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# Phytochemical composition, antioxidant and antifungal activity of essential oils and crude extracts of *Dittrichia viscosa maritime* (L.), an aromatic and medicinal plant from Northern Morocco

**Fatima Ezzohra Eddardaki[a] | Amina Benoutman[a] | Hajar Ettakifi[a] | El Hadi Erbiai[abc] | Jamila El Abboudi[a] |Mounir Legssyer[a] | Rabah Saidi[a] | Zouhaire Lamrani[a] | Joaquim C. G. Esteves da Silva[b] | Abdelfettah Maouni[a]**

[a]Biology, Environment, and Sustainable Development Laboratory, ENS, Abdelmalek Essaadi University, Tetouan 93000, Morocco.
[b]Centro de Investigação em Química (CIQUP), Instituto de Ciências Moleculares (IMS), Departamento de Geociências, Ambiente e Ordenamento do Território, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal.
[c]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Department of Agronomy, University of Trás-os-Montes and Alto Douro, 5000-801, Vila Real, Portugal.

**Abstract.** *Dittrichia viscosa*, a perennial herb belonging to the Asteraceae family, was collected in a coastal site located in northern Morocco. This study focused on exploring the phytochemical composition and biological activities of essential oils (EOs) and solvents (methanol and acetone) extract of leaves, stems, and flowers. Total phenolic (TPC) and total flavonoid (TFC) contents were determined, and the result showed a high level of TPC and TFC in leaves with the values of 127.61 mg GAEs/g and 117.82 mg CEs/g, respectively. HPLC-MS analysis highlighted 18 phenolic acids and flavonoids, with notable quantities of apigenin 7-glucoside (2233 µg/g) and chlorogenic acid (1429 µg/g). GC–MS analysis of EOs allowed the identification of 27 biologically active compounds, dominated by decanoic acid (20.98%) and toluene (19.66%). Regarding the biological activities, antioxidant activity was evaluated through the DPPH test, while antifungal activity was tested using the microdilution method. The most potent antioxidant effect was observed in the methanol extract of leaves, with a value of 0.18 mg/mL, while the weakest effect was noted in the acetone extract of flowers, registering a value of 1.63 mg/mL. Similarly, the antioxidant activity of methanolic extracts surpassed that of acetonic extracts in each part of *D. viscosa*. Concerning antifungal activity, the findings reveal that all extracts demonstrated important capacity against the tested pathogens. The methanolic extract displayed high efficacy compared to the acetonic one, with MFC values ranging between 1.94-3.38 mg/mL and 2.88-4.75 for *Candida albicans, Aspergillus niger* and *Trichophyton rubrum*, respectively. However, for *Fusarium oxysporum f. sp. albedinis,* the MFCs were higher (7.5-8.5 mg/mL). Regarding EOs, the MFCs were more favorable for the three human pathogenic fungi, ranging between 1.88 and 3.35 µL/mL, compared to *F. oxysporum,* which has an MFCs of 3.5 µL/mL. In summary, the findings demonstrated that *D. viscosa* could be considered a useful alternative and reliable source of bioactive compounds for the pharmaceutical and agricultural industries.

**Keywords:** *Dittrichia viscosa*, bioactive compound, antioxidants, antimicrobial activity

## 1. Introduction

Medicinal and aromatic plants are recognized for their therapeutic potential due to the presence of natural compounds such as flavonoids, phenols, alkaloids, and terpenes (Stan et al., 2021). These compounds possess several bioactivities, such as antibacterial, antifungal, antioxidant, anti-inflammatory, analgesic, anticancer, and other biological activities that are beneficial to human and plant health (Stan et al., 2021; Bouyahya et al., 2017). In addition, these natural compounds are widely used in the manufacture of medicines and cosmetic products. For centuries, essential oils (EOs) and plant extracts have been used for their medicinal properties and are effective against common strains of bacteria, viruses, and fungi (Raut & Karuppayil, 2014; Ben Sassi et al., 2008; Tariq et al., 2019). In addition, essential oils can act synergistically with antibiotics to help fight resistant infections (Bhattacharya et al., 2021; Tahri et al., 2021). Currently, there is increasing

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interest in analyzing the chemical composition and evaluating the biological properties of aromatic and medicinal plants (Labiad et al., 2020; Talib et al., 2012) to discover new interesting compounds for human benefit. Fungal infections are a public health problem that can be very contagious and can affect a large number of people. Pathogenic fungi can cause skin, nail, respiratory, and serious systemic infections in people with a weakened immune system (e.g., people with HIV/AIDS, diabetic patients, and those taking immunosuppressive drugs to prevent rejection of transplanted organs or those receiving chemotherapy) due to difficulties in fighting the infection (Ben Sassi et al., 2008). The overuse of conventional antifungal agents can lead to increasing resistance of fungi to drugs currently available on the market, which makes it more difficult to treat fungal diseases (Sousa et al., 2020). Therefore, the search for alternative natural products is a promising way to find new and more effective treatments for fungal infections (Tariq et al., 2019).

Compared with synthetic drugs, natural products also offer the advantage of having fewer side effects, which makes them safer to use. As a result, an increasing number of scientists are turning to natural sources to discover new antifungal drugs, and many natural products have already shown effectiveness against different types of fungi (Ben Sassi et al., 2008). This underlines the importance of researching natural products and continuing to explore their potential therapeutic effects (Ramawat et al., 2009).

