (the first dome); intermediate concentrations ranging from 0.5 µg/ml to 0,0156µg/ ml are obtained.

14.2. Preparation of bacterial inoculum

- Prepare from a 24-hour pure culture a suspension of the bacterial strain to be studied in 5 ml of strictest siological water of a density equivalent to 0.5 Mc Ferland (108 CFU/ml);
- Distribute 05uL of bacterial inoculum into each cupula ;
- Distribution of the bacterial inoculum: must be done within 15 minutes of preparation of the inoculum ;
- For each micro plaque, one must make a test without gloves oil and in the presence of the

inoculum; this line serves as a positive test, and one without the bacterial inoculum in the Presence of an extract serves as a negative test ;

- Incubate the microplate for 24H at 37°C. in the stove.

a. Reading

- The presence of a halo or a deposition at the bottom of the dome indicates the existence of bacterial growth, which means bacteria resistance;
- The absence of a halo or deposition at the bottom of the cupula indicates the lack of bacterial growth, which means bacteria sensitivity;
- So the MIC of each extract corresponds to the concentration of the first clear dome (no culture relative to the negative test);
- According to the result, classify bacteria in the resistant (R) or sensitive (S) category.

III. Results and discussion

1. Gelatin Extraction

After extracting the gelatin from the chicken feet, the extraction yield was calculated based on the ratio of the weight of the gelatin powder to that of the chicken's feet. As indicated **(Table 01)**, the extraction yield was approximately **11.22%.** This value is higher than that found by (Fatima & al,2022) (Between 3.50 % and 7.65 %) and too high compared to the yield of (Suparno & Prasetyo, 2019**) (0.14 %)**. In addition, the value is also higher than the gelatin yield of (Chakka & al, 2017) **(10.16%)** on both sides and higher than the 2 values **(6.59% and 8.51%)** presented by the same reference.

The extraction method using acetic acid has the advantage of producing collagen relatively fast, requiring little equipment, making it continuous, producing little waste, and reducing production costs. The difference in yield can be caused by the difference between extraction methods, solution concentration, material type, and extraction temperature and time (Zaelani & al, 2019).

Table 7: % gelatin yield extracted from chicken feet by various acid treatments.

Source and extraction method	**The yield in %**
Our result	11,22%
Extraction à partir des pattes de poulet par l'acide acétique **(Chakka & al, 2017).**	6.59 % 8,51% 10,16%
Extracting from chicken feet by acetic acid **(Fatima & al,2022).**	0,14 %
Hydro-extraction from chicken feet **(Suparno & Prasetyo, 2019).**	Between 3.50 % et 7.65 %

2. Sensory analysis of gelatin

The sensory test results (color, smell, appearance) for gelatin extracted from chicken feet are presented in the following table:

Table 08: Sensory characteristics of gelatin extracted from chicken feet.

Variables	Criteria
Color	Light yellow
Appearance	Solid Powder (Cristaux)
Smell	No smell

The gelatin extracted from the chicken feet was light yellow, without taste or smell, and a powder formed from very fine crystals.

We note that the properties of the gelatin extracted from chicken feet are entirely identical to the gelatine sold on the market.

3. Essential oil of Eugenia caryophyllus

3.1. sensory characteristics of essential oil of Eugenia caryophyllus

Essential oils are usually liquid and volatile at room temperature, with aromatic odors distinguishing them from fixed oils. They are colored (AFNOR, 2000).

The results of the organoleptic properties of clove Girofle oil of *Eugenia caryophyllus* are presented in the following table:

Table 09: sensory characteristics of essential oil of *Eugenia caryophyllus*.

Essentials oils	Organoleptic characteristics of oil		
	Appearance	**Color**	**Odor**
Clove oil	Liquide	yellow	aromatic, spicy, and robust, with a rising aldehyde odor.

