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Antimicrobial activity of by-products from mint essential oil extraction

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Dedication

To my cherished parents,

You are the silent architects of my dreams and the steady light in every moment of doubt.

Your values, your resilience, and your unconditional love shaped the person I am today.

This achievement stands as a humble tribute to all that you have given me without asking for anything in return.

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Dedication

To my dearest parents,

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Every page I wrote carries a part of your story, for without you, none of this would have been possible.

With all my gratitude and love this is for you.

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Summry

Abstract

The extraction of essential oils from aromatic and medicinal plants, such as Mentha spicata (spearmint), generates large quantities of by-products, including hydrosols, liquid residues, and solid biomass, which are often neglected or discarded. This study aims to evaluate the antimicrobial potential of these by-products resulting from the hydrodistillation process. The findings, based on disc diffusion assays and minimum inhibitory concentration (MIC) tests, revealed a moderate antimicrobial activity, highlighting the possibility of valorizing these co-products as bioactive agents. Phytochemical screening revealed the presence of bioactive compounds such as tannins, flavonoids, terpenoids, and saponins in methanolic extracts and liquid waste. These findings suggest that the valorization of these plant-derived residues as natural antimicrobials offers a sustainable and environmentally friendly solution within the essential oil industry.

Keywords: antimicrobial activity, by-products, essential oil extraction, *Mentha spicata*, phytochemicals.

Résume

L'extraction des huiles essentielles à partir de plantes aromatiques et médicinales, telles que *Mentha spicata* (menthe verte), engendre des sous-produits en grande quantité, notamment des résidus solides, des extraits liquides et des hydrolats, qui demeurent largement inexploités ou éliminés. La présente étude vise à évaluer le potentiel antimicrobien de ces co-produits issus du processus d'hydrodistillation. Les résultats obtenus, à travers des tests de diffusion sur disque et la détermination de la concentration minimale inhibitrice (CMI), révèlent une activité antimicrobienne modérée, suggérant un intérêt pour leur valorisation en tant qu'agents bioactifs. Le criblage phytochimique a révélé la présence de composés bioactifs tels que des tanins, des flavonoïdes, des terpénoïdes et des saponines dans l'extrait méthanolique et le résidu liquide. Ces résultats suggèrent que la valorisation de ces résidus végétaux en tant qu'antimicrobiens naturels offre une solution durable et respectueuse de l'environnement dans l'industrie des huiles essentielles.

Mots-clés : activité antimicrobienne, extraction d'huile essentielle, composés phytochimiques, *Mentha spicata*, sous-produits.

ملخص

تُنتج عملية استخلاص الزيوت الأساسية من النباتات العطرية والطبية، مثل *Mentha spicata* (النعناع الأخضر)، كميات كبيرة من المنتجات الثانوية، تشمل الهيدولات، والمخلفات السائلة والصلبة، والتي غالباً ما تُهمل أو يتم التخلص منها. تهدف هذه الدراسة إلى تقييم الفعالية المضادة للميكروبات لهذه المنتجات الثانوية الناتجة عن عملية التقطير المائي. وقد أظهرت النتائج، من خلال اختبارات الانتشار على الأقراص وتحديد التركيز المثبط الأدنى(CMI) ، فعالية مضادة للميكروبات بدرجة متوسطة، مما يشير إلى إمكانية استغلالها كمركبات ذات نشاط بيولوجي . أظهرت المنتجات الثانوية فعالية معتدلة، وهو ما أكده اختبار الانتشار القرصي واختبار الحد الأدنى للتركيز المثبط (MIC). كشف الفحص الكيميائي النباتي عن وجود مركبات نشطة بيولوجيًا مثل العفص والفلافونويدات والتربينويدات والصابونين في المستخلصات الميثانولية و البقايا السائلة. تشير هذه النتائج إلى أن تثمين هذه المخلفات المشتقة من النباتات كمضادات طبيعية للميكروبات يوفر حلاً مستدامًا وصديقًا للبيئة في صناعة الزيوت العطرية.

الكلمات المفتاحية: النشاط المضاد للميكروبات، المنتجات الثانوية، استخلاص الزيوت العطرية، مينثا سبيكاتا، المواد الكيميائية النباتية.

List of figures

List of photography

List of tables

List of abbreviations

- **aq.**: Aqueous
- **BHI**: Brain Heart Infusion
- **CFU**: Colony Forming Units
- **CLSI**: Clinical and Laboratory Standards Institute
- **DMSO**: Dimethyl Sulfoxide
- **DW**: Distilled Water
- **EO**: Essential Oil
- **EtOH**: Ethanol
- **MH**: Mueller-Hinton (agar or medium)
- **MHB**: Mueller-Hinton Broth
- **MIC**: Minimum Inhibitory Concentration
- **NaCl**: Sodium Chloride
- **PBS**: Phosphate-Buffered Saline
- **pH**: Potential of Hydrogen
- **T°**: Temperature
- **UV**: Ultraviolet
- **v/v**: Volume per Volume
- **w/v**: Weight per Volume
- **WHO**: World Health Organization

Table of contents

Table of contents

Table of contents

Table of contents

Table of contents

Introduction

1. General introduction

Medicinal plants have long played a crucial role in traditional medicine systems worldwide, owing to their diverse bioactive compounds and minimal side effects when used correctly **(Debuigne & Couplan, 2013; Bousta & Ennabili, 2011).** These plants serve as a source of secondary metabolites, including flavonoids, alkaloids, terpenoids, and phenolics, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties **(Chabrier, 2010; Ramos, 2007; Elise, 2019).**

In particular, *Mentha spicata* L. (spearmint), a member of the Lamiaceae family, is widely cultivated and appreciated for its aromatic essential oils and its uses in both traditional medicine and modern industries **(Askari & Mohtashami, 2022; Gupta *et al.*, 2023).** The essential oils from mint are rich in compounds such as carvone, limonene, and 1,8-cineole, which have been shown to possess significant biological activity, especially antimicrobial **(El *et al.*, 2022; Chrysargyris *et al.*, 2021).**

However, the extraction of essential oils through methods like hydrodistillation generates large volumes of by-products such as hydrolats, liquid waste, and solid residues. These by-products are typically discarded, despite their potential to contain residual bioactive compounds with antimicrobial effects **(Zaccardelli *et al.*, 2021; Almeida *et al.*, 2024).**

Given the increasing interest in sustainable practices, the valorization of these by-products aligns with circular economy principles. Reintegrating them into antimicrobial formulations not only reduces waste but also offers new alternatives to synthetic antibiotics, especially in the face of growing bacterial resistance **(Di Maro *et al.*, 2024; Lopes *et al.*, 2024)**

This research focused on evaluating the antibacterial potential of by-products from the extraction of mint essential oil, specifically: hydrolate, liquid waste, and solid residues. Thus, this Master thesis is structured in different chapters:

- Chapter I presents a general overview of medicinal plants and their therapeutic uses;
- Chapter II describes the properties, uses, and chemical composition of essential oils;
- Chapter III focuses on the by-products of essential oil extraction, their environmental impact, and valorization potential;
- Chapter IV details the botany, uses, and chemical characteristics of *Mentha spicata*;

- Chapter V explains the materials and methods used, including phytochemical screening and antibacterial testing;
- Chapter VI discusses the results, highlighting the comparative antimicrobial efficacy of the tested by-products;
- Conclusion.

CHAPTER I

General Information About Medicinal Plants

1. What is a medicinal plant?

More generally, a medicinal plant is a plant endowed with a therapeutic effect on the body without being toxic at normal doses. It is important above all that reasonable use with the aim of curing a declared disease does not cause harm **(Debuigne and Couplan, 2013).** Moreover, a medicinal plant is not only a plant, but can also be a tree, a bush, a mushroom, a vegetable, a root, an *algae*, etc **(Bousta and Ennabili, 2011).**

Obviously, a medicinal plant contains one or more substances that can be used for therapeutic purposes or are precursors for the synthesis of useful medications **(Sofowora, 2010),** and whose therapeutic properties are scientifically or empirically proven through the use in traditional medicine **(Neffati and Sghaier, 2014).**

2. Chemical composition of medicinal plants

The chemical composition of aromatic plants is complex. Most scientists define natural substances as chemical compounds found in many plant families and species **(Iserin *et al.*, 2001).** Currently, the development of various extraction, isolation, and identification techniques has allowed the use of these compounds in various industrial fields, such as pharmaceuticals, food, cosmetics, and perfumery **(Boughrara, 2018).**

The list of plants that precisely fall within this framework is exhaustive. The majority of medicinal plants are used in the form of herbal teas, extracts, or preparations that contain complex substances; to this day, it is really difficult to define the molecules responsible for the action in all these preparations. Although some pharmacological effects of these plants are tested on experimental subjects, they have been attributed to compounds such as alkaloids and their derivatives, terpenes, and polyphenolic compounds **(Chabrier, 2010; Boughrara, 2018).**

In the lines below, the importance of some secondary metabolite components is given.

