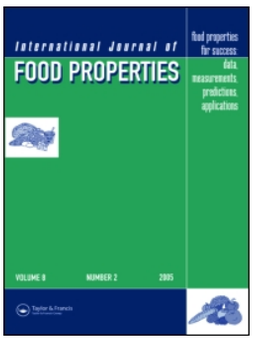

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Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts

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ABSTRACT

This study aimed to assess the biological activities and secondary compound composition from Algerian *Crithmum maritimum* and, therefore, to better characterize local medicinal plants. The aerial parts were investigated regarding their mineral, antioxidant and essential oil content, and composition. The *C. maritimum* aerial parts contained high levels of potassium, calcium, manganese, and iron. The phenolic fraction was composed by at least 10 identified hydroxycinnamic acids that exhibited scavenging activity against DPPH and $ABTS^{+}$. Moreover, the *C. maritimum* essential oil had a strong antimicrobial activity. The essential oil was composed by a least 10 molecules comprising monoterpene hydrocarbons and oxygenated monoterpenes.

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KEYWORDS
Crithmum maritimum; Antioxidants; Phenolic profiles; Antioxidant activity; Essential oil; Antimicrobial activity

Introduction

Crithmum maritimum L. is a halophyte plant, belonging to the Apiaceae family. This plant is known as sea fennel or rock samphire[1] and grows on maritime cliffs and sand in the European Atlantic coasts, Azores, Madeira, Canarias Islands, Mediterranean and Black Sea coasts, north-west Africa, and western Asia.[2] *C. maritimum* is known to have several medicinal properties, especially its succulent aerial part and young branches which exhibit antiscorbutic, carminative, diuretic, digestive, purgative, and vermifuge properties. Furthermore, *C. maritimum* is also commonly known in the coastal areas as an excellent season salad used to accompany fish dishes.[3]

According to the available literature on its phytochemical composition, *C. maritimum* is known as a source of proteins, amino acids, vitamin C, minerals (potassium, sodium, calcium, and magnesium), phenolic compounds, and flavonoids.[4–10] In addition, in the last decades, different studies have contributed to characterize the composition of *C. maritimum* essential oil (EO) and described their antimicrobial activities.[2,3,11–14] The phenolic composition of *C. maritimum* has only been analyzed in a few studies. Coumarins were first identified in the fruits and the aerial parts.[15] Seven hydroxycinnamic

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acids were identified in aqueous extracts of *C. maritimum* aerial parts, chlorogenic acid being the major one,[9,16] whereas catechin and flavonoid were also reported in acetone extract of the stalk.[17]

In Algeria, *C. maritimum* is little known and not exploited despite its richness in different compounds and its medicinal properties. In order to provide more information about the composition of secondary compounds of local *C. maritimum* and promote the medicinal plants of Algeria, we propose to study, for the first time, this plant harvested in the Bejaia region (Algeria). This study aimed (1) to quantify the content in macro- and micro-elements together with the total content in carotenoids and phenolic compounds; (2) to identify and quantify the individual phenolic compounds in a hydro-methanolic extract of the aerial parts by high-performance liquid chromatography (HPLC)-diode array detector (DAD)-ESI-mass spectrometry (MS); (3) to determine the chemical composition of *C. maritimum* EO by gas chromatography (GC)-MS method; and (4) to evaluate its antioxidant and antimicrobial activities.

Materials and methods

Plant material

The aerial parts of *C. maritimum* plant were harvested in Tighzert region (Bejaïa, Algeria: latitude N36° 51′ 01.05, longitude E4° 52′ 59.93, altitude above sea level 30 m, a Mediterranean bioclimatic zone) in July 2014. At the laboratory, the plant was manually separated from bad weeds and washed with distilled water to remove dust and soil particles. The aerial parts were then separated into two portions; one was dried at 50°C in an oven before being ground and reduced to a fine powder of 250–500 µm diameter using a blender type A100 (Ika, Germany). The powder obtained was used for mineral, pigment, phenolic measurements, and characterization processes. The second portion was air-dried in darkness at room temperature until constant weight and then cut in small pieces and used for the extraction of the EOs.

