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Exploring Natural Remedies in the Region of Boumerdes and Investigating the Chemical Profile and Biological Potency of Two Extracts from *Inula viscosa L. Roots*

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Dedication

This thesis is dedicated to my forever role model and hero, ***"Mum"*** *I would never reach anything I reached today if it wasn't for you . May Allah have mercy on your beautiful soul , I'll keep doing my best to be always the girl you raised .*

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NOUMIDIA

Dedication

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*To all **my friends** and to all the people who love me*

ASMAA

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%	Percentage
°C	DegreesCelsius
µL	Microliters
µm	Micrometer
AlCl3	Aluminumchloride
BHT	Butylatedhydroxytoluene
BPH	Benignprostatichyperplasia
C	Concentration
Do	Opticaldensity
DPPH	2,2-diphenyl-1-picrylhydrazyl
ED	Erectiledysfunction
EHM	Hydromethanolicextract
GC-FID	Gaschromatographyflameionizationdetector
GC-MS	Gaschromatographymassspectra
HCl	Hydrochloricacid
HPLC	High-performanceliquidchromatography
I.V	*Inulaviscosa*
IC50	Half-maximalinhibitoryconcentration
Kg	Kilogram
LD50	Medianlethaldose
LOH	Late-onsethypogonadism
LPS	Lipopolysaccharide
MeOH	Methanol
mg	Milligrams
MH	MullerHinton
MIC	Minimuminhibitoryconcentration
min	Minute
mL	Milliliters
mm	Millimetre
NaCl	Sodiumchloride
nm	Nanometer
PDA	Potatodextroseagar
R	Root

TAC	Totalantioxidantcapacity
GAE	gallic acid equivalent
EC	equivalent catechin
EQ	equivalent quercetin

Table of contents

Introduction

Natural remedies, especially medicinal plants, have served as the mainstay of traditional medicine since ancient times. However, the rise of the modern pharmaceutical industry has led to substantial advancements in contemporary medicine, enabling effective treatment of numerous potentially life-threatening illnesses. (El Rhaffari and Zaid, 2004).

Algeria is the largest country bordering the Mediterranean. It is renowned for its diverse variety of medicinal and aromatic plants, as well as their various popular uses throughout the country's territories. These are ancestral skills passed down from generation to generation among the population, mostly rural (Sahi, 2016). However, these vast knowledge, traditions, and ancestral skills, primarily transmitted orally, are currently held by few individuals, with high illiteracy rates. Moreover, they are rarely conveyed to the younger generation, who typically show little interest in nature-related subjects. (Meddour et al., 2009).

Today, there is an urgent need to gather ethnobotanical and especially ethnomedicinal information before it's too late, by compiling as comprehensive an inventory as possible of the plants still used by rural populations. Preserving this knowledge is crucial for the conservation and valorization of natural resources on one hand, and for safeguarding cultural heritage on the other (Rebbas et al., 2012).

Plant crude extracts are increasingly recognized for their potential as sources of bioactive natural compounds. Research is focusing on their potential application as alternatives for crop protection (Boudjemaa, 1999; Chabon, 2000; Benabedelkrim, 2009; Guerrida, 2010).

The *Asteraceae* family offers a wide range of genera and species, each with its own distinct characteristics. This plant family holds plants that serve various purposes; some are valued for their medicinal properties, leading to extensive research on therapeutic molecules (Rolnik et al., 2009; Carvalho et al., 2018). Among these species is the widely spread *Inula viscosa* L. in the Mediterranean region (Quezel and Santa, 1963), known for its use in treating various ailments including inflammatory and infection disorders. Our aim is to investigate treatments derived from this plant that exhibit significant biological properties.

Due to the lack of research on extracts from its subterranean part, we have chosen to study only the root system of the Algerian species *Inula viscosa* L. Our objective in this study is to conduct research on the natural remedies used in the Boumerdes province, gathering information on herbalists (their level of experience, etc.), and specifying the nature and usage

of natural remedies (their origin, preparation methods, etc.). Additionally, we aim to research the secondary metabolism (phenolic and terpenic compounds) and to evaluate some biological effects (antioxidant, antimicrobial, cytotoxic and antalgic) of crude extract and hydrosol from the roots of *Inula viscosa* for therapeutic purposes.

- The initial section consists of three chapters. The first chapter provides in-depth literature reviews covering phytotherapy and medicinal plants. Following that, the second chapter delves into the active components found in medicinal plants. Lastly,the third chapter focuses on detailing the chosen plant, *Inula viscosa* .

- The second part covers material and methods. It explains the plant and animal material used, as well as all the techniques and methods applied for the phytochemical, and biological study.
- The third part will present our results, interpret them, and discuss them in comparison to existing literature. Finally, we will provide a general conclusion and suggest future perspectives.

Bibliographic synthesis

1. Phytotherapy

1.1Definition

The term "Phytotherapy" is derived from two Greek roots: "phyto," which means plant, and "therapy," which means treatment. It refers to treatment using plants (Baba, 2000). The idea behind this was brought forth by the physician Henri Leclerc, who coined the term for the first time in 1913 (Heinrich, 2013).

Interest in phytotherapy is increasing in both Asian and Western nations due to its potential for preventing and managing diseases, enhancing overall health, and combating aging. (Woong Kim, 2012).

1.2Different types of phytotherapy

Different types of plant therapies can be distinguished:

a- Aromatherapy

Aromatherapy, just as Homeopathy, allows the chosen active substance to be used in a range of different concentrations. In Aromatherapy, the essential oils function according to the concentration chosen for the physical, mental and emotional aspects, from a higher to a lower concentration, respectively (Napoleão, 2011).

Example: **Lavender oil** is commonly used in aromatherapy for its calming and relaxing effects. It can be used in a diffuser to help alleviate stress, anxiety, and promote better sleep.

b- Gemmotherapy

Gemmotherapy, which utilizes bud macerates as a natural remedy, has recently become more prominent in the domain of dietary supplements (Bréard et al., 2023).

Example: **Argan oil** (*Argania spinosa*), used for its cosmetic benefits, can also fit within the natural remedies used in gemmotherapy.

c- Herbalism

Herbalism, also known as herbology or botanical medicine, encompasses the study, cultivation, gathering, and distribution of herbs renowned for their medicinal benefits (Ameh et al., 2011).

Example: **Pomegranate peel** (*Punica granatum*) is used in herbalism for its ability to lower cholesterol levels.

d- Pharmaceutical phytotherapy

It involves using plant-derived products obtained through extraction and diluted in ethyl alcohol or other solvents. These extracts are measured in quantities adequate to provide quick and sustained effects. They come in forms such as syrups, drops, capsules, and more (Strang, 2006). Example: **Valerian herb** (*Valeriana officinalis*) is used in pharmaceutical phytotherapy for its sedative and anxiolytic properties, often available in capsule form for treating insomnia and anxiety.

2. Medicinal plants

Medicinal plants, the earliest form of medicine, have been utilized for millennia in traditional practices across various cultures globally. The accumulated wisdom regarding their positive impacts has been passed down through generations within human societies over centuries (Marrelli, 2021).

Around 170 herbal drugs were officially acknowledged in the U.S. Pharmacopeia and National Formulary. The Director of WHO's Traditional Medicine division noted in 1993 that approximately 80% of the global population primarily depend on traditional medicine, predominantly plant-based, especially for their primary healthcare requirements (Indrayan, 2004).

2.1Ethnobotanical survey

Ethnobotany is a scientific discipline that explores how humans across different geographical areas interact with and understand plants. It delves into the traditional knowledge and practices of local communities regarding plants and investigates the impact of historical and contemporary human activities on the plant environment (Crozat, 2001).

2.2Methods of preparation and use of medicinal plants

a- **Infusion:** Herbal tea is primarily crafted from plant flowers and leaves. The method entails pouring boiling water over the plant and allowing it to steep for 10 to 20 minutes. Infusions can be refrigerated for up to 48 hours, and it's advised not to add sweeteners (Nogaret-Ehrhart and Anne-Sophie, 2003) (Examples include : Lemon balm (*Melissa officinalis L.),* Marjoram (*Origanum majorana*)

b- **Decoction:** Decoction entails boiling either dried or fresh plants, chopped beforehand, in water; subsequently straining the resulting liquid, which is called the decoction. It can be enjoyed either warm or chilled (Chevallier, 2001). (Examples include : Wormwood (Artemisia absinthium) , Indian costus (Dolomiaea costus)

c- **Maceration:** It involves soaking plants in a solvent such as water, alcohol, or oil at cold temperatures for an extended duration, ranging from hours to weeks, depending on the solvent and plant component. This process should be carried out in a container protected from both air and light lumière (Charbier, 2010). (Examples include : Sesame oil (Sesamum indicum), Argan oil (Argania spinosa)

d- **Cataplasms:** Poultices, made from plant preparations, are applied to the skin. They alleviate muscle pain and neuralgia, aid in the relief of sprains and fractures, and assist in drawing out pus from infected wounds, ulcers, and boils (Chevallier, 2001). Examples include : Earthnut (Bunium mauritanicum) , Indian costus (Dolomiaea costus)

e- **Vegetable oil:** To produce vegetable oil, mix a handful of dried or fresh herbs with olive, almond, or walnut oil in a sealed container and leave it undisturbed for 2 to 3 weeks (Nogaret-Ehrhart, 2003). (Examples include : Castor oil (Ricinus communis), Sesame oil (Sesamum indicum)

f- **Essential oils:** Essential oils and hydrosols are derived from aromatic plants using hydrodistillation or steam distillation methods. They contain vital active compounds found in plants, often oxygenated and sometimes terpenoid in origin, with aromatic characteristics. Hydrosols, on the other hand, are the water byproduct that retains the plant's properties during the hydrodistillation process (Iserin et al., 2001).

Examples include : Marjoram (Origanum majorana) , Lemon balm (Melissa officinalis L.)

g- **Ointment:** An ointment is made by blending a selected plant, either powdered or in juice form, with a fatty substance like petroleum jelly, coconut oil, olive oil, almond oil, or even animal fats (Garber, 2015). (Examples include :

Valerian herb (Valeriana officinalis) , Frankincense (Boswellia sacara)

h- **Powder:** A powder is acquired by crushing a plant, either using a coffee grinder or a mortar and pestle. Alternatively, it can be made by briefly heating the plant in a low-temperature oven (Morigane, 2007). (Examples include : Earthnut (Bunium mauritanicum) , Indian costus (Dolomiaea costus)

2.3 Active principles

The plant contains naturally occurring active principles, which are typically found in very small amounts (Aoudahi, 2010). It holds therapeutic significance for both humans and animals, offering potential curative and preventive benefits (Chabrier, 2010).

These substances are present throughout the plant, but their distribution varies and they exhibit distinct properties (Adouane, 2010). It is present in a botanical drug or a formulation derived from botanical sources. It is utilized in the manufacturing of pharmaceutical drugs (Pelt, 1980).

2.4 Plant metabolism

One of the remarkable characteristics of plants is their capacity to synthesize a broad range of diverse natural substances. In addition to the well-known primary metabolites like carbohydrates, proteins, lipids, and nucleic acids, they often accumulate secondary metabolites whose exact physiological roles may not always be clear. However, these secondary metabolites serve as a valuable source of molecules utilized by humans in various domains, including pharmacology and the agri-food industry (Bekkouche, 2007).

2.4.1 Primary metabolites

Primary metabolites are molecules that are typically permanent or constitutive and serve important physiological or intrinsic functions within an organism. These molecules play a direct role in the fundamental metabolic pathways of the cell, contributing to the plant's development, reproduction, and growth. As such, they are essential for the plant's survival, just as they are for any other living organism.

Primary metabolites comprise:

• Proteins: amino acids that serve a structural function and contribute to the regeneration of plant tissues.

• Lipids: fatty acids, which are characterized as water-insoluble molecules but soluble in non polar organic solvents.

• Carbohydrates: also referred to as sugars, they represent a crucial group of biomolecules in terms of both quality and quantity, following the generic formula: Cn(H2O)n.

2.4.2Secondary metabolites

The term "secondary metabolite" was possibly coined by Albrecht Kossel in 1891 (Yezza et Bouchama, 2014) , These molecules are essential for the plant's defense against external threats, produced in minimal amounts, and display a remarkable structural diversity with over 200.000 defined structures (Hartmann, 2007). Secondary metabolites have a relational function, and their composition and concentrations can be readily influenced by abioticfactors and biotic factors-induced molecules. These metabolites can be categorized into several prominent families, including phenolic compounds, terpenoids and alkaloids (Houël, 2012).

2.4.2.1 Phenolic compounds

1- Definition

Phenolic compounds are vital secondary metabolites found in plants, playing crucial roles in growth, lignification, pigmentation, pollination, and defense against pathogens, predators, and environmental stresses (Duthie et al., 2003). They are a diverse group of natural substances characterized by an aromatic ring containing one or more hydroxyl groups (Barlecher and Graca, 2020). Natural polyphenols include both simple molecules such as phenolic acids (C_6; C_6-C_1 or C_6-C_3), complex like flavonoides (C_6-C_3-C_6), and highly polymerized compounds like tannins $(C_{15})_n$. As of now, there are approximately 8000 identified compounds (Cory et al., 2018).

2- Role of polyphenols

Polyphenols exhibit a range of biological functions contingent upon their chemical makeup. They represent a significant group of antioxidants found in plants, fruits, and vegetables, totaling over 6000 molecules. In contrast to synthetic antioxidants like butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), polyphenols pose no adverse health effects on humans (Bounatirou et al., 2007). These compounds display various activities including anti-carcinogenic, anti-inflammatory, anti-atherogenic, anti-thrombotic, analgesic, antibacterial, antiviral, anticancer (Babar et al., 2007), anti-allergic, vasodilatory (Falleh et al., 2008), and antioxidant properties (Gomez-Caravaca et al., 2006).

Moreover, polyphenols play a vital role in regulating plant growth and development by interacting with different plant growth hormones. They enable plants to defend themselves against harmful ultraviolet radiation. Certain polyphenols, like isoflavonols, function as phytoalexins, aiding in the fight against fungal and bacterial infections (Makoi and Ndakidemi, 2007).

3- Classification

3.1Phenolic acids

Phenolic acid is a label used for organic compounds possessing a phenolic hydroxyl group and a carboxylic function, a classification particularly applied in phytochemistry to refer to derivatives of cinnamic acids and benzoic acids (Bruneton, 1993).

3.2Flavonoids

Flavonoids belong to the broader category of polyphenols. Typically, they are comprised of two aromatic rings, each containing at least one hydroxyl group, linked together by a three-carbon "bridge" to form a six-member heterocyclic ring (Figure 1) (Beecher, 2013). This group represents the most extensive category of polyphenolic compounds. They are categorized into various subgroups, including flavones, isoflavones, flavonols, flavanols, flavanones, chalcones, and anthocyanins (Derbel et Ghedira, 2005). Their role in the advancement of modern medicine has been crucial.

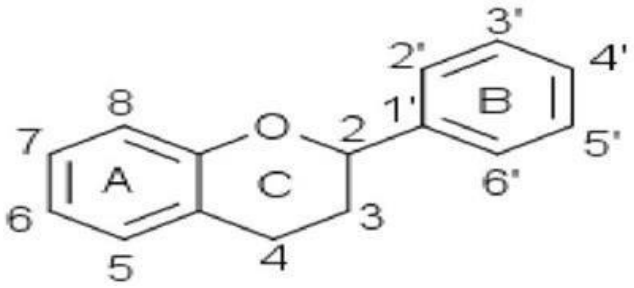

Figure 1: The basic structure of flavonoids (Lhuilier, 2007).

3.3**Tannins**

Tannins form a notable group of secondary plant substances initially utilized in the leather industry to treat animal hides (Sieniawska and bag, 2017). Tannins are employed for various purposes including treating diarrhea, constricting blood vessels, and controlling bleeding. However, their primary application lies in protecting veins during the treatment of varicose veins and hemorrhoids. They are also widely utilized in the leather industry, particularly in the production of varnishes and paints (Keil et al., 2004).

2.4.2.2Terpenic compounds

Terpenes constitute a broad family of compounds, typically lipid-soluble, originating from the condensation of basic units of 5-carbon isoprene. Their extensive diversity arises from the varying numbers of these basic units forming the main chain, with the formula (C5H8)n. Depending on this number, we distinguish between monoterpene, sesquiterpene, diterpene, and triterpene compounds (Wichtl and Anton, 2003).

Over 4000 triterpenes have been identified. While some are found independently, others exist as glycosides (known as saponins) or in specialized combinations (Jiri, 2003). Triterpenes promote collagen and glycosaminoglycan synthesis, exhibit antioxidant properties, and contribute to UV protection (Puziah, 2011).

2.4.2.3Alkaloids

According to Badiaga (2011), an alkaloid, is a nitrogen-containing organic substance originating from plants, characterized by its alkalinity and complex heterocyclic molecular arrangement.

Many alkaloids, like morphine, quinine, cocaine, and atropine, are notably important analgesics. They directly affect the body, showing sedative effects and influencing conditions such as Parkinson's disease (Bruneton, 1999; Iserin, 2001).

3. Study of the chosen species "*Inula viscosa.* L"

3.1 Etymology

Inula is derived from the Greek word "Ineo," which means "I purge," alluding to a therapeutic property of the plant (Fauron and Moati, 1983). *Viscosa* can have two different meanings, viscous or elecampane (Fournier, 1947). Other names are listed in the following table 1 for this species.

Table 01: Other names of *Inula viscosa*.

	Names	Bibliographical references
Scientific name	*Dittrichia viscosa* (L) Greut,	Bartel, 1997
Vernaculair name	Magrammane, magramen, terhala or mersit	Baba , 1999; Zeggwagh et al., 2006 ; Zeguerrou et al., 2013
Common name in english	Stichky fleabane, slimy inula	Halimi, 1997 ; Kanan and AL-Najar, 2009
Common name in french	Inule visqueuse	Bonnier 1990; Léger, 2007

3.2 Classification

According to the studies conducted by Quezel et Santa, 1963 and Dupont et al., 2007, the systematic categorization of *Iunla viscosa* can be described as follows (Table 02).

Table 02: The classification of *Inula viscosa* L.

Ranks	Classification
Reign	plantae
Phylum	Spermatophytes
Subphylum	Angiosperms
Class	Dicotyledons
Under class	Asterids
Order	Asterales

Family	Asteraceae
Genus	Inula
Species	*Inula viscosa* L.

3.3 Geographical distribution

The herbaceous plant *Inula viscosa*, which is an annual, can be found throughout the Mediterranean basin in abundance (Moussi, 2022). Is naturally found in the southern regions of Europe, including Spain, Greece, Italy, Bulgaria, as well as in the Middle Eastern countries of Jordan, Syria, and Turkey (Ulubelenet, 1987; Parolin et al., 2014). Is extensively utilized in North Africa (Rekkal and Ourida, 2016). There are more than a hundred species that are widely distributed in the temperate regions of Europe and Asia (Ana ML Seca et al., 2014). Is mainly confined to the coastal areas of the Adriatic Sea (Gianfranco et al., 2014), Growing in the wild in southern Turkey (Frank et al., 1995). It was harvested in different regions of Morocco (Messaoudi et al., 2016). It is found in the western part of Algeria (Senhadji et al., 2023)

3.4 Botanical description

Inula viscosa (L.) is a sticky and glandular herbaceous plant that completes its life cycle within a year (Bakkaraetal, 2008). With its simple or branched upright stems, it can grow upto a height of 120cm (Bartels, 1997). The plant's stems bear dense foliage and, as they mature,they develop a woody and dark base. At the base, they are frutescent and range in height from 40 to 100 cm, featuring reddish branches (Quezel and Santa, 1963).

The leaves are alternate, elongated, and lanceolate, measuring 3 to 7 cm in length and 6 to 12 mm in width (Figure 2A), becoming smaller towards the top (Bayer et al., 1990), are characterized by their rough texture and are coated with sticky glands on both surfaces (Bensegueni, 2001). A distinctive and powerful odor is emitted by them (Baba, 1999).

From the month of September onwards, the plant starts to bloom, displaying long clusters of inflorescences (Bensegueni, 2001; Rameau et al., 2008). The plant's flowers are arranged in capitula, measuring around 10-20mm in diameter. These capitula are clustered together to form panicles, supported by multiple branches of the main stem, resulting in a pyramid-like shape (Reeb, 2010). The flowers exhibit a vibrant yellow hue (Figure 2B) (Jiofack et al., 2010), Each of them is fertile (Ait youcef, 2006).

The fruits consist of hairy achenes adorned with a grayish tuft (Figure 2C) (Bensegueni, 2001), possesses a woody taproot that can grow up to 30 cm in length (Kaddem, 1990).

