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Comparative study of *Crithmum maritimum* L. richness on natural antioxydants: Effects of plant organ and geographic variation

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Abstract
Due to their negative side effects on human health, synthetic antioxidants and preservatives are increasingly being replaced by natural antioxidants, produced primarily by plants, such as phenolic compounds, essential oils, and vitamins. *Crithmum maritimum* L. is an edible halophyte potentially rich in these biomolecules. The present work aimed to investigate the richness of this plant on different classes of natural antioxidant (total polyphenols, flavonoids, condensed tannins, anthocyanins, carotenoids, essential oils, and ascorbic acid), and to study the effect of plant organ (root, stem, leaf, and umbel) ant geographic origin on the amount of these molecules. Colorimetric quantification divulged the high amounts of all the investigated compound classes except the anthocyanins, whose contents were weak. Results showed that leaves contained the highest amounts of phenolic compounds, ascorbic acid, and carotenoids; while umbels were the richest on essential oils and anthocyanins. Besides, the production of these molecules was significantly dependent on the geographic and bioclimatic conditions, underling their implication in biotique and abiotic stress defence. Thus, and due to its richness on natural antioxidants and many other nutrients, *C. maritimum* represent a potential candidate that can replace conventional foods in the Mediterranean diet and can promote human health and life quality.

Keywords: Natural antioxidants, *Crithmum maritimum*, phenolic compounds, essential oils, geographic origin

Introduction
Crithmum maritimum L. (Linné, 1753), also known as see fennel, is a perennial facultative halophyte belonging to the Apiaceae family. It is widely thriving g on the Atlantic coasts of Europe, the coasts of the Mediterranean, the Black Sea, and Macaronesia where it is consumed pickled, as condiment or as salad ingredient [1, 2]. This plant was also consumed by sailors since ancient time to prevent scurvy due to its richness on vitamin C [3]. It is a promising food and medicinal plant, having ethnopharmacological uses, and rich on iodine, carotenoids, phenolic compounds, essential oils, minerals, lipids and fatty acids, proteins and amino acids [1, 3].
In recent years, there has been growing concerns about the potential health risks posed by changes in culinary habits imposed by the accelerated pace of life and globalization. One of the most important factors of these risks is the use of synthetic preservatives and antioxidants to extend shelf life of foods [4]. For this, shift toward natural alternatives is the new wave invading food and food processing industries. These natural alternatives, derived from plants and other sources, are being explored for their ability to extend food shelf life by inhibiting microbial growth and lipid oxidation, while also offering potential health benefits. Plant derived antioxidants have a high degree of nutritional and pharmaceutical values, and have a great potential in promoting human health [5]. The antioxidant mechanism of plants is composed of molecules of diverse origins and natures. The non-enzymatic component, the other being composed of antioxidant enzymes, encompasses a large number of molecules such as phenolic compounds, vitamins C and E, carotenoids, and essential oils [3, 6, 7].
Phenolic compounds are secondary metabolites derived in plants from the pentose phosphate, shikimate, and phenylpropanoid pathways [6]. They can be grouped into numerous classes according to the complexity of their basic skeleton, among which phenolic acids, flavonoids, and tannins are considered the major phenols in food [6]. The fundamental structural element that characterizes them is the presence of at least one benzene ring to which at least one hydroxyl group is directly bonded, either free or involved in another functional group: ether, ester, or heteroside. It is this chemical reactivity that gives phenolic compounds their antioxidant character [8].

The antioxidant properties of phenolic compounds have also been widely demonstrated [2, 7, 9]. Generally, phenolic compounds block the propagation reactions of the membrane lipid oxidation chain. They can also inhibit oxidations indirectly, by deactivating $^1O_2$ or by chelating transition metals (Fe^{2+},Cu^{2+}), while some are inhibitors of oxidation enzymes, in particular lipoxygenase or cyclooxygenase [10].

Among polyphenols, anthocyanins are water-soluble pigments responsible for the red, pink, mauve, purple, blue or violet colouring of most flowers and fruits, but also present in vegetative organs [8]. Anthocyanins are glucosides of the anthocyanidins, derivatives of the flavylium cation, which underlines the belonging of these molecules to the vast group of flavonoids. They are present in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits [10].

Anthocyanins have a large spectrum of beneficial effects on human health, largely due to their antioxidant potential. When consumed regularly as dietary antioxidants, anthocyanins may be very helpful in managing diseases like inflammation, diabetes, cancers, and cardiovascular accidents [8].

Carotenoids are natural lipophilic pigments synthesized by all photosynthetic organisms and some bacteria, algae and fungi [11]. They are mainly C40 terpenoids deriving from the isoprenoid pathway (Sathasivama 2020). They play important roles in plant growth and development since they are directly implicated in photosynthesis, pigmentation, photoprotection, the production of phytohormones, and signaling [11, 12].

Due to the large spectrum of nuances they give and to their high antioxidant potential, carotenoids are frequently used as food colorants and preserving ingredient, as they are exploited in cosmetic and pharmaceutic industries [5]. They are also very important to human health due to their role as precursors of vitamin A, and their direct implication in the protection of many diseases like cancer, cardiovascular and neurodegenerative diseases [12]. Most of these biological activities of carotenoids were linked to their high antioxidant potential and their capacities to alleviate oxidative stress and related damages [5]. They ensure detoxification by quenching or scavenging the main antioxidant triggering $^1O_2$ and stabilizing photochemical redox reactions [11].

