RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2024, **14**, 22548

GC-MS analysis, phytochemical composition of *Hertia cheirifolia* L. essential oil with pharmacological assessments: antioxidant, antibacterial, and antifungal activities†

Wassila Benabderrahmane,[a] Hamza Fadel,[b] Ines Sekhara,[b] Imad Mennai,[b] Imed Eddine Kadi,[c] Mahmoud Helal,[d] Rokayya Sami, *[e] Hala M. Abo-Dief,[f] Ruqaiah I. Bedaiwi,[g] Mohammad A. Alanazi,[g] Helal F. Al-Harthi,[h] Roqayah H. Kadi,[i] Suzan A. Abushal,[j] Tasahil S. Albishi,[k] Alaa T. Qumsani[l] and Sameer H. Qari[l]

The genus *Hertia*, which belongs to the Asteraceae family, is a flowering genus with 12 species found in Africa, North and South. Among the species present in Algeria, *Hertia cheirifolia* L. is distributed in the eastern regions of Algeria. The aim of this study is to evaluate its phytochemical composition with following pharmacological assessments: the antioxidant, antibacterial, and antifungal activities of *Hertia cheirifolia* L. essential oil (EO). GC-MS analysis was used to analyze the chemical constituents of *H. cheirifolia* essential oil. The antioxidant capacity was assessed using DPPH, FRAP, and H_2O_2 tests. The EO was also tested for its ability to inhibit six strains of microorganisms, including two Gram (+) and four Gram (−) strains. The antifungal activity was tested by analyzing the effect of the EO on the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* (FOL) fungi. Results showed that primary volatile components were α-pinene (32.59%), 2-(1-cyclopent-1-enyl-1-methylethyl) cyclopentanone (14.62%), (−)-germacrene D (11.37%), and bakkenolide A (9.57%). *H. cheirifolia* EO showed inhibitory effects against DPPH, H_2O_2, and FRAP (IC_{50} = 0.34 ± 0.1, 0.053 ± 0.1, and 0.047 ± 0.01 mg mL^{-1}, respectively). The EO also exhibited moderate antibacterial effects against *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Streptococcus pneumoniae* ATCC 49619 (*S. pneumoniae*), and *Enterobacter aerogenes* ATCC 13048 (*E. aerogenes*), as well as significant antioxidant potential and varied antifungal activity based on dosage and fungal strain. To our knowledge, no previous research has examined the antifungal capacity of *H. cheirifolia* oil and oil-mycelial development of the FOL relationship. To fully explore the benefits of *H. cheirifolia* EO, more *in vivo* research is necessary, along with more testing on other bacterial and fungal strains.

Received 15th May 2024
Accepted 28th June 2024

DOI: 10.1039/d4ra03578j

rsc.li/rsc-advances

Introduction

Around 1911 genera and 32 913 species make up the Asteraceae family.[1,2] This family is among the biggest worldwide, with members across all continents, including the Mediterranean basin.[3,4] Many Asteraceae species are used in various medicinal contexts and contain volatile oils that give them their distinctive scents.[5–7] This family includes the genus *Hertia*, with 12

[a]*Department of Chemistry, Faculty of Sciences, University of 20 Août 1955-Skikda, PO Box 26, El-Hadaiek Road, Algeria. E-mail: b_wassila84@yahoo.fr*

[b]*Research Unit Valorization of Natural Resources, Bioactive Molecules, and Physicochemical and Biological Analyses, University of the Mentouri Brothers, Constantine1, Aïn El Bey Road, 25000, Constantine, Algeria. E-mail: hamzafadel64@yahoo.com; sekharaines@gmail.com; mennai_imad@umc.edu.dz*

[c]*Research Unit in Medicinal Plants (URPM. 3000, Laghouat) Attached to the Research Centre of Biotechnology (CRBt. 25000, Constantine), Algeria. E-mail: kadi.imed.eddine@gmail.com*

[d]*Department of Mechanical Engineering, Faculty of Engineering, Taif University, PO 11099, Taif 21944, Saudi Arabia. E-mail: mo.helal@tu.edu.sa*

[e]*Department of Food Science and Nutrition, College of Sciences, Taif University, PO Box 11099, Taif 21944, Saudi Arabia. E-mail: rokayya.d@; tu.edu.sa*

[f]*Department of Science and Technology, University College-Ranyah, Taif University, PO Box 11099, Saudi Arabia. E-mail: h.abodeif@tu.edu.sa*

[g]*Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, PO Box 741, Tabuk 71491, Saudi Arabia. E-mail: rbedaiwi@ut.edu.sa; m.alenezi@ut.edu.sa*

[h]*Department of Biology, Turabah University College, Taif University, 21995, Saudi Arabia. E-mail: h.alharthy@tu.edu.sa*

[i]*Department of Biological Sciences, College of Science, University of Jeddah, Jeddah 21959, Saudi Arabia. E-mail: rhkadi@uj.edu.sa*

[j]*Program of Food Sciences and Nutrition, Turabah University College, Taif University, PO 11099, Taif 21944, Saudi Arabia. E-mail: s.aboshal@tu.edu.sa*

[k]*Biology Department, College of Sciences, Umm Al-Qura University, Makkah, Saudi Arabia. E-mail: tsbishi@uqu.edu.sa*

[l]*Department of Biology, Al-Jumum University College, Umm Al-Qura University, Makkah, Saudi Arabia. E-mail: atqumsani@uqu.edu.sa; shqari@uqu.edu.sa*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra03578j

flowering species found in Africa, North and South and Central and Southwestern Asia.[8,9] It has pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, cytotoxic, spasmolytic, anthelmintic, and acaricidal effects, because of its bioactive phytochemicals.[10,11] *Hertia cheirifolia* L., also known as "Kherchoun"[12] or "Barbary ragwort",[13] is an evergreen perennial woody plant. It bears fleshy, bluish oval leaves on creeping stems, and yellow flowers in the capitulum, 2 or 3 cm in diameter, appear during the spring. This plant can withstand various environmental conditions, including wind, sea spray, cold, and drought. It is endemic to North Africa, including the Algerian Aures area and is also widely distributed in gardens.[14–16] Historically, traditional healers have used it to treat hemorrhoids, spasms, inflammation, diarrhea, and a range of gastrointestinal ailments.[17] In fact, literature studies indicate that *H. cheirifolia* possesses biological activities.[18–25] Moreover, it is recommended for use as a mosquito control since it is successful against larval stages.[26] To the best of our knowledge, however, no research has investigated the phytochemical constituents and pharmacological properties of the EO (essential oil) found in the aerial parts of *H. cheirifolia* growing in the Aures area (Algeria), and, in particular, no prior studies have looked at the antifungal potential of the EO of this species, and its association with the mycelial growth of FOL. Therefore, given the valuable chemical and biological properties of this plant, the purpose of this study was to examine the chemical makeup and biological characteristics of the EO of *H. cheirifolia*, which was collected for the first time from a mountainous region of Algeria. The study also aimed to compare the results with those of other studies conducted on same species to identify any similarities or differences in outcomes and factors that contributed to them.

Experimental

Materials

Chemicals. Ethanol (95%) was purchased from Sigma-Aldrich (Deisenhofen, Germany), DPPH and H_2O_2 were obtained from Sigma-Aldrich (St Louis, USA), and analytical standards ascorbic acid and α-tocopherol were obtained from Sigma-Aldrich, USA (purity ≥ 98%). DMSO was purchased from Merck-Millipore (Darmstadt, Germany). Ultrapure water was acquired with a Milli-Q system (Merck-Millipore, Algeria). Agar culture medium (MHA) was obtained from Oxoid (Basingstoke, Hampshire, UK). Culture media and reagents used to examine biological properties were acquired from Merck (Darmstadt, Germany). The selected antibiotic for this investigation was purchased from Sigma-Aldrich.

Plant samples

Aerial portions of *H. cheirifolia* were gathered in March 2023 from the Ain Kercha region of Algeria's Aures zone (HC-AKA); (35° 55′ 16″ north, 6° 41′ 58″ east, altitude: 875 m). A voucher specimen (HC/128/VAR/04-23) has been deposited in the herbarium of the research unit VARENBIOMOL of the University of Constantine 1.

Sample preparation. Aerial sections of *H. cheirifolia* (leaves, flowers, and stems) weighing 645 g were distilled using steam distillation. This process involved running water vapor through plants at 100 °C, destroying cells, and releasing aromatic compounds into a cooling coil. As the vapors cool, they revert to liquid form, resulting in an azeotropic combination of water and essential oil. This process was completed in three hours. The essential oil and floral water were distinguished by simple density difference. Finally, we extracted 1.32 g ($Y = 0.2\%$) of *H. cheirifolia* EO. The EO extraction yield was expressed in % (v/w) of the dry plant material using the following equation:

$$\%\ \text{EO yield} = (\text{weight of the extracted EO (g)}/\text{weight of the dried plant material (g)}) \times 100.$$

The obtained essential oil was subjected to GC-MS analysis to establish its chemical makeup. Fig. 1 presents an outline of the work carried out.