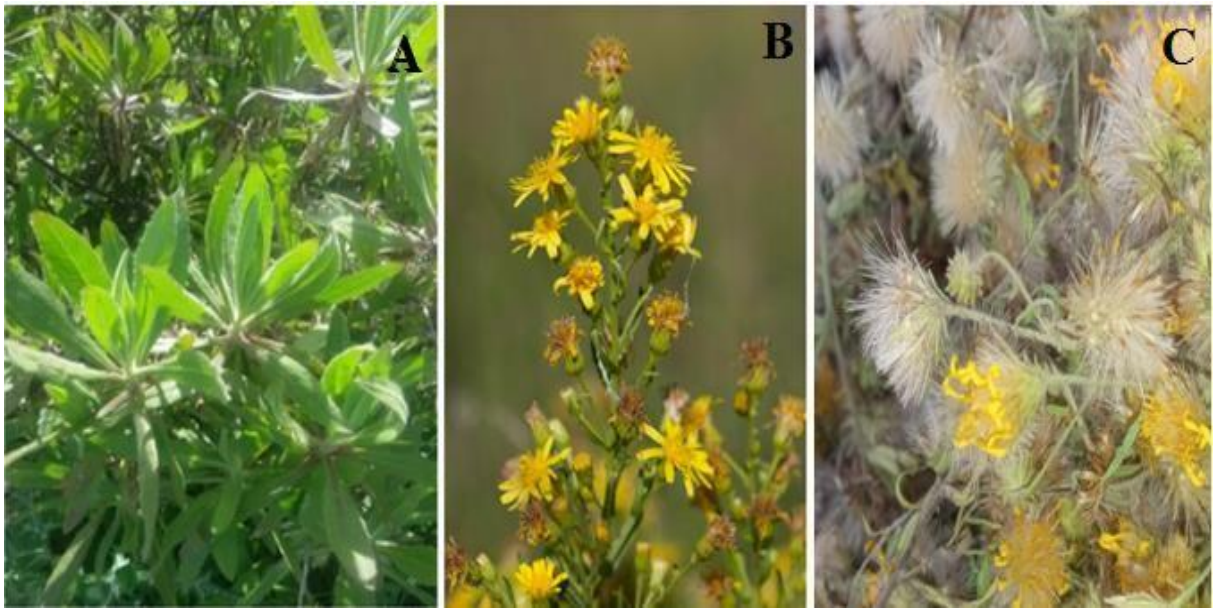

Figure 02: Aerial part of *Inula viscosa*; A: Leaves, B: Flowers, C: Fruits (Original).

The roots of *Inula viscosa* are characterized by their glandular and viscous nature, emitting a strong odor. At the base, they become woody and form a robust, lignified taproot (Figure 3) that can reach up to 30 cm in length (Touhari et al ., 2020)

Figure 03: The underground part of *Inula viscosa* (original).

3.5Habitats

I. viscosa is commonly found in various habitats such as dried riverbeds, neglected fields, road verges, hiking paths, and even urban areas. It demonstrates adaptability by thriving in both clayey and sandy soils (Bensegueni, 2001), can be observed in rocky coastal areas, natural marshes, and other wetland habitats. It thrives in well-lit environments and is known to inhabit areas with elevated levels of magnesium and nitrogen in the soil (Parolin et al., 2014).

3.6 Traditional use

The *Inula* genus, comprising more than 100 species, holds great importance, especially because various of its isolated pure secondary metabolites have demonstrated significant biological activities, making it a valuable resource in traditional medicine (Seca, 2015),antioxidant and anticancer (Chahmi,2014), This substance demonstrates effectiveness in the treatment of neoplasms, wounds, freckles, and dandruff, along with its potential for addressing conditions like bronchitis, diabetes, fever, hypertension, and diverse forms of inflammation. It is widely utilized to target a broad spectrum of ailments, primarily respiratory, digestive, inflammatory, dermatological, cancerous, and microbial disorders, showcasing its cytotoxic, anti-inflammatory, antimicrobial, antidiabetic, and insecticidal properties (Seca, 2014), Demonstrating efficacy against human breast cancer cells MCF-7 (Gharred, 2022). Typically

displays inhibitory properties on the germination of different plant species, serving to stimulate and protect plants (Prisa, 2022), antifungal, analgesic, antibiotic resistance (Jerada, 2024), hypoglycemic and anti-hyperglycemic (Djedioui et al., 2010), providing relief from rheumatic pain (Guedri, 2019).

Material and methods

1. Survey on natural remedies (Feb – June , 2024)

This part of our thesis investigates the personal and pharmacological data employed by 34 herbalists operating within various towns of Boumerdes Wilaya.

The focal points of our investigation include in the first part the personal data of herbalists, to assess the educational backgrounds of herbalists; to discern the modes through which herbalists acquired their expertise (formal training, self-instruction or knowledge by inheritance) and to document the duration of their practical experience in herbal medicine.

The second part of this survey concerns the natural remedies, starting by gauging the demand for medicinal plants among clientele, documenting the geographical origins of sourced herbs, identifying the scientific and family names of used herbs, and determining the preferred plant organs utilized in remedies. Followed by analyzing the varied applications of herbal preparations (therapeutic, culinary, or cosmetic), delineating the modes of administration (oral ingestion, topical application, or as incense), elucidating the diverse methods of preparation employed by herbalists, quantifying the frequency of herb intake per treatment regimen, establishing recommended doses for therapeutic efficacy, cataloging the range of ailments treated with herbal interventions. For ending we evaluated the compatibility of herbs with other ingredients in formulations, and categorized remedies as plant-based or incorporating non-plant elements.

2. Biological Material

2. 1 Plant material

This study focuses on an investigation of the belowground portion (roots) of *Inula viscosa*, our work was about plant crude extract and hydrolat.

2.2 Animal material

The *In vivo* study of antalgic test was conducted using Balbussi mice (*Mus musculus*) with weights varying from 25 to 35 g. These mice are provided by the USTHB animal store. Mice were acclimated to the conditions of the animal facility, or had free access to water and food.

They were kept at constant temperature (24±2°C) and subjected to a 12/12 hour light/dark cycle.

2.3 Tested microbial strains

The chosen bacterial and fungal strains are pathogenic agents renowned for their rapid development of antibiotic resistance. These specific strains are indicated in the accompanying table 03. The study was carried out at the Laboratory of Microbial Systems Biology, ENS-Kouba.

Table 03: Tested bacterial and fungal strains .

		Name of strains	Reference of strains
Bacterial	Gram postif	*Listeria monocytogenes*	ATCC 13932
		Bacillus subtilis	ATCC 6633
		Bacillus cereus	ATCC 14579
		Staphylococcus aureus	ATCC 6538
		Staphylococcus aureus	MRSA 639c
		Staphylococcus aureus	ATCC 43300
	gram- negative	*Salmonella enteMLrica serovar Typhi*	ATCC 14028
		Micrococcus luteus	ATCC 9314
		Klebsiella pneumonia	E 40
		Escherichia Coli	ATCC 8739
		Pseudomonas aeruginosa	ATCC 9027A
Yeast		*Candida albicans*	ATCC 10237
Filamentous fungi		*Fusarium*	
		Aspergillus niger	
		Aspergillus flavus	NRRL 3251
		Penicillium expansum	

3. Preparation of crude extract and hydrolat

3.1Extraction by maceration (solid/liquid)

In this study, we utilized a hydro-methanolic solvent-based maceration as the primary extraction technique (Figure 04). The effectiveness of this extraction method relies on the ratio of methanol to water (70:30 v/v), as water-soluble glycosylated phenolic compounds and methanol-soluble aglycones exhibit differential solubilities. The application of boiling water during maceration serves the purpose of safeguarding glycosidic flavonoids against enzymatic degradation by deactivating the glycosidase enzyme (Gangwal, 2013).

10 grams of powdered plant material.

Soaked in 30 ml of boiling distilled water + 70 ml of absolute methanol.

After 48 hours.

Filtration using Whatman filter paper and renow the solvent three times.

Recovery of the three filtrates.

Vacuum evaporation using a rotary evaporator at 90 rpm and 40°C.

Recovery of the dried extract to obtain 100 mg/ml concentration.

Figure 04: Solid-liquid extraction steps of crude hydro-methanolic extract of roots of *Inula viscosa* L.

The crude extract obtained was stored in a tightly closed dark vial at 4°C until use.

3.2Hydrodistillation

In our thesis, we focused on hydrosols due to the meager yield of essential oil from the roots of Inula viscosa. Using a Clevenger apparatus, we hydrodistilled 100 grams of Inula viscosa roots with 1 liter of distilled water for 3 hours, carefully controlling temperature and pressure. After ending the hydrodistillation process, a few essential oil droplets dissociate spontaneously from distilled water for their immiscibility. however, the hydrosol was a small fraction of polar, oxygenated, and hydrophilic compounds.

To obtain the hydrosol, we performed a liquid-liquid extraction using an organic solvent and then dried the organic phase with anhydrous sodium sulfate. We used a rotary evaporator at temperatures below 34°C to remove the solvent. The extracted hydrosol was stored under conditions similar to those used for EHM (Matulyte et al., 2020, Moukhles et al., 2022).

4. Total polyphenol dosage by spectrometry

4.1 Total polyphenol content

Principle

Polyphenol content was measured using a colorimetric method with the Folin Ciocalteureagent, as described by Singleton and Rossi (1965). This reagent is composed of phosphotungstic acid and phosphomolybdic acid, which react with phenols to produce blue tungsten and molybdenum oxides. The intensity of the blue color is directly related to the concentration of phenolic compounds, with maximum absorption occurring at 760 nm.

Experimental protocol

In a test tube, we mix 4ml of each extract (diluted to 10 mg/mL), 3.75 mL of distilled water, and 250 µL of FolinCiocalteu reagent. After 3 minutes, 750 µL of 20% sodium carbonate solution is added. The mixture is then incubated in a water bath at 40°C for 40 minutes and the absorbance is measured against a blank made from distilled water. Phenolic compound concentrations are determined using the calibration equation derived from the gallic acid standard. Results are reported as mg of gallic acid equivalent per gram of dry extract. Each test is conducted in triplicate.

4.2 Total flavonoid content

Principle

The technique outlined by Lamaison and Carnet (1990), involving aluminum chloride (AlCl3), is employed to assess flavonoid content in our extracts. This method relies on the development of an aluminum- flavonoid complex, which shows peak absorbance at 440 nm.

Experimental protocol

In a test tube, 1.5 µL of $AlCl_3$ solution was combined with an equivalent volume of the methanol-diluted extract. The mixture was then agitated, and after a 10-minute incubation period, absorbance was measured at 440 nm.

4.3Tannin dosage

Condensed tannin levels were assessed using the vanillin method in acidic conditions (Price et al., 1978). Each extract (10 mg/mL) was combined with 50 µL of a 4% vanillin/methanol solution, followed by the addition of 50 µL of concentrated HCl after agitation. After a 20 minutes incubation, absorbance was measured at 550 nm against a blank. A range of concentrations (0 to 1000 µg/mL) prepared from a catechin stock solution will be used to construct the calibration curve. Condensed tannins are reported in mg of catechin equivalents per gram of dry extract.

5. Chemical analysis

5.1 High Performance Liquid Chromatography (C.L.H.P)

High Performance Liquid Chromatography, is an exceptionally useful and dependable method for effectively separating minute amounts of non-volatile or thermally labile substances, such as polyphenols, within highly intricate mixtures. This exceptional capability is directly attributed to the compact column size and the finely divided particles of the stationary phase (Hostettman et al., 1998). The chromatographic system operates by propelling the mobile phase through it using a high-pressure pump. The mixture under analysis is injected and carried through the system. Within the system, the compounds in the solution segregate based on their affinity for the mobile and stationary phases. As the compounds exit the column, a detector capable of discerning them generates peaks corresponding to each solute. The resulting peaks collectively form a chromatogram, which serves as a graphical representation of the separated components (Louis Cuq, 2001).

5.1.2 Experimental conditions:

The samples were analyzed using an Agilent 1260 Infinity CLHP system equipped with a Diode Array Detector (DAD). The analyses were conducted under the following experimental conditions:

The column: Reverse phase with a C18 column (5 µm, 250 x 4.6 mm).
The temperature: 22±01°C.

The flow rate: 1 ml/min.

The injection volume: 5µl

The elution conditions (Table 04): Two phases, A and B, were used.

Mobile Phase: Solution A: Bi-distilled water with 1% Acetic Acid

Solution B: CLHP-grade methanol.

Table 04 : CLHP Elution Gradient.

Time (min)	P .A	P.B
0	95 %	5%
55	5%	95%
60	95%	5%

5.2 Gas chromatography analysis

5.2.1 Gas chromatography (GC-MS) analysis

GC-MS analysis was conducted using a Trace Ultra GC connected to a DSQII mass spectrometer. The analysis utilized a DB-5 capillary column with dimensions of 30 m × 0.32 mm and a film thickness of 0.25 µm. The temperature program started at 60°C for 3 minutes and then increased at a rate of 3°C per minute until reaching 240°C, where it was held for 3 minutes. Helium (He) was employed as the carrier gas with a flow rate of 1 mL per minute, and the injector heater was set to 250°C. The mass spectrometer operated under electron impact (EI) conditions, utilizing an electron energy of 70 eV and a source temperature of 250°C. The acquisition mass range spanned from 40 to 450 m/z20 (Senani et al ,2024)

5.2.2 Gas chromatography-flame ionization detector (GC-FID) analysis

GC-FID analysis was conducted using a Shimadzu GC17A chromatograph equipped with a fused silica capillary column containing a DB-5 stationary phase. The column had fixed parameters of 30 m length, 0.32 mm diameter, and a 0.25 μm film thickness. The temperature program involved a gradual increase from 60°C to 240°C over a period of 3 minutes at a rate of 3°C per minute. Both the injector and detector were set at 250°C. Nitrogen (N2) was employed as the carrier gas with a flow rate of 1 mL/minute in split mode (1:50), and an injection volume of 0.2 μl was used. Quantitative data were obtained by electronically integrating the area percentages without utilizing any correction factors 20.

In order to calculate the retention indices (RIs), a set of n-alkane mixtures (C5-C28) were examined using a DB-5 column under identical operating conditions. The RIs of the samples were determined using the formula developed by Van den Dool and Kratz21, which is as follows:

$$RI(x) = 100\,nz + 100\left[\frac{(RT(x) - RT(nz))}{(RT(nz+1) - RT(nz))}\right]$$

where: $RT(nz) \leq RT(x) \leq RT(nz+1)$ (Senani et al ,2024)

6. Antioxidant activity

6.1 DPPH

6.1.1 Principle

6.1.2 Preparation of DPPH

Initially, a methanolic solution of DPPH at a concentration of 0.004% was prepared. This involved dissolving 4 mg of DPPH powder in 100 mL of MeOH. The solution was then stored in a refrigerated amber bottle covered with aluminum foil to maintain its integrity. The absorbance of this solution, designated as initial Do, should fall within the range of 0.53 to 0.58. The absorbance measurement was performed at 517 nm.

6.1.3 Preparation of Dilution Range:

Starting from the previously prepared extracts at a concentration of 100 mg/mL, we create

extracts at 1 mg/mL, this time generating a dilution range for each extract studied (5µL,10 µL, 20 µL to 1000 µL). These dilutions are made in test tubes, followed by the addition of 1 mL of the DPPH solution to each tube. Additionally, a blank is prepared with 1 mL of DPPH solution and 1 mL of methanol. The tubes are then placed in darkness for 30 minutes, after which the optical densities are measured at a wavelength of 517 nm. The antioxidant capacity of the samples, or percentage inhibition, is calculated using the formula: I(%)=[(Abs control−Abs sample) /Abs control)]×100.

β6.2-Carotene Bleaching Test

6.2.1 Principle of the test

This analytical method utilizes spectrophotometry to assess the fading of β-carotene color resulting from its oxidation by the breakdown products of linoleic acid. The oxidation process generates peroxide radicals, which subsequently react with β-carotene, leading to the loss of its red color. This color change is monitored using spectrometry at 470 nm. However, the presence of an antioxidant can effectively neutralize the free radicals derived from linoleic acid, thereby preventing the oxidation and subsequent fading of β-carotene (Tepe et al., 2006).

6.2.2 Experimental protocol

3 mg of β-carotene dissolved in 10 ml of chloroform, 1ml of this solution was pipetted into a round-bottom flask containing 20 mg of linoleic acid and 200 mg of Tween 20 (after total evaporation of chloroform by vacuum evaporator). Then, 50 mL of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. Blank solution was prepared in a similar way except that addition of -carotene was omitted.

4.8 mL of the obtained emulsion were transferred into different test tubes containing 0.2 mL of extract (2 mg/mL). BHT was used as standards.

In order to assess the antioxidant activity of our extracts, we performed a time-dependent degradation kinetics study using the following time points: T0 = 0 min, T1 = 30 min, T2 = 60 min, T3 = 90 min, and T4 = 120 min, following the methodology outlined by Kelen and Bektas (2008).

6.3 Total anti-oxidant capacity

6.3.1 Principle

The evaluation of the total antioxidant capacity (TAC) was conducted in accordance with the procedure outlined by Prieto et al. (1999). This method, extensively documented in the literature, serves as a reliable means of quantifying the antioxidative potential of plant extracts. The fundamental principle underlying this assay involves the reduction of molybdenum (VI) to molybdenum (V) by the antioxidant compounds present in the extracts. This chemical reaction serves as a proxy for the overall antioxidant activity of the samples, providing valuable insights into their capacity to neutralize harmful free radicals and oxidative stress. By employing this established methodology, we aimed to gain a comprehensive understanding of the antioxidative properties exhibited by the plant extracts under investigation.

6.3.2 Experimental protocol

In the course of this study, precise quantities of sodium phosphate and ammonium molybdate were meticulously weighed and subsequently dissolved in distilled water to achieve desired

concentrations. Following the preparation of these solutions, the required amount of sulfuric acid was added to each solution. The contents of both beakers were combined into a single bottle, ensuring thorough mixing to achieve homogeneity. This prepared solution was then securely stored in the designated container, safeguarding its integrity for subsequent utilization in experimental procedures.

A portion of plant extracts (0.2 mL) at a concentration of 1 mg/mL was added to a reagent solution consisting of 2 mL containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes were then incubated at 95°C for 90 minutes. Following incubation, the samples were allowed to cool to room temperature, and the resulting green coloration was measured at 695 nm against a blank. The antioxidant capacity was quantifiedas milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

7. Antimicrobial activity

Two tests were conducted to evaluate the antimicrobial effectiveness of the studied extracts

from *Inula viscosa*: one focused on antibacterial activity and the other on antifungal activity, utilizing distinct techniques (disk diffusion and minimum inhibitory concentration method).

Muller-Hinton is the designated culture medium for bacteria and yeast strains, PDA as the culture medium for fungi. The agar needs to be heated in a water bath until fully dissolved, followed by pouring the medium into Petri dishes at a volume of 20 mL per dish and allowing it to cool.

To prepare microbial suspensions, 3 to 5 isolated and identical colonies are collected and transferred into 10 mL of sterile physiological water containing 9 g/L NaCl. The mixture is then vortexed to achieve an inoculum with a cell concentration ranging from 10^7 to 10^8 cells / mL. Next, the turbidity of the suspension is measured using a spectrophotometer, and the standard should exhibit an absorbance between 0.08 and 0.1 at 625 nm. However, the turbidity of the fungal suspension is measured by transmittance at 530 nm using a spectrophotometer, aiming for a range between 68% and 82%. It's important to utilize the prepared inoculum within 15 minutes.

7.1 Disc diffusion method

Antimicrobial tests of extract and hydrolat were first screened for their inhibitory zone by agar disc diffusion method. Once Petri dishes solidified, 100 µL of microbial suspension is added to each dish. Using a twisted pasteur pipette in a raking motion, the bacterial and fungalstrains are evenly spread in three opposite directions. Next, pre-cut disks made from sterile Whatman filter paper No. 3, soaked with 10 µL of each extract and hydrolat, and are placed on the agar surface. The dishes are then left to rest at refrigerator for 2 hours, followed by incubation at 37°C/ 24 and 25°C/ 72 hours for bacteria and fungi, respectively. Following specific contact duration between the antibacterial compounds and the strain, the antibacterial product's impact becomes evident as an inhibition zone, allowing classification of the strain assensitive, highly sensitive, extremely sensitive, or resistant.

We evaluate the antimicrobial activity of our extract and hydrolate by measuring the diameter of the inhibition zone (in mm) around the disks containing the tested extract and we calculate the average values from three measurements for each extract against pathogenic microorganisms.

7.2 Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an extract capable of inhibiting the growth of a particular strain of microorganisms. We conduct the test on extracts showing antimicrobial activity, using the solid medium macrodilution protocol outlined by Ebrahimabadi et al. (2010).

We conducted a series of dilutions at a 1/2 ratio using 10 mL of a stock solution with a concentration of 25 mg/mL. To prepare the stock solution, we mixed 2.5 mL of the plant extract and hydrosol with 7.5 mL of Mueller-Hinton for bacteria and PDA culture medium forfungi. The dilution process involved pouring 5 mL of the stock solution into the first Petri dish and homogenizing it through rotational movement. The remaining 5 mL were then diluted with an additional 5 mL of culture medium. Subsequently, 5 mL of the resulting dilution were poured into a second Petri dish, and we repeated this procedure to obtain a rangeof dilutions of the extract in the culture medium.

Using a micropipette, we proceed to place 1µL of the inoculum from various strains onto the surface of the solidified agar medium.

The results are read by visually observing the presence or absence of microbial growth, comparing it to the growth on a control plate without extract. After 24 hours of incubation at 37°C and 72 hours of incubation at 25°C for bacteria and fungi, respectively.

8. Cytotoxicity activity by Brine shrimp test

The Brine shrimp called also Artemia cytotoxicity test has been widely utilized in numerous studies to assess the pesticidal, anticancer, larvicidal, antiparasitic, and cytotoxic activities of plant extracts (Quignard et al., 2003).

