Trop J Nat Prod Res, February 2025; 9(2): 653 - 663 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Comprehensive Quality Assessment of *Dittrichia viscosa* (L.) from Southeast Morocco: Physicochemical Properties, Phytochemical Composition, Microbiological Profile and Mineral Content Analysis

Rania Jerada[1]*, Abdelmoula El Ouardi[2], Rachid Ben Aakame[3], Abdeljalil Er-Rakibi[4], Najia Ameur[2], Rim Bougassa[3], Achraf Hamik[6], Nour-Eddine Loud[7], Hanane Benzeid[1], Brahim Mojemmi[1], Anass Doukkali[1]

[1]*Laboratory of Analytical Chemistry, Faculty of Medicine and Pharmacy, Team of Formulation and Quality Control of Health Products, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.*
[2]*Department of Food Microbiology and Hygiene, National Institute of Hygiene. Av. Ibn Batouta, 27, B.P. 769 Rabat, Morocco.*
[3]*Department of Toxicology, Industrial and Environmental Hygiene and Forensic Research-National Institute of Hygiene, Av. Ibn Batouta, 27, B.P. 769 Rabat, Morocco.*
[4]*Computer Science, Artificial Intelligence and Cyber Security Laboratory (2IACS), ENSET Mohammedia, Hassan II University of Casablanca, Casablanca, Morocco.*
[5]*Laboratory of Medicinal Chemistry, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.*
[6]*Laboratory of pharmacognosy, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.*

ARTICLE INFO

Article history:
Received 16 December 2024
Revised 21 December 2024
Accepted 10 January 2025
Published online 01 March 2025

ABSTRACT

Dittrichia viscosa (L.) Greuter, is a perennial herb renowned for its therapeutic properties. The present study aimed to carry out a comprehensive assessment of the quality of *D. viscosa* aerial parts. The aerial parts of *D. viscosa* were sourced from the Ouarzazate region in southeast Morocco. Dried powdered *D. viscosa* aerial parts were extracted using aqueous ethanol, and cyclohexane by various extraction techniques. Essential oil was obtained by hydrodistillation in a Clevenger-type apparatus. The quality of the plant material, including its essential oil was assessed by evaluating various parameters including extraction yields, organoleptic and physicochemical properties, phytochemical composition, mineral content, and microbiological profile. Results showed that the yields of the essential oil and other extracts from *D. viscosa* aerial parts significantly depends on the extraction solvents and techniques used. The hydroethanol extract produced the highest levels of total phenol and total tannin contents, whereas the cyclohexane extract showed the highest total flavonoid content. The organoleptic analysis indicated a slimy, dark yellow oil, with an herbaceous camphoraceous odour The essential oil was predominantly composed of sesquiterpenes, including γ-Muurolene, *α*-Muurolene, and *β*-Cadinene. The quality assessment indices and physicochemical properties suggested a commendable quality of the oil, with properties comparable to or exceeding those of other species within the Asteraceae family. Microbiological analysis showed that *D. viscosa* conformed to WHO standards for herbal medicines, which suggests that the plant is safe for consumption. However, the mineral analysis showed elevated cadmium level, hence the need for caution in its medicinal use.

Keywords: *Dittrichia viscosa*, Quality assessment, Phytochemical analysis, Microbiological analysis, Mineral content.

Introduction

Dittrichia viscosa (L.) Greuter, commonly known in Morocco as "Tarehla", "Magramane", and "Safsag",[1] is a perennial herb extensively found throughout the Mediterranean basin. It thrives in humid areas, on hillslopes, and along roadsides.[2] This plant is renowned for its therapeutic properties, it has been traditionally used in folk medicine to treat a variety of conditions, in which it is used as an antimicrobial, antioxidant, antidiabetic, antihypertensive, analgesic, and anti-inflammatory remedy.

*Corresponding author. Email: rania.jerada@um5r.ac.ma
Tel: +212 6 76 32 78 28

Citation: Jerada R, El Ouardi A, Aakame RB, Er-Rakibi–A, Ameur N, Bougassa R, Hamik A, Loud N, Benzeid H, Mojemmi B, Doukkali A. Comprehensive Quality Assessment of *Dittrichia viscosa* (L.) from Southeast Morocco: Physicochemical Properties, Phytochemical Composition, Microbiological Profile and Mineral Content Analysis. Trop J Nat Prod Res. 2025; 9(2): 653 – 663 https://doi.org/10.26538/tjnpr/v9i2.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

These medicinal properties are attributed to the bioactive compounds present in the plant, including polyphenols, tannins, flavonoids, and other phytochemicals found in essential oils.[3-6]The present study aims to provide a comprehensive assessment of the phytochemical composition of *D. viscosa* using the traditional colorimetric methods alongside GC-MS/GC-FID analysis. Atomic absorption spectroscopy was also employed to elucidate the mineral composition, offering insights into the trace elements and heavy metals profile of the plant. Additionally, microbiological analysis was conducted to ensure the microbiological safety and quality of the plant material. This research represents the first investigation into the organoleptic, physicochemical, and oil quality of *D. viscosa*.

Materials and Methods

Collection and identification of plant material

The aerial parts of *D. viscosa* were collected in May 2022 from the Ouarzazate region in southeast Morocco. The plant was identified by Dr. Ouafae Benkhnigue at the Botany Department of the Scientific Institute of Rabat. A voucher specimen was deposited in the herbarium under the code RAB113637.

Determination of organoleptic and physicochemical properties
Moisture content

The moisture content of the plant material was determined according to standard method.[7] The initial weight (W_f) of the fresh powdered aerial parts of *D. viscosa* was noted. The sample was dried in a ventilated isothermal oven at 130°C under standard atmospheric conditions until a constant weight (W_d) was achieved. The moisture content was calculated using the following formula (Equation 1).

Moisture content (%) = $(W_f - W_d)/W_f$ x 100 ---------- Eq. 1

Ash content

The ash content was determined following standard method.[8] One gram (1 g) of the air-dried aerial part of *D. viscosa* was weighed into a crucible that had previously been dried and tared. The plant sample was then incinerated at 900°C in a muffle furnace (Biobase®) until a light gray ash was obtained or the weight remained constant. The ash content was calculated using the formula shown in equation 2 below.

Ash content (%) = weight of Ash / weight of sample x 100 ---------- Eq. 2

pH and total titratable acidity

The pH of the powdered, air-dried aerial parts of *D. viscosa* diluted tenfold (1:10) in distilled water was measured using a pH meter (HANNA edge®) previously calibrated with buffer solutions of pH 4 and pH 7.[9]

The total titratable acidity of the plant sample was determined by the titrimetric method as previously described.[10] The macerated air-dried aerial parts of *D. viscosa* (10 mL) was titrated with NaOH (0. 1 N). The titratable acidity was expressed in millilitre (mL) of NaOH added per gram of the test sample (meq/g).

