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First Report on the Chemical Profile and Antioxidant Activity of Moroccan *Crithmum maritimum* Essential Oils

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Abstract

Sea fennel (*Crithmum maritimum* L.) is a wild halophyte valued for its culinary, medicinal, and cosmetic applications owing to its rich nutritional profile and distinctive sensory properties. This study provides the first investigation of essential oils (EOs) extracted from different parts of Moroccan *C. maritimum* (leaves, inflorescences, and stems) using hydrodistillation, with the aim of evaluating their phytochemical composition and bioactive potential. The oils were characterized by gas chromatography–mass spectrometry (GC–MS), while total phenolic content (TPC) and antioxidant activity (DPPH and FRAP assays) were assessed spectrophotometrically.

The highest EO yield was obtained from inflorescences (0.59%), followed by leaves (0.44%) and stems (0.28%). GC–MS analysis identified more than 30 compounds, with dillapiole (16.41–32.47%), γ-terpinene (18.31–32.34%), and thymol methyl ether (19.76–22.33%) as the major constituents. Stems and leaves exhibited the highest phenolic contents (17.97 ± 1.07 and 17.64 ± 0.75 mg GAE/g EO,

respectively), while inflorescences contained significantly lower amounts (15.17 ± 0.09 mg GAE/g EO). Antioxidant evaluation showed that leaf oil possessed the highest radical-scavenging capacity (DPPH), whereas stem oil demonstrated the greatest reducing power (FRAP). Molecular docking calculations were conducted to model the interaction between the six major constituents of *C. maritimum* EOs (dillapiole, *γ*-terpinene, thymol methyl ether, *p*-cymene, *β*-terpinene, and terpinen-4-ol), to evaluate their potential antioxidant activity. Among the identified *C. maritimum* EOs compounds, thymol methyl ether exhibited the most favorable binding profile with both target proteins NAD(P)H oxidase (PDB ID: 2CDU) and nitric oxide synthase (PDB ID: 6NGI), highlighting its potential as a broad-spectrum antioxidant. Overall, despite organ-dependent variations in yield and composition, all Moroccan *C. maritimum* EOs were rich in bioactive compounds with recognized biological properties. These findings highlight their potential for sustainable applications in nutraceutical, cosmetic, and food industries, supporting future valorisation of this halophyte through eco-friendly processing technologies.

Keywords: *C. maritimum*; Antioxidant activity; Total phenolic content; Natural products; Molecular docking.

Introduction

Dry and highly saline environments, such as wetlands and coastal areas, are widespread around the world and these conditions are expected to increase in the future as a result of climate change [1]. These conditions are unfavourable for plant growth and reproduction, causing osmotic stress, ion toxicity and oxidative stress due to water deficiency and high salinity [2]. In general, these environments pose significant physiological and biochemical challenges to plants, affecting water availability, nutrient uptake, ion balance and cellular functions, leading to reduced plant fitness and reproductive capacity [3].

There is a group of plants called halophytes that have evolved specialized physiological and molecular mechanisms allowing them to persist and grow in environments characterized by high salinity and water scarcity [4,5]. Halophytes possess sophisticated internal defence mechanisms to mitigate oxidative stress generated in response to salt and drought stress. Notably, halophytes tend to accumulate higher levels of

phenolic compounds and flavonoids compared to plants that grow under normal salinity and water conditions, which form part of their protective pathways against oxidative damage. Additionally, these plants can synthesize diverse secondary metabolites, including EOs, alkaloids, saponins and bitter compounds, which contribute to their tolerance to salinity and oxidative stress [1,6-9]. Owing to these adaptive traits, halophytes have garnered considerable attention as renewable sources of bioactive compounds for applications in medicine and functional foods. This has led to growing interest in salt-tolerant halophytic crops species. Nowadays one of the most widespread and well-researched edible halophytes is *C. maritimum*. It is known by a variety of vernacular names across different regions, reflecting its long history of traditional use. In Greece it is called “Kritmo” or “Kritamo”, while in England it is referred to as Rock Samphire, Samphire, or St. Peter’s herb a designation linked to Saint Peter, the patron saint of fishermen. Other common names include funcho do mar in Portugal, hinojo marino in Spain, criste marine in France, finocchio marino in Italy, motar in Croatia, denizteresi in Türkiye, and shanar bahariya in Arabic-speaking regions. The genus name *Crithmum* originates from the Greek word “κρῑθή” (krithe), meaning barley in reference to the resemblance between its fruits and barley grains, while the term *'maritimum'* is derived from the Latin word for 'maritime'. Indicating the growth of this plant in proximity to the sea. Notably, a distinctive feature of this plant is its status as the sole species within the genus *Crithmum* of the family Apiaceae [10-12].

Its natural distribution extends along the coastal regions of the Mediterranean and Black Seas, as well as the Atlantic Ocean. The succulent leaves of *C. maritimum* are thick, deeply divided, fleshy, and display a gray-olive coloration. Flowering occurs between July and September. Its inflorescences form umbels bearing white-greenish flowers, while the fruits are schizocarps divided into two compartments containing numerous seeds [11].