2.1. Flavonoids

Flavonoids, also known as polyphenolic compounds, are widely distributed in the plant kingdom **(Ramos, 2007; Elise, 2019).** These compounds constitute the colored pigments of plants, ranging from yellow to red, giving these organisms the multitude of colors they exhibit. They are always present in significant quantities in fruits, vegetables, but also in beverages such as tea and coffee, so we regularly find them on our tables during meals **(Elise, 2019).**

Moreover, flavonoids are part of the composition of various herbal remedies used in traditional medicine, such as *Combretum micranthum, Aloe vera sp*., *Ageratum conyzoides*, and *Cylicodiscus gabunensis*. They are used in the treatment of 73 diseases, among which malaria, typhoid fever, dermatoses, jaundice, kidney pain, colic, diabetes, cardiovascular diseases, and hypertension are the most important. These plants appear as powerful antioxidants and antiradicals **(Kidik Pouka *et al.,* 2015).**

2.2. Alkaloids

Alkaloids are natural and organic substances primarily derived from plants and that contain at least one nitrogen atom in their chemical structure, with a varying degree of basicity **(Yinyang *et al.* 2014).** Since the identification of the first alkaloid, namely morphine, from opium in 1806, **Yinyang *et al*. (2014)** stated that more than ten thousand alkaloids have been isolated from plants; some of which are well-known drugs with proven therapeutic virtues, for example: a derivative of Madagascar periwinkle (*Vinca rosea Syn Catharanthus roseus)* used to treat certain types of cancer.

2.3. Terpenoids

Terpenoids form a class of natural organic substances, many of which are encountered daily and whose names often reflect this familiar nature. They include menthol, which is responsible for the smell of crushed mint leaves, cedrene, which is responsible for the smell of wooden pencils, abietic acid, an important component of pine resin, betulin, the white pigment of birch bark, β-carotene, the orange pigment of carrots and many berries, and rubber **(Masyita *et al*., 2022).**

2.4. Steroids

Plant sterols, steroid hormones, and brassinosteroids (BRs), are compounds that exert a wide range of biological activities. They are essential for plant growth, reproduction, and responses to various abiotic and biotic stresses. Given the importance of sterols and BRs in these processes, engineering their biosynthetic and signaling pathways offers interesting potentials for improving crop yield **(Vriet *et al.*, 2012).**

3. Use of medicinal and aromatic plants

According to a Burkinabe botanist: "no plant on earth is foreign to human needs and contributes to them, whether it concerns his home, his clothing, his roof, his pleasures, or even simply his food" **(FAO, 1996).**

Aromatic and medicinal plants (AMP) are used like all plants in food, spices, additives, ornamental, toxic, pastoral and forage, crafts (basketry, tanning, carpentry), beverages, condiments, cosmetics, hygiene, industrial incense because they contain components with therapeutic value. Thus, these plants have been used for centuries as remedies for human and animal diseases **(Chaachouay, 2020).** Thus, these plants have been used for centuries as remedies for human and animal diseases **(Chaachouay, 2020).**

In reality, about 30% of the medications prescribed by doctors are of natural origin, while this proportion is 50% for over-the-counter medications. Among these plant-derived medications, there is taxol, isolated from the yew tree (*Taxus baccata L.*), which is used in the treatment of gynecological cancers **(Sofowora, 2010).**

In order to present the importance of the therapeutic use of some medicinal plants, Table 1 includes the name of the plant, its most utilized part, and its use.

Table 1. Main medicinal plants and their medicinal uses **(Iserin et *al.*, 2001)**

Plants	Use in traditional medicine
Great chamomile (*Tanacetum Parthenium L*)	Fresh leaves or tincture for migraines and headaches.
Melisse (*Melissa officinalis L*)	Infusion for anxiety, difficulty sleeping, indigestion. Herpes lotion.
Souci (*Calendula officinalis L*)	Cream for cuts and scrapes. Infusion against fungal infections
Peppermint (*Mentha piperita* L.)	Infusion for headaches and indigestion. Lotion for itching.
Rosemary (*Rosmarinus officinalis L*)	Infusion as a tonic for the nervous system and against difficult digestion.
Sage *(Salvia officinalis L)*	Infusion against sore throats, mouth ulcers and diarrhea.
Thym (*Thymus vulgaris L*)	Infusion against coughs, colds and infections.

4. Classification of medicinal plants according to their therapeutic effects

There are many medicinal and aromatic plants used for their therapeutic effects, and it is difficult to classify them all according to their properties. **Table 2** includes the classification of medicinal and aromatic plants according to their therapeutic effects.

It is important to note that these plants can have side effects and interact with other medications, so it is recommended to consult a healthcare professional before using them for medicinal purposes **(Kosh-Komba *et al*., 2021).**

According to the reading of Table 2, we can say that *C. nobile* "Roman chamomile" is the most used plant in the field of phytotherapy. This medicinal plant has been used for centuries to treat a wide range of health issues. This plant has soothing, anti-inflammatory, digestive, and antispasmodic therapeutic effects. Out of the 10 plants presented, we find that the following six plants: *M. pipertia, Z. officinale, Curcuma* sp., *Eucalyptus* sp., *Thymus* sp., *G. glabra* have almost the same therapeutic effect on several diseases. Furthermore, *V. officinalis* remains the only plant that can treat anxiety and induce sleep compared to the other plants.

Table 2. Presentation of the therapeutic virtues of some medicinal plants **(Petrovska, 2012; Kosh-Komba *et al.*, 2021).**

Therapeutic effect	Some medicinal plants									
	Chamaemelum nobile	*Lavandula* sp.	*Mentha piperita*	*Zingiber officinale*	*Curcuma* sp.	*Eucalyptus* sp.	*Thymus* sp.	*Glycyrrhiza glabra*	*Salvia rosmarinus*	*Valeriana officinalis*
Apaisante	X									
Anti-inflammatory	X			X	X	X	X	X		
Digestive	X		X							
Antispasmodic	X		X					X		
Calming		X								X
Sedative		X								X
Antiseptic		X					X		X	
Healing		X						X		
Analgesic			X	X	X					
Immune booster				X	X		X			
Anti-nausea										
Antioxydant					X	X			X	
Expectorant						X	X	X		
Decongestant						X				
Calmante	X									
Anxiolytic										X
Hypnotic										X

5. The precautions for using medicinal plants

To treat numerous health issues, the use of medicinal plants is still crucial. When using them, it is important to take precautions, as they can also have undesirable side effects or interact with other medications **(ANSM, 2020).**

Undoubtedly, the commonly held idea that herbal medicines, because they come from nature, are necessarily devoid of any risk, is dangerous because not everything natural is harmless. In this regard, traditional medicine remedies must be used wisely, like any other medication, with full awareness of the risks of toxicity or drug interactions **(Lehmann, 2013).** Table 3 includes two plants that have toxic effects on human health.

This type of risk may occur due to the exploitation of medicinal plants containing complex mixtures of different potentially toxic molecules, such as cardiotonic glycosides, specific alkaloids, and coumarins. In some cases, toxic contaminants such as pesticides, heavy metals, pollen, microscopic fungi, or molds can cause toxic and/or allergic reactions and also chemically alter preparations of medicinal plants **(ANSM, 2020).**

Table 3. The different effects of medicinal plants (**ANSM, 2020).**

Medicinal plant	The different effects
St. John's wort *Hypericum perforatum* L. (air section)	Cause a decrease or an increase in the therapeutic effect of other medications through drug interactions. By enhancing the effect of enzymes involved in the metabolism of certain medications.
Ginkgo *Ginkgo biloba* L. (feuilles)	Ginkgo leaf extracts can either increase the activity of other medications (such as anticoagulants) or decrease it (such as the antiretroviral efavirenz).

CHAPTER II

General Information About Essential Oils

1. Histories

Going back deeply into history, it is seen that the usage of natural essences, especially to benefit from their scents, was thought to have a curative effect that went beyond explanation, while there was no concrete knowledge or information that could be strictly supported by scientific data. Such applications suggested the involvement of certain rules in general or ideas in the minds of people concerning the use of natural substances **(Ansari *et al.*, 2023).** These applications were generally used to state that these essences would have been useful for the body and soul at regular intervals in the lives of individuals, particularly those that would provide serious benefits in the short term before large gatherings, weddings, or death ceremonies **(Noreen *et al.*, 2023; Elachouri *et al.*, 2021).**

According to some trustworthy sources regarding the use of natural essences, these essences may have significantly contributed to this purpose through combinations that may have evoked feelings of elation or other mood states in one's mind. As soon as the rules that emerged as a product of reasonable and plausible combinations began to be disclosed gradually, such interventions were transformed into psychotherapy **(Hikal *et al.*, 2021).** On behalf of the people whose knowledge about natural essences was accumulated in history and reached abundance over time, the assessment of scientific data to provide the best projection for developing and guiding innovations in order to enjoy the healing and rejuvenating effects suggested by the potential of these essences through empirical research was seen as sufficient and valuable as a bridge **(John *et al.*, 2022).**

2. Definition of essential oils

Aromatherapy utilizes plant phytochemicals to enhance health and promote emotional relaxation. Essential oil is crucial in this practice for massage, bathing, and inhalation, influencing the central nervous system **(Almeida *et al.,* 2024).** Extracted through steam distillation from various plant parts and storage structures, essential oils serve multiple roles in medicine, deodorization, sterilization, and more **(Epelle *et al.,* 2023).** Despite being called "oil," they are water-insoluble and essential **(El Amrani, 2023)**, reflecting a plant's essence, taste, aroma, and function. Four essential oil types exist: extracted substances, volatile oils containing the plant's essence, and mixtures formed by secondary metabolism, differing by extraction method and plant part. The complex chemical composition of essential oils is diverse and varies among species, with most being intricate mixtures rather than single compounds **(Park *et al.,* 2023; Bharti *et al.,* 2022)**.

