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Evaluation of the Antibacterial Properties of *Inula Viscosa* Essential Oil

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Dedication

To myself…

To the girl who fell but stood back up, who faced hardships yet never gave up, To the one who believed in her dreams and walked the path with strength and hope…

I dedicate this work to you because you truly deserve it. To my dear parents, You are the light that guided my steps, the prayers that protected me in silence, You are my strength and my blessing… thank you for everything, with all my heart.

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HIBA

Dedication

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This graduation is not mine alone... It belongs to all of you for with you the journey began, and through your love, I arrived

HALA

Abstract

This study aims to evaluate the antibacterial activity of the essential oil extracted from the leaves of *Inula viscosa,* a medicinal plant traditionally used in folk medicine.

The plant material was collected from the Kenoua region (Skikda province, Algeria) during the month of March, and the essential oil was extracted by hydrodistillation using an "Alambic" apparatus .

The antibacterial activity was tested against two strains of *Staphylococcus aureus:* a reference strain (ATCC 25923) and a clinical isolate, using two in vitro methods: Aromatogram (agar well diffusion method) with pure essential oil and two dilutions (1/2 and 1/4), where the results showed varying inhibition zones depending on the concentration and strain tested. Antibiotic susceptibility test (disc diffusion method) using vancomycin and penicillin on Mueller-Hinton agar.

The results revealed strong activity of vancomycin, particularly against the reference strain, while penicillin showed weak effectiveness, especially against the clinical strain, suggesting potential resistance. The essential oil of *Inula viscosa* demonstrated significant antibacterial effects, particularly in its pure form, with a level of inhibition comparable to that of vancomycin. These findings support the potential use of this natural oil as an alternative antimicrobial agent, especially in the context of rising antibiotic resistance.

Keys Words : *Inula Viscosa****,*** *Staphylococcus Aureus* , Antibiotic , Essential Oil , Antibacterial Activity , Aromatogram.

Résumé

Cette étude vise à évaluer l'activité antibactérienne de l'huile essentielle extraite des feuilles de *Inule visqueuse*, une plante médicinale traditionnellement utilisée en médecine populaire.

Le matériel végétal a été récolté dans la région de Kenoua (wilaya de Skikda, Algérie) au mois de mars, et l'huile essentielle a été extraite par hydrodistillation à l'aide d'un appareil de type « Alambic ».

L'activité antibactérienne a été testée in vitro contre deux souches de *Staphylococcus aureus* : une souche de référence (ATCC 25923) et une souche clinique, en utilisant deux méthodes : La méthode de l'aromatogramme (diffusion sur gélose) avec de l'huile pure ainsi que deux dilutions (1/2 et 1/4), les résultats ont montré des zones d'inhibition variables selon la concentration et la souche testée. La méthode de diffusion des antibiotiques (test de sensibilité) avec la vancomycine et la pénicilline sur gélose Mueller-Hinton.

Les résultats ont révélé une forte activité de la vancomycine, notamment sur la souche de référence, tandis que la pénicilline a montré une faible efficacité, surtout vis-à-vis de la souche clinique, suggérant une éventuelle résistance. L'huile essentielle de *Inule visqueuse* a démontré un effet antibactérien notable, en particulier à l'état pur, avec un niveau d'inhibition comparable à celui de la vancomycine. Ces résultats appuient l'intérêt potentiel de cette huile naturelle comme agent antimicrobien alternatif, notamment dans le contexte de la résistance croissante aux antibiotiques.

Mots Clé : *Inule Visqueuse, Staphylococcus Aureus* , Antibiotiques , L'huile Essentielle, 'Activité Antibactérienne , Aromatogramme.

الملخص

تهدف هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا للزيت الأساسي المستخلص من أوراق *Inula viscosa* وهو نبات طبي يُستخدم تقليديًا في الطب الشعبي.

تم جمع المادة النباتية من منطقة قنواع (ولاية سكيكدة، الجزائر) خلال شهر مارس، وتم استخراج الزيت العطري بواسطة التقطير المائي باستخدام جهاز "Alambic".

تم اختبار النشاط المضاد للبكتيريا ضد سلالتين من Staphylococcus aureus السلالة المرجعية (ATTCC 25923) و سلالة سريرية و ذلك باستخدام طريقتين في الوسط المخبري

طريقة الانتشار على الوسط الصلب Aromatogram باستعمال الزيت النقي وتخفيفين (2/1 و4/1) ، حيث أظهرت النتائج مناطق تثبيط متفاوتة تبعًا لتركيز الزيت ونوع السلالة باستخدام مضادي البينيسلين والفانكومايسين.

طريقة اختبار حساسية المضادات الحيوية بالأقراص Antibiotic على وسط Mueller-Hinton وقد أظهرت النتائج فعالية قوية للفانكومايسين خاصة ضد السلالة المرجعية، في حين أبدى البنسلين فعالية ضعيفة خصوصًا على السلالة السريرية ، مما يشير إلى احتمال وجود مقاومة للمضاد .

أظهر الزيت العطري لنبات *inula viscosa* نشاطًا مضادًا ملحوظًا للبكتيريا، خاصة في حالته النقية، وكان تأثيره قريبًا من تأثير الفانكومايسين. تدعم هذه النتائج إمكانية استخدام هذا الزيت كمضاد حيوي طبيعي بديل، خصوصًا في ظل تزايد مقاومة البكتيريا للمضادات التقليدية.

الكلمات المفتاحية الطيون , المكورات العنقودية , مضاد حيوي , الزيوت الاساسية , نشاط مضاد للبكتيريا.

List Of Abbreviations

AFNOR: Association Francaise de Normalisation.

ATCC: American Type Culture Collection

C°: Degrees Celsius

C_{10}: Monoterpenes

C_{15}: Sesquiterpenes

C_{20}: Diterpenes

CLSI: Clinical and Laboratory Standards Institute

Cm: Centimeter

CO_2: Carbon dioxide

CP : Phenolic compounds.

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic Acid

EO: Essential oils.

EUCAST: European Committee on Antimicrobial Susceptibility

g: gram

GC-MS: Gas Chromatography- mass spectrometry.

h: hours.

HPLC: High Performance Liquid Chromatography

IV: *Inula Viscosa*

kg: Kilogram

M: mass

Mm: Millimeter

MRSA: Methicillin Resistant *Staphylococcus aureus*

NaCl: Soduim Chloride

P: Penicillin

PEN^S: penicillin-sensitive

PIA : Pasteur Instite Of Algeria

RNA: Ribonucleic Acid

S. aureuse: *Staphylococcus aureus*

Staph C*:* Clinical *Staphylococcus*

Staph R: Reference *Staphylococcus*

VAN: Vancomycin

Y : yeild

α: Alpha

β: beta

%: Percent

µL: Microliter

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introduction

Introduction

Over the past few decades, interest in the study and utilization of plants has increased significantly across various regions of the world **(Muchuete et al. 2006).**

In the continuous battle against microbial infections, bacteria and fungi rank among the most harmful organisms that cause health issues. Although antibiotics have long been the preferred treatment, interest in phytotherapy (herbal medicine) has increased due to the emergence of antibiotic resistance in many species and genera as well as the frequently disregarded adverse effects of synthetic medications. Natural materials have been widely used by humans in a variety of industrial sectors, such as the food, cosmetic, and pharmaceutical industries **(Tagiolmukani and Moayedi, 2015)**.

A large number of aromatic and medicinal plants are used in various fields such as medicine, pharmacy, cosmetics (such as perfumes and soaps), as well as in agriculture **(Twigg and Hassan, 2022)**. The evaluation of their therapeutic properties, especially as antioxidants and antimicrobial agents, is an increasingly expanding and important area of research **(Nastro et al., 2002).** These therapeutic properties are mainly attributed to the active compounds contained in these plants **(Tehic et al., 2007).**

There are many well-known medicinal plants including chamomile, ginger, turmeric, fennel, fennel, lavender, mint, parsley, mugraman .... etc. The latter is known in northern Algeria as Moghraman, a perennial species that is widespread in Mediterranean regions **(Mahmoudi et al., 2016).** The aerial parts of the plant are traditionally used as a decoction to treat kidney disease, diabetes, and hypertension **(Oyaizu, 1986).**

In-depth studies on the chemical components of plant species haveled to the identification of several substances, including essential oils, with interesting antibacterial and antioxidant activity.