C. maritimum has been traditionally used as a culinary herb in Mediterranean coastal regions. Where its fleshy, aromatic leaves are appreciated for their characteristic saline, slightly bitter flavour which resembles of fennel and celery. Traditionally, it is consumed raw in salads, pickled or incorporated as a

seasoning for fish and seafood dishes. In recent years, the interest in this halophyte has increased, driven by the growing popularity of wild-foraged and functional foods. Beyond its gastronomic value, *C. maritimum* is a rich source of essential oils (EOs), polyphenols, vitamins and minerals conferring both sensory and potential health-promoting properties. This combination of nutritional, functional and organoleptic attributes positions *C. maritimum* as a promising ingredient for both traditional Mediterranean diets and modern gourmet cuisine [13,14]. Numerous research has identified *C. maritimum* as an inexpensive raw material of high nutritional worth and functional potential, suitable for producing natural, bioactive and health-enhancing food ingredients [3]. Infusions prepared from the aerial parts of *C. maritimum* have traditionally been used to treat prostatic inflammation and nephritis, while leaf infusions have been employed for the relief of colic and for liver cleansing. Additionally, the leaves are reported to possess antiscorbutic, tonic, carminative, diuretic, depurative, and vermifuge properties in traditional medicine [15-18]. Numerous studies have documented the use of *C. maritimum* EOs in ethnomedicine [15,19,20]. Beyond their carminative and anti-inflammatory applications, these oils are also widely incorporated into cosmetic formulations [19]. The primary volatile constituents identified in the plant's EOs were γ-terpinene, thymol methyl ether, dillapiole, *α*- and *β*-pinene, cymene, *β*-phellandrene, limonene, sabinene, thymyl methyl oxide. However, environmental factors such as salinity, temperature, humidity, light intensity, soil composition, rainfall have been shown to induce substantial qualitative and quantitative variations in the composition of *C. maritimum* EOs [9,11,15,19-22]. In addition to experimental antioxidant assays, molecular docking is widely employed as an in silico approach for elucidating the molecular mechanisms underlying the biological activities of plant-derived compounds [23]. By predicting ligand–enzyme interactions and binding affinities, docking studies provide insight into the potential antioxidant behaviour of EO constituents through their interaction with key oxidative stress-related enzymes [24].

The present study represents the first investigation of *C. maritimum* growing in Morocco, with a focus on the chemical composition of EOs derived from different plant parts and their antioxidant potential. The volatile constituents of the leaves, stems and inflorescences were characterized using GC-MS analysis and

the resulting chemical profiles were compared to previously reported data from other geographic regions. In addition, the antioxidant activities of the EOs were evaluated through in vitro assays, while molecular docking analyses were performed to investigate the interactions between the major EO constituents and selected oxidative enzymes. This integrated experimental and computational approach provides valuable insights into the phytochemical diversity, biological activity, and potential applications of Moroccan *C. maritimum* EOs.

Materials and Methods

2.1 Plant material

The aerial parts of *C. maritimum* were collected during the summer of 2024 in the early morning from the coastal area of Fnideq, Morocco (35°49′30″ N, 5°21′08″ W). The collection of *C. maritimum* complied with all relevant institutional, national, and international guidelines and legislation, including receiving permission from the corresponding national authorities. Furthermore, the study was conducted in accordance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Botanical identification and authentication were carried out by Dr. Mohamed Kadiri, (botanist at the Laboratory of Applied Botany, Abdelmalek Essaâdi University, Morocco). The plant material was air-dried in the shade at ambient temperature for 15 days. A voucher specimen was deposited in the Herbarium of the Biology Department, Faculty of Sciences, Abdelmalek Essaâdi University, Tétouan, Morocco under the code LAB-BIO-SF-2025-073.

2.2 Isolation of EOs

EOs were obtained from the aerial parts of *C. maritimum*, fresh leaves, stems, and inflorescences were separately ground. Hydrodistillation was performed for a minimum of 3 h using a Clevenger-type apparatus, under identical conditions for each plant part. For each extraction, 100 g of plant material was used. The obtained EOs were dehydrated using anhydrous sodium sulphate and subsequently stored in

tightly sealed amber glass vials at 4°C under dark conditions until further analysis. Yields (%) were determined in triplicate based on the calculation of the ratio between the obtained weight of the EO and the dry weight of the corresponding sample.

2.3 GC-MS analysis of EOs

GC–MS analysis of the EOs was performed following the procedure previously described by [25], using a PerkinElmer Clarus 580 GC coupled to an SQ8S quadrupole mass spectrometer under identical analytical conditions.

2.4 Quantification of the total phenolic content of EOs

The total phenolic content (TPC) of the EOs was quantified using the Folin–Ciocalteu colorimetric assay reported by Manian, et al. [26] with some modifications. Briefly, 50 µL of each EO sample was combined with 450 µL of distilled water in test tubes, than 250 µL of Folin–Ciocalteu reagent (1/10) was added. The reaction mixture was vortex-mixed and allowed to stand for 5 min before the addition of 1.25 mL of sodium carbonate solution (20%, w/v). The tubes were then shaken and incubated in the dark at room temperature for 40 min. Absorbance was recorded against the reagent blank at 725 nm using a spectrophotometer. A standard calibration curve was established with gallic acid at different concentrations, and the results were expressed as milligrams of gallic acid equivalents per gram of essential oil (mg GAE/g EO).