GC-MS analysis. The sample was analyzed at the Physico-Chemical Analysis Technical Platform (PTAPC-CRAPC) in Algeria (Laghouat), using Shimadzu GCMS-MSQP2020 equipment, employing a fused Rxi®-5ms capillary column (phase: Crossbond® 95% dimethylpolysiloxane/5% diphenyl) with following settings: thickness of the film (0.25 μm) and dimensions (0.25 mm × 30 m). This column has an equivalent phase to these other columns: DB-5ms, DB-1msUI, HP-1ms, HP-1msUI, DB-1ms, Ultra-1, VF-1ms, and ZB-1ms, which are all comparable to USP G1, G2, and G38 phases. The temperature of the column was held at 50 °C for 2 minutes and gradually increased to 310 °C at 3 °C min^{-1} and held for 2 minutes. The temperatures of the detector and injector were kept at 310 °C and 250 °C, respectively. Helium (99.995% purity) was used as the carrier gas, at a flow rate of 1 mL min^{-1}. Electron ionization (EI) mass spectrometer settings were: temperature of the ion source 200 °C, mass range 45–600 m/z and 70 eV ionization voltage. A volume of 0.5 μL of the EO was injected in split mode (1 : 80).[27]

Identification of essential oil composition. For each component, linear retention indices (LRIs) were calculated and compared to a homologous series of *n*-alkanes (*n*-C8-C33). Compounds were identified by comparing their calculated LRI to that found in the literature,[28,29] and their mass spectra to those registered by the NIST and Wiley libraries (NIST17.lib, W11N17MAI, and FFNSC1.2.lib).

Antioxidant activity. The antioxidant capacity of the oil was assessed using DPPH, FRAP, and H_2O_2 assays. The results are expressed in mg mL^{-1} using vitamin C (DPPH), ascorbic acid (FRAP), and α-tocopherol as references.

DPPH radical-scavenging test

The ability of *H. cheirifolia* essential oil to decrease DPPH was evaluated using the approach outlined by Gargouri *et al.*[30] Aliquots of 0.1 mL of the essential oil with concentrations ranging from 31.25 to 1000 μg mL^{-1} were combined with 2 mL

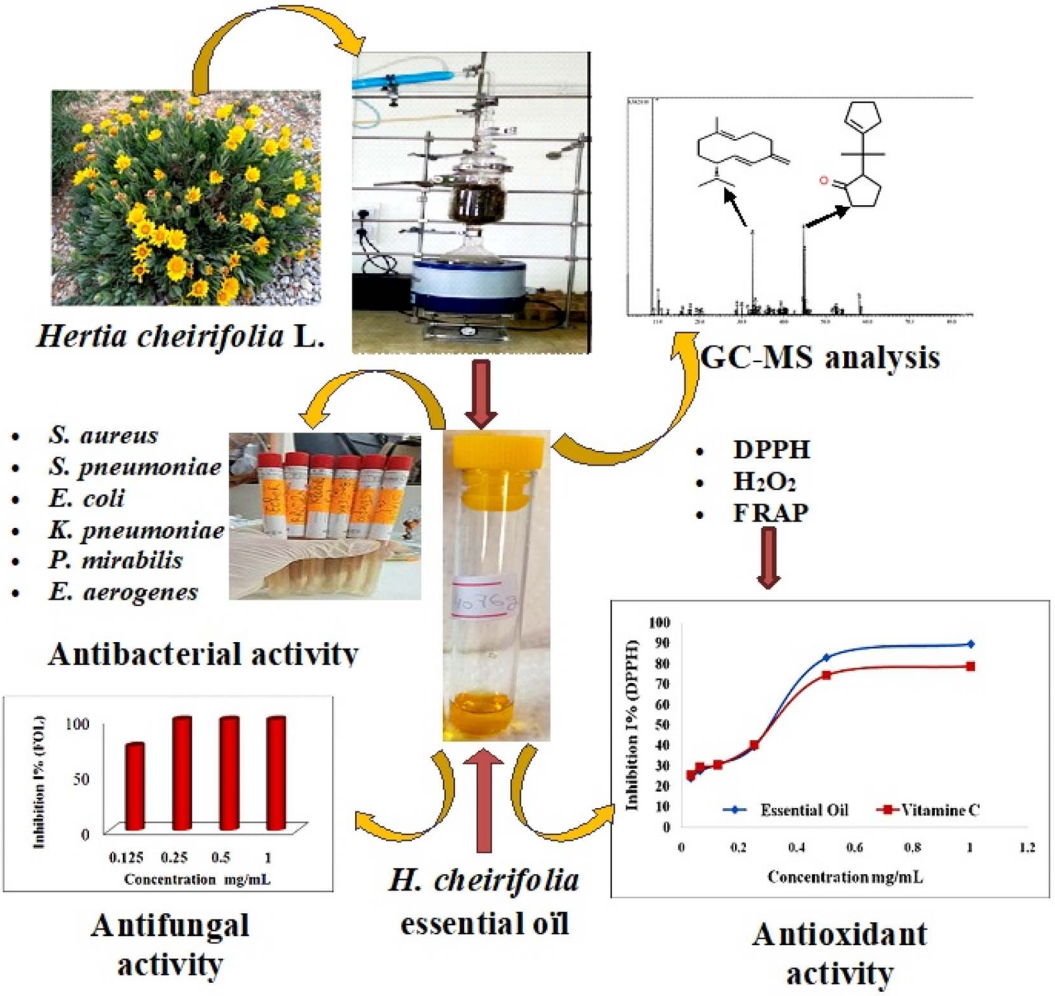

Fig. 1 Summary of the research work.

of DPPH solution (0.4%) in ethanol and vigorously shaken. After 30 minutes of incubation at 30 °C in the dark, the absorbance at 517 nm was measured, with a blank sample as reference. The following equation was used to measure the scavenging capacity of the oil:

$$(\%) = (A_0 - A_1)/A_0 \times 100$$

A_0 represents the absorbance of the control after 30 minutes, while A_1 represents the absorbance by the sample after the same time period. All the samples underwent three replications.

Hydrogen peroxide scavenging testing

The EO scavenging ability against H_2O_2 was measured based on the technique developed by Remigante *et al.*[31] A 40 mM solution of deionized H_2O_2 at pH 7.4 in a phosphate buffer was prepared. The oil was made up at different concentrations, ranging from 1 to 0.0312 mg mL^{-1}, in H_2O_2 solution and allowed to react for about ten minutes at ambient temperature. The reference, α-tocopherol, was subjected to the same procedure. The absorbance of H_2O_2 solutions was measured at 240 nm and compared to an oil-free control. The H_2O_2 inhibition rate (IR%) was determined using the following formula:

$$\mathrm{IR}\% = \frac{(\mathrm{Ac} - \mathrm{Ae})}{\mathrm{Ac}} \times 100$$

in which Ae is the absorbance of the tested sample, and Ac is the absorbance of the control.

Ferric reducing antioxidant power (FRAP) assay

Approximately 30 μL of diluted EO (2 mg mL^{-1}) were mixed with 90 μL of distilled water and added to 900 μL of FRAP reagent and incubated for 30 minutes at 37 °C. The ferric reducing power was determined from the $FeSO_4 \cdot 7H_2O$ calibration curve and its corresponding linear regression equation.[32]

Results are expressed as mean values ± standard errors ($n = 3$). Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA) was used for all statistical analyses.

Antibacterial capacity

Microorganism strains. The antibacterial activity of *H. cheirifolia* EO was tested against two Gram-positive microorganism

strains (*Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumoniae* ATCC 49619) and four Gram-negative microorganism strains (*Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 4330, *Proteus mirabilis* ATCC 7002, and *Enterobacter aerogenes* ATCC 13048).