3. Localization or distribution of essential oils

3.1. Introduction to the essential oils distribution

Most essential oils derived from plants. The plants are distributed from the Arctic to the Antarctic. They are tropical, subtropical, or temperate in nature and are distributed worldwide **(Bolouri *et al*., 2022)**. For every food, many species vary from cultivated agricultural crops to the wild relatives in their original natural environment around the world **(Maurya *et al*., 2021)**.

Localization is a very critical concern, as it has been shown that oil from such a natural and wild environment is of better quality because the oil composition is influenced by the climate and pedo-climate variations. In these climatic conditions, only a few of these wild relatives grow at the top of the quality of the product worldwide **(Ni et *al.,* 2021)**.

3.2. Climate and Pedo-Climate characteristics

Climate and pedo-climate are the primary factors that affect the composition of the management of the germs and the essential oils. At each end of the world, where different climates and pedo-climate conditions exist **(Lechhab *et al*., 2021)**, different oils with different amounts of components can exist in native species, for example, in citrus, lavender, and rose **(Gong *et al.*, 2024)**. In essential oils derived from species that inhabit somewhat separated environments, rich connections between environmental factors and chemical composition have been found **(Oualad El Majdoub, 2023)**.

4. Methods of obtaining essential oils

To obtain essential oils, extraction from natural sources is crucial. Notable processes include improved extraction techniques (Figure 1) and certification, along with production by fungi and chemical composition analysis **(de *et al*., 2022)**. Techniques apply to biosynthetic systems, like plant multiplication for aromatic plants. Steam distillation, used since ancient times, remains relevant in the industry **(Ayub *et al.,* 2023)**. A modern method, supercritical fluid extraction with diffusion cells, enables quicker extraction of essential oils than hydrodistillation **(Machado *et al.,* 2022).**

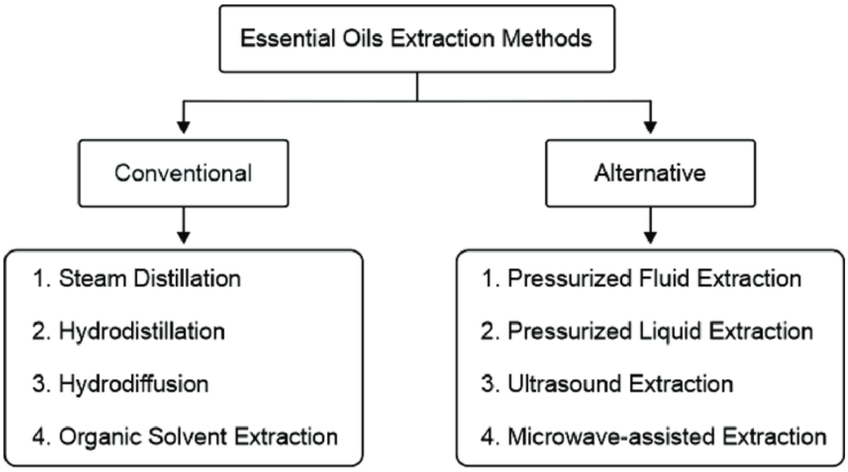


Figure 1. Extraction methods used for obtaining essential oils **(Almeida *et al.,* 2024).**

5. Chemical composition of essential oils

Essential oils are complex mixtures of volatile organic compounds primarily composed of terpenes and terpenoids, which determine their aroma and biological activity. Monoterpenes (e.g., limonene, pinene) and sesquiterpenes (e.g., caryophyllene) provide antibacterial, antifungal, and anti-inflammatory properties. Oxygenated derivatives, including alcohols (e.g., linalool, menthol), ketones (e.g., camphor, carvone), aldehydes (e.g., citral, cinnamaldehyde), esters (e.g., linalyl acetate), phenols (e.g., thymol, eugenol), and oxides (e.g., 1,8-cineole), contribute to their therapeutic effects **(Eslahi *et al.,* 2017; Qaderi *et al.,* 2023)**. Additional components like coumarins, furanocoumarins, and sulfur-containing compounds influence their medicinal and industrial applications **(Guarino *et al.,* 2021)**.

The chemical composition of essential oils varies based on plant species, environmental conditions, extraction methods, and storage, affecting their potency and usability in aromatherapy, medicine, cosmetics, and food industries **(Ganteaume *et al.,* 2021)**.

6. Properties and use of essential oils

Essential oils have antimicrobial, antioxidant, anti-inflammatory, and analgesic properties. The anti-mutagenic activity of these oils is also highlighted, showcasing their significance in health improvement research. Additionally, they have emerging trends in aromatherapy benefits and traditional food preservation roles **(Wainer *et al.,* 2022)**.

Formulation of essential oil for preservation: The effectiveness of essential oils against fruit spoilage is influenced by their application method. Some essential oils can be used for fumigation to control fungal growth **(Freche *et al.,* 2022 ; Pandey *et al.,* 2022)**.

Adding glyceryl water or alcohol enhances the antifungal properties of essential oils during storage **(Xylia *et al.,* 2021)**. While pure essential oils are an option, they are often not cost-effective, prompting researchers to develop value-added formulations **(Perumal *et al.,* 2022)**. For instance, an emulsion of cinnamon oil was created with varying hydrocolloids; a mixture of maltodextrin and xanthan gum with 0.5% cinnamon showed effective fungal inhibition. Additionally, combining chitosan with clove oil improved the durability of red seaweed cakes **(Freche *et al.,* 2022 ; Perumal *et al., 2022*)**.

CHAPTER III

By-products from Essential Oils extraction

1. Nature and composition of residues

1.1. Definition of waste from extraction

The essential oil manufacturing process involves the extraction of essential oil from plant materials by different methods **(Sadgrove *et al.*, 2022)**. Essential oil can be extracted from various plant materials such as flowers, seeds, bark, leaves, wood, roots, or fruits, depending on the prevailing method. Early essential oils were obtained primarily by steam distillation and are referred to as hydrodistilled essential oils **(Bolouri *et al.* ,2022).**

Later on, two further different extraction methods were developed: cold pressing and solvent extraction, which is also known as enfleurage. The cold pressing process involves oil extraction by exposure to high pressure. Meanwhile, the solvent extraction method involves various solvents, namely, hexane, ethanol, ether, or an acetone mixture using percolation or maceration (**del *et al.* ,2021 ; Krakowska-Sieprawska *et al.*, 2022)**.

Essential oil extraction waste can contain organic, inorganic, or hazardous substances. Organic components usually from 65 to 85% of the wastes and generally disappear by natural decomposition **(Yang *et al.*, 2023)**. The essential oil extraction wastes' organic components can be classified as rhizomes, peels, rinds, twigs, etc. Inorganic substances can be composed of minerals, rocks, snow, and/or chemical substances of an inorganic nature **(Russo & Palla, 2023)**. Hazardous wastes display a poisonous/toxic nature attributed to chemical and biological aspects. These potential environmental threats mainly manifest themselves through waste-land, water, and air pollution **(Bhatt *et al.*, 2022; Shahi *et al.*, 2023)**.

1.2. Types of by-products (plant residues, hydrolats, press cakes)

There is a wide range of by-products regarding the kind of extraction method employed **(Maqbool *et al.*, 2023 ; Zaccardelli *et al.*, 2021)**. While steam distillation, water, and hydrodistillation can make hydrolats and after the essential oil extraction press cakes mostly remain, the use of solvent, supercritical fluid, or, more recently, microwave solvents extractions additionally enable oil phases as by-product streams **(Marcelino *et al.*, 2023; Turrini *et al.*, 2021 ; Güçlü, 2025)**.

Plant residues are, by far, the largest component. Plant residues from the essential oil industry are made up of the leftover biomass of the distilled plants, which is the raw material of the essential oil extraction process **(Nannaware *et al.*, 2022)**. It is estimated that after the extraction of the marketable products—essential oils, fruits, roots, or flowers—steam distillation can produce between 70% and 90% of residues per weight of the species used on a lab scale. For each kg of extracted essential oil, 150–250 kg of material residue will be created by the plant distillation **(Zaccardelli *et al.*, 2021)**.

Due to the large scale of the industry, a massive amount of leftover biomass is generated, generally stretching from 1.5 t to 8 t per t of essential oil, depending on the species. However, an average of 3 to 5 t of distillation waste per t of essential oil is suggested. Many of the 3000 species of vascular plants worldwide cultivated for their essential oils may have high amounts of by-products generation after steam distillation, which are becoming a concern of sustainable awareness, as a switch from the high-yield demand for the essential oil industry **(Truzzi *et al.*, 2022; Xiao *et al.*, 2021)**.

1.3. Chemical Composition of waste (fibers, polyphenols, traces of essential oil)

Plant material yields an aromatic, bioactive fraction and oil through steam distillation, leaving behind fibrous residues made mainly of cellulose, which are crucial to plant waste. Native fibers possess optimal strength, though they often appear degraded. Market waste fibers enable new applications beyond textiles and paper, such as reinforcement in biodegradable materials **(Khalati *et al.*, 2023).** Vascular plants contain polyphenols, which are primary phytochemicals with significant antioxidant properties beneficial for food conservation and health. Some polyphenols can bind chemical contaminants like heavy metals and pesticides, but not all are equally effective, and they may also reduce bioavailability of these contaminants. Traces of essential oils can emerge in plant residues post-extraction, presenting commercial and economic potential **(Li *et al.*, 2022; Inthalaeng *et al.*, 2023)**.