Vitamin C, or L-(+)-threo-ascorbic acid (AsA) is structurally one of the simplest vitamins, exclusively synthesized by eukaryotes. It is an essential element for Humans, which are unable to synthesize it and so, must afford it from diet intake[13]. Ascorbic acid is the most potent predominant antioxidant of the hydrophilic phase of plant cells [14]. It pays an important role in plant development and protection. Its functions as a vital enzymatic cofactor and as a potent antioxidant make it essential for both plants and animals [13]. It also interfere in collagen and carnitine production, and it constitute an important element of skin, tendons, ligaments and blood vessels [15]. The acidity of the molecule and its reducing character are linked to its enediol structure, which is readily oxidized by O_2^-, O_2, and H_2O_2 to a bicyclic structure, dehydroascorbic acid (DHA) [6]. This structural specificity of AsA is at the origin of its strong antioxidant properties like neutralizing reactive oxygen species, preventing the generation of new free radicals, and assisting in the recycling of other antioxidants [16]. AsA is able to detoxify reactive oxygen species at the level of hydrophilic territories of tissues, protecting proteins and DNA from oxidative damage and ensuring prevention against lipid peroxidation [14]. AsA can be rapidly oxidized to DHA. It must be immediately recycled through the AsA pathway to maintain efficient AsA levels and redox state in cells [13].

Essential oils are volatile and naturally occurring secondary metabolites characterized by a strong odor. They are complex and highly variable mixtures of constituents deriving almost exclusively from terpenoid and phenylpropanes groups. Generally, these mixtures contain mono- and sesqui-oxygenated terpenes, phenols, and other compounds [1, 4]. Essential oils exhibit significant antioxidant activity, depending on the presence of components able to quench peroxy radicals, which is strongly correlated, in most cases, with their content of monoterpenes, ketones, and aldehydes, but particularly with their content of phenolic compounds [17]. Essential oils exhibit a large number of biological acivities as antimicrobial, anti-inlammatory, antidiabetic, and antihypertensive. They also have been recommended as good alternatives in food industries to prevent food spoilage [4].

The production of antioxidant molecules in plants is influenced by both endogenous and exogenous factors. Indeed, different plant species, organs, and growth stages can exhibit different antioxidants levels [13]. Especially, environmental stresses have been reported to induce profound changes in the redox status of these molecules [13, 16]. In this context, phenolics, carotenoids, and flavonoids (including anthocyanins) contribute in maintaining a balanced physiological state in their respective tissues [7]. Thus, it has been shown that pigment synthesis enhances the antioxidant response of plants to maintain a stable physiological state in tissues directly or indirectly affected by biotic and abiotic stresses and underlines an ecological adaptation to environmental changes [11, 17, 18]. Further, plants growing under high light conditions, for example, have significantly higher levels of ascorbate. This vitamin helps plants to cope with many stressful conditions such as salinity, drought, high temperature and intense luminosity [13].

To the best of our knowledge, previous work reported the amounts and biological activities of phenolic compounds and essential oils of *C. maritimum*. However, there is a lake of investigation of the real richness of this plant on many other antioxidant molecules. In the present work, we proposed a detailed investigation of the amounts of different classes of natural antioxidants (phenolic compounds, anthcyanins, carotenoids, ascorbic acid, and essential oils) and their variation among plant organs and as a function of bioclimatic habitats conditions.

Material and methods

1. Chemical and reagents

All chemicals and solvents used during the following experiments were HPLC grade and purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Water was bi-distilled.

2. Plant sampling

L. crithmoïdes L. was collected at the end of September 2024 at three different Tunisian localities belonging to different bioclimatic stages. The choice of the collecting sites depended on the disponibility of the plant and the variability of the climatic conditions. *C. maritimum* L. was collected in Tabarka (CM1) (in the North-West), Kelibia (CM2) (in the North-Est), and Monastir (CM3) (in the Center-Est) (Figure 1). Geographic coordinates of the collection sites are resumed in Table 1. The harvested plants were identified at the Biotechnology Center at the Technopark of Borj-Cédria by the expert botanist Professor Abderrazek Smaoui, and a voucher specimen [PLM80] was deposited at the Herbarium of the Laboratory.

