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Sea fennel (*Crithmum maritimum* L.) leaves and flowers: Bioactive compounds, antioxidant activity and hypoglycaemic potential

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ABSTRACT

Sea fennel (*Crithmum maritimum* L.) leaves and flowers from Italian wild and cultivated populations were herein investigated for their content of carotenoids and tocopherols using ultra-high-performance liquid chromatography coupled with the photodiode array and fluorimeter detectors to assess their functional value. Moreover, aqueous extracts were prepared to explore *in vitro* bioactivities never tested in this halophyte herb. Thus, chlorogenic acid-enriched sea fennel extracts were evaluated for their major bioactive compounds, antioxidant potential, pancreatic lipase, and carbohydrate hydrolase inhibitors. Neoxanthin, violaxanthin, lutein, zeaxanthin, β-carotene, and α- and γ-tocopherols were identified. *C. maritimum* can be considered a high source of lutein, vitamin A, and vitamin E up to 19.1, 1.85 and 52.81 mg/100 g of dried leaves, respectively. Despite a low TPC content, a promising $ABTS^{+\cdot}$ radical scavenging activity (CON-L-WT, IC_{50} value of 3.83 μg/mL) and the highest FRAP value were observed in the wild leaves extract of Conero Park of Marche Region. The water extract from the wild Sicilian leaves was the most active against pancreatic lipase. The evidence herein suggests that sea fennel extract might be potentially used in the formulation of nutraceuticals for the prevention of diseases associated with oxidative stress and hyperglycemic conditions.

1. Introduction

Sea fennel (*Crithmum maritimum* L.) is an emerging crop whose scientific relevance is increasing. It is a facultative halophyte, meaning that it can tolerate saline environments. *C. maritimum* grows spontaneously in cliffs along rocky or sandy coasts of the Mediterranean, Western Europe, North America, and Central-Western Asia (Renna, 2018). In the Mediterranean countries, sea fennel aerial parts are used in cuisine and folk medicine for their aromatic, antiscorbutic, diuretic, digestive, and carminative properties (Giordano et al., 2021; Meot-Duros et al., 2008). Traditionally, succulent, and crunchy leaves are consumed as a condiment in salads, sauces, soups, and pickled in vinegar or oil (Kraouia et al., 2023; Renna, 2018). Recently, innovative foods have been developed, such as fermented preserves (Maoloni et al., 2021; Özcan et al., 2019), co-fermented preserves with other vegetables (Maoloni, Cardinali, Milanović, Osimani, Garofalo, et al., 2022), Maoloni, Cardinali, Milanović, Osimani, Verdenelli, et al., 2022rtificially acidified preserves (Maoloni, Cardinali, Milanović, Osimani, Verdenelli, et al., 2022), powders (Renna et al., 2017), and functional beverages (Pedreiro et al., 2023; Pereira et al., 2017).

To preserve biodiversity and avoid indiscriminate harvesting, sea fennel is a protected floral species in some areas like the Conero Regional Park (Marche, Italy) (Kraouia et al., 2023). Therefore, an increasing number of small and medium enterprises in the Mediterranean basin started to cultivate this crop. Sea fennel has recently been claimed as a "cash crop" and "emerging crop" for its high potential in terms of adaptation to salinization and erosion of soils, and short-term water drought (Kraouia et al., 2023). Sea fennel is also considered a good source of dietary fiber, proteins, polyunsaturated fatty acids (e.g., linoleic, and linolenic), minerals, vitamins (C, A, E), and bioactive compounds such as polyphenols (hydroxycinnamic acids, flavonoids) and essential oils, the last two already well characterized. To the authors' knowledge, the profile of sea fennel carotenoids and tocopherols, respectively with provitamin A and vitamin E activity, has never been

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investigated.

