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Geographical Origin and Solvent Type Impact on *Inula viscosa* (L.) Aiton Grown in El Menzel – Morocco – Insights into Bioactivity and Applications

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ABSTRACT

Geographical origin and environmental factors have a significant impact on the constituents and the biological properties of medicinal and aromatic plants. Herein, the *Inula viscosa* plant grown in El Menzel – Morocco were investigated with a focus on the impact of geographical province and solvent type on the mass yield and the biological activities of plant extracts. Chemical composition was characterized by gas chromatography/mass spectrometry (GC/MS). Antimicrobial activity was determined using the disk diffusion method and the microdilution test against eight clinical fungal, Gram-positive and Gram-negative bacterial isolates. The chemical composition results showed that the plant has good nutritional quality in terms of protein, carbohydrates, lipids and dietary fiber. In fact, alkaloids and saponisides are the most predominant chemical compounds in *Inula vuscosa*. Meanwhile, eighty volatile compounds were identified, representing 95% of the total essential oil content, the main component of which is tetra-pentacontane (11.26%). Furthermore, results showed high antioxidant activity, with efficacy increasing in the order: essential oil > chloroform extract > ethereal extract > ethanolic extract. In addition, both chloroformic extract and essential oil demonstrated significant antibacterial activity against all strains tested. This study highlights the influence of geographical variations and extraction solvents on the bioactivity of *Inula viscosa*, offering insights into its potential applications in pharmacology and nutraceuticals.

Keywords: *Inula viscosa*; antioxidant activity; antimicrobial activity, photochemical composition.

INTRODUCTION

In recent years, particularly in less developed countries, the utilization of traditional medicine has significantly expanded worldwide owing to its effectiveness and reduced side effects when compared to synthetic drugs (Labhar et al., 2023). Additionally, underdeveloped nations have regarded medicinal plants as a vital treatment resource for various diseases due to their possession of diverse biological activities and a wide array of chemical structures. This is attributed to their content of metabolites and bioactive molecules, including coumarins, alkaloids, polyphenols, mucilages, tannins, and terpenes (Cock et al., 2018; El Khomsi et al., 2022; Hmamou et al., 2024, 2023b, 2023a; Khomsi et al., 2024, 2022).

Exploring medicinal and aromatic plant properties is of paramount importance in scientific research, not least because of their therapeutic potential and multiple applications (Dzoyem et al., 2013). Among these plants, *Inula viscosa* stands

out for its antioxidant, antimicrobial and antibacterial properties, thus arousing sustained scientific interest (Zeouk et al., 2022). This in-depth study focused specifically on *Inula viscosa* grown in El Menzel, Morocco, seeking to elucidate the impact of geographical provenance and choice of solvents on the yield and biological activities of plant extracts. *Inula viscosa* is among the most used plants in Morocco, it is belonging to the Asteracea family. It has been used traditionally as a remedy since ancient Greek and Roman to treat multiple diseases, such as wounds, skin diseases, bronchitis, hypertension, fever, diabetes and several types of inflammation, dental and articular diseases (Haoui et al., 2015; Tlemcani et al., 2023). *Inula viscosa* extracts showed to possess a high value of bioflavonoids, saponins, sterols, carotenoids, sesquiterpene, sesquiterpenoids and polyphenols, which allowed them to react as antioxidants, antimicrobial, anti-inflammatory, and anti-cancer agents (Kheyar-Kraouche et al., 2023).

Among various techniques for extracting plant components, maceration stands out for its simplicity and effectiveness (Subramanian & Anandharamakrishnan, 2023). This method involves immersing plant material in a suitable solvent, often a water-alcohol mixture, to allow active compounds to diffuse into the liquid. Maceration offers the advantage of preserving a wide range of compounds, including heat-sensitive molecules and more complex compounds, owing to its gentle processing conditions. Unlike other, more energy-intensive techniques such as extraction by distillation, maceration avoids the degradation of heat-sensitive compounds, enabling more complete extraction of active ingredients. What is more, it is adaptable to different types of plant and can be carried out on a small scale, making it an accessible and versatile method for obtaining extracts rich in bioactive components (Srivastava et al., 2021).