Oxidative stress is the main initial cause of several diseases, such as cancer, cardiovascular and neurodegenerative diseases (Parkinson's disease and Alzheimer's disease), and certain allergies and other chronic diseases (Sousa et al., 2020). There is currently renewed interest in phytochemicals as potential sources of natural antioxidants. Free radicals produced during cellular respiration can damage DNA, proteins, and lipids, resulting in an alteration of normal cellular function (Ramawat et al., 2009; Tahri et al., 2021). Oxidative stress can be caused by several factors, including exposure to environmental pollutants, a diet deficient in antioxidants, infections, chronic inflammation, a lack of physical exercise, and aging (Mishra et al., 2020). Therefore, antioxidants are recommended, and the most commonly used antioxidants are those used for synthesis. At present, the use of available synthetic antioxidant molecules is being questioned due to the potential health risks and the toxicity that causes them (Kohen & Nyska, 2002; Raut & Khullar, 2023).

Currently, the study of the antioxidant activities of plants has become mandatory; for this purpose, the investigation of plants represents an invaluable potential for the discovery of new substances with greater antioxidant and antifungal power (Kohen & Nyska, 2002; Mishra et al., 2020). Most of the antioxidants isolated from plants are phenolic compounds. However, there is currently renewed interest in phytochemical compounds as potential sources of natural antioxidants; the objective is to use them in foods and pharmaceutical preparations to replace synthetic antioxidants (BHA, BHT, etc.) (Makhaik et al., 2021). In addition, medicinal plants are known to have powerful antioxidant activities due to their composition of phytochemicals such as polyphenols, flavonoids, carotenoids, phenolic acids, and vitamins (Benoutman et al., 2022; Pammi et al., 2023). These plants are rich in antioxidants, and we can help prevent these health problems and maintain good overall health. (Raut & Khullar, 2023).

*Dittrichia viscosa* maritime (L.) Greuter, 1973 (or *Inula viscosa* (L.) Aiton, as a scientific synonym), known as false yellow head, is an aromatic and medicinal plant of the Asteraceae (or Compositae) family; it is an annual, herbaceous, perennial, viscous, and glandular species with a strong odor (Lardry & Haberkorn, 2007; Parolin, Scotta, Bresch, et al., 2014). It is appreciated for its therapeutic properties and is widely used in pharmaceutical products, cosmetics, and traditional medicine (Benayache et al., 1991; Gharred et al., 2019). Ethnopharmacological surveys conducted in different Moroccan regions have made it possible to inventory several recipes used to treat different pathologies (Benayache et al., 1991; Bouyahya et al., 2018). This species blooms from late summer to early autumn and can reach up to 1.50 m in height; it is cosmopolitan and less frequent in tropical rainforests (Haoui, 2011). Widespread throughout the Mediterranean basin, the coasts of southern Europe (France, Spain, Greece, Italy, Bulgaria), Turkey, the Middle East (Israel, Jordan, and Syria) and North Africa (Algeria, Egypt, Libya) (Gharred et al., 2019; Parolin, Scotta, & Bresch, 2014). It is predominant in the Mediterranean region, comprising more than 100 species (Asraoui et al., 2021), and widely distributed in northern Morocco (Hammada et al., 2009), where our study site, Oued El Maleh-Martil-Cabo Negro, belongs (a preforest formation belonging to the RAMSAR site). *D. viscosa* is a plant used medicinally against lung ailments and headaches. It is known for its antiseptic properties and effectiveness against skin inflammation, and it is an anti-scabies, anti-inflammatory, renal anti-lytic, antihypertensive, diuretic, healing, and disinfectant of the first order (Bekkara et al., 2008; Bouchouka, 2016). The EOs of *D. viscosa* are used for the treatment of bronchitis, diabetes, rheumatism, injuries, and diseases of the urinary and digestive system (Bekkara et al., 2008; Talib et al., 2012). The extracts of *D. viscosa* contain a variety of phenolic compounds to which various biological activities are attributed, including antioxidant and antifungal activities (Ghazghazia et al., 2013; Mahmoudi et al., 2016). This medicinal plant contains pharmacologically active compounds, particularly flavonoids and polyphenols (Ghazghazia et al., 2013; Trimech et al., 2014), as well as EOs (Gharred et al., 2019; Kheyer et al., 2014). It is rich in essential oil, the content of which varies according to the different parts of the plant. Previous studies have shown that the contents of phenolic compounds are responsible for high antioxidant activities (Chahmi et al., 2015; Karima et al., 2012; Sengul et al., 2009). *Dittrichia viscosa* inhibits the growth of several fungi, such as *Microsporum cookei L.*, *Trichophyton mentagrophyte L.*,

and other fungi pathogenic to humans, such as *Candida albicans* (Bensegueni-Tounsi, 2001; Maoz & Neeman, 2000; RAMLI, 2013).

In this study, the primary aim was to explore the chemical composition of both essential oils and crude extracts obtained from *Dittrichia viscosa* sourced from the coastal Ramsar site located in northern Morocco. Additionally, the secondary objective involved assessing the antioxidant properties through the DPPH test and examining the antifungal activity against three human pathogenic fungi, and the fourth was *Fusarium oxysporum* f. sp. *albedinis*, the main causative agent of Bayoud disease in Moroccan date palms.

## 2. Materials and Methods

### *2.1. Reagents and Standards*

L-ascorbic acid, Folin-Ciocalteu phenol reagent, dimethyl sulfoxide (DMSO), alkane standards ($C_8$–$C_{20}$ and $C_{21}$–$C_{40}$), phenolic standards and RPMI-1640 medium were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethyl acetate, diethyl ether and acetonitrile were obtained from Merck KGaA (Darmstadt, Germany). 2-Diphenyl-1-picrylhydrazyl acid (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Acetone, n-hexane and hexane were purchased from CABLO ERBA Reagent, S.A.S. (Val de Reuil Cedex, France), and Sabouraud dextrose agar (SDA) was purchased from Bio-Mérieux (Marcy L'Etoile, France). Methanol and all other chemicals and solvents were acquired from Honeywell (St. Muskegon, MI, USA).