2.5 Antioxidant assay

2.5.1 DPPH free radical scavenging activity

The antioxidant activity of *C. maritimum* EOs was assessed following the method described Benguedouar, et al. [27]. The EOs were diluted in methanol to prepare solutions with concentrations ranging from 5.49 to 186.4 mg/mL. Subsequently, 3 mL of each concentration was mixed with 1 mL of a DPPH

solution (1 mM) in glass tubes. The mixtures were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm.

The antioxidant activity was expressed using IC_{50} value, defined as the concentration of antioxidant required to reduce the initial concentration of the target compound by 50%. A lower IC_{50} indicates a stronger antioxidant capacity.

2.5.2 Reducing power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was carried out following the procedure previously reported by Bouchfara, et al. [25], with slight modifications. Absorbance readings were recorded at 700 nm, using Rutin as a reference antioxidant. The antioxidant capacity was expressed as IC_{50} values, defined as the EO concentration required to reach an absorbance of 0.5; lower IC_{50} values correspond to stronger reducing power [28].

3 Statistical analysis

The results obtained from triplicate experiments were analysed using Statgraphics Centurion XVI. One-way analysis of variance (ANOVA) followed by post hoc multiple comparisons against the positive controls was performed, with significance set at $p < 0.05$. Results are presented as mean ± SD (n= 3).

4 Molecular docking studies

Molecular docking calculations were conducted using AutoDock Vina [29] to model the interaction between the three major constituents of *C. maritimum* EOs (dillapiole, *γ*-terpinene, and thymol methyl ether) and three other important compounds (*p*-cymene, *β*-terpinene, and terpinen-4-ol) also detected in EOs. The target proteins were NAD(P)H oxidase (PDB ID: 2CDU) [30] and nitric oxide synthase (PDB ID: 6NGI) [31]. Both targets were selected to study the antioxidant activity of the compounds because they are key enzymes involved in oxidative stress regulation and reactive oxygen species detoxification, making them suitable molecular targets for evaluating potential antioxidant interactions [32]. Prior to docking, all ligands were optimized using the B3LYP [33] density functional theory (DFT) method combined with the 6-

31G(d,p) basis set [34], as implemented in Gaussian 16 [35]. The optimized structures were used for ligand preparations. The crystal structures of both enzymes were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org).

For 2CDU, a grid box of 126 × 126 × 126 Å was centred at x= 10.141, y= 0.694, and z= 6.107, while for 6NGI, the grid box was centred at x= 117.347, y= 249.585, and z= 359.605, using the same box dimensions. The position of the grid boxes was defined by using the original innate ligands of the receptors. The exhaustiveness parameter was set to 8. The receptors were also prepared and during this process water molecules were removed from the downloaded crystal structures, and the corresponding PDBQT files were created by using AutoDock Tools [36]. For each ligand, up to nine binding modes generated. The binding modes with the most negative binding energy were selected as most preferred structures. The resulting complexes were visualized and analysed using LigPlot+ [37] and PyMOL [38] to identify noncovalent interactions and key residues involved in ligand binding.

5 Results and Discussion

5.1 Yields of *C. maritimum* EOs

The extraction of EOs from the dried aerial parts of *C. maritimum* (leaves, inflorescences, and stems) revealed a clear variability and a statistically significant difference in oil yield among the plant organs *($p < 0.05$)*. The inflorescences provided the highest yield (0.59%), followed by the leaves (0.44%). Whereas the stems yielded the lowest amount (0.28%) (**Figure 1**). These findings indicate that the EO yields affected by the selected organ.

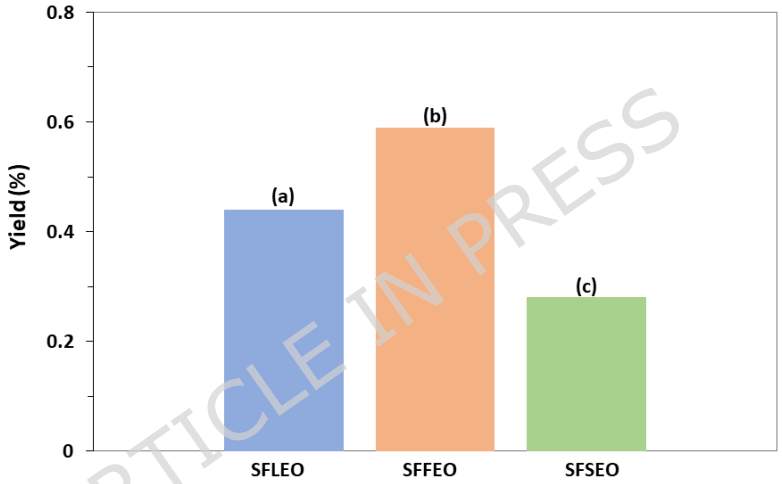

Figure 1. The yield content of EOs from different parts of *C. maritimum*. SFLEO, SFFEO and SFSEO indicate EOs from leaves, inflorescence and stems, respectively. a, b and c indicate that the three yields are statistically different (p-value < 0.05).