Determination of mineral content

The determination of macro- and micro-elements of the *C. maritimum* aerial parts was assessed using the method previously described by Carbonell-Barrachina,[18] with slight modifications. Dried powder (1 g) was digested for 12 h at 130°C in a multi-place digestion block (Block Digest 20, Selecta, Barcelona, Spain) using 5 mL of 65% nitric acid (HNO_3). Samples were left to cool at room temperature and then transferred to volumetric flasks. Dilutions of 1:25, 1:100, and 1:200 (v/v) were prepared using ultrahigh-purity deionized water. Samples were stored at 4°C until analysis. Quantification of calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in the previously mineralized samples was performed using a Solaar 969 atomic absorption–emission spectrometer (Unicam Ltd., Cambridge, UK). K was analyzed by atomic emission, while the other elements were analyzed by atomic absorption. Instruments were calibrated using certified standards. Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.997$). Analyses were run in triplicate.

Determination of carotenoid content

The total carotenoid content of *C. maritimum* aerial parts was evaluated using the method described by Sass-Kiss,[19] with slight modifications. One gram of dried powder was placed in 50 mL flask and extracted in darkness with 20 mL *n*-hexane/acetone/ethanol (2/1/1, v/v/v). The mixture was shaken for 10 min. Then, the upper layer was pipetted into 50 mL flask and the procedure was repeated twice again. The collected extracts were gathered and placed in 100 mL separating funnel. One volume of 10% potassium hydroxide (KOH) in ethanol was added to settle the chlorophylls[20] and then the mixture was washed with distilled water. The *n*-hexane layer was recovered, filtered through filter paper and the absorbance of the yellow-colored extract was carried out at 450 nm. The results were expressed as mg β-carotene per kilogram dry weight [dw] mg β-car/kg dw).

Preparation of hydro-methanolic extract from C. maritimum aerial parts

The method of Chun[21] was adopted to extract phenolic compounds from *C. maritimum*. One gram of plant powder was mixed with 100 mL of 80% aqueous methanol. After shaking during 24 h at room temperature, the mixture was centrifuged at 4000 rpm for 20 min and then filtered. The extract obtained was used for the determination of total phenolic compounds, total flavonoid and condensed tannin contents, antioxidant activities and phenolic profiles by HPLC-DAD-ESI-MS analysis.

Determination of total phenolic content (TPC)

Determination of TPC was assessed by the method described by Djeridane,[22] using Folin–Ciocalteu reagent. One hundred microliters of the hydro-methanolic extract were dissolved in 500 µL (1/10) of the Folin–Ciocalteu reagent and 1000 µL of distilled water. After 1 min, 1500 µL of 20% sodium carbonate (Na_2CO_3) solution were added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of the blue complex formed was read at 765 nm using a Shimadzu-1240 ultraviolet-visible (UV-Vis) spectrophotometer (Germany). The amount of the TPC was deduced from the linear equation of a standard curve prepared with gallic acid. The results were expressed in gram of gallic acid equivalent (GAE) per kilogram of dw (g GAE/kg dw).

Determination of total flavonoid content (TFC)

TFC was determined according to the method of Zhishen.[23] One hundred microliters of the hydro-methanolic extract were first dissolved in 400 µL of distilled water. After 5 min, 30 µL of 5% of sodium nitrite was added to the solution and subsequently 30 µL of 10% aluminum chloride ($AlCl_3$) were added. After 6 min, 200 µL of 1 M sodium hydroxide (NaOH) were added to the mixture. The final volume of the reaction mixture was made up to 1 mL with distilled water and mixed thoroughly. Absorbance of the reaction mixture was measured at 510 nm against a blank. A standard calibration curve of catechin was plotted to calculate the flavonoids concentration. Results were expressed as gram of catechin equivalents per kilogram of dw (g CE/kg dw).

Determination of condensed tannins content (CTC)

CTC was determined according to the method of Vermerris.[24] In brief, 250 µL of the hydro-methanolic extract were mixed with 2.5 mL of an acidic solution of ferrous sulfate (77 mg of ferric ammonium sulfate: $Fe_2(SO_4)_3$ dissolved in 500 mL of [3:2 *n*-butanol:HCl]). After 50 min of incubation at 95°C, absorbance at 530 nm was measured against a blank. Condensed tannins were calculated using the following equation:

$$\frac{A_{530nm} \times \mathrm{DF} \times \mathrm{MW}}{\times l}$$

where DF is the dilution factor, MW the molecular weight of the cyanidin (287 g/mol), and ε the molecular extinction coefficient (34,700 L/mol.cm). The condensed tannins were expressed as gram of cyanidin equivalents per kilogram of dw (g CE/kg dw).