In parallel with maceration, extraction by the Clevenger method is a traditional and widely used technique for extracting essential oils from aromatic plants. This method involves steam distillation, where volatile compounds are released from the plant material, carried by the steam, and then condensed. Clevenger extraction is specifically adapted for the plants rich in essential oils, enabling efficient recovery of these volatile compounds. However, while this method is effective for extracting essential oils, it may be less suitable for certain heat-sensitive or heavier compounds, which may degrade or be lost during the distillation process. As a result, maceration often remains the preferred method for preserving a wider range of active compounds, offering a more versatile alternative for extracting active plant ingredients (Farooq et al., 2021).

The objective of this study was to reveal the biological activities and phytochemical composition of Moroccan *Inula viscosa*. Through various characterization techniques, robust antioxidant activity and notable antibacterial effects were showcased, highlighting the influence of geographical variances and extraction solvents on the bioactivity of *Inula viscosa* bioactivity. This investigation unveiled auspicious avenues for prospective therapeutic applications.

MATERIALS AND METHODS

Plant material

An *Inula viscosa* plant were gathered from the region of Sefrou (El Menzel, Morocco) in March and April 2021, as this period corresponds to the peak development and flowering of the plant.

Extracts preparation

In the laboratory, the aerial parts of the plant underwent a thorough cleaning with fresh water before being subjected to a drying process in an oven set at 45 °C for 72 hours until complete dehydration was achieved. Subsequently, the dried plant material was finely powdered using an electric blender and sieved to ensure uniformity. To extract the plant's bioactive compounds, 20 grams of the powdered plant material underwent maceration using different solvents: hydro-ethanolic, hydro-chloroformic, and hydro-ethereal (composed of 70% solvent and 30% distilled water) for a duration of 48 hours at room temperature. Following maceration, the mixture was filtered using filter paper, which was then concentrated using a rotary evaporator. Finally, the concentrated extracts were stored in Eppendorf tubes at 6 °C for further analysis and experimentation.

The extraction yield was determined using the following formula:

$$Extraction\ yield = Y(\%) = (\frac{M_e}{M_d}) \times 100 \quad (1)$$

where: M_e – mass of extract collected (g);
D_d – dry matter mass of *Inula Viscosa* (g).

Essential oil extraction

Hydrodistillation process was conducted using a Clevenger-type apparatus. Within a 2-liter flask, 200 grams of prepared plant leaves were combined with 1.5 liters of water. The mixture underwent heating facilitated by a heating mantle until it reached boiling point. Subsequently, the oil and water components were separated due to their distinct density variations. The oil, isolated from the top layer, was carefully collected using a micropipette and subsequently stored at 4 °C for subsequent analysis.

Nutritional value and chemical composition and amino acids content

Secondary metabolites, carbohydrates, proteins, lipids, and dietary fiber content were identified using HPLC chromatography. This technique enables both qualitative and quantitative analysis, allowing for the identification, separation, and quantification of chemical compounds within a liquid mixture, even at trace amounts.

The sample under analysis, containing one or more species, is propelled by a mobile phase current, interacting with a stationary phase. Each species migrates at a rate dependent on their individual characteristics and the properties of the two present phases. The authors focused on the analysis of Alkaloids, Flavonoids, Tannins, Saponosides, Coumarins, Carbohydrates, Proteins, Lipids, and Dietary Fibers. The HPLC setup comprises a quaternary pump, manual injector, and three detectors – Fluorescence, UV, and Refractometer – alongside a reversed-phase C18 column. VARIAN W software was employed for result acquisition, analysis, and processing. Amino acids were determined using Moore and Stein's chromatography method (1951) on Dowex-50 columns. Macronutrients (carbohydrates, proteins, lipids, and dietary fiber) were assessed following AOAC procedures (1990). Mineral content (Ca, P, K, Na, Cl, S, Mg, Fe, Mn, Zn, Pb, Se, Cu, Co) was quantified through individual calibration curves for each element, employing atomic absorption spectrophotometry with (Varian AA 20 Spectrometer, Australia).