The organoleptic properties of the oil, including its appearance, color, and odor, align closely with the typical characteristics of a clove of Girofle oil (*Eugenia caryophyllus)*, as noted in the literature (Doe & Roe, 2015). The consistent findings across studies suggest high reliability in assessing these sensory attributes. Additionally, the recommended storage conditions identified in our analysis are consistent with established guidelines for preserving essential oils (White & Black, 2017). These findings validate existing knowledge and offer practical insights for storing and maintaining the Organic essential oil of *Eugenia caryophyllus*. By corroborating our results with those reported in academic research, this study contributes valuable information to understanding the composition and conservation practices relevant to clove essential oil.

3.2. Composition essential oil of *Eugenia caryophyllus*

The composition of clove Girofle oil of *Eugenia caryophyllus* , in gas phase chromatographic analysis results are presented in the table:

Table 09: Composition of essential oil *Eugenia caryophyllus.*

Verification performed	**Measurement result**
Density	Traces
Eugenol	++++++++++++++++++++
Beta-caryophyllene	+
Acetate eugenol	++
Methyleugenol	Traces
Furfural	Traces

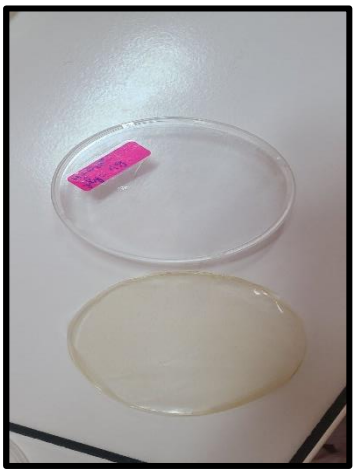
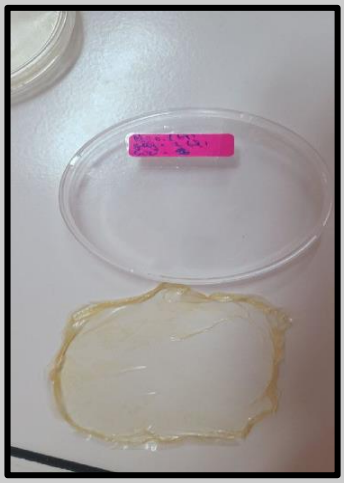
The essential oil clove Girofle of *Eugenia caryophyllus* analysis revealed a rich chemical composition, with eugenol comprising the major, followed by eugenol acetate and beta-caryophyllene . These findings align closely with the typical composition of essential oil of *Eugenia caryophyllus* reported in the literature. Studies by Li & al, (2017) have highlighted that eugenol content in clove essential oil generally falls within the 70% to 90% range, which is well-reflected in the dominant presence of eugenol in the Organic Girofle oil. Furthermore, the beta-caryophyllene content in the Organic clove Girofle oil of *Eugenia caryophyllus* is consistent with the average levels of 3% to 10% reported in Clove girofle oil by Mahboubi (2019). Similarly, the proportion of eugenol acetate in the clove Girofle oil of *Eugenia caryophyllus* aligns with typical ranges of 5% to 15% observed in clove essential oil, as Chevallier & al, (2018) indicated. The trace amounts of methyleugenol and Furfural found in the colve Girofle oil of *Eugenia caryophyllus* align with the minor presence of these compounds commonly reported in clove essential oil across various studies (Patel & al, 2020).

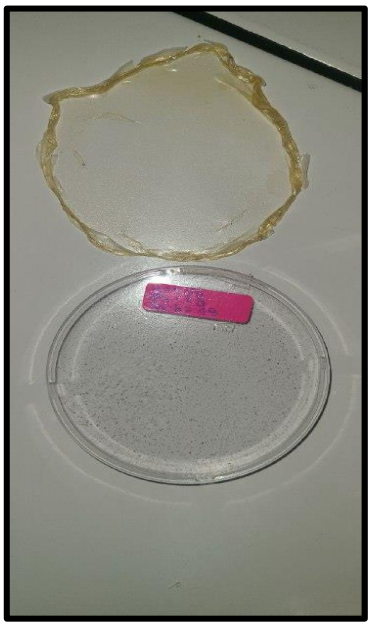
4. Sensory characteristics of biofilm

The sensory characteristics of different developed biofilms are presented in Table 03 below.

