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Chemical Composition, Antioxidant and Anti-acetylcholinesterase activities of Tunisian *Crithmum maritimum* L. Essential oils

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Abstract: acetylcholinesterase properties of the aerial parts and roots essential oils from *Crithmum maritimum* L., Apiaceae, gathered in the area of Monastir (Tunisia). The essential oils have been analysed by GC and GC-MS. Remarkable differences were found between the constituent percentages of the different studied organs. The most important compounds from the aerial parts were: *γ*-terpinene (39.3%), methylcarvacrol (21.6%) and *p*-cymene (11.8%). In the roots oil, the main components were terpinolene (36.9%), dillapiole (26.8%) and *γ*-terpinene (21.9%). The antioxidant activity of the two oils was evaluated by employing 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging tests. The results showed that the antioxidant capacity assessed by different *in vitro* tests were moderate, the ABTS assay after 20 min (IC_{50} = 0.051 mg/mL) of aerial parts and (IC_{50} = 0.643 mg/mL) of the roots, appeared to be more potent than that for the DPPH assay (IC_{50} = 0.92 mg/mL) of aerial parts and (IC_{50} = 0.048 mg/mL) of roots. In addition, the examined oils showed the highest AchE inhibitory 1 mg/mL activity (31.16% and 26.35%, for the aerial parts and roots, respectively).

Keywords: *Crithmum maritimum* L., Essential oil composition, γ-Terpinene, Terpinolene, Dillapiole, Anti-acetylcholinesterase activity, Antioxidant activity.

Introduction

Crithmum maritimum L. is a spontaneous plant of the family Apiaceae. Its geographical distribution extends along the European Atlantic coasts, Azores, Madeira and Canary Islands,

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Mediterranean and Black Sea coasts, NW Africa and W Asia. It grows on rocky sea-cliffs under the influence of salt-rich sprays. It is rarely found on sand or gravel[1]. The flowering period of this taxon ranges from May to October. Several uses of *C. maritimum* L. are known for culinary purposes and its leaves have been also used for aromatic and medicinal purposes[2] as a tonic, diuretic, carminative and vermifuge[3]. It has been shown that oil extracted from *Crithmum maritimum* L. seeds is of good nutritional quality, and could be used for human consumption[4]. Moreover, the essential oil is used also for the formulation of cosmetics with slimming properties[2]. Their young leaves and fermented products are used as salad with yogurt, especially in Mediterranean regions[5]. It is also known that all parts of the plant contain essential oil[6]. The main constituents are variable and depend on the region of growth[5].

The essential oil of this plant collected from several areas of Campania (Southern Italy)[2], Portugal[1], Antalya and Mersin (Turkey)[5] has been the subject of previous studies showing similarities and sometimes significant differences. Two types of oils may be distinguished: those dominated by *β*-phellandrene and thymol methyl ether[5] and those containing *γ*-terpinene and dillapiole as the major components or in appreciable amounts[5].

As a part of our contribution to the chemical and biological studies of Tunisian medicinal and aromatic plants[7, 8] and within the aim of valorizing *C. maritimum* growing in Tunisia, we report here the investigation of the chemical composition of its aerial parts and roots essential oils. The antioxidant and anti-acetylcholinesterase activities were studied and gave significant results.

Results and Discussion

Chemical composition.

The hydrodistillation of fresh aerial parts and roots of *Crithmum maritimum* L. furnished colorless oils, in 0.19 and 0.17 % (w/v) yields, respectively, both with a pleasant, characteristic odour. The composition of the oils was determined by GC and GC-MS. The percentage composition, with Linear Retention Indices (L.R.I) calculated for each compound, and the identification methods are reported in Table1. A total of 21 and 18 constituents, accounting for 99.79% and 99.8% of the whole oils, respectively, were identified.

The essential oil of the aerial parts was rich in monoterpenes, phenylpropanoids and phenols. Indeed, *γ*-terpinene (39.3%) was, by far, the major components of the essential oil obtained from the aerial parts. Methyl carvacrol, dillapiole and *p*-cymene (21.6%; 19.7% and 11.8%) were also important compounds of this oil. The occurrence of methyl carvacrol as one of the main components of the oil isolated from aerial parts seems unusual by comparison with literature data[2,4,5,9]. Other constituent detected in lesser amounts were terpinolene (1.3%) and α-pinene (1.1%). The phenylpropanoid fraction was represented, besides dillapiole, by small amounts of myristicin (0.2 %). The composition of the essential oil of the roots was quite different. Indeed, it was rich in monoterpene hydrocarbons (69.4%), terpinolene (36.9%) being the major constituent of this oil, together with *γ*-terpinene (21.9%). *p*-cymene, *α*-pinene and limonene (4.6%; 2.5% and 2.0%, respectively) were detected in smaller amounts.

The phenylpropanoid fraction was constituted by a major component, dillapiole (26.8%) and a minor one, myristicin (0.3%). Phenols were represented only by small percentages of methyl carvacrol (2.8%) and trace amount of methyl thymol. Another important difference between the two oils was in the content of oxygenated monoterpenes that reached 23.3% in the aerial parts

and only 3.3% in the roots.