The pharmaceutical, cosmetic, flavor, and fragrance sectors find value in these natural extracts, possibly increasing oil recovery through optimized extraction conditions and raw material choices. Investigations show essential oil traces in various plant materials; however, some studies found no traces after extracting rosemary oil **(Mukherjee *et al.*, 2023)**. The presence of these traces impacts the release patterns observed in bilinear asymptotes of release curves. Chemical analyses post-extraction involve gas chromatography techniques for quantifying components. After steam distillation, both monoterpene compounds were analyzed, revealing varying volatility and loss of higher weight compounds. Certain components, like α-pinene, dominate the total area in chromatograms **(Hedayati *et al.*, 2025**; **Li *et al.*, 2022)**.

Eucalyptus essential oils underwent extensive extraction using n-hexane, adjusting parameters like extraction time and dried material ratios. Notably, iridoids such as 1,8-cineole weren't detected in essential oil traces from *Eucalyptus globulus*, raising questions about the retention of these compounds in extraction vessels. Essential oil traces have similarly been extracted from pepper, differing by containing higher molecular weight compounds like terpenes **(Kırkıncı *et al.*, 2024; de *et al.*, 2024)**.

2. Environmental impacts of unused waste

The beneficial effects of the utilization of essential oils are very well known and wide-ranging. However, the large amount of waste produced that is unused, called exhausted waste or useless waste, is inevitably generated in the whole process of the utilization of essential oils. Therefore, the accumulation of this waste exerts a severe impact on our ecosystem, the biotic (flora and fauna) and abiotic environment it imbalances **(Skendi *et al.*, 2022)**.

Especially, since a great part of the waste is plant-derived, characteristic organic pollution is considered **(Tomić*et al.*, 2023)**. The composition of waste is relatively complex and the substances are mainly biomacromolecules consisting, from left after macromolecular cleavage **(Wei *et al.*, 2024)**. This causes a slow process of degradation. As for this, pollution chemicals remain long in nature and may continuously weaken the defense force and regulation ability of ecosystems **(Priyadarshanee *et al.*, 2022; Crișan *et al.*, 2023)**.

Essential oils accumulate in drops on the external epidermis of secretory cells. Once excreted from mother cells, they are shielded by wax, leading to minimal waste compared to other plant metabolites like flavonoids, tannins, and alkaloids. Consequently, significant waste enters the environment, and wax's small pores inhibit vaporization and degradation of these oils, in contrast to volatile compounds. Airborne free drops may variolize within days, while surface-deposited drops can take years to variolize **(Ghosh *et al.*, 2022; Akhtar *et al.*, 2021)**.

This disordered accumulation disrupts ecosystems, causing local fields and forests to experience soil and water pollution **(Ogidi & Akpan, 2022)**. Additionally, biotope plants near oceans face threats from organic pollution. Herbivorous pests also suffer, leading to reduced plant irrigation and resistance, creating adverse situations. This contamination triggers a chain reaction affecting plants, pests, fauna, and water bodies, ultimately impacting humans. Systematic contamination damages biocoenosis, necessitating attention to agricultural and aquatic pollutants, significantly raising costs and reducing economic returns from contaminated areas **(Siddiqua *et al.*, 2022 ; Khan *et al.*, 2023)**.

2.1. Organic pollution and slow biodegradation

The complex nature of essential oil waste, combined with its slow biodegradation, impedes microbial activity, leading to waste accumulation and persistence. This waste often contains hydrocarbons and heavy metals, creating barriers to microorganism-mediated degradation **(**Karishma ***et al.*, 2024)**. The prolonged presence of waste can increase organic loading and toxicity in the ecosystem through leachate effluent, contributing to issues like eutrophication and habitat disruption. Ineffective degradation may release toxic substances, harming both terrestrial and aquatic ecosystems. These effects are intensified by the complex composition of waste and suboptimal biodegradation conditions **(Elumalai *et al.*, 2024; Kebede *et al.*, 2021)**.

The degradability is impacted by material inertness and barriers to surface adhesion, alongside protective factors like shade and low oxygen supply. The waste manifests as macro- or micro-debris and foam ionic material **(Karishma *et al.*, 2024)**. Macro waste typically results from post-harvest or post-processing activities, while micro debris originates from inadequate recovery methods in natural settings. Foam ionic debris arises from cleaning processing surfaces **(Upadhyay *et al.*, 2023; Zahri *et al.*, 2021)**.

The accumulation of organic and occasionally toxic material in treated and prepared essential oil waste can be considered as resistant, potential source of medium and long-term soil perturbation **(Innocent *et al.*, 2024)**. The kind of potential damage can vary in the wide range from diffuse wild vegetation disappearing for quite efficient localization of the dumping site. The principal active dump-pollutants paths may include: volatile emanating substances, falling onto the topsoil and causing drought, pronounced toxic and allelopathic influence on germination ability of many wild species seeds **(Dakhli, 2017)** and groundwater contamination in case of waste water residue penetration **(Chakraborty *et al.*, 2023; Franchi & Fusini, 2021)**.

2.2. Economic and ecological consequences

Waste is generated at all stages of processing in the essential oil industry, from cultivation to post-consumer use. This leads to complex economic and ecological consequences in a global multi-billion-euro industry. The economic risks of poor waste management include clean-up costs for contaminated sites, decreased agricultural productivity, and potential health risks from household and medical waste. High resource consumption and waste production disrupt ecosystems, resulting in decreased efficiency in waste processing and ecosystem destruction necessary for natural waste recycling. **(Marcelino *et al.*, 2023; Xiaojie *et al.*, 2022)**.

This culminates in loss of biodiversity and critical ecosystem services like water and air purification and carbon sequestration. Most essential oil waste is improperly disposed of, leading to environmental accumulation and distribution that intensifies long-term impacts, increasing the fragility of ecosystems **(Kolawole *et al.*, 2024)**.

3. Waste valorization methods

Worldwide, plant origin wastes are generated in a significantly good amount; these wastes possess various valuable gifted resources that are beneficial to human life. The potential of these wastes has been utilized in developing many value-added compounds for diverse applications **(Benek & Tiryaki, 2025)**.

The waste extracted compounds are being used for functional food, pharmaceutical industry, cosmetic industry, and agriculture sector as well. Using these plant origin waste compounds, the development of new biodegradable packaging materials has been initiated **(Kichonge & Kivevele, 2023)**. The world is trembling currently with the environmental pollutants generated by waste material; in this aspect, the developed biopolymers possess a unique perspective to biodegrade in the environment which ultimately results in the protection of the earth's environment **(Singh & Kaur, 2024; Sah *et al.*, 2022)**.

3.1. Use in agriculture

The waste can serve as a resource in agriculture, enhancing soil fertility and decreasing reliance on chemical fertilizers. Research demonstrates that using fruit and vegetable waste (FVW) and deoiled cold-pressed rapeseed cake (DCRC) as organic nutrients improves maize germination rates. Furthermore, their application boosts maize growth and yield while reducing chemical fertilizer use. These discoveries will enhance the value of essential oil processing and promote environmental sustainability **(Yadav *et al.*, 2024)**.

3.2. Use in animal feed

One major challenge in sustainable essential oil production is managing waste from oil extraction. Three aspects must be addressed for sustainable value: maximizing waste valorization, minimizing waste generation through improved extraction methods, and optimizing farming practices with Good Agricultural Collection Practices (GACP) **(Ratna *et al.*, 2021)** and environmentally sound techniques. Distillation residue is often hazardous, characterized by high Chemical Oxygen Demand (COD), necessitating treatment prior to disposal or recycling **(Soto *et al.*, 2021)**.

This wastewater may contain toxic phenolic compounds that persist in the environment. Using residues for mulch is impractical due to their potential for burns and contamination of soil, as well as their role in pathogen proliferation **(Kovo *et al.*, 2023)**. Without eco-friendly strategies, the advantages of sustainable practices are diminished **(Liu *et al.*, 2022)**. The environmental impact of herbs is crucial from collection through processing and use. Shock-harvested plants may struggle to recover or even perish, which affects sustainable yields **(Meneceur *et al.*, 2023)**.

Moreover, waste contaminates water sources and contributes to ecological degradation. Current stakeholder engagement is insufficient for promoting sustainable herb harvesting **(Wessels Wells, 2023)**.

3.3. Industrial valorization

Industrial valorization of essential oil waste aims to extract commercially valuable compounds. Recent studies have investigated various processing methods, including oxidation, pyrolysis, and gasification, to isolate compounds like bio-oil, fertilizers, and biochar. These have applications in food, pharmaceutical, and cosmetic industries, with their market values exceeding those of the original biomass. This justifies investments in research on essential oil waste valorization **(Skendi *et al.*, 2022; Truzzi *et al.*, 2022)**.

The economic and environmental benefits of waste repurposing further enhance feasibility. However, there remains a substantial amount of biomass to valorize, necessitating innovative technologies like hydrodynamic cavitation and microwave-assisted methods. A key challenge is the limited literature on scaling these processes for commercial production **(Kumar *et al.*, 2025)**. Additionally, techno-economic assessments for this valorization are underexplored, hindering interest from farmers and investors. Future research should address energy demands, environmental impacts, and scaling technologies for effective valorization in industry **(Gautam & Kumar, 2025; Gonzalez-Rivera *et al.*, 2023)**.