Identification and quantification of individual phenolic compounds by HPLC-DAD-ESI-MS

For their identification, the phenolic compounds contained in the hydro-methanolic extract, were separated and analyzed on a HPLC-DAD-ESI-MS^n system as described in Chougui.[25] Raw data were processed using the XCALIBUR software program (version 2.1, http://www.thermoscientific.com). Experimental exact masses and MS^2 fragmentation data were compared to metabolomics data banks (ReSpect: http://spectra.psc.riken.jp/, DNP:http://dnp.chemnetbase.com, Mass Bank:

http://www.massbank.jp) and other available data from the literature in order to identify the nature of the metabolites. For their quantification, the phenolic compounds were separated on a U-HPLC system as described in Larbat.[26] Briefly, one microliter of the hydro-methanolic extract was separated on a C18 Kinetex (100 mm × 2.1 mm) column (Phenomenex, USA) by using a gradient elution from 1 to 60% MeOH for 6.8 min, then 90% MeOH for 2.7 min with a flow rate of 300 µL/min. The column was rinsed for 1 min with 90% MeOH and reequilibrated to 1% MeOH for 2 min prior to the next run. Compound quantification was based on measurement of area under peak determined at 320 nm and expressed relative to calibration curves with ferulic acid (for 5-FQA), *p*-coumaric acid (for 3-CoQA, 5Co-QA) and chlorogenic acid (for 3-CQA, 5-CQA, 1-CQA, 3,4-DCQA, 3,5-DCQA, and 4,5-DCQA).

Determination of antioxidant activities

2,2-diphenylpicrylhydrazyl (DPPH) scavenging activity

The hydrogen atom or electron donation abilities of the hydro-methanolic extract was measured from the bleaching of the purple-colored MeOH solution of 2,2-diphenylpicrylhydrazyl (DPPH), according to method described by Gachkar.[27] Fifty microliters of the hydro-methanolic extract were added to 5 mL of a 0.004% MeOH solution of DPPH. BHA and BHT, stable antioxidants, were used as synthetic references. After a 30 min incubation period at room temperature, the absorbance was read against a control at 517 nm. Inhibition of free radical DPPH in percent (I[%]) was calculated using the following equation:

$$I\% = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control reaction (containing all reagents except the extract), and A_s is the absorbance of the assayed extract. The concentration of extract which scavenges 50% of the DPPH free radical (IC_{50}, mg/mL) was deduced from the percentage inhibition curve of the DPPH free radical obtained with different concentrations.

ABTS test

The total antioxidant activity (TAA) of the *C. maritimum* extract was evaluated by the Trolox equivalent antioxidant capacity (TEAC) assay according to the method given by Re.[28] In this test, the relative capacity of antioxidants to scavenge the $ABTS^{\bullet+}$ radical was evaluated by comparison with the antioxidant potency of Trolox which is used as a standard.

ABTS was dissolved in water at a concentration of 7 mM. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the darkness at room temperature for 12–16 h before use. For the assessment of the extract, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm and equilibrated at 30°C.

The spectrophotometer is preliminary blanked with ethanol. After addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution (A_{734nm} = 0.700 ± 0.020) to 10 µL of antioxidant compounds or Trolox standards (final concentration 0–1.5 M) in ethanol, the absorbance reading was taken at 30°C after 6 min of initial mixing. The percentage of inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data.

Extraction and characterization of C. Maritimum EO

The extraction of EO was made by hydro-distillation method, using a Clevenger-type apparatus for 3 h. The EO was collected, dried over anhydrous sodium sulfate and stored at –20°C until use. Analysis of volatile oil was carried out by GC and by GC-MS. The *C. maritimum* EO was diluted in ethyl acetate before analysis. Analytical GC was performed using Shimadzu QP2010 GC-MS, equipped with a

ZB5MS column (30 m × 0.25 μm × 0.25μm). The carrier gas was helium at flow rate of 1.03 mL/min. One microliter of each diluted sample was injected in the split-less mode using following conditions: injection temperature at 230°C, the oven temperature program was initially at 60°C and increased at rate of 3°C/min to 240°C, then held at 240°C for 5 min. GC/MS conditions were the same as described previously with the use of electronic impact mode at 70 eV, ion source temperature at 200°C and the interface temperature at 245°C.

The constituents were identified by comparison of their GC Kovats retention indices (RI), determined with reference to an homologous series of C_5–C_{28} *n*-alkanes and with those of authentic standards. The identification was confirmed when possible by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries).