Table 1. Chemical composition of the aerial parts and roots of *Crithmum maritimum* L.

Compound	L.R.I (HP-5)	L.R.I (Lit.)[19-21] (HP-5)	Fennel oil		Identification
			%[c] A. parts	% Roots	
α-Thujene	931	936	0.4	0.1	RI, MS
α-Pinene	939	941	1.1	2.5	RI, MS
Sabinene	977	978	0.8	0.2	RI, MS
β-Pinene	980	981	tr[a]	0.1	RI, MS
Myrcene	992	992	0.7	0.5	RI, MS
α-Phellandrene	1005	1002	-[b]	0.3	RI, MS
α-Terpinene	1018	1013	0.5	0.3	RI, MS
***p*-Cymene**	1027	**1030**	**11.8**	**4.6**	RI, MS
β-Phellandrene	1031	1032	0.4	-	RI, MS
Limonene	1032	1033	-	2.0	RI, MS
1,8-Cineole	1034	1033	0.1	tr	RI, MS
γ-Terpinene	1062	**1064**	**39.3**	**21.9**	RI, MS
Terpinolene	1089	1082	1.3	**36.9**	RI, MS
α-Thujone	1104	1117	0.3	0.2	RI, MS
Camphor	1145	1145	0.3	0.1	RI, MS
4-Terpineol	1178	1179	0.2	0.1	RI, MS
p-Cymen-8-ol	1185	1180	-	0.1	RI, MS
Dill ethet	1187	1189	0.2	-	RI, MS
Methyl thymol	1235	1235	0.6	tr	RI, MS
Methyl carvacrol	1244	**1244**	**21.6**	2.8	RI, MS
Carvacrol	1300	1309	0.2	-	RI, MS
Myristicin	1520	1520	0.2	0.3	RI, MS
Dillapiole	1622	**1625**	**19.7**	**26.8**	RI, MS
Monoterpenes, Oxygenated		23.3	3.3		
Phenylpropanoids		20.1	27.1		
Total identified		99.7	99.8		

[a] tr: traces (< 0.1%). [b] : Not identified. [c] %: Percentage calculated by GC-FID on non-polar capillary column HP-5.

Other important differences regarded the two main components of the oils: γ-terpinene (39.3% and 21.9% in aerial parts and roots, respectively) and dillapiole (19.7% and 26.8%). They were found to be also the main volatile compounds of both aerial parts and roots oils of *C. maritimum* collected in Mersin (Turkey), (24% and 21%, respectively)[4]. Always γ-terpinene was present in high amount during all seasonal variation (66.2% in May, 47.1% in June, 68% in July, (41.1% during fruit ripening and 58.9% during only fruits) in September and 48.8% in December), while dillapiole reached its maximum value in December (10%) of essential oil from *C. maritimum* grown in Liguria (Italy) [9].

Conversely, methyl thymol, which accounted for 28.8% in the aerial parts oil of *Crithmum maritimum* L. from Sorrento (Italy)[2] 26% in Catania (Italy)[5], in 25% Antalya (Turkey)[5] and 17.7% in Liguria (Italy)[9], was detected in very small amounts in our samples (0.6% and traces). Finally, *p*-cymene an important components (11.8%) of our aerial parts, was also present in appreciable percentages in the essential oils from Antalya (Turkey) 13%, Mersin (Turkey) 7%[5] and Sorrento (Italy) 9.6%.[2]

Antioxidant activity.

The antioxidant activity of the essential oils extracted by hydrodistillation from the aerial parts and roots of *C. maritimum* has been determined by two different test systems, namely the DPPH and ABTS assays. As a reference antioxidant, Trolox, a water-soluble analog of vitamin E[10], was used. The results are resumed in Table 2.

According to the DPPH assay, the two essential oils of *C. maritimum* showed a moderate antioxidant activity. The most active one was the oil obtained from the roots, with an IC_{50} = 0.048 ± 0.002 mg/mL; the IC_{50} for the essential oil of the aerial parts was 0.91 ± 0.10 mg/mL.

On the contrary in the ABTS assay, the aerial parts essential oil (IC_{50} = 0.0514 ± 0.002 mg/mL) was found to be more active than that of the roots (IC_{50} = 0.643 ± 0.034 mg/mL). In fact, this relatively high activity (case of ABTS assay) may due to the particular structure of the ABTS radical cation by comparison to that of the DPPH. It is known that the antioxidant activity of aerial parts and roots essential oils of *Crithmum maritimum* L. has been previously reported in the literature[11,12]. The relatively high activity of our essential oils by comparison to that cited in the literature and isolated from *C. maritimum* could be explained by the difference of the chemical composition and the proportion of some common compounds.