As a result, essential oils are beginning to attract a great deal of interest as a potential source of bioactive natural molecules **(Bruneton, 1999; Teuscher et al, 2005).** They are being studied for their potential use as an alternative treatment for infectious diseases **(Chalchat et al, 1997; Baser et al, 2001)** and for the protection of foodstuffs against oxidation **(Deans et al, 1994; Miguel et al, 2003)**

As part of our study, we have chosen to explore the properties of *Inula Viscosa*, a plant belonging to the Asteraceae family, due to its richness in compounds of medicinal interest. This plant is widely used in traditional medicine in Mediterranean countries for its multiple therapeutic virtues. It is mentioned in ancient Roman writings (notably by Pliny the Elder), as well as in Hebrew and Arabic texts. Dried inflorescences and leaves are used in infusions, and essential oils recognized for their medicinal properties are also extracted **(Reeb, 2010).**

The aim of this work is to enhance one of the medicinal value of *Inula Viscosa*, by highlighting the efficacy of its essential oil as an antimicrobial agent.

Literature review

1.Phytotherapy And Medicinal Plants

Plants have always been essential to human life, used for food, healing, and rituals. The medicinal use of plants dates back to ancient civilizations such as China, India, and the Near East **(Benkiki, 2006).**

In recent decades, scientific interest in phytotherapy has increased, evolving into pharmacognosy a key field in modern medicine. Traditional knowledge from herbalists remains a rich source of therapeutic potential **(Bensegueni, 2001).**

1.1 The Different Types Of Phytotherapy

Phytotherapy encompasses several therapeutic approaches based on the use of plants. A distinction is made between:

1.1.1 Classical Phytotherapy

Based on the use of different plant parts (roots, leaves, flowers or the whole plant), in various galenic forms such as infusions, decoctions, powders or dry extracts.

1.1.2 Gemmotherapy

This branch uses plant buds and young shoots, considered rich in active principles at an early stage of development **(vernex-lozet, 2011).**

1.1.3 Aromatherapy

Essential oils extracted from plants by hydrodistillation or other methods are used for their powerful therapeutic properties **(vernex-lozet, 2011).**

1.1.4 Pharmaceutical Phytotherapy

Uses concentrated plant extracts obtained by extraction in solvents such as ethyl alcohol. These extracts, standardized and highly dosed, are formulated as syrups, drops, capsules or tablets, and enable rapid, targeted action **(Strang, 2006).**

1.2 Definition Of Medicinal Plants

A medicinal plant is defined as one that contains active compounds in its organs that can be used therapeutically, either directly as medicine or as precursors for drug synthesis. This definition, according to the **WHO (2000),** helps distinguish scientifically validated medicinal plants from those known only through traditional use.

1.3 Origin Of Medicinal Plants

There are two main soures of medicinal plants :wild and cultured

- **Wild Medicinal Plants**

  also known as" forage",develop organically and spontaneously in their natural habitats without any assistance from humans. Usually, meadows, mountains, forests, andother untamed areas are used to harvest these plants. Because they are exposed to natural environmental stressors, which can increase the production of secondary metabolites that give them their therapeutic qualities, they are thought to be rich in bioactive compounds. **(Chabrier, 2010).**

- **Cultured Medicinal Plants**

  Are propagating specifically under controlled agronomic conditions,for medicine. Cultivationgives an enduring source, standardization of active constituents, and enhanced quality control. It reduces the risk of pollution with polluants or adulterants as well and allowes research and selection of high-yielding or potency strains Cultivated sources have become increasingly important, especially in pharmaceuticals,where consistency and purity are vital **(Zhao et al.;2011).**

2.Essential Oils

Essential oils extracted from plants are used in various fields. In the food industry, they are employed to enhance flavor, aroma, and color, with orange essential oil being one of the most widely used **(Swiss Confederation, 2009).**

In the cosmetic field, they have been used for centuries in the production of perfumes and soaps, and are valued for their antiseptic properties, such as citronella oil **(Al-Hibb, 2011).**

In the pharmaceutical field, they are used in different forms such as sprays, tablets, and inhalations to treat respiratory issues or soothe the throat, as well as in alternative medicine like aromatherapy and homeopathy **(Turgeon, 2001; Elhaib, 2011).**

Essential oils are in high demand due to their important biological properties; some possess recognized therapeutic effects, while others are used as perfume bases or food additives **(Marghache et al., 2009).**

2.1 Definition

AFNOR standard (1998) defines essential oil as “a product obtained from a plant raw material, either by hydrodistillation, or by mechanical processes from the epicarp of citrus fruits, or by dry distillation **(Bruneton,1999).**

2.2 Location And Distribution

Essential oils, once synthesized in the cytoplasm of cells, accumulate within specialized cells called granular cells. They are then stored in specific structures called secretory pockets **(Bruneton, 1999; Hazzit, 2002; Boz et al., 2009).**

2.3 Chemical Composition

Essential oils (EOs) are complex mixtures of volatile organic compounds primarily classified into two major groups based on their biosynthetic origin: terpenoids and phenylpropanoids. Terpenoids, derived from isoprene units through the mevalonate and methylerythritol phosphate (MEP) pathways, represent the largest fraction of EO constituents. These include monoterpenes (C_{10}), sesquiterpenes (C_{15}), and, to a lesser extent, diterpenes (C_{20}), occurring in both hydrocarbon and oxygenated forms such as alcohols , aldehydes , ketones , esters , and oxides . Phenylpropanoids, on the other hand, are aromatic compounds synthesized via the shikimic acid pathway and include molecules such as eugenol, cinnamaldehyde, and anethole. In addition to these main classes, EOs may contain minor constituents such as phenols , amines, amides, sulfur- and nitrogen-containing compounds, and heterocyclic structures. This structural diversity underpins the wide range of biological properties attributed to essential oils, including antimicrobial, antioxidant, anti-inflammatory, and insecticidal activities**. (sadgrove & phumthum,.2022)**

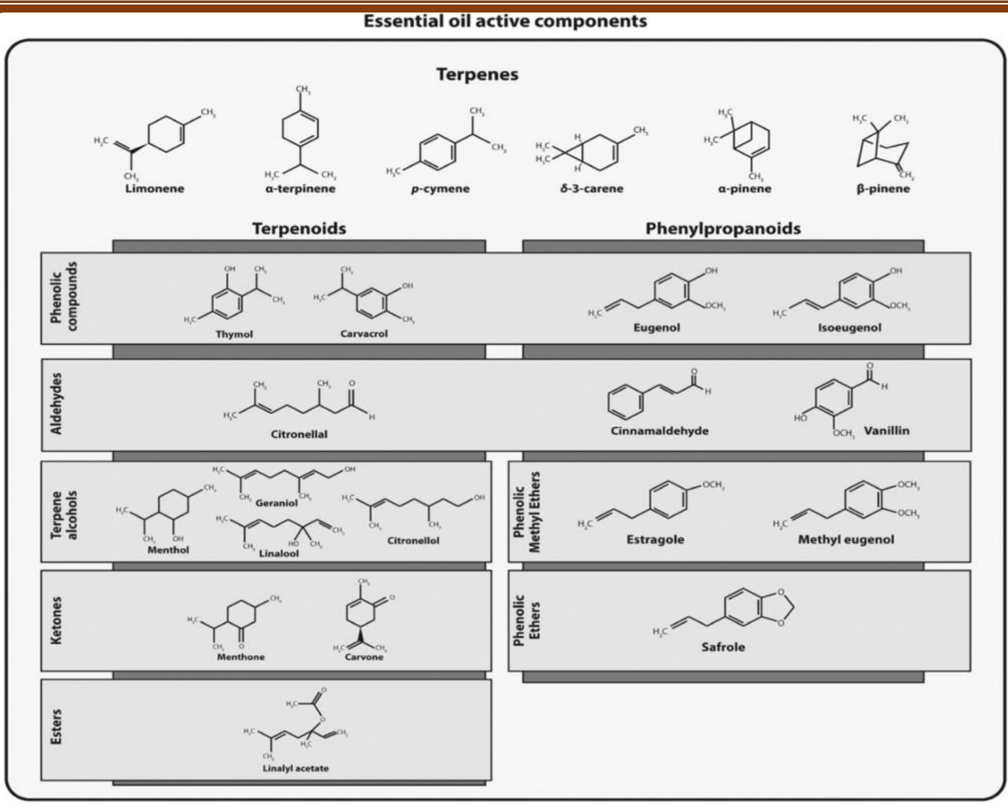

Figure 01: Chemical structure of some common EO active components **(sadgrove & phumthum,.2022)**