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8.1 Experimental protocol

A rectangular plastic jar with a capacity of 1 L was used to culture 1 g of *Artemia salina*

(*Linnaeus*) cysts obtained from CNRDPA, Algeria. The jar was filled with seawater, which was prepared by dissolving 36 g of sea salt in 1 L of distilled water. Positioned near a 60 W lamp, the system provided direct light and maintained a constant temperature of 27 ± 01°C. After an incubation period of 10-12 hours, newly hatched nauplii, distinguished by their pink color and active swimming, were collected from the outlet at the bottom of the jar. These nauplii were then allowed to mature for a duration of two days.

The experimental procedure involved preparing an assay system using 2.5 mL of seawater containing different concentrations of hydro-methanolic crude extract and hydrosol obtained from the roots of *Inula viscosa* (1000, 100, 10, 1 µg/mL). Each concentration was tested with

10 nauplii, and the system was placed under constant illumination for a duration of 24 hours. The number of deceased nauplii was recorded to determine the percentage of mortality. Based on the obtained mortality percentages, the median lethal concentration (LC50) of the plant extract, which indicates the concentration at which 50% of the brine shrimp nauplii experienced mortality, was calculated. To ensure reliability, three replicates were prepared for each concentration, and a negative control was included using the same saline solution without the plant extract.

9. Analgesic activity

This procedure involves causing a pain-inducing effect by administering 1% acetic acid to Balbussi mice (Mus musculus) intraperitoneally. The injection triggers a feeling of pain in guinea pigs, which is expressed through stretching of the hind legs and twisting of the dorso-abdominal muscles, commonly known as abdominal cramps (Kang et al., 2008).

The analgesic effect is evaluated by tallying the occurrence of cramps within a 30 minute period following the administration of the pain-inducing substance (Ouedraogo et al., 2012; Ochieng et al., 2013).

9.1 experimental protocol

Thirty minutes prior to acetic acid injection, batches of mice were intraperitoneally administered 0,5 ml with the following treatments:

-Batch1 negative control: The mice in this batch receive physiological saline solution.

-Batch 2 positive control: The mice in this batch receive paracetamol (reference product) at 100mg/kg.

-Two batch 3 and 4 extract: The mice receive EHM from *I. viscosa* root at doses of 400 and 800mg/kg.

-Batch 5 hydrolat: The mice receive hydrolat of *I. viscosa* root.

Percentage of protection PP% = (mean cramps of batch C- mean cramps of batch E/ mean cramps of batch C) x 100;

With C: control, E: experimental.

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10. Statistical analysis

The results are presented as the mean value plus or minus the standard deviation. XLSTAT software was used for the statistical analyses, which included a one-way analysis of variance (ANOVA), followed by the Tukey test for comparing multiple groups and determining the significance levels. Mean values are considered to be significantly different when the p-value is less than 0.05.

The correlations between the total content of polyphenols and flavonoids and various tests (antioxidants and antimicrobials) were determined using ANOVA and quantified in terms of determination coefficients (R^2).

Results and discussion

1. Analyzing surveys conducted with herbalists

Through a combination of interviews, surveys, and observational studies, we aim to elucidate the intricate dynamics of natural remedies utilization in Boumerdes wilaya.

By exploring the interactions between herbalists and their clientele, as well as the various factors influencing the selection and administration of medicinal plants, our study aims to provide a comprehensive understanding of traditional healing practices on a public scale. For this, a non-exhaustive list has been established of the 50 natural remedies, particularly those most requested and least known by customers (see appendix 1). Furthermore, by contextualizing our findings within the broader framework of herbal medicine research, we seek to contribute valuable insights to the advancement of this field, potentially informing future initiatives aimed at integrating traditional and modern medical approaches.

Discussion :

Additionally, our study involved visits to different regions including Koursou, Boudouaou, Yesser, Thenia, among others. It's worth noting that we encountered some challenges during interviews, as certain herbalists refused to answer specific questions, citing concerns about the personal nature of inquiries, especially regarding educational backgrounds. In some instances, herbalists declined to provide any information, stating a lack of knowledge. Moreover, some regions have only one herbalist, which posed logistical challenges in data collection

1.1 Analysis of personal data

1.1.1 Herbalist's educational background

From our questioning of 34 herbalists regarding their educational background, we deduce that the majority of them have a college level of education with 63.33% (Figure 05), followed by those who have a university diploma (26.66%) and a level secondary education (10%). On the other hand, no herbalists have a primary education level or are illiterate (0%).

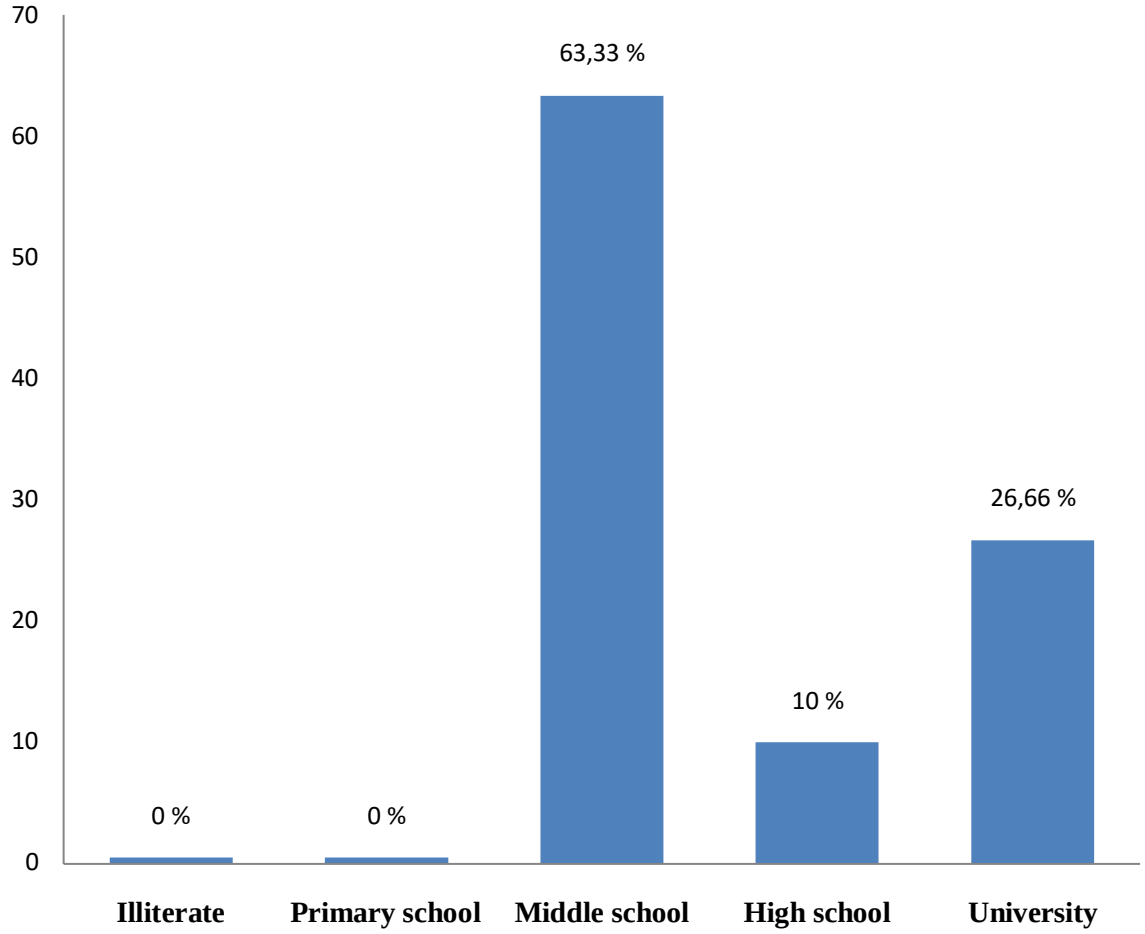

Figure 05: Herbalist's educational background.

1.1.2 Source of Knowledge

From the sample of 34 herbalists interviewed in Boumerdes who responded to our survey, about 81% of herbalists are self-taught (Figure 06), indicating that they are enthusiasts with a strong desire for independent learning and skill acquisition within the profession. This shows that many herbalists have honed their expertise through personal exploration and experimentation. On the other hand, 13% of herbalists are solely dealers who buy and sell natural remedies, this indicates the inability to help clients with the choice and correct use of remedies.

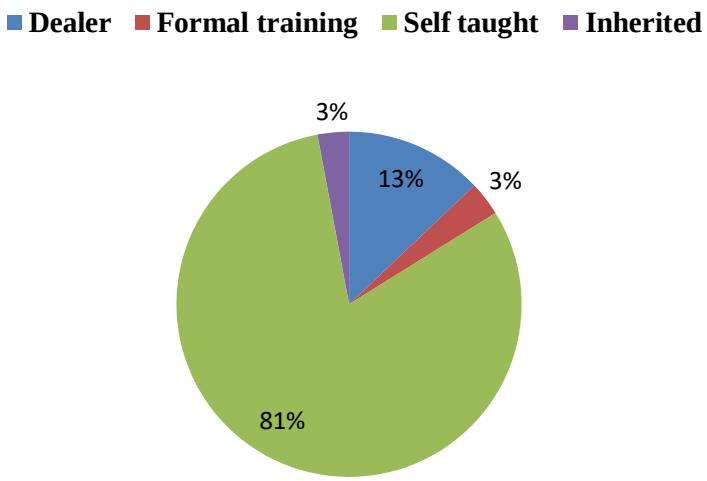

Figure 06: Source of Knowledge

In contrast, barely 3% of herbalists have inherited knowledge from their ancestors, indicating that the transmission of botanical information and traditional healing practices from generation to generation is gradually decreasing. This indicates a shift in interest among new generations, leading to a great loss of the heritage of family and traditional healing practices.

Ultimately, approximately 3% of herbalists have received formal training, which implies a limited number of structured training opportunities in herbalism in the wilaya of Boumerdès. Notably, one herbalist we interviewed mentioned receiving training in the Algerian desert, highlighting unconventional but valuable sources of training in the field. With the aim of improving this sector, it would be desirable to propose to the organizations concerned the

opening and implementation of specialties aimed at training specialist advisors in herbalism and plant-based health products. This type of approved training provides the skills necessary to advise and ensure consumer safety, particularly through technical and regulatory knowledge of products.

1.1.3 Duration of experience

The histogram presented in Figure 07 provides a clear visualization of the distribution of exercise durations between herbalists in Boumerdes.

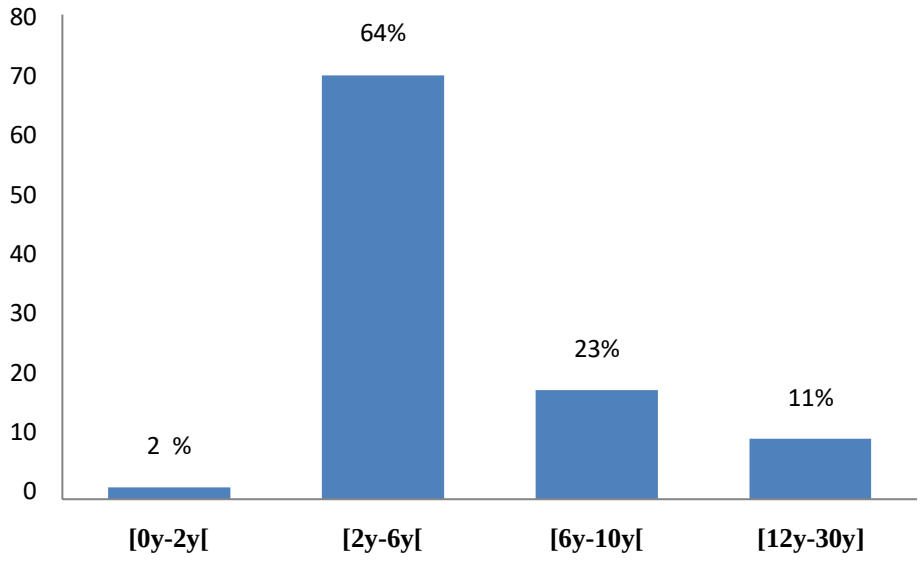

Figure 07: Duration of experience by years

The responses obtained reveal a strong preponderance with 64% in the range of 2 to 6 years, followed by the brackets of 6 to 10 years and 12 to 30 years with 23% and 11%, respectively. The lowest percentage goes to herbalists who have been practicing for less than two years.

However, several herbalists interviewed refuse to answer this question. Which could be explained by the following reasons; concerns about privacy or confidentiality, reluctance to disclose personal information, time constraints, or simply a lack of interest in participating in the survey.

1.2 Analysis of pharmacological data.

1.2.1Type of remedy

The graph shown in Figure 08 simply illustrates the predominance of herbal remedies with 91%, compared with non-plant remedies such as beehive products, salts, or shellfishs (propolis, alum, cauris, etc.), which represents one-tenth of the natural remedies at the Boumerdes stations.

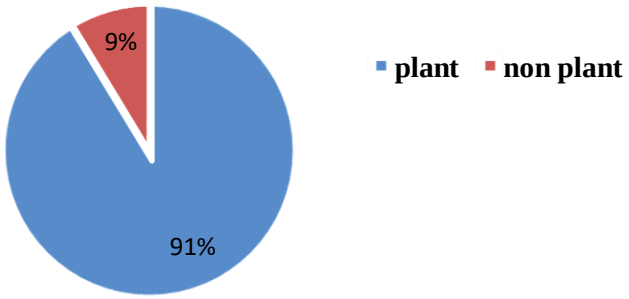

Figure 08: Type of remedy

1.2.2 Origin Countries of Natural Remedies

The survey results demonstrate that various countries are actively engaged in initiatives to import natural remedies, with the following distribution: China: 38.09%, Algeria: 23.8%, India: 16.52%, Egypt: 12.04%, Africa: 4.12%, Pakistan: 2.82%. , Asia: 2.28%, America: 1.44% and Morocco: 1.14% (Figure 09). The highest percentage of imported remedies comes from China, including ginseng, cinnamon, chamomile, green tea and cloves. Followed by Algeria where the different Wilayas of the country bring their goods to certain centers or wholesale markets in particular, Semmar and Maghnia.

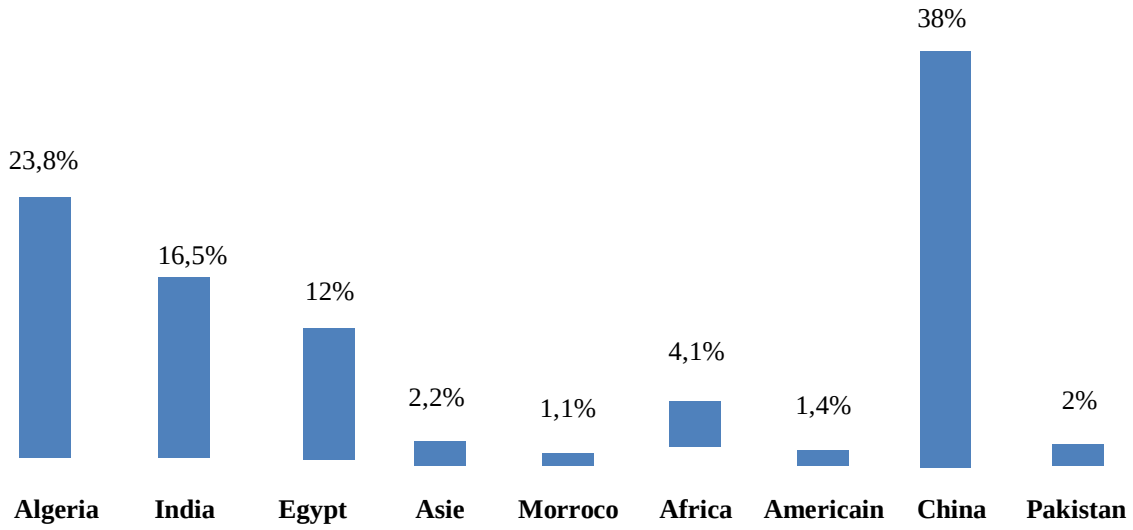

Figure09: Origin Countries of Natural Remedies

However, this raises intriguing questions about the potential for cultivating medicinal and aromatic plants in Algeria. Could we grow more of these valuable plants domestically instead of relying heavily on imports? Exploring the possibilities of growing medicinal herbs like pumpkin and many other species locally, could reduce dependence on foreign sources and strengthen the national economy. What strategies and resources would be needed to improve the local cultivation of these natural remedies? These questions deserve further discussion andinvestigation, given Algeria's economic and agricultural expertise.

1.2.3 Natural remedies usage

According to this circle (Figure 10), we see that 67% of the total sample of remedies are used for therapeutic purposes, and 22% as well as 11% use it in the context of food (culinary) and cosmetics, respectively.

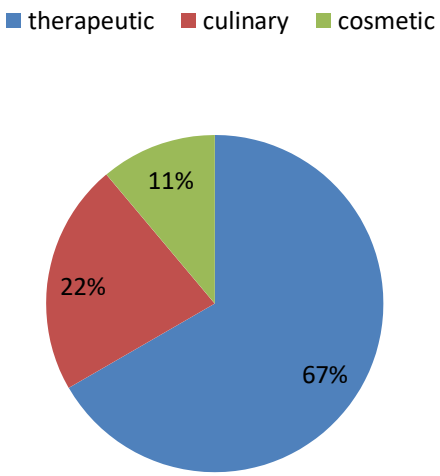

Figure 10: Usage of natural remedies

1.3.3 Administration mode

The use of natural oral remedies is the majority (90%) compared to other ways of use (Figure 11). As for remedies for external use or by massage and those that are fumigated by aspiration, their use is infrequent, with respective percentages of 8% and 2%.

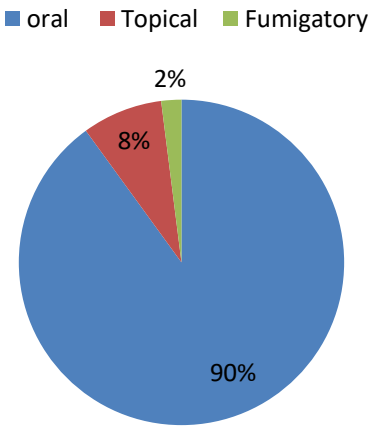

Figure 11: Administration mode

1.3.4 More common botanical family

The most frequently encountered botanical family among the herbs found in Boumerdès herbalists is the Lamiaceae family. This family includes a wide variety of aromatic herbs, including mint, sage, rosemary and thyme, which are commonly used for culinary, medicinal and aromatic purposes. Their prevalence among herbalists suggests their traditional and culinary importance.

1.3.5 Customer demand

Based on the results provided, we can deduce the following regarding the demand for natural remedies by customers and the market in many towns of Boumerdes.

High demand represented by 64% (Figure 12) of remedies and plants which are widely recognized and used for various purposes. Examples include fennel seed, clove, rosemary, mint, garlic oil, alum, flaxseed, thyme and ginger. It should be noted that in this area, some remedies become very popular for a while due to media advertisements which are intended to capture the attention of the consumer and user in order to encourage them to purchase. In particular, Ginkgo biloba and Moringa.

A common use of 25% natural remedies although not as universally popular, these plants are regularly used for specific applications. Here we cite some examples such as chia seeds, lavender oil, barberry and St. John's wort.

A rare use of 11% of remedies which have limited popularity and are not commonly sought after such as the case of shilajit and ginseng.

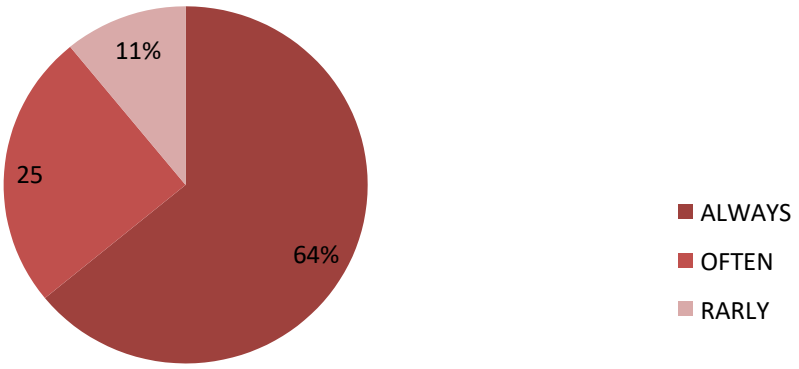

Figure 12: Customer demand

1.3.6 Used organ

The proportions of the use of the different plant organs of medicinal plants are as follows (Figure 13): the leaves are the most frequently used organ with 28% (rosemary, mint, mugwort, etc.), followed by the seeds represented by 26% (seeds of fennel, chia seeds, pumpkin seeds). Branches, roots as well as flowers (valerian, chamomile) are also used, with percentages very close to 10% and 8% respectively. Several other organs are also mentioned with low percentage of use including stems, resin, tree bark and different parts of fruits. It should be noted that the choice of plant organ for medicinal use may depend on the concentration of targeted active ingredients and traditional practices.