3.4. Energy and biofuel production

Sustainable resource use and waste minimization are pressing global issues. The industrial demand for plant-derived natural substances has grown due to a trend favoring eco-friendly products over synthetic ones **(Hedayati *et al.*, 2025)**. Traditional disposal methods, like burning, are no longer favored for their ecological impact **(Gupta *et al.*, 2024)**. Herbal waste, being durable and aromatic, is hard to decompose. Pyrolysis presents a potential solution, enabling chemical decomposition at low temperatures. This process generates bio-oil, pyrolytic gas, and biochar. Still, the bio-oil from waste plants contains more phytotoxic components than that from fresh plants, making the former less desirable for essential oil extraction **(Tăbărașu *et al.*, 2023; Mavandi *et al.*, 2021)**.

CHAPTER IV

Mentha spicata

1. Classification and botanic description

1.1.Taxonomic classification

The mint group comprises about 19 genera and over 7,700 species, mainly in tropical regions. The Lamiaceae family, also known as Labiatae, includes familiar plants like sage, thyme, basil, and mint. Mentha consists of valuable species recognized since Ancient Greece and Egypt as common garden herbs. Mint varieties, including peppermint and spearmint, have been cultivated for their culinary and medicinal uses **(Vining *et al.,* 2020)**. These herbaceous, rhizomatous, perennial, and aromatic plants feature smooth underground stems, though hairy forms exist. Leaves of cultivated plants are at least 2 cm long, arranged in opposite pairs, and flowers appear in terminal clusters from leaf axils, exhibiting bilateral symmetry with tubular shapes, fused petals forming an upper hood and lower lip (Figures 2-3) **(Nazar *et al.,* 2022; Askari and Mohtashami, 2022)**

Cultivated spearmint, known as *M. spicata* L. var. spicata var. crispa Benth, faces classification challenges due to species determination difficulties and hybridization used for commercial flavors. Willdenow's classification in 1809 includes many mint accessions without specific description. Over 3,000 epithets have been reported for Mentha, and the Global Biodiversity Information Facility lists over 740,000 locality data points from 454 taxa, with few verified. Wild mints hybridize easily, creating minor diversities that don’t often qualify for species status **(İsfendiyaroğlu *et al.*, 2024)**.

Figure 2. Leaf of *Mentha spicata*

Figure 3. Flower Description of *Mentha spicata* **(Parker, 2015)**

2. Distribution and Habitat

Most commercially valuable genera are native to Europe or Asia, including 13 species like apple mint and peppermint. American natives include 10 spearmints (e.g., *M. arvensis* L., *M. canadensis* L.) and 13 wild mints (e.g., *M. pulegium* L.) with lesser volatile values. Many North American natives are commercially viable, especially for oil production **(Vining *et al.*, 2020).**

3. Properties and chemical composition

3.1. Active compounds (essential oils, flavonoids, tannins)

3.1.1. Essential oils

The popularity of mint essential oils is undoubtedly due to the fresh and pleasant flavor they add to a variety of food and beverages. The commercial demand for mint oil both as a flavoring agent and as an ingredient in confectionary, oral products, and medicines has been increasing **(Gupta *et al.,* 2023).**

3.2. Medicinal properties

Leaves and essential oil of *Mentha spicata* are used for a wide range of medicinal purposes; as digestive and sedative, antipruritic, for rheumatism pain, antispasmodic, ophthalmic, refrigerant, colitis, carminative etc. The plant contains tannins, mucilage, terpenes, and essential oil rich in carvone, limonene, dihydrocarveol, and 1,8-cineol and showed antioxidant properties **(El *et al.*, 2022)**.

Different *Mentha* spp. show antioxidant properties, particularly in methanol extracts, which may be effective in food protection systems **(Appolloni *et al.*, 2022)**.

3.3.Other uses

Mentha spp. is a well-known and widely cultivated herb with a long history of medicinal use for many chronic and acute diseases, including cancer and microbial infections (**Ji Park *et al.*, 2016**).

4. Uses and applications of mint

4.1.Culinary use

In cooking, mint is prized for its freshness and refreshing taste. It is commonly used in infusions and herbal teas, renowned for their digestive and soothing properties. In gastronomy, fresh or dried leaves are used in a variety of dishes, including salads, sauces, soups, desserts and Oriental dishes such as tabbouleh. It is also used as a condiment, notably to flavor drinks (such as mint tea in the Maghreb countries) or to accompany meats and vegetables **(Frolova, 2022)**.

4.2.Use in phytotherapy and aromatherapy

In phytotherapy, mint is recognized for its antispasmodic, carminative and analgesic properties. Peppermint essential oil (*Mentha × piperita*), rich in menthol, is used to relieve digestive disorders, headaches and muscular pains. In aromatherapy, inhalation of this essential oil has shown beneficial effects on memory and attention **(Moss *et al.*, 2008).**

4.3.Industrial applications

In the food industry, mint essential oils are used for their antimicrobial properties, contributing to food preservation. They are also incorporated into cosmetic and pharmaceutical products for their refreshing and antiseptic effects. In addition, mint is used in the manufacture of products such as toothpastes, mouthwashes and chewing gums, due to its pleasant aroma and beneficial properties for oral hygiene **(Ozcan, 2006).**

CHAPTER V

Material and methods

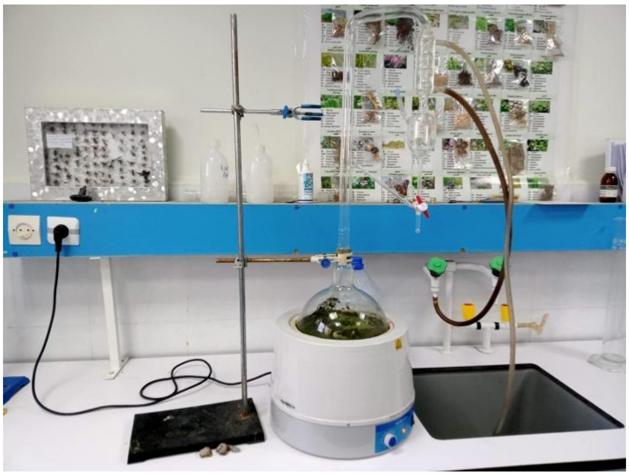
1. Plant material, by-products, and extracts preparation

1.1. Plant collection

Fresh mint was collected from the market at Khenchela city, rinsed by tab water and air dried for 30 days.

1.2. Essential oil extraction and recovery of by-products

The plant leaves were ground very finely and subjected to hydro-distillation using a Clevenger-type device (Photography 1). The operation consists of immersing 300 g of the plant mass in a large glass flask containing 3 liters of distilled water. The mixture is brought to the boil using a flask heater. The vapors pass through the vertical tube, then through the refrigerator then through the condenser, where condensation takes place. The droplets thus produced accumulate in the tube previously filled with distilled water. Hydrolate was separated from the essential oil and conserved at 4˚C. Solid water was separated from liquid waste by filtration, air dried, and immediately processed for methanolic extraction. Liquid waste was conserved at 4˚C.



Photography 1. The hydrodistillation process

1.3. Preparation of methanolic extracts

Organic extracts were prepared from mint powder and solid waste obtained from essential oil extraction. Methanolic extracts were prepared by maceration of 10 g of biomass in 200 ml of methanol 80% (V/V) for 24 h. The operation was repeated twice. After filtration, the extracts were mixed, dried by evaporation **(SCILOGEX, Figure 5)** at 40°C and stored at 4°C until use.

Photography 2. Rotovap apparatus **(SCILOGEX)**

2. Antibacterial activity

2.1. Bacterial strains

Bacterial strains used in this work were isolated from urine samples of patients with urinary tract infections at Hospital Kais (Khenchela) during February 2025.

2.2. Antibiotic resistance testing

The disc diffusion method was used for antibiotic resistance testing. The following antibiotics were used:

- Ofloxacin (5 µg)
- Netilmicin (30 µg)
- Penicillin tazocin (15 µg)
- Tétracycline (30 µg)
- Sulfamethoxazole (25 µg)

Bacterial suspension, equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), were prepared in physiological water from overnight cultures. Bacterial suspensions were spread on Mueller-Hinton Agar plates using sterile swabs, then antibiotic discs were placed on the agar plates. After 16-18 hours of incubation at 37°C for 16–18 hours, the diameters of the inhibition zones were measured. Bacteria were classified into three categories: susceptible, intermediate, or resistant according to the interpretive standard provided by the Clinical and Laboratory Standards Institute (CLSI).

2.3. Antibacterial activity

2.3.1. Disc diffusion method

Antibacterial activity was assessed using the disc diffusion method with Muller-Hinton (MH) medium. After seeding, 6 mm diameter discs were filled with 20 µl of each methanolic extract prepared with dimethyl sulfoxide (DMSO) at a concentration of 300 mg/ml, liquid waste and hydrolate. The plates were then incubated for 3 h at 4°C and then at 37°C for 16-18 hours. Aztreonam (30 µg), Ofloxacin (5 µg) and DMSO were used as positive and negative controls, respectively. The diameters of the inhibition zones surrounding the discs were measured after 24 h.