Agar diffusion technique (ADT). ADT was utilized to measure the antibacterial capacity, following NCLLS recommendations. Sterile MHA was placed on Petri plates and allowed to set. Cell-culture dishes were infected with microorganisms. To create the inoculum, test microorganisms were cultured overnight and adjusted to a turbidity of 0.5 McFarland. Sterile discs (6 mm) were filled with 1/8, 1/4, 1/2, or 1 mg mL^{-1} of *H. cheirifolia* EO and later put on agar dishes harboring one of the microorganisms. These concentrations were produced by diluting the oil with DMSO (1/1, 1/2, 1/4, and 1/8 v/v), with DMSO and gentamicin serving as negative and positive controls, respectively. The plates were incubated at 37 °C for 24 hours after being kept for 30 minutes at room temperature. All disc diffusion or antibacterial assays were conducted with at least three repetitions per species. Three measurements were performed for the disc diffusion study on each bacterium to confirm the results. The antibacterial ability of *H. cheirifolia* essential oil was assessed by measuring the width of its inhibitory zones.[19]

Antifungal activity. The effect of *H. cheirifolia* essential oil on the mycelial growth of phytopathogenic FOL fungi was also analyzed. The toxicity of FOL fungi 4287 was investigated by inhibiting mycelial growth using phytopathogenic drugs. The inhibitory action of the essential oil on the development of the phytopathogenic agent mycelium was measured on PDA, including the tested sample. To conduct the experiment, at 60 ° C, 100 mL of sterilized PDA medium was blended with 1 mL of DMSO solution containing 1 mg of freeze-dried essential oil before being dispersed in 4 Petri plates. Similarly, 100 mL of PDA medium was added to 1 mL of DMSO as a positive control. The negative control was the PDA medium without oil.[33] To conduct the experiment, a 5 mm diameter disk from a small fungal culture was aseptically placed in the center of a Petri plate with PDA medium and EO. The procedure was performed three times for each treatment. A millimeter scale was used to measure the mycelial growth of the phytopathogenic agent following six days of incubation at 25 °C. Findings were expressed as the percentage of essential oil-induced growth inhibition relative to the average colony diameters of fungi cultured in control media.

The inhibitory activity was represented as a percentage and computed using the formula: $IR = (C\text{-}T/C) \times 100$. In this equation, IR represents the inhibition rate as a percentage, C represents the radial growth of the phytopathogenic agent in millimeters on DMSO (control) with PDA medium, and T represents the radial growth of the phytopathogenic agent in millimeters on PDA medium with the tested complex. The EO was tested at various concentrations (1/8, 1/4, 1/2, and 1 mg mL^{-1} of PDA) to determine the lowest inhibitory concentration for controlling FOL growth.

Statistical analysis. All analyses were performed in triplicate and the results are expressed as the mean values ± standard deviation (SD). Group means were compared by one-way ANOVA and Tukey test significance ($p < 0.05$) among groups. Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA) was used for all statistical analyses.

Results and discussion

GC-MS analysis

GC-MS analysis led to experimental retention indices that were compared with literature values,[34] and each peak of the EI mass spectrum was compared to available mass spectra libraries (Fig. 2).

In this way, sixty-eight compounds (100%) were detected by GC-MS analysis. Table S1† shows that compounds, α-pinene, 2-

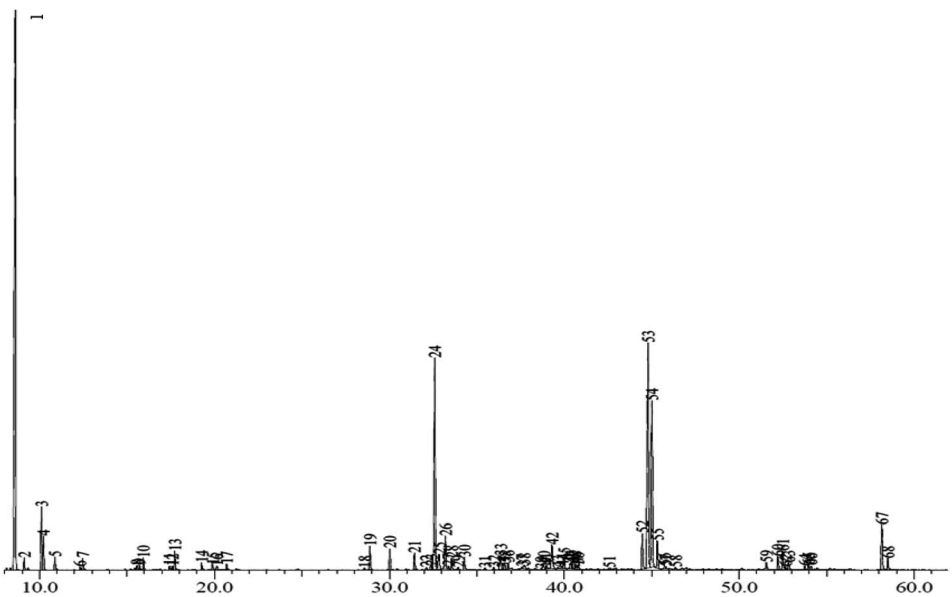

Fig. 2 Chromatogram of the phytochemical component of *H. cheirifolia* EO.

(1-cyclopent-1-enyl-1-methylethyl) cyclopentanone, (−)-germacrene D and bakkenolide A, are the principal volatile components of *H. cheirifolia* with ratios of 32.59%, 14.62%, 11.37%, and 9.57%, respectively. Oil-contained monoterpenoids (40.76%) and sesquiterpenoids (24.32%), with sesquiterpenoids (17 compounds) and oxygenated sesquiterpenoids (14 compounds) dominating their respective groups (21.06% and 3.26%, respectively), as well as 20 other substances having percentages between 0.08 and 14.62% (Fig. 3). Several investigations in Algeria found variations in the chemical makeup of *H. cheirifolia* essential oil. Samples of *Hertia cheirifolia* were collected from different regions of Algeria: Oum El Bouaghi (HC-OEB1, HC-OEB2), Batna (HC-BA), Setif (HC-SE), and Tunisia (HC-TU) and consequently coded.

As an example, sixty-two compounds were found in HC-OEB-EO,[22] representing 78.3% of the total components of this EO. Monoethylhexyl phthalate, valeranone, (−)-drimenin, and *tert*-butylbenzene were determined to be major volatile compounds, but were not found in our sample from the Ain Kercha region of the Algeria's Aures zone, coded HC-AKA. In a previous study[19] on the same species (HC-OEB2), forty-seven compounds were identified, accounting for 95.2%. Main constituents were α-pinene, β-phellandrene, and 2-(1-cyclopent-1-enyl-1-methylethyl) cyclopentanone. Ounoughi *et al.*[20] identified fifty-seven volatile compounds in HC-BA-EO, accounting for 99.3% of the total *H. cheirifolia* EO. Primary components were spathulenol, α-pinene, and α-campholene aldehyde, whereas in HC-SE-EO, fifty-five components (98.57%) were identified, including α-pinene, germacrene D, caryophyllene oxide, and spathulenol.[20] Notably, HC-AKA, our *H. cheirifolia* EO, differed from previously published investigations in terms of the presence of α-pinene. HC-SE-EO and HC-BA-EO samples had higher rates of α-pinene, while HC-AKA had a lower content (53.8, 48.5, and 32.6%, respectively). We identified fourteen common chemicals in HC-AKA compared to HC-SE and HC-BA and sixteen compounds compared to the HC-SE-EO sample. HC-AKA-EO has fifty-two components that were lacking in the HC-SE sample, and fifty components that were absent in the HC-BA-EO sample. HC-SE-EO had thirty-nine missing components, whereas HC-BA-EO had forty-three missing components. Variations might be due to harvesting time, local conditions, or a distinct chemotype.[35–37] HC-TU-EO showed diversity in volatile chemical makeup across many areas. HC-TU essential oil, collected from Sousse, was found to be high in germacrene D, α-pinene, and drimane-type sesquiterpene lactones.[21] In another HC-TU sample, α-pinene and α-cadinol were found to be main volatile components.[16] Moreover, thymol and 2,6-dimethoxy-phenol were found to be predominant chemicals in the HC-TU-EO South-West Kef sample,[24] with just nine identified compounds accounting for 93.1%. Indeed, our HC-AKA sample differed from the Tunisian sample in that it contained only thymol and 2,6-dimethoxyphenol, while HC-TU-EO contained camphor, 1,8-cineole, terpinene-4-ol, *p*-cymen-8-ol, myrtenol, α-terpineol, and 6-methyl-α-ionone.[24] The variance in the composition of EOs from *H. cheirifolia* might be explained by a number of factors, including distillation technique, location, age and cultivar of the plant, environment, and equipment.[21,38] Furthermore, some published research found that the essential oil content of the species may fluctuate during their growth cycle[39] and may be influenced by environmental factors[34,40] as well as ecological and genetic factors, such as the properties and type of soil.[41] In addition, plant oil content differs substantially depending on genotype, collection season, and geographical origin.[42–48] Therefore, through the results obtained and comparing them with the results of previous studies on the same plant species from different regions, it can be concluded that there are obvious differences in quantity and quality in the relative contents of the chemical components between *H. cheirifolia* EOs. In addition, there is a difference in its main constituents, and if there is a similarity, there is a difference in amount, even if it is a small percentage.

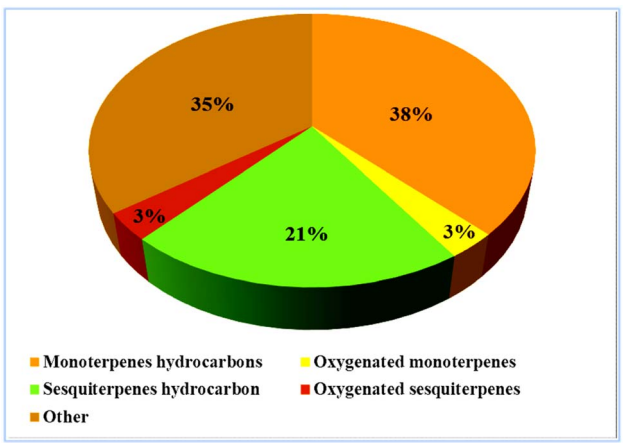

Fig. 3 Phytochemical composition of *H. cheirifolia* essential oil.