The antioxidant activities of α-pinene[13] and *p*-cymene[14] have been ready determined, and they did not show any appreciable antioxidant activity, while *γ*-terpinene was found very active[15]. The antioxidant activity of our two essential oils is probably due to the presence of *γ*-terpinene (21.9-39.3%) and phenolic compounds such as dillapiole (19.7-26.8%), for which we can hypothesize the donation of a $H^{\cdot}$ by the methylene of the allyl moiety in dillapiole, leading to the formation of a conjugated stable tertiary radical, which may explain the note activity.

Table 2. Antioxidant activity (DPPH and ABTS Assays) of aerial parts and roots essential oils of *Crithmum maritimum* L.

	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
Aerial parts	0.91 ± 0.10	0.0514 ± 0.002
Roots	0.048 ± 0.002	0.643 ± 0.034
Trolox	0.014 ± 0.004	0.2 ± 0.001

Determination of anti-acetylcholinesterase activity.

Acetylcholine is a compound released at the synaptic gap as a neurotransmitter. Neurotransmitter disturbances and insufficient cholinergic functions are identified among the pathological features in central nervous system disorders. The most important changes observed in the brain are a decrease in cortical levels of the neurotransmitter acetylcholine. Inhibition of

acetylcholinesterase therefore can restore the level of acetylcholine in the brain[16]. Plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated nowadays with Alzheimer's disease[17]. Most of the drugs used in Alzheimer therapy are formed by an enzyme inhibitor, e.g. galantamine, isolated from extracts of snowdrop[18]. The AChE inhibitory activity of the essential oils of the aerial parts and roots of *C. maritimum* has never been reported before. The results as acetycholinesterase inhibitor are resumed in Table 3.

The AChE inhibition of the essential oil of aerial parts was stronger than that of the roots. The former exhibited moderate inhibitory properties (31.16% and 28.27% enzyme inhibition) at 1 mg/mL and 0.5 mg/mL, respectively. The essential oil of the roots showed a lesser effectiveness, even though differences are not so striking (26.35 % and 21.54% enzyme inhibition at 1mg/mL and 0.5 mg/mL).

Table 3. Anti-acetylcholinesterase activity of aerial parts and roots essential oils from *Crithmum maritimum* L.

Enzyme inhibition in %		
	Concentration (mg/mL)	
	1	0.5
Aerial parts	31.16 ± 0.012	28.27 ± 0.09
Roots	26.35 ± 0.37	21.54 ± 0.002

Conclusion

In this work, we were able to show that the chemical composition of essential oil varies according to the treated party. So, essential oils stemming from the aerial parts of *Crithmum maritimum* L. are dominated by the γ-terpinene (39.3 %) and the methylcarvacrol (21.6 %), those of the roots are rich in terpinolene (36.9 %) and the dillapiole (26.8 %).

C. maritimum is an edible plant with interesting medicinal activities like tonic, diuretic, carminative and vermifuge. Essential oil showed antioxidant activity measured by either DPPH or ABTS tests and moderate inhibitory activity of acetylcholinesterase. This dual activity supports the medicinal use of this plant by local populations.

Experimental Section

Plant Material. *Crithmum maritimum* L. was collected in October 2010 in Monastir (Tunisia). Identification was performed at the laboratory of Plant Biology and Botanic, High Institute of Biotechnology of Monastir, University of Monastir, Tunisia. A voucher specimen (C.M-10) has been deposited in our laboratory.

Extraction of essential oils. Fresh aerial parts and roots, were separately cut in small pieces, weighed before extraction and subjected to hydrodistillation for 3h using a Clevenger-type apparatus. The essential oils were collected by decantation, dried over sodium sulphate, weighed and stored in sealed glass vials in a refrigerator at 4-5 °C until future use.

Analytical GC. Gas chromatograph: HP 5890-series II equipped with flame ionization detector (FID), HP-5 (30m × 0.25 mm ID, 0.25 µm film thickness) fused silica capillary column, carrier

gas nitrogen (1.2 mL/min). The temperature oven was programmed from 50°C (1 min) to 280°C at 5°C/min (1 min). I injector and detector temperatures 250°C and
280°C, respectively. Volume injected: 0.1µL of 1% hexane solution. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by mean of their Linear Retention Indices (L.R.I.) relative to the series of *n*-hydrocarbons.

Analytical GC-MS. GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data[22,23]. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing.

Antioxidant activity. Antioxidant activity was measured with the DPPH and ABTS methods, as described by Hatano et al. and Re et al., respectively[24,25].

Acetylcholinesterase inhibition. Inhibition of AChE by plant extracts was evaluated as described by Ellman et *al*[24] with some modifications as detailed by Moyo et *al*[25]. The assay is based on the pectrophotometric measurement of the increase in yellow color produced by thiocholine when it reacts with the dithiobisnitrobenzoate ion. Eserine was used as a positive control and water served as a negative control. The increase in absorbance value due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of the reaction before adding the enzyme from the rate after the enzyme addition. Percentage inhibition by extracts and eserine were calculated using the equation below.
Inhibition (%) = [1-sample reaction rate/Blank reaction rate]*100

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