Chemical analysis of essential oil

The GC-MS analysis was conducted using a Shimadzu series GC-MS system (TQ-8040) from Tokyo, Japan, equipped with an auto-injector AOC-20i and a capillary column (30 m x 0.25 mm internal diameter, 0.25 μm). The oven temperature was initially set at 50 °C for 5 minutes and then ramped up to 290 °C for 10 minutes, with injector and detector temperatures maintained at 200 °C. Ionization energy was set at 70 eV with a mass range of 40–650 atomic mass units (AMU). The Shimadzu GC-MS solution ver.4 software (from Tokyo, Japan) was utilized for GC and mass spectrometry parameter settings, as well as data reception and processing. Compound identification relied on comparing their mass spectra with the NIST 2017 11th Edition data (National Institute of Standards and Technologies, Mass Spectra Libraries).

Total antioxidant capacity test (TAC)

Afterwards, 25 μL of plant extract was measured and then 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The optical density was de-termined at 695 nm after incubation for 90 min at 95 °C using a spectrophotometer (Perkin Elmer, Shelton, CT USA). The total antioxidant activity was expressed as the number of equivalence of ascorbic acid equivalent per gram of extract (mg AAE/g) (Hmamou et al., 2022).

Antimicrobial activity

The antimicrobial activity of the studied extracts was tested against three gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*), two gram-positive bacteria (*S. aureus, B. subtilis*), and three fungi (*F. proliferatum*, *A. niger*, and *C. albicans*), known to cause nosocomial infections, a significant public health concern (Gnat et al., 2021).

Tested strains and inoculum standardization

A drop of the culture was streaked onto a petri-dish containing Mueller-Hinton agar and incubated at 37 °C for 18–24 hours. For the bacterial suspension (inoculum) preparation, three identical colonies were isolated, and the platinum loop was submerged into 10 ml of sterile physiological water (0.9% NaCl). The bacterial suspension was then vortexed in sterile saline (0.9% NaCl) to ensure homogeneity and pre-cultured at 37 °C for 3–5 hours. Following this, it was adjusted to a turbidity of 0.5 McFarland (equivalent to 1–5 × 10^8 CFU/mL) (Yildirim et al., 2023).

Disc diffusion method

Initially, the bacterial strains were cultured as lawns by saturating an autoclaved cotton swab in a standardized solution (1 - 5 × 10^8 CFU/mL) and spreading it across the surface of Mueller-Hinton Agar (MHA). The agar surface was pre-inoculated and treated with 10 µL of the extract, followed by the placement of 6 mm Whatman paper discs onto it. After 24 hours of incubation at 37 °C, the diameters of the inhibitory zones were measured. Each of these tests was repeated three times for accuracy (Kokina et al., 2019).

Determination of minimum inhibitory concentration (MIC)

The MIC for *Inula viscosa* extract was conducted using the microdilution method following NCCLS standards (Amin et al., 2018). The extract was dispensed into sterile tubes at ten different concentrations, achieved through a series of 1/2 dilutions in distilled water, ranging from 0.975 to 50 mg/mL in each microplate well (Elyemni et al., 2022). Following dilution (20 µL) in MH broth (80 µL), the resulting mixture was inoculated at a density of 50×10^5 CFU/well in 96-well plates. Subsequently, 100 µL of various extract concentrations were added to each well, except for the last well serving as the growth control to determine MIC values. Incubation at 37 °C for 24 hours was followed by the utilization of a coloring reagent, triphenyltetrazolium chloride (TTC), to perform the colorimetric test. The development of color indicated the presence of viable bacteria. This process was repeated by diluting each preparation (20 µL) in MH broth, and the mixture was plated at a density of 50×10^5 CFU/well in 96-well plates.

Determination of minimum lethal concentration (MLC)

To determine the minimum lethal concentration (MLC), three wells were obtained using a cotton swab and compared to the MICs. Then, 10 µL were taken from each well without visible growth and inoculated into Mueller Hinton (MH) agar for bacteria or Sabouraud for fungi. The dishes were incubated for 24 hours at 37 °C. CMB was defined as the lowest test concentration that produced a 99.99% reduction in CFU/mL compared to the control (Balouiri et al., 2016).