Antioxidant capacity

Using DPPH, H_2O_2, and FRAP techniques, the antioxidant effect of *H. cheirifolia* EO was checked (Table 1 and Fig. 4). In fact, the oil demonstrated a greater antioxidant capacity (89.55% ± 0.3) than the vitamin C standard (78.9% ± 0.1) at 1 mg mL^{-1} with significant DPPH activity (IC_{50} = 0.34% ± 0.1 mg mL^{-1}). Regarding the H_2O_2 test, the results indicated a high level of antioxidant capacity with an IC_{50} of 0.052 ± 0.1 mg mL^{-1}. Inhibitory rates increased progressively with the addition of concentrations of 1/32, 1/16, 1/8, 1/4, 1/2, and 1 mg mL^{-1}. Among these concentrations, 0.25 and 0.5 mg mL^{-1} tested oils demonstrated the highest level of activity, exhibiting inhibition rates of 74.9% ± 0.2 and 87.9% ± 0.1, respectively. At 1 mg mL^{-1}, the maximal activity (92.3% ± 0.3) of the essential oil was achieved, while the highest dosage of the reference compound α-tocopherol (1 mg mL^{-1}) inhibited 90.4% ± 0.1 of the reaction. In the presence of antioxidants, the ability of plant fractions to convert ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) can be measured by FRAP testing, frequently used to assess the overall antioxidant efficacy of extracts. HC-AKA-EO possessed substantial antioxidant capacity (IC_{50} = 0.047 ± 0.09 mg mL^{-1}) in comparison to the ascorbic acid standard reference (IC_{50} = 0.09 ± 0.01 mg mL^{-1}), at 93.4% ± 0.1. This allowed the oil to catalyze

Table 1 Statistical analysis of the antioxidant effect of *H. cheirifolia* EO and standards, using three assays: DPPH, H_2O_2, and FRAP

	DPPH test		H_2O_2 test		FRAP	
Concentration (mg mL^{-1})	% EO	% Vitamin C	% EO	% α-tocopherol	% EO	Ascorbic acid
1	89.55 ± 0.3	78.86 ± 0.1	92.34 ± 0.3	90.37 ± 0.1	94.52 ± 0.1	88.58 ± 0.1
0.5	83.04 ± 0.1	74.42 ± 0.2	87.86 ± 0.1	85.63 ± 0.2	89.12 ± 0.1	80.38 ± 0.1
0.25	39.44 ± 0.2	40.25 ± 0.1	74.89 ± 0.2	73.53 ± 0.0	79.74 ± 0.1	68.79 ± 0.1
0.125	30.93 ± 0.01	30.45 ± 0.0	53.8 ± 0.1	51.84 ± 0.2	61.56 ± 0.1	52.37 ± 0.1
0.0625	27.61 ± 0.4	29.24 ± 0.1	47.31 ± 0.0	40.84 ± 0.1	44.26 ± 0.1	48.65 ± 0.1
0.0312	24.01 ± 0.2	25.36 ± 0.1	34.34 ± 0.0	31.15 ± 0.1	28.54 ± 0.1	33.23 ± 0.1
IC_{50} (mg mL^{-1})	**0.34 ± 0.1**	**0.39 ± 0.1**	**0.053 ± 0.1**	**0.041 ± 0.1**	**0.047 ± 0.01**	**0.09 ± 0.01**

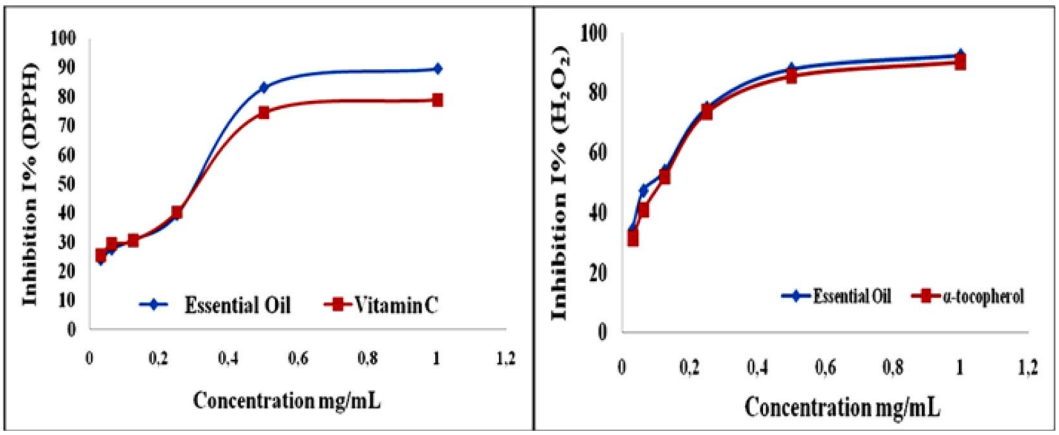

Fig. 4 Graphic curve of the antioxidant activity of *H. cheirifolia* EO (DPPH and H_2O_2 tests) at different concentrations.

the reduction of Fe^{3+} to Fe^{2+}. When compared to previously published research, *H. cheirifolia* essential oil (HC-AKA) showed the greatest amount of scavenging ability using the DPPH assay.[22,49] The presence of hydroxylated chemicals, such as monoterpenes (α-pinene, camphene, sabinene, limonene, linalool, and verbenol) and sesquiterpenes (germacrene D, α-muurolene, and spathulenol) in the sample could account for the potent inhibitory ability of the essential oil.[50–53] Compared with the results of previous studies on the same plant,[11,18,21,22,25] we note that there is a difference in the results obtained. However, according to previous studies, these differences in antioxidant capacity could be due to several factors, including the molecular nature of bioactive compounds.[41,54–56] In fact, the major compound (α-pinene) is known for its strong antioxidant proprieties.[54,55] In contrast, minor compounds, such as limonene and β-pinene, can interact directly to create a complex of biological properties.[54] In addition, antioxidant capacity was reported to be linked to the molecular structure of the EO, such as hydroxyl group substitution in phenolic aromatic rings, contributing to oxygenated monoterpenes (α-pinene oxide, linalool, and verbenol), a blend of mono and sesquiterpene hydrocarbons, and their hydrogen-donating ability.[54]

According to the impressive antioxidant findings, *H. cheirifolia* EO showed the greatest level of antioxidant capacity in the FRAP, H_2O_2, and DPPH tests. Furthermore, with regard to *H. cheirifolia* extracts, several investigations have demonstrated that the fractions of this species contained phenolic compounds[17,25,57] and demonstrated its antioxidant capacities using ABTS and DPPH assays. Additionally, the best rate of inhibition and strongest iron-reducing power were noted with the β-carotene test.[18,58] Therefore, the species is regarded as a trustworthy source of natural antioxidant.

Antibacterial activity

The antibacterial effect of *H. cheirifolia* EO was assessed using the disc technique. After a 24 hour period of incubation at 37 °C, the inhibition zone diameter (IZD) of the disc was measured to determine the findings (Table 2). In comparison to the standard antibiotic (positive control), the findings show that the EO has significant antibacterial capacity. The findings demonstrated that the antibacterial potential of the essential oil varied depending on the type of bacteria tested. It was found to be less effective against *E. coli* and *Proteus* and be more active against Gram-(+) bacteria (IZD = 16 ± 0.01 mm to 7 ± 0.07 mm) than Gram-(−) bacteria (IZD of 13 mm to 2 mm). Additionally, IZD values showed that *H. cheirifolia* essential oil had the best antibacterial action in fighting *S. aureus* (IZD = 12 ± 0.06 mm for 0.25 mg mL^{-1}), but its ability to combat *K. pneumoniae* and *E. coli* was lower (IZD = 1 ± 0.05 mm for 0.5 mg mL^{-1} and 2 mm for 0.25 mg mL^{-1}).

Table 2 Inhibition zone of *H. cheirifolia* EO and selected antibiotic against six bacterial strains

	IZD (mm)						
	Control		Concentration (mg mL^{-1})				
Bacterial strains	DMSO	G	1	1/2	1/4	1/8	S[a]
S. aureus	0	21 ± 0.09	16 ± 0.01	15 ± 0.01	12 ± 0.20	8 ± 0.04	++
S. pneumoniae	0	12 ± 0.06	15 ± 0.01	13 ± 0.07	7 ± 0.04	7 ± 0.06	++
E. coli	0	16 ± 0.12	3 ± 0.05	1 ± 0.05	0 ± 0.01	0 ± 0.32	−
K. pneumoniae	0	14 ± 0.07	9 ± 0.04	4 ± 0.01	2 ± 0.06	0 ± 0.01	+
P. mirabilis	0	10 ± 0.01	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.01	−
E. aerogenes	0	0 ± 0.35	13 ± 0.00	10 ± 0.08	10 ± 0.18	7 ± 0.02	+

[a] S = sensitivity: (+) sensitive; (++) very sensitive; and (−) not sensitive.