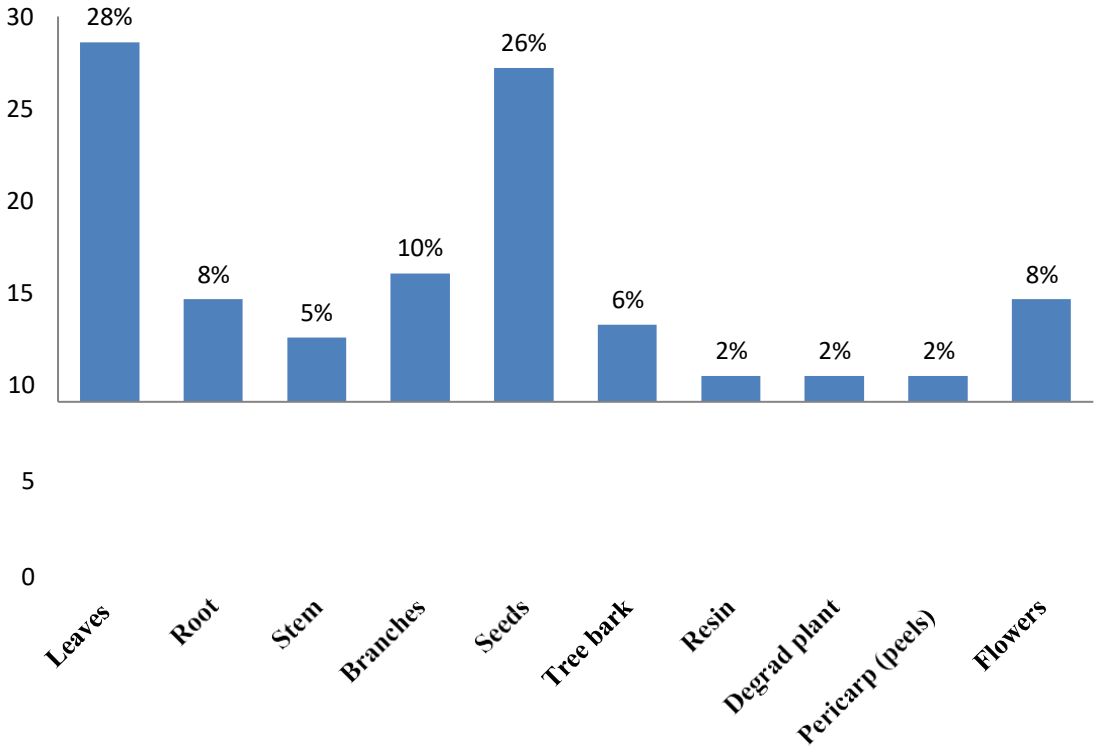

Figure 13: Used organ

1.3.7 Method of preparation

The most commonly used method of plant preparation is infusion representing 66% of cases

(flax seeds, valeian, ginko, tiger nuts, etc.)(Figure 14). Infusion involves steeping the plant material in hot water to extract the desired components. Maceration, which represents 14% of cases (castor oil, sesame oil, argan oil, etc.), consists of using a solvent to dissolve the active constituents of the plant material and is often used to create tinctures or concentrated extracts. Dusting of plant material represents 10% of cases. This method involves grinding or pulverizing the plant parts into a fine powder, which can be used in a variety of applications. Decoction accounted for 4% of cases (Fenugreek) and involves boiling the plant material in water for a longer period, this method is commonly used for harder, less soluble plant parts, such as roots or bark. Other methods, including external application, dilution and heating

each accounted for 2% of cases (shilajit). These methods have specific applications depending on the desired use and properties of the plant material.

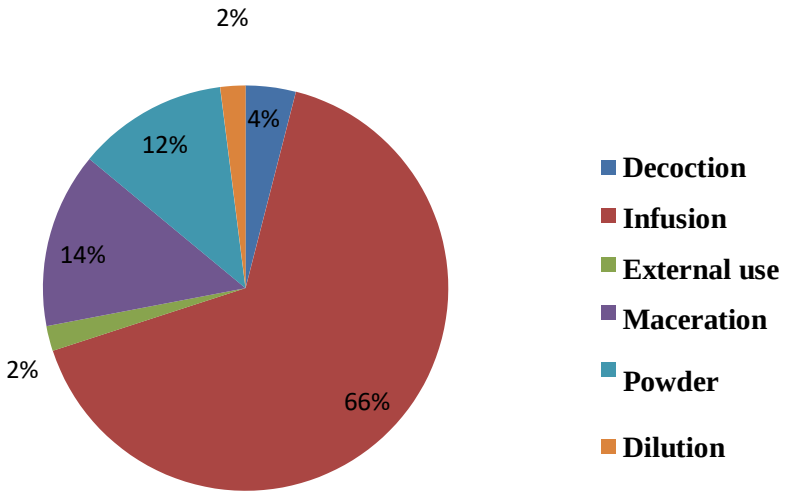

Figure 14: Method of preparation

1.3.8 Number of intakes

Based on the information provided by this relative circle (Figure 15), it can be inferred that the majority of herbal medicines are usually taken once a day (56%), followed by a frequency ranging from once to twice a day and occasional use as needed with 19% and 16%, respectively. Finally a less common regimen of one to three times per day (9%).

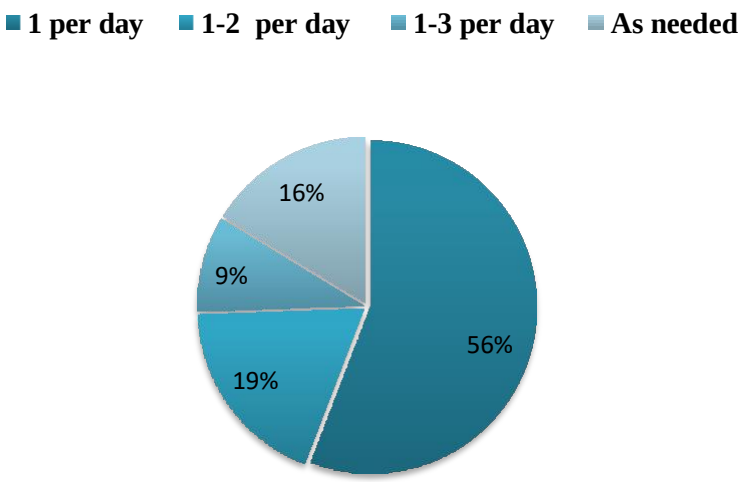

Figure 15: The number of intakes

Some herbal remedies are usually taken once daily, including psyllium seeds, garlic oil, flaxseed, valerian, rosemary, and ginger. Overuse of these remedies can lead to unwanted side effects due to their powerful active compounds. For example, excessive consumption of psyllium seeds can cause gastrointestinal problems such as bloating and diarrhea. At the same

time, high doses of garlic oil can lead to digestive issues and an increased risk of bleeding. If consumed in large quantities, flaxseeds can cause digestive issues and hormonal imbalances.

Overuse of valerian can lead to dizziness, headaches, and dependence, and too much rosemary can cause gastrointestinal irritation and pose risks to pregnant women. Ginger, takenin large quantities, can cause heartburn and nausea. On the other hand, some remedies are taken as needed to treat specific discomforts rather than for ongoing treatment. Mint, ginger, fennel seed, barberry, and mugwort are used to relieve acute symptoms like indigestion, nausea, bloating, and breathing problems. These occasional uses help prevent potential side effects and ensure that remedies are only used when necessary, thereby avoiding excessive use and associated risks.

1.3.9 Mixture of natural remedies

Based on the survey findings, the classification of natural remedies reveals two distinct categories.

1- The first category comprises the majority of remedies, accounting for 84% of the total, and these remedies can be supplemented with additional ingredients (Table 5, Figure 16).
2- The second category represents a smaller proportion, estimated at 16%, and consists of natural remedies that can't be combined with other substances, we quote here: sagebrush, psyllium seeds common centaury, wormwood and ephedra.

Table 05 : Natural remedies can be mixed with other ingredients

Natural remedies	Ingredients
Ginseng	Honey.
Marjoram	Other herbs like basil, thyme, or rosemary for seasoning blends, olive oil.
Argan oil	Other oils like sesame oil.
Sesame oil	Other cooking oils, marinades, dressings with herbs like basil or garlic.
Basil	Tomatoes, garlic, olive oil in various culinary dishes.
Pomegranate peels	Other dried herbs like mint or chamomile.

Fennel seeds	Cumin, coriander, honey for digestive remedies.
Garlic oil	Olive oil for cooking, other essential oils for topical applications.
Flax seed	Other seeds like chia seeds for a nutritious blend.
Rosemary	Other herbs like thyme and oregano, olive oil.
Ginger	Other herbs like turmeric or lemongrass, honey.
Mint	Other herbs like lemon balm or chamomile.
Thyme	Garlic, lemon, olive oil.
Chia seeds	Other seeds and grains like sunflower seeds, pumpkin seeds.
Lavender oil	Other essential oils.
St. John's Wort	Other calming herbs like chamomile or valerian.
Fenugreek	Honey

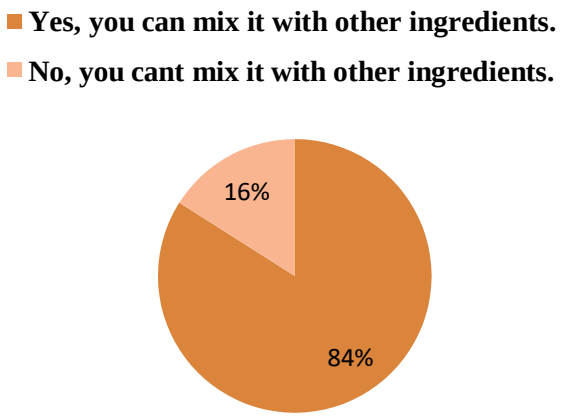

Figure 16: Mixture of natural remedies

Certain natural remedies, such as ephedra, wormwood, sagebrush, green tea, and common centaury, should not be mixed with other ingredients due to their strong effects and potential toxicity when combined with other substances. Ephedra contains ephedrine, a powerful stimulant that can significantly increase heart rate and blood pressure, posing severe risks when mixed with other stimulants like caffeine, potentially leading to hypertension, heart attack, stroke, and seizures. Wormwood and sagebrush both contain thujone, a neurotoxic compound and mixing them with other thujone containing substances can heighten the risk of

neurotoxicity, causing seizures and other serious neurological issues. Green Tea, commonly used for weight loss, is rich in caffeine and antioxidants. Adding other ingredients like honey, which can contribute to weight gain, is avoided to ensure the effectiveness of Green Tea for weight loss. Common Centaury, with its potent bitter compounds that stimulate digestion, can cause excessive gastrointestinal stimulation and alter medication absorption when mixed with other strong digestive stimulants or gastrointestinal medications.

The potent effects and possible toxicities of these remedies require careful usage and professional supervision to prevent harmful interactions and health issues.

1.3.10 The toxicity threshold of plants

Although each plant has inherent medicinal properties, it is crucial to recognize that misuse can result in varying degrees of toxicity. According to the lived experience of an herbalist who told us and which remains to be scientifically approved, that people suffering from eczema could experience an exacerbation, such as an increase in acne, after consuming ginseng.

2. Phytochemical study

2.1 Yield of extraction

Extraction by maceration of *Inula viscosa* root powder in the methanol-water solvent mixture (70% v/v) allowed us to obtain a hydromethanolic EHM extract with an average yield of 16.15%. The hydrodistillation process applied to the roots of *Inula viscosa* resulted in a fragrant hydrosol. The resulting hydrosol, enriched with aromatic compounds inherent to *Inula viscosa*, presents a promising resource for further scientific research. Initially, we wanted to work with essential oil from the roots of *Inula viscosa*, but as the yield of this was very low (0.01% w/w), we decided to work instead with hydrosol.

The yield and effectiveness of extraction methods can vary greatly depending on the technique, solvent, and the specific parts of the plant used. Comparing the results of this study with those of otherresearch highlights these differences. In a previous study, it was indicated that the extraction with pure methanol in herb and root of *Inula viscosa*, reported respectively its yields of 15% and 21% for samples collected from Turkey.

In contrast, the yield of our hydromethanolic extract could be attributed to the soil type and geographical

conditions. In parallel, the composition of the roots could differ significantly from that ofthe leaves because they contain different types and concentrations of bioactive compounds.

2.2 Quantification of polyphenols, flavonoids, and tannins content

Phenolic compounds contribute to the overall biological activities of plants, so that the hydromethanolic extract (EHM) and hydrosol of the roots of *Inula viscosa* were analysed for their total phenolic, flavonoids, and tannins content by the Folin Ciocalteu, $AlCl_3$ and vanillic test.

In our study, we used spectrophotometric method to determine the concentrations of researched compounds previously cited from the linear regression equations of three calibration curves made with gallic acid, quercetin, and catechin respectively (See appendix 2). The amounts were reported in milligrams equivalent to the standard used per gram of extract.

2.2.1 Polyphenol content

Hydromethanolic extract of the roots (EHM) revealed a total polyphenol content of 34.58 mg GAE/g extract, which means a highly significant presence of polyphenols. However, the hydrosol, which is a different type of extract, showed a lower polyphenol content with 0.16 mg GAE/g extract.

These findings highlight the variation in polyphenol content between different types of extracts of the same part of *Inulaviscosa*.

In the results of our study, the observed differences were found to be statistically significant, with a p-value of less than 0.05 ($p<0.05$*)

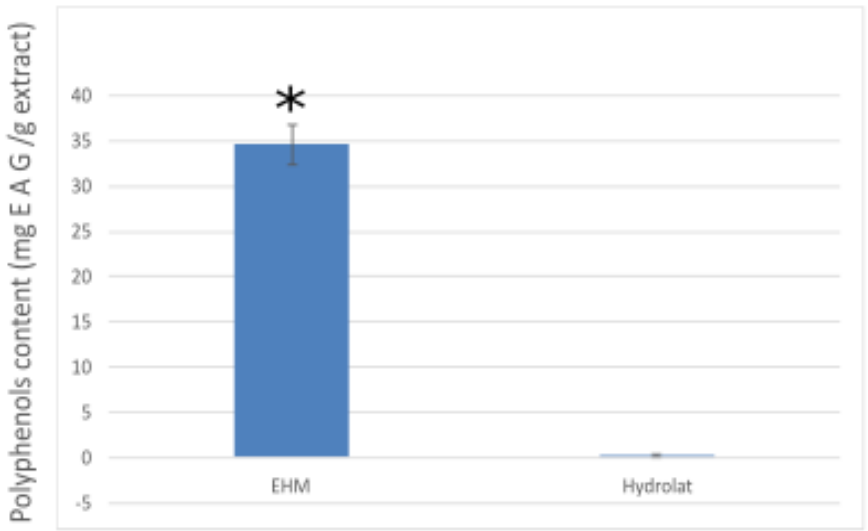

Figure 17: Phenolic contents

2.2 .2 Flavonoids content

The flavonoid content in EHM and hydrolat of I.viscosa roots was found to be similar. This indicates that both extracts of our study contain a poor amount of flavonoids (Figure 18).

In the results of our study, the observed differences were found to be statistically unsignificant, with a p-value superior to 0.05 ($p>0.05$)

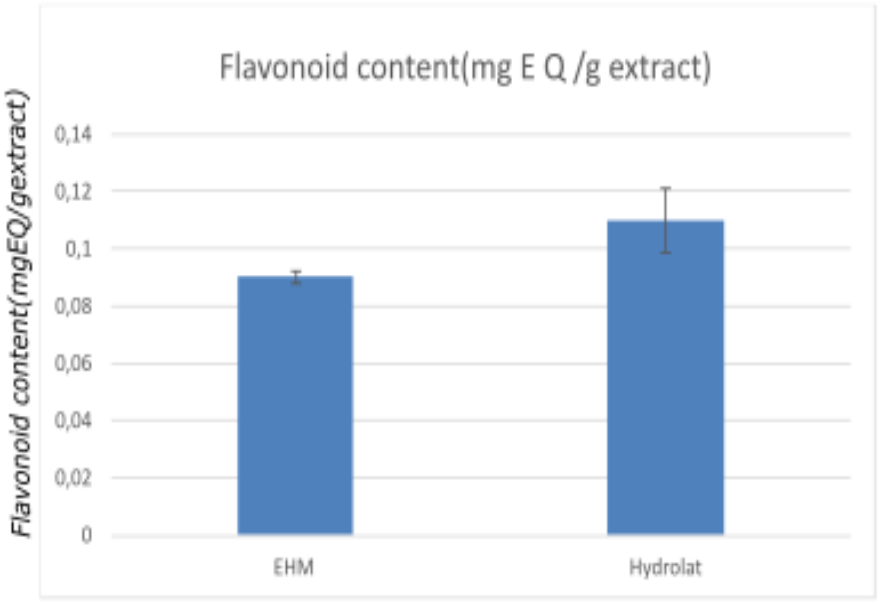

Figure18: Flavonoid content

2.2.3 Tanins content

The EHM sample has a tannin content of 0.5 mg CE/g extract, while the hydrolat sample has a significantly higher tannin content of 5.93 mg CE/g extract (Figure19). This indicates that the hydrolat sample contains a substantially greater amount of tannins compared to the EHM sample.

Our study's results revealed statistically significant differences, indicated by a p-value of less than 0.001 ($p<0.001$***)

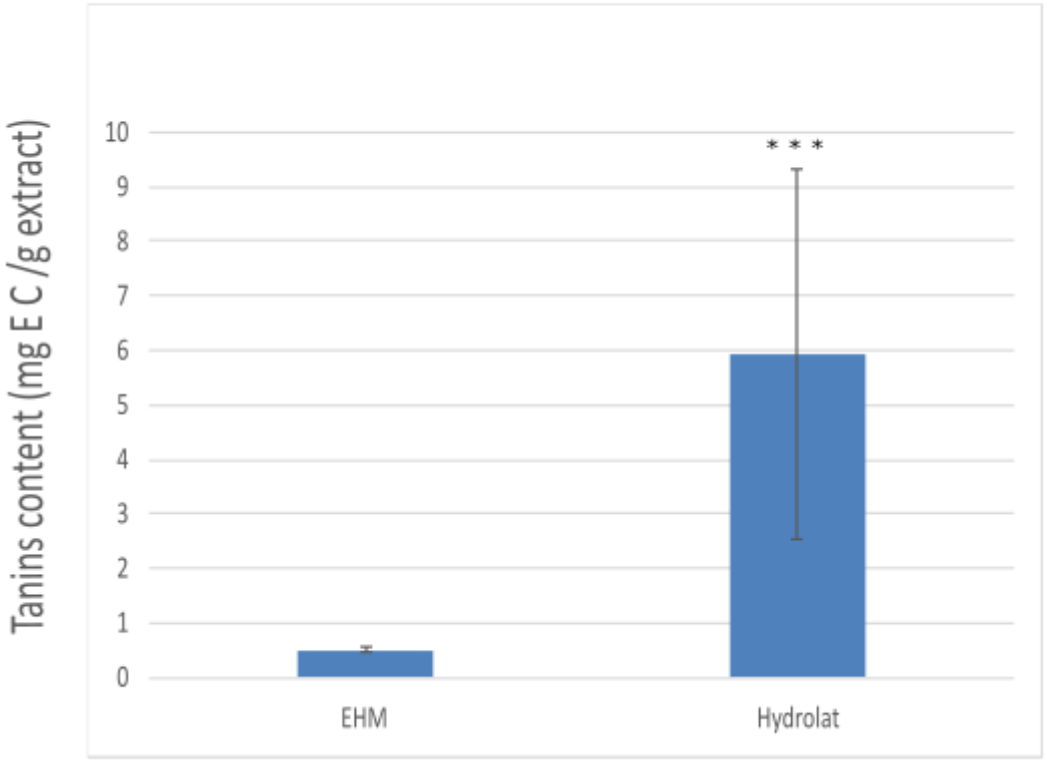

Figure19: Tanin content

Discussion

The results reveal that the EHM extract is significantly richer in polyphenols. However, the hydrolat extract exhibits high tannin content. Furthermore, the total flavonoids present in our studied extracts represent the lowest proportions, this makes it possible to note that these compounds do not represent the majority class of total polyphenols.

Our study confirms that methanolic extraction efficiently yields higher concentrations of polyphenols from *I. viscosa* roots compared to hydrodistilation method, consistent with previous research highlighting methanol's effectiveness in extracting phenolic compounds (Ben Mrid et al., 2022).

Gokbulut et al. (2013) showed that the level of total phenols of three *Inula* studied (*I. viscosa*, *I. montbretiana* and *I. helenium*) varies depending on the organs tested. Indeed, they revealed that methanolic root extracts of *I. viscosa* and *I. montbretiana* have the highest contents followed by the methanolic extracts of the aerial part, while the extract of the roots of *I. helenium* are the least rich in these compounds.

In contrast, previous studies report that in other Asteraceae species, they were in the range concentration where the phenolic content ranged from 15.98 to 227.93 mg GAE/g dw, whereas flavonoid content ranged from 1.13 to 182.56 mg CE/g dw (Mahmoudi et al ., 2015; Rhimi et al ., 2019; Özkan et al., 2019). These values are high compared to our results. These differences in phenolic and flavonoid concentration in *D. viscosa* extracts could be strongly

related to environmental conditions. They can also be influenced either by the extraction method or other physiological parameters, such as the growth stage at harvest time (Ben Mrid et al., 2022).