2.4 Physiological Role Of Essential Oils In Plants

The volatile compounds of the essential oils, earlier classified as secondary metabolites, have a variety of physiological functions in plants they majority of which are still not fully understood **(Obame, 2009).**

Due to Their volatility and inherent smell, these compounds facilitate plants to communication, aiding pollination and spore dispersal. They also have important defense functions, including protection of plants against a range of pathogens like microorganisms, insects, fungi and herbivores **(Ariba et al., 2020)**. Essential oils are also involved in allelopathic interaction , which are biochemical plant interactions that can influance plant growth and survival .they are aslo engaged in tri-trophic interactions- complex ecological interactions between the plant, herbivores , and predators of the herbivores. Under some condition , particularly where photosynthesis is not enough , essential oils can even serve as alternative energy reservoires **(Deschepper, 2017).**

2.5 The Biological Activities

Essential oils have been known for their antimicrobial properties since ancient times. The first scientific studies began in France as early as **1885**. In **1888**, researchers Cadeac and Meunier published their findings in the Annals of the Pasteur Institute. **Later, René-Maurice Gattefossé** considered the father of aromatherapy highlighted the significant progress made in this field in his book Essential Antiseptics **(Gattefossé,1938).** Over time, numerous studies, particularly in vitro experiments, have confirmed the efficacy of essential oils against microorganisms, including antibiotic-resistant strains. Researchers have also been able to explain the mechanisms of action of certain components and determine their broad spectrum of activity, which includes bacteria, fungi, yeasts, and even viruses **(Zhiri, 2006)** Beyond their antimicrobial action, essential oils exhibit a wide range of biological activities, such as:

- **Antimicrobial properties** Essential oils are capable of inhibiting the growth of several pathogenic micro-organisms. They exert a bactericidal or bacteriostatic action thanks to certain
- active compounds present in their composition. **(Hanana et al., 2014; Hamrouni et al., 2014**; **Parveen et al., 2014).**
- **Antioxidant effects :**Many essential oils have the ability to neutralize free radicals, helping to protect cells against oxidative stress and premature aging **(Arab et al., 2014).**
- **Anticancer activity:** Some essential oils have shown cytotoxic action in in vitro conditions, inhibiting cell proliferation or inducing apoptosis in abnormal cells. **(Millet, 2014).**
- **Anti-inflammatory effects:** Thanks to their active compounds, essential oils can reduce inflammatory reactions by modulating certain inflammatory mediators **(Kim et al., 2014).**
- **Insecticidal properties:** Some essential oils act as repellents or toxicants against various insects, making them natural alternatives to chemical pesticides. **(Vera et al., 2014).**
- **Analgesic activity:** Essential oils can alleviate pain by acting on sensory receptors or inhibiting the transmission of painful signals **(Hajjaj et al., 2014).**
- **Sedative, antispasmodic, and local anesthetic effects :**Some oils have a relaxing effect on the nervous system, reducing stress and anxiety, or promoting sleep and muscle relaxation. **(Bakkali et al., 2008).**
- **Antiasthmatic properties:** Certain essential oils can improve breathing by reducing bronchial spasms and airway inflammation **(Shirole et al., 2014).**

- **Cytotoxic activity:** in certain contexts Some oils demonstrate targeted toxicity on abnormal cells under certain experimental conditions, which can be exploited in therapeutic research **(Gazim et al., 2014).**
- **Antibacterial activities: Dalia and colleagues (2012) ,**reported that phenolic compounds **(CP)** possess potent antibacterial properties. Notable among these are phenolic acids, such as cinnamic acid and caffeic acid, which show remarkable efficacy against many types of bacterial infections.

He explained that flavonoids, with their multiple classes, are characterized by their ability to resist a wide range of bacteria. The strength of their effect varies depending on the type of microorganism and its environment. These compounds show efficacy in inhibiting the growth of different types of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus coli*, *Enterococcus faecalis, Proteus mirabilis*, and others **(Akrum et al., 2011).**

On the other hand**, (Chaouch ,2014)** emphasizes that tannins play a pivotal role in enhancing the ability of plants to adapt to environmental stresses, especially against pathogens. Their mechanism of action lies in their interaction with the bacterial cell membrane, which leads to morphological changes in the cell and modifies the functions of certain proteins, including transporter proteins and adhesion proteins.

2.6 Toxicity Of Essential Oils

Essential oils are not products that can be used without risk. Some essential oils are dangerous when applied to the skin because of their irritant (oils rich in thymol or carvacrol), allergenic (oils rich in cinnamaldehyde or cinnamaldehyde or phototoxic (citrus oils containing furocoumarins). Other essential oils have a neurotoxic effect: ketones such as a-thujone are particularly toxic to nervous tissue **(Guba, 2001).**

2.7 Essential Oil Extraction Techniques

Various methods are employed to extract essential oils from plants. The choice of method depends on factors such as the type of plant, the physical and chemical characteristics of the oil, and its intended application.

2.7.1 Cold Expression Primarily:

used for citrus fruits, this mechanical method involves pressing the peels to extract the oil without heat. As a result, the volatile compounds remain chemically unaltered **(Obame, 2009).**

2.7.2 Solvent Extraction :

In this technique, plant materials are immersed in a solvent that dissolves the aromatic compounds. It is particularly suitable for delicate flowers that are sensitive to the high temperatures of steam distillation **(Gregory, 2013).**

2.7.3 Hydrodistillation:

Hydrodistillation is one of the most widely used techniques for extracting essential oils from aromatic plants. It is based on the principle of co-distillation of water and volatile compounds. This process can be carried out using two main approaches. In the first method, the plant material is fully immersed in water within a distillation apparatus and heated to boiling. The resulting steam, enriched with essential oil vapors, condenses upon contact with a cold surface. The oil is then separated from the water based on density differences. In laboratory settings, this method is commonly performed using a Clevenger-type apparatus **(Goore,2017).** In the second approach, steam is passed directly through the plant material, which is not in contact with boiling water, typically using a device known as an alembic. The heat and moisture from the steam facilitate the release of volatile oils from plant tissues. The steam-oil mixture condenses on a cooling surface, and the oil is then separated from water through decantation **(Bruneton,1993).** Despite the operational differences between these two methods, both are considered forms of hydrodistillation and rely on the same fundamental physical principles to isolate volatile compounds from plant material.

3. The *Inula Viscosa*

Inula Viscosa is a highly medicinal plant traditionally used in folk medicine to treat a wide range of ailments. It is widely distributed throughout the world and is member of the asteraceae family. The plant distinguished by its yellow , sometimes purplish, flowers, which are actually capitula made up of many tiny florests encircled by a bract involucre. A pappus, which is usually cinstricted and then widened at the apex , is placed on top of the fruit and is composed of bristles arranged in a single row. In species of the genus Dittrichia , the leaves are arranged alternately **(Levrault, 2007).**

Inula Viscoosa is abundant in secondary metabolites, such as phenolic acids, flavonoids, terpenoid compounds. According to phytochemical research. Numerous pharmacological characteristics, including antibacterial, antitumoral, antifungal, anti-inflammatory effects, are displayed by these bioactive compounds. A number of bioactive compounds, including inuviscolide, tomentosine, and fokienol, have beenfound and isolated through bioguided screening of *Inula Viscosa (L.)* extracts indicating potential for a range of therapeutic uses.

3.1 Etymology

According to **Fournon and Moate (1983),**

Inula is derived from Greek:

Inéo meaning I am purified,alluding to one of the plant's therapeutic properties,

viscosa meaning sticky or sticky elecampane **(Fournier,., 1947).**

Scientific name: *Inula viscosa Ait* **(Bartels, 1997)**

Arabic name: *Tebek, Teyun*

French name: *Inule visqueuse*, Aunée visqueuse

English name: Rock Flea-bane **(Halimi, 1997)**

In Morocco, it is found under the name Trehla **(Ziguag et al., 2006).**

In Algeria it is commonly known as" magramane " **"ماغرمان "**

3.2 Botanical Classification Of *Inula Viscosa*

According to **Judd et al., (2002**) *Inula Viscosa* belongs to the family ***Asteraceae***, which is one of the largest families of flowering plants. This species is classified within the order ***Asterales,*** class ***Magnoliopsida*****,** and division ***Magnoliophyta***. It is known for its aromatic properties and is widely distributed across the Mediterranean region.