Swelling index

The swelling index refers to the volume (in milliliters) occupied by one gram of the plant material after swelling under specified conditions. Dried and finely ground aerial parts of *D. viscosa* (10 g) were placed in a 250 mL glass-stoppered measuring cylinder containing 100 mL of water. The mixture was stirred carefully every 10 minutes for 1 hour. Thereafter, the mixture was allowed to stand for 3 hours at room temperature. The volume (mL) occupied by the plant material including any sticky mucilage present was taken as the swelling index.[11]

Foam index

The pulverized air-dried aerial parts of *D. viscosa* (1 g) was placed in a 250 mL conical flask containing 100 mL of distilled water. This mixture was boiled gently for 30 minutes. Subsequently, the decoction was apportioned into 10 sealed test tubes in incremental volumes of 1 mL to 10 mL, with each volume being adjusted to 10 mL using distilled water. Each tube was agitated continuously for 15 seconds before being left undisturbed for 15 minutes. The height of the foam produced was measured, and the foam index (X) was calculated using the formula below.[11]

$$X = 1000 / \alpha$$ ---------- Eq. 3

Where; α denotes the volume in milliliters of the decoction required to achieve a foam height of 1 cm in the test tube.

Extraction of essential oil from the aerial parts of D. viscosa

The essential oil (EO) extraction was carried out by hydrodistillation using a Clevenger type apparatus according to the method described by Dorsaf *et al*. (2016).[12] Briefly, a quantity, denoted as M_0, equivalent to 100 g of the aerial parts of *D. viscosa* was immersed in distilled water and subjected to hydrodistillation for 3 h. The resulting essential oil (EO) was dried using anhydrous magnesium sulfate ($MgSO_4$) and stored in a glass container, shielded from light, and kept in a refrigerator at +4°C. The essential oil yield was calculated using the following formula:

$$R\ (\%) = \frac{Weight\ of\ the\ essential\ oil\ in\ grams\ (g)}{Weight\ of\ plant\ material\ in\ grams\ (g)} \times 100$$ ---------- Eq. 4

Physicochemical characterization of the essential oil
Acid value

The acid value denotes the quantity of potassium hydroxide, expressed in milligrams, required to neutralize the free acids in 1 g of essential oil. In this procedure, a 2 g sample of the oil was measured and transferred into a 100 mL flask, followed by the addition of 5 mL of 95% ethanol and 5 drops of 0.2% phenolphthalein indicator. The resultant mixture was titrated with 0.1 mol/L of potassium hydroxide until a purple colour was observed. The acid value was calculated using the following formula.[13]

$$\text{Acid value} = \frac{C\ x\ V\ x\ 5.61}{W}$$ ---------- Eq. 5

Where;

V = Volume in milliliters of potassium hydroxide solution used for the titration
C = Exact concentration in moles per liter of the potassium hydroxide solution
W = Weight in grams of the test sample.

Saponification value

The oil sample (2 g) was accurately weighed into a clean, dry conical flask, followed by the addition of 25 mL of alcoholic potassium hydroxide (KOH) solution. The flask was equipped with a reflux condenser, and the mixture was heated for one hour with intermittent shaking. The completion of the saponification process was indicated by the appearance of a clear solution. Subsequently, 1 mL of a 1% phenolphthalein indicator was added, and the excess hot alkali was titrated against 0.5 M hydrochloric acid (HCl) until the endpoint, indicated by a colour change to colorless. A blank titration was carried out concurrently under the same conditions. The saponification value was calculated using the formula below.[14]

$$\text{Saponification value} = \frac{b - a}{W}\ x\ 28.05$$ ---------- Eq. 6

Where;

b = 0.5 N HCl required (mL) by the blank
a = 0.5 N HCl required (mL) by the sample
W = Weight in grams of the test sample.

Ester value

The ester value is the amount of potassium hydroxide, expressed in milligrams, required to neutralize the acids formed through the hydrolysis of esters in 1 g of essential oil. A 2 g sample of the essential oil was accurately weighed and transferred into a conical flask, followed by the addition of 25 mL of potassium hydroxide solution, along with small pieces of pumice stone. The flask was heated for one hour on a heating mantle. After cooling, 20 mL of distilled water was added to the mixture, followed by 5 drops of phenolphthalein indicator. The residual potassium hydroxide was titrated with hydrochloric acid solution. A blank experiment was carried out simultaneously under the same conditions. The ester value was then calculated using the following formula.[15]

$$\text{Ester value} = \frac{28.05\ x\ (V0 - V1)}{W}$$ ---------- Eq. 7

Where;

V_0 = Volume in mL of the HCl solution (0.5 mol/L) used for the blank

V_1 = Volume in mL of the HCl solution (0.5 mol/L) used for the sample the essential oil

W = Weight in grams of the test sample

Iodine value

A 0.2 g sample of essential oil was accurately weighed and transferred into a conical flask. To the sample were added 10 mL of chloroform and 20 mL of Wijs solution. The mixture was allowed to stand in the dark at room temperature for 30 minutes. Subsequently, 15 mL of a 10% potassium iodide solution and 100 mL of distilled water were added to the flask. The resulting solution was titrated with 0.1 M sodium thiosulfate ($Na_2S_2O_3$), using starch mucilage as an indicator, until the

endpoint was reached, indicated by the disappearance of the initial colour. A blank titration was conducted simultaneously, starting with 10 mL of chloroform. The iodine value was calculated using the following formula.[16]

$$\text{Iodine value} = \frac{(B - S)\ x\ N\ x\ 12.69}{W} \quad \text{---------- Eq. 8}$$

Where;

B = 0.1 N sodium thiosulfate required (mL) by blank
S = 0.1 N sodium thiosulfate required (mL) by sample
N = Normality of sodium thiosulfate solution
W = Weight in grams of the test sample

Relative density
A clean and dry 1.8 mL cryotube was weighed using an electronic balance (Mettler Toledo®) with an accuracy of 10^{-1}. The tube was zeroed, and 1 mL quantity of essential oil was added and weighed.[17]

Refractive index
The refractive index of the essential oil sample was determined using an Abbe refractometer®. Two drops of the essential oil were applied onto the surface of the prism using a syringe, and the prism was closed and secured by tightening the screw head. The apparatus was allowed to stabilize for 5 minutes before the refractive index was recorded from the display screen.[18]

Optical rotation
The optical rotation of the EO of *D.viscosa* was measured using a (Schmidt + Haensch ®) Polarimeter with a 2 dm tube at 20 °C and a sodium D-line light source. Specific optical rotation was calculated according to the European Pharmacopoeia. Methanol solutions of the oil at concentrations of 10%, 5%, and 2.5% were prepared, and their optical rotation was recorded. [19]

Quantitative phytochemical analysis of D. viscosa aerial parts

The amount of phytoconstituents in the aerial parts of *D. viscosa* was determined using standard methods. Extracts of the plant material were obtained by hot extraction with distilled water

(both infusion and decoction) and cold maceration using solvents of varying polarity (distilled water, ethanol (70% v/v), and cyclohexane), and used for the analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the aerial parts of *D. viscosa* was determined using the Folin-Ciocalteu colorimetric method.[20] Specifically, 200 µL of each extract was combined with 1000 µL of 10% Folin-Ciocalteu reagent, followed by the addition of 800 µL of 7.5% (w/v) sodium carbonate (Na_2CO_3). The reaction mixture was thoroughly shaken and then incubated in the dark for 30 minutes. The absorbance of the solution was measured at 765 nm against a blank using a UV-Vis spectrophotometer (Peak Instruments® C-7200A). A calibration curve of gallic acid was prepared. The TPC was estimated from the linear regression equation of the calibration curve, and expressed as mg gallic acid equivalent per gram of the extract dry weight (mg GAE/g extract).