### *2.2. Plant Material*

The studied plant *Dittrichia viscosa* was collected during the flowering period of October-December 2018 in the region of Oued El Maleh-Martil, a coastal ecological interest site (35°38’30 N, 05°17’00 W). The plants were identified according to the Manual of Determination of Vascular Plants (Fennane & Rejdali, 2018; Najem et al., 2019). The different parts of the *D. viscosa* plant (leaves, flowers and stems) were weighed, cut into small pieces, dried on paper for 15 days at room temperature away from light and humidity, crushed and sieved in powder form, and stored in airtight dark glass bottles until use.

### *2.3. Extraction of Essential Oils and Crude Extracts*

The extraction of essential oils was carried out from the aerial parts of *D. viscosa* through hydrodistillation for 4 hours using a Clevenger-type apparatus. In this process, 100 grams of finely powdered material was immersed in a 1000 mL flask containing distilled water. The resulting oil was then stored in a glass vial in the dark at -20°C until use.

The crude extract was prepared from three plant organs (stems, leaves, and flowers) using two solvents (methanol and acetone). One gram of crushed plant material was macerated in 10 ml of the respective solvents for 12 hours with stirring at room temperature, followed by centrifugation for 10 min at 3000 rpm and filtration through Whatman N°4 paper. The filtration residue underwent two additional extractions using the same method. The resulting crude extracts were evaporated under reduced pressure at 40°C with a rotary evaporator. The dried extracts were weighed and stored at -20°C for future use.

### *2.4. Quantifying the Content of Bioactive Compounds*

The total phenolic content (TPC) and total flavonoid content (TFC) in the *D. viscosa* extracts were determined via spectrophotometric methods.

The TPC was determined using the Folin-Ciocalteu (FC) method. Briefly, in glass hemolysis tubes, a volume of 1 ml of each plant extract (1 mg/mL) was mixed with 5 ml of Folin-Ciocalteu reagent diluted 10 times and 4 ml of a solution of $Na_2CO3$ (7.5%). The tubes were vortexed for 15 s and kept in the dark for 30 min at 40°C. The absorbance was measured at 760 nm against a blank. All the samples were tested three times to ensure the reliability of the results. A calibration curve was generated in parallel under the same operating conditions using gallic acid (GA) as a standard at different concentrations [7.81 to 125 µg/ml]. The concentration of TPC in each extract was expressed in mg of gallic acid equivalent (GAE/g) per gram of dry extract (DE).

The TFC was determined using the aluminum chloride method (Najem et al., 2019). In glass hemolysis tubes, 250 µL of each plant extract (1 mg/mL) was added to 1100 µL of distilled water and 75 µL of $NaNO_2$ (5%) solution. After 5 minutes of incubation, 75 µl of $AlCl_3$ (10%) solution was added. After 6 minutes of incubation, a volume of 1000 µL NaOH (4%) was added. After 15 min of incubation, the absorbance was measured at 510 nm against the blank. Thus, all the samples were tested three times. A calibration curve was generated in parallel under the same operating conditions using catechin at different concentrations (15.62 to 250 µg/l). The total concentration of flavonoids in each extract was expressed as mg of catechin equivalent (CE/g of DE).

### *2.5. Analysis of Essential Oil by GC–MS*

The essential oil extracted from *D. viscosa* leaves was subjected to chemical analysis using gas chromatography (GC) (Trace 1300; Thermo Fisher Scientific, Waltham, MA, USA) linked to a mass spectrometry (MS) system (ISQ single quadrupole mass spectrometer; Thermo Fisher Scientific) following the procedure described by the authors (Erbiai et al., 2023). A volume of 10 µL of *D. viscosa* essential oil was diluted in 90 µL of n-hexane and analyzed by GC–MS. A TG5-MS capillary column (60 m × 0.25 mm i.d.; film thickness of 0.25 µm) was fitted with the GC. The stationary phase consisted of 95% dimethylpolysiloxane and 5% phenyl. The oven temperature was increased at a rate of 5°C per minute from 40°C to 350°C. As the carrier gas, helium was used at a flow rate of 1.2 mL/min. The chemical components of *D. viscosa* essential oil were identified using spectral data from the National Institute of Standard and Technology (NIST) databases and retention indices (RIs) compared to a homologous series of known alkane mixture standards ($C_8$–$C_{20}$ and $C_{21}$–$C_{40}$) (Dalton et al., 1998). Thermo Xcalibur TM 2.2 SP1.48 and NIST MS Search 2.2 Library 2014 software were used to collect and analyze the data.

### *2.6. Extraction and Analysis of Phenolic Compounds by HPLC–MS*

Phenolic compound extraction from *D. viscosa* aerial parts was carried out by following the procedure described by Erbiai et al. (2021a), with some modifications (Ettakifi et al., 2023). One gram of the fine plant powder was extracted with 20 mL of methanol/water (80:20, v/v) at -20°C for 2 h. After stirring for 1 h in the dark using a magnetic stirrer, centrifugation was carried out for 10 min at 3000 rpm. The obtained mixture was subsequently filtered using Whatman N°4 paper. Subsequently, the residue was re-extracted using identical conditions, and the combined extracts were evaporated at 40°C under reduced pressure to remove the methanol. Afterward, the aqueous phase underwent liquid–liquid extraction twice, using 20 mL of diethyl ether and 20 mL of ethyl acetate. Anhydrous sodium sulfate was added to the combined organic phase, and the resulting extract was filtered through Whatman N° 4 paper and evaporated at 40°C until dry. Five milligrams of the obtained phenolic extract was dissolved in 1 mL of methanol/water (80:20, v/v) and filtered through a 0.22 µm disposable LC filter disc for subsequent HPLC analysis.