Table 01: Botanical classification of *inula viscosa*

TAXONOMIC RANK	**NAME**
KINGDOM	**Plantae (Plants)**
PHYLUM (DIVISION)	***Spermatophyta* (Seed plants)**
SUBPHYLUM	***Angiospermae* (Flowering plants)**
CLASS	***Magnoliopsida* (Dicotyledons)**
ORDER	***Asterales***
FAMILY	***Asteraceae* (Compositae)**
SUBFAMILY	***Asteroideae***
TRIBE	***Inuleae***
GENUS	***Inula***
SPECIES	***Inula viscosa***

3.3 Characteristics of *Inula viscosa (L.)*

- Height up to 150 cm.
- The stem is erect, fan-shaped and highly branched. Over time, it becomes woody and darker.

Figure 02: *Inula Viscosa* from Kanoua (Original)

- At the base ,it is covered with a glandular hair, releasing a sticky, strong-smelling resin (camphor odor), present all over the plant.
- **The root:** strong, viscous, glandular, odoriferous, can reach up to 30 cm in length and has the peculiarity of being swiveling **(Bartels, 1997)**

Figure 03: Glandular hair in *Inula Viscosa* (Original)

- **The flowers** are grouped in inflorescences, forming clusters of capitula between 10 and 20 mm in diameter. They are of two types: yellow-orange flowers in tubes and yellow flowers with petals.

The first (tubulated) are located in the center of the flower head and the second (ligulate) on the outside. **(Baydar, 1998 and Garbari F, 2007).** Depending on the region where it grows, *Inula Viscosa (L.)* ' flowers from August-September until the end of October and sometimes even Novemberof being swiveling .

Figure 04: The flowers of *Inula Viscosa* (Original)

- **Leaves** are medium-sized (5 mm wide), dense, alternate, lanceolate, glandular. *Inula Viscosa* leaves are stalkless. They are attached directly to the stem**. (Bssaibiset al., 2009)**
- **The fruit** a dry fruit containing a single seed (hence the name achene), 2 mm long. Fruits are grouped together on the flower head. The seed is topped by a capitulum. The seed is surmounted by a pappus (a small tuft of hairs) **(BabaAissa, 1991).**

Figure 05: The leaves of *Inula Viscosa* (Original)

Figure 06: The fruits of *Inula Viscosa* **(Marseille ,2011)**

Table 02: Characteristic of *Inula Viscosa* **(julve,2024)**

CHARACTERISTIC	DESCRIPTION
BIOLOGICAL TYPE	Cha Mephytes- Suffrutescents
PLANT FORMATION	Hemicryptophyta
CHOROLOGY	Mediterranean
INFLORESCENCE	Raceme Of Flower Heads
SEXUALITY	Hermaphroditic
POLLINATION	Entomogamous
DISSEMINATION	Anemochorous

3.4 Geographical Distribution

Inula Viscosa (L.), also known as *Dittrichia viscosa*, is a plant species widely distributed in the Mediterranean region, particularly in Spain, France, Algeria and Morocco. It is also found in Asia, notably in China, Japan and Korea **(Quézel & Santa, 1963).** In Algeria, this plant colonizes various types of environment: it is found on rocky and clayey soils **(Benayache et al., 1991)**, as well as on saline soils, wet meadows and along watercourses **(Quézel & Santa, 1963).**

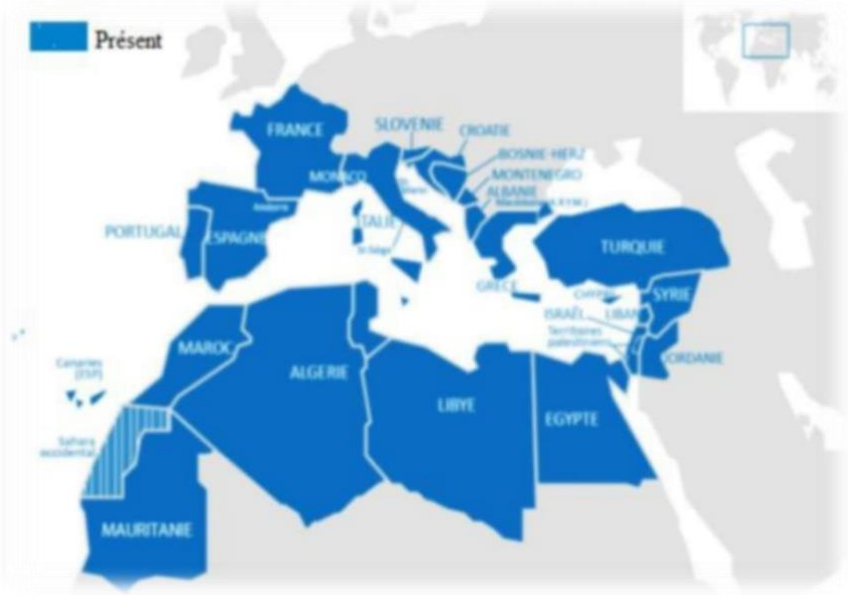

Figure 07 : Geographical distribution of *Inula Viscosa* in algeria and the mediterranean bassin **(Benyahia,2014)**

3.5 Chemical Composition Of *Inula Viscosa*

According to study by **Vuko et al., (2021)**published in the journal Plants (Basel), the essential oil of *Inula Viscosa* was analyzed using GC-MS and HPLC techniques. The analysis identified about 20 compounds, which together accounted for **96.7%** of the total oil composition. The major constituents were:

Table 03: Chemical constituents of *Inula Viscosa* essential oil **(vuko et al .,2021)**

Compound	**Percentage(%)**	**type**
1,8-Cineole	16.41%	Oxygenated monoterpene
α-Terpinylacetate	13.92%	Oxygenated monoterpene ester
Caryophyllene oxide	15.14%	Oxygenated sesquiterpene
α-Muurolol	13.75%	Oxygenated sesquiterpene alcohol
Linalool	6.62%	Monoterpene alcohol
cis-Sabinene hydrate	4.23%	Oxygenated monoterpene
Bornyl acetate	2.71%	Monoterpene ester

The oil was found to be rich in oxygenated terpenoids, which made up about **53.41%** of the total composition. These components are responsible for much of the plant's biological activity, including antimicrobial, antioxidant, and anti-inflammatory properties.

3.6 Therapeutic effect of *Inula Viscosa*

Inula Viscosa is widely used in traditional medicine to treat many diseases and many therapeutic practices related to this plant - such as the part used, method of application, and dosage administered - have been documented in several countries around the world **(Sikka et al. 2014).** The plant is known for its anti-inflammatory **(Aldissi et al., 2001; Barbetti et al., 1985)** antipyretic **(Leroux and Roulet, 1990),** antidiabetic **(Yaniv et al., 1987),** chronic anti-inflammatory **(Lev and Amar, 2000),** and used in the treatment of gastric and duodenal diseases **(Alarcon et al., 1993).** These therapeutic properties are related to the plant's richness in active secondary compounds. Several phytochemical studies conducted on this plant have revealed the presence of phenolic, terpene and lactone compounds **(Ali Shteh and Abu-Ghadib, 1999).**

In Morocco, this plant is used in human herbal medicine, and several ethnophysiological studies have been conducted on it **(Hammamouchi, 2001; Texidor et al., 2016).**

Similarly, in Algeria , the plant has been investigated for its medicinal applications and phytochemical composition in traditional practices **(Benmehdi et al., 2012).**

4. Bacterial Strains And Antibiotics Studied

4.1 Bacterial Stains *" Staphylococcus aureus (S. aureuse)"*

Staphylococci are a group of spherical-shaped bacteria (*cocci*) that are Gram-positive and facultative aerobes/anaerobes, meaning they can survive in both the presence and absence of oxygen. These bacteria are well known for their high resistance to environmental conditions and their low nutritional requirements in culture media. One of the most clinically significant species is *Staphylococcus aureus*, commonly known as "*golden staphylococcus*", which is coagulase-positive. This species was named by **Rosenbach in 1884,** due to its production of carotenoid pigments that give its colonies a distinctive golden color **(Licitra, 2013).**

S. aureus is considered a commensal bacterium, naturally colonizing the skin and mucous membranes of humans. Its primary colonization site is the anterior nares (nostrils)

(Wertheim et al., 2005), although it may also be found in other areas such as the pharynx, intestines, perineum, armpits, and skin. Although humans represent the main reservoir of this bacterium, it is also found in the surrounding environment (water, air, surfaces, and food) and in animals, particularly farm animals. The primary route of entry for *S. aureus* is through the skin, and the bacterium possesses a wide array of virulence factors, including adhesion molecules, enzymes, and toxins, which enable it to cause a variety of infections. Among these, enterotoxins play a major role in foodborne intoxications **(Shallcross et al., 2013).**