Determination of Total Flavonoids Content (TFC)

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric assay.[21] Exactly 500 µL of each extract was added to a test tube containing 500 µL of 2% aluminum chloride ($AlCl_3$) solution. The reaction mixture was incubated at room temperature for 1 h, after which the absorbance was measured at 420 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A). A calibration curve of quercetin was prepared. The TFC was estimated from the linear regression equation of the quercetin calibration curve, and expressed as mg quercetin equivalent per gram of the extract dry weight (mg QE/g extract).

Determination of Total Tannins Content (TTC)

The total tannin content was quantified following the method described by Julkunen-Tiitto (1985).[22] A volume of 50 µL of each extract was combined with 1500 µL of a 4% methanol vanillin solution and 750 µL of concentrated hydrochloric acid (37%). The mixture was allowed to stand for 20 minutes at room temperature, after which the absorbance was measured at 500 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A), with methanol serving as the blank. Catechin was used as the reference standard, and the tannin content was expressed in milligrams of catechin equivalent per gram of extract (mg CE/g extract).

Chromatographic analysis of the essential oil using GC-MS/GC-FID
The essential oil was prepared for GC-MS analysis following the AOCS method.[23] A 0.60 g quantity of the oil was weighed into screw-capped test tube of 5 mL capacity, followed by the addition of 4 mL of iso-octane to dissolve the oil. Subsequently, 0.20 mL of a 2 N KOH solution in methanol was added to the tube. The tube, equipped with PTFE-sealed cap, was vigorously shaken to ensure proper mixing. After phase separation, the upper layer containing the methyl esters was isolated and further extracted with hexane. For gas chromatographic analysis, 1 µL of the extract was injected into a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID). The system consisted of a CP-Wax 52 CB column (30 m in length, 0.25 mm internal diameter) with helium as the carrier gas at a flow rate of 1 mL/min. The column temperature was initially set at 170°C and gradually increased to 230°C at a rate of 4°C/min. The injector and detector temperatures were maintained at 230°C. Data analysis was performed using Varian Star Workstation v6.30 (Varian Inc., Walnut Creek, CA, USA).[23]

Determination of mineral content of D. viscosa aerial parts
The concentrations of metallic elements of *D. viscosa* aerial parts were determined using atomic absorption spectrophotometer (Varian GTA 120 Graphite Tube Atomizer paired with a PSD 120) with plasma spectroscopy detector. Before analysis, the powdered aerial parts of *D. viscosa* was incinerated with 4 mL of nitric acid in an oven at 200°C. After complete mineralization, the solution was filtered through Whatman filter paper. A blank solution of nitric acid was prepared. These solutions were then analyzed using atomic absorption spectroscopy to determine the concentrations of nickel (Ni), chromium (Cr), cadmium (Cd), copper (Cu), and lead (Pb). The concentrations of the metals were determined based on calibration curves established from standard solutions.[24]

Microbiological analysis
Dilution
Twenty-five grams (25 g) each of three replicate samples were placed into Stomacher BagMixer interscience® Sterile Pouches. A 100 mL volume of buffered peptone water (Ref OXOID - 2435555) was added to each pouch. The contents were then mixed for 2 minutes, followed by a series of dilutions according to ISO 6887-1:2017.[25]

A. Enumeration of hygienic flora
Total aerobic mesophilic flora (FMAT)
The Total Aerobic Mesophilic Flora (FMAT) enumeration was performed after suitable dilutions of the sample in water broth buffered peptone (Ref OXOID – 2435695). The sample was subsequently inoculated on Plate Count Agar (PCA) (Ref OXOID - 2435555) and incubated at 30°C for 72 h (ISO 4833-1:2013).[26]

Total and faecal coliforms
Total coliform enumeration was conducted on Crystal Violet Neutral Red Lactose Bead (VRBL) Agar (Ref OXOID - 2861995). It was incubated at 30°C for total coliforms and 44°C for faecal coliforms. After 24 h of incubation, the red colonies were counted (NF V08-050).[27]

B. Enumeration of toxigenic and pathogenic flora
Staphylococcus aureus
Staphylococcus aureus enumeration was conducted on Baird Parker medium (Ref OXOID 3410579). The samples were incubated at 37°C for 24 h (ISO 6888-1:2021).[28]

Salmonella spp

For *Salmonella spp* detection, pre-enrichment was done using buffered peptone water (Ref OXOID 1847595) for 12 h at 37°C. This was followed by an enrichment using Rappaport Vassiliadis Soya peptone broth (RVS) (Ref OXOID 2341537) for 24 h at 37°C. Enumeration and isolation were then done on Xylose Lysine Desoxycholate Agar (X.L.D) medium (Ref OXOID 2911881) after a 24 h incubation at 37°C (ISO 6579-1:2017).[29]

Bacillus cereus

Bacillus cereus was counted on the *Bacillus cereus* selective agar base (MYP) (Ref OXOID 2160195) after incubating at 37°C for 24 h (ISO 7932:2004).[30]

Clostridium perfringens

Clostridium perfringens enumeration was done on Tryptone-Sulfite Cycloserine (TSC) Agar (Ref OXOID 2435720) and incubated at 46°C for 24 h (ISO 15213-2:2023).[31]

Yeasts and molds

Yeasts and molds were enumerated on Sabouraud dextrose agar (Ref OXOID 2393644) after incubation at 30°C for 24 h (NF V08-059).[32]

Statistical analysis

Each experiment was conducted in triplicate, with the results expressed as the mean ± standard deviation of three independent measurements. Statistical analyses were carried out using Microsoft Excel 2016®.

Results and Discussion

Extraction yields

The yields for the essential oil and other solvent extracts from the aerial parts of *D. viscosa* are presented in Table 1. For the essential oil, a percentage yield of 0.085% was obtained following hydrodistillation. This yield falls within the medium yield range compared to the yields previously reported by other researchers. For example, a percentage yield of 0.05% was obtained for the essential oil of *Inula viscosa*,[33] while a percentage yield of 0.15% has been reported for the essential oil of Tunisian *D. viscosa*.[34] A much higher yield of 0.23%, has also been reported for the essential oil of *Inula viscosa*.[35] The variations in the essential oil yields can be attributed to differences in geographical regions where the plant is cultivated. For instance, the study of Nadia *et al*. (2022) reported yields ranging from 0.05% to 0.20% for the essential oil of *D. viscosa* from various Algerian locations.[36]

Table 1: Extraction conditions and yields of *D. viscosa* extracts

Extract Code	Extraction method	Solvent used	Yield (%)
E_1	Hydrodistillation (3 h)	Distilled water	0.085
E_2	Maceration (24 h)	Distilled water	19.5 ± 0.200
E_3	Maceration (24 h)	Distilled water/Ethanol (80 :20)	35.167 ± 2.444
E_4	Maceration (24 h)	Cyclohexane	9,386 ± 0,127
E_5	Infusion (30 min)	Distilled water	26.267 ± 2.756
E_6	Decoction (1 h)	Distilled water	25.183 ± 1.511

E_1: Essential oil, **E_2:** Macerated water extract; **E_3**: Hydroethanol extract; **E_4**: Cyclohexane extract; **E_5**: Infused water extract; **E_6**: Decocted water extract.