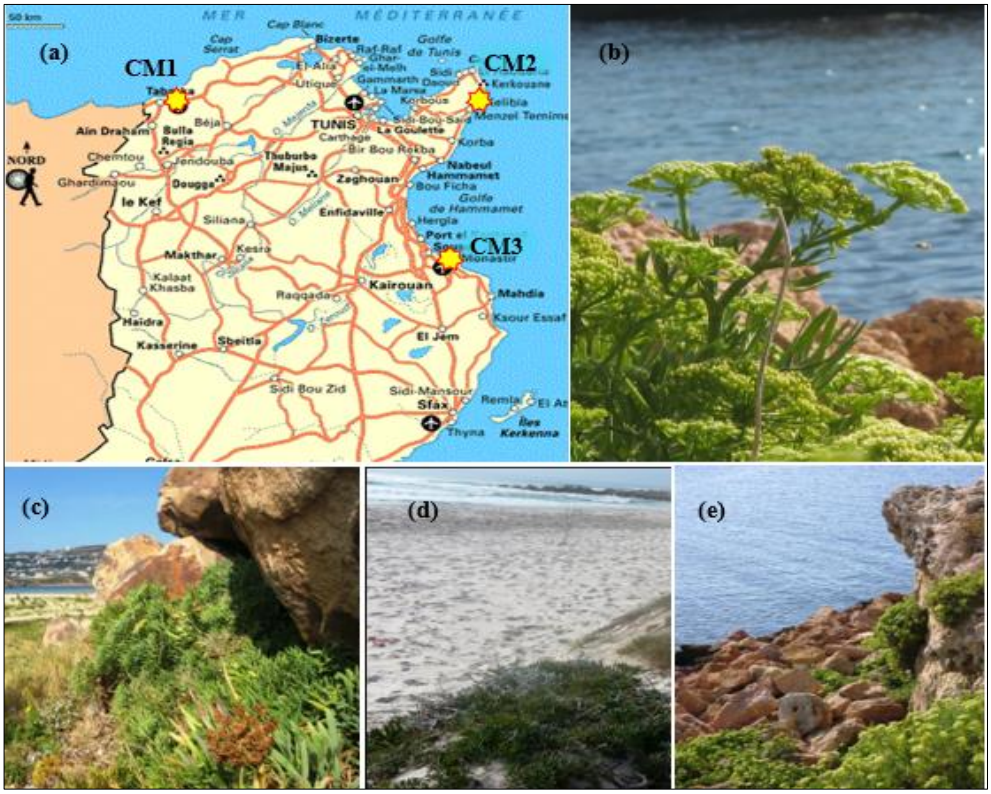

Fig 1: (a) Localization of the collection sites of *C. maritimum* L. on the map of Tunisia; (b) general aspect of the plant; (c), (d), and (e) *C. maritimum* in the three native harvesting ecosystems.

Table 1: Bioclimatic zones of collection sites defined according to Emberger's rainfall coefficient[19].

Geographic origin	Bioclimatic stage	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm/year)
CM1 (Tabarka)	Lower Humid	N36°57'11	E08°45'62	4	1029
CM2 (Kelibia)	Sub-Humide	N36°50'19	E11°07'88	1	710
CM3 (Monastir)	Lower Semi-Arid	N35°46'79	E10°50'22	4	350

The bioclimatic stages were defined according to the rainfall coefficient of Emberger [19] as followed: $Q2=2000P/(M^2-m^2)$, with P: annual precipitations (mm), M: average maxima of the hottest month (K°), m: average minima of the coldest month (K°), 1K=T°+273.

3. Samples preparation

Samples were rinsed with distilled water and organs were separated (root, stem, leaf, flower), then divided in two batches. The first one was well drained then kept in zip-lock bags at -80°C to be used as fresh material (FM) for carotenoids, anthocyanins, and vitamin C quantification. The second batch was oven dried at 37 °C for 1-3 days depending on the organ texture. The dry material (DM) was partially smashed (for the extraction of essential oils) or reduced to a fine powder (for the extraction of phenolic compounds).

4. Extraction and phenolic compounds determination

4.1. Extraction

Twenty grams of each organ were added to 100 mL acetone at 70%, sbjected to a frequent agitation for one hour, then filtrated through Whattman filter paper N°4. The obtained filtrates were evaporated under vacuum, and the residual aqueous phases were subjected to a liquid/liquid extraction with the petroleum ether (V/V) to eliminate waxes and chlorophyll. Petroleum ether phases were discarded and aqueous fractions were evaporated under vacuum at 35°C until complete dryness, dissolved in water and freeze dried then kept at 4°C until use.

4.2. Determination of phenolic compound contents

Total phenolics content (TPC) Colorimetric quantification of total phenolics was determined as described by Dewanto *et al* [20], using the Folin-Ciocalteu reagent. A calibration curve using the Gallic acid (ranging from 0 to 400 µg.mL^{-1}, r^2 = 0.99), was used to calculate the TPC, expressed as milligram gallic acid equivalent per gram dry weight (mg GAE.g^{-1} DW). Three replicates of each sample were analyzed.

*Total flavonoid content (TFC)*Total flavonoids were measured by the colorimetric assay described in Jallali *et al* [2]. Results were expressed as milligram catechin equivalent per gram dry weight (mg CE.g^{-1} DW), calculated using the calibration curve of (+)-catechin ranging from 0 to 400 µg.mL^{-1} (r2 = 0.99). All samples were analyzed in triplicates.

Condensed Tannins Content (CTC) Condensed Tannins Content were measured using the vanillin assay according to Jallali *et al*.. Results were expressed as mg CE.g^{-1} DW, calculated using the calibration curve of (+)-catechin ranging from 0 to 400 µg.mL^{-1} (r^2 = 0.99). All samples were analyzed in triplicates.