According to these results, *H. cheirifolia* EO had the same effect against Gram-(+) bacteria, especially against *S. aureus*, which was the bacterium that was most sensitive to *H. cheirifolia* EO in earlier studies on the same plant from various regions.[19,20] The results of Segueni *et al.*[22] showed considerable activity of *H. cheirifolia* EO against *E. coli* and *K. pneumoniae*, with the strongest inhibition zone diameter value (23–12 mm for 2000–25 μg mL^{-1}) and (IZD = 22.33–8.6 mm for 2000–25 μg mL^{-1}). However, our study revealed that the EO had a very weak-to-no effect on *E. coli* (IZD = 3–0 mm for 1–0.25 mg mL^{-1}) and *K. pneumoniae* (IZD = 9–0 mm for 1–0.15 mg mL^{-1}). Nevertheless, HC-AKA-EO was active against Gram-(−) microorganisms (*E. coli* and *E. cloacae*), in contrast to the results of Segueni *et al.*,[22] who reported that the sample was ineffective against these bacterial species. The same observation pertains to an investigation carried out by Majouli *et al.*,[16] which demonstrated the antibacterial ability of the Tunisian sample against Gram-positive bacteria, specifically *S. aureus* (MIC of 0.078 to 0.312 mg mL^{-1}), as opposed to Gram-(−) bacterial strains. Furthermore, the *H. cheirifolia* extracts were ineffective against *E. coli*, *P. aeruginosa*, and *S. aureus*, according to the findings of Bousselsela *et al.*[25] According to Rahali *et al.*,[21] fluctuation in EO content may be the primary cause of these variations. Additionally, several prior studies have shown how well α-pinene works to inhibit and eradicate a broad range of microorganisms, Gram (+) and Gram (−). It has also been used to alter the resistance of bacteria to antibiotics.[56,59–61] According to Rahali *et al.*,[21] an examination of the correlation between main compounds with biological capacities, and the EO revealed that the quantities of drimenin, sesquiterpenes, lactones, and drimane-type compounds were negatively correlated with the inhibition of β-carotene bleaching, whereas a favorable correlation was observed between the contents of α-pinene and monoterpene hydrocarbons. Nevertheless, the biological activities of an essential oil rely on interactions between primary and the minor compounds, which can result in antagonistic or synergistic reactions, rather than on the concentration of the main molecule alone.[62–64] As a result, the lack of a meaningful relationship between the amounts of some phytochemical components, and the degree of activity does not rule out their potential benefits because the biological capacities of these compounds have been revealed in several studies and on a large scale.

Previous studies have shown that α-pinene,[54,65] β-pinene,[73] sabinene,[66,71] germacrene-D,[74] and linalool,[67,76] are volatile compounds present in many EOs extracted from several plant species with several recognized biological properties. These substances have demonstrated antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and allelopathic effects.[10,12,54,65,67,70]

The possible mechanisms of action of these compounds are described below:

α-Pinene is a bicyclic monoterpene, whose isomer is β-pinene. It is present in EOs from plants belonging to the Asteraceae family (*H. Cheirifolia*,[12,16,21] *Matricaria recutita* L.[68,69] and *Achillea millefolium* L.[70,77]) This substance is known for its antimicrobial[65,67,73] and antioxidant activity.[54,55] Some investigations also showed that α-pinene had hypoglycemic and anti-inflammatory activities *in vivo*.[72] Conversely, its isomer, β-pinene, is known for its good biological properties in several species of medicinal plant.[73] Enaide *et al.*[69] confirmed this, since they demonstrated that this natural compound presented hypoglycemic and hypolipidemic effects. This appears to entail, at least partially, the blockage of ATP-dependent K^+ channels and activation of LPL, respectively. Additionally, the administration of β-pinene treatment demonstrated a significant anti-inflammatory effect, potentially through the inhibition of inflammatory mediators involved in the second phase of the process and/or a reduction in leukocyte migration, suggesting a connection between the action of the compound and suppression of cytokine production. It is crucial to emphasize that further research is required to fully understand the processes underlying the hypoglycemic, hypolipidemic, and anti-inflammatory benefits of β-pinene in diabetic rats. In contrast, a study by Ana Cristina *et al.*[65] showed that only the positive enantiomers of pinene have an antimicrobial effect against MRSA, C. neoformans, R. oryzae, or C. albicans.

Germacrene-D is a hydrocarbon belonging to the class of sesquiterpenoid germacranes. This sesquiterpene has five isomers: germacrene A, B, C, D, and E. It is known for its antibacterial properties.[70] According to several previous studies, this organic compound is present in the EOs of several plants (*L. camara*, *L. alba*, *A. gratissima*, and *L. montevidensis*).[69] This substance can be applied as an adjuvant agent when applying

azoles and aminoglycosides since it possesses antifungal and antibacterial properties.[67]

Sabinene is a natural organic compound classified as a monoterpene found in the EOs of various plants.[67,73,77] EO antimicrobial activity is highly correlated with the composition of monoterpenes compared with other chemical families and in oxygenated molecules against hydrocarbons. Indeed, this monoterpene (sabinene) showed promising antibacterial and antifungal activities.[67,73,75]

Linalool is a terpene alcohol, an unsaturated tertiary alcohol, with a floral and fresh odor. It is found in a majority of essential oils, notably those of *L. angustifolia*, *L. Officinalis*, *A. Millefolium* and *S. rosmarinus*.[54,66,72,76] This natural product is one of the major constituents of some of these EOs, and it has been shown in previous investigations to improve the permeability of fungal cells and negatively charged membranes.[78,79] Owing to their molecular makeup, alcohols have significant affinity for attaching to various molecular structures, including proteins and glycoproteins. Because of this, they have a strong affinity for cell membranes and have a high potential to penetrate cell walls and release cytoplasm.

Antifungal activity

The effect of HC-AKA-EO on the mycelial growth of phytopathogenic FOL (*Fusarium oxysporum* f.sp. *lycopersici*) fungi was evaluated. FOL was 100% sensitive to oil over the six-day incubation period, as indicated by data (Table 3; Fig. 5), which also showed that the essential oil was a very efficient inhibitor. In contrast to the control (8.8 ± 0.1 cm, data is expressed as mean ± SEM, $n = 3$), the sample fully suppressed the development of FOL at three doses (1/4, 1/2, and 1 mg mL^{-1}), while the maximum mycelial growth rate (2.1 ± 0.1 cm; with inhibition effect % = 76.13 ± 0.09) was recorded at a 0.125 mg mL^{-1} concentration. These results show that FOL responded to increasing concentrations of HC-AKA-EO, which increased the rate of inhibition and slowed mycelial development. Conceivably, these results are linked mainly to the richness of HC-AKA-EO in the bioactive compounds, especially monoterpenes and sesquiterpenes. Indeed, many studies have shown that monoterpenoids and sesquiterpenoids are considered excellent alternatives for antifungal and antibacterial agents due to their wide-reaching bioactivities and abundance of structural skeletal types.[80–82] To the best of our knowledge, no prior research has looked into how the essential oil of this sap affects the mycelial growth of FOL. This work is the first to address this activity, and it is interesting to see that HC-AKA-EO has a strong antifungal effect against FOL.

Table 3 Growth inhibition of FOL treated with *H. cheirifolia* essential oil

Colony diameter (cm)		Concentration EO (mg mL^{-1})	Inhibition effect (%)
C	T		
8.8 ± 0.1	2.1 ± 0.1	1/8	76.13 ± 0.09
	0.0 ± 0.0	1/4	100 ± 0.14
	0.0 ± 0.0	1/2	100 ± 0.48
	0.0 ± 0.0	1	100 ± 0.07

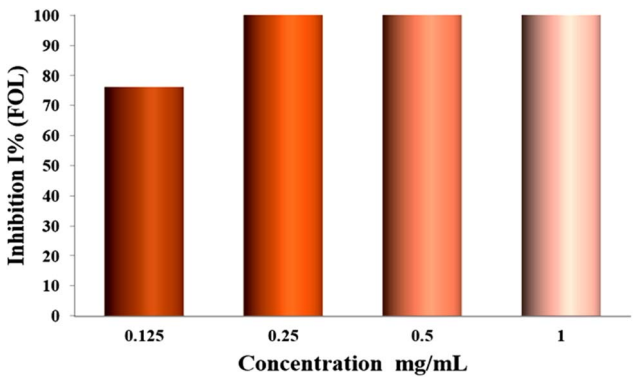

Fig. 5 Effect of *H. cheirifolia* essential oil of different concentrations on FOL.