4.2 Antibiotics

4.2.1 Definition Of Antibiotics

The word antibiotic comes from the Greek anti meaning "against" and bios meaning "life". It refers to natural, hemisynthetic or synthetic substances capable of either inhibiting bacterial growth or destroying bacteria **(Veyssière et al., 2019).** Antibiotics are classified as chemotherapeutic agents, and can be obtained either from producing microorganisms, or by chemical synthesis without direct microbial intervention **(Chebbah et al., 2010).**

4.2.2 Antibiotic Modes Of Action

Antibiotics generally exert their antimicrobial effect in a specific way, targeting precise structures within the bacterial cell. There are five main mechanisms of action:

- Inhibition of peptidoglycan synthesis, an essential component of the bacterial wall, which weakens the cell and leads to its lysis.
- Direct alteration of the cell wall, disrupting its integrity and protective function.
- Blockage of protein synthesis, through interaction with bacterial ribosomes, preventing the production of proteins required for bacterial survival.
- Inhibition of nucleic acid synthesis, disrupting DNA replication or RNA transcription.
- Competitive inhibition, where the antibiotic acts by mimicking a natural substrate, thus blocking the action of an essential bacterial enzyme **(Mokrani & Hamdani,2017)**

4.2.3 Definition Of Antibiotic Resistance

Antibiotic resistance, also known as bacterial resistance, refers to the ability of certain bacteria to survive or continue to proliferate despite exposure to antibiotics or biocides intended to eliminate them or curb their growth **(Sophie, 2014**). There are two main forms of antibiotic resistance:

a) Natural (or Intrinsic) Resistance: This is a stable genetic trait shared by all bacteria belonging to the same species or genus. This type of resistance thus delimits the spectrum of action of a given antibiotic **(Azmoun, 2016).**

b) Acquired Resistance: This concerns only certain strains within a bacterial species, but can spread to other bacteria. This resistance can result either from spontaneous genetic mutation, or from the acquisition of transferable resistance genes from other micro-organisms **(El Brahmi, 2013; Aboya, 2013).**

4.3 The Disk Diffusion Method (Antibiogram)

The disk diffusion method is one of the most commonly used techniques to determine the susceptibility of bacterial strains to antibiotics. This method involves spreading a standardized bacterial suspension over the surface of a solid culture medium (typically Mueller-Hinton agar). Paper disks impregnated with known concentrations of different antibiotics are then placed on the inoculated surface. After incubation, clear zones appear around certain disks, referred to as inhibition zones, where bacterial growth is prevented. The diameter of these zones is measured to assess the effectiveness of each antibiotic against the tested strain, based on standardized guidelines provided by expert committees (such as **EUCAST** or **CLSI**). This technique serves as a fundamental tool in laboratory diagnostics and in guiding appropriate antibiotic therapy **(Leysour, 2020).**

4.4 The Well Difusion Method (Aromatogram)

This is the same procedure as the disk method, except that 6mm-diameter wells are cut on Mueller Hinton agar. using a sterile pipette. Then, using a micropipette, the wells were filled with 50 µl of essential oil. Incubation at 37°C for 24 hours..**(Djenane et al.,2011).**

Materials and methodes

Experimental Sites

The study was conducted in two separate structures:

- The extraction of *Inula viscosa* essential oil was carried out in a local aromatic and medicinal plant processing workshop **“BioVerma”,** located in Chreaïa (Skikda).
- Evaluation of its antibacterial activity was carried out at the laboratory of **“The Berdoudi Clinic”** in El-Harrouch (Skikda).

Materials and Methodes

1.Materials

1.1 Plant Material

This study was based on only one plant species, *Inula viscosa*. The leaves of this plant were collected during the month of **March 2025** from Kanoua area, located in the western part of Skikda province.

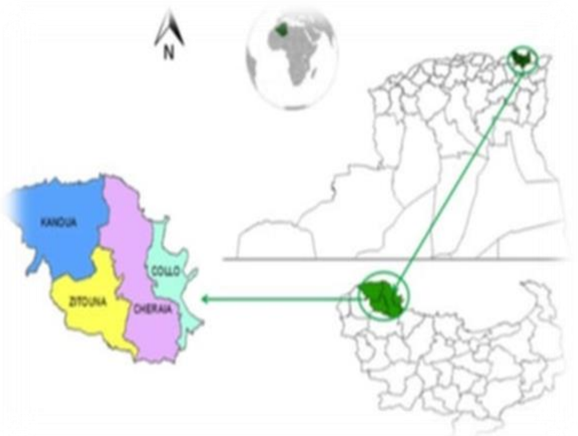

Figure 08 : Map showing the collection site of *Inula Viscosa* (Original)

Figure 09: *Inula viscosa (*original)

2.Methodes

- An estimated 12 kg of *Inula viscosa* leaves were collected and washed thoroughly with distilled water to remove microorganisms on the surface.
- The leaves were then placed in a dark and dry place, at room temperature, and left to dry for 20 days to preserve their natural properties.
- At the end of the drying period, the dry leaves weighed about 3 kg.
- leaves were then transported to the **Bio Verma** laboratory (Chiraya, Skikda), where the essential oil was extracted using the hydrodistillation.

Figure 10: Processing steps of *Inula Viscosa* leaves for essential oil extraction (original)

2.1 Essential Oil Extraction From *Inula Viscosa*

Extraction Principle

- Hydrodistillation was used to extract the essential oil from the aerial parts of the *Inula viscosa* plant. A traditional distillation device known as the Système Alambic was used, which operates similarly to the Clevenger apparatus.
- The setup consists of a 70-liter metal vessel (similar to a pressure cooker) placed on a natural gas stove.
- The rising vapors are transferred through a copper tube to a condenser made of a coiled copper tube placed inside a cooler, where cold water is continuously pumped to facilitate condensation.

- The condensed mixture, which contains both the essential oil and aromatic water (hydrosol), is collected in a special receiving vessel (asincer).
- The two phases are then separated using a separating funnel, allowing the essential oil to be isolated from the aqueous phase.

Figure 11: The Hydrodistillation Unit (Original)

Practical Procedure Plant Material:

- 3kg of dry aerial parts of *Inula viscosa*
- **Solvent:** 10 liters of distilled water
- **Apparatus Used:** Traditional hydrodistillation unit (Système Alambic)
- **Distillation Time:** 3 continuous hours Outcome The distillation process resulted in the separation of two main substances: Essential oil (organic phase) Aromatic water (hydrosol) (aqueous phase)
- The essential oil is stored in dark glass bottles at low temperatures to protect it from external factors.

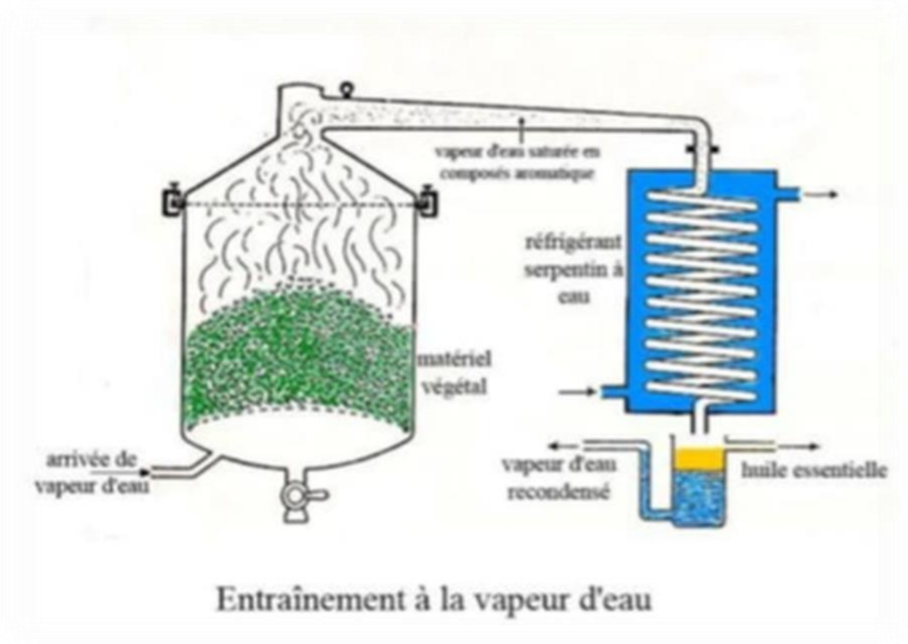

Figure 12: Hydrodistillation Device **(Harborn.,1998)**

2.2 Yield

The yield in essential oil extraction is the relationship between the amount of oil produced and the weight of plant material used. This yield is calculated as a percentage using the following formula:

$$\mathbf{Y\ (\%)} = \frac{\mathbf{M\ oil}}{\mathbf{M\ of\ plant}} \times \mathbf{1OO}$$

Y: Yield of essential oil extracted. (%)

M oil : Mass of essential oil (g)

M plant : Plant mass.(g)

2.3 Preparation Of Essential Oil By Macrodilution

According to **Kalemba and Kunicka (2003),** macrodilution in tubes is a reliable and widely used method for the preparation of essential oil dilutions, particularly in view of their low solubility in water. In our study, dimethyl sulfoxide **(DMSO)** was used as the diluting solvent.