5. Carotenoid Assay

Carotenoid contents were estimated according to the method of Gould *et al* [21]. Aliquot of 0.2 g of fresh material was ground with liquid nitrogen and then incubated in 80% acetone in the dark at 4°C for 72 hours. The mixture was then centrifuged at a speed of 5000 x *g* for 10 min, then the supernatant was collected. Absorbance was measured at three different wavelengths: 663, 470, and 647 nm. The carotenoid

concentration was calculated using formula established by Lichtenthaler [22]:
Carotenoids (µg.mL^{-1}) = 5 * A470 + 2.846 * A663 + 14.876 * A647

6. Anthocyanin Determination
The anthocyanin contents were determined according to the method of Gould *et al*. [21] with a slight modification. Aliquot of 0.2 g of fresh material was ground with liquid nitrogen and incubated in the presence of a solvent mixture: HCl-H_2O-MeOH (1/3/16) in the dark at 4°C for 72 hours. The mixture was then centrifuged at 5000 x *g* for 10 min. Two different wavelengths are used for absorbance readings, namely 653 and 530 nm, and the anthocyanin content was estimated using the following formula:
Anthocyanins (µg.mL^{-1}) = A530 - 0.24 *A653
The anthocyanins soluble in this solution exhibit a maximum absorption spectrum at 530 nm; the subtraction of 0.24 * A653 compensates for the excess absorption at 530 nm due to the presence of chlorophylls.

7. Extraction and Ascorbic Acid Determination
7.1. Extraction
Fresh tissues of each organ were ground into a fine powder using liquid nitrogen in a mortar placed in ice. They were then deproteinized with 6% (w/v) trichloroacetic acid (TCA). The mixture was rapidly returned in ice until the TCA crystallizes. After vigorous stirring, mixture was centrifuged at 4°C for 10 min at 15.000 x *g*.

7.2. Ascorbic Acid Determination
Amounts of ascorbic acid were estimated according to the method of Kampfenkel *et al* [23]. The determination was based on the reduction, in an acidic medium, of Fe^{3+} to Fe^{2+} by the ascorbic acid present in the samples and the spectrophotometric detection of the Fe^{2+}-dipyridyl complex at a wavelength of 525 nm. The total amount of ascorbate (reduced form AsA + oxidized form DHA) was measured after pre-incubation of the samples in the presence of Dithiothreitol or DTT (10 mM), which reduced the oxidized fraction of ascorbate. Excess of DTT was removed with N-ethylmaleimide (NEM).
Aliquot of 0.2 mL of the supernatant obtained after extraction was mixed with 0.2 mL of DTT and 0.4 mL of phosphate buffer (0.2 M at pH 7.4). The mixture was vigorously stirred and then incubated for 15 min at 42°C. After that, 0.2 mL of 0.5% NEM was added, followed by a second stirring and incubation for 1 min at room temperature. One mmilliliter of 10% TCA, 0.8 mL of 42% orthophosphoric acid (H_3PO_4), 0.8 mL of 4% 2,2'-pipyridyl, and 0.4 mL of 3% $FeCl_3$ were added and mixed sequentially. The last reagent was added while vortexing to prevent turbidity. A final incubation of 40 min at 42°C was performed before measuring the absorbance at 525 nm at 25°C. Vitamin C concentrations were determined using a standard range of ascorbic acid concentrations (0.1-1mM).

8. Essential oils Extraction
Triplicate samples (100g) of each organ were extracted by a Clevenger apparatus. Kinetic studies to determine the appropriate extraction time for each organ were conducted (data not shown) and according to which, hydrodistillation process required 4 hours for the leaves, stems and umbels, and 6 hours for the root to ensure a complete extraction of the plant material. The obtained oils were dried over anhydrous sodium sulphate, filtrated and stored at +4 °C. Essential oil yield was expressed as percent of the plant dry material.

9. Statistical analyses
All analyses were done in triplicate and data were expressed as mean±SD (standard deviation). One-way analysis of variance (ANOVA) was performed followed by Newman-Keuls multiple comparison test (P<0.05). Analyses were computed on the data using XLSTAT software[24] (trial version, www.xlstat.com).

Results and discussion
1. Total Polyphenols, Flavonoids and Condensed Tannin Contents
Comparison of the results of phenolic compounds quantification showed significant variability of the amounts of these bioactive molecules among the different studied samples and as a function of the tested parameters (Table 2). In fact, TPC varied between 4.9 et 41.3 mg GAE.g^{-1} DM. The highest amounts were recorded in CM1 leaves and umbels, the lowest ones in CM1 stems. For CM1 and CM2, the leaves produced the highest amounts of polyphenols followed by the umbels, contrary to CM3 were the stems were the most provided with these natural antioxidants. The effect of the geographic origin on TPC was also significant. An increasing gradient going from north to south was observed when comparing samples from the three collection sites. Plants belonging to the upper humid region (CM1) were the richest on these compounds, followed by CM2, then CM3.
Amounts of TFC were very interesting (Table 2). They ranged from 48.6 to 1.8 mg CE.g^{-1} DM. Differences between these two extents reflected the significant variability of the amounts of these compounds among the studied samples. Amounts of CTC in *C. maritimum* extracts were moderate to week (Table 2). They ranged between 13.7 and 1.2 mg CE.g^{-1}. The distribution of TFC and CTC was almost the same as TPC.
Changes in the phenolic compounds composition were reported in several previous studies [2, 3, 9]. Authors attributed these changes to intrinsic and extrinsic factors. Among them, environmental factors seemed to have a great impact on the phenology, biosynthetic pathway, amounts and quality of phenolic compounds [2, 9]. The works of Jallali *et al* [2, 3, 25] demonstrated that amounts and related biological activities of *C. maritimum* phenolic compounds varied significantly as according to biotic and abiotic factors such as physiological stage, extraction method, geographic origin and plant part. This variability is essentially due to the implication of these secondary metabolites in the defense response to biotic and abiotic stresses.