Table 10: The sensory characteristics of different developed biofilms.

Samples	Color	Smell	Texture	Surface	Figure of samples
Sam 1:	Light yellow	Smell of Oil of clove	Elastique	smooth	
Samp 2:	Light yellow	Smell of Oil of clove	Elastique	smooth	

Sam 3:	Transparent	Smell of Oil of clove	Very Elastique	smooth	
Sam 4:	Transparent	Smell of Oil of clove	Elastique	smooth	
Sam 5:	Transparent	Smell of Oil of clove	Very Elastique	smooth	

Sam 6:	Transparent	Smell of Oil of clove	Very Elastique	smooth	
Sam 7:	Light yellow	Smell of Oil of clove	Elastique	smooth	

Sam 8:	Transparent	Smell of Oil of clove	Very Elastique	smooth	

As for the presence or absence of incense essential oil, it only affects the smell. In its presence, the film acquires the smell of clove Girofle oil of *Eugenia caryophyllus*. Changing the concentration of oil did not affect the texture or surface.

The film created from sorbitol (samples 3,5,6,8) is clear and transparent, making it ideal for packaging food while allowing for easy visibility. The food film is made from glycerol (sample 1,2,4,7). It is supple, flexible, and tear-resistant, protecting packaged food well.

5. Antimicrobial activity

We studied the antimicrobial activity of the essential oils of lemon and shrimp using diffusion wells in the medium MH against 03 strains of gram-negative bacteria (*E. coli, Kluyvera sp, Enterobacter cloacae*). The results obtained are shown in the table.

Table 11: Diameter of inhibition zones (mm) illustrating the antibacterial activity of clove Girofle oil of *Eugenia caryophyllus* .

Bacterial strain	**Diameter of inhibition zones**	**Results**
Gram -	**Girofle**	**Girofle**
E.coli 214	**22**	+++
Kluyvera sp153	**14**	++
Enterobacter cloacea 9	**19**	+++

No effect: -, Medium: +, Good: ++, Very good: +++.

According to the results, clove Girofle oil of *Eugenia caryophyllus* has a significant inhibitory effect **(14)** on *Kluyvera sp* but less than *Enterobacter cloacae* **(19)**, and *E. coli* appears very sensitive.

A study (Bachiri & Nassiri, 2016) showed that the essential oil of clove Girofle (*Eugenia caryophyllus)* is A subsequent study by Boukartaba and Hammoum in 2019 revealed that *Bacillus cereus* was most sensitive to the essential oil of Girofle, with an area inhibition, followed by *Escherichia coli*, with 21 mm. These results are less than the ones we have obtained.

Benfekih's work 2015 showed an average activity of essential oil of *Eugenia caryophyllus* against Gram-negative bacteria, with an inhibition area of 10.5 mm to 15 mm. These results are inferior to the results we have obtained.

An essential oil's microbial effectiveness is due to its various constituents' nature and content. Previous studies have indicated that polyphenolic compounds are responsible for the antimicrobial activity of plant extracts (Funatogawa & al, 2004; Buzzini & al, 2008; Min & al, 2008). In addition, many authors have found that the change in the chemical composition of HES directly affects their biological properties. (Celiktas & al, 2007; Van Vuuren & al,

2009). The sensitivity of a microorganism to the essential oil depends on the properties of the oil and the micro-organism (Farah & al, 1999).

A 1995 study by Leclerc & al Found that the external membrane of lipopolysaccharides in Gram-negative bacteria constituted a waterproof barrier to hydrophobic substances, making them less likely to grow. In contrast, Gram + positive bacteria do not have this external membrane, and because the peptidoglycan layer is located outside, they are more sensitive to contact with essential oils (Burt, 2004). In addition, Gram-negative bacteria often have membrane proteins called efflux pumps, which expel the antimicrobial agents from the cell before they can act, as Loucif pointed out in 2011.