Essential oil yield is also affected by the extraction method, as well as duration of extraction. For example, the application of microwave-ultrasonic agitation has been shown to enhance essential oil yield.[37]The yields for the other extracts varied depending on the solvent used and the extraction method employed. The highest yield of 35.16% was achieved by hydroalcoholic maceration over 24 hours. Conversely, the lowest yield of 9.38% was obtained from 24-hour maceration in cyclohexane. A moderate yield of 19.6% was achieved by aqueous maceration for 24 hours. On the other hand, yields of 26.26% and 25.18% were obtained from infusion in water for 30 min and decoction for one hour, respectively. These yields are consistent with results obtained from previous studies. For example, a yield of 22.5% from the maceration of *Inula viscosa* leaves in methanol for 24 hours.[38] Comparable results of 22.5% and 21% were reported for the 72-hour maceration of *D. viscosa* leaves in acetone and ethanol, respectively.[39] The variability in the extraction yields have been shown to be influenced by the polarity and the nature of the solvents, as well as the extraction technique used.[40, 41]

Organoleptic and physicochemical properties of D. viscosa essential oil and dry matter

The essential oil derived from the aerial parts of *D. viscosa* exhibited organoleptic and physicochemical characteristics indicative of its quality. The results obtained for these properties are summarized in Table 2. The essential oil showed a slimy appearance with an herbaceous camphoraceous odour and a dark yellow colour. Similar studies in Morocco reported yellow and light-yellow colours for the essential oils of *Inula viscosa* and *D. viscosa*, respectively.[33, 34] These properties suggest that the essential oil of *D. viscosa* has unique properties that can be recognizable by sensory evaluation. To the best of our knowledge, this is the first report on the physicochemical properties of *D. viscosa* essential oil and dry matter. The essential oil had an acid value of 12.05 mg KOH/g. Acid value is a measure of the oil rancidity, with higher levels of free fatty acids indicating reduced quality.[42] Zekri *et al*. (2023) reported acid values between 21.91 and 28.07 mg KOH/g for three *Mentha* species in Morocco,[43] while a value of 0.5945 mg KOH/g was noted for *Eucalyptus globulus* in Algeria.[19] The saponification value was found to be 30.8 mg KOH/g, which was lower than values obtained for six other *Asteraceae* species, which ranged from 180.70 mg KOH/g oil for *milk thistle* and 221.38 mg KOH/g oil for *safflower* seed.[44] The ester value which is the difference between the saponification value and the acid value was found to be 27.97 mg KOH/g. The iodine value, which is a reflection of the number of double bonds and the level of unsaturation of the fatty acids,[43] was found to be 62.836 g I_2/100 g oil. This is lower than the iodine values obtained for *Mentha* and *Asteraceae* species, but slightly higher than that of *Eucalyptus globulus*.[4, 19, 43, 44] These indices suggest that *D. viscosa* essential oil quality surpasses that of *Mentha* and *Asteraceae* species, but inferior to that of *Eucalyptus globulus*.

For the physical properties, *D. viscosa* essential oil obtained in this study had a relative density of 0.80 g/cm³, which falls within the range found for other *Asteraceae* species (0.79 to 0.87 g/cm³), but less than that of *Eucalyptus globulus* (0.919 g/cm³).[19, 44] On the other hand, the observed refractive index at 26.6°C was higher for *D. viscosa* essential oil (1.5137) compared to that of *Eucalyptus globulus* essential oil (1.46933).[19] The optical rotation of *D. viscosa* essential oil was -3.5°, which is in contrast with the value (+1.5956°) reported for *Eucalyptus globulus* essential oil.[19]

With respect to the physicochemical properties of *D. viscosa* dry plant material, the moisture content was recorded as 57.52%. This value is higher than the value obtained for *Mentha* species in Morocco, which ranged from 16 to 46%.[43] The higher moisture content may indicate a shorter shelf life for *D. viscosa* dry matter, as microorganisms tend to thrive in environments with high moisture content. The total ash content was found to be 23.80%, exceeding the ranges reported for *Mentha* species in previous study (11.25% – 21.87%). Total titratable acidity of 0.025 mol/L and a pH of 5.66 were recorded for *D. viscosa* dry matter. The pH value is comparable to values obtained for *Mentha* species in Morocco (5.45 - 5.95), but less than the value obtained for *Eucalyptus globulus* (pH = 4.9).[19, 43]

Table 2: Organoleptic and physicochemical properties of *D. viscosa*

	Parameter	Observation/Value
Organoleptic properties of the EO	Colour	Dark yellow
	Odour	Herbaceous, Camphoraceous
	Appearance	Slimy
Chemical Properties of the EO	Acid value	12.05 mg KOH/g
	Saponification value	30.86 mg KOH/g
	Ester value	18.81 mg KOH/g
	Iodine value	62.836 g I/100 g oil
Physical Properties of the EO	Relative density	0.800 g/cm³
	Refractive index	1.5137, 26.6°C
	Optical rotation	-3.5°
Physicochemical Analysis of the dry matter	Moisture content	57.52 %
	Total ash content	23.80 ± 5.01%
	pH	5.66 ± 0.026
	Total titratable acidity	0.025 mol/L
	Swelling index	305.0 mL
	Foam index	107.407 ± 4.938

EO: Essential Oil.

Furthermore, the swelling index was found to be 305 mL, which is significantly higher than the value (7 mL) recorded for other *Asteraceae* species like *Parthenium hysterophorus.*[45]

The foam index for *D. viscosa* dry matter was recorded as 107.40. The foam index is an indicator of the presence of saponins, which are responsible for the formation of persistent foam when an aqueous decoction is agitated. The foam index obtained for *D. viscosa* in the present study is comparable to the value (100) obtained for *Parthenium hysterophorus*, but significantly lower than that of *Blumea malcolmii* Hook f. with a foam index of 2000.[45, 46]

This study serves as an initial investigation into the physicochemical and the quality properties of *D. viscosa* essential oil and dry matter. Further studies are necessary to deepen the understanding of these properties and facilitate more comprehensive comparisons.