Comparable results have been reported in previous studies. For instance, Šunić, et al. [39] observed similar trends in *C. maritimum* collected in Montenegro, where the inflorescences exhibited the highest yield (0.83%), while leaves and stems showed lower contents of 0.52% and 0.08%, respectively. Likewise. Generalić Mekinić, et al. [17] reported yields of approximately 2.42% in flowers, and 0.55%-0.19% in the leaves and stems of *C. maritimum* from Croatia. By contrast, despite Tunisia being geographically close to Morocco, Houta, et al. [15] reported different findings: the EO extracted from the leaves had the highest content (0.50%), while the flowers and stems presented lower values of 0.30% and 0.28%, respectively.

Such differences in yield across regions may be attributed to environmental factors, including climate, soil type, and salinity, as well as the period of harvesting. Indeed, seasonal variation and harvesting time have been previously shown to significantly influence both the content and composition of EOs in aromatic plants [39-41].

5.2 Chemical profile of *C. maritimum* EOs from different parts

The chemical composition of EOs obtained from different aerial parts of *C. maritimum* was investigated using gas chromatography–mass spectrometry (GC-MS). A total of 13, 25, and 30 compounds were identified in the leaf, inflorescence, and stem oils, respectively. All three oils were primarily characterized by hydrocarbon monoterpenes (35.12–54.45%), oxygenated monoterpenes (24.66–28.49%), and oxygenated non-terpenes (17.16–34.59%) **(Table 1).** The GC–MS chromatograms of the EOs obtained from leaves, inflorescences, and stems of *C. maritimum* are provided in the Supplementary Material **(Figures S1–S3)**.

Among the identified constituents, dillapiole (16.41–32.47%), *γ*-terpinene (18.31–32.34%), and thymol methyl ether (19.76–22.33%) were consistently the most abundant across all oils (**Table 1)**. Notably, dillapiole exhibited the highest concentrations in both the leaf and stem oils. Accounting for 23.99% and 32.47%, respectively. In contrast, *γ*-terpinene was the predominant compound in the inflorescence oil, with a relative abundance of 32.34% **(Table 1)**.

Significant variations were observed in the minor constituents across the different plant parts. Spatulenol (0.14%), hexadecane (0.18%), nonadecane (0.20%), eicosane (0.24%) and falcarinol (0.11%) were exclusively detected in the inflorescence oil. Additionally, ocimenol (0.16%) was identified solely in stem oil (**Table 1)**. These findings highlight substantial differences in the EO profiles of *C. maritimum* depending on the plant part being analysed, suggesting that the distribution of volatile compounds may be influenced by the specific physiological functions or ecological roles of each part.

Table 1: Chemical composition of *C. maritimum* EOs from the leaves, inflorescences and stems.

N°	Compound	Class	RI^{Exp}	$RI^{Lit39, 42}$	Content %		
					Leaves	Inflorescences	Stems
1	*α*-thujene	HO	926	924	0.73	0.63	ND
2	*α*-pinene	HO	929	932	0.81	2.79	0.72
3	*β*-Terpinene	HO	1028	-	12.88	6.39	8.16
4	*δ*-3-Carene	HO	1009	1008	1.67	0.10	1.06
5	*p*-Cymene	HO	1022	1020	6.41	12.15	6.87
6	*γ*-Terpinene	HO	1060	1054	20.31	32.34	18.31
7	cis-*β*-Terpineol	OM	1144	1140	0.22	0.60	ND
8	Cosmene	HO	1131	-	0.04	0.05	ND
9	Terpinen-4-ol	OM	1182	1174	7.65	2.39	4.42
10	*α*-Terpineol	OM	1190	1186	0.41	0.18	ND
11	Ocimenol	OM	1174		ND	ND	0.16
12	Thymol methyl ether	OM	1235	1232	19.76	20.55	22.33
13	cis-*p*-mentha-1(7).8-dien-2-ol	OM	1252	1227	0.21	0.20	ND
14	Menth-2-en-1.4-diol	OM	1272	1266	0.07	0.12	ND
15	Thymol	OM	1291	1289	0.13	0.43	0.07
16	2-Acetyl-4-methylphenol	OO	1316	-	0.04	0.02	0.02
17	Myrtenyl acetate	OM	1327	1324	0.01	0.16	ND
18	Lilac aldehyde D	OM	1169	-	0.03	0.03	ND
19	+3-Carene-2-acetyle methyl	HS	1400	-	0.58	0.13	ND
20	*γ*-Elemene	HS	1435	1434	0.14	ND	0.04
21	*α*-Curcumene	HS	1483	1479	0.03	0.06	ND
22	*α*-Zingiberene	HS	1495	1493	ND	0.32	ND
23	*β*-Bisabolene	HS	1509	1505	0.08	0.27	ND
24	*β*-Sesquiphellandrene	HS	1524	1521	0.13	0.36	ND
25	Dehydronerolidol	OS	1562	-	0.11	0.04	ND
26	Elemicin	ON	1554	1555	0.05	0.62	2.10
27	Spathulenol	OS	1576	1577	ND	0.14	ND
28	Hexadecane	HN	1600	1600	ND	0.18	ND
29	Dillapiole	ON	1626	1620	23.99	16.41	32.47