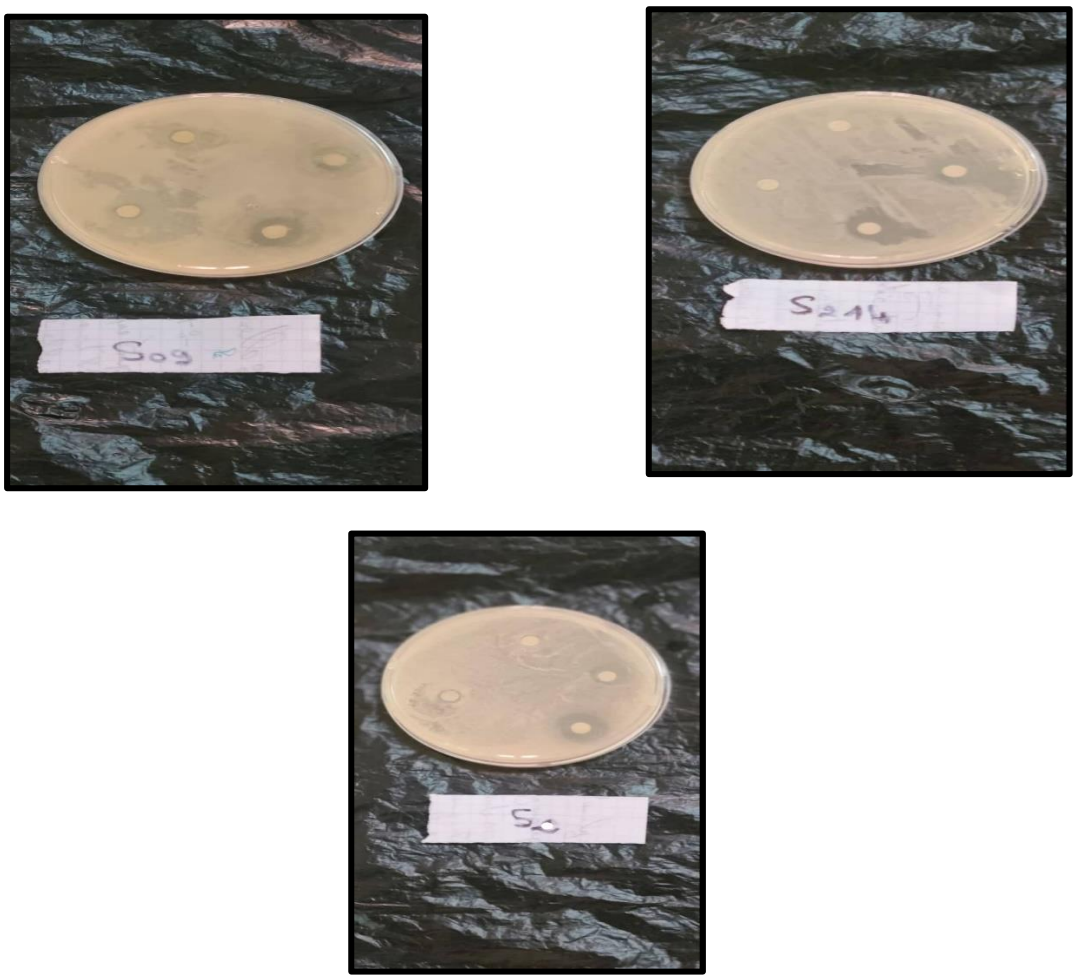


Figure 26: Inhibitory effect of the essential oil of Clove Girofle f *Eugenia caryophyllus* .

6. Test for minimum inhibitory concentration and minimum bactericidal concentration

The MIC and BMC values for the essential oils of *Eugenia caryophyllus* are presented in the table below.