The obtained phenolic extract was analyzed by high-performance liquid chromatography–mass spectrometry (HPLC-MS) to identify and quantify the individual phenolic compounds present in *D. viscosa* following a protocol previously established by Erbiai et al. (2021a) and used by Ettakifi et al. (2023) with the same settings and HPLC equipment. The separation was performed using an Acclaim™ 120 reversed-phase C18 column (3 µm, 150 × 4.6 mm) maintained at 35°C, and the peak was detected at 280 nm, which is the recommended wavelength. The mobile phase consisted of 100% acetonitrile and 1% acetic acid. Detection was carried out in a photodiode array detector (PDA), with 280 nm being the preferred wavelength. The mass spectra, retention times, and UV–Vis spectra of the samples were used to identify the individual phenolic compounds and characterize them in comparison with authentic commercial standards. The peak areas measured at 280 nm were quantified by comparing them to the standards for each compound as µg per gram of dry weight (DW).

### *2.7. Determination of Antioxidant Activity by DPPH*

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging was subsequently performed (Erbiai et al., 2021a). Three hundred microliters of the plant samples or ascorbic acid at concentrations varying between 4 and 0.25 mg/mL and between 1 and 0.06 mg/mL were mixed with 2.7 mL of a methanol solution of DPPH. The combination was stored in the dark at room temperature for 30 min. Afterwards, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer against a blank. The reduction of DPPH radical (% of inhibition of DPPH) was calculated as follows:

$$\%\ \text{of inhibition} = \left[\frac{A_{DPPH} - A_{Sample}}{A_{DPPH}}\right] \times 100$$

The concentration ($IC_{50}$) that provides 50% DPPH radical scavenging ability was graphically calculated. In this investigation, ascorbic acid was used as the reference standard.

### *2.8. Pathogens and Determination of Antifungal Activity*

The antifungal tests were carried out on four pathogenic fungi. Three human pathogen fungal strains*, Candida albicans*, *Aspergillus niger,* and *Trichophyton rubrum,* were collected from the microbiological analysis center at the University Hospital of Rabat*,* and the other fungus*, Fusarium oxysporum* f. sp. *albedinis*, which causes Bayoud disease, was isolated from the Moroccany date palm, the same strain used by Ettakifi et al. (2023).

The antifungal activity of *D. viscosa* was determined by the broth microdilution method described by the Laboratory Research Team (Erbiai et al., 2021 a). Briefly, dimethyl sulfoxide (DMSO) was used to dissolve the dry extract (methanolic and acetonic) and EOs, which were then serially diluted in RPMI 1640 medium to achieve the concentrations to be evaluated (DMSO not to exceed 5% in the final solution created). A 96-well plate was filled with 100 µL of each dilution. The fungal inoculum was made in physiological water and was standardized for yeasts using spectrophotometric analysis and for filamentous fungi using spore counting. One hundred microliters of the fungal suspension was added to each well after the prepared inoculum was diluted in RPMI to achieve a final suspension of $10^3$ to $10^4$ colony-forming units (CFU)/mL. For

*Candida* and *Aspergillus,* the plates were incubated for 48 hours at 37°C, while *Trichophyton* and *Fusarium* was incubated for 7 days at 25°C.

In a Petri medium SDA plate, 10 µL of wells without turbidity were inoculated to determine the minimum fungicidal concentration (MFC). The concentration that generated no apparent growth was termed the minimum inhibitory concentration (MIC). The lowest concentration of MFC completely stopped development under the previously mentioned incubation conditions.

## 3. Results and Discussion

### *3.1. Extraction Yield*

The extraction yields of the methanolic extract (ME), acetonic extract (AE) and essential oils (EOs) of the *D. viscosa* aerial parts are presented in Table 1. For the crude extracts, the yield was greater in the leaves with a value of 30.54% for ME, while it was lower in the stems with a value of 12.2% for AE. The yield of EOs was greater in the leaf and flower organs with values of 0.23% and 0.19%, respectively, while it was lower in the case of stems (0.08%).

**Table 1** Extraction yield (%) of the methanolic extract, acetonic extracts and essential oils of *D. viscosa*.

| | Plant organ | | |
|---|---|---|---|
| Extract | Leaves | Flowers | Stems |
| ME | 30,54 ± 0,64 | 28,30 ± 0,94 | 13,15 ± 1,79 |
| AE | 30,45 ± 0,37 | 21,65 ± 1,56 | 12,2 ± 0,58 |
| EOs | 0,23 ± 0,01 | 0,19 ± 0,03 | 0,08 ± 0,06 |

The values are expressed as the means ± standard deviations (SD) of three independent measurements.

The ME values of the leaves and flowers were similar to those obtained by (Bssaibis et al., 2009; Samira & Loubna, 2016). With respect to the extraction of EO, diverse findings indicate comparable, diminished, and heightened yields, as reported by (Kheyer et al., 2014; Haoui, 2011).

### *3.2. Contents of Bioactive Compounds*

Table 2 presents the total phenolic content (TPC) and total flavonoid content (TFC) in MEs and AEs derived from the three parts (leaves, stems, and flowers) of *D. viscosa*. The results were determined from the linear regression equation of the calibration curve and expressed in mg GAEs/g DE and mg CEs/g DE. The MEs and AEs from the leaves exhibited significantly greater TPC than those from the flowers and stems, with values of 127.61 and 126.04 mg GAEs/g DE, respectively.

**Table 2** Bioactive compounds (total phenolics and flavonoids) in the DVME and DVAE.

| | | Plant organ | | |
|---|---|---|---|---|
| Bioactive compound | | Leaves | Flowers | Stems |
| TPC (mg GAEs/g DE) | ME | 127,61 ± 0,05 | 77,51 ± 0,07 | 50,74 ± 0,34 |
| | AE | 126,04 ± 0,32 | 49,63 ± 0,23 | 19,53 ± 0,02 |
| TFC (mg CEs/g DE) | ME | 117,82 ± 0,13 | 93,04 ± 0,12 | 23,04 ± 0,08 |
| | AE | 105,86± 0,31 | 56,46 ± 0,38 | 14,13 ± 0,04 |

The values are expressed as the means ± standard deviations (SD) of three independent measurements.