Determination of antimicrobial activity from *C. maritimum* EO

Strains and growth conditions

The antibacterial activity tests of *C. maritimum* EO, included five foodborne pathogenic bacteria and one species of yeast supplied by Pasteur institute (Algiers, Algeria), identified with the ATCC number (American Type Culture Collection). The Gram-negative bacteria: *Escherichia coli* ATCC 25922 and the Gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, Methicillin-Resistant *S. aureus* ATCC 43300 (MRSA), *Lysteria innocua* CLIP 74915, and *Candida albicans* ATCC 10231 were studied. All strains were grown in brain heart infusion (BHI) agar and incubated at 37°C for 18–24 h until the stationary growth phase was reached.

Antimicrobial screening

Antibacterial activity of *C. maritimum* EO was determined by the disc diffusion method described by Belaiche,[29] which is usually used as a preliminary check for antibacterial activity. The six strains selected were grown on the nutrient agar plate at 37 ± 1°C for 18–24 h for bacteria and at 30 ± 1°C for 24–48 h for *Candida albicans* in order to obtain freshly cultured microbial suspensions for tests. Sterile filter paper discs (6 mm in diameter) were impregnated with 20 μL of the oil and then placed onto Mueller Hinton plates (25 mL, pH 7) previously inoculated with a microbial suspension (10^7 CFU/mL) using a sterile cotton swap. After incubation for 2 h at 4°C, the treated Petri dishes were incubated at 37 ± 1°C for 18–24 h for bacteria and at 30 ± 1°C for 24–48 h for yeast. The antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zone around the discs (including the diameter [6 mm] of the paper disc) with an electronic calliper. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was calculated.

Minimum inhibitory concentration (MIC)

The MIC values were determined for the microbial strains which were sensitive to the EO in disc diffusion assay. MIC was determined by the microdilution plate method as recommended by NCCLS.[30] All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween 80 (final concentration of 0.5% [v/v]), except for yeast (Sabouraud dextrose broth: SDB + Tween 80). Bacterial strains were grown overnight at 37°C in MHA and *Candida albicans* at 30°C for 48 h for in SDB. Assayed strains were suspended in MHB to give a final density of 5×10^5 colony forming units (CFU)/mL and these were confirmed by viable counts.

Different dilutions ranging from 0.05 to 100 μL/mL of the EO were prepared in a 96-well plate. In each well, 95 μL of MHB were dispensed followed by 5 μL of the inoculum and then by 100 μL of the diluted solution of EO.A negative control was prepared by mixing 195 μL of nutrient broth without EO and 5 μL of the inoculums on each strip. The final volume in each well was 200 μL. The plate was covered with a sterile plate sealer and incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and at 30°C for 48 h for *Candida albicans*. The bacterial growth was indicated by the

presence of a white pellet on the well bottom. The EO tested in this study was screened three times against each microorganism. The MIC was defined as the lowest concentration of the oil to inhibit the microorganism growth.

Statistical analyses

All assays were run in triplicate. The data were recorded as mean ± standard deviation, and analyzed by STATISTICA program V 5.5. Analysis of variance (ANOVA) was performed to detect the least significant difference (LSD) between means at level of 5% ($p \leq 0.05$).

Results and discussion

Minerals content

The contents of macro- and micro-elements from the aerial parts of *C. maritimum* are presented in Table 1. According to these results, the edible portion of *C. maritimum* can be considered as an important natural source of macro-elements, such as K (5.2 ± 2.4 g/kg) and Ca (23.3 ± 0.4 g/kg), and micro-elements, such as Fe (360 ± 12 mg/kg) and Mn (144 ± 1 mg/kg). On the other hand, *C. maritimum* contained low amounts of Mg and Cu, with values of 3.4 ± 0.2 and 24.7 ± 0.1 mg/kg, respectively. Ben Amor[8] reported the contents of macro-elements from the aerial part of *C. maritimum* which were 50.3, 58.5, and 8.2 g/kg for K, Ca, and Mg, respectively, which are higher than those obtained in the present study. These differences might be explained by the plant age and the growth conditions. Indeed, the plants used by Ben Amor[8] were only about 2-months old and were hydroponically grown, using nutrient solutions, whereas the plants we studied were harvested in a natural environment.

Carotenoids content

C. maritimum aerial parts had a carotenoids content of 62.6 ± 3.8 mg β-car/kg dw (Table 2) which is quite low compared to data from Spanish ecotype (338 mg/kg dw)[31] and also the leaf carotenoid content reported on other Apiaceous species, like *Hydrocotyle asiatica, Daucus carota*, and *Coriandrum sativum* (90, 121, and 675 mg β-car/kg dw, respectively).[32] The differences observed in carotenoids content may be related to species variations, climatic, and geographic conditions.