The tested strains are:

- *Escherichia coli* R59
- *Escherichia coli* R81
- *Escherichia coli* R82
- *Escherichia coli* R100
- *Escherichia coli* R101
- *Pseudomonas sp*
- *Staphylococcus aureus*
- *Corenybacterium sp*
- *Lactobacillus sp*
- *Klebsiella pneumoniae*
- *Proteus mirabilis*

2.3.2. Determination of the minimal inhibitory concentration (MIC)

In order to better assess antibacterial activity, a more detailed study was carried out by determining MICs for strains sensitive to the extracts tested, using the dilution method. The MIC is defined as the lowest concentration capable of inhibiting all microbial growth visible to the naked eye.

A test tube containing 5ml Muller-Hinton broth (MHB) was inoculated with 100 µl of bacterial suspension, to which 100 µl of different extract concentrations **(**300 mg/ml -150 mg/ml - 75 mg/ml - 37.5 mg/ml - 18.75 mg/ml - 9.37 mg/ml - 4.68 mg/ml -2.34 mg/ml - 1.17 mg/ml -

0.58mg/ml) have been added. Then and after incubation at 37° C for 24h, the reading was taken according to the growth of the inoculum in the BMH, which is indicated by the broth turbidity, and the lowest concentration of extract that inhibited the growth of the test organism was taken as the MIC.

3. Phytochemical screening

Colorimetric assays were used for the phyto-chemical screening of different bioactive compounds in the methanolic extract of solid waste and the liquid waste.

3.1. Solid waste

3.1.1. Saponins

About 200 mg of extract obtained from the plant sample was put into test tubes. A 10 mL distilled water was added to it and boiled. The froth appeared persistent for more than three minutes showing the presence of saponin **(Shanmugam, 2014).**

3.1.2. Flavonoids test

200 mg of each extract was dissolved in NaOH and then HCl was added to it. Turning the solution colorless from yellow confirmed the existence of flavonoids **(Shanmugam, 2014).**

3.1.3. Tannins test

Distilled water was mixed up with a small quantity of sample and then boiled. Filtrate was obtained and a few drops of Ferric chloride were added to the filtrate. The appearance of blackish-green color indicated the tannin's availability **(Shanmugam, 2014).**

3.1.4. Terpenoids test

200 mg extract from each sample was mixed with 2 mL of chloroform along with 3 mL fully concentrated H_2SO_4 added to forming a layer. The interface appeared to be reddish-brown which indicated the presence of terpenoids **(Shanmugam, 2014).**

3.2. Liquid waste

3.2.1. Saponins

2 ml of extract was added to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins **(Shanmugam, 2014).**

3.2.2. Flavonoids test

To a portion of the dissolved extract, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus **(Shanmugam, 2014).**

3.2.3. Tannins test

About 0.5 gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration **(Shanmugam, 2014).**

3.2.4. Terpenoids test

0.5 gram of each extract was added to 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids **(Shanmugam, 2014).**

3.2.5. Quinones test

Alcoholic solution (Ethanol)of potassium hydroxide was added to 1ml of sample (liquid waste). Appearance of red color precipitate indicate the presence of quinones **(Shanmugam, 2014).**

CHAPTER VI

Results and discussion

1. Yield extraction

The extraction of essential oil yields not only the volatile oil but also by-products such as hydrosols, liquid and solid residues.

From 300 mg of plant material and 3L of distilled water, the distillation process produced 350 mL of hydrosol, 0.62 g of methanolic extract from the solid waste representing a percentage of 3.1٪, and 3.1٪ mg of methanolic extract from the raw plant with a percentage of 6.5٪.

2. Antibiotic resistance

Table 4 shows the results for antibiotic susceptibility against a set of five antibiotic agents. Two strains, *E. coli* and *Lactobacillus* sp., exhibited resistance to two antibiotics tetracycline and trimethoprim/sulfamethoxazole, *Pseudomonas* sp. and *K. pneumonae* exhibited resistance against pristinamycin, while *Pseudomonas* sp. was also resistant to netilmicin and trimethoprim/sulfamethoxazole. *P. mirabili*s showed resistance only against trimethoprim/sulfamethoxazole.

The isolated bacteria in this study belong to the most widespread bacteria in the etiology of urinary tract infections namely: *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. in the group of Gram-negative bacteria, and *Enterococcus* spp. and *Staphylococcus* spp., in the Gram-positive group **(Manges *et al.*, 2006).**

As indicated in our results, both worldwide and in Europe, the highest resistance was obtained against trimethoprim-sulfamethoxazole. Previous results have indicated that in both worldwide and in Europe, the highest resistance rates of uropathogens were represented by trimethoprim-sulfamethoxazole and aminoglycosides **(Li *et al.*, 2022).** More importantly, antimicrobial resistance is considered a major global health issue; this phenomenon is responsible for approximately 700,000 deaths per year worldwide **(Mestrovic *et al.*, 2022).**

Table 4. Antibiotic susceptibility of the studied strains

	Ofx 5	Net 30	Pt 15	Te 30	Sxt 25
***Pseudomonas* sp.**	3	R	R	15	R
K. pneumoniae	31	18	R	14	21
***Lactobacillus* sp.**	32	26	36	R	R
***Corynebacterium* sp.**	26	19	15	16	25

P. mirabilis	22	16	18	26	R
S. aureus	29	19	33	17	25
E. coli	33	18	21	R	R

3. Antibacterial activity

3.1.Antibacterial efficacy of extracts from different plant fractions

3.1.1. Disc diffusion method

Table 8 presents the antibacterial activity of different plant extracts, including hydrolate, liquid waste, and methanolic extract of solid waste, against elevene bacterial strains, including both Gram-negative (*E.coli, Klebsiella pneumoniae,Pseudomonas sp*.) and Gram-positive *(Corenybacterium sp , Lactobacillus sp, Staphylococcus aureus)*

- **Hydrolat and liquid waste:** these fractions demonstrated reduced but notable inhibitory effects (typically 7–12 mm), especially on *E. coli* R82, *Pseudomonas* sp., *Corynebacterium* sp., and *Lactobacillus* sp. However, some resistance was observed, namely: *E. coli* R59, *E. Coli* R81, *P. mirabilis,* and *S. aureus*.
- **Solid Waste:** interestingly, the solid waste, although a by-product, maintained moderate antimicrobial properties against the most tested strains, especially *E. coli R82* and *Lactobacillus* where inhibition zone diameters between 12 and 15 mm were observed.

Hydrolates have attracted a great interest this last decade by the scientific community. Oral *et al.* (2008) showed that hydrosol obtained from mint from Turkey has antibacterial activity against *P. fluorescens* and *P. aeruginosa* and that *E. coli* was the most resistant strain. While the hydrolate obtained in our study was ineffective against *S. aureus*, hydrolates from Mentha species from Morocco showed inhibitory efficacy against *S. aureus* with inhibition zone diameters varying between 10 and 35 mm **(Zekri *et al.*, 2022).** Also, **Sutour *et al.*, (2008)** have observed that the hydrosol of *Menta suaveolens* was active with regard to *S. aureus* and the IZ diameter was 20 mm. **Al-Turky (2007)** examined peppermint hydrosols for their potential to inhibit *Bacillus cereus* and *Salmonella enterica* serotype *Enteritidis*, commonly encountered in foods as pathogens. All herbal spice extracts showed inhibitory effects against the tested bacteria. **Zekri *et al.* (2014)** suggested that the strong inhibitory effect of Mentha hydrolates against *S. aureus* strains was due mainly to the presence of carvacrol and piperitenone, 1,8-cineole and camphor. Previous studies reported that these compounds exhibited high antibacterial capacity **(El Moussaoui *et al.*, 2023;**

Mahboubi *et al.*, 2008). Moreover, **Berktas and Cam (2021),** showed that hydrosol of peppermint contained valuable amount of phenolics (840.1 mg/L). According to **Kırkıncı *et al.* (2024),** phenolic extracts can be employed against pathogenic microorganisms' spoilage and to control lipid oxidation.

Regarding the raw plant material, previous study showed the maximum zone of inhibition against *S. aureus*, *E. coli*, *Streptococcus pyogenes*, *P aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *A. clavatus* over the control **(Patel *et al.*, 2021).** More specifically, the ethyl acetate leaf extract of *Mentha piperita* showed pronounced inhibition than chloroform, petroleum ether and hexane. The strong antibacterial activity was observed against *Bacillus subtilis*, *S. aureus* and *Proteus vulgaris* than *E. coli*, *Streptococcus pneumonia* and *K. pneumonia* **(Sujana *et al.*, 2013).**

Table 5. Antibacterial activity of the different extracts on the bacterial strains

	Inhibition zone diameter (mm)					
Strains	**Plant extract**	**Hydrolate**	**Liquid waste**	**Solid waste**	**- control**	**+ control**
E. coli R59	R	R	R	6	R	24
E. Coli R81	R	R	R	R	R	25
E. Coli R82	13	8	12	15	R	25
E. Coli R100	15	9	10	9	R	22
E. Coli R101	15	9	10	9	R	22
Pseudomonas sp.	9	9	10	8	R	38
P. mirabilis	7	R	8	10	R	30
S. aureus	7	R	R	R	R	37
K. pneumoniae	8	9	7	10	R	30
Corynebacterium sp.	10	11	10	10	R	37
Lactobacillus sp.	8	10	11	12	R	40

(R) Resistance

3.2. Determining the minimum inhibitory concentration (MIC)

The MIC test was conducted on *E. Coli* R82, *E. Coli* R100, *E. Coli* R101, *Pseudomonas* sp., *P. mirabilis*, *K. pneumoniae*, *Corynebacterium* sp., and *Lactobacillus* sp. with the methanolic extract of solid waste. Visual analysis of the test tubes indicates that bacterial growth was completely inhibited at concentrations of 150 mg/ml, as evidenced by the clarity of the broth (Photography 3-7).