Our results were superior to those reported by Rhimi et al. (2017) and Sqalli et al. (2007) and inferior to those reported by (Aidel Manel et al., 2021).

Concerning the TFC, the highest content was observed in the leaf ME, with 117.82 mg of CEs/g DE, and the lowest content was in the stem ME, with 14.13 mg CEs/g DE. Our findings were superior to those of previous studies (Benabdallah et al., 2016; Boussouf et al., 2017; Mahmoudi et al., 2016; Trimech et al., 2014).

### *3.3. GC–MS Analysis*

The findings from the GC–MS analysis, as illustrated in Figure 1 and summarized in Table 3, revealed 99.73% of the total volatile compounds, comprising 27 distinct compounds in the EOs of *D. viscosa*. The identified biomolecules are categorized into five groups: aromatic hydrocarbons (34.11%), alkanes (29.85%), monoterpenes (22.48%), sesquiterpenoids (9.82%), and carboxylic acids (3.47%). The main compounds identified included decanoic acid (20.98%), toluene (19.66%), p-cymene (12.34%), m-xylene (9.92%), carvacrol (7.85%), α–cadinene (5.73%), and acetic acid (3.13%).

Our results were consistent with those of (Mahmoudi et al., 2016), who reported that nonterpene molecules and sesquiterpene hydrocarbons were the major constituents of EOs. In line with findings from other researchers (Haoui et al., 2015; Benchohra et al., 2011; Silva et al., 2003), the EOs of *D. viscosa* did not consistently exhibit identical compositions. The varied composition of the biomolecules in the EO of *D. viscosa* appears to be associated with factors such as harvesting,

storage conditions, and extraction procedures. These factors influence the plant's vegetative cycle and contribute significantly to the chemical diversity of volatile compounds.

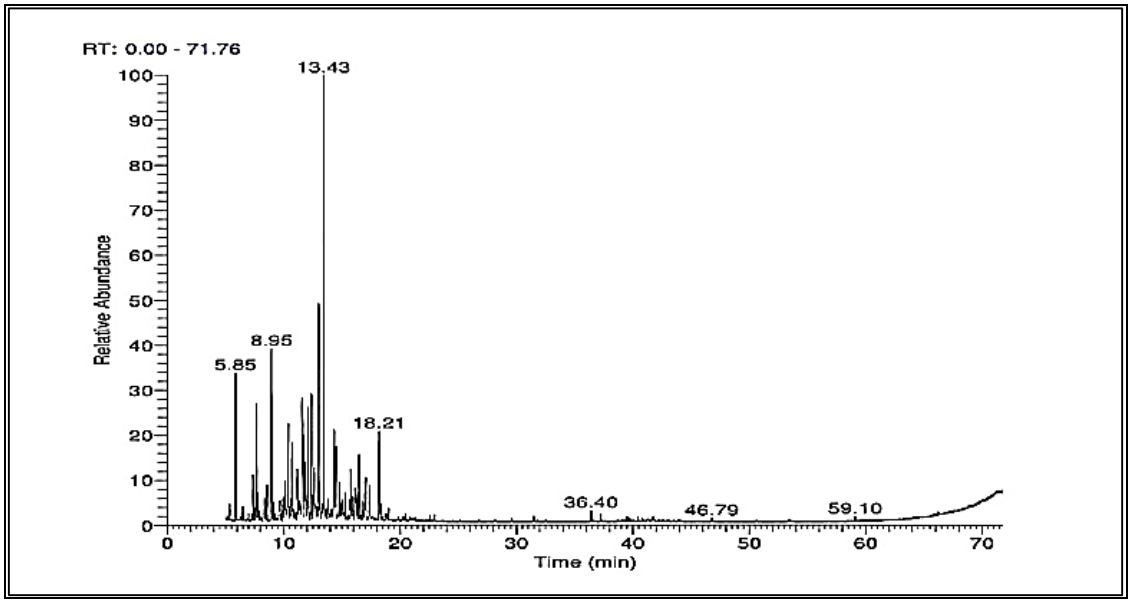


**Figure 1** Chromatogram of *D. viscosa* essential oil by GC–MS.

**Table 3** The chemical constituents of the essential oil of *D. viscosa L*. obtained by GC–MS analysis.

| Picks | Compound Name | KI* | Area % |
|---|---|---|---|
| 1 | Hexacosan | 718 | 0.33% |
| 2 | Acetic acid | 816 | 3.13% |
| 3 | Cyclohexane | 843.16 | 1.33% |
| 4 | m-xylene | 867 | 9,92% |
| 5 | Ethylbenzene | 878 | 1.20% |
| 6 | Cyclohexane, 1, 1, 2,3-tetramethyl | 881.65 | 1.43% |
| 7 | P-xylene | 894 | 1.64% |
| 8 | Toluene | 883 | 19,66% |
| 9 | Nonane | 899 | 1.12% |
| 10 | α-Terpineol | 901 | 2.18% |
| 11 | Mesitylene | 905.2 | 1.58% |
| 12 | α-Pinene | 939 | 2.97% |
| 13 | Heptane, 3-ethyl-2-methyl | 942 | 1.79% |
| 14 | Decanoic acid | 956.17 | 20.98% |
| 15 | Butane, 1-cyclohexyl | 1000.37 | 1.53% |
| 16 | α-Cadinene | 1044 | 5.73% |
| 17 | p-cymene | 1058 | 12.34% |
| 18 | 1-Undecene, 4-methyl | 1076.13 | 0.84% |
| 19 | Undecane | 1131.22 | 0.27% |
| 20 | Benzyl alcohol | 1113 | 0.06% |
| 21 | Caproic acid | 1326.79 | 0.26% |
| 22 | 1,5,5-Trimethyl-6-methylene-cyclohexene | 1642.18 | 0.11% |
| 23 | Cis-5, 8, 11, 14,17-Eicosapentaenoic acid | 1777.37 | 0.08% |
| 24 | Carvacrol | 1967.70 | 7.85% |
| 25 | β- caryophyllene | 1978.46 | 1.12% |
| 26 | 2-Myristynoyl pantetheine | 2300 | 0.05% |
| 27 | 1-Propylundecyl trifluoroacetate | 2400 | 0.23% |
| | Total | | 99.73% |
| | Aromatic hydrocarbons | | 34,11% |
| | Saturated hydrocarbons: Alkanes | | 29.85% |
| | Monoterpenes | | 22,48% |
| | Sesquiterpenes | | 9,82% |
| | Carboxylic acids | | 3.47% |