Phytochemical constituents of D. viscosa aerial parts

The quantification of Total Polyphenol Content (TPC), Total Flavonoid Content (TFC), and Total Tannin Content (TTC) of the hydroethanol and cyclohexane extracts of *D. viscosa* aerial parts was performed, and the values were estimated from the calibration curves of gallic acid, quercetin, and catechin, respectively. As indicated in Table 3, the hydroethanol extract exhibited the highest total polyphenol and total tannin contents, while the cyclohexane extract demonstrated the highest flavonoid content. The total polyphenol content in the hydroethanol extract was 219.68 mg GAE/g extract, this was comparable to the findings of Qneibi *et al*. (2021),[37] for the same solvent. Using cyclohexane, the TPC was recorded as 99.71 mg GAE/g extract. The findings from the present study for the hydroethanol extract surpasses those from studies in Morocco, Algeria, Palestine and Turkey, which found TPC ranging from 85 to 180 mg GAE/g extract, using different solvents such as methanol, ethanol, acetonitrile and ethyl acetate.[39, 47-51]

Table 3: Total polyphenols, flavonoids, and condensed tannins contents of *D. viscosa*

Extract	TPC (mg GAE/g DW)	TFC (mg EQ/g extract)	TTC (mg EC/g extract)
Hydroethanol	219.68 ± 0.16	47.07 ± 0,1	31.8 ± 0.1
Cyclohexane	99.71 ± 0.32	61.20 ± 0,61	6.0 ± 0.1

TPC: Total Phenolic Content; **TFC:** Total Flavonoid Content; **TTC**: Total Tannin Content

For the total flavonoid content, the cyclohexane extract showed the highest content of 61.20 mg QE/g extract, which was compared to 47.07 mg QE/g extract for the hydroethanol extract. The TFC obtained in the present findings are higher than the TFC reported in previous studies for the methanol, ethanol, acetonitrile and diethyl ether extracts, which had TFC ranging from 30.02 to 32 mg QE/g extract.[2, 39, 50, 52] Conversely, TPC reported for ethyl acetate (74 mg QE/g extract) was higher than that obtained in previous study.[52]

The present study showed that the hydroethanol extract contained five times more tannins (31.8 mg CE/g extract) than the cyclohexane extract, which had total tannin content of 6 mg CE/g extract. In the study of Rhimi *et al*. (2017), TTC ranging between 7.05 and 27.15 mg CE/g extract were reported for the methanol, ethanol, 80% ethanol, and butanol extracts of *D. viscosa* leaves, with the methanol extract having the highest TTC.[50] Another study reported TTC of 109.19 and 127.57 mg AAE/g for the acetonitrile and ethanol extracts of *D. viscosa* leaves, respectively.[39]

The variability in the concentrations of phytochemical compounds among the different extracts can be attributed to the influence of the extraction solvent, the parts of plant used, the location of plant collection, the climatic conditions, soil characteristics and the season of harvest of the plant material.[6, 37, 51]

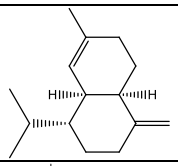
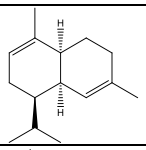
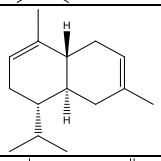
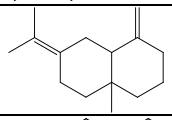
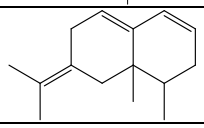
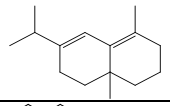
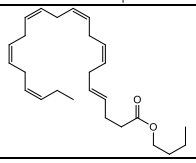
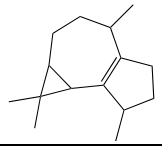
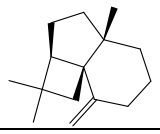
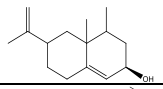
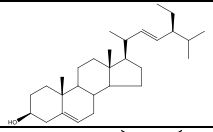
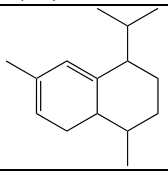
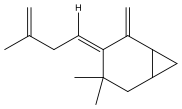
Chemical constituents of the essential oil of D. viscosa aerial parts

The study investigated the chemical composition of the essential oil derived from the hydrodistillation of *D. viscosa* aerial parts using Gas Chromatography Mass Spectrometry (GC-MS) alongside Gas Chromatography with Flame Ionization Detection (GC-FID). The results of the chromatographic analysis showed a variety of volatile compounds in *D. viscosa* essential oil (Figure 1, Table 4).

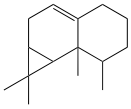
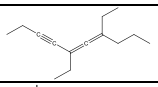
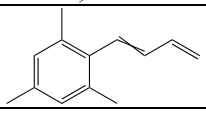
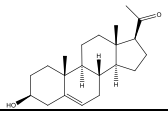
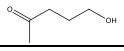
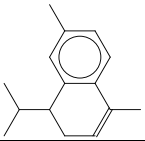
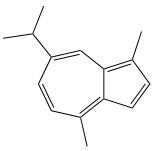
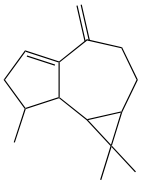
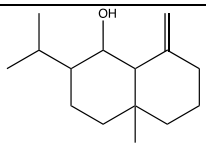
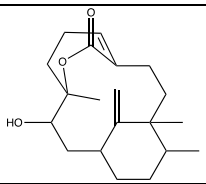
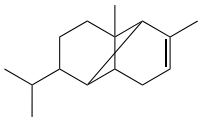
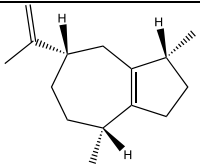
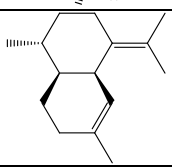
A total of twenty-six (26) compounds were identified, accounting for 93.41% of the total mass of the oil. The most predominant compounds are sesquiterpenes representing 81.529% of the total composition. This group included notable compounds such as γ-Muurolene (24.124%), α-Muurolene (17.957%), and *β*-Cadinene (7.377%), among others, which are known for their robust aromatic profiles and potential therapeutic properties.[53] Monoterpenes, although in a significantly lower concentration, contributed 1.823% to the oil's composition, with 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4,1,0]heptane being a representative compound. Steroids, including Stigmasterol and Pregnenolone, constituted 3.760% of the oil, suggesting a potential for diverse biological activities.[54]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Chemical composition of essential oil of *D. viscosa* aerial parts

S/N	Compound Name	RT (min)	Abundance (%)	Structure
1	γ-Muurolene	42.186	24.124	
2	*α*-Muurolene	41.809	17.957	
3	*β*-Cadinene	38.227	7.377	
4	γ-selinene	42.283	6.709	
5	*β*-vatirenene	43.674	6.187	
6	δ-Selinene	41.235	5.329	
7	Butyl 4,7,10,13,16,19-docosahexaenoate	40.261	3.256	
8	Isoledene	41.909	3.244	
9	*β*-Panasinsene	41.976	2.896	
10	*β*-Nootkatol	41.436	2.566	
11	Stigmasterol	39.087	2.536	
12	Cis-Muurola-3,5-diene	38.023	2.006	
13	4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4,1,0]heptane	44.141	1.823	

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

14	(-)-Aristolene	37.533	1.410	
15	5,6-Decadien-3-yne, 5,7-diethyl-	40.138	1.312	
16	Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	39.531	1.278	
17	Pregnenolone	40.935	1.224	
18	2-Pentanone, 4-hydroxy-4-methyl	12.323	1.128	
19	*α*-Calacorene	38.921	1.053	
20	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	42.536	0.927	
21	Aromadendrene, dehydro-	41.075	0.921	
22	Junenol	41.676	0.900	
23	Cleomeolide	38.087	0.736	
24	*α*-Copaene	38.71	0.648	
25	*α*-Guaiene	40.842	0.568	
26	Cis-muurola-4(14),5-diene	36.826	0.504	
	Total composition		**93.415**	