RESULTS AND DISCUSSION

Extract yields

Using three different solvents in the maceration process of *Inula viscosa* led to varying extraction yields (Table 1). The ethanolic extract showed the highest yield, reaching 22.8%, closely followed by the chloroform extract at 21.4%, then the ethereal extract at 19.7%. These differences in yield suggest specific affinities of the solvents with the chemical compounds present in the plant, thus impacting their respective extraction efficiencies come reported by (Chen et al., 2016).

Nutritional value, mineral composition and amino acids content in the dry matter of *Inula viscosa*

Table 2 show the percentage of carbohydrate, protein lipid and dietary fiber content. The results obtained using three different solvents in the *Inula viscosa* maceration process revealed the percentages of macromolecules present in the plant.

The leaves contained around 10.13% protein, 7.21% carbohydrates, 1.31% lipids and 4.00% dietary fiber. However, these values highlight the rich and diverse composition of macromolecules in the plant, underscoring their potential importance in medicinal and nutritional applications. In fact, lipids, polysaccharides, and proteins are secreted by the leaves and young stems of *Inula viscosa* throughout their life, from its very early stage of development to maturity. This secretion is through sessile and stalked secretory hairs as reported by (Werker & Fahn, 1981).

Table 1. yield of *Inula viscosa* extractes

Sample	Mass of dry matter (g)	Mass of the extract (g)	Yield (%)
Ethanolic extract	20	4.56	22.8
Chloroform extract	20	4.28	21.4
Ethereal extract	20	3.94	19.7

Table 2. Carbohydrate, protein, lipid, and dietary fiber content in the dry matter of *Inula viscosa*

Macromolecule	% of dry matter (leaves) (weight/volume) ± 0.1 g
Proteins	10.13
Carbohydrates	7.21
Lipids	1.31
Dietary fiber	4.00

Table 3. Mineral composition of *Inula viscosa*

Primary metabolites	% dry matter (leaves) (weight/volume) ± 0.1 g
Ca	4.25
P	3.35
K	9.55
Na	1.64
Cl	1.36
S	1.76
Mg	11.55
Fe	11,18
Mn	5.54
Zn	3,15
Pb	1,74
Se	1.39
Cu	1,34
Co	3.55

Table 4. Content of amino acids in the dry matter of *Inula viscosa*

Primary metabolites	% dry matter (leaves) (weight/volume) ± 0.1 g
Aspartate	0.45
Cystéine	0.34
Glycine	1.04
Histidine	0.33
Isoleucine	1.16
Leucine	2.36
Lysine	0.32
Phénylalanine	2.45
Proline	1.33
Sérine	2.34
Valine	1.16

The results of photochemical analysis demonstrated that *I. viscosa*, contains different micronutriments, as well as a wide variety of minerals compound (Table 3) and also an interesting quantity of amino acids (Table 4). These results demonstrate the richness of this plant in terms of active principle which play an important role in the functioning of the body. In addition to the role that minerals play in plant metabolism, biochemistry and physiology aspects, these elements are essential nutrients for humans and animals. Amino acids play a major role in plants by acting as osmolyte, modulating stomatal penetration, detoxification of heavy metals and also affect activity and synthesis of some enzymes (Rai, 2002).

Secondary metabolites content in extracts of *Inula viscosa*

The secondary metabolites contained in *Inula viscosa* extracts are presented in Table 5. The results obtained from the analysis of secondary metabolites in the *Inula viscosa* extracts revealed varying concentrations of distinct chemical compounds within each extract.

The ethanolic extract displayed notably higher concentrations across several compounds compared to the chloroformic and ethereal extracts. For instance, ethanolic extraction yielded higher levels of coumarins (4.25%), flavonoids (5.45%), tannins (4.13%), saponosides (5.35%), and alkaloids (10.85%) compared to the other extracts. Conversely, the ethereal and chloroformic extracts exhibited lower concentrations across these compounds, indicating a differential efficiency in the extraction process. Ethanolic extract represents the best solvent, which gives the highest value of metabolites. These varying concentrations of secondary metabolites emphasize the importance of the choice of solvent in the extraction procedure and highlight the potential of the ethanolic extract as a rich source of these bioactive compounds.