KI*: Kovats retention indices.

**Table 4** Phenolic compounds identified and quantified in the hydromethanolic extract of the aerial parts of *D. viscosa L.*

| N° | Phenolic Compounds | RT | Content (µg/g of dry weight) |
|---|---|---|---|
| 1 | Gallic acid | 6,09 | 6.60 ± 0.02 |
| 2 | Protocatechuic acid | 10,8 | 105 ± 2.19 |
| 3 | Chlorogenic acid | 13,44 | 1429 ± 12.39 |
| 4 | Catechin | ND | ND |
| 5 | *p*-Hydroxybenzoic acid | 14,74 | 127.9 ± 0.68 |
| 6 | Caffeic acid | 15,29 | 12.14 ± 0.18 |
| 7 | Vanillic acid | ND | ND |
| 8 | Syringic acid | 16,14 | 77.42 ± 0.52 |
| 9 | Rutin | ND | ND |
| 10 | Ellagic acid | ND | ND |
| 11 | Luteolin 7-glucoside | 18,83 | 1263 ± 1.11 |
| 12 | *p*-Coumaric acid | 19,17 | 575.20 ± 0.50 |
| 13 | Vanillin | ND | ND |
| 14 | Ferulic acid | 20,31 | 592.90 ±0.21 |
| 15 | Naringin | 20,83 | 787.80 ± 1.64 |
| 16 | Apigenin 7-glucoside | 21,27 | 2233 ± 3.97 |
| 17 | Rosmarinic acid | 22,44 | 100.40 ± 0.45 |
| 18 | Salicylic acid | 23,87 | 842.7 ± 1.87 |
| 19 | Methyl paraben | ND | ND |
| 20 | Luteolin | 30,9 | 495.90 ± 0.87 |
| 21 | Quercitin | 31,49 | 1203 ± 1.32 |
| 22 | Apigenin | 34,31 | 162.10 ± 0.20 |
| 23 | Kaempferol | 34,67 | 1206 ± 1.70 |
| 24 | Isorhamnetin | 35,34 | 543.50 ± 3.96 |

The values are expressed as the means ± standard deviations (SD) of three independent measurements. RT = Retention time, ND = Not detected.

*D. viscosa* is a hardy plant that can withstand harsh environmental conditions. To combat this problem, plants have evolved defense mechanisms, including specialized enzymes that enable them to metabolize and breakdown toluene. Plants can selectively sequester these poisonous molecules in specific areas, including the roots, because of the enzymes that help them convert toluene into less harmful compounds or beneficial metabolic components. This reinforces the plant's cuticle and inhibits toluene and m-xylene from penetrating its tissues, preventing cellular damage and limiting harm to the plant's vital organs.

### *3.4. Analysis of Phenolic Compounds through HPLC–MS*

Figure 2 displays the HPLC-MS chromatogram, delineating the peaks corresponding to phenolic compounds found in the aerial part of *D. viscosa*, as presented in Table 4. Among the 18 compounds identified, 10 were phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, syringic acid, ferulic acid, *p*-coumaric acid, rosmarinic acid and salicylic acid), and 8 were flavonoids (naringin, luteolin 7-glucoside, apigenin 7-glucoside, luteolin, quercetin, apigenin, kaempferol and isorhamnetin). This phenolic fraction of the methanolic extract of *D. viscosa* was mainly composed of apigenin 7-glucoside (2233 µg/g), chlorogenic acid (1429 µg/g), luteolin 7-glucoside (1263 µg/g), kaempferol (1206 µg/g), and quercetin (1203 µg/g). In contrast, gallic acid and caffeic acid were detected in the lowest quantities in the methanolic extract, with values of 6.60 and 12.14 µg/g, respectively. However, catechin, vanillic acid, rutin, ellagic acid, vanillin, and methylparaben were not detected in the hydromethanolic extracts of the aerial parts of *D. viscosa.*

HPLC-MS analysis (Rhimi et al., 2017) of the Tunisia plant also revealed 18 phenolic compounds in the methanolic extracts of *D. viscosa*. These phenolic compounds were dominated by derivatives of caffeoylquinic acid, such as chlorogenic acid; isomers of dicaffeoylquinic acid and caffeoyl glucose; and other hydroxycinnamic acids, such as coumaric acid and caffeic acid derivatives. However, in a study by Kheyar-Kraouche et al. (2018) in Algeria, HPLC was used to identify 47 phenolic compounds of *I. viscosa*, such as chlorogenic acid derivatives and sesquiterpenes, of which 11 are phenolic acids, 23 flavonoids, one lignan, and 12 terpenoids. Twenty-six of these compounds were identified, and they belong to different families of compounds. Caffeoylquinic acid derivatives are detected in the methanolic extracts of *I. viscosa* in Israel (Bar-Shalom et al., 2019), Turkey (Ozkan et al., 2019), Morocco (Chahmi et al., 2015), and Tunisia (Rhimi et al., 2017). However, in another study, chlorogenic acid and cynarine acid were the main phenolic compounds identified in the leaves of *D. viscosa* (Hakkou et al., 2017).