2 .3 The chemical composition of crude phenolic extract and hydrolate by HPLC

In order to separate the phenolic compounds from the hydromethanolic extract and the hydrosol, which have a wide range of polarity, it is often necessary to use an elution gradient to avoid co-elution and ensure the elution of all compounds. To do this, the elution profile was monitored at wavelengths of 254 nm, 280 nm and 320 nm, which are characteristic wavelengths of phenolic compounds. Flavonoids, in particular, have a distinct absorption spectrum with two characteristic bands in the UV- Vis region. Band I corresponds to the absorption of cycle B between 310 and 520 nm, while band II corresponds to the absorption of cycle A between 240 and 300 nm.

The profiles obtained by injection of the crude extract (EHM) and the hydrosol present several peaks. By comparing the retention times and UV spectra of these peaks with those of the standards injected under the same chromatographic conditions, we were able to identify 4 compounds in the crude extract (EHM) and 3 compounds in the hydrosol (Table06).

Table06: Chromatographic characteristics of the compounds present in the hydromethanolic extract (EHM) and hydrolat of the roots of *Inula viscosa (L.)*.

	Peak number	Retention time (mm)	Identified molecule	Class	Relative content %
EHM	18	32 .447	Naringenin	flavanone	**10 .55**
	20	49.315	Kaemferide	flavonol	1 .08
	21	51.688	**Tangeritine**	**flavone**	0.36
	22	56.626	Hesperin	flavanone	**29.05**
Hydrolat	7	32.413	Naringenin	flavanone	**23.12**
	8	49.325	Kaemferide	flavonol	5 .09
	9	56.690	Hesperin	flavanone	**63.79**

According to the results obtained by HPLC, the compounds identified in the extract are Naringenin, Kaemferide, Tangeritin and Hesperin. Mean while, the compounds identified in the hydrosol are Naringenin, Kaemferide and Hesperin.

Discussion

We observed the presence of Naringenin, Kaemferide, and Hesperin in both the hydro-methanolic extract and hydrolate of *I. viscosa*. Howover, Tangeritine was specifically identified in the crude extract, characterized as a polymethoxylated flavone primarily found inthe peels of mandarins and other citrus fruits. Its function involves reinforcing the plant's cell wall and serving as a defense mechanism against pathogens that contribute to various diseases. Additionally, Tangeritine demonstrates significant potential as an anticancer agent (Ihammichene and Zemouri, 2023)

In comparison with our study, the research of Ihammichene and Zemouri (2023) gave similar results concerning the presence of tangeritin and hesperin in the ethanolic extracts of the different organs of the plant studied. However, other compounds were found to be absent in our extracts, including apigenin and chrysin. On the other hand, our study revealed the presence of naringenin and kaempferide. While, Gokbulut et al. (2013) showed the presenceof chlorogenic acid and caffeic acid in the methanolic extract of the roots of *I. viscosa.*

Several phenolic compounds have been identified and cited in various papers of alcoholic extracts of the aerial part of *I. viscosa*. Specifically, the hydroxycinnamic acid derivatives comprised, p-coumaric acid, caffeoyl derivatives, mainly chlorogenic acid derivatives, as well as flavonoids of the class of flavonols and flavanones such as quercetin derivatives, including quercetin galactosylrhamnoside and rutin-O-pentoside, and others such as padmatin, hispidulin, cirsiliol, luteolin and isorhamnetin (Mahmoudi et al., 2015; Rhimi et al., 2019;Ben Mrid.R et al., 2022; Rechek et al., 2023). Indeed, it is possible that one of these flavonoids is present but it was not identified due to lack of standards.

2 .4 The chemical composition of hydrolate by GC- FID and GC-MS

The chromatographic analysis by GC-FID and GC-MS of the hydrosol of *Inula viscosa* roots obtained after hydrodistillation using a Clevenger apparatus revealed the presence of 31 compounds representing 46,67 % of the total chemical composition of the hydrosol. The components are presentedin the table according to their elution order (Tableau 07).

The hydrosol is predominantly composed of β-Acorenol, which accounts for 11.36%, followed by bis(2-methylpropyl) ester of 1,2-benzenedicarboxylic acid at 9.83%. Sesquiterpenes were found to be highly abundant, representing 53.19 % of the identified compounds in the hydrosol, while the monoterpene fraction accounted for less than 1.56 %.

In addition to that, it was observed that certain compounds were present in the hydrosol at moderate percentages: 6-methyl-6-(3-methylphenyl)heptan-2-one (4.67%), 2-undecanone (2.50%), methyleugenol (2.40%), β-himachalene (1.79%), β-acoradienol (1.34%), α-cadinol (0.98%), and guaiol acetate (0.59%).

Discussion

A recent and unique study conducted by Aissa et al. (2019) analyzed fresh essential oil and its

fractions derived from the root of *I. viscosa* using GC/FID and GC/MS analysis. The main constituents were found to be oxygenated monoterpenes (50.5%), oxygenated sesquiterpenes (37.5%), and sesquiterpene hydrocarbons (7.6%). Through the analysis, the prominent compounds determined to be (Z)-neryl isovalerate (17.5–29.8%), 1,10-di-epi-cubenol (19.1–27.2%), and 2,5-dimethoxy-p-cymene (5.9–17.7%). This analysis is in agreement with our results concerning the presence of (Z)-neryl isovalerate and 2,5-dimethoxy-p-cymene.

According to the bibliography, these compounds found in hydrosol, exhibit various beneficial activities. These include anti-inflammatory, antimicrobial, anesthetic, antifungal, calming, antihypertensive, venous and lymphatic congestion, antiallergic, antipruritic, and analgesic properties.

Table 07 : Chemical composition of the roots hydrolat of *Inula viscosa* L.

No	Apex RT	Compound name	Formula	RI cal	RI ref	% Area
1	36,342	ND	-	1814	-	26,00
2	33,843	ND	-	1718	-	15,39
3	31,702	**β-Acorenol**	$C_{15}H_{26}O$	1640	1637	**11,36**
4	37,582	**1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester**	$C_{16}H_{22}O_4$	1863	1863	**9,83**
5	31,484	6-methyl-6-(3-methylphenyl)Heptan-2-one	$C_{15}H_{22}O$	1632	1640	4,67
6	35,996	ND	-	1800	-	3,66
7	20,681	2-Undecanone	$C_{11}H_{22}O$	1289	1294	2,50
8	25,225	Methyl eugenol	$C_{11}H_{14}O_2$	1414	1403	2,40
9	27,369	β-Himachalene	$C_{15}H_{24}$	1495	1500	1,79
10	27,807	Butylated Hydroxytoluene	$C_{15}H_{24}O$	1508	1515	1,40
11	35,347	β-Acoradienol	$C_{15}H_{24}O$	1770	1763	1,34
12	30,927	Sesquithuriferol	$C_{15}H_{26}O$	1610	1605	1,12
13	31,879	α-Cadinol	$C_{15}H_{26}O$	1648	1652	0,98
14	32,213	Cedr-8(15)-en-10-ol	$C_{15}H_{24}O$	1658	1652	0,88
15	31,042	epi-Cedrol	$C_{15}H_{26}O$	1616	1618	0,87
16	39,855	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	1955	1961	0,80
17	25,074	Cymene <2,5-dimethoxy-ρ->	$C_{12}H_{18}O_2$	1419	1424	0,73
18	33,535	Guaiol acetate	$C_{17}H_{28}O_2$	1711	1724	0,59
19	34,08	Vetiselinenol	$C_{15}H_{24}O$	1727	1730	0,58
20	32,849	3H-Benz[e]indene, 2-methyl-	$C_{14}H_{12}$	1666	1669	0,51
21	34,319	Fumaric acid, 3-heptyl isobutyl ester	$C_{15}H_{26}O$	1736	1740	0,41
22	32,03	Allohimachalol	$C_{15}H_{26}O$	1654	1661	0,41
23	25,7	α-Himachalene	$C_{15}H_{24}$	1442	1449	0,40
24	31,381	trans-Isolongifolanone	$C_{15}H_{24}O$	1628	1625	0,38
25	30,508	5-epi-7-epi-α-Eudesmol	$C_{15}H_{26}O$	1598	1607	0,37
26	29,77	α-Cedrene epoxide	$C_{15}H_{24}O$	1572	1574	0,37
27	32,426	Cadalene	$C_{15}H_{18}$	1669	1675	0,36
28	32,791	(E)-10,11-Dihydroatlantone	$C_{15}H_{26}O$	1676	1669	0,35
29	29,983	Neryl isovalerate	$C_{15}H_{26}O_2$	1579	1582	0,34
30	34,791	Khusimol	$C_{15}H_{26}O$	1750	1742	0,28
31	28,181	γ-dehydro-ar-Himachalene	$C_{15}H_{22}$	1523	1530	0,21
31	26,638	9-epi-(E)-Caryophyllene	$C_{15}H_{24}$	1468	1464	0,16
32	30,717	cis-Isolongifolanone	$C_{15}H_{24}O$	1606	1612	0,15
33	26,329	allo-Aromadendrene	$C_{15}H_{24}$	1459	1460	0,14

3. Antioxidant activity

3.1 DPPH

In our investigation of how our extracts inhibit DPPH, we measured their IC_{50} values. This value represents the concentration where 50% of DPPH is inhibited. A lower IC_{50} indicates a stronger antioxidant effect against the free radical DPPH. We compared the results from the plant extracts (EHM, hydrosol) with each other and with those of the standards we used: BHT, vitamin E, and vitamin C.

Our findings demonstrate that the hydrolate is statistically significant

Values of IC_{50} (µg/mL) corresponding to the antioxidant activity of root extract, Hydrosol of *I. viscosa* and standards

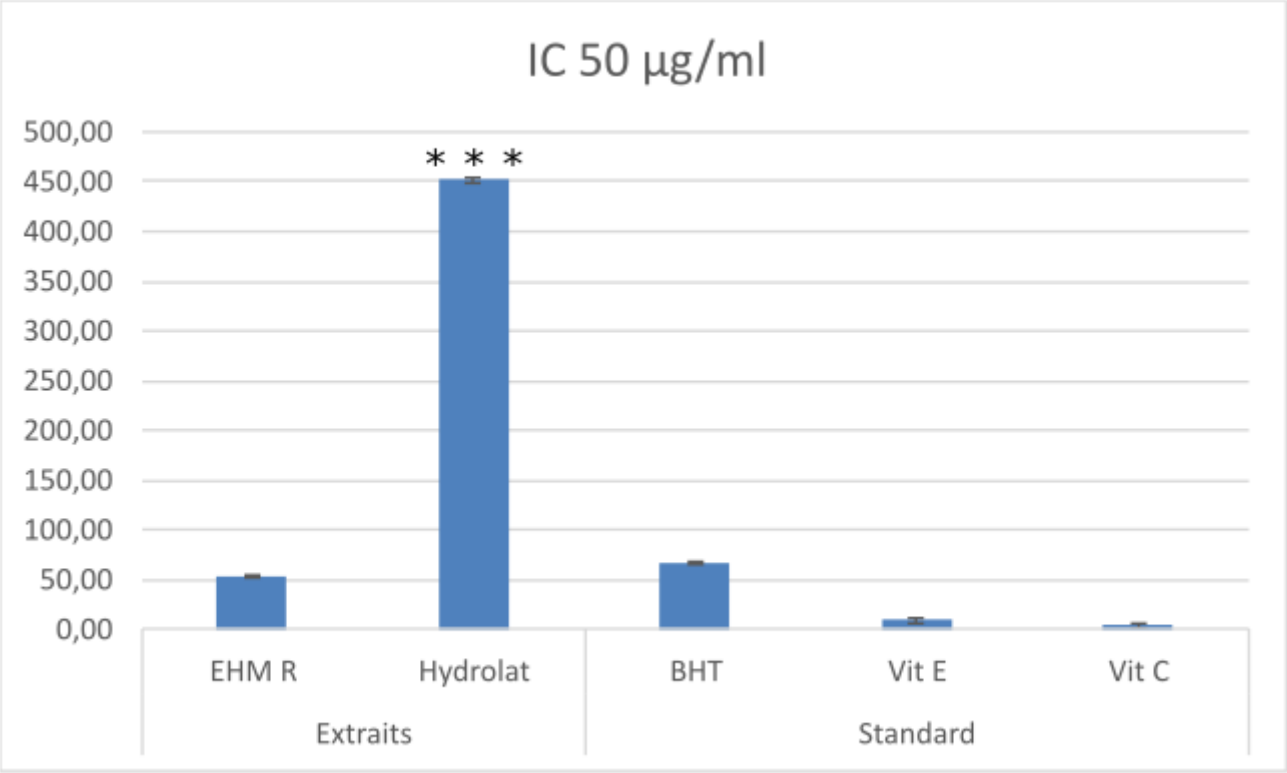

Figure 20: The IC_{50} values (µg/mL) corresponding to the antioxidant activity of the crude extract, hydrosol of *I. viscosa* and standards

Our results indicate that the two extracts of *Inula viscosa* roots are capable of scavenging the

DPPH radical depending on their concentrations. The DPPH trapping percentages reveal that the crude extract (EHM) is the most active, because it neutralizes 50% at a concentration of around 53.69 µg/ml, followed by the hydrosol which has an IC_{50} of 452.36 µg/ml (Figure 20). In comparison with the standards, the anti-radical activity of the crude extract (EHM)was found to be stronger than that of BHT (67.90 µg/ml). These results suggest that although *Inula viscosa* extracts possess antioxidant properties, they are less effective than Vitamin E and Vitamin C. These results provide valuable information on the antioxidant potential of *Inula viscose* and contribute to the understanding of its pharmacological properties.

Discussion

In comparison with previous work, we noted the presence of several scientific papers which report the anti-radical activity against DPPH of *Inula viscosa* extracts (Albano et al. (2011); Brahmi et al. (2019); Mohti et al. (2020); Asraoui et al. (2021); Qneibi et al. (2021) and Uckaya (2022)). The majority found that alcoholic extracts of *Inula viscosa* strongly inhibited the DPPH radical, which is consistent with our work.

A single study carried out by Gokbulut et al (2013), where the root extracts of the chosen plant were evaluated, according to this same study the methanolic extract of the roots showed an IC_{50} of 400 µg/ml which reveals that our crude extract is eight times stronger against DPPH compared to the latter. Our results are to some extent in agreement with these studies, but show lower IC50 values with 53.69 µg/ml, indicating strong antioxidant activity in our *Inula viscosa* extracts. This discrepancy could be due to differences in extraction methods, harvesting or experimental conditions.

Our results agree to some extent with these studies, but show higher IC_{50} values, indicating variable antioxidant activity in our *Inula viscosa* extracts. This discrepancy could be due to differences in extraction methods, harvesting or experimental conditions.

3.2 Beta-carotene Bleaching

The antioxidant potential of the extracts was evaluated by determining their ability to inhibit the oxidative degradation of B-carotene, which is visually observed through its discoloration caused by the oxidation products of linoleic acid. Our extracts consistently demonstrate antioxidant activity by effectively neutralizing the free radicals derived from linoleic acid, thereby preventing the oxidation and bleaching of β-carotene.

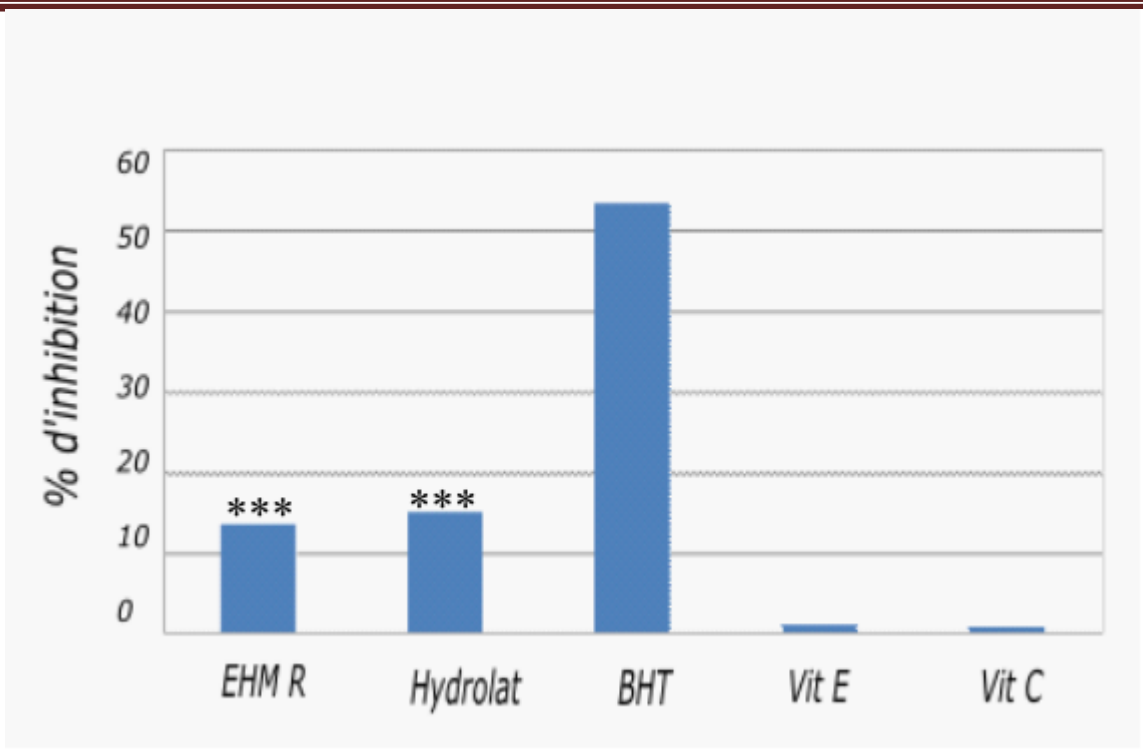

Figure 21 : Antioxidant activity of extract and hydrosol of *Inula viscosa* and some standards in the β-carotene/linoleic acid system.

From our results (Figure 21), the crude extract (EHM) and the hydrosol of the roots of *Inula viscosa* tested effectively inhibit the coupled oxidation of linoleic acid and β-carotene compared to the control negative with inhibition percentages of 13.32% and 14.95% respectively.
Extract and hydrosol derived from this plant exhibit substantial inhibitory effects on β- carotene

bleaching, although their potency is slightly lower than that of BHT, a commonly used synthetic antioxidant. It should be noted that vitamins E and C, known for their antioxidant properties, demonstrate relatively lower activity in this β-carotene/linoleic acid system.
Our findings indicate that both HMEr and hydrolate are statistically significant .

These results highlight the antioxidant potential of *Inula viscosa* as a natural source of antioxidants and suggest further exploration of its components for various applications in the field of oxidative stress and food preservation.

Discussion

To our knowledge, no study has evaluated the inhibitory activity of linoleic acid oxidation coupled to β-carotene of *Inula viscosa* root extracts. For this reason, it isn't easy to compare our results.

Furthermore, a study carried out by Rhimi et al., (2019) on the ethnolic extract of *Inula viscosa* leaves, confirmed the good activity of inhibiting lipid peroxidation with an inhibition percentage of 54.01%.

3.3 Total anti-oxidant capacity

The total antioxidant capacity of the crude extract and hydrolat of *Inula viscose* is expressed in gallic acid equivalents based on a calibration curve established using gallic acid as a reference (Appendix 2).

This molybdate phosphate assay is a direct test used primarily to measure the potential and potency of non-enzymatic antioxidants. The estimation of the total antioxidant capacity of our extracts showed slight variability depending on the type of extraction. Indeed, the EHM extract demonstrated a content of 471.30 mg EAG/g of extract and the hydrosol presented a content of 443.52 mg EAG/g of extract (Figure22).

Our study found that the observed differences were statistically unsignificant, with a p-value exceeding 0.05 (p>0.05)

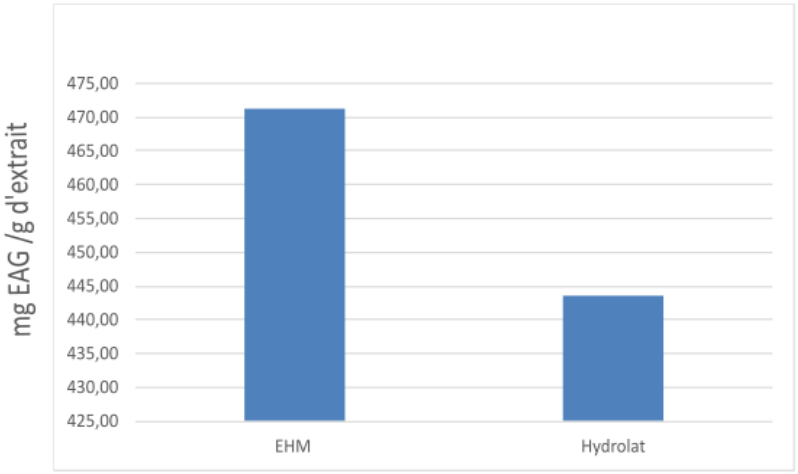

Figure 22: Total anti-oxidant capacity

Discussion

To the best of our knowledge, there is no data on the β-carotene and TAC method of *I. viscosa* root extracts. However, the TAC and IC_{50} (DPPH) are in good agreement with thoseof Morocco and Tunisia *I.viscosa* leaves extracts (Chahmi et al., 2015; Rhimi et al., 2019). However, the percentage of β-carotene inhibition was 54.01%, this result does not corroborate with our results.