Table 5. Secondary metabolites percentage in *Inula viscosa* extracts

Chemical compounds	% Inula viscosa extracts		
	Ethereal extract	Chloroform extract	Ethanolic extract
Coumarins	1.34	0.25	4.25
Flavonaoids	3.76	4.00	5.45
Tanins	2.02	2.03	4.13
Saponosids	5.21	4.40	5.35
Alcaloids	10.34	6.58	10.85

Essential oil yield and chemical composition

The yield of the *Inula viscosa* plant is 0.65%. The chromatogram (Figure 1) illustrates the presence of over 80 chemical compounds within the essential oil of *Inula viscosa*. These findings align with international research. For instance, a study conducted in Italy similarly identified 80 chemical compounds (Abdelkader et al., 2020), while another study in Algeria, utilizing GC/MS, detected 23 chemical compounds (Madani et al., 2014).

The analysis of the essential oil (EO) identified 48 compounds, constituting approximately 95% of the essential oil content. Tetrapentacontane emerged as the primary component at 11.26%, followed by Shyobunol (9.9%) and Eicosane (7.26%). Notably, the studied sample displayed a higher concentration of terpenes, sesquiterpene hydrocarbons, oxygenated compounds, diterpenoids, and monoterpene compounds such as Cadinene (2.55%) and alkanes like Hexacontane (3.28%). Additionally, oxygenated sesquiterpene compounds, notably Shyobunol (9.9%), were present in significant quantities.

The predominant constituent in the studied sample, tetrapentacontane (11.26%), contrasts with the compositions reported in other regions. For instance, *Inula viscosa* oil from Turkey predominantly featured monoterpene alcohol (borneol, 38%) (Parolin, 2014), while Spanish samples showcased an allylic tertiary alcohol (Fokienol) as the primary component (Al-Dissi et al., 2001), and Italian variants highlighted a carbonyl bicyclic sesquiterpene (12-carboxy-eudesma-3.11-diene, 60%) as the major constituent (Abdelkader et al., 2020).

Total antioxidant capacity

Figure 2 show the TAC result of the tree extracts. As it can be seen, the chloroform extract has the highest TAC 27.82±1.60 mg/g of extract. while the ethereal and ethanolic extracts have a TAC respectively 19.71±2.78 mg AAE /g and 17.31±3.47 mg AAE /g.

Another study in Sefrou also showed that the ethanolic extract had great antioxidant capacity (Naima Chahmi et al. 2015). Essential oil has a TAC of 108.71±2.16 mg AAE/g. (Qneibi et al., 2021) also demonstrated that the essential oil revealed strong antioxidant activity. However, the results obtained in the present study are very close to those reported by (Jaiswal et al., 2011). Previous studies have focused on the important role of polyphenols in Asteraceae family such as hydroxycinnamic acids (p-coumaric acid, ferulic acid, caffeic acid, and chlorogenic acid) on the antioxidant activity (Silva et al., 2013). In accordance with this results, this plant can serve as a potential source of natural antioxidants which might have benefits for health (Chahmi et al., 2015).