The richness of the methanolic extracts of the aerial parts of this perennial plant by chlorogenic acid and kaempferol may be due to its specific geographical distribution in this Mediterranean area, which is humid with salty soil and appreciable by abandoned and characteristic vegetation. From these results, it appears that *D. viscosa* could be a potential source of

bioactive components, thus exerting antioxidant and antifungal effects due to the radical scavenging properties of DPPH (Asraoui et al., 2021).

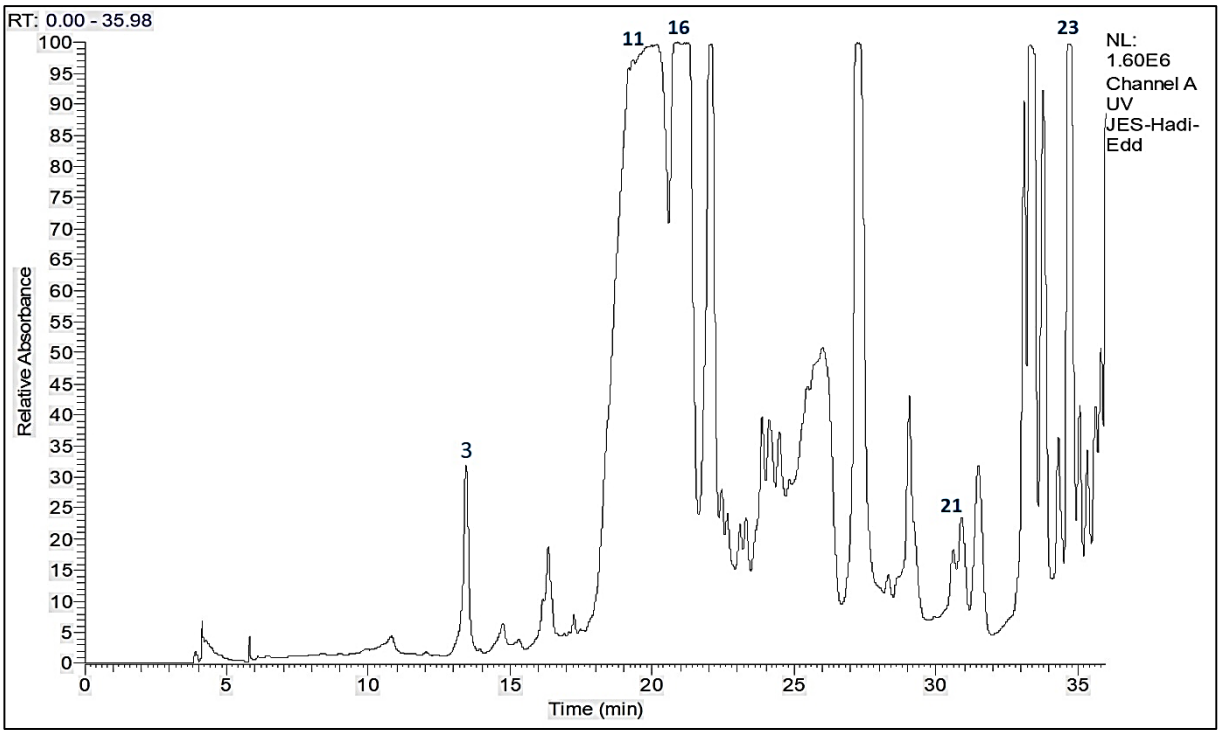


**Figure 2** HPLC-MS chromatogram of phenolic compounds in the hydromethanolic extract of the aerial parts of *D. viscosa* detected at 280 nm.

### *3.5. Antioxidant Activity*

As indicated in Table 5, the majority of crude extracts demonstrated a significant level of antioxidant capacity, consistent with expectations due to the heightened levels of total phenolic and total flavonoid contents in each respective extract. The $IC_{50}$ values ranged between 0.18 and 1.63 mg/mL. The strongest antioxidant effect was shown in the ME of leaves, with a value of 0.18 mg/mL, and the weakest effect was observed in the AE of flowers, with a value of 1.63 mg/mL. Similarly, the antioxidant activity of the methanolic extracts was greater than that of the acetonic extracts in each part of *D. viscosa*.

**Table 5** $IC_{50}$ (mg/mL) values of the antioxidant activity of the *D. viscosa* methanolic and acetonic extracts. Mean ± SD (n=3).

| Extracts | $IC_{50}$ (mg/ml) | | |
|---|---|---|---|
| | Leaves | Stems | Flowers |
| ME | 0.18 ± 0.01 | 0.42 ± 0.01 | 0.87 ± 0.01 |
| AE | 0.21 ± 0.03 | 0.33 ± 0.02 | 1.63 ± 0.01 |

The results are expressed as the mean ± SD of three replicates. ND: not detected.

Comparing our results with those of others, it appears that our findings are generally in concordance with them. The differences in $IC_{50}$ values are not very significant for the methanol extract of leaves, as reported by different authors (Sevgi et al., 2021; Gökbulut et al., 2013; Samira & Loubna, 2016).

Comparing our results with those of others, it seems that our findings are generally consistent with theirs, with some minor variations. Mohti et al. (2020) revealed that the methanolic extract of *D. viscosa* leaves had the highest free radical scavenging activity ($IC_{50}$=0.14 mg/mL). In addition, Mahmoudi et al. (2016) determined the antioxidant activity of *D. viscosa* leaves using a methanolic extract, which gave the best $IC_{50}$ value of 0.02 mg/mL, which was better than our result. In the same context, Gökbulut et al. (2013) evaluated the antioxidant capacity of the flowers, leaves, and roots of *D. viscosa* using water, methanol, and ethyl acetate as extraction solvents, and the aqueous extract of the flowers showed the highest activity, with an $IC_{50}$ = 0.28 mg/mL. However, Trimech et al. (2014) evaluated the antioxidant properties of the methanolic extract of *D. viscosa,* and the results revealed that the $IC_{50}$ of the extract in the stems was greater (0.19 mg/mL) than that in the flowers and leaves (0.21 mg/mL and 0.26 mg/mL, respectively), which is different from our findings (the results for the leaves were better than those for the stems and flowers). This result could be explained by the variation in solvent, the

choice of the organ or synergistic effects of polyphenols that make the antioxidant activity of the methanolic and acetonic extracts weaker or much stronger than that of the isolated bioactive compounds.