Table 2: Comparison of Total Polyphenol Contents (TPC), Total Flavonoid Contents (TFC), and Condensed Tannin Contents (CTC) in *C. maritimum* organs collected at three different geographic origins in Tunisia

Geographic origin	Plant part	**TPC** (mg GAE.g^{-1} DM)	**TFC** (mg CE.g^{-1} DM)	**CTC** (mg CE.g^{-1} DM)
Tabarka (CM1)	Root	18.7±0.9 ^{cd}	20.3±0.8 ^{d}	1.2±0.2 ^{e}
	Stem	4.9±0.5 ^{g}	4.3±0.9 ^{h}	2.3±0.4 ^{d}
	Leaf	41.3±1.1 ^{a}	48.6±1.8 ^{a}	5.6±0.8 ^{c}
	Umbel	39.0±2.3 ^{a}	48.1±1.2 ^{a}	4.3±0.5 ^{c}

Kelibia (CM2)	Root	7.3±0.7 [f]	5.7±0.2 [gh]	1.3±0.2 [e]
	Stem	7.1±0.9 [f]	8.1±0.6 [f]	4.7±0.3 [c]
	Leaf	30.1±1.5 [b]	43.4±1.2 [b]	6.0±0.9 [b]
	Umbel	10.5±0.7 [e]	39.0±1.5 [c]	6.7±0.6 [b]
Monastir (CM3)	Root	8.6±0.9 [f]	1.8±0.4 [d]	2.3±0.3 [d]
	Stem	21.3±0.8 [c]	19.8±1.4 [d]	7.1±0.9 [b]
	Leaf	17.9±1.3 [d]	13.2±1.6 [e]	13.7±0.8 [a]
	Umbel	11.2±0.9 [e]	5.8±1.5 [g]	2.2±0.3 [d]

Results are means of three replicates. Means followed by the same letter in each column are not significantly different at $P<0.05$ using Newman-Keuls test; CE: g^{-1} catechin equivalent; GAE: gallic acid equivalent; DM: dry matter.

1. Essential oil yields

Comparison of the amounts of essential oils extracted from *C. martimum* are represented in Figure 2. They varied significantly according to the organ and geographic origin and they ranged from 0.13 to.75% DM. Amounts recorded in the umbels were very distinguished as compared to the other organs, independently from the collection site. They were comprised between 1.4 and 1.75% DM. Percentages recorded in the other organs were statistically not different and ranged from 0.13 to 0.27%. The effect of geographic origin was less accentuated, especially when comparing root, stem and leaf contents.

Intraspecific variability of the amounts of these molecules is intimately linked to the environmental factor specificity of each bioclimatic stage. In fact, and similarly to phenolic compound biosynthesis, it seems that production of essential oils is inversely correlated to the severity of climatic conditions. Samples from the northern region produced the highest amounts, while those collected from the southern one contained the lowest amount, probably due to the to the hardest climatic (low pluviometry, high temperatures) of this locality as compared to the two other ones. Underlying genetic or physiological adaptations to these differences in the edapho-climatic conditions in the native habitats of this plant could directly explain the phytochemical variations observed. Several previous studies indicated biotic and abiotic conditions deeply affect the biosynthesis and accumulation of secondary metabolites [13, 16]. Besides, investigations of the amounts and chemical composition of *C. maritimum* essential oils among the Mediterranean territories like Turkey (0.85%)[26], Italy (0.15%) [27], and Greece (0.08-0.99%) [28] confirmed this intraspecific variability, and suggested that fluctuations in the biosynthesis of these compounds was dependent on the specific conditions of each location, as their production was significantly enhanced as a response to biotic and abiotic stresses to overcome their negative effects.