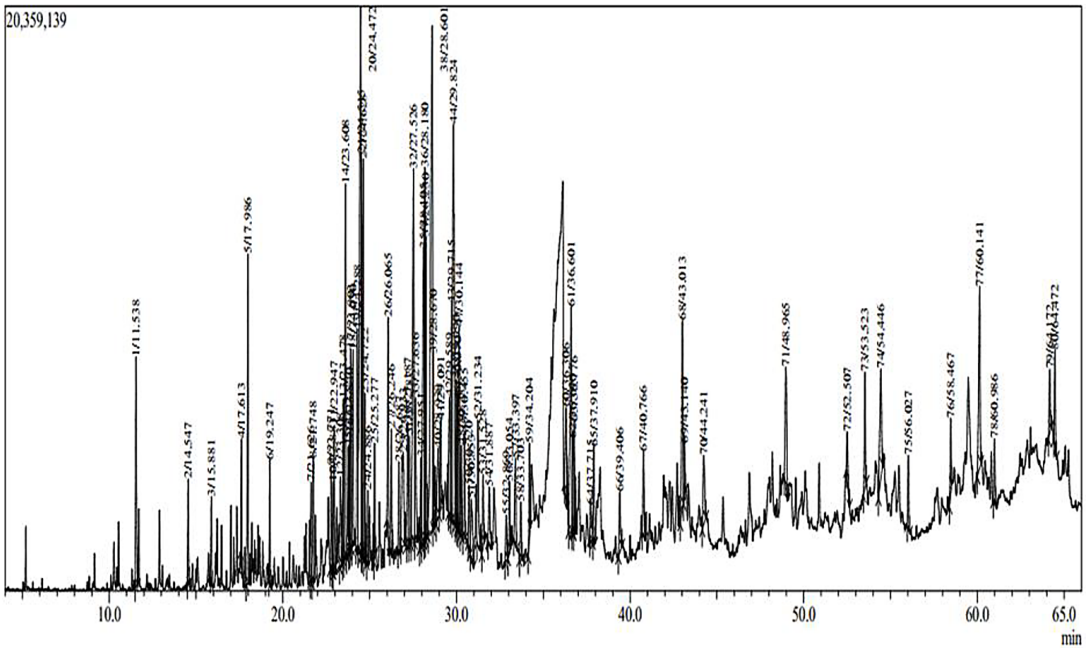

Figure 1. Chromatogram of *Inula viscosa* essential oil

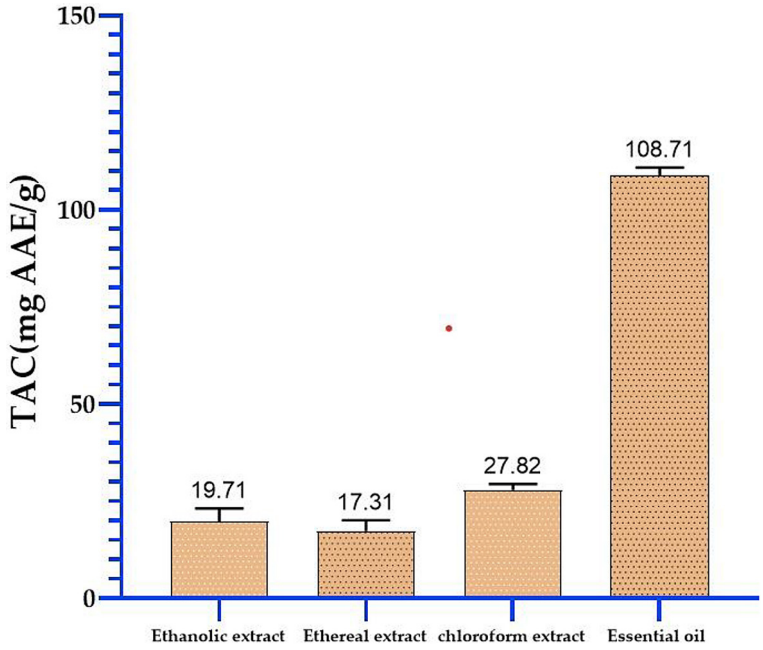

Figure 2. TAC of *Inula viscosa* maceration extracts and essentiel oil

Antimicrobial activity

Disc inhibitory assay

The disc diffusion test evaluated the antibacterial efficacy of the *Inula viscosa* extracts against eight pathogenic strains, Table 6 summarizing the measured diameters of the inhibitory zones (DIZ). The studied samples generally exhibited sensitivity across most strains, showcasing the DIZ values ranging from 7 mm to 16 mm. Notably, the essential oil and chloroform extract displayed superior DIZ against all tested strains, while many strains are resistant to ethereal and ethanol extracts. Upon comparison with common antibiotics, it was observed that several harmful bacterial strains showed resistance to Fluconazole and Ampicillin. Mssillou et al. (2022) also found that the essential oil from *I. viscosa* was active on *S. aureus* (31.0 ± 1.5 mm), *E. coli* (9.5 ± 0.5 mm) and *C. albicans* (20.4 ± 0.5 mm). In turn, another study revealed that the aerial parts of turkish I. viscosa inhibit only Gram-positive bacteria (S. aureus, S. epidermidis and S. pyogenes) with aqueous and methanol extracts (Erva et al., 2019).