- A stock solution was prepared using (100%) pure essential oil ,without the addition of any solvent.

Successive dilutions were then made:

- 1/2 dilution (50%): 1 mL stock solution + 1 mL DMSO.
- 1/4 dilution (25%): 1 mL of the previous dilution (1/2)+ 1 mL DMSO.

Each solution was homogenized after dilution by vortexing for 10-15 seconds to ensure good dispersion. Solutions were stored at room temperature, protected from light, until use in antimicrobial tests.

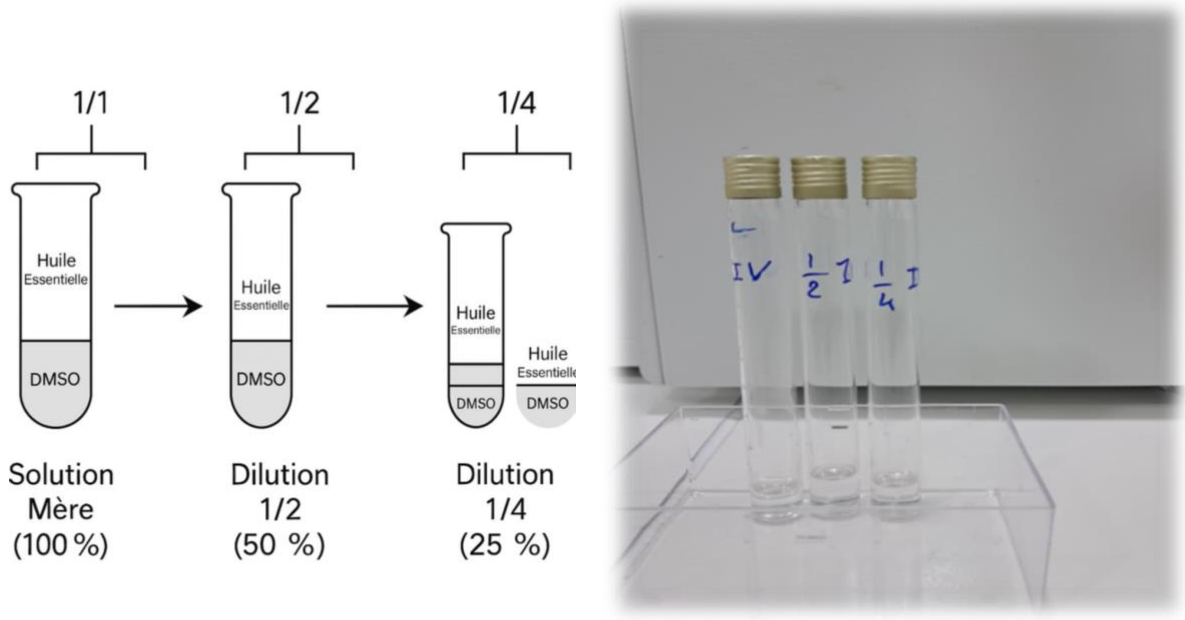

Figure 13: Serial dilutions of *Inula Viscosa* essentiel oil (original)

2.4 Evaluation Of Antibacterial Activity

- **Source And Selection Of Bacterial Strains**

The bacterial strains under in this study were selected based of their scientific relevance and involvemant in common human infections .two types of stains were included in this analysis.

A) Reference Stains: obtained from "Pasteur Instite Of Algeria **(PIA)** " ,used as standards to assess the efficacy of antimicrobial substances.

B) Clinical Stains: The bacterial strains used in this study were clinical isolates obtained from samples taken from patients hospitalized at hospital **" Abed Razak Bouhara of Skikda"**. These isolates were selected to reflect the real-life antibiotic resistance profile encountered in a hospital environment. Their use in antimicrobial susceptibility testing (both antibiogram and aromatogram) provides relevant data regarding the therapeutic potential of essential oils in the context of increasing antibiotic resistance.

To preserve their viability and efficacy,the stains were periodically grown on selective culture media, then incubated at 37°C for 24 hours. The study involed two 2 bacterial stains belonging to **Gram-positive bacteria** *Staphylococcus.*

- **Choosing The Culture Medium**

The appropriate culture medium was selected based on the nature of the experiment and the type of bacterial strains studied. The Muller-Hinton agar medium was used to assess the sensitivity of the bacteria to both antibiotics and essential oils.

- **Antibiotic and Essential Oil Susceptibility Test (Antibiogram and Aromatogram)**

To assess the effectiveness of antibiotics and essential oils against the studied bacterial strains, the agar diffusion method was used in two variations:

the disk diffusion method (for antibiotics) and both disk and well diffusion methods (for essential oils).

2.4.1 Antibiotic Susceptibility Test (Antibiogram)

A) Preparation of the Inoculum:

- Mature colonies (3 to 5), from a pure culture aged 24 hours on solid medium, were collected.
- Colonies were transferred into a sterile tube containing 5 to 10 mL of sterile physiological saline (0.9% NaCl).
- The suspension was homogenized to ensure even distribution of bacteria, then the turbidity was adjusted to match approximately McFarland standard 0.5.

B) Preparation of the Medium:

- Mueller-Hinton agar was melted and poured into sterile Petri dishes to achieve a uniform thickness of 4 mm. Plates were left to cool and solidify.

C) Inoculation:

- A sterile swab was dipped into the bacterial suspension, and excess liquid was removed by pressing it against the inner wall of the tube.
- The swab was then spread over the agar surface in three directions, rotating the plate 60° each time, to ensure homogeneous inoculation.

D) Placement of Antibiotic Disks:

- Using sterile forceps, antibiotic-impregnated paper disks were gently placed onto the inoculated agar surface.
- Care was taken to position the disks with adequate spacing to prevent overlapping zones.

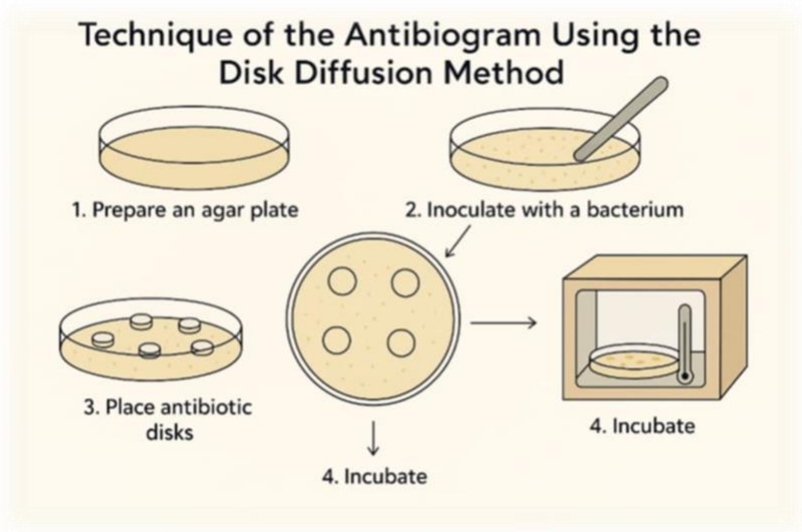

Figure 14: Technique of antibiogram using **(Bakkali et al.,2008)**

E) Incubation And Reading:

After incubation, the diameters of the inhibition zones around each disk were measured in millimeters and interpreted . According to standard guidelines to classify the strain **(Ponce et al., 2003)(table 04)**

Table 04: Diameters of inhibition zone of antibiogram and aromatogram

Diameters (D) Of Inhibition Zone (Mm)	Transcription	Germ Sensitivity
D < 8 Mm	-	Resistant
9 Mm > D > 14 Mm	+	Sensitive
15 Mm > D > 19 Mm	++	Moderately Sensitive
D ≥ 20 Mm	+++	Highly Sensitive

2.4.2 Antibiotic Susceptibility Test (Aromatogram)

A) Well Diffusion Method:

- The same Mueller-Hinton agar inoculation procedure as described above was followed.
- Using a sterile tool, wells of **6 mm** diameter were cut into the inoculated Mueller-Hinton agar.
- Each well was filled with **50 µL** of essential oil or its dilutions using a micropipette , Pure essential oil (IV pur) , (½) and (¼)
- DMSO was also added in a separate well as a negative control.
- The plates were incubated at 37°C for 24 hours, and the diameters of the inhibition zones were recorded.