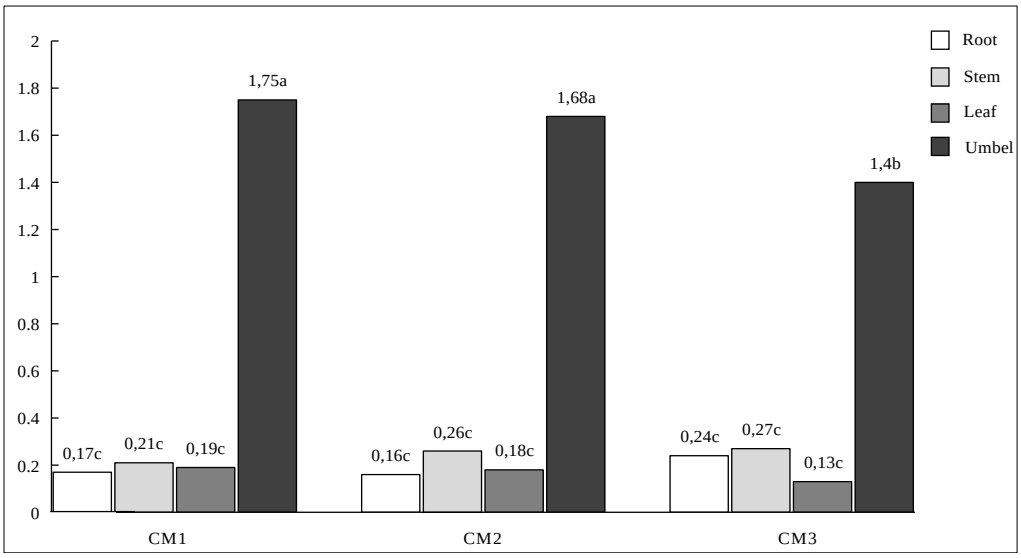

Fig 2: Essential oil contents in *C. maritimum* organs collected at three different bioclimatic stages in Tunisia.

1. Carotenoid contents

The results of carotenoids quantification in the different organs and provenances of sea fennel are shown in Figure 3. They revealed the richness of this species on these lipophilic antioxidant pigments with varying amounts between 25.1 and 379.5 $mg.100^{-1}g$ FM. They also divulged the great variability in the biosynthesis of these pigments according to the plant organ and the geographic origin. In fact, when comparing the amounts of carotenoids, the same hierarchical order of organs was recorded, independently from the collection site. Leaves were the richest on these terpenic antioxidants, followed by the stems, the umbels, and finally the roots. The comparison of our results to the literature showed that recorded levels of carotenoids closely resemble, and even exceed, those found in some conventional fruits and vegetables such as apples and carrots (0.3 to 0.5 and 0.32 to 17 $mg.100^{-1}g$ FM, respectively) [29]. The high concentration of these secondary metabolites, especially in leaves and stems of *C. maritimum*, may be due to the essential role these molecules play as key components of the photosynthetic membrane, involved in light energy collection, photoprotection, and as antioxidants, in addition to their role in attracting pollinators [5, 12]. Sathasivama *et al*. [11] indicated that the nature and content of carotenoids varied greatly according to plant parts (flowers, fruits, seeds, vegetables, etc.). They showed that most of the plant tissues contain these pigments, with higher percentages and diversified forms in green tissues (essentially leaves and stems). They also indicated that accumulation of carotenoids by non phothosynthetic tissues (like flowers, fruits and seeds) can be modulated by many biotic and abiotic factors [11].

The effect of geographic origin was also significant (Figure 3). An increasing gradient of the amounts of carotenoids was correlated to the severity of climatic conditions (decrease of precipitations, increase in temperature) going from CM1 to CM2, then CM3. Indeed, when environmental factors such as temperature, water, and nutrient availability are optimal for photosynthesis, the majority of absorbed light is used for photosynthesis. This contrasts with periods and habitats where factors such as nutrient limitation, drought, and high soil salinity decrease the rate of photosynthesis, forcing plants to dissipate excess energy through heat, which is associated with an increase in carotenoids concentration [30]. The high levels of these metabolites detected under stress conditions demonstrate their involvement in leaf protection mechanisms [30]. This fact is supported by numerous studies that have demonstrated the influence of light on carotenoid synthesis, and that have shown that most plants can modulate their composition of these antioxidant pigments in response to the variability of environmental factors [5, 12, 29]. Thus, in higher plants, the function of carotenoids in photoprotection is essential for the survival of the photosynthetic apparatus under aerobic conditions.

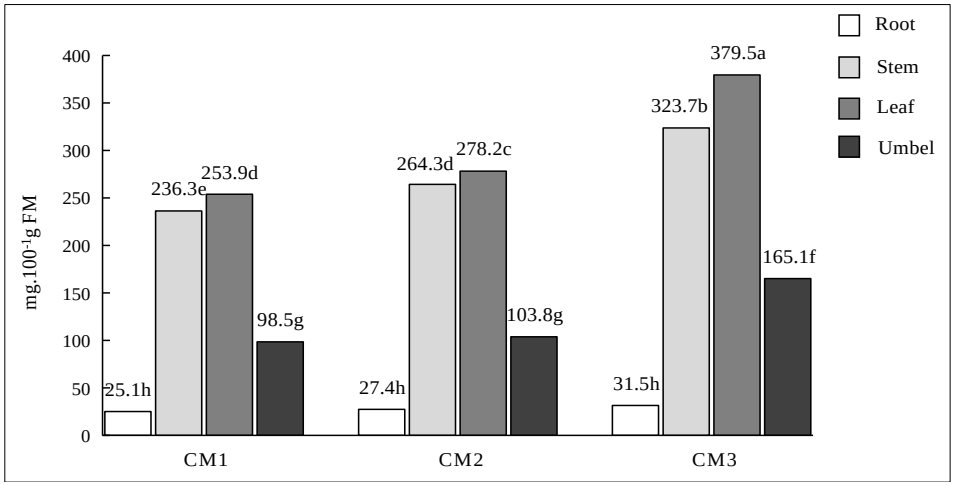

Fig 3: Carotenoid contents in *C. maritimum* organs collected at three different bioclimatic stages in Tunisia.