30	Nonadecane	HN	1900	1900	ND	0.20	ND
31	Falcarinol	ON	1997	2035	ND	0.11	ND
32	Eicosane	HN	2000	2000	ND	0.24	ND
	Total identified				**96.49**	**98.44**	**96.73**
	Hydrocarbon monoterpenes				**42.85**	**54.45**	**35.12**
	Oxygenated monoterpenes				**28.49**	**24.66**	**26.98**
	Hydrocarbon sesquiterpenes				**0.96**	**1.14**	**0.04**
	Oxygenated sesquiterpenes				**0.11**	**0.18**	**0.00**
	Oxygenated non-terpenes				**24.08**	**17.16**	**34.59**
	Hydrocarbon non-terpenes				**0.00**	**0.62**	**0.00**

N°: Compounds listed in order of elution; **RI^{Exp}** Retention index calculated experimentally on Rtx-5MS column; **RI^{Lit}** Retention index ^{in} literature; **ND** Compound not detected in the oil; **HM** Hydrocarbon monoterpenes; **OM** Oxygenated monoterpenes; **HS** Hydrocarbon sesquiterpenes; **OS** Oxygenated sesquiterpenes; **ON** Oxygenated non-terpenes; **HN** Hydrocarbon non-terpenes

A similar study on *C. maritimum* EO from Tunisia reported γ-terpinene (19.34–30.62%), thymol methyl ether (20.62–40.4%) and dillapiole (14.27–40.25%) as major constituents [43]. Likewise, research from France revealed comparable results with dillapiole (55.7%), γ-terpinene (14%) and thymol methyl ether (11.8%) dominating the oil profile [11]. A recent investigation conducted in Libya by Ismail, et al. [3] revealed that thymol methyl ether, γ-terpinene, and ledene oxide were the dominant components of *C. maritimum* EO, with relative abundances of 56.86%, 16.17%, and 4.32%, respectively.

In Turkey the EO composition of *C. maritimum* leaves and branches was predominantly characterized by γ-terpinene (32–36%), β-phellandrene (21–22%), and sabinene (13–9%) [44]. Meanwhile, in Montenegro, EOs extracted from different plant parts (leaves, inflorescences, stems, and umbels with seeds) were dominated by limonene (62.4–72.0%)[39]. In contrast, *C. maritimum* EO from Croatia was chiefly composed of sabinene (42.55–51.47%) and limonene (36.28–43.58%) [45].

A noteworthy observation arises from the comparison of *C. maritimum* EO compositions between Morocco and Algeria, two regions in close geographical proximity. While these countries share proximity, the volatile profiles of *C. maritimum* oils show differences. In Algeria, the oil was dominated by γ-terpinene (50.48%), thymol methyl ether (33%), and *p*-cymene (12.57%), and lacked the presence of certain compounds found in Moroccan oils such as dillapiole [46]. This divergence suggests that despite the geographical closeness of these two regions, distinct climatic, edaphic and biotic factors may contribute to the variation in the secondary metabolite profiles of *C. maritimum*.

The chemical variability observed in *C. maritimum* EOs among different geographic origins is likely associated with differences in environmental conditions and developmental stages, which influence the biosynthesis of volatiles terpenes. Previous studies have shown that temperature, salinity and edaphic conditions can modulate metabolic pathways involved in EO production, leading to qualitative and quantitative variations in chemical profiles[47,48]. In addition, phenological stage has been reported as a key determinant affecting the accumulation of specific volatile constituents. These findings highlight the existence of distinct chemotypes within *C. maritimum* populations and support the importance of comparative regional investigations to better elucidate the ecological and biochemical drivers of EO composition.

5.3 Total phenolic content of *C. maritimum* EOs

Phenolic compounds are among the most important secondary metabolites contributing significantly to the antioxidant activity of plant extracts and EOs. The quantification of the total phenolic content (TPC) in *C. maritimum* EOs revealed appreciable levels in all three plant parts. The highest TPC values were observed in stem (17.97 ± 1.07 mg GAE/g EO) and inflorescence oils (17.64 ± 0.75 mg GAE/g EO), followed by leaf oil (15.17 ± 0.09 mg GAE/g EO), as shown in **Figure 2**.

To the best of our knowledge, only one previous study has reported the TPC of *C. maritimum* EOs. In that work, Pasias, et al. [49] reported a significantly lower value of TPC (7.5 mg GAE/g) in samples from Greece when comparing with our results. Furthermore, when compared to other EOs from plants of the Apiaceae family, our results appear to be relatively high. For example, Lin, et al. [50] reported that *Elettaria cardamomum (*caraway) and *Anethum graveolens* (dill weed) EOs showed low total phenolic contents, with values of only 1.93 and 4.20 mg GAE/g EO, respectively.