Photography 3. MIC of the strain *K. pneumoniae*

Photography 4. MIC of the strain *Lactobacillus* sp.

Photography 5. MIC of the strain *E. coli* 101

Photography 6. MIC of the strain *E. coli* 82

Photography 7. MIC of the strain *Corynebacterium* sp.

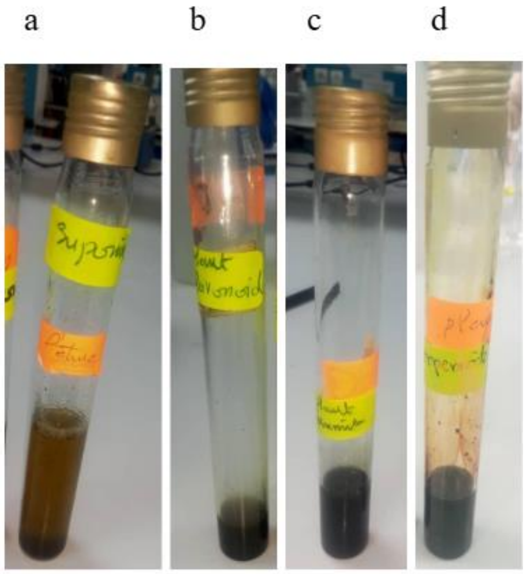
- The results of the MIC of different extracts prepared from the raw material obtained in previous studies were different from the results obtained in our study with the methanolic extract from the solid waste. **Afrin *et al.* (2023)** showed that the MIC for *S. aureus* and *E. coli* were 200 and 400µg/ml in aqueous extract of mint leaves respectively. The MIC of the methanolic peppermint extract against clinical isolates of *E. coli*, *Acinetobacter*, *S. aureus* and two fungi such as *Candida albicans*, *C. glabrata* was 3.125 µg/ml **(Pramila *et al.*, 2012).** However, from the study of **Saravani *et al.* (2021),** the MIC of peppermint methanol extract for *E. coli* was 6.25 mg/mL.

4. Phytochemical screening

4.1. Solid waste

The phytochemical screening of the methanolic extract of solid waste from essential oil extraction revealed the presence of several bioactive compounds, as indicated by distinct color changes in specific tests (Photography 8).

- **Saponins:** the appearance of a forth for more than 3 minutes indicated the presence of saponins.
- **Flavonoids:** a negative result was obtained with this test of flavonoid. The color did not turn colorless after the addition of hydrochloric acid.
- **Tannis :** the appearance of a blackish-green color after addition of ferric chloride confirmed the presence of tannins.
- **Terpenoids:** a positive result was indicated by the appearance of an interface with a reddish-brown color.

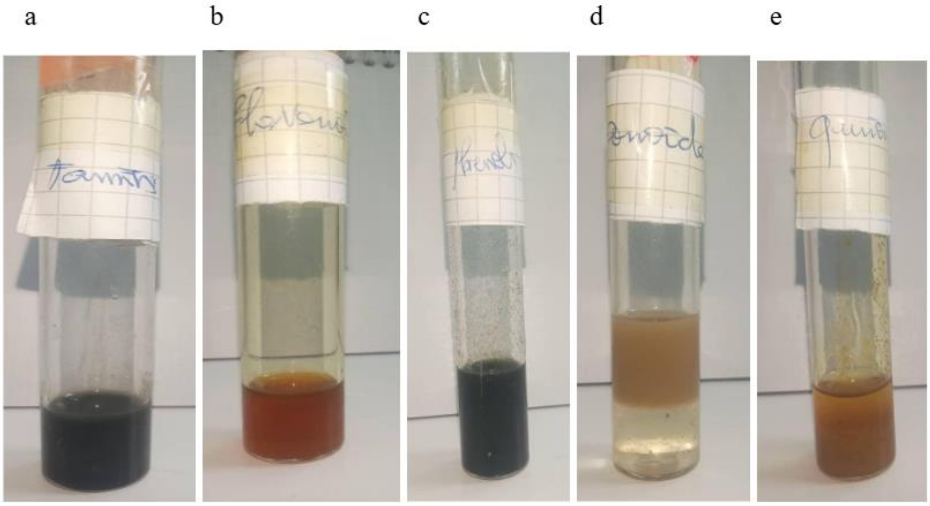


Photography 8. Results of the phytochemical screening of the methanolic extract of solid waste. a) Saponins, b) flavonoids, c) tannins, d) terpenoids

4.2. Liquid waste

The phytochemical screening of the liquid waste indicated the presence of the following bioactive compounds (Photography 9).

- **Flavonoids:** the formation of a yellow color indicated the presence of flavonoids.
- **Tannins:** the presence of tannins were confirmed by the presence of a brownish green coloration.
- **Terpenoids :** the appearance of reddish-brown color at the interface indicated the presence of terpenoids.
- **Quinones:** a positive result was obtained by the appearance of red color precipitate.
- **Phenols:** the formation of bluish-green color indicated the presence of phenols.



Photography 9. Results of the phytochemical screening of the liquid waste. a) Tannis, b) flavonoids, c) phenols, d) terpenoids, e) quinones.

Overall, the dark-coloured decoction obtained from the production of essential oil by steam distillation contains plant particles, various organic acids, proteins and flavonoids **(Farrel, 1985).** A recent study conducted by **Berktas and Cam (2021),** demonstrated that extracts from distilled leaves of peppermint prepared with ethyl acetate and ethanol were rich in phenolic compounds. **Patel *et al.* (2021),** demonstrated that the methanolic crude leaves extracts of *Mentha arvensis* L were composed of flavonoids, tannins, and terpenoids. These extracts had antimicrobial activities against *S. aureus*, *E. coli*, *S. pyogenes*, *P. aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *A. clavatus* over the control. However, The methanolic extraction of peppermint showed the presence of phenols and tannins, flavonoids, carbohydrates, glycosides and alkaloids. However, the sample extracted didn't show any antibacterial activity.

Abdul-Alim *et al.* (2019), using the cold maceration approach, prepared extracts of *Mentha piperita* in n-hexane, acetone, dimethyl ether, petroleum ether, chloroform, ethanol, and water. The resulting extracts were then qualitatively screened for a few secondary metabolites. Depending on the extraction solvent, it was discovered that the *M. piperita* leaf extract contained alkaloids, flavonoids, tannins, steroids, glycosides, terpenoids, saponins, and phenols. According to **Paikara and Pandey (2018),** the results of the phytochemical screening of alcoholic extract of Mint leaves (*Mentha spicata*), found that alkaloids, phenol, glycoside, flavonoids, and protein were present in leaves of Mint. The results obtained by **Rosmalena *et al.* (2022)** showed that ethanolic extract revealed the presence of flavonoids, alkaloids, steroids, and tannin**.** **Sujana *et al.* (2013)** also evaluated the phytochemical analysis of organic extracts, prepared with ethanol, methanol, ethyl acetate, chloroform, hexane and petroleum ether, for the presence of various secondary metabolites. The analysis revealed the presence of alkaloids, flavonoids, steroids, tannins, and phenols.

Conclusion and Perspectives

Conclusion and perspectives

This study highlighted the significant antimicrobial potential of by-products obtained from *Mentha spicata* essential oil extraction namely hydrolate, liquid residue, and methanolic extract of solid waste. Hydrolate and liquid waste demonstrated moderate antibacterial effects (7–12 mm), owing to the presence of water-soluble compounds like phenolic acids and alcohols, while the solid waste showed residual but noteworthy activity (8–15 mm) against several clinical strains, explained by the presence of tannins, flavonoids, terpenoids, and saponins, as confirmed by phytochemical screening. Interestingly, the determination of Minimum Inhibitory Concentrations (MICs) revealed a concentration of 150 mg/ml. Beyond the promising biological results, this research carries substantial scientific and environmental relevance. It reinforces the idea that by-products from essential oil distillation, often considered waste, can be valorized for their antimicrobial properties, offering a sustainable alternative in the fight against antibiotic resistance. As such, the study contributes not only to the field of applied microbiology but also to sustainable waste management strategies in the essential oil industry.

Perspectives

- Solid waste, liquid waste and hydrolats are effective in maintaining blood pressure balance.
- They can be used in several areas: health, medicine, cosmetics, agriculture, nutritional supplements, and more.
- They contain essential and secondary compounds essential for building the human body's immunity.