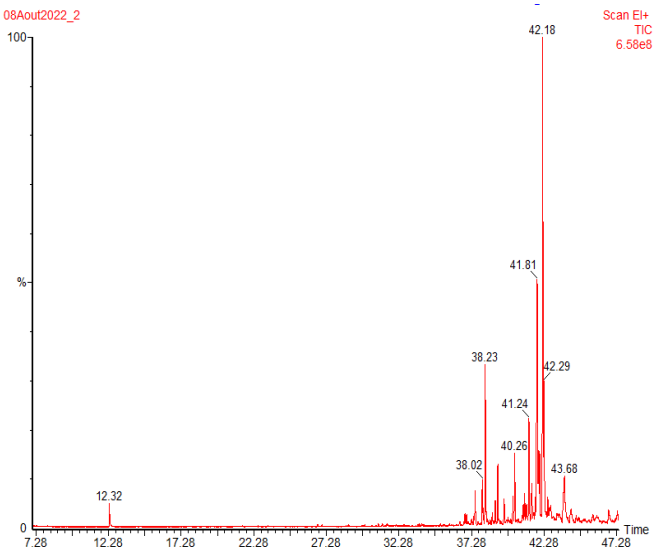


Figure 1: Chromatogram of the essential oil of *D. viscosa* analyzed by GC-MS/GC-FID

The presence of a fatty acid ester, Butyl 4,7,10,13,16,19-docosahexaenoate, was also notable, comprising 3.256% of the oil, indicative of its potential contribution to the oil's functional properties. Alkynes and aromatic hydrocarbons were present in smaller amounts, accounting for 1.312% and 1.278% of the oil's composition, respectively, with 5,6-Decadien-3-yne, 5,7-diethyl- and Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl- being the predominant compounds. Ketones, specifically 2-Pentanone, 4-hydroxy-4-methyl, constituted 1.128% of the oil. Other compounds, including *β*-Nootkatol, Azulene, Junenol, and Cleomeolide, collectively accounted for 5.129% of the oil.

Many other studies that investigated the phytochemical profile of *D. viscosa* confirmed the dominance of sesquiterpenes in its essential oil composition. Nevertheless, significant differences have been noted regarding the compounds found.[4, 5, 34, 36] Nadia *et al*. (2022) reported the presence of nineteen compounds in the essential oil of *Inula viscosa*, where sesquiterpenes represented 87.3% of the total oil composition. The major compounds in this category were α-bisabolol (16.0%), (E)-Z-Farnesylacetone (13.2%), (E)-nerolidol (15.5%), α-Cadinol (11.6%), Caryophyllene oxide (10.6%), and τ-Muurolol (9.8%).[36] A similar study also reported sesquiterpenes as the major component of *Inula viscosa* essential oil, accounting for 75% of the oil, with the primary compounds being isocostic acid (56.83%) and Fokienol (14.60%).[4] Oxygenated sesquiterpenes such as (E)-nerolidol has been reported as a predominant component of *Inula viscosa* essential oil, representing 40.7% of the oil.[34]

The variation in the essential oil composition may be attributed to several factors including geographical location, cultivation conditions, extraction methods, plant parts used, and analytical techniques employed. This diverse phytochemical profile underscores the complex nature of the essential oil of *D. viscosa*, highlighting its potential for various applications in aromatherapy, pharmacology, and natural product research.

Mineral content of D. viscosa aerial parts

The World Health Organization (WHO) categorizes mineral elements into three groups based on their significance and/or toxicity to the human body. The first group consists of essential trace elements, including chromium, copper, zinc, selenium, molybdenum, and iodine, which are crucial for various physiological functions. The second group comprises trace elements that are potentially essential, such as manganese, silicon, nickel, boron, and vanadium. The third category includes elements that are potentially toxic, some of which may have essential roles, such as fluoride, lead, cadmium, mercury, arsenic, aluminum, lithium, and tin.[55] The study of the mineral content of the aerial parts of *D. viscosa* was conducted using atomic absorption spectroscopy. The contents are expressed as the mean values in mg/kg, as shown in Table 5.

Table 5: Mineral content of *D. viscosa* aerial parts

Element	Concentration (mg/kg)
Lead (Pb)	0.33
Cadmium (Cd)	3.11
Nickel (Ni)	1.405
Copper (Cu)	2.65
Chromium (Cr)	0.069

The concentrations of potentially toxic elements such as lead (Pd), and Cadmium (Cd) were found to be 0.33 mg/kg, and 3.11 mg/kg, respectively. The WHO sets the limits for toxic metals in herbal medicines at 10 mg/kg for lead (Pb) and 0.3 mg/kg for cadmium (Cd). Hence, the concentration of cadmium in *D. viscosa* aerial parts was outside the acceptable limit.[56] The concentrations of other elements were 1.405 mg/kg for nickel (Ni), 2.65 mg/kg for copper (Cu), and 0.069 mg/kg for chromium (Cr). In contrast to the findings from this study, a study on the mineral content of *D. viscosa* leaves collected in Morocco detected no cadmium in the plant material, while the concentrations of copper and chromium were higher, with values of 12.69 mg/kg, and 0.43mg/kg, respectively.[57] Significant variations were also observed in the concentrations of other minerals such as calcium, potassium, magnesium, sodium, phosphorus, iron, cobalt, manganese, zinc, and selenium.[57]

The disparity in trace element concentrations between the present study and that previously reported may be ascribed to differences in environmental conditions, geographical location, and the parts of the plant used. Nevertheless, soil contamination also plays a significant role in the mineral composition of *D. viscosa*. Two studies conducted in Spain and Italy showed that samples of *D. viscosa* collected in areas close to mines had significantly higher concentrations of heavy metals compared to the samples collected from unpolluted areas.[58, 59]

Microbiological profile of D. viscosa aerial parts

To ensure the microbiological safety of food products and herbal medicines, various sets of regulatory standards have been established by organizations such as The World Health Organization and Pharmacopeias from different countries. As depicted in Table 6, the microbiological examination of the dried powder obtained from the aerial parts of *D. viscosa* was conducted. The results demonstrated an exemplary microbiological profile that complies with the microbiological quality standards set by the WHO and international pharmacopeias. The microbiological analysis of the sample indicated the absence of total coliforms, *Escherichia coli*, *Salmonella*, *Bacillus cereus*, and *Staphylococcus aureus*. Other microorganisms such as FMAT, sulfite-reducing *Clostridia*, yeasts, and molds showed counts below the permissible thresholds (colony-forming units per gram (UFC g^{-1}). These findings align with the WHO guidelines for permissible limits of microbial contamination in herbal medicines intended for internal use.[56] Such results imply a favorable safety profile for *D. viscosa*. The absence of harmful or pathogenic microorganism from *D. viscosa* samples used in this study, is a reflection of the hygienic condition of the plant's growth environment, and the hygiene practices during cultivation, harvesting and production processes[56, 60] To the best of our knowledge, this study appears to be the first to investigate the microbiological quality of *D. viscosa*, underscoring the need for similar studies to compare the resulting microbiological profiles.