Table 1. Mineral content of the aerial parts of *C. maritimum*.

Macro-elements (g/kg dw)	
Mg	3.4 ± 0.2
Ca	23.3 ± 0.4
K	5.2 ± 2.4
Micro-elements (mg/kg dw)	
Mn	144 ± 1
Cu	24.7 ± 0.1
Zn	2.5 ± 0.4
Fe	360 ± 12

Table 2. Contents in carotenoids, total phenolics, flavonoids, and condensed tannins from the *C. maritimum* extract.

Parameter	Content
Carotenoids (mg β-carotene/kg dw)	62.6 ± 3.8
Total phenolic compounds (g GAE/kg dw)	47.1 ± 0.1
Flavonoids (g CE/kg dw)	17.3 ± 0.7
Total condensed tannins (g CE/kg dw)	0.04 ± 0.01

Other factors such as the maturity stage, season, and storage conditions that could also affect carotenoids level in vegetables aerial parts, were reported by several authors.[19,33,34]

TPC, TFC, and CTC

The TPC of the *C. maritimum* hydro-methanolic extract was 47.1 ± 0.1 g GAE/kg, whereas the TFC and CTC were 17 ± 0.7 and 0.04 ± 0.01 g CE/kg, respectively (Table 2). The TPC and TFC measured in our study were higher than those registered in acetone extracts from Tunisian *C. maritimum* (from 4 to 12 times and from 3 to 6 times, respectively),[14,17] and in methanol extracts from Tunisian (from 3 to 5 times) and French (from 1.5 to 2 times) ecotypes.[9,10,35] However, the CTC amount obtained in our study was very low compared to those reported in the literature for the same species. Indeed, values of 1.4 and 0.6 g CE/kg were, respectively, registered in pure methanol and 80% acetone extracts of Tunisian ecotypes.[10,14]

Identification and quantification of phenolic compounds by HPLC-DAD-ESI-MS

The HPLC-DAD-ESI-MS^n analysis allowed the identification of 10 compounds that were separated and characterized regarding their retention time, λ_{max} and *m/z* for monoisotopic and fragment ions (Fig. 1 and Table 3). All of them were hydroxycinnamic acids, however, only nine of the 10 compounds could be firmly identified. They corresponded to (1) 3-caffeoylquinic acid; (2) 3-coumaroylquinic acid; (3) 5-caffeoylquinic acid (chlorogenic acid); (4) 1-caffeoylquinic acid; (5) 5-coumaroylquinic acid; (6) 5-feruloylquinic acid; (7) 3,4-dicaffeoylquinic acid; (8) 3,5-dicaffeoylquinic acid; and (10) 4,5-dicaffeoylquinic acid. Compound 7 had a monoisotopic *m/z* corresponding to coumaroylquinic acid, with a fragmentation pattern similar to that of 5-coumaroylquinic acid. This compound was tentatively identified as *cis*-5-coumaroylquinic acid.

Chlorogenic acid was the major phenolic compound with a concentration of 6366 mg/kg dw. On the opposite, 3-coumaroylquinic acid was the less concentrated compound with a value 121 mg/kg dw. Overall, the phenolic profile from the hydro-methanolic extract of the *C. maritimum* aerial parts compared with that determined in aqueous infusions of stalks,[16] with the addition of two molecules which are reported for the first time: 3-coumaroylquinic acid and putative cis-5-coumaroylquinic acid.

Acetone extraction of *C. maritimum* aerial parts had also led to the identification of other compounds: gallic acid, rutin, catechin, epigallocatechin, vanillic acid, rosmarinic acid, querticin-3-galactoside, *p*-coumaric acid, and *trans*-2-hydroxycinnamic acid, with epigallocatechin as major compound.[17] The differences observed with regard to previous data could be explained by several factors, such as the extraction method, the nature of the solvent used and the time of extraction. Chirinos[36] showed that