Nevertheless, it is important to emphasize that the presence of phenolic compounds in EOs does not necessarily correspond to strong antioxidant activity, since biological effects depend not only on total content but also on the structure and reactivity of individual compounds. Therefore, these results should

be interpreted in conjunction with the antioxidant assays (DPPH and FRAP), which indicate that the overall antioxidant activity of the oils is assay-dependent and characterized by relatively weak radical-scavenging capacity.

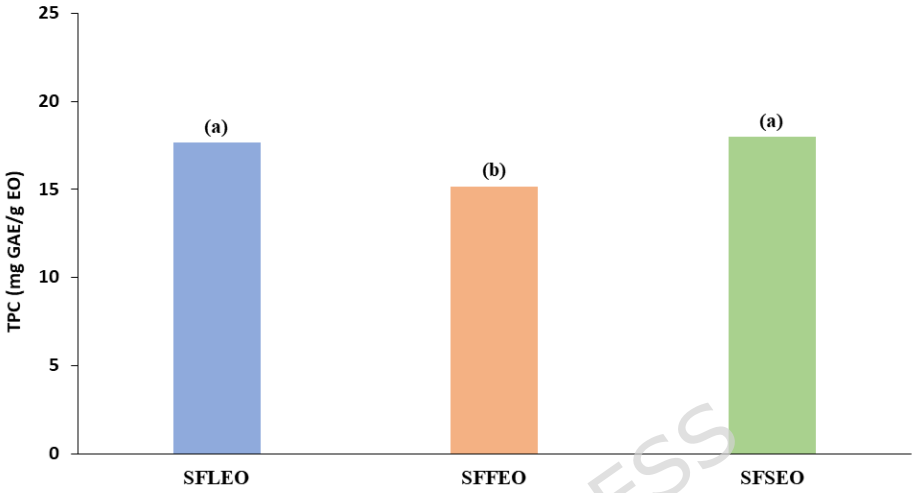

Figure 2: The total phenolic content (TPC) content of EOs from different parts of *C. maritimum*. SFLEO, SFFEO and SFSEO indicate leaves, inflorescence and stems, respectively. Different letters indicate a significant difference between values (p-value < 0.05).

5.4 *In vitro* antioxidant activity

The results of the antioxidant activities of *C. maritimum* EOs evaluated by DPPH and FRAP assays are shown in **Table 2**. The DPPH assay revealed significant differences among the EOs obtained from the three plant parts ($p < 0.05$). Leaf EO exhibited the highest radical-scavenging activity (IC_{50}= 52.17 ± 1.48 mg/mL) followed by stem oil (IC_{50} = 60.28 ± 0.74 mg/mL) and inflorescence oil (IC_{50}= 63.20 ± 0.12 mg/mL). However, all samples showed considerably lower antioxidant efficiency compared to the synthetic standard BHT (IC_{50}= 0.11 ± 0.01 mg/mL). Our findings are different from those reported by Šunić, et al. [39], who described higher IC_{50} values (lower antioxidant activity) for *C. maritimum* leaf EO 58.30 mg/mL than other parts, the IC_{50} value (52.17 ± 1.48) mg/mL of our leaf oil was slightly high, indicating a somewhat stronger radical-scavenging activity than the leaves collected in Montenegro. In the same paper [39] much lower IC_{50} values were reported for stem 5.04 mg/mL and inflorescence oils IC_{50}

=14.53 mg/mL, demonstrating higher antioxidant capacity than those observed in our study. In contrast Jallali, et al. [43] revealed that EOs from aerial parts of *C. maritimum* aerial parts collected from Tunisia have much higher antioxidant activity IC_{50} (0.47-3.3) mg/mL than it was found in the current study.

For the FRAP assay, evaluation of the ferric-reducing antioxidant power revealed that the leaf and inflorescence oils displayed moderate and statically identical reducing activity, with IC_{50} values of 0.204 ± 0.019 and 0.197 ± 0.010 mg/mL, respectively. In contrast, stem oil exhibited the strongest activity, with an IC_{50} value of 0.022 ± 0.004 mg/mL, which was lower even than that of rutin (positive control).

These IC_{50} values of different parts indicate stronger antioxidant activity than those reported for the EOs of aerial parts of *C. maritimum* from Tunisia (0.53–1.87) mg/mL [51]. Furthermore, our results revealed better antioxidant potential than the ethanolic extract of *C. maritimum* leaves described by Souid, et al. [52], who reported an IC_{50} of 1.82 mg/mL.

Table 2: IC_{50} values of *C. maritimum* EOs from the different plant parts by using DPPH and FRAP.

Sample	**IC_{50} (mg /mL)**	
	DPPH	**FRAP**
Leaves	52.17 ± 1.48[a]	0.204 ± 0.019[a]
Inflorescences	63.20 ± 0.12[b]	0.197 ± 0.010[a]
Stems	60.28 ± 0.74[c]	0.022 ± 0.004[b]
BHT	0.11 ± 0.01[d]	-
Rutin	-	0.040 ± 0.002[c]

a, b, c and d: numbers in column marked with different letters are significantly different (p-value < 0.05).