1.1. Anthocyanins contents

Unlike carotenoids, the anthocyanins contents of the different organs of *C. maritimum* was week (Figure 4), ranging from 0.15 to 0.95 mg.100^{-1} g FM. Several papers reported the determination of anthocyanins in diverse biological samples such as plants extracts, food, and agricultural samples [8, 10]. Amounts recorded in *C. maritimum* extracts were very low compared to those measured in strawberries, blueberries, blackberries, blackcurrant, redcurrant and raspberries (100 to about 700 mg/100 g FM) [10]. Such results can be inferred from the absence of pigmentation belonging to the red, blue, and purple degradation in the different organs of *C. maritimum*. The low levels detected probably represented the red outlines of achenes contained in the umbels or the leaf blade of some samples collected at CM2 and CM3. Their presence in these organs testified to the role they play in photosynthetic tissues, as well as to the presence of metabolic reactions associated with the intensity and nature of the captured light. Indeed, several studies have proven that the appearance of red color in plants was induced by UV-B light and that anthocyanins constitute protective shields against this light [18]. By absorbing at the same wavelength as chlorophyll b, anthocyanins play an auxiliary role in tissue protection and allow for the re-uptake of nutrients during senescence when chlorophyll is degraded[10]. In addition, anthocyanin-rich tissues are less frequently attacked by fungi and herbivores [18]. These molecules are stress metabolites since high temperatures and water deficit are associated with increased anthocyanin biosynthesis in some species [18]. In addition to the multiple and important functions that these hydrophilic pigments perform within the plant, anthocyanins enjoy powerful antioxidant properties and can express a wide variety of benefits for human health [8, 10].

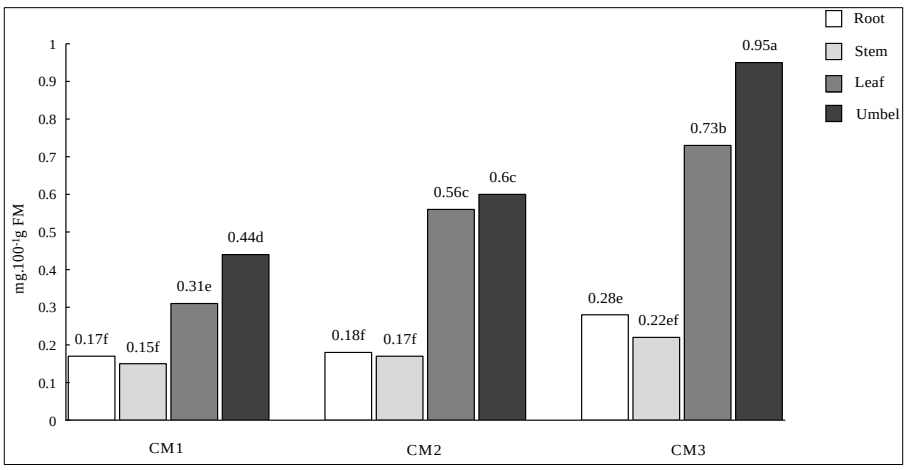

Fig 4: Anthocyanin contents in *C. maritimum* organs collected at three different bioclimatic stages in Tunisia.

2. Ascorbic acid amounts

The study of Total AsA content and its two forms revealed the richness of *C. maritimum* on this natural antioxidant (Table 3). Amounts of Total AsA varied between 109.3 and 22.5 mg.100 g^{-1} FM among studied organs and localities. This hydrophilic antioxidant consisted of two fractions: reduced AsA and oxidized AsA known as dehydroascorbate (DHA). The former constituted the major component of vitamin C, independently from the studied parameters, with levels comprised between 76.5 and 18.1 mg.100 g^{-1} FM. The latter, was the difference between the amount of Total AsA and that of the reduced fraction. Independently from the geographic origin, leaves were the richest organs on Total AsA, followed by the umbels, the stems and finally the roots. This distribution of AsA levels was directly linked to the physiologic function of each organ. In fact, this hydrophilic antioxidant is found primarily in the mitochondria of plant cells, where it is produced in response to stress. It then circulates to other organelles, such as chloroplasts, where it plays important roles as an antioxidant and coenzyme factor, and by interfering in the photosynthesis process, the hormone biosynthesis and in the redox reactions [16]. Variation in its contents among plant tissues was reported and it was demonstrated that AsA is more concentrated in parts of the plant exposed to sunlight, such as the leaves and flowers. Non-photosynthetic organs, like roots, flowers and young fruits, present a localized accumulation of AsA in their meristematic tissues [16].