The higher antioxydant potential of the crude extract (EHM) and hydrolat of *I.viscosa* roots could be explained with regard to the presence of significant amount of Naringenin, Kaempferide, Tangeritin, and Hesperidin (detected by HPLC). As well as the presence of α-Cedrene epoxide, Cadalene, Khusimol, β-Himachalene, Guaiol acetate, and Vetiselinenol (which detected in the hydrolate by GC-MS).

4. Antimicrobial activity

The antimicrobial effectiveness of crude extract and hydrolate obtained from the roots of*Inulaviscosa* was evaluated through the measurement of inhibition zone diameters (DD) and determination of minimum inhibitory concentration (MIC) values. These values represent the lowest concentration of the extract required to inhibit the growth of microbial strains. According to the scale provided by Chaouche et al. (2013), antimicrobial activity can be classified into three classes based on the diameters of the inhibition zones. A diameter greater than 15 mm represents strong antimicrobial activity, while a diameter between 9-14 mm indicates moderate activity. If the inhibition zone diameter is less than 8 mm, the activity is considered weak. (Table 08 and 09)

4.1 Antibacterial activity

The obtained results showed that the antibacterial activities varied depending on the strains and extracts tested.

The assessment of antibacterial efficacy against bacteria revealed varying activity levels for hydro-methanolic extract, hydrosol and standards (Levofloxacin for bacteria and Nystatin for

yeast) (Table 8).

The hydro-methanolic extract of the root exhibited moderate activity against *Escherichia coli,Micrococcusluteus,Klebsiella pneumonia, Staphylococcus aureus* MRSA 639c, and *Salmonella enterica* serovar Typhinurium with values ranging from 8 to 11 mm. However, it showed low activity against the remaining bacteria and the yeast.

The hydrolat of the root exhibited moderate activity against, *Klebsiella pneumonia* and *Salmonella enteric* serovar*Typh*i with values ranging from 9 to 10.5 mm. However, it exhibited low activity against the remaining bacteria and the yeast

Table 08: Antibacterial activity of crude extract and hydrolate of *Inula viscosa* roots

		Microbial strains	EHM		Hydrolat		Standards	
			DD (mm)	MIC (mg/ml)	DD (mm)	MIC (mg/ml)	DD (mm)	MIC (mg/ml)
Bacteria	Gram +	*L monocytogenes*	7± 0.1	3.125	7 ± 0.5	12.5	31.3± 1.5	0.01
		B subtilis	5 .5± 0.2	> 25	5.5± 0.2	> 25	39± 2.2	0.01
		B cereus	5.5± 0.7	1.56	5.5± 0.1	> 25	-	-
		S aureus ATCC 65	7± 0.5	3.125	7± 0.3	> 25	-	-
		S aureus MRSA 63	9± 0.4	3.125	5 .5± 0.1	> 25	25.3± 0.5	0.01
		S aureus ATCC 43	5.5± 0.2	> 25	5.5± 0.2	> 25	-	-
	Gram-	*K pneumonia*	8.5± 1.5	3.125	9± 0.5	12.5	25± 0.4	0.09
		E Coli	8± 0.9	12.5	7.5± 0.5	> 25	39± 1.5	0.02
		M luteus	8± 0.5	3.125	5.5± 0.3	> 25	-	-
		P aeruginosa	7± 0.7	3.125	7± 0.1	> 25	24± 0.4	0.02
		S Typhimurium	11± 1.0	12.5	10.5± 0.5	> 25	35± 1.5	0.05
Yeast		*C albicans*	5.5± 1.1	> 25	5.5± 0.25	> 25	20± 0.7	0.12

In terms of minimum inhibitory concentrations (MICs), the crude root extract demonstrated a value of MIC of 1.56 mg/mL against *Bacillus cereus*, which represents the lowest concentration

observed to inhibit the growth of this bacterium. The MIC values of 3.125 mg/mL were recorded for the same extract against *Staphylococcus aureus* ATCC 65, *Staphylococcus aureus* MRSA 639c, *Micrococcus luteus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Listeria monocytogenes.*

On average, the minimum inhibitory concentrations (MICs) recorded for the crude root extract are lower than those for the root hydrosol.

Discussion

When comparing our findings to those of Ramli (2013) regarding the methanol/water extract obtained through leaching, it was noted that there were no observed inhibition zones for *Escherichia coli* and *Pseudomonas aeruginosa*. This suggests that these bacteria exhibit resistance to the plant constituents found in *Inulaviscosa.*

Based on the studies by Abdoune (2012) and Benhammou (2006), it has been found that this plant does not show any impact on *Pseudomonas aeruginosa*. However, it demonstrates significant inhibitory effects on *Staphylococcus aureus* and mild inhibitory effects on *E. coli.*

The antibacterial activity results indicate that both the aqueous and ethanolic extracts have MICs ranging from 0.858 ± 0.29 to 66.66 ± 0.00 mg/ml, with the alcoholic extract demonstrating strong activity. The alcoholic extract exhibited a more potent inhibition of bacterial growth against Gram-negative strains compared to Gram-positive strains. On the other hand, the decoction generally displayed similar lower activity against both types of Gram-positive and Gram-negative bacteria, with a MIC of 33.33 ± 0.00 mg/ml (Ennacerie et al., 2019).

The observed activity is influenced by factors such as the extract's organ, the solvent used, the strain being tested, and the prevailing climatic conditions.

In addition, the findings of this study, which examined the sensitivities of Gram-positive strains and the resistance of Gram-negative strains, support the conclusions of Smith (2001) and Mohamedi (2006) validating that Gram-positive bacteria are more susceptible than Gram-negative bacteria. This variation in susceptibility can be attributed to the differences in the cell wall structure between Gram-positive and Gram-negative bacteria. The presence of a lipopolysaccharide (LPS) layer in the cell wall of Gram-negative bacteria enables the formation of an outer membrane, which serves as an effective barrier against various biomolecules (Bagamboula et al., 2004).

In comparison to the study conducted by Aissa et al.(2019), which observed that the essential oil derived from *Inulaviscosa* roots displayed lower activity against the tested Gram-negative bacteria (MIC) compared to its high activity against the Gram-positive strains, our extract and hydrosol yielded similar results. Moreover, our hydrosol demonstrated a substantial antibacterial effect (MIC) against *E. coli*, *P. aeruginosa* and *S. aureus*, aligning with the findings of Aissa et al., (2019) where all fractions (R1-R10) exhibited the same effect.

Compared to the study conducted by Ben Mrid et al. in 2022, which examined the same bacteria as our study, we observed differences in the results. Their study demonstrated that theextract of *D. viscosa L*. leaves exhibited variable antimicrobial activity against the tested bacterium *Bacillus subtilis* with DD and MIC values were 10.3 ± 0.6 mm and 1.56 $.10^{-16}$ mg/ml, respectively. However, in our study, this bacterium did not show sensitivity to our extract (5.5 mm). On the other hand, *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* were found to be resistance to their extracts. However, in our study, these bacteria were shown to be sensitive to our extract.

In a previous study, it was indicated that *Inulaviscosa* was one of the most active of plant extracts against *C.albicans*, Gökbulut et al. (2013) confirm this, and also show the root extract of *Inulaviscosa* to be more active against *Candida* species than the leaf and flower extracts.This result suggests that the low anticandidal activity of our extracts could be explained by the strong resistance of the yeast tested in our study .

In comparison with Ozkan et al. (2019), who found that only Gram-positive bacteria (*S. aureus, S. epidermidis, and S. pyogenes*) were sensitive to aqueous and methanol extracts of *I.viscosa*, our study revealed that the bacterium *S. aureus* showed moderate sensitivity to both the extract and hydrolat.

The antibacterial activity of the extracts studied could be due and favored by the presence of molecules such as Naringenin and Kaempferide, 2-Undecanone and Methyl eugenol. Which have various properties.

The molecules Naringenin and Kaempferide, identified by HPLC in the hydrosol and extract in our study , were not identified by in the works of (Rhimi et al., 2019, Ozkan, 2019, Rechek et al., 2023, and Gökbulut et al., 2013). However, the study by Ben Marid et al., (2022) identified "Naringenin" in their work using HPLC. and the molecules 2-Undecanone and Methyl eugenol,

identified by GC-MS in the hydrosol were not identified by in the work of (Aissa et al.,2019), exhibit antibacterial activity properties

4.2 Antifungal Testing

The antifungal activity of crude extract and hydrolat of *I.viscosa* root was determined by disk diffusion and agar dilution methods against various fungi species is detailed in follow in Table 09.

Table 09: The antifungal activity of the crude extract and hydrosol of *Inula viscosa*.

Fungi	**EHM**		**Hydrolat**		**Standard**	
	DD (mm)	**MIC (mg/ml)**	**DD (mm)**	**MIC (mg/ml)**	**DD (mm)**	**MIC (mg/ml)**
Fusarium	7± 0.5	12.5	7± 1.3	> 25	15 ± 1.5	3.12
A . niger	10± 2.1	12.5	7± 0.5	> 25	-	-
A. flavus	5.5± 1.2	12.5	5.5± 2.1	> 25	17 ± 1.9	1.56
Penicillium expansum	5.5± 1.3	12.5	5.5± 1.4	> 25	19 ± 1.73	0.39

The results were qualitatively assessed by the presence or absence of inhibition zone (DD) showed that the crude extract exhibited moderate antifungal activity against *Aspergillus niger* (10 mm).

In both extracts of *I. viscosa* we obtained a similar response against *Fusarium*, *Aspergillus flavus* and *Penicilliumexpansum*with inhibition zone less to 7 mm. On the other hand, the MIC values of crude extract were better than those of hydrolat for all tested fungi with 12,5 and more then 25mg/ml, respectively.

These results suggest that the EHM crude extract possesses antifungal properties, while the hydrolat extract exhibits less potent inhibitory effects against the tested fungal strains. These findings contribute to the understanding of the potential applications of *Inula viscosa* extracts in combating fungal infections.

Discussion

The antifungal activity of Inulaviscosa extracts has been evaluated in both our study and previous literature. Our work represents the first investigation into the antifungal properties of root extracts, focusing specifically on Fusarium, Aspergillus niger, Aspergillus flavus, and Penicillium expansum, simultaneously. In contrast, Mahmoudi et al. (2015) studied the antifungal activity of leaf methanolic extracts of *I. viscosa* , examining various (5) species of the Fusarium genera and others.

In Mahmoudi et al.'s study (2015), various *Fusarium* species exhibited diverse responses to I. viscosa extracts. For instance, *Fusarium polyphialidicum* showed considerable resistance with 46.84% inhibition at 40 µg/mL, while *Fusarium accuminatum* displayed higher sensitivity with 63.63% inhibition at the same concentration. This variability in response underscores the complexity of antifungal activity in *I. viscosa* extracts, influenced by specific fungal species' characteristics.

In contrast, our study focused on a narrower set of fungal strains including *Fusarium*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium expansum*. While our results demonstrated robust inhibitory effects against these strains with EHM extract, Mahmoudi et al.'s broader exploration of Fusarium species suggests a potential benefit in studying a wider array of fungal strains. The varied responses observed by Mahmoudi et al. (2015) may be indicative of specific interactions between *I. viscosa* extracts and different *Fusarium* species, highlighting the potential for enhanced efficacy with a more comprehensive fungal panel in future studies. This approach could provide deeper insights into optimizing *I. viscosa* extracts for combating fungal infections effectively.

Given the presence of Methyl Eugenol, detected by GC-MS in the hydrosol, it is likely that this compound is responsible for the observed antifungal activity since Methyl Eugenol is known for its antifungal properties

5. Cytotoxicity by brine shrimp

The *Artemia* "Brine shrimp" test provides a quick, cost-effective, and straightforward approach for assessing the biological activity of plant extracts, which is generally well correlated with their cytotoxic and antitumor properties (McLaughlin et al., 1991). We utilized this method to assess the cytotoxic potency of crude extract and hydrolat derived of the species under investigation. We exposed nauplius stage larvae of the *Artemia* species to different concentrations of the tested extracts for a duration of 24 hours.

The cytotoxic impact of the analyzed extracts is depicted in Figure (23). The results are conveyed as the lethal dose (LD_{50}), which denotes the concentration of the extract necessary to induce a 50% mortality rate in the larvae. The LD_{50} is calculated through linear regression, where the percentages of mortality are correlated with the logarithm of the tested extract concentrations.

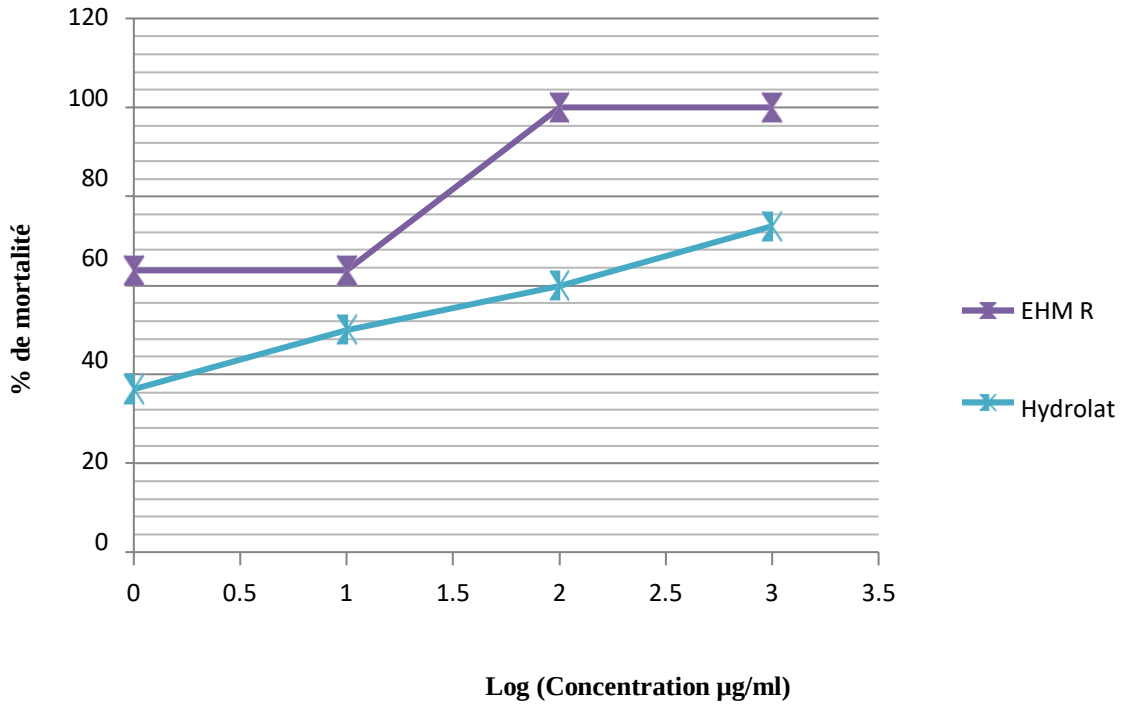

Figure 23 : Curves of mortality percentages as a function of the logarithm of the tested hydro-methanolic extract and hydrolat concentrations.

In accordance with Meyer et al.'s approach (1982), our results were interpreted. According to

this reference, plant extracts are considered non-toxic (inactive) when the LD_{50} exceeds 1000 µg/mL. Additionally, cytotoxic activity is categorized as weak when the LD_{50} ranges between 1000 and 500 µg/mL, moderate when the LD_{50} ranges between 500 and 100 µg/mL, and strong when the LD_{50} is below 100 µg/mL. The LD_{50} values we observed for the hydro- methanolic extract and hydrolat of *I. viscosa* were found to be <1 µg/mL and 10 µg/mL, respectively. These values are considerably lower than 100 µg/mL, indicating the presence of highly cytotoxic compounds responsible for the observed activity against *Artemia salina* larvae.

Discussion

The analysis of brine shrimp lethality serves as a general toxicity indicator and can also be a predictive factor for effects on cancer cells (Laughlin et al., 1991). A positive correlation has been observed between the cytotoxicity of *Artemia* and human nasopharyngeal carcinoma. Furthermore, *Artemia*is considered a pre-screening analysis for various activities, including antimicrobial, antifungal, insecticidal, antitumor, and antimalarial properties (Meyer et al., 1982).

Several studies have suggested that methanolic extracts of various species exhibit enhanced cytotoxicity, as demonstrated in the research conducted by Kanegusuku et al. (2001) on *Rubus* species (Rosaceae). In a separate study conducted in Brazil, 60 species of medicinal plants were evaluated for cytotoxicity using Brine shrimp. Only 10% of the species demonstrated a

IC50 value below 1000 µg/ml (Maria et al., 2000). Furthermore, Jacques et al. (2003) conducted screening of 226 methanol and water extracts for larval lethality, successfully identifying several cytotoxic species as a preliminary test.

To the best of our knowledge, there is no data on the Brine shrimp method of *I. viscosa* extracts. Consequently, the assessment of cytotoxicity for the methanolic extract of *I. viscosa* leaves against peripheral blood mononuclear cells (PBMCs) revealed an IC_{50} value greater than 50 µg/mL. Consistent with expectations from the National Cancer Institute (NCI), the *I. viscosa* methanolic extract exhibited no cytotoxic effects on normal cells (Ben Mrid., 2022) Findings support future clinical use of these compounds for cancer treatment, with selective cytotoxicity against cancer cells. Understanding their effects on cell proliferation and telomerase can aid in developing effective and safe cancer therapies.

These results suggest that the compounds mentioned in the *I. viscosa* ethanolic extract,

particularly 1,5-O-caffeoylquinic acid , may have antiproliferative effects on cancer cells. These compounds could act on intracellular pathways such as the IL6/JAK2/PI3 K pathway, there by inhibiting the growth of cancer cells. These findings could have potential applications in the field of cancer treatment and the development of new anticancer drugs (Rechek et al .,2023).

6. analgesic activity

The obtained results are as follows. Figure (24) presents the results of the Writhing Test, indicating the protective percentage of the extract from our species at two different concentrations (400mg/kg and 800mg/kg), along with the hydrolats. The Intraperitoneal administration was used for each extract, while the reference batch treated with paracetamol had a concentration of 100mg/kg.

HME at 800 mg/kg showed statistically significant results ($p<0.05$*), whereas both HME at 400 mg/kg and hydrolate did not show statistically significant results ($p>0.05$)

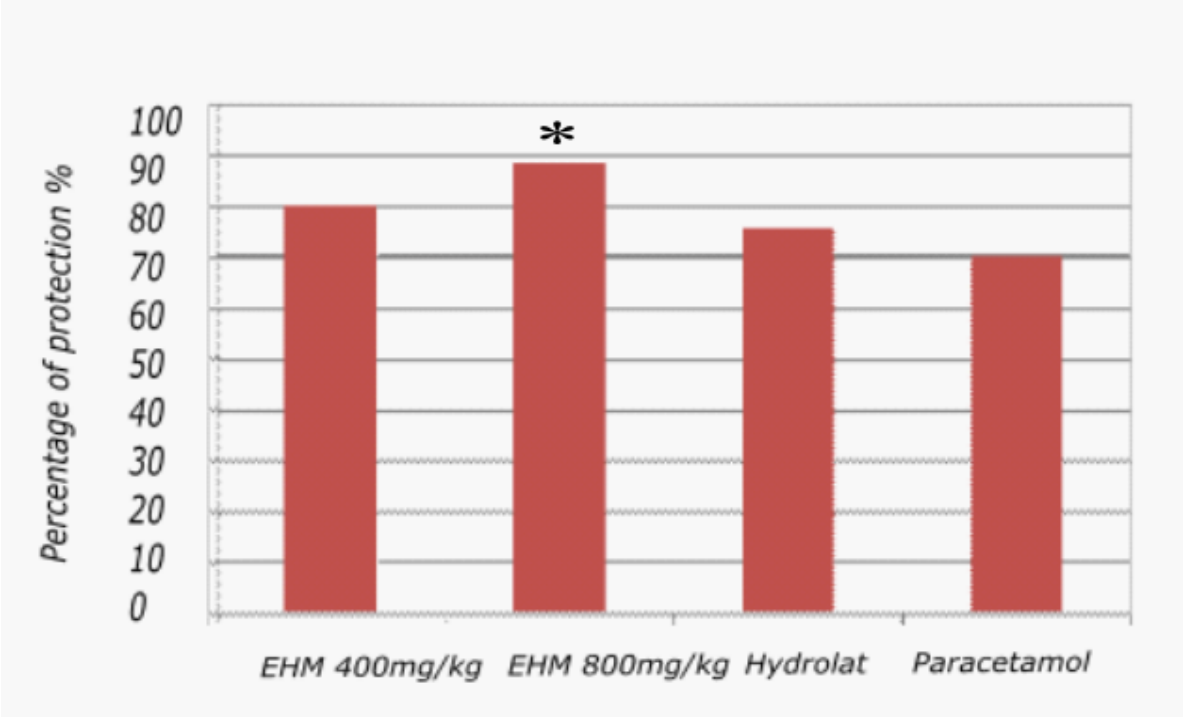

Figure 24: The percentage of protection provided by extracts of *I. viscosa* and paracetamol**.**

By administering crude extract (EHM) and hydrolat from the underground part of our species intraperitoneally at different doses of 400 and 800 mg/kg followed by the injection of acetic acid, a significant reduction in the occurrence of abdominal cramps is observed compared to the control group.

According to the obtained results, the hydromethanolic extract of the root exhibits a strong protection with a value of 79.90% and 88.35% at a dose of 400 and 800mg/kg, respectively. Followed by significant values revealed in the hydrolats with 75.55%.