The bioactive compounds of sea fennel are responsible for its functional traits, such as the antioxidant, antimicrobial, anti-inflammatory, and anti-proliferative activity with great potential in the nutraceutical and pharmaceutical sectors (Giordano et al., 2021). To date, different water and ethanol extracts have been studied for health properties targeting the phenolic components. Generalić Mekinić et al. (2016) partially attributed a vasodilatory effect in rat aortic rings to chlorogenic acid occurring in ethanolic extracts of sea fennel flowers. Alemán et al. (2022) demonstrated the anti-inflammatory activity of chlorogenic acid-enriched water extract inducing the secretion of IL-10 cytokines in macrophages. Souid et al. (2020) found a hepatoprotective effect in liver rats after administering water suspension of sea fennel leaves. Chlorogenic acids have also anti-hypoglycemic and hypolipidemic effects (Nartea et al., 2022; Naveed et al., 2018). The disproportions between pro-oxidant species, physiological processes and antioxidants generates cellular damage at various levels. In the case of metabolic syndrome, oxidative stress is both the cause and the consequence of obesity and associated disorders such as type 2 diabetes mellitus and cardiovascular diseases (Carrier, 2017). It contributes to impaired inflammation, vascular function, and atherosclerosis (Monserrat-Mesquida et al., 2020).

Several plants and herbs have been assayed for use in herbal teas, functional foods, or nutraceutical formulations to counteract obesity and hyperglycemia status. The inhibition of pancreatic lipase and the enzymes involved in the digestion of carbohydrates α-amylase and α-glucosidase are possible solutions to counteract obesity and hyperglycemia (de Sales et al., 2012; Dirir et al., 2022; Rajan et al., 2020). However, to the best of the authors' knowledge, no studies for sea fennel have been carried out, yet.

Within this framework, the present study was mainly aimed at characterizing carotenoids and tocopherols in leaves and flowers of sea fennel wild populations to disclose their functional value. Secondly, the study aimed at preparing economically affordable and eco-friendly extracts exploring never tested *in vitro* assays in *C. maritimum,* such as inhibitors of lipase and carbohydrate-hydrolyzing enzymes. Sea fennel water extract has already been assayed for anti-inflammatory and hepatoprotective effects. Thus, chlorogenic acid-enriched extracts from *C. maritimum* were evaluated for the content of major hydroxycinnamic acids, total polyphenols and flavonoids, inhibitors of lipase and carbohydrate-hydrolyzing enzymes with metabolic disease implications, as well as for their antioxidant potential. Since optimizing carotenoids and tocopherols-enriched extracts requires organic solvents, a selective aqueous extraction towards phenolics was considered the most appropriate and green choice.

2. Materials and methods

2.1. Sampling of wild and cultivated C. maritimum populations

Leaves (L; $n = 7$) and flowers (F; $n = 6$) were collected at the plant flowering period in August/September 2023 from wild sea fennel populations ($n = 7$) growing spontaneously in Italian coastal areas: Calabria (CAL); Marche, Conero Regional Park (CON); Marche, Porto Potenza Picena (MAR); Apulia (APU); Sardinia (SAR); Sicily (SIC); Tuscany (TUS). *C. maritimum* plant species were identified by the botanists of the Department of Agricultural, Food, and Environmental Science, Università Politecnica delle Marche, using morphological traits and geographical distribution of *C. maritimum*. About 500 g of fresh tender leaves were sampled by mixing leaves/sprouts from different individuals of the same population; about 500 g of flowers (umbels) from each population were also sampled. Samples of leaves and flowers were stored separately in sterile plastic bags, transported to the laboratory under refrigerated conditions, and kept at −20 °C till the freeze-drying stabilization for long-term storage until further analysis.

Moreover, a sample of organic *C. maritimum* crop produced in an open field in the Marche region (Central Italy) was collected to compare wild (WT) and cultivated (C) *C. maritimum* populations in terms of phytochemical potential. Leaves from organic sea fennel crop were manually harvested, air-dried in a De Cloet Dryer at < 40 °C till reaching RH% < 15, milled to obtain a powder, and stored at 20 °C.

2.2. Preparation of aqueous extracts from leaves and flowers

An aliquot (0.5 g) of dried powder from organic sea fennel crop and wild sea fennel (leaves or flowers) was extracted with 10 mL of deionized water, following the ratio 1:20 w/v, as reported by Alemán et al. (2019), at room temperature (21 °C) in dark conditions, under agitation for 18 h, centrifuged (1370×*g*, 5 min, 21 °C, 4800×*g* maximum), stabilized at −18 °C for 2 h, and filtered on regenerated cellulose filter (0.45 μm) and kept at −20 °C for one day. Selected extracts (n=5) were freeze-dried and stored at −20 °C before further analysis.