Table 6: The microbiological quality of the analyzed *D. viscosa*

Microorganism	Sample 1	Sample 2	Sample 3	Reference value
Mesophilic flora (FMAT)	≤ 1	≤ 1	≤ 1	< 5 x 10^5 UFC⁄g
Escherichia coli	Abs	Abs	Abs	< 10 UFC⁄g
Total Coliforms	Abs	Abs	Abs	< 1 x 10^3 UFC⁄g
Staphylococcus aureus	Abs	Abs	Abs	< 1 x 10^2 UFC⁄g
Salmonella spp	Abs	Abs	Abs	Absence per 1 g
Bacillus cereus	Abs	Abs	Abs	< 1 x 10^3 UFC⁄g
Sulfite-Reducing Clostridia	≤ 1	≤ 1	≤ 1	< 1 x 10^2 UFC⁄g
Yeasts and molds	≤ 1	≤ 1	≤ 1	< 1 x 10^3 UFC⁄g

Abs: absence

Conclusion

This study provided a comprehensive assessment of essential oil, dry matter, and various extracts derived from *D. viscosa* collected in southeast Morocco. Insights were gained into the extraction yield, physicochemical properties, phytochemical and mineral compositions, as well as the microbiological profile of the plant. The results indicated that the yield and phytochemical content significantly depended on the choice of solvent used for the extraction, with the hydroethanol extract having the highest amounts of phenolics and tannins. Comparative analysis suggests that the yield and phytochemical profiles of the plant are also influenced by geographic location, climatic conditions, harvest season, and extraction method. GC-MS/GC-FID analysis showed that the essential oil of *D. viscosa* is predominantly composed of sesquiterpenes, with γ-Muurolene, *α*-Muurolene, and *β*-Cadinene as the principal components. This investigation marks the beginning of a detailed exploration into the sensory and physicochemical attributes of *D. viscosa*, highlighting the need for further research to enhance the understanding of its quality attributes. The microbiological analysis has shown that *D. viscosa* meets the WHO guidelines for microbial quality, confirming its safety for use in food products and herbal medicines. However, the mineral content analysis revealed a high cadmium level, suggesting the need for careful monitoring and sourcing of the plant material.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Boulos L. Medicinal plants of North Africa. Medicinal plants of North Africa. 1983.
2. Brullo S, de Marco G. Taxonomical revision of the genus Dittrichia (Asteraceae). Port Acta Biol. 2000; 19(1):341-54.
3. Mssillou I, Agour A, Allali A, Saghrouchni H, Bourhia M, El Moussaoui A, Salamatullah AM, Alzahrani A, Aboul-Soud MAM, Giesy JP, Lyoussi B, Derwich E. Antioxidant, antimicrobial, and insecticidal properties of a chemically characterized essential oil from the leaves of *Dittrichia viscosa* L. Molecules. 2022;27(7):2282.
4. Madani L, Derriche R, Haoui IE. Essential oil of Algerian Inula viscosa leaves. J Essent Oil-Bear Plants. 2014; 17(1):164-168.
5. Haoui IE, Derriche R, Madani L, Oukali Z. Analysis of the chemical composition of essential oil from Algerian Inula viscosa (L.) Aiton. Arab J Chem. 2015; 8(4):587-590.
6. Jerada R, Er-Rakibi A, Cherkani Hassani A, Benzeid H, El Ouardi A, Harhar H, Goh BH, Yow YY, Ser HL, Bouyahya A, Mojemmi B, Doukkali A. A comprehensive review on ethnomedicinal uses, phytochemistry, toxicology, and pharmacological activities of *Dittrichia viscosa* (L.) Greuter. J Tradit Complement Med. 2024;14(4):355-380.
7. AFNOR. NF EN ISO 712 Cereals and cereal products - Determination of moisture content - Routine reference method. AFNOR Editions; 2010.
8. AFNOR. NF EN ISO 2171 Cereals, pulses and by-products - Determination of ash yield by incineration.: AFNOR Editions; 2010.
9. Horwitz W, Latimer GW. Official methods of analysis of the Association of Official Analytical Chemists. . 18 ed. Gaithersburg: AOAC International; 2005.
10. Lecoq R. Manuel d'analyses alimentaires et d'expertises usuelles. 1965.
11. WHO. Quality control methods for medicinal plant materials: World Health Organization; 1998.
12. Dorsaf BH, Hanen BI, Chokri J, Larbi KM, Manef A. Chemical composition of some Tunisian Eucalyptus essential oils as obtained by hydrodistillation using Clevenger type apparatus. Biosci Biotechnol Res Asia. 2016; 7(2):647-656.
13. ISO. NF ISO 1242 Essential oils - Determination of acid value by two titration methods, manual and automatic.: AFNOR Editions; 2023.
14. ISO. ISO 3657:2023 Animal and vegetable fats and oils Determination of saponification value. 2023.
15. ISO. ISO 706:2001 Essential Oils - Determination of Ester Value. 2001.
16. ISO. ISO 3961:2018 Animal and vegetable fats and oils Determination of iodine value. 2018.
17. ISO. NF ISO 279 Essential oils. Determination of relative density at 20 °C — Reference method. AFNOR Editions; 1999.
18. ISO. NF EN ISO 6320 Animal and vegetable fats and oils - Determination of refractive index. AFNOR Editions; 2017.
19. Boukhatem MN, Amine FM, Kameli A, Saidi F, Walid K, Mohamed SB. Quality assessment of the essential oil from Eucalyptus globulus Labill of Blida (Algeria) origin. Int Lett Chem Phys Astron. 2014; 17(3):303-15.
20. Poh-Hwa T, Yoke-Kqueen C, Indu Bala J, Son R. Bioprotective properties of three Malaysia Phyllanthus species: An investigation of the antioxidant and antimicrobial activities. Int Food Res J. 2011; 18(3).