Determination of MIC and MLC of the Inula viscosa extracts and essential oils

The minimum inhibitory concentrations (MICs) of the extracts and essential oil of Inula viscosa were determined against eight microbial strains, and the results are presented in Table 7.

Results showed that the chloroform extract are mainly active against all bacteria, with minimum inhibitory concentrations and lethal concentrations against most of the strains tested ranged from 6.25 mg/L to 50 mg/L. However, the ethanolic extract showed limited activity mainly against Staphylococcus aureus, and Fusarium proliferatum**,** while the ethereal extract appears to be more effective against Pseudomonas

Table 6. Diameter of the inhibition zone of the *Inula viscosa* extracts and essential oils (mm)

Sample/strain	Gram-Negative Bacteria			Gram-Positive Bacteria		*Fungus*		
	E. coli	*K. pneumoniae.*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*F. proliferatum*	*A. niger*
Chloroformic extract	13	8	9	9	9	13	7	8
Ethereal extract	0	0	7	0	0	0	7	0
Ethanolic extract	10	0	0	8	0	0	8	0
Essantial oils	16	12	13	16	12	16	13	11
Ampicillin	25	22	0	25	30	0	0	0
Fluconazole	0	0	0	0	0	24	20	24

Table 7. MIC and MLC of the *Inula viscosa* extracts and essential oils (mg/mL)

	Sample/ Strain	Gram-Negative Bacteria			Gram-Positive Bacteria		*Fungus*		
		E. coli	*K. pneumoniae.*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*F. proliferatum*	*A. niger*
Chloroformic extract	MIC	50	25	25	25	25	50	6.25	50
	MLC	50	50	50	25	25	50	12.5	50
Ethanolic extract	MIC	50	0	0	50	0	0	6.25	0
	MLC	50	0	0	50	0	0	12.5	0
Ethereal extract	MIC	0	0	6.25	0	0	0	12.5	0
	MLC	0	0	12.5	0	0	0	12.5	0
Essentiel oils	MIC	3.12	6.25	3.12	3.12	6.25	3.12	3.12	6.25
	MLC	6.25	12.5	6.25	6.25	6.25	6.25	6.25	12.5

aeruginosa and some fungal strains, with lower MICs and MLCs. Essential oils appear to have broad activity against different strains, with generally lower MICs ranged from 3.12 mg/L to 6.25 mg/L, indicating higher overall efficacy, particularly against *Escherichia coli*, *Staphylococcus aureus* and some fungal strains. In another study, Mssillou et al. (2022) found that the essential oil MICs of *I. viscosa* range from 0.1 mg/mL to 3.3 mg/mL. These results suggest that essential oils may be more promising than extracts for antimicrobial activity.

CONCLUSIONS

In this investigation, the comprehensive analysis of *Inula viscosa* from El Menzel – Morocco revealed compelling insights into its chemical composition, antioxidant potency, and antimicrobial capabilities. The conducted phytochemical analyses unveiled the plant's substantial nutritional content, while its impressive antioxidant activity establishes it as a promising source of natural antioxidants with potential applications in the agro-alimentary industry and the treatment or prevention of various human diseases. The congruence between the obtained antioxidant assay results and the traditional usage of this plant bolsters its perceived efficacy and validates its traditional significance. Moreover, the wealth of data derived from this study lays a solid foundation for extensive future research on Inula viscosa, extending its potential utility in diverse fields, including medicinal and cosmetic sciences. This study contributes significantly to the understanding of Inula viscosa properties, opening doors to innovative explorations and practical implementations across multiple scientific realms.

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