2.3. Simultaneous determination of carotenoids and tocopherols in leaves and flowers

Freeze-dried samples (100 mg) were extracted in acetone (5 mL, 4 °C), kept at 4 ± 1 °C (15 min), vortexed (5 min), and centrifuged (210×*g*, 10 min, 4 °C), repeating the acetone extraction twice. The supernatant was filtered (0.45 μm, Sartorius Regenerated Cellulose Membrane), dried, re-suspended in 0.5 mL in a solvent mixture composed of acetonitrile (75%), dichloromethane (10%), and methanol (15%), and injected in an Acquity Ultra Pressure Liquid Chromatographic (UPLC) H-class system (Waters Corporation, Milford, US), equipped with photodiode array (PDA) and fluorimeter (FLD) detectors and an Ascentis column UPLC C18 (2.1 mm × 100 mm, 1.7 μm). Chromatographic conditions are reported in Nartea et al. (2023). Retention time and absorbance spectrum of pure standards were used to identify the carotenoids. Their quantification was performed by external calibration reaching correlation coefficients (R^2) of 0.999 for lutein and β-carotene spanning in the range of 1–100 μg/mL and 0.05–100 μg/mL, respectively. FLD was set with at 290 nm excitation wavelength and 330 nm emission wavelength to detect tocopherols. Tocopherols were identified by comparison of the retention time with pure standards and quantified with external calibration. Standard stock solutions of each tocopherol (α-, γ-, β-, δ-tocopherol) were prepared in the range of 0–100 μg/mL, and good correlation coefficients were obtained for the calibration curves ($R^2 = 0.9836–0.9965$).

2.4. Major hydroxycinnamic acids in leaves, flowers, and extracts

Aliquots (50 mg) of freeze-dried leaves and flowers were extracted in 0.5 mL of methanol 70% with 0.1% formic acid, with ultrasounds (15 min), vortexed (5 min), and centrifuged (2264×*g*, 2 min, 21 °C). The supernatant was filtered (0.45 μm, PTFE membrane), and injected (1 μL) in an Acquity UPLC H-class system (Waters Corporation, Milford, US), equipped with PDA and an Ascentis column UPLC C18 (2.1 mm × 100 mm, 1.7 μm). Freeze-dried extracts were dissolved in 0.1 mL of methanol 70% with 0.1% formic acid, centrifuged, and injected. The gradient solvent consisted of water with 0.1% formic acid, and acetonitrile as it is reported by Martins-Noguerol et al. (2022). The flow rate was 0.4 mL/min, column temperature was controlled at 35 °C, and PDA was set at 330 nm. The major hydroxycinnamic acids were identified by comparison of the retention time and absorbance spectrum with pure standards and quantified with external calibration based on chlorogenic acid ($R^2 > 0.999$ in the range of 1–200 μg/mL).

2.5. Total phenol, flavonoids, and β-carotene contents in extracts

The total content of some phytochemicals was analyzed. In particular, the Total Phenols Content (TPC), Total Flavonoids Content (TFC), and Total Carotenoids Content (TCC) were the object of our study. TPC

was evaluated as previously described by Gao et al. (2019). An extract was mixed with Folin-Ciocalteu reagent and 20% Na_2CO_3 solution.

The absorbance of the extract was read after 2 h using an UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, Milan, Italy). The results were expressed as mg gallic acid equivalents (GAE)/g dry weight (DW). TFC was determined following the flavonoid-aluminium complex methodology (Yoo et al., 2008). The extract was mixed with 2% aluminium chloride solution (25 °C, 15 min). The TFC was expressed as mg quercetin equivalents (QE)/g DW plant material. For TCC, the methodology proposed by Fish et al. (2002). Results were expressed as equivalent mg β-carotene (βC)/g DW plant material.

2.6. Radical scavenging activity

The radical scavenging activity was tested using: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as previously reported by Sottile et al. (2023). In the DPPH test, a solution of DPPH and extracts at different 1–1000 μg/mL concentrations were mixed. After 30 min of incubation at 25 °C, the absorbance was read.