Besides, the biosynthesis of this antioxidant was enhanced when going from the northern bioclimattic stage (high pluviometry) to the southern one (low pluviometry). This results was concomitant with previous studies indicating that both endogenous and exogenous factors may induce profound variability on the enzymatic activity of AsA biosynthesis [13]. The reduced water availability, for example, was correlated to the decrease in AsA levels [13].

Furthermore, the ascorbic acid content of *C. maritimum* exceeded that of several conventionally consumed species, such as carrots (6 mg.100 g^{-1} DM), bananas (10-30 mg.100 g^{-1} DM), citrus fruits (40-50 mg.100 g^{-1} DM), spinach (51 mg.100 g^{-1} DM), and tomatoes (20-25 mg.100 g^{-1} DM) [14, 16]. This result demonstrated the importance of sea fennel as a natural source of this vitamin, since consuming 100 g of this plant covered an adult's daily requirement for this metabolite (recommended intake: 65 to 100 mg/day).

In addition to its importance as an essential vitamin molecule for human health, ascorbate is a major metabolite of chloroplasts in higher plants, recognized as a photoprotectant involved in the clearance of excess photon energy. Ascorbate is also known as a "terminal antioxidant" because the redox potential of the reduced ascorbate/monodehydroascorbate pair (280 mV) is lower than that of most bioradicals[30]. These detoxification reactions can be considered an integral part of the housekeeping functions required for the aerobic existence of eukaryotic cells [14]. Consequently, its presence in such significant quantities in the aerial parts of *C. maritimum* could enhance the antioxidant pool and ensure the protection of the photosynthetic process, especially in the leaves.

On the other hand, the results of quantifying the different fractions of ascorbic acid showed that the oxidized form (DHA) represented 15.1 to 30% of the Total AsA pool. This result was consistent with previously established work that showed that DHA contributed 14% and 8% of the total vitamin C content in cauliflower and broccoli, respectively, while it represented the dominant form of vitamin C in cabbage, with levels four times higher than those of reduced AsA[31]. In this context, several authors found that the oxidized / reduced AsA ratio reflected the physiological state of the plant. Indeed, the redox status of ascorbate was a good indicator of the degree of stress experienced by plants [14]. Efficient recycling of the oxidized form of ascorbate minimized DHA degradation and reduced the need for *de novo* ascorbate synthesis [15]. In fact, ascorbic acid levels and their regenerative enzymes depended on a variety of environmental factors (or stresses) such as excessive light, high temperatures, excess salt, drought, and the presence of air pollutants, metals, and herbicides [14]. In this context, Ben Hamed [32] quantified the two forms of vitamin C in *C. maritimum* grown under controlled conditions and subjected to different salt concentrations. Results showed that in the presence of 100 to 300 mM NaCl, the ascorbate pool was dominated by the reduced form. Conversely, at 400 mM, the species was more sensitive to stress conditions, as both the total ascorbic acid pool and the ratio between the reduced and oxidized forms decreased.

Table 3: Comparison of Total ascorbic acid contents and its reduced and oxidized forms in *C. maritimum* organs collected at three different geographic origins in Tunisia

Geographic origin	Plant part	Reduced AsA (mg.100g^{-1} FM)	% of Reduced AsA	DHA (mg.100g^{-1} FM)	% of DHA	Total AsA (mg.100g^{-1} FM)
Tabarka (CM1)	Root	18.1±0.4 ^{g}	80.4	4.4±0.2 ^{d}	19.6	22.5±0.2 ^{i}
	Stem	38.3±0.9 ^{d}	84.9	8.3±0.5 ^{e}	15.1	46.6±0.5 ^{g}
	Leaf	60.4±1.0 ^{b}	80.8	14.4±0.3 ^{d}	19.2	74.8±0.9 ^{d}
	Umbel	54.1±0.7 ^{c}	82.2	9.7±0.5 ^{e}	17.8	63.8±0.8 ^{e}
Kelibia (CM2)	Root	19.2±0.3 ^{g}	74.8	5.3±0.3 ^{f}	25.2	24.5±0.9 ^{i}
	Stem	32.3±1.3 ^{e}	75.9	9.3±0.4 ^{e}	24.1	41.6±0.6 ^{g}
	Leaf	62.5±1.2 ^{b}	70	16.8±0.9 ^{c}	30	79.3±1.1 ^{c}
	Umbel	56.4±0.6 ^{c}	75	14.1±0.3 ^{d}	25	70.5±1.4 ^{b}
Monastir (CM3)	Root	27.3±0.7 ^{f}	78.4	9.2±0.6 ^{e}	21.6	36.5±0.3 ^{h}
	Stem	40.3±0.6 ^{d}	80	13.4±0.5 ^{d}	20	53.7±0.7 ^{f}
	Leaf	76.5±1.4 ^{a}	78.8	32.8±0.9 ^{a}	21.2	109.3±1.4 ^{a}
	Umbel	74.2±1.2 ^{a}	77.6	23.5±0.7 ^{b}	22.4	97.7±1.2 ^{b}

Results are means of three replicates. Means followed by the same letter in each column are not significantly different at $P<0.05$ using Newman-Keuls test; Reduced AsA: reduced form of ascorbic acid; DHA: dehydroascorbate; Total AsA: total ascorbic acid; FM: fresh matter

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