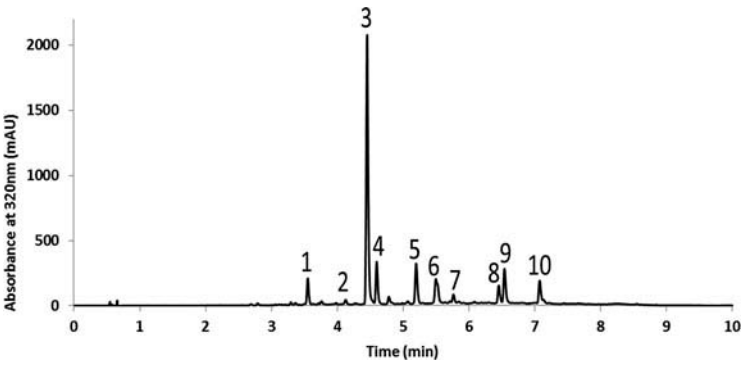

Figure 1. HPLC chromatograms of soluble phenolics from the *C. maritimum* extract. The profile was recorded at 320 nm. Peak numbers match those of Table 3.

Table 3. Phenolic compounds identified in the *C. maritimum* extract by LC/MS analysis.

Peak	Rt (min)	λ_{max}	M-	M+	MS2 -	MS2+	Compound	Abbreviation	Content (mg/kg dw)
1	3.552	300, 325	353,082	355,101	191 (100), 179 (50), 135 (12), 173 (4)	163 (100), 145 (8)	3-caffeoylquinic acid	3-CQA	629 ± 54
2	4.127	311	337,087	339,107	163 (100), 191 (15), 173 (5)	147 (100)	3-coumaroylquininc acid	3-CoQA	121 ± 10
3	4.453	300, 325	353,082	355,101	191 (100), 179 (10), 173 (10)	163 (100)	5-caffeoylquinic acid	5-CQA	6366 ± 531
4	4.598	300, 325	353,082	355,101	191 (100), 179 (10), 173 (10)	163 (100), 145 (8)	1-caffeoylquinic acid	1-CQA	1033 ± 78
5	5.198	312	337,087	339,107	191 (100), 173 (15), 163 (5)	147 (100)	5-coumaroylquininc acid	5-CoQA	1040 ± 84
6	5.498	300, 326	367,097	369,117	191 (100), 173 (5)	177 (100), 145 (10)	5-feruloylquinic acid	5-FQA	1045 ± 78
7	5.764	307	337,087	339,106	191 (100), 173 (15), 163 (5)	147 (100)	cis-5-coumaroylquininc acid	*cis*-5-CoQA	243 ± 19
8	6.453	325	515,111	517,132	353 (100), 173 (20),179 (15), 335 (15) 191 (10)	319 (100), 163 (90)	3,4-dicaffeoylquinic acid	3,4-DCQA	753 ± 55
9	6.540	325	515,111	517,132	353 (100), 191 (5)	–	3,5-dicaffeoylquinic acid	3,5-DCQA	1637 ± 99
10	7.074	325	515,111	517,132	353 (100), 173 (20), 179 (10)	319 (100), 163 (90)	4,5-dicaffeoylquinic acid	4,5-DCQA	1033 ± 73

Peak number corresponds to that found in Fig. S1.

λ_{max} is the absorbance maxima in the UV/visible range.

The reported fragments were observed in source and verified through MS/MS analysis of the parental ion in both negative and positive mode. Abundance of each fragment is indicated between brackets.

the content in phenolic compounds increased significantly when the extraction time was increased from 5 to 60 min. After 60 min, increasing the extraction time did not affect the content in phenolic compounds. The time chosen by Jallali[17] and Siracusa[16] was only 30 min. Other factors like the region and the period of harvesting could also affect the quality and the quantity of phenolic compounds.[9]

Antioxidant activities

The antioxidant activities have been attributed to phenolic compounds. Several studies showed the implication of these compounds in the prevention of different kinds of diseases. This action is a result of their inhibitory effect on the oxidized compounds and free radicals through electron transfer mechanism.[37,38]

DPPH radical scavenging activity

The concentration of *C. maritimum* extract, necessary to scavenge half of the DPPH free radical, was 7.6 ± 0.2 mg/mL. The antioxidant activity of the sample was 10 and 19 times less effective than those of the standards BHT and BHA, respectively. Compared to previous studies, the ability of *C. maritimum* extract to scavenge the DPPH free radical was lower than those obtained by Jallali[14,17] who found IC_{50} values of 0.056, 0.050, 0.059, and 0.146 mg/mL from 80% acetone of *C. maritimum*, harvested at different periods (vegetative and flowering) and regions (Kelibia and Monastir), respectively. It was also lower than those obtained by Meot-Duros[9] which values were varying, according to the origin (Sand Hills, Cliffs) and the season, between 0.15 and 1.21 mg/mL and by Houta[10] with value of 0.5mg/mL.