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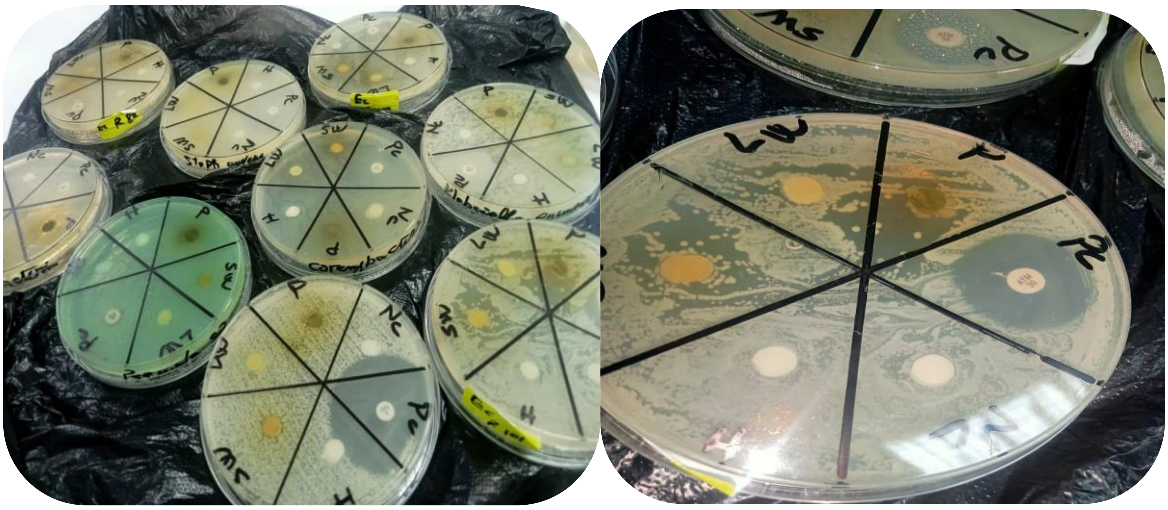
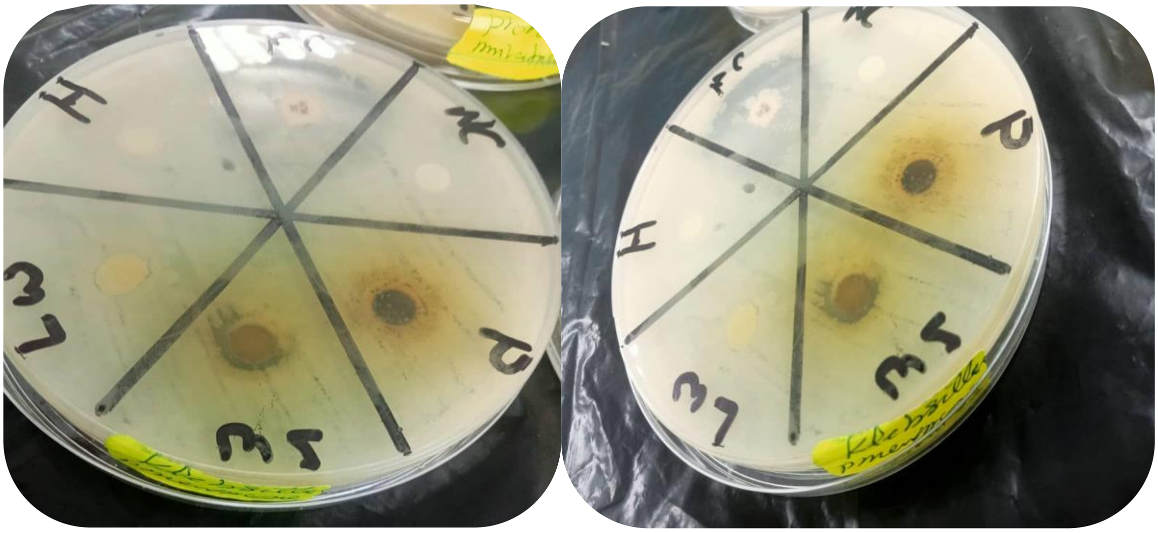
Annexes

Annexes

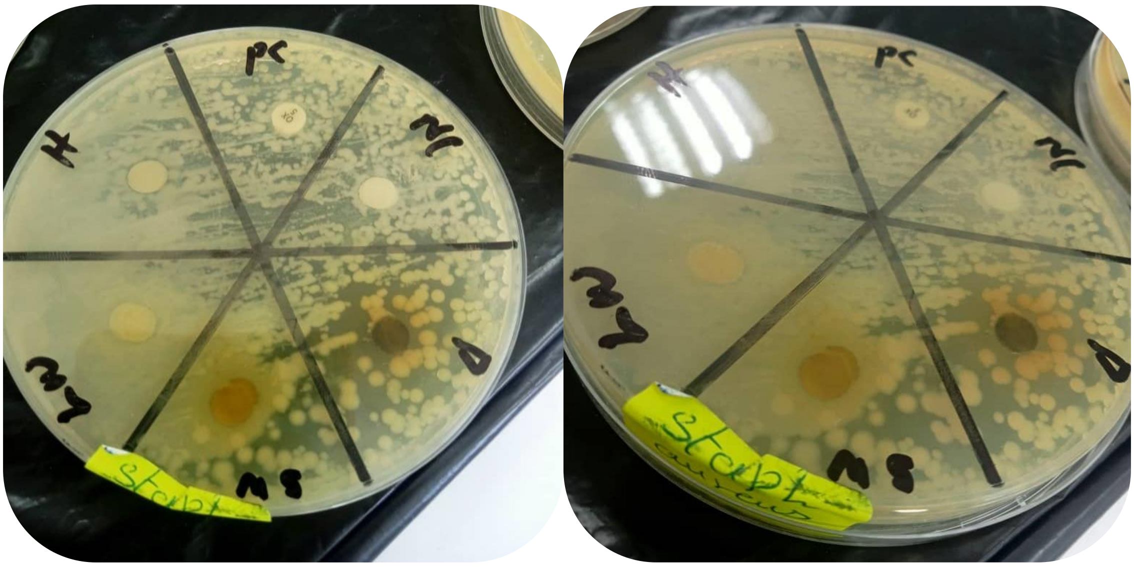
Result of the antimicrobial activity



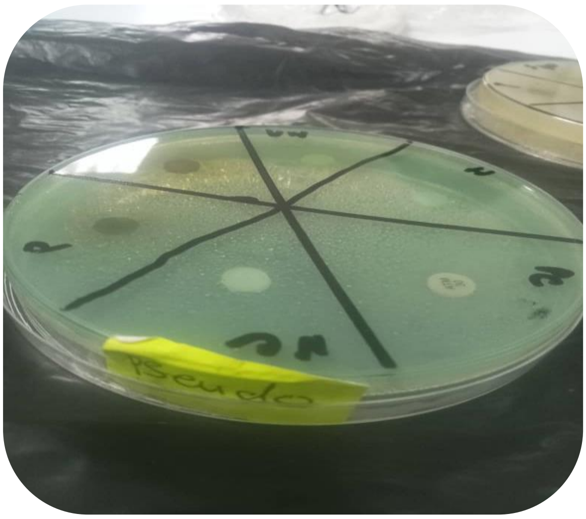
A. Bacteria with mint extracts



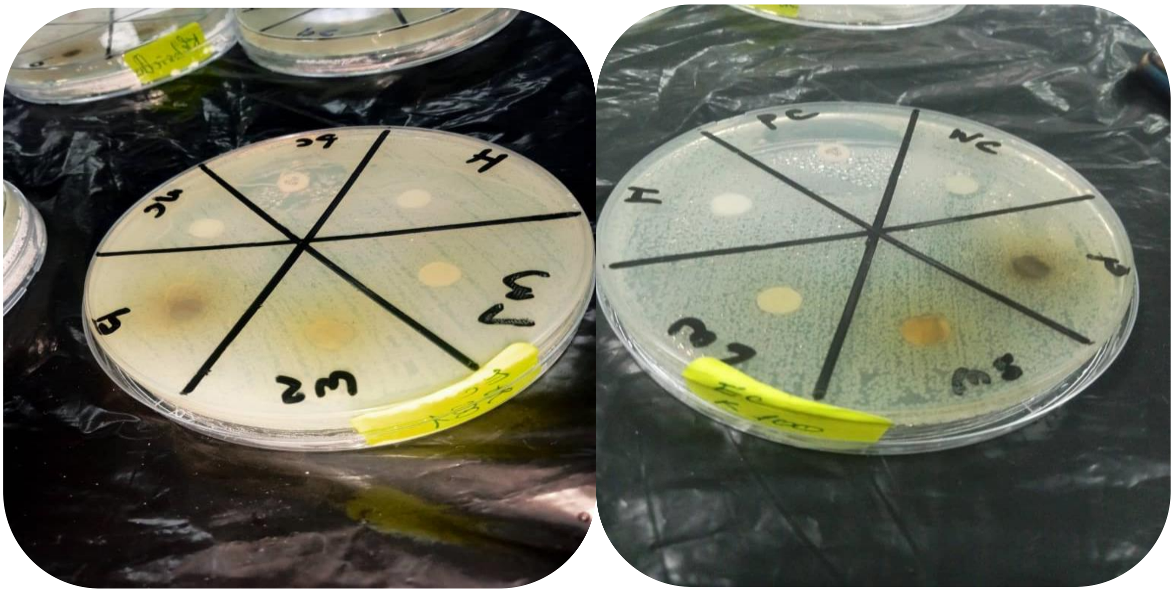
B. *Klebsiella pneumoniae* with mint extracts



C. *Staphylococcus aureus* with mint extracts



D. *Pseudomonas sp* with mint extracts



E. *Escherichia coli* R100 with mint extracts

F. *Escherichia coli* R82 with mint extracts

Photography 10. Antimicrobial activities of the studied extracts against the tested bacteria **(Annexes)**