Conclusions

These findings indicate that the essential oil of *H. cheirifolia* may be a readily available, natural source of chemicals with antioxidant, antimicrobial, and antifungal properties. They can be applied to cosmetics, food preparation and preservation, and pharmacological treatments. Furthermore, in comparison to earlier research, it can be said that, to acquire oil of superior quality and value, the right time period for the growth of this plant as well as section from which the oil is to be harvested must be chosen. However, to fully explore the benefits of *H. cheirifolia* EO, more *in vivo* research is necessary, along with more testing on different bacterial and fungal strains, which could contribute significantly to the discovery and development of new antimicrobial and antifungal agents based on *H. cheirifolia* EO. In addition, we hope in future studies to examine the essential oils separately from leaves, flowers, and stems to find out which part is rich in the bioactive compounds. Additional investigations are needed to determine the compounds responsible for antioxidant, antibacterial, and antifungal activities.

Abbreviations

EO	Essential oil
GC-MS	Gas chromatography coupled with mass spectrometry
EI	Electron ionization
LRI	Linear retention index
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
H_2O_2	Hydrogen peroxide
FRAP	Ferric reducing antioxidant power
Y	Yield
IC_{50}	Half-maximal inhibitory concentration
MHA	Mueller-Hinton Agar
ADT	Agar diffusion technique

IZ	Inhibition zone diameter
PDA	Potato, dextrose, and agar
FOL	*Fusarium oxysporum* f.sp. *lycopersici*
USA	United States of America
UK	United Kingdom
G	Gentamicin

Data availability

The manuscript includes all the research data used in this study.

Author contributions

Wassila Benabderrahmane, Hamza Fadel, Ruqaiah I. Bedaiwi, Mohammad A. Alanazi, Helal F. Al-Harthi: conceptualization, methodology, and software. Ines Sekhara, Imad Mennai, Tasahil S. Albishi, Alaa T. Qumsani, Rokayya Sami, Hamza Fadel: funding acquisition. Rokayya Sami, Hala M. Abo-Dief, Sameer H. Qari, Wassila Benabderrahmane, Mohammad A. Alanazi: validation, formal analysis, investigation, and resources. Imed Eddine Kadi, Mahmoud Helal, Tasahil S. Albishi, Alaa T. Qumsani, Mohammad A. Alanazi, Hala M. Abo-Dief, Sameer H. Qari: writing—original draft preparation. Ruqaiah I. Bedaiwi, Helal F. Al-Harthi, Wassila Benabderrahmane, Imed Eddine Kadi, Mahmoud Helal, Roqayah H. Kadi, Suzan A. Abushal: data curation, writing—review and editing, and visualization. Roqayah H. Kadi, Suzan A. Abushal, Wassila Benabderrahmane, Rokayya Sami, Ines Sekhara, Imad Mennai: software and drawings.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-79). The Center for Scientific and Technical Research in Physico-Chemical Analyzes (CRAPC)-PTAPC-Laghouat, Algeria's Miss Kherifi Hadda, is much appreciated by the authors for her analysis of the essential oil sample. Further, the authors thank Marta Lores for her great efforts in editing.

References

1 A. Hussain, Potential Medicinal Uses of Plants From the Asteraceae (Compositae) Family in Pakistan: A Literature Review Based Meta-analysis, *J. Herb. Med.*, 2024, **45**, 100871.
2 A. Rolnik and B. Olas, The plants of the Asteraceae family as agents in the protection of human health, *Int. J. Mol. Sci.*, 2021, **22**(6), 3009.
3 M. Denisow-Pietrzyk, L. Pietrzyk and B. Denisows, Asteraceae species as potential environmental factors of allergy, *Environ. Sci. Pollut. Res.*, 2019, **26**, 6290–6300.
4 G. V. Ccana-Ccapatinta, M. Monge, P. L. Ferreira and F. B. Da Costa, Chemistry and medicinal uses of the subfamily Barnadesioideae (Asteraceae), *Phytochem. Rev.*, 2018, **17**, 471–489.
5 E. Piątkowska, W. Biel, R. Witkowicz and J. Kępińska-Pacelik, Chemical composition and antioxidant activity of Asteraceae family plants, *Appl. Sci.*, 2022, **12**(23), 12293.
6 R. M. K. Yien, A. P. d. Santos Matos, A. C. C. Gomes, D. d. A. Garófalo, R. Santos-Oliveira, N. K. Simas and E. Ricci-Júnior, Nanotechnology promoting the development of products from the biodiversity of the Asteraceae family, *Nutrients*, 2023, **15**(7), 1610.
7 M. Petrova, K. M. Georgieva and M. P. Geneva, Influence of abiotic and biotic elicitors on organogenesis, biomass accumulation, and production of key secondary metabolites in Asteraceae plants, *Int. J. Mol. Sci.*, 2024, **25**(8), 4197.
8 M. R. Akhgar, M. Shariatifar, A. R. Akhgar, M. Moradalizadeh and A. Faghihi-Zarandi, Chemical composition and antibacterial activity of the leaf essential oil from *Hertia Intermedia*, *Chem. Nat. Compd.*, 2012, **48**, 329–331.
9 D. Iamonico and R. El mokni, Hertia cheirifolia and H. maroccana (Asteraceae), two species endemic to North Africa: nomenclatural notes, morphology, distribution, and IUCN Red List assessments, *Collect. Bot.*, 2021, **40**, 009.
10 S. Soltanian, M. Sheikhbahaei, N. Mohamadi, A. Pabarja, M. F. S. Abadi and M. H. M. Tahroudi, Biosynthesis of zinc oxide nanoparticles Using *Hertia intermedia* and evaluation of its cytotoxic and antimicrobial activities, *J. Bionanosci.*, 2021, **11**, 245–255.
11 K. Majouli, M. B. Hlila, A. Hamdi and A. Kenani, Phytochemical and pharmacological properties of *Hertia* L. genus, *SOJ Pharm. Pharm. Sci.*, 2018, **5**(2), 1–3.
12 M. Cherni, S. Dallali, D. Dallali, R. Mouhbi, H. Bouraoui, S. Jdidi and H. Selmi, Allelopathic activity and chemical constituents of extracts from leaves and stems of Kherchoun (*Hertia cheirifolia*), *J. Int. Res. Med. Pharm. Sci.*, 2023, **18**(2), 1–12.
13 A. S. Gubb, La flore Algérienne naturelle et Acquise, *Imprimerie Adolphe Jourdan, place du gouvernement*, Alger, 1913.
14 G. Pottier-Alapetite, *Flore de la Tunisie. Imprimerie officielle de la république Tunisienne*, 1981, vol. 11.
15 N. T. Beniston and W. S. Beniston, *Fleurs d'Algérie F.N.1*, Alger, 1984.
16 K. Majouli, M. B. Hlila, G. Flamini, H. B. Jannet and A. Kenani, *In vitro* antibacterial activity of the *Hertia cheirifolia* L. essential oils, *J. Coastal Life Med.*, 2016, **4**(11), 865–867.
17 H. Bouriche, S. Kada, A. M. Assaf, A. Senator, F. Gül and I. Dimertas, Phytochemical screening and anti-inflammatory properties of Algerian *Hertia cheirifolia* methanol extract, *Pharm. Biol.*, 2016, **54**(11), 2584–2590.
18 K. Abdelouhab, T. Guemmaz, M. Karamać, D. E. Kati, R. Amarowicz and L. Arrar, Phenolic composition and correlation with antioxidant properties of various organic

fractions from *Hertia cheirifolia* extracts, *J. Pharm. Biomed. Anal.*, 2023, **235**, 115673.

19 N. Bassa, S. Boulechfar, S. Boutellaa, M. M. Senoussi and A. Zellagui, Essential oils of *Hertia cheirifolia* Leaves in fructification stage with high anti bacterial activity, *Ann. Rom. Soc. Cell Biol.*, 2022, **26**(1), 3784–3791.

20 A. Ounoughi, M. Ramdani, T. Lograda, P. Chalard and G. Figueredo, Chemical composition, antimicrobial activity and chromosome number of *Hertia cheirifolia* L. from Algeria, *Acta Sci. Nat.*, 2020, **7**(2), 31–43.

21 N. Rahali, S. Mehdi, F. Younsi, M. Boussaid and C. Messaoud, Antioxidant, α-amylase, and acetylcholinesterase inhibitory activities of *Hertia cheirifolia* essential oils: influence of plant organs and seasonal variation, *Int. J. Food Prop.*, 2017, **20**(S2), S1637–S1651.

22 N. Segueni, A. Zellagui, S. Boulechfar, K. Derouiche and S. Rhouati, Essential oil of *Hertia cheirifolia* leaves: chemical composition, antibacterial and antioxidant activities, *J. Mater. Environ. Sci.*, 2017, **8**(2), 551–556.