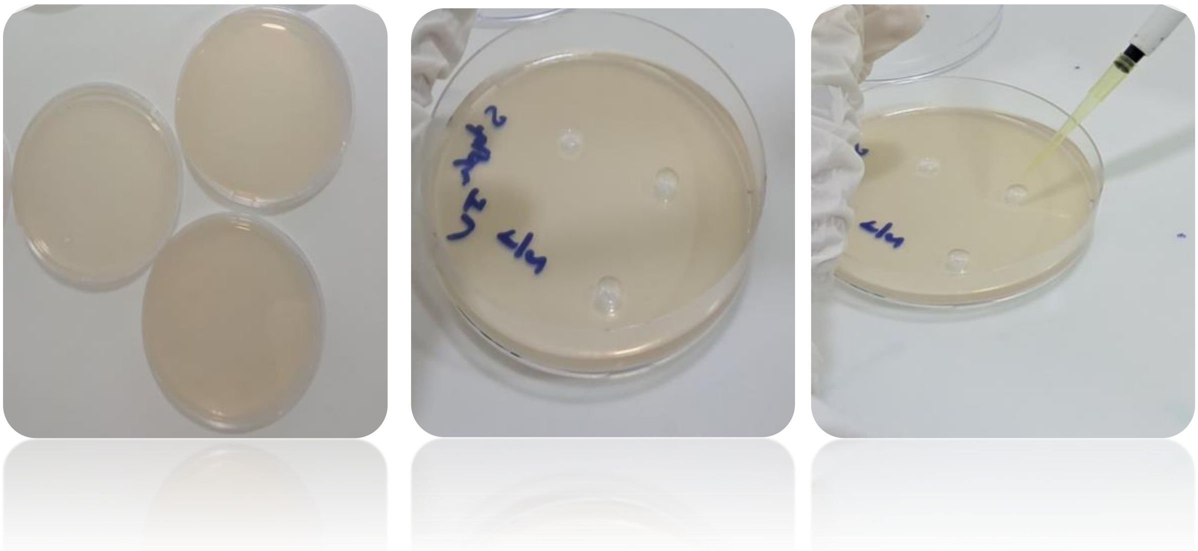

Figure 15: well diffusion methods (original)

Results and disscusion

1. **Extraction Yield**

Hydrodistillation of *inula viscosa* yielded a pale yellow, clear liquid oil ,with a characteristic spicy odour. The yields obtained for our oils are shown in **Table 05.**

Table 05: *Inula Viscosa* extraction yield results.

The Mass Of Essential Oil In (G)	Plant Material In (G)	The Essential Oil Yield (%)
1.6	3000	0.05 %

In the present study, the essential oil of *Inula Viscosa*, extracted via hydrodistillation from 3000 g of fresh aerial parts, showed a yield of **0.05%.** Although relatively low, this result aligns with the lower range of yields reported in previous research.

For instance, **Ounoughi et al. (2020)** obtained a notably higher yield of **0.23%** from *I. viscosa* collected in Algeria, using the same hydrodistillation method. Similarly, **Benine Lamine et al. (2022)** reported a yield of **0.23%** from leaves collected in Sidi Bel Abbès (Algeria), underlining the influence of plant part and geographical origin.

A more recent work, **jerada et al. (2025)**, recorded a slightly higher yield of **0.085%,** which remains within the same general range, further confirming that *Inula Viscosa* can produce relatively low essential oil quantities depending on specific environmental and methodological parameters.

Moreover, **Vuko et al. (2021)** demonstrated a wider variability, with yields ranging from **0.05%** to **0.30%,** highlighting the significant influence of factors such as climate, soil, seasonal stage of the plant, and the extraction technique used. Their study also considered alternative methods like supercritical CO_2 extraction, which can increase yields compared to traditional hydrodistillation.

Overall, while the yeild obtained in this study is modest, it falls within the range expected for this species and supports the notion that *Inula Viscosa* remains a relevant plant for essential oil production, especially when proper optimization measures are implemented.

2. Evaluation Of Antibacterial Activity

2.1 Antibiotic Susceptibility Test " Antibiogram"

The aim of the susceptibility test is to evaluate the sensitivity of two *Staphylococcus aureus* stains a reference strain **(ATCC)** and a clinical isolate to two antibiotics: Vancomycin **(VAN)** and Penicillin **(P).** The test is performed using the standard disk diffusion method on Mueller-Hinton agar. Antibiotic-impregnated discs are placed on the surface of the agar, which is previously inoculated with the bacterial strains.

According to the diameters of the inhibition zones around the antibiotic discs **(table 04 in materials and methodes)**, bacterial susceptibility is classified as follows:

Table 06: Diameters of antibiotic inhibition zones using (*staph R)*

	Antibiotic	**Median ±Standard Deviation**	**Result**
Reference Staphylococcus aureus	Penicillin	11.50.7	S+
	Vancomycin	15.5±0.7	S++

Figure 16: Evaluation of the antibacterial activity of *IV* extract against S*taph R*

The susceptibility test results show that the *Staphylococcus R* strain exhibited an inhibition zone of 11.5 mm with penicillin and 15.5 mm with vancomycin. We observe the following:

Penicillin (11.5 mm) falls under the weak sensitivity (+) category, indicating that the strain is not fully resistant, yet the effectiveness of penicillin is limited. Therefore, penicillin is not considered a reliable therapeutic option for severe or persistent infections.

This aligns with studies such as **Siddiqui et al. (2022),** which reported reduced penicillin efficacy against **MRSA (Methicillin Resistant *Staphylococcus aureus*)** isolates due to the production of the β-lactamase enzyme, which degrades the antibiotic. Vancomycin (15.5 mm) falls within the moderate sensitivity (++) range, suggesting that the strain responds to vancomycin at a moderate level. While it does not show high sensitivity, vancomycin remains clinically effective in most cases. This is supported by studies like **Lee et al. (2023),** which showed that over **95%** of **MRSA** strains are still susceptible to vancomycin.

Table 07: Diameters of essential oil inhibition zones using the well difusion method (*staph R*)

	DMSO	The average *staph R*	Result
IV pure	00	12±**0.05**	S+
IV 1/2	00	10±**0.05**	S+
IV 1/4	00	08±**0.05**	R-

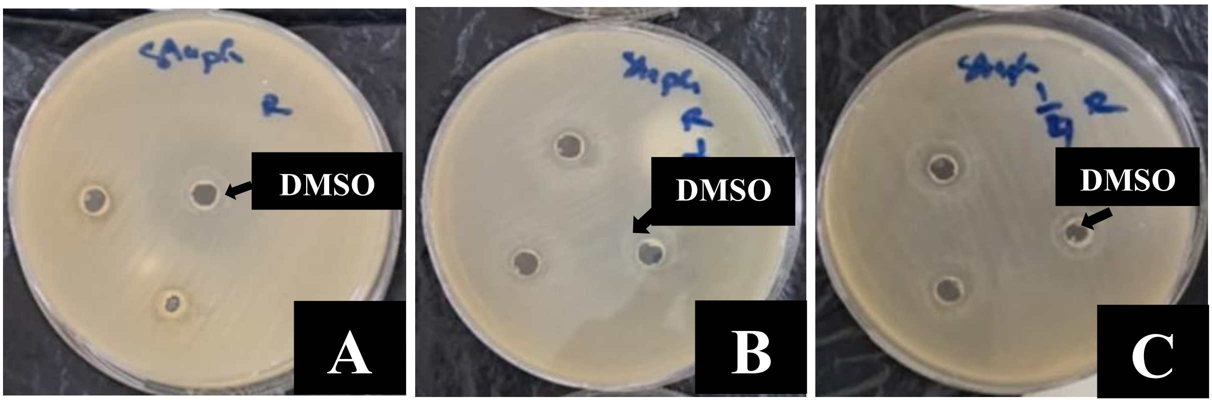

Figure 17: Results of the aromatogram using the well diffusion method (*Staph R*)

A:Pure **B:**1/2 **C**:1/4

The results of the susceptibility test showed that the extracted essential oil exhibited antibacterial activity against the resistant *Staphylococcus aureus* strain (*Staph R),* with effectiveness clearly dependent on concentration:

When using the pure essential oil (IV Pure), the inhibition zone reached 12 mm, which falls under the category of weak sensitivity (+). This indicates the presence of antibacterial activity, although limited. When the oil was diluted to half (IV 1/2), the inhibition zone decreased to 10 mm, which is classified as Sensitivity (+), suggesting that the oil retains a moderate antibacterial effect at this concentration. At a quarter dilution (IV 1/4), the inhibition zone remains at 8 mm, which is classified as resistant (−), meaning that this concentration is no longer sufficient to produce a noticeable inhibitory effect.