In this study, the reference product used was paracetamol, which showed a protection value of 75.25% at a dose of 100mg/kg. The hydromethanolic extract (EHM) of the root at a dose of 400mg/kg, along with the hydrolat, demonstrated higher percentages of protection compared to the reference.

When mice were given pretreatment of hydro methanolic extract and hydrosol of *Inula viscosa* via intraperitoneal administration, a remarkably significant reduction in abdominal cramps was observed compared to the control group. This reduction in the painful effects caused by acetic acid is believed to be due to the analgesic properties of the compounds foundin the extracts (polyphenols, flavonoids and terpenoids), which are already recognized for their anti-inflammatory properties.

From our results, we observed the independence between the dose and analgesic efficacy in EHM. The findings reveal that both hydro-methanolic extract and hydrolat of the root of *Inula viscosa* and exhibit a highly potent protective capacity.

Discussion

To our knowledge and based on our research, this work is original and no research on the analgesic activity of *Inula viscosa* roots has been conducted.

The findings of the study by Soro (2009) support the results of the aforementioned studies, indicating that the aqueous extract's effect on inhibiting acetic acid-induced abdominal cramps in rats follows the following order, *Ajuga iva* 85.39% ≥ buprofen 77.53%. Additionally, the studies demonstrate that morphine exhibited 100% inhibition, followed by *Ximenia americana* with 61.1 ± 2.1% inhibition, and Phenyl butazone with 51 ± 2.3%inhibition (Rouibi et al., 2012).

In comparing with Ouahchia et al. (2020), the objective of this study was to evaluate the anti-inflammatory and analgesic effects of methanolic extracts and decoctions derived from *Inula viscosa* leaves and flowers. The extracts were orally administered to mice and/or rats at doses of 400, 600, and 800 mg/kg. The results demonstrated that the leaf methanolic extract at a dose of 800 mg/kg exhibited the highest inhibition of writhing (93.39%; $P < 0.001$). These results are consistent with our study where we found that our extract at a dose of 800 mg/kg showed the highest inhibition of writhing (88.35%). The potent analgesic activity observed in this study of leaves and flowers for *I. viscosa* frequently linked to the presence of phytochemicals such as flavonoids, phenolic acids, and terpenoids in the extracts. These

compounds are thought to inhibit the synthesis of prostaglandins and influence the central nervous system, thereby contributing to their strong analgesic effects (Side larbi et al., 2016).

Interestingly, phenolic compounds of the roots of *I.viscosa* contain a variety of flavanone (Naringenin and Hesperidin), flavone (Tangeritin) and flavonol (Kaempferide); were isolated from hydromethanolique extract and hydrolat. With diversity of contents in the total phenolics, methanolic extract have promising a potent analgisic, antioxidant activity and antimicrobial effects.

Based on the molecules identified by GC-MS in the hydrolate several have analgesic effects such as α-Himachalene, α-Cedrene epoxide, Cadalene, (E)-10,11-Dihydroatlantone, Nerylisovalerate, Khusimol, γ -dehydro-ar-Himachalene, 9-epi- (E)-Caryophyllene, cis-Isolongifolanone, allo-Aromadendrene, β-Acorenol, β-Himachalene, β-Acoradienol, Sesquithuriferol, α-Cadinol, Cedr-8(15)- en-10-ol, epi-Cedrol, Guaiol acetate, Vetiselinenol, Allohimachalol, trans-Isolongifolanone and 5-epi-7-epi-α-Eudesmol. Where the analgesic activity could be due to the effect of a few most effective compounds or the synergy of several of them.

The plant possesses significant bioactivities like antioxidant, antimicrobial, anti- inflammatory, analgesic, anticancer, antidiabetic, anti-Alzheimer's disease, insecticidal, hepatoprotective, antimalarial, and antiobesity. Extensive research is necessary to explore the detailed mechanism of action of study extracts and finded compounds to design effective medicines, herbal products, and functional foods.

Conclusion and perspectives

Natural remedies form a diverse and economically significant group, serving not only as direct remedies but also finding applications in various industries including pharmaceuticals, food, cosmetics, and more.

Inula viscosa is a plant that represents a valuable resource in traditional medicine due to its anti-inflammatory and healing wound properties. It has been used in the treatment of various conditions such as neoplasms, bronchitis, and diabetes. To enhance its efficacy or other purposes, it is often combined with other plants such as (mint, sage, and cloves)

Our study had two primary objectives. Firstly, we aimed to conduct a comprehensive survey of natural remedies in different towns of Boumerdes Wilaya. Secondly, we sought to investigate the phytochemical composition of the underground part (roots) of *Inula viscosa* and evaluate its *In vivo* and *In vitro* biological activities.

The survey carried out provided us with personal and professional information about herbalists, namely the level of education, the source of knowledge and the length of time in the activity, as well as information on the remedies sold. The set of preliminary results obtained are; the level of education of herbalists is middle school (63%), more than 80% of herbalists are self-taught, the seniority in the profession of herbalists is mainly marked in the interval of 2 to 6 years with 64%. The preponderance of natural remedies is plant-based with 91%. Three main countries importing natural remedies are China with 38.09%, India and Egypt with 16.52% and 12.04% respectively.

The results of the phytochemical study and the research of the biological activities of the extracts of the roots of *Inula viscosa* are listed:

The quantification of total content of polyphenols, flavonoids, and tannins revealed significant levels in the hydromethanolic extract and hydrolate.

Utilizing HPLC-DAD analysis, the crude extract of *I. viscosa* roots was examined to identify its constituent molecules. The results revealed the presence of Naringenin, Kaempferide, and Hesperin in both the extract and hydrosol. However, Tangeritin was exclusively detected in the crude extract. In addition, the analysis of the hydrolate obtained by hydrodistillation using GC-FID and GC-MS chromatography techniques revealed that β-Acorénol (11.36%) and 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (9.83%) are the predominant identified compounds. Furthermore, notable proportions of sesquiterpene compounds which are represented by 53.19% of the total compounds identified in the hydrosol.

Our study also revealed the antioxidant activity of *Inula viscosa* through various tests: DPPH scavenging assay demonstrated that the crude extract exhibited predominantly higher activity than BHT. β-carotene bleaching inhibition assay showed that the extract and hydrolate had stronger activity compared to vitamin E and C. Calculation of the content in mg EAG/g extract for determining the total antioxidant capacity (TAC) suggested that *Inula viscosa* is a significant source of molecules with antioxidant activity, in hydro-methanolic extract and hydrolate.

Moreover, the findings indicate that *Inula viscosa* exhibits promising potential as an antimicrobial agent, specifically as an effective antibacterial and antifungal agent in both the hydromethanolic extract and hydrolate.

In addition, our findings revealed that the extracts derived from the roots of *Inula viscosa* exhibited cytotoxic activity against *Artemia salina*, with LD50 values of <1 µg/mL and 10 µg/mL for hydro-methanolic extract and hydrolat, respectively.

Furthermore, it is noteworthy to mention the substantial analgesic potency of the roots part, which displayed pain protection percentages that were consistently higher compared to those demonstrated by paracetamol. This suggests that *Inula viscosa extracts* hold significant promise as a potent analgesic agent.

Finaly, comprehensive and advanced research is essential to uncover the underlying mechanisms driving these outcomes. This research could potentially expand the applications of Inula viscosa across various sectors, such as agri-food and cosmetics industries, by harnessing its effectiveness as a preservation agent with antioxidant and antimicrobial properties. Furthermore, exploring its potential in the phytopharmaceutical industry holds promise for discovering novel uses and practices. Moreover, conducting such research would provide compelling scientific evidence to validate the traditional uses of Inula viscosa, affirming its significant therapeutic potential.

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Appendix

Appendix 01: Analyzing surveys conducted with herbalists

Table1:Monographs of fifty natural remedies mentioned in the Boumerdes

Natural Remedy 01	
Scientific name	*Saliva hispanica*
Common name in french	Noix de karite
Common name in english	Chia seeds
Common name in arabic	بذور الشيا
Family	*Lamiaceae*
Used organ	Seeds
Method of preparation	Decoction
Treated diseases	Improves digestion, acts as a dietary supplement and aids in weight loss

Natural Remedy 02	
Scientific name	*Lagenaria siceraria*
Common name in french	Les graines de citrouille
Common name in english	Pumpkin seeds
Common name in arabic	بذور اليقطين
Family	*Cucurbitaceae*
Used organ	Seeds
Method of preparation	You can eat a seed
Treated diseases	Treats prostate disease

Natural Remedy 03	
Scientific name	*Oleum cucurbita pepo*
Common name in french	Huile de citrouille
Common name in english	Pumpkin seed oil
Common name in arabic	زيت بذور اليقطين
Family	*Cucurbitacea*
Used organ	Seed
Method of preparation	Extraction
Treated diseases	Treats prostate disease and serves as a dietary supplement

Natural Remedy 04	
Scientific name	
Common name in french	Alun
Common name in english	Alum
Common name in arabic	الشب
Family	*Amaryllidaceae*
Used organ	Rocky stones
Method of preparation	For external use only
Treated diseases	Heals wounds and closes pores .

Natural Remedy 05	
Scientific name	*Goeniculum vulgare*
Common name in french	Graines de fenouil
Common name in english	Fennel seeds
Common name in arabic	زريعة البسباس
Family	*Apiaceae*
Used organ	Seeds
Method of preparation	Infusion
Treated diseases	Treats colon issues.

Natural Remedy 06	
Scientific name	*Lavandula coronopifolia*
Common name in english	Lavender oil
Common name in french	Huile de lavande
Common name in arabic	زيت الخزامة
Family	*Lamiaceae*
Used organ	Flowers
Method of preparation	Extraction
Treated diseases	Treats dandruff itching and scalp inflammation.

Natural Remedy 07	
Scientific name	*Matricaria*
Common name in french	Camomille
Common name in english	Chamimole
Common name in arabic	البابونج
Family	*Asteraceae*
Used organ	Flowers
Method of preparation	Infusion
Treated diseases	Anti stress

Natural Remedy 08	
Scientific name	*Syzygium aromaticum*
Common name in french	Clou de girofle
Common name in english	Clove
Common name in arabic	القرنفل
Family	*Myrtaceae*
Used organ	Flowers
Method of preparation	Infusion
Treated diseases	Serves as a cough suppressant and soothes tooth pain .

Natural Remedy 09	
Scientific name	*Salvia rosmarinus*
Common name in english	Rosemary
Common name in frenchromarin	Romarin
Common name in arabic	إكليل الجبل
Family	*Lamiaceae*
Used organ:	Leaves
Method of preparation	Infusion
Treated diseases	Promotes hair strength, prevents hair loss and benefits colon health .

Natural Remedy 10	
Scientific name	*Aquilaria malaccensis*
Common name in english	Agarwood
Common name in french	BOIS DE OUD
Common name in arabic	عود غريس
Family:	*Thymelaeaceae*
Used organ:	Tree barks
Method of preparation:	Eaten ground with honey
Treated diseases	Adresses kidney disease

Natural Remedy 11	
Scientific name	*Berberis vulgaris Lry*
Common name in english	Barberry
Common name in french	Epine vinette
Common name in arabic	برستم
Family	*Cucurbitacees*
Used organ	Tree barks
Method of preparation	Eaten ground with honey
Treated diseases	Regenerates dead cells (against cancerous diseases).

Natural Remedy 12	
Scientific name	*Mentha*
Common name in english	Mint
Common name in french	La menthe
Common name in arabic	النعناع
Family	*Lamiacees*
Used organ	leaves
Method of preparation	Infusion
Treated diseases	Acts as a soothing agent for the digestive system inflammations.

Natural Remedy 13	
Scientific name	*Hypericum perforatum*
Common name in english	St.John's wort
Common name in french	Millepertuis
Common name in arabic:	عشبة القديسين
Family	*Hypericaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	A soothing agent against stress and depression.

Natural Remedy 14	
Scientific name	*Allium oleraceum*
Common name in english	Male garlic oil
Common name in french	Huile d'ail mâle
Common name in arabic	زيت ذكر الثوم
Family	*Alliaceae*
Used organ	Garlic cloves
Method of preparation	Extraction
Treated diseases	For treating dandruff hair thickening and alopecia .

Natural Remedy 15	
Scientific name	*Salvia officinalis*
Common name in english	Sagebrush
Common name in french	La sauge
Common name in arabic:	المريمية
Family	Lamiaceae
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Relieves stress and anxiety

Natural Remedy 16	
Scientific name	*Linum usitatissimum*
Common name in english	Flax seed
Common name in french:	Lin cultivé
Common name in arabic	زريعة الكتان
Family	*Linaceae*
Used organ	Seeds
Method of preparation	Infusion
Treated diseases	Reduces the risk of heart's disease . Type 2 diabetes (lowers blood sugar). Enhances bone thickness .

Natural Remedy 17	
Scientific name	*Atriplex halimus*
Common name in english	Mediterranean saltbrush
Common name in french	Arroche halime
Common name in arabic	القطف
Family	*Chenopodiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Good for menstrual cycles and goiter (inhibits hyperthyroidism)

Natural Remedy 18	
Scientific name	*Trigonella foenum-graecum*
Common name in english	Fenugreek
Common name in french:	Fenugrec
Common name in arabic	الحلبة
Family	*Fabaceae*
Used organ	Seeds
Method of preparation	Decoction
Treated diseases	May aid with psychological disorders , increases appetite and increases maternal milk production.

Natural Remedy 19	
Scientific name	*Thymus vulgaris*
Common name in english	Thyme
Common name in french	Thym
Common name in arabic	الزعتر
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Helps to alleviate cough and removes toxins derived from food.

Natural Remedy 20	
Scientific name	*Plantago ovata*
Common name in english	Psyllium seed
Common name in french	Graines de psyllium
Common name in arabic	بذور القطونة
Family	*plantaginaceae*
Used organ	Seeds
Method of preparation	Infusion
Treated diseases	Facilitates relief from constipation.

Natural Remedy 21	
Scientific name	*Zingiber officinale*
Common name in english	Ginger
Common name in french:	Gingembre
Common name in arabic	زنجبيل
Family	*Zingiberaceae*
Used organ	Roots
Method of preparation	Infusion
Treated diseases	Has calming effects on stomach discomfort, nausea and vomiting , also used to relieve common cold symptoms.

Natural Remedy 22	
Scientific name	*Centaurium erythraea*
Common name in english	Common centaury
Common name in french:	Petite centaurée
Common name in arabic	مرارة الحنش
Family	*Gentianaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Treats digestive disorders, hemorrhoids, activates liver and gallbladder, and manages diabetes.

Natural Remedy 23	
Scientific name	*Cyperus esculentus L*
Common name in english	Tiger nut
Common name in french	Noix tigrée
Common name in arabic	حب العزيز
Family	*Cyperaceae*
Used organ	Tubers
Method of preparation	Infusion
Treated diseases	Enhances digestion and aids in appetite regula

Natural Remedy 24	
Scientific name	*Valeriana officinalis*
Common name in english	Valerian
Common name in french	valériane
Common name in arabic	عشبة الناردين
Family	*Caprifoliaceae*
Used organ	roots
Method of preparation	Infusion
Treated diseases	Promotes better sleep and reduces stress.

Natural Remedy 25	
Scientific name	*Ginkgo biloba*
Common name in english	Ginkgo
Common name in french:	Ginkgo
Common name in arabic	الجنكة
Family	*Ginkgoaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Enhances brain performance and intelligence.

Natural Remedy 26	
Scientific name	*Melissa officinalis L*
Common name in english	Lemon balm
Common name in french	Mélisse
Common name in arabic	مليس
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Addresses jaundice.

Natural Remedy 27	
Scientific name	origanum majorana
Common name in english	Marjoram
Common name in french	Marjolaine
Common name in arabic	بردقوش
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Manages cholesterol levels and reduces liver fat.

Natural Remedy 28	
Scientific name	*Argania spinosa*
Common name in english	Argan oil
Common name in french	huile d'argane
Common name in arabic	زيت الارغان
Family	*Sapotaceae*
Used organ	Seeds
Method of preparation	Extraction
Treated diseases	Repairs hair split ends.

Natural Remedy 29	
Scientific name	*Sesamum indicum*
Common name in english	Sesame oil
Common name in french	Huile de sésame
Common name in arabic	زيت السمسم
Family	*Pedaliaceae*
Used organ	Seeds
Method of preparation	Extraction
Treated diseases	Addresses hair, skin, and stomach conditions.

Natural Remedy 30	
Scientific name	*Ocimum basilicum*
Common name in english	basil
Common name in french	Basilic
Common name in arabic	الريحان
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Relieves intestinal bloating

Natural Remedy 31	
Scientific name	*Dolomiaea costus*
Common name in english	Indian costus
Common name in french	Costus indien
Common name in arabic	القسط الهندي
Family	*Saussurea*
Used organ	Roots
Method of preparation	By grinding
Treated diseases	Addresses gastritis, asthma, and bronchitis.

Natural Remedy 32	
Scientific name	*Bunium mauritanicum*
Common name in english	Earth chestnut
Common name in french	Chtaigne de terre
Common name in arabic	تالغودة
Family	*Apiaceae*
Used organ	Treats thyroid gland disorders
Method of preparation	By grinding
Treated diseases	Tuber

Natural Remedy 33	
Scientific name	*Artemisia absinthium*
Common name in english	Wormwood
Common name in french	Armoise
Common name in arabic	الشيح
Family	*Asteraceae*
Used organ	Stems
Method of preparation	Infusion
Treated diseases	Alleviates digestive issues.

Natural Remedy 34	
Scientific name	*Punica granatum*
Common name in english	Pomegranate peel
Common name in french	Pelure de grenade
Common name in arabic	قشور الرمان
Family	*Punicaceae*
Used organ	Peels
Method of preparation	Infusion
Treated diseases	Reduces cholesterol levels.

Natural Remedy 35	
Scientific name	Camellia sinensis
Common name in english	Green tea
Common name in french	Thé vert
Common name in arabic	الشاي الاخضر
Family	*Theaceae*
Used organ	leaves
Method of preparation	Infusion
Treated diseases	Aids weight loss, lowers cholesterol and boosts memory

Natural Remedy 36	
Scientific name	*Senna alexandrina*
Common name in english	Senna leaves
Common name in french	Feuilles de Séné
Common name in arabic	سنامكي
Family	*Fabaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Detoxifies the intestines and alleviates constipation.

Natural Remedy 37	
Scientific name	*Asphaltum punjabianum*
Common name in english	Shilajit
Common name in french	Chilajit
Common name in arabic	شيلاجيت
Family	*Asphaltum punjabianum*
Used organ	Mineral pitch
Method of preparation	Dilution
Treated diseases	Energetic

Natural Remedy 38	
Scientific name	*Vitex agnus castus*
Common name in english	Chaste tree
Common name in french	Gattilier
Common name in arabic	عشبة مريم
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Eases menstrual cycle troubles.

Natural Remedy 39	
Scientific name	*Moringa oleifera*
Common name in english	Moringa
Common name in french	Moringa
Common name in arabic	المورينغا
Family	*Moringaceae*
Used organ	Leaves
Method of preparation	Infusion
Treated diseases	Addresses anemia, rheumatism, and diabetes.

Natural Remedy 40	
Scientific name	*Cinnamomum verum*
Common name in english	Cinnamon
Common name in french	Cannelle
Common name in arabic	القرفة
Family	*Lauraceae*
Used organ	Sticks
Method of preparation	Infusion
Treated diseases	Acts as an antimicrobial, and provides antioxidants.

Natural Remedy 41	
Scientific name	*Teucrium polium*
Common name in english	Felty germander
Common name in french	Germandrée tomenteuse
Common name in arabic	الخياطة
Family	*Lamiaceae*
Used organ	Leaves
Method of preparation	infusion
Treated diseases	Alleviates gastrointestinal issues.

Natural Remedy 42	
Scientific name	*Citrullus colocynthis*
Common name in english	Ephedra
Common name in french	Ephedra
Common name in arabic	العلندة
Family	*Ephedraceae*
Used organ	Branches and stems
Method of preparation	Infusion
Treated diseases	Aids in cancer treatment.

Natural Remedy 43	
Scientific name	*Boswellia sacara*
Common name in english	Frankincense
Common name in french	Oliban
Common name in arabic	لبان
Family	*Burseraceae*
Used organ	Resin
Method of preparation	Extraction
Treated diseases	Addresses hair and skin issues.

Natural Remedy 44	
Scientific name	*Plantago ovata*
Common name in english	psyllium
Common name in french	Plantain des indes
Common name in arabic	قاطونة
Family	*Plantaginaceae*
Used organ	Seeds
Method of preparation	Infusion
Treated diseases	Treats colon problems and aids weight loss.

Natural Remedy 45	
Scientific name	*Ricinus communis*
Common name in english	Castor oil
Common name in french	Huile de ricin
Common name in arabic	زيت الخروع
Family	*Ephorbiaceae*
Used organ	Seeds
Method of preparation	Extraction
Treated diseases	Accelerates hair growth.

Natural Remedy 46	
Scientific name	
Common name in english	propolis
Common name in french	propolis
Common name in arabic	العكبر اوالعقبة
Family	*Rhytismataceae*
Used organ	Beewax
Method of preparation	Extraction
Treated diseases	Treats cancer, asthma, colds, and skin issues.