Table 12: Minimum inhibitory (MIC) and bactericidal (BMC) concentrations of essential oils of clove Girofle (*Eugenia caryophyllus).*

Bacterial strain	**GIROFLE**		**CMB / CMI**
Gram -	**CMI**	**CMB**	**Girofle**
E.coli	**5**	**6**	**1.2**
Kluyvera sp	**3**	**4**	**1.3**
Enterobacter cloacea	**4**	**5**	**1.25**

+: total growth CMB / CMI = 4: Bactericidal

CMB / CMI = 8 - 16: bacteriostatic

Based on the results, the essential oil appears to have antimicrobial activity against *E. coli, Kluyvera sp,* and *Enterobacter cloacae* of gram-. The BMC of essential oil of *Eugenia caryophyllus* is between **3 mg/ml** in *Kluyvera sp, E. coli* **5mg/ml** and **4 mg/mL** for *Enterobacter cloacae*.

This suggests that the shrimp essential oil of *Eugenia caryophyllus* has a bactericidal effect on this group of bacteria. According to Rhayour (2002), the essential oil of *Eugenia caryophyllus* exercises a predominantly bactericidal activity due to its majority constituent, eugenol, which belongs to the phenol family.
According to Hermal (1993), the sensitivity of microorganisms may vary depending on the germ tested as an essential oil, which may be bactericidal to specific strains, bacteriostatic to others, or have no effect.
In general, the variability of the results is probably due to the influence of several factors, such as the methodology, the tested microorganisms and the essential oils used (Pattnaik & al, 1996)

Our results are superior to those you get, meaning peppermint's essential oil has a significant

effect against Gram-negative.

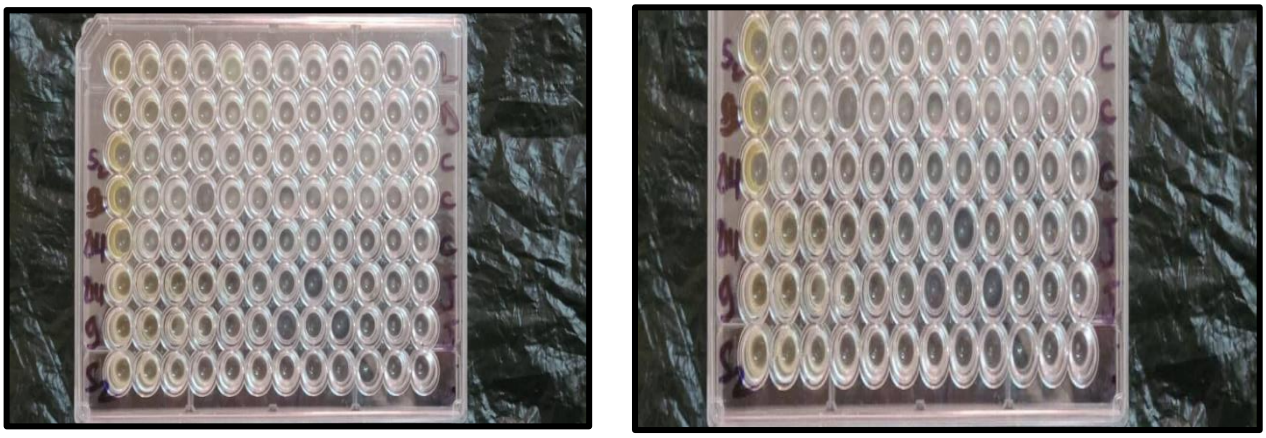


Figure 27: Minimum inhibitory concentration of essential oil of *Eugenia caryophyllus* .