The low effectiveness in antioxidant properties of the *C. maritimum* extract could be explained by the low concentration in condensed tannins compared to reported results.[10,14] The concentration but also the nature of the bioactive molecules influence the antioxidant acivities and themselves are governed by other factors including harvesting time climatic conditions, extraction method (temperature, time, etc.), the polarity of the solvents used, the structure of the compounds contained in the plant and their physicochemical properties. So depending on these factors, extracts obtained from the same species may vary widely with respect to their concentration and activities.[36,39–42]

ABTS radical scavenging activity

C. maritimum showed ability to scavenge the $ABTS^{+}$ radical cation generated in the assay system with value of 0.43 ± 0.01 mg Trolox equivalents/mL (Table 4). These values are about four times lower than values reported from *C. maritimum* extracts harvested in summer in sand hills and cliffs in France (0.112 and 0.139 mg /mL, respectively).[9] In the mentioned study, the ABTS scavenging activity was correlated to the evolution of chlorogenic acid content over the year. Interestingly, the chlorogenic acid concentration measured in our study (6366 mg/kg dw) is about two to three times less than in the plant reported in,[9] which is consistent with a major role of chlorogenic acid concentration in the radical scavenging activity of the extract. In addition to chlorogenic acid, the

Table 4. Antioxidant activities of the *C. maritimum* extract.

Parameter	Value
DPPH (IC_{50}, mg/mL)	7.59 ± 0.20[a]
BHA (IC_{50}, mg/mL)	0.40 ± 0.01[b]
BHT (IC_{50}, mg/mL)	0.72 ± 0.12[b]
ABTS (mg Trolox equivalent/ml)	0.43 ± 0.01

Means followed by the same letter are not significantly different at $p \leq 0.05$ (only applied for the DPPH test).
A lower DPPH IC_{50} value indicates a higher antioxidant activity.

other hydroxycinnamic acids composing the hydro-methanolic extract of *C. maritimum* might contribute to the radical scavenging activity. Hence, 3-caffeoylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5 di-*O*-caffeoylquinic acid were reported to be major contributors to the ABTS free radical scavenging activity in gardenia fruit extracts.[43]

C. maritimum EO

EO yield

The EO yield from the *C. maritimum* aerial parts was 0.12%. This yield was lower than those obtained by several authors for the same species collected in different regions. Indeed, the EO yield ranged from 1 to 3.2% for *C. maritimum* harvested in Spain, France, and Poland,[15] whereas, it varied from 0.12 to 1.64%, according to the vegetative stage in Italian ecotype.[11]

Chemical composition of C. maritimum EO

The chemical composition of *C. maritimum* EO, determined by GC-MS analysis, revealed the presence of 10 compounds which are summarized in Table 5. *C. maritimum* EO was characterized by about two-thirds of monoterpene hydrocarbons (65.3%) and one-third of oxygenated monoterpenes (33.7%). The major compounds found were ɣ-terpinene (50.5%), thymolmethyl ether (33.6%), and *ρ*-cymene (12.6%). In comparison to the available literature, this chemical composition is peculiar and different from the EO extracted from Mediterranean and Atlantic ecotypes.[2–4,11,13] Indeed, the EO analyzed in our study was characterized by a high amount of ɣ-terpinene, thymol methyl ether, and the absence of dillapiole. Interestingly, the amount of dillapiole was proved extremely variable among ecotype, from absence to about 40%.[3,4,12,13] The variations observed in the EO content may be due to the difference in the environmental factors and period of harvesting of the plants. Indeed, it has been demonstrated on Italian *C. maritimum*, that there is a variation in the EO composition due to the different period of plants harvesting.[11]

Antimicrobial activity

The results of the antimicrobial activity of *C. maritimum* EO extracted by hydrodistillation method are summarized in Table 6. The disc diffusion method highlighted that *C. maritimum* EO exhibited

Table 5. Chemical composition (%) of *C. maritimum* essential oil extracted by the hydrodistillation method.