These results clearly demonstrate that the antibacterial effectiveness of the essential oil significantly decreases with dilution. This observation is consistent with the findings of **Ounoughi et al. (2020),** which reported that the antibacterial activity of *Inula Viscosa* essential oil is concentration-dependent. This effect is attributed to active compounds such as thymol, carvacrol, and eugenol, which possess physiological properties capable of disrupting bacterial membrane permeability and inhibiting key enzymes **(Benbelaïd et al., 2014; Bouyahya et al., 2017).**

As for DMSO, used as a solvent, it showed an inhibition zone of 0 mm in all treatments, indicating that it has no antibacterial activity and confirming that the observed effect is entirely due to the essential oil.

Table 08: Diameters of antibiotic inhibition zones using *(staph C)*

	antibiotic	**Median ±Standard Deviation**	**Result**
Clinical *Staphylococcus aureus*	Penicillin	33.5±3.5	S+++
	Vancomycin	18.5±3.5	S++

Figure 18: Evaluation of the antibacterial activity of IV extract against *staph C*

The results of the antibiotic susceptibility test for the *Staphylococcus C* strain were as follows:

It is observed that: Penicillin (33.5 mm) falls under the category of high sensitivity (+++), indicating that the strain responds excellently to this antibiotic. This reflects the absence of resistance mechanisms commonly associated with **MRSA** isolates, such as the production of β-lactamase. Vancomycin (18.5mm) falls under the moderate sensitivity (++) category, indicating a moderate level of effectiveness, which is adequate in clinical treatment, especially in cases where acquired resistance is suspected. These results are consistent with **CLSI** guidelines and are supported by studies such as **Siddiqui et al. (2022),**

Furthermore, these findings align with a multicentre study conducted in Spain by **Cercenado et al. (2021),** which investigated penicillin susceptibility among MSSA isolates from bloodstream infections. Out of 1011 isolates collected from 16 hospitals, 156 strains were confirmed to be penicillin-sensitive **(PEN^S).** The study also demonstrated that the presence of the *blaZ* gene, typically associated with *β-lactamase*-mediated resistance, does not necessarily result in phenotypic resistance. This supports our observation and reinforces the clinical relevance of penicillin as an effective treatment option for certain MSSA infections.

Furthermore, **Lee et al. (2023)** confirmed the continued effectiveness of vancomycin against most *Staphylococcus* isolates, making it a reliable therapeutic option, particularly in the context of suspected resistance. Therefore, both penicillin and vancomycin can be considered effective treatment options for this strain. However, the final therapeutic decision should take into account the patient's clinical condition, prior antibiotic exposure, and the potential risk of infection with resistant strains such as **MRSA.**

Table 09: Diameters of essential oil inhibition zones using the well difusion method (*staph C*)

	DMSO	**The average *staph C***	**Result**
IV pure	**00**	**11±0.05**	**S+**
IV 1/2	**00**	**08±0.05**	**R-**
IV 1/4	**00**	**07±0.05**	**R-**

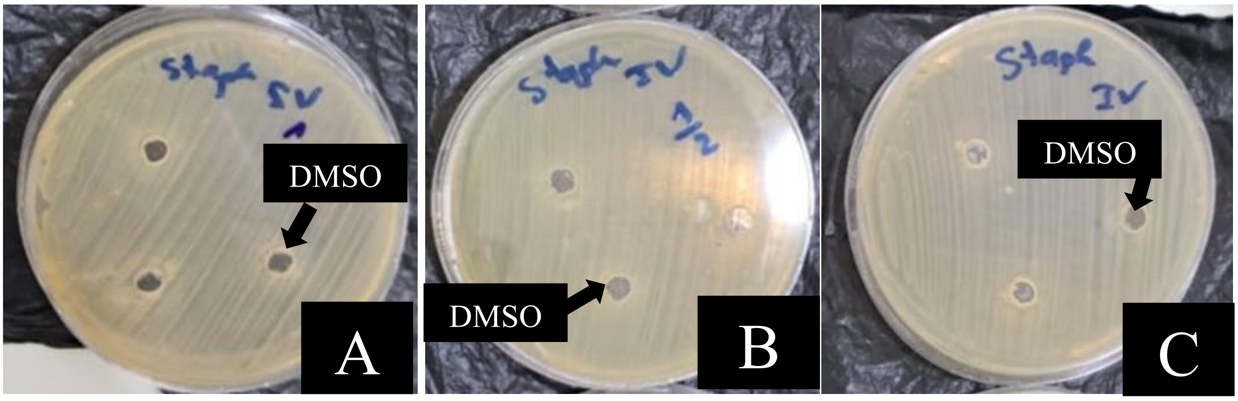

Figure 19: Results of the aromatogram using the well diffusion method (*staph C*)

A:Pure **B**:1/2 **C**:1/4

The results of the susceptibility test showed that the essential oil extracted from *Inula Viscosa* possesses antibacterial activity against the resistant *Staphylococcus aureus* strain (*Staph C*), with its effectiveness clearly dependent on concentration:

When using the pure essential oil (IV Pure), the inhibition zone diameter measures 11 mm, which falls under the category of weak sensitivity (+). This indicates the presence of antibacterial activity, although limited. When the oil is diluted to half strength (IV 1/2), the inhibition zone decreases to 8 mm, classifying the strain as resistant (−), suggesting that this concentration is insufficient to produce a significant inhibitory effect. Upon further dilution to a quarter strength (IV 1/4), the inhibition zone decreases further to 7 mm, maintaining the strain's classification as resistant (−).

These results highlight that the antibacterial effectiveness of the essential oil decreases significantly with dilution, which aligns with previous studies such as **Ounoughi et al. (2020) and Burt (2004),** both of which confirmed that the antimicrobial activity of essential oils gradually diminishes as concentration decreases.

The different responses of **ATCC** and clinical *Staphylococcus aureus* strains to *Inula Viscosa* essential oil and antibiotics are attributed to variations in genetic background, environmental exposure, and membrane structure.

The **ATCC** strain, maintained under controlled laboratory conditions, is more genetically stable and lacks adaptive resistance mechanisms, which may explain its slightly higher sensitivity to the essential oil. Essential oils act non-specifically by disrupting bacterial membranes, making them broadly effective unless structural adaptations occur **(Bakkali et al., 2008; Burt, 2004).**

In contrast, the clinical strain, exposed to hospital environments, may develop resistance traits such as efflux pumps and altered membrane composition reducing its susceptibility to essential oils **(Nazzaro et al., 2013; Bouyahya et al., 2017).** However, it shows greater sensitivity to antibiotics like Penicillin and Vancomycin, likely due to the absence of resistance genes such as mecA or vanA, which are often present in MRSA strains **(Siddiqui et al., 2022; CLSI, 2023).**

conclusion

Conclusion

The present study highlights the antibacterial potential of Inula viscosa essential oil, which is extracted by hydrodistillation and tested against both clinical and reference bacterial strains. Although the oil yield is relatively low (0.05%), the essential oil shows measurable antibacterial activity, particularly in its pure form. The inhibition zones display a clear concentration-dependent response, confirming the influence of dilution on its efficacy.

The essential oil demonstrates antibacterial effects against Staphylococcus aureus, with the ATCC strains generally showing greater sensitivity than the clinical isolates. When compared to conventional antibiotics, the essential oil proves to be less effective overall, yet it exhibits promising inhibitory action especially in cases where antibiotic resistance presents a clinical concern.

These findings support the potential use of Inula viscosa essential oil as a complementary natural antimicrobial agent. However, its lower efficacy relative to standard antibiotics and the limited extraction yield indicate the need for further research.

- Future studies focus on isolating the active constituents, optimizing extraction methods, and assessing safety and therapeutic potential through in vivo evaluations.

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