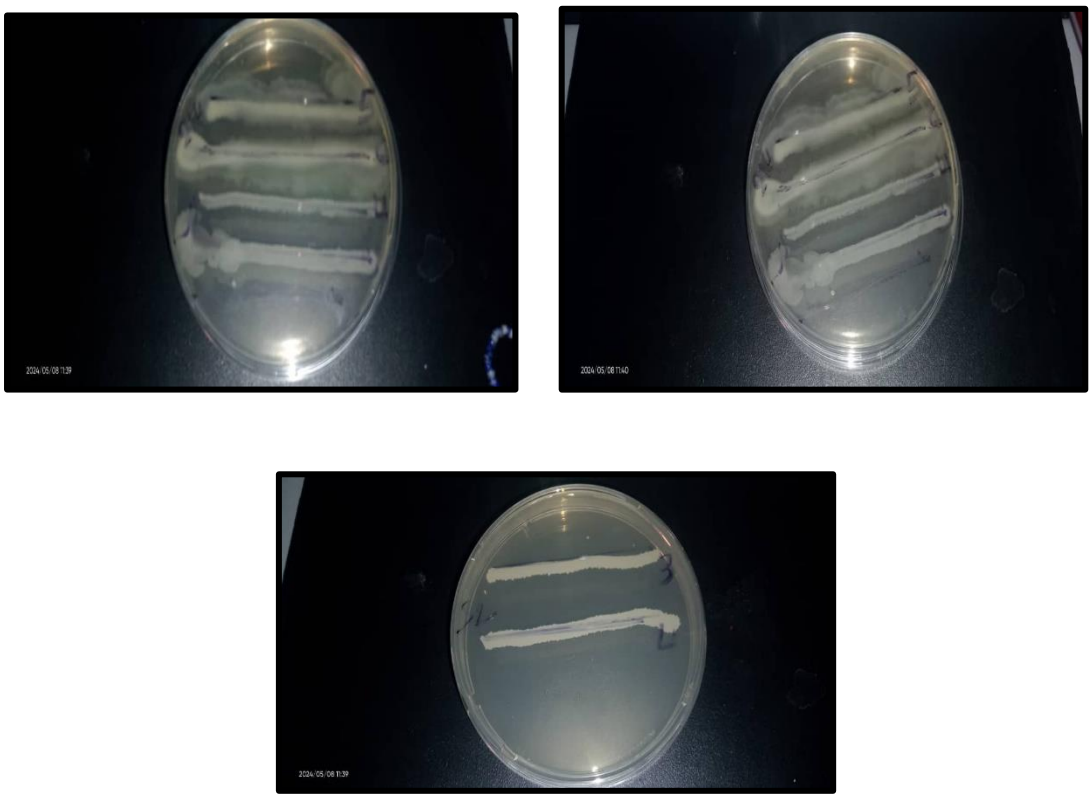


Figure 28: Minimum bactericidal concentration of essential oil of *Eugenia caryophyllus*.

Conclusion

Food packaging is essential in storing, protecting, and preserving the food it contains from manufacture until consumed. However, consumers are increasingly concerned about some current practices, and waste management is a major issue.

Our work focused on the phytochemical screening of biomolecules (essential oil of glove *Eugenia caryophyllus)* used in the creation of a new organic food packaging based on gelatin, glycerol, and sorbitol. The gelatin was extracted from chicken feet using acetic acid. Different combinations of these biopolymers, in addition to the essential oil, have been tested.

The yield of gelatin extracted from 100 g of chicken legs was estimated at 11.22%. The elatin extracted from the chicken feet was an excellent crystal powder, light yellow and without smell.

The organoleptic properties of the clove oil include its appearance as liquid, color as yellow, and odor as aromatic and spicy.

The essential oil of *Eugenia caryophyllus* revealed a rich chemical composition, with phenol comprising the major , followed by esters and sesquiterpenes.

We evaluated the antibacterial activity of the essential oil of clove Girofle of *Eugenia caryophyllus* against multi-resistant gram-negative bacteria. The results show that the clove oil positively affects the gram-negative. The essential oil of clove Girofle of *Eugenia caryophyllus* has a strong antimicrobial effect; it inhibits all bacteria (*E. coli, Kluyvera sp, Enterobacter cloacae*).

In perspective, it will be interesting to study the more biological activities of the essential oil of Eugenia caryophyllus used in the preparation of biofilm and also test the bioactivity of the biofilm.

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