23 K. Majouli and A. Kenani, Bioactivity of essential oil from *Hertia cheirifolia* L. flowers in the control of bacteria, *Trends Phytochem. Res.*, 2017, **1**(1), 23–26.

24 S. Attia, K. L. Grissa, A. C. Mailleux, S. Heuskin, G. Lognay and T. Hance, Acaricidal activities of *Santolina africana* and *Hertia cheirifolia* essential oils against the two-spotted spider mite (Tetranychusurticae), *SCI Pest Manag. Sci.*, 2012, **68**, 1069–1076.

25 H. Bousselsela, A. Benhouda, M. Yahia, S. Benbia, A. Ghecham and A. Zidani, *In vitro* evaluation of antioxidant and antibacterial activities of extracts of *Hertia cheirifolia*'s leaves, *Nat. Sci.*, 2012, **4**(11), 825–831.

26 A. Khedidja, C. Touahria, N. E. H. Djeghader and H. Boudjelida, Laboratory study of the larvicidal efficacy of a local plant *Hernia cheirifolia* against the most abundant mosquito species, in Algeria, *J. Entomol. Zool. Stud.*, 2018, **6**(1), 258–262.

27 B. Boulanouar, K. Cheraif, A. Gherib and M. Rezzoug, GC-MS Analysis of essential oil of Lavandula officinalis (Lamiaceae) from Laghouat, Algeria, *South Asian J.*, 2022, **12**(2), 232–236.

28 V. I. Babushok, P. J. Linstrom and I. G. Zenkevich, Retention indices for frequently reported compounds of plant essential oils, *J. Phys. Chem. Ref. Data*, 2011, **40**(4), 043101.

29 S. Mahcene, F. Elhouiti, I. Mennai, D. C. G. A. Pinto, D. Tahri, M. Ouinten and M. Yousfi, A comparative study on the chemical composition, anti-microbial, antiparasitic, and cytotoxic activities of *Rhanterium adpressum* Coss. & Dur. and *Rhanterium suaveolens* Desf. essential oils from Algeria, *J. Essent.Oil Bear. Plants*, 2024, **27**(2), 327–340.

30 O. D. Gargouri, B. Gargouri, S. K. Trabelsi, M. Bouaziz and R. Abdelhédi, Synthesis of 3-O-methylgallic acid a powerful antioxidant by electrochemical conversion of syringic acid, *Biochim. Biophys. Acta, Gen. Subj.*, 2013, **1830**(6), 3643–3649.

31 A. Remigante, S. Spinelli, E. Straface, L. Gambardella, D. Caruso, G. Falliti, S. Dossena, A. Marino and R. Morabito, Antioxidant activity of quercetin in a H_2O_2-induced oxidative stress model in red blood cells: functional role of band 3 protein, *Int. J. Mol. Sci.*, 2022, **23**(19), 10991.

32 M. Chokri, A. Laabidi and M. Boussaid, *Myrtus communis* L. Infusions: the effect of infusion time on phytochemical composition, antioxidant, and antimicrobial activities, *J. Food Sci.*, 2012, **77**(9), 941–947.

33 W. Song, L. Zhou, C. Yang, X. Cao, L. Zhang and X. Liu, Tomato Fusarium wilt and its chemical control strategies in a hydroponic system, *Crop Prot.*, 2004, **23**(3), 243–247.

34 R. P. Patel, R. Singh, B. R. R. Rao, R. R. Singh, A. Srivastava and R. K. Lal, Differential response of genotype×environment on phenology, essential oil yield and quality of natural aroma chemicals of five *Ocimum* species, *Ind. Crops Prod.*, 2016, **87**, 210–217.

35 S. F. K. Doozakhdarreh, J. Khorshidi and M. R. Morshedloo, Essential oil content and components, antioxidant activity and total phenol content of rosemary (*Rosmarinus ofcinalis* L.) as affected by harvesting time and drying method, *Bull. Natl. Res. Cent.*, 2022, **46**(1), 199.

36 O. S. Tsiftsoglou, R. Stagiopoulou, N. Krigas and D. Lazari, Exploring the ecological preferences and essential oil variability in wild-growing populations of the endangered local greek endemic *Thymus holosericeus* (Lamiaceae), *Plants*, 2023, **12**(2), 348.

37 F. Z. Benomari, M. Sarazin, D. Chaib, A. Pichette, H. Boumghar, Y. Boumghar and N. Djabou, Chemical variability and chemotype concept of essential oils from Algerian wild plants, *Molecules*, 2023, **28**(11), 4439.

38 O. Izuegbuna, G. Otunola and G. Bradley, Chemical composition, antioxidant, antiinflammatory, and cytotoxic activities of *Opuntia stricta* cladodes, *PLoS One*, 2019, **14**(1), e0209682.

39 Z. J. Ni, X. Wang, Y. Shen, K. Thakur, J. Han, J. G. Zhang, F. Hu and Z. J. Wei, Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils, *Trends Food Sci. Technol.*, 2021, **110**(1), 78–89.

40 P. Ramak, S. K. Osaloo, M. Sharifi, H. Ebrahimzadeh and M. Behmanesh, Biosynthesis, regulation and properties of plant monoterpenoids, *J. Med. Plants Res.*, 2014, **8**(29), 983–991.

41 F. Z. Kamal, G. D. Stanciu, R. Lefter, V. V. Cotea, M. Niculaua, D. C. Ababei, A. Ciobica and A. Ech-Chahad, Chemical Composition and Antioxidant Activity of Ammi visnaga L. Essential Oil, *Antioxidants*, 2022, **11**(2), 347.

42 H. Fadel, F. Benayache, J. C. Chalchat, G. Figueredo, P. Chalard, H. Hazmoune and S. Benayache, Essential oil constituents of *Juniperus oxycedrus* L. and *Cupressus sempervirens* L. (Cupressaceae) growing in Aures region of Algeria, *Nat. Prod. Res.*, 2019, **35**(15), 2616–2620.

43 S. Walia, S. Mukhia, V. Bhatt, R. Kumar and R. Kumar, Variability in chemical composition and antimicrobial activity of *Tagetes minuta* L. essential oil collected from different locations of Himalaya, *Ind. Crops Prod.*, 2020, **150**, 112449.

44 N. Farhadi, K. Babaei, S. Farsaraei, M. Moghaddam and A. G. Pirbalouti, Changes in essential oil compositions,

total phenol, flavonoids and antioxidant capacity of *Achillea millefolium* at different growth stages, *Ind. Crops Prod.*, 2020, **152**(8), 112570.

45 S. Daghbouche, I. Ammar, D. M. Rekik, Z. E. Djazouli, B. Zebib and O. Merah, Effect of phenological stages on essential oil composition of *Cytisus triflorus* L'Her, *J. King Saud Univ., Sci.*, 2020, **32**(4), 2383–2387.

46 M. Afshar, S. Najafian and M. Radi, The effect of harvest time on the natural product of *Rosmarinus officinalis* L. from South Iran (Fars province), *Nat. Prod. Res.*, 2021, **36**(10), 2637–2642.

47 S. Srivastava, R. K. Lal, R. Maurya, A. Mishra, A. K. Yadav, G. Pandey, P. K. Rout and C. S. Chanotiya, Chemical diversity of essential oil among basil genotypes (*Ocimum viride* Willd.) across the years, *Ind. Crops Prod.*, 2021, **173**(3149362), 114153.

48 S. Rathore, S. Mukhia, S. Kapoor, V. Bhatt, R. Kumar and R. Kumar, Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya, *Sci. Rep.*, 2022, **12**(1), 1–13.

49 K. Majouli, M. B. Hlila, A. Hamdi, G. Flamini, H. B. Jannet and A. Kenani, Antioxidant activity and α-glucosidase inhibition by essential oils from *Hertia cheirifolia* (L.), *Ind. Crops Prod.*, 2015, **82**, 23–28.

50 A. Kadri, Z. Zarai, I. B. Chobba, N. Gharsallah, M. Damak and A. Békir, Chemical composition and *in vitro* antioxidant activities of *Thymelaea hirsute* L: essential oil from Tunisia, *Afr. J. Biotechnol.*, 2013, **10**, 2930–2935.

51 A. Assaeed, A. Elshamy, A. E. El Gendy, B. Dar, S. Al-Rowaily and A. Abd-El Gawad, Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis*exhibited antioxidant activity and allelopathic effect on weeds, *Agronomy*, 2020, **10**(3), 399.

52 D. V. Sudarikov, Y. V. Krymskaya, A. K. Melekhin and O. G. Shevchenko, Synthesis and antioxidantactivity of monoterpenen itrobenzylidene sulfenimines, *Chem. Pap.*, 2021, **75**(6), 1–7.

53 I. Y. Chukicheva, I. V. Fedorova, O. G. Shevchenko and A. V. Kutchin, Monoterpene derivatives of sesamol. Synthesis and evaluation of antioxidant properties, *Russ. Chem. Bull.*, 2023, **72**, 2215–2223.