Natural Remedy 47	
Scientific name	*Hibiscus rosa-sinensis*
Common name in english	Hibiscus
Common name in french	Hibiscus
Common name in arabic	الكركدية
Family	Malvaceae
Used organ	Fower
Method of preparation	Infusion
Treated diseases	Treats anemia and regulates blood pressure.

Natural Remedy 48	
Scientific name	*Urtica dioca L*
Common name in english	Nettles
Common name in french	Orties
Common name in arabic	القرايص او الحرايق
Family	*Urticaceae*
Used organ	Leaves and stem
Method of preparation	Infusion
Treated diseases	Alleviates anemia.

Natural Remedy 49	
Scientific name	*Panax ginseng*
Common name in english	Ginseng
Common name in french	Ginseng
Common name in arabic	الجنسنغ
Family	*Araliaceae*
Used organ	Roots
Method of preparation	Grated
Treated diseases	Boosts energy and appetite.

Natural Remedy 50	
Scientific name	*Withania somnifera*
Common name in english	Ashwagandha
Common name in french	L'ashwagandha
Common name in arabic	شواغاندا
Family	*Solanaceae*
Used organ	Branches
Method of preparation	Infusion
Treated diseases	Energetic.

Appendix 02

1)- Calibration curve of gallic acid for measuring total antioxidant capacity

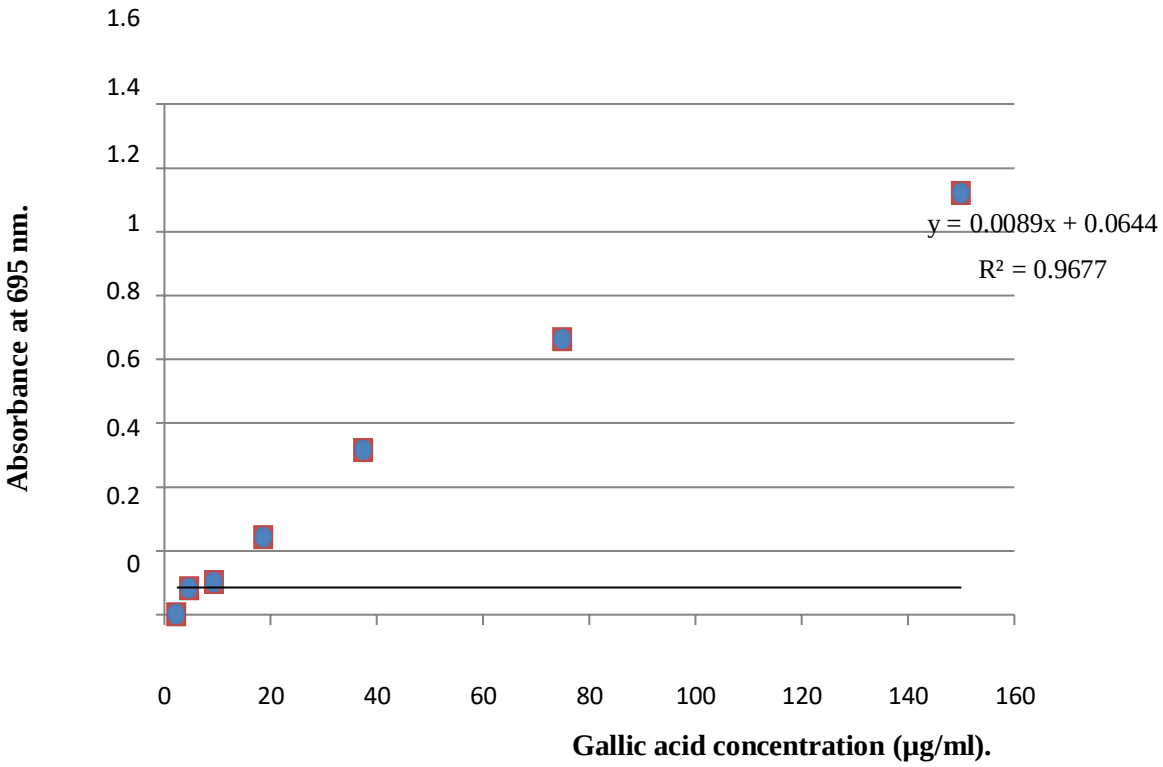

Figure 01: Calibration curve of gallic acid.

2) - Measurement of total polyphenols, flavonoids and tanins dosage.

Spectrophotometric assay is the most commonly used technique for the quantification of total polyphenols (Figures 02a), total flavonoids (Figures 02b) and tannins (Figures 03). The contents were reported as milligrams equivalent of the standard used per gram of dry extract.

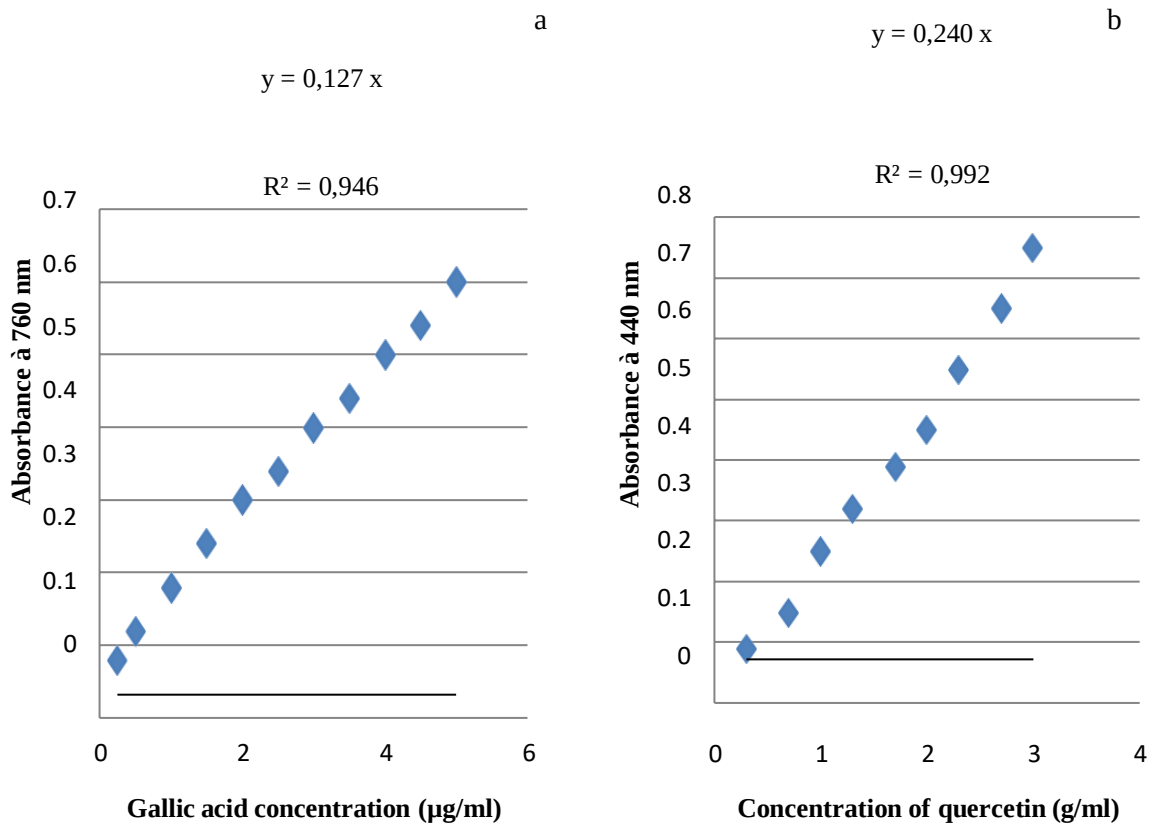

Figure 02.. Calibration curves of gallic acid and quercetin for the quantification of total polyphenols and total flavonoids, respectively

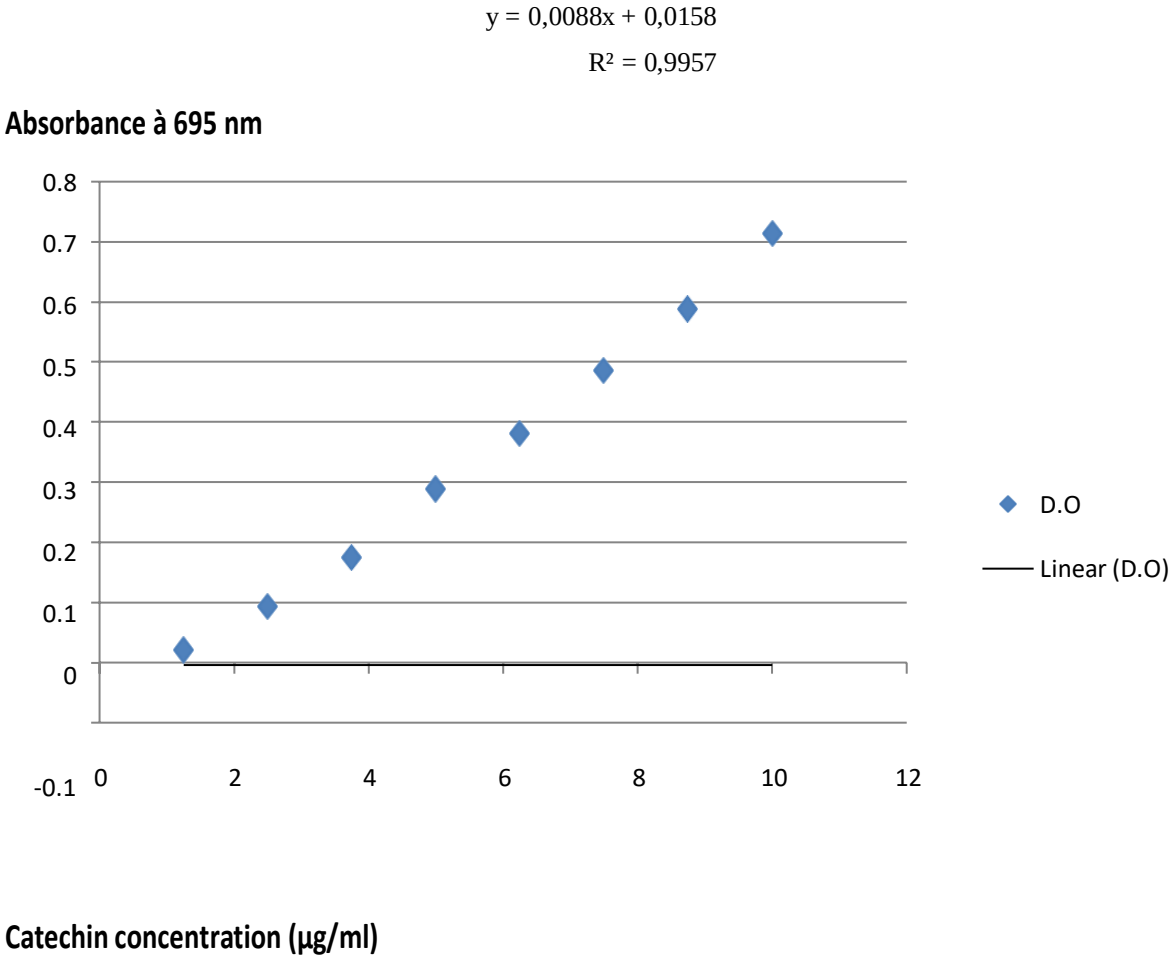

Figure 3: The calibration curve of catechin for tannin quantification

Appendix 03: H.P.L.C

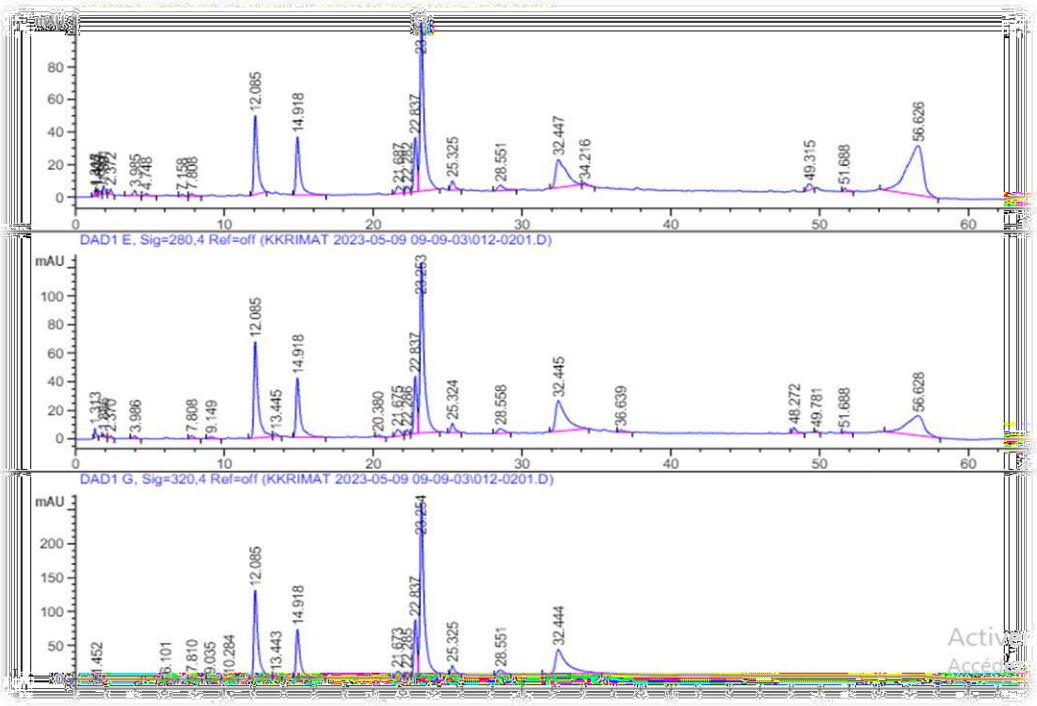

Figure 04: Chromatogram of the hydro-methanolic extract of *Inula viscosa* root

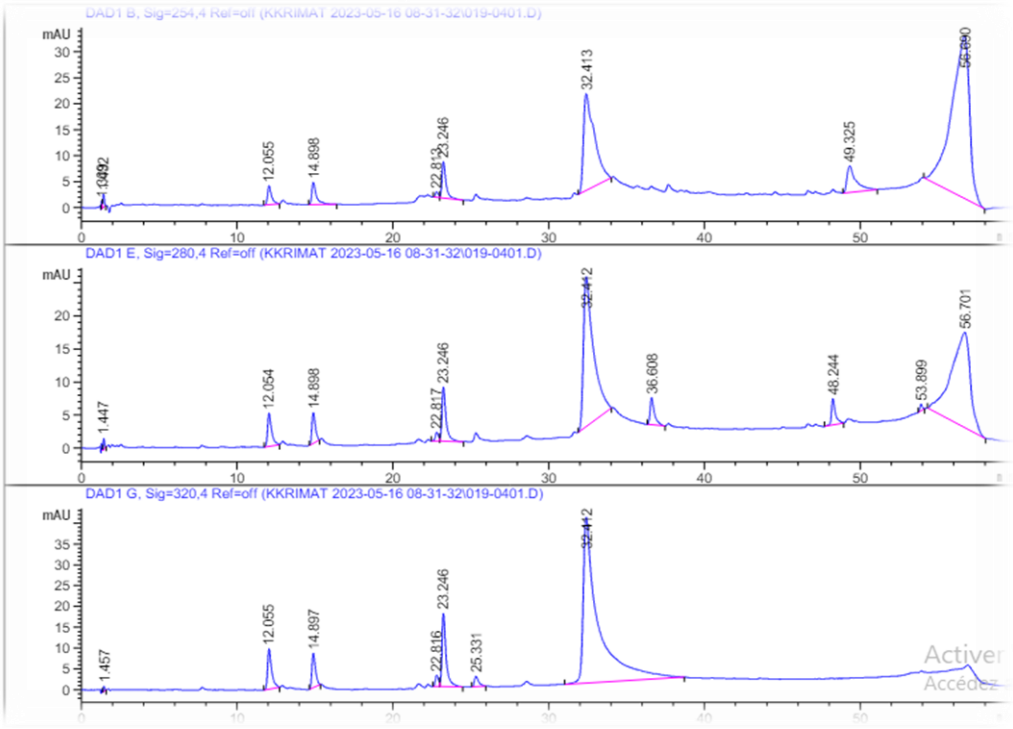

Figure 05: Chromatogram of the hydrolate of *Inula viscosa* root.

Annexure – 04 **QUESTIONNAIRE**

Documentation of herbalists' Knowledge on Natural Remedies

Date: Feb-june , 2024

Personal and Professional Background

1. **What is your educational background?**
   - ☐ No formal education
   - ☐ Primary education
   - ☐ Middle school
   - ☐ High school
   - ☐ Higher education (specify field)

2. **What is your source of knowledge about natural remedies?**

- ☐ Traditional family knowledge
- ☐ Formal training (e.g., courses, certifications)
- ☐ Self-taught through books and research
- ☐ Other (please specify)

3. **How long have you been practicing as an herbalist?**
   - ☐ Less than 2 year
   - ☐ 2-6 years
   - ☐ 6-10 years

- More than 10 years

Types of Remedies and Their Origins

4. **What type of remedy do you sell the most?**
   - Plant-based remedies
   - Non-plant-based remedies

5. **Where are these remedies brought from?**
   - Locally sourced
   - Other regions within the country
   - Imported from abroad (please specify countries)

Natural Remedies Usage and Administration

6. **For what conditions or purposes are natural remedies most commonly used by your customers?**
   - Common ailments (e.g., colds, headaches)
   - Chronic conditions (e.g., diabetes, arthritis)
   - Skin conditions
   - Digestive issues
   - Other (please specify)

7. **What is the mode of administration for the remedies you provide?**
   - Oral
   - Topical

- ☐ Inhalation
- ☐ Other (please specify)

8. **How would you rate the customer demand for these remedies?**
   - ☐ Always
   - ☐ Often
   - ☐ Rarely

Preparation and Usage Instructions

9. **Which part of the plant or material is most commonly used in your remedies?**
   - ☐ Leaves
   - ☐ Roots
   - ☐ Flowers
   - ☐ Seeds
   - ☐ Other (please specify)
10. **What is the common method of preparation for these remedies?**
    - ☐ Infusion
    - ☐ Decoction
    - ☐ Maceration
    - ☐ Powder
    - ☐ Other (please specify)
11. **How many times should the remedy be taken or applied per day?**
    - ☐ Once
    - ☐ Twice
    - ☐ Three times

- ☐ Other

12. **Can the natural remedy be mixed with other ingredients?**
    - ☐ Yes
    - ☐ No
    - ☐ If yes, please specify which ingredients

Safety and Efficacy

13. **What is the toxicity threshold of the remedies you provide?**
    - ☐ Known safe dosage (please specify)
    - ☐ Unknown
    - ☐ Variable, depending on the remedy and condition

Abstract

This study contributes to understanding traditional healing practices and explores the phytochemical and biological properties of Inula viscosa L. extracts. It has two main objectives. Firstly, we conducted a comprehensive survey of natural remedies in various towns of Boumerdes Wilaya, focusing on herbalist practices, preparation techniques, and remedy origins to enhance our understanding of traditional healing methods and contribute insights to herbal medicine research. Secondly, our study investigates the phytochemical and biological properties of crude extracts and hydrosols from Inula viscosa L. Our findings highlight significant levels of polyphenols, flavonoids, and tannins, as well as potent antioxidant, antimicrobial, and analgesic effects. These results suggest promising avenues for further research in herbal medicine.

Résumé

Ce travail contribue à la compréhension des pratiques de guérison traditionnelles et à l'exploration des propriétés phytochimiques et biologiques des extraits d'Inula viscosa L

Nous avons étudié les remèdes naturels à Boumerdes, en analysant les données des herboristes, les techniques de préparation et l'origine des remèdes. Nos analyses ont révélé des niveaux élevés de polyphénols, flavonoïdes et tanins dans les extraits bruts et les hydrolats, ainsi que des composés spécifiques comme la Naringénine et la Kaempféride. Ces extraits ont montré une forte activité antioxydante, antimicrobienne et analgésique. Ces résultats ouvrent de nouvelles perspectives pour la recherche en médecine herboriste.

الملخص

يهدف هذا العمل إلى فهم ممارسات الشفاء التقليدية واستكشاف الخصائص الفيتوكيميائية والبيولوجية لمستخلصات نبات inula viscosa L قمنا بإجراء مسح شامل للمعالجات الطبيعية في مناطق مختلفة من ولاية بومرداس، مع التركيز على ممارسات الاعشاب وتقنيات التحضير وتفضيلات العملاء.

تدرس دراستنا الخصائص الفيتوكيميائية والبيولوجية لمستخلصات نبات Inula viscosa. كشفت التحليلات عن مستويات عالية من البوليفينولاتوالفلافونويدات والتانينات. أظهرت المستخلصات نشاطا مضادًا للأكسدة ومضادًا للميكروبات بالإضافة إلى تأثيرات مسكنة قوية. تم تحليل المستخلص الخام بواسطة HPLC-DAD ، واحتوى على نارنجينوكامفيرايد. كشف تحليل الهيدرولات للجذور باستخدام تقنيات GCMS على المركبات الرئيسية واكتشاف كميات كبيرة من المركبات غير المعروفة.

تفتح هذه النتائج آفاقا واعدة للبحث في مجال الطب بالأعشاب.