### *3.6. Antifungal activity*

The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of the essential oils (EOs) and leaf extracts from *D. viscosa* against the four fungal strains are listed in Table 6. The results indicate that the methanolic extract exhibited greater efficacy than the acetonic extract, with MFC values ranging between 1.94-3.38 mg/mL and 2.88-4.75 for *C. albicans, A. niger*, and *T. rubrum, respectively.* However, for *F. oxysporum*, the MFCs were greater, ranging between 7.5 and 8.5 mg/mL. Concerning EOs, the MFCs were better for the three human fungi, with values ranging between 1.88-3.35 µL/mL, than for *F. oxysporum*, which has an MFC of 3.5 µL/mL.

**Table 6** Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *D. viscosa* leaf extracts

| Fungal Strains | EOs (µL/mL) | | ME (mg/mL) | | AE (mg/mL) | |
|---|---|---|---|---|---|---|
| | MIC | MFC | MIC | MFC | MIC | MFC |
| *Candida albicans* | 1.56 ± 1.33 | 2.2 ± 0.35 | 1.78 ± 0.66 | 1.94 ± 0.45 | 2.25 ± 0.89 | 2.88 ± 0.88 |
| *Aspergillus niger* | 1.88 ± 0.88 | 3.35 ± 0.21 | 2.56 ± 0.33 | 3.38 ± 0.18 | 2.88 ± 0.88 | 4.75 ± 1.06 |
| *Trichophyton rubrum* | 0.35 ± 0.06 | 1.88 ± 0.88 | 1.62 ± 0.44 | 2.15 ± 0.75 | 1.78 ± 0.66 | 3.5 ± 1.77 |
| *Fusarium oxysporum* | 2.88 ± 0.88 | 3.5 ± 0.00 | 4.26 ± 1.34 | 7.5 ± 1.00 | 6.20 ± 1.34 | 8.5 ± 1.00 |

The results are expressed as the mean ± SD of three replicates.

Other studies have revealed that EOs have activity against *Aspergillus niger, Aspergillus oryzae*, and *Fusarium solani* (Tabti et al., 2014). Chebouti-Meziou (2016) reported that the methanolic extract of the aerial part of *D. viscosa* has antifungal activity against *A. niger.* observed that aqueous extracts from *D. viscosa* leaves exhibited significant antifungal efficacy against *C. albicans* and *T. rubrum* (Maoz & Neeman, 1998).

These results are consistent with those of Cafarchia et al., who obtained four essential oils by steam distillation of the leaves, flowers, whole plant, and plants without flower extracts of *D. viscosa*. All extracts were found to have significant antifungal activity against *Microsporum canis, Trichophyton mentagrophytes, Microsporum gypseum and Trichophyton terrestre*. The highest antifungal activity was detected in the leaves, followed by extracts from whole plants and plants without flowers. The flower extracts had marginally lower inhibitory effects than the other extracts (Cafarchia et al., 2002). Ali-Shtayeh and Abu Ghdeib reported that aqueous extracts from *D. viscosa* leaves inhibit specific dermatophytes, including *Trichophyton mentagrophytes* (92.8 µl/mL), *Trichophyton violaceum* (100 µl/mL), and *Microsporum canis* (88.4 µl/mL) (Ali-Shtayeh & Abu Ghdeib, 1999). An investigation conducted by Benhammou and AtikBekkara revealed that the EOs of *D. viscosa had strong inhibitory effects on T. rubrum and C. albicans at concentrations of 20 and 5.0 µg/mL*, respectively (Benhammou & AtikBekkara, 2005).

The most robust antifungal properties are identified in leaf extracts, and their heightened activity may be attributed to the elevated concentration of sesquiterpenes they contain (Andreu et al., 2018). Phenolic substances, including coumaric acid or derivatives of hydroxycinnamic acids (such as caffeoylquinic acid and chlorogenic acid), are also recognized as highly effective agents against *C. albicans* (Rhimi et al., 2017). Nonetheless, both the EOs and extracts of *D. viscosa* are emerging as potential sources of natural bioactive molecules.

## 4. Conclusions

Pathogenic fungi cause many diseases in both humans and plants, leading to substantial financial losses, particularly in the realms of human health and agriculture. Consequently, there is an urgent need to explore and develop new natural compounds with the potential to combat these pathogenic fungi. For the first time, the present study evaluated the phenolic composition of crude extracts derived from three parts of *D. viscosa* in the coastal region of northern Morocco. The results demonstrated that the *D. viscosa* crude extract contained an important amount of TPC and TFC. In addition, the leaf and methanol were the best organ and solvent for extracting the bioactive compounds, respectively. The phytochemical characterization showed the presence of 27 major compounds in the essential oil by GC–MS and 18 phenolic compounds by HPLC-MS, which were responsible for the strong antioxidant and antifungal properties of the plant *D. viscosa*. *D. viscosa* is a resistant plant capable of adapting to the difficult environmental conditions of the study region. Overall, *D. viscosa* could be considered a useful alternative and reliable source of bioactive compounds for the pharmaceutical and agricultural industries.

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## Ethical considerations

Not applicable.

## Conflict of interest

The authors declare no conflicts of interest.

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