54 M. Chraibi, A. Farah, O. Elamin, H. M. Iraqui and K. Fikri-Benbrahim, Characterization, antioxidant, antimycobacterial, antimicrobial effects of Moroccan rosemary essential oil, and its synergistic antimicrobial potential with carvacrol, *J. Adv. Pharm. Technol. Res.*, 2020, **11**(1), 25–29.

55 B. B. Akacha, M. Michalak, I. G. Mekinić, M. Kačániová, M. Chaari, F. Brini, R. B. Saad, W. Mnif, S. Garzoli and A. B. Hsouna, Mixture design of α-pinene, α-terpineol, and 1,8-cineole: a multiobjective response followed by chemometric approaches to optimize the antibacterial effect against various bacteria and antioxidant activity, *Food Sci. Nutr.*, 2023, **12**(1), 574–589.

56 J. K. Nagy, Á. M. Móricz, A. Böszörményi, Á. Ambrus and I. Schwarczinger, Antibacterial effect of essential oils and their components against *Xanthomonas arboricola* pv. *pruni* revealed by microdilution and direct bioautographic assays, *Front. Cell. Infect. Microbiol.*, 2023, **13**, 2023.

57 A. Benslama, S. Boumerfeg, A. Beghiani, S. Aouachria, S. Khennou and L. Arrar, Total phenolic contents and antioxidant activities of *Hertia cheirifolia* leaves extracts, *Proceeding of the 2nd African Congress on Biology and Health University Ferhat Abbas Setif1*, 2012, vol. 93.

58 M. Menakh, D. Mahdi, S. Boutellaa, A. Zellagui, M. Lahouel and C. Bensouici, In vitro antioxidant activity and protective effect of *Hertia cheirifolia* L. *n*-butanol extract against liver and heart mitochondrial oxidative stress in rat, *Acta Sci. Nat.*, 2020, **7**(1), 33.

59 A. C. R. da Silva, P. M. Lopes, M. M. B. Azevedo, D. C. M. de Costa, C. S. Alviano and D. S. Alviano, Biological activities of α-pinene and β-pinene enantiomers, *Molecules*, 2012, **17**(6), 6305–6316.

60 B. Salehi, S. Upadhyay, I. E. Orhan, A. K. Jugran, S. L. D. Jayaweera, D. A. Dias, F. Sharopov, Y. T. N. Martins, N. Baghalpour, W. C. Cho and J. Sharifi-Rad, Therapeutic potential of α- and β-Pinene: a miracle gift of nature, *Biomol*, 2019, **9**(11), 738.

61 A. Joshi, U. Sharma, K. Samani, V. Surendra, G. Swamy and P. Manjunath, Review on therapeutic activity of pinene ($C_{10}H1_6$): an essential oil, *Int. J. Adv. Res. Pharm. Edu.*, 2020, **2**(1), 28–33.

62 S. Aazza, B. Lyoussi and M. G. Miguel, Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds, *Molecules*, 2011, **16**(9), 7672–7690.

63 J. H. Tak, E. Jovel and M. B. Isman, Comparative and synergistic activity of *Rosmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, Trichoplusiani (Lepidoptera: Noctuidae), *Pest Manage. Sci.*, 2016, **72**(3), 474–480.

64 B. Tohidi, M. Rahimmalek and A. Arzani, Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran, *Food Chem.*, 2017, **220**, 153–161.

65 A. C. d. S. Rivas, P. M. Lopes, M. M. A. Barros, D. C. C. Machado, C. S. Alviano and D. S. Alviano, Biological Activities of α-Pinene and β-Pinene Enantiomers, *Molecules*, 2012, **17**(6), 6305–6316.

66 S. Ramos, L. B. Rojas, M. E. Lucena, G. Meccia and A. Usubillaga, Chemical Composition and Antibacterial Activity of *Origanum majorana* L. Essential Oil from the Venezuelan Andes, *J. Essent. Oil Res.*, 2011, **23**(5), 45–49.

67 K. Diass, M. Merzouki, K. Elfazazi, H. Azzouzi, A. Challioui, K. Azzaoui, B. Hammouti, R. Touzani, F. Depeint, A. A. Gotor and L. Rhazi, *Plants*, 2023, **12**(7), 1571.

68 M. Kazemi, Chemical Composition and Antimicrobial Activity of Essential Oil of *Matricaria recutita*, *Int. J. Food Prop.*, 2015, **18**, 1784–1792.

69 I. Salamon, A. Ibraliu and M. Kryvtsova, Essential Oil Content and Composition of the Chamomile Inflorescences (*Matricaria recutita* L.) Belonging to Central Albania, *Horticulturae*, 2023, **9**(1), 47.

70 P. S. Daniel, E. L. B. Lourenço, R. M. S. Cruz, C. H. D. S. Gonçalves, L. R. M. D. Almas, J. Hoscheid, C. D. Silva, E. Jacomassi, L. B. Junior and O. Alberton, Composition and antimicrobial activity of essential oil of yarrow (*Achillea Millefolium* L.), *AJCS*, 2020, **14**(3), 545–550.

71 W. Zaibet, H. Laouer, S. amira, G. Flamini, M. Ramdani and S. Akkal, Chemical composition and biological activities of *Daucus aureus* essential oils from eastern algeria, *J. Chil. Chem. Soc.*, 2015, **60**(4), 2721–2728.

72 H. Özbek and B. S. Yılmaz, Anti-inflammatory and Hypoglycemic Activities of Alpha-pinene, *Acta Pharm. Sci.*, 2017, **55**(4), 7–14.

73 E. S. Santos, G. L. A. Coelho, Y. K. S. F. Loula, B. L. S. Landim, C. N. F. Lima, S. T. S. Machado, M. J. P. Lopes, A. D. S. Gomes, J. G. M. d. Costa, I. R. A. d. Menezes, H. D. M. Coutinho, B. Kim, C. F. B. Felipe, S. d. A. Neves and M. R. Kerntopf, Hypoglycemic, Hypolipidemic, and Anti-Inflammatory Effects of Beta-Pinene in Diabetic Rats, *Evidence-Based Complementary Altern. Med.*, 2022, 8173307.

74 C. M. P. Zamora, C. A. Torres and M. B. Nuñez, Antimicrobial Activity and Chemical Composition of Essential Oils from Verbenaceae Species Growing in South America, *Molecules*, 2018, **23**(3), 544.

75 R. Arunkumar, S. A. Nair, K. B. Rameshkumar and A. Subramoniam, The Essential Oil Constituents of *Zornia diphylla* (L.) Pers, and Anti-Inflammatory and Antimicrobial Activities of the Oil, *Recent Nat. Prod.*, 2014, **8**(4), 385–393.

76 K. Pokajewicz, M. Białoń, L. Svydenko, R. Fedin and N. Hudz, Chemical Composition of the Essential Oil of the New Cultivars of Lavandula angustifolia Mill. Bred in Ukraine, *Molecules*, 2021, **26**(18), 5681.

77 S. T. Chou, H. Y. Peng, J. C. Hsu, C. C. Lin and Y. Shih, *Achillea millefolium* L. Essential Oil Inhibits LPS-Induced Oxidative Stress and Nitric Oxide Production in RAW 264.7 Macrophages, *Int. J. Mol. Sci.*, 2013, **14**(7), 12978–12993.

78 W. S. Alviano, R. R. Mendonça-Filho, D. S. Alviano, H. R. Bizzo, T. Souto-Padrón, M. L. Rodrigues, A. M. Bolognese, C. S. Alviano and M. M. G. Souza, Antimicrobial activity of Croton cajucara Benth linalool-rich essential oil on artificial biofilms and planktonic microorganisms, *Oral Microbiol. Immunol.*, 2005, **20**(2), 101–105.

79 F. Silva, S. Ferreira, J. A. Queiroz and F. C. Domingues, Coriander (Coriandrum sativum L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry, *J. Med. Microbiol.*, 2011, **60**, 1479–1486.

80 F. G. Pereira, R. Marquete, L. T. Domingos, M. E. N. Rocha, A. Ferreira-Pereira, E. Mansur and D. L. Moreira, Antifungal activities of the essential oil and its fractions rich in sesquiterpenes from leaves of *Casearia sylvestris* Sw, *An. Acad. Bras. Cienc.*, 2017, **89**(4), 2817–2824.

81 H. Li, X. Song, H. Li, L. Zhu, S. Cao and J. Liu, Sesquiterpenes and monoterpenes from the leaves and stems of *Illicium simonsii* and their antibacterial activity, *Molecules*, 2022, **27**(3), 1115.

82 H. L. Li, W. Q. Yang, X. Z. Zhou, F. Shao, T. Shen, H. Y. Guan, J. Zheng and L. M. Zhang, Antibacterial and antifungal sesquiterpenoids: chemistry, resource, and activity, *Biomol.*, 2022, **12**(9), 1271.