The obtained results from the two antioxidant assays (DPPH and FRAP) revealed notable differences in the response of *C. maritimum* EOs depending on the model applied. In the DPPH assay, the EO from leaves exhibited the strongest radical-scavenging activity among the tested oils, followed by stems and inflorescences. Nevertheless, all samples displayed relatively weak radical-scavenging activity when

compared with the synthetic antioxidant BHT and with several previously reported C. *maritimum* EOs. In contrast, the FRAP assay indicated that leaf and inflorescence oils had a similar and significantly lower reducing power compared to the stem oil. This discrepancy highlights that the antioxidant activity of EOs is not uniform across different mechanisms of action: while DPPH reflects the capacity to scavenge free radicals via hydrogen atom or electron donation, FRAP measures the ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The difference in the ranking of samples between the two assays may suggest that various compounds may contribute differently to radical scavenging versus reducing activities, depending on their chemical structure and reactivity.

The observed activities may be linked to the hydro gen-donating ability of certain constituents. Commonly associated with the presence of reductones [17,53,54]. Although the overall antioxidant potential was good, this may be attributed to the chemical diversity of the EOs. The major compounds dillapiole, *γ*-terpinene and thymol methyl ether are known to influence antioxidant activity. Studies have shown that aromatic compounds with electron-donating substituents can enhance antioxidant capacity [55,56]. Dillapiole and thymol methyl ether have been reported to exhibit notable antioxidant effects [51,57,58]. Moreover, the presence of phenolic compounds such as thymol, even at relatively low concentrations in our oils (**Table 1**), may play a role in the radical-scavenging and reducing effects observed. since thymol is recognized as a potent antioxidant [59,60].

Overall, the results indicate that *C. maritimum* EOs exhibit weak free-radical scavenging activity but a comparatively stronger ferric-reducing capacity, particularly in stem oil. These effects are likely attributable to the combined action of major and minor constituents present in the oils.

5.5 Molecular docking

Many potent antioxidant agents are derived from natural sources. To investigate the antioxidant potential of *C. maritimum* EOs, molecular docking calculations were conducted using six major constituents (dillapiole, *γ*-terpinene, thymol methyl ether, *p*-cymene, *β*-terpinene, and terpinen-4-ol) (>6%) and their

interactions with the active sites of NAD(P)H oxidase (PDB ID: 2CDU) and nitric oxide synthase (PDB ID: 6NGI) were analysed. The study aimed to elucidate their potential binding and their affinities within the active sites of the selected enzymes. The most favourable binding poses and corresponding binding energies (E_A, kcal/mol) for each ligand–enzyme complex were further analyzed (**Table 3**). It was revealed that the binding affinities cover a range between –4.9 to –7.5 kcal/mol.

The complexes of the studied species with the two enzymes include various noncovalent interactions (**Table 3**). Among all compounds, thymol methyl ether showed the strongest binding affinity toward nitric oxide synthase (–7.5 kcal/mol) which can be attributed to the formation of a conventional hydrogen bond with Gln540, alongside multiple hydrophobic interactions involving Ile541, Pro518, Asp523, Val524, Glu548, Val546, and Pro543 (**Figure 3.A**). These interactions stabilize the enzyme–ligand complex, suggesting its potential as an efficient antioxidant agent through effective binding.

Regarding the interaction with NAD(P)H oxidase, terpinen-4-ol formed hydrophobic interactions with Ser41, Ala300, Thr112, Ala11, His10, Ala303, and Thr9, along with hydrogen bonding interactions with Lys134 and Asp282 (**Figure 3.B**). *β*-terpinene established hydrophobic interactions via His10, Val304, Phe14, Thr13, Lys17, and Thr62 with the receptor (**Table 3**). In all interactions analyzed for both proteins, binding was established by hydrophobic contacts and hydrogen bonding. Overall, among the compounds of *C. maritimum* EOs, thymol methyl ether emerged as the most promising antioxidant due to its favourable binding profile with both target proteins, indicating potential broad-spectrum antioxidant activity.

Table 3. Molecular docking results of selected compounds from *C. maritimum* EOs associated with nitric oxide synthase (PDB ID: 6NGI) and NAD(P)H oxidase (PDB ID: 2CDU)

	nitric oxide synthase			**NAD(P)H oxidase**		
Compounds	**Binding affinities (E_A, kcal/mol)**	**Hydrophobic interactions**	**Hydrogen bonding interactions**	**Binding affinities (E_A, kcal/mol)**	**Hydrophobic interactions**	**Hydrogen bonding interactions**

dillapiole	-4.9	Glu699, Phe696, Trp681, and Val628,	Met700	-5.2	Ser401, Asp397, Vall404, Gln400, Tyr435, Asp430, Phe429, and Leu424	Asn434
***γ*-terpinene**	-5.6	Phe709, Cys420, Ala417, Ser418, Tyr711, Arg419, and Met575	-	-5.8	Pro298, Ala300, Ala303, His10, Ala11, Asp282, Thr9, and Leu299	
thymol methyl ether	-7.5	Ile541, Pro518, Asp523, Val524, Glu548, Val546, and Pro543	Gln540	-5.4	Asn36, Ile37, Phe61, Arg58, Ser63, and Pro65	
***p*-cymene**	-6.3	Sys420, Ala417, Trp414, Leu429, Phe589, and Phe709	-	-5.1	Gly180, Ile243, Pro120, Lys213, Ile122, Val214, Ile155, and Asp179	
***β*-terpinen**	-5.5	Cys420, Phe589, Lei429, Ser590, Ala463, Trp414, and Gly591	-	-6.1	His10, Val304, Phe14, Thr13, Lys17, and Thr62	

terpinen -4-ol	-5.6	Ala417, Met575, Phe709, and Arg704	Ser418, and Arg419	-6.2	Ser41, Ala300, Thr112, Ala11, His10, Ala303, and Thr9	Lys134, and Asp282