N°	Compounds	KIL[a]	KIC[b]	*C. maritimum* (%)
1	α-Thujene	931	926	0.49
2	α-Pinene	939	933	0.28
3	Sabinene	976	973	0.65
4	β-Myrcene	991	989	0.61
5	α- Terpinene	1018	1017	0.12
6	ρ-Cymene	1027	1024	12.57
7	ɣ-Terpinene	1061	1058	50.48
8	p-cymenene	-	1090	0.11
9	Thymol methylether	1230	1235	33.65
10	Carvacrol methylether	1246	1244	0.10
	Total			99.06
	Monoterpene hydrocarbons			65.31
	Oxygenated monoterpenes			33.75
	Sesquiterpenes			0.0
	Others			0.0
	Yield % (v/m)			0.12

[a]KIL: literature Kovats index.
[b]KIC: calculated Kovats index relative to C_5–C_{28} *n*-alkanes on the ZB5MS column.

Table 6. Anti-microbial activity of *C. maritimum* essential oil expressed by the diameter inhibition zones and MIC methods.

Micro-organisms	DD (mm)	MIC (µL/mL)
Bacillus subtilis	6.00 ± 0.00[c]	—
Staphylococcus aureus	21.33 ± 0.16[b]	0.11[a]
Methicillin-resistant *Staphylococcus aureus*	24.09 ± 0.08[b]	0.11[a]
Listeria innucia	23.22 ± 0.06[b]	0.11[a]
E. coli	21.21 ± 0.16[b]	0.11[a]
Candida albicans	33.63 ± 0.12[a]	0.11[a]

DD: agar disc diffusion method.
Diameter of inhibition zone (mm) including diameter paper disc of 6 mm.
MIC: minimum inhibitory concentration.
Values given as µL/mL.
Means followed by the same letter are not significantly different at $p \leq 0.05$.

an antimicrobial activity against all the germs tested but *B. subtilis*. Regarding the inhibition zone diameter (IZD), *C. albicans* was the most sensitive strain, followed by Methicillin-resistant *Staphylococcus aureus, Listeria innucia, Staphyloccocus aureus*, and *Escherichia coli*.

All the EO sensitive strains had the same sensitivity level as demonstrated by minimum inhibitory content (MIC; 0.11 ± 0.01 µL/mL).

The strong antimicrobial activity of *C. maritimum* EO investigated in this study is probably related to its major compounds (thymol methyl ether, ɣ-terpinene, and *ρ*-cymene). Indeed, phenolic components (thymol and carvacrol) and their precursors (*ρ*-cymene and γ-terpinene) present in EO are found to be a strong antifungal.[44] γ-terpinene was also shown to exert bactericidal effect against both gram positive and gram negative bacteria.[45] These effects are linked to the capacity of these molecules to disrupt cell membranes.

The antimicrobial activity of *C. maritimum* EO has been investigated previously.[2,11] Interestingly, EO from Italian *C. maritimum* exhibited antimicrobial activity against *B. subtilis* and two other germs. This activity was proposed to be linked to the level of sabinene, since the use of the pure molecule had the same inhibitory effect.[11] In addition, the antimicrobial activities of EO from *C. maritimum* harvested along the Atlantic coast were attributed to the EO levels in dillapiole. The oil with the higher amount of dillapiole (64.2%) was found to be the most active with MIC values ranging from 0.08–0.32 µl/mL.[2] In our study, the EO from Algerian *C. maritimum* was devoid of sabinene and dillapiole, which may explain the lack of activity against *B. subtilis*. By contrast, our EO from Algerian *C. maritimum* was able to inhibit growth of *E. coli* and *S. aureus*, which was not the case of the EO from Italian ecotype. This difference may be related to higher proportion of thymol methyl ether, ɣ-terpinene, and *ρ*-cymene, but antimicrobial assays using these pure molecules should be done to confirm this assumption.

Conclusion

In conclusion, both the hydro-methanolic extract of aerial parts and EO from *C. maritimum* exhibited biological activities, as antioxidant and antimicrobial, respectively. The plant aerial parts possess a high content of soluble phenolic compounds, mainly hydroxycinnamic acids derivatives, in particular chlorogenic acid. Our findings revealed also the presence of 10 compounds *in C. maritimum* EO, classified as monoterpene hydrocarbons and oxygenated monoterpenes withɣ-terpinene, thymol methyl ether, and ρ-cymene as major compounds. Besides, the *C. maritimum* EO showed a strong antimicrobial activity against a range of germs including *Candida albicans* and *Staphylococcus aureus. C. maritimum* may be considered as a promising food plant for the future as it contains bioactive natural substances that may be used as nutraceuticals or agro-food supplements to increase their shelf life. Further, other supplementary studies *in vivo* are necessary to complete this work.

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