21. Ordonez A, Gomez J, Vattuone M. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem. 2006; 97(3):452-8.
22. Julkunen-Tiitto R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J Agric Food Chem. 1985; 33(2):213-7.
23. AOCS. AOCS (ed) Official methods & recommended practices of the American Oil Chemists Society. Champaign, IL1993.
24. Paul B, Chanda S, Das S, Singh P, Pandey B, Giri S. Mineral assay in atomic absorption spectroscopy. The beats nat sci. 2014; 4(1):1-17.
25. ISO. ISO 6887-1:2017 Microbiology of the food chain Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions2017.
26. ISO. ISO 4833-1:2013 Microbiology of the food chain Horizontal method for the enumeration of microorganisms Part 1: Colony count at 30 °C by the pour plate technique 2013.
27. AFNOR. NF V 08-050 Microbiology of food and animal feeding stuffs - Enumeration of presumptive coliforms by colony-count technique at 30 °C. AFNOR Editions; 2009.
28. ISO. ISO 6888-1:2021 Microbiology of the food chain Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1: Method using Baird-Parker agar medium2021.
29. ISO. ISO 6579-1:2017 Microbiology of the food chain Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp2017.
30. ISO. ISO 7932:2004 Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of presumptive Bacillus cereus. Colony-count technique at 30 degrees C2004.
31. ISO. ISO 15213-2:2023 Microbiology of the food chain Horizontal method for the detection and enumeration of Clostridium spp. Part 2: Enumeration of Clostridium perfringens by colony-count technique2023.
32. ISO. NF V08-059 Microbiology of food animal feeding stuffs - Enumeration of yeasts and moulds by colony-count technique at 25 °C - Routine method. AFNOR Editions; 2002.
33. Aissa I, Nimbarte VD, Zardi-Bergaoui A, Znati M, Flamini G, Ascrizzi R, Jannet HB. Isocostic Acid, a Promising Bioactive Agent from the Essential Oil of *Inula viscosa* (L.): Insights from Drug Likeness Properties, Molecular Docking and SAR Analysis. Chem Biodivers. 2019;16(4):e1800648.
34. Gharred N, Dbeibia A, Falconieri D, Hammami S, Piras A, Dridi-Dhaouadi S. Chemical composition, antibacterial and antioxidant activities of essential oils from flowers, leaves and aerial parts of Tunisian Dittrichia Viscosa. J Essent Oil Res. 2019; 31(6):582-9.
35. Ounoughi A, Ramdani M, Lograda T, Chalard P. Chemotypes and antibacterial activities of Inula viscosa essential oils from Algeria. Biodiversitas. 2020; 21(4).
36. Nadia B, Mesli F, Zahra BF, Merad-Boussalah N, Radja A, Muselli A, Djabou N, Dib MEA. Chemical composition variability and vascular endothelial growth factor receptors inhibitory activity of Inulaviscosa essential oils from Algeria. J Biomol Struct Dyn. 2020:1-19.
37. Qneibi M, Hanania M, Jaradat N, Emwas N, Radwan S. Inula viscosa (L.) Greuter, phytochemical composition, antioxidant, total phenolic content, total flavonoids content and neuroprotective effects. Eur J Integr Med. 2021; 42:101291.
38. Ounaissia K. Total Phenol Content, Antioxidant And Anti-Inflammatory Activities Of Inula Viscosa From Guelma-Algeria. Int J Agric Nat Sci. 2021; 14(3):186-93.
39. Mssillou I, Agour A, Slighoua M, Tourabi M, Nouioura G, Lyoussi B, Derwich E. Phytochemical characterization, antioxidant activity, and in vitro investigation of antimicrobial potential of *Dittrichia viscosa* L. leaf extracts against nosocomial infections. Acta Ecol Sin. 2023;43(1):1–8. doi:10.1016/j.chnaes.2022.12.001.
40. Truong D-H, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. J Food Qual. 2019; 2019.
41. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus coccifera L. and Juniperus phoenicea L. fruit extracts. Food Chem. 2007; 105(3):1126-34.
42. Katkade M, Syed H, Andhale R, Sontakke M. Fatty acid profile and quality assessment of safflower (Carthamus tinctorius) oil at region. J Pharmacogn Phytochem. 2018; 7(2):3581-5.
43. Zekri N, Elazzouzi H, Ailli A, Gourich AA, Radi FZ, El Belghiti MA, Zair T, Nieto G, Centeno JA, Lorenzo JM. Physicochemical Characterization and Antioxidant Properties of Essential Oils of M. pulegium (L.), M. suaveolens (Ehrh.) and M. spicata (L.) from Moroccan Middle-Atlas. *Foods*. 2023;12(4):760.
44. Ghiasy-Oskoee M, AghaAlikhani M. Towards utilizing Asteraceae alternative oilseed species on marginal lands: Agronomic performance, fatty acid composition, oil biocompounds, and oil physicochemical properties of Asteraceae species. J Agric Food Res. 2023; 14:100799.
45. Khaliq HA, Chaudhary BA. Pharmacognostic and phytochemical studies on Parthenium hysterophorus L. J Biomed Pharm Res. 2016; 5(1):65-75.
46. Suryawanshi VR, Yadav HR, Surani HC. Pharmacognostic and phytochemicals evaluation of Blumea species. Phytochemistry. 2021; 26:2079-83.
47. Asraoui F, Kounnoun A, Cacciola F, El Mansouri F, Kabach I, Oulad El Majdoub Y, Alibrando F, Arena K, Trovato E, Mondello L, Louajri A. Phytochemical Profile, Antioxidant Capacity, α-Amylase and α-Glucosidase Inhibitory Potential of Wild Moroccan *Inula viscosa* (L.) Aiton Leaves. *Molecules*. 2021;26(11):3134. doi:10.3390/molecules26113134
48. .Gökbulut A, Özhana O, Satılmiş B, Batçioğlu K, Günal S, Şarer E. Antioxidant and antimicrobial activities, and phenolic compounds of selected Inula species from Turkey. Nat Prod Commun. 2013; 8(4):1934578X1300800417.
49. Mahmoudi H, Hosni K, Zaouali W, Amri I, Zargouni H, Ben Hamida N, Kaddour R, Hamrouni L, Ben Nasri M, Ouerghi Z. Comprehensive phytochemical analysis, antioxidant and antifungal activities of Inula viscosa Aiton leaves. J Food Saf. 2016;36(1):77–88.
50. Rhimi W, Ben Salem I, Immediato D, Saidi M, Boulila A, Cafarchia C. Chemical composition, antibacterial and antifungal activities of crude Dittrichia viscosa (L.) greuter leaf extracts. Molecules. 2017; 22(7):942.
51. Salim H, Rimawi WH, Mjahed A. Analysis of extracts from Palestinian Inula viscosa for their phenolic, flavonoid and lipid contents, antioxidant and antibacterial activity. J Chem Biochem. 2017.
52. El Ouariachi E, Bouyanzer A. Antioxidant activity of solvent extracts of Inula viscosa from Morocco. Arabian Journal of Chemical and Environmental Research. 2014; 1(1):33-40.
53. Ma G-H, Chen K-X, Zhang L-Q, Li Y-M. Advance in biological activities of natural guaiane-type sesquiterpenes. Med Chem Res. 2019; 28(9):1339-58.
54. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and Medicinal Uses of Terpenes. In: Joshee N, Dhekney SA, Parajuli P, editors. Medicinal Plants: From Farm to

Pharmacy. Cham: Springer International Publishing; 2019. p. 333-59.
55. WHO. Trace elements in human nutrition and health: World Health Organization; 1996.
56. WHO. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues: World Health Organization; 2007.
57. Ben Mrid R, Bouchmaa N, Kabach I, Zouaoui Z, Chtibi H, El Maadoudi M, Kounnoun A, Cacciola F, Oulad El Majdoub Y, Mondello L, Zyad A, Nhiri M. Dittrichia viscosa L. Leaves Dittrichia viscosa L. Leaves: A Valuable Source of Bioactive Compounds with Multiple Pharmacological Effects. Molecules. 2022; 27(7):2108.
58. Buscaroli A, Zannoni D, Menichetti M, Dinelli E. Assessment of metal accumulation capacity of Dittrichia viscosa (L.) Greuter in two different Italian mine areas for contaminated soils remediation. J Geochem Explor. 2017; 182:123-31.
59. Martínez-Sánchez MJ, García-Lorenzo ML, Pérez-Sirvent C, Bech J. Trace element accumulation in plants from an aridic area affected by mining activities. J Geochem Explor. 2012; 123:8-12.
60. Cicero N, Gervasi T, Durazzo A, Lucarini M, Macrì A, Nava V, Giarratana F, Tardugno R, Vadalà R, Santini A. Mineral and Microbiological Analysis of Spices and Aromatic Herbs. Foods. 2022;11(4):548