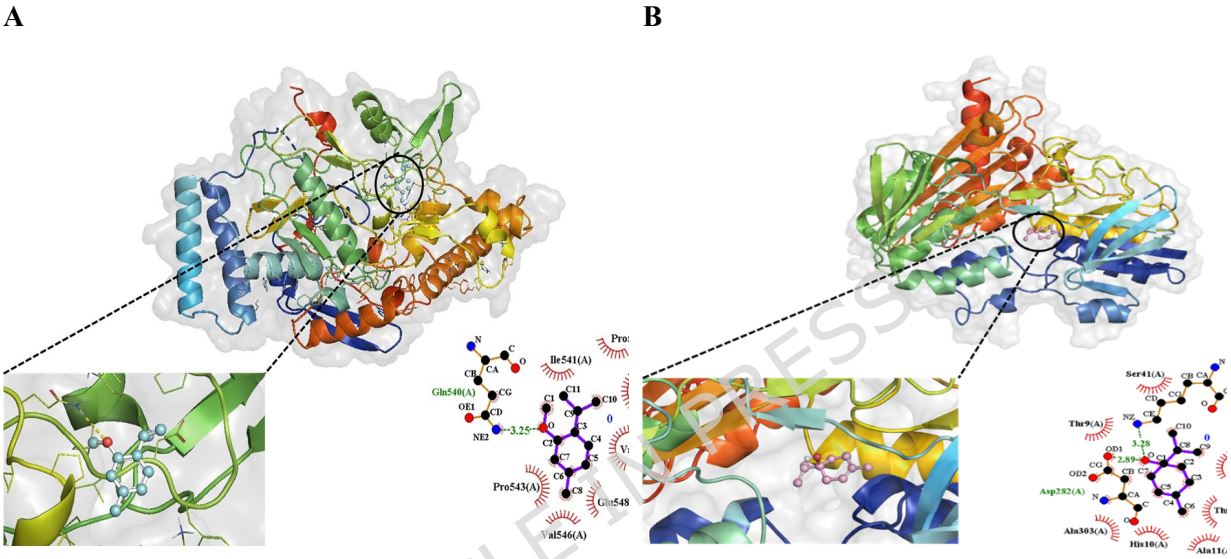

Figure 3. 3D and 2D visualisation of interactions of thymol methyl ether with nitric oxide synthase (PDB ID: 6NGI) and terpinen-4-ol with NAD(P)H oxidase (PDB ID: 2CDU) (A and B), respectively. The complexes were prepared by using molecular docking. Non-bonded contacts are defined within a 3.90 Å distance.

6 Conclusion

This work represents the first comprehensive investigation of EOs obtained from different organs (leaves, inflorescences, and stems) of *C. maritimum* growing wild in Morocco. The results demonstrated organ-dependent variations in oil yield, with inflorescences producing the highest amounts, and revealed a characteristic chemotype dominated by dillapiole, *γ*-terpinene, and thymol methyl ether. The EOs contained detectable levels of phenolic compounds and exhibited measurable antioxidant activity. However, the DPPH results indicated relatively weak radical-scavenging activity compared with the

reference antioxidant BHT, while the FRAP assay revealed moderate reducing power, particularly for stem oil. These findings suggest that the antioxidant properties of Moroccan *C. maritimum* EOs are dependent on both the plant organ and the mechanism of action evaluated. In addition, molecular docking analyses provided mechanistic insight into the antioxidant potential of major oil constituents. The selected compounds exhibited favourable binding affinities toward NAD(P)H oxidase and nitric oxide synthase, mainly through hydrophobic interactions and hydrogen bonding. Notably, thymol methyl ether displayed the strongest binding profile with both enzymes, supporting its key role in the observed antioxidant activity. Overall, beyond advancing phytochemical knowledge. Overall, this study expands the phytochemical knowledge of Moroccan *C. maritimum* and highlights its EOs as a source of bioactive volatile compounds with potential applications in cosmetic, nutraceutical, and food-related formulations. Further investigations are warranted to explore additional biological activities and practical applications of these oils. The outcomes may also stimulate industrial interest in cultivating this halophytic species in Moroccan coastal ecosystems to support sustainable production of EOs with distinctive profiles and biological activities.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author Contributions

B.S., and M.N. conceived the scientific idea. A.B., H.Z., and M.E.B. wrote the main manuscript text. B.S., M.N., R.B., H.Z., and B.F. carried out editing. B.S., B.F., and M.N. carried out supervision activity. A.B., H.Z., and M.E.B., carried out experiments, calculations and characterisation of the samples. All authors participated in the formal analysis of data.

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