For the ABTS test, ABTS radical cation solution was prepared and then diluted with ethanol to reach an absorbance of 0.70 at 734 nm. This $ABTS^+$ solution was added to extracts at different concentrations (1–400 μg/mL) and left to react for 6 min at room temperature (21 °C) before absorption reading.

2.7. Ferric reducing ability power (FRAP)

For FRAP evaluation, FRAP reagent, acetate buffer (pH 3.6), $FeCl_3$, HCl, and tripyridyltriazine were mixed and added to the extract at a 2.5 mg/mL concentration. The positive control was butylated hydroxytoluene (BHT) as reported by Sottile et al. (2023).

2.8. Carbohydrate-hydrolyzing enzymes inhibitory activity

The α-amylase and α-glucosidase inhibitory activity tests were assessed as detailed by Leporini et al. (2020). In the α-amylase inhibitory assay, a starch solution was added to extract at different concentrations (25–1000 μg/mL) and left to react with the α-amylase (EC 3.2.1.1) at room temperature (21 °C) for 5 min.

The enzyme (EC 3.2.1.20) was mixed with maltose and *o*-dianisidine solutions and peroxidase/glucose oxidase system-colour reagent in the α-glucosidase inhibitory activity assay. After that, the extract (25–1000 μg/mL) was added, and the mixture was left to react for half an hour at 37 °C in the water bath. For both assays, acarbose was chosen as positive control.

2.9. Lipase inhibitory activity

For pancreatic lipase inhibitory activity, the previously published protocol was adopted (Leporini et al., 2020). Briefly, extracts at different concentrations (2.5–40 mg/mL) were mixed with lipase, Tris-HCl buffer (pH 8.5), and 4-nitrophenyl octanoate. After 30 min at 37 °C, the absorbance was read. Orlistat was used as a positive control.

2.10. Statistical analysis

Results are reported as means of three experiments ± standard deviation. Prism GraphPad Prism version 4.0 for Windows, GraphPad Software (San Diego, CA, USA) was used to build the concentration-response curve and calculate the inhibitory concentration of 50% (IC_{50}) and to perform a one-way analysis of variance (ANOVA). Significant differences ($p < 0.05$) were calculated according to Tukey's multiple-range tests. MetaboAnalyst 5.0 online software was employed for the Principal Component Analysis (PCA) conducted to discriminate the two groups (leaves and flowers). Box plots were used to visualize the variation of the different carotenoids and tocopherols.

3. Results and discussion

3.1. Profiling of carotenoids and tocopherols

In sea fennel leaves and flowers, the simultaneous analysis of carotenoids and tocopherols performed by using UPLC-PDA/FLD allowed to identify four xanthophylls (neoxanthin, violaxanthin, zeaxanthin, and lutein), one carotene (β-carotene) as reported in Fig. 1, and β + γ-tocopherol and α-tocopherol (Fig. 2).

Regarding carotenoids, in all the samples, the predominant compound was lutein, followed by β-carotene. In the window of elution of xanthophylls, especially from 1 to 4 min, five minor peaks were reported as non-identified, presenting a similar spectrum of absorbance to the identified xanthophylls; thus, they could be minor isomers (i.e., lutein epoxide). The carotenoid profile of medicinally green leafy vegetables, including apiaceous species such as coriander leaves, Indian pennywort, and carrot greens, agrees with our results since neoxanthin, violaxanthin, lutein, zeaxanthin, and β-carotene were identified as well (Raju et al., 2007).

For tocopherols, the major form was the α-tocopherol followed by β+γ-tocopherol since reversed phase does not allow the separation between β- and γ-tocopherol. In agreement, Portuguese *Salicorniaceae* halophytes contained α-tocopherol as the dominant isomer, followed by γ-tocopherol (Barreira et al., 2017), and this distribution is typical of halophytes (Ksouri et al., 2012). While, in *S. ramosissima*, *S. fruticosa,* and *M. nodiflorum* only α-tocopherol was analyzed (Castañeda-Loaiza et al., 2020).

Several halophytes are known to be rich in carotenoids (e.g., β-carotene, lutein) and tocopherols (e.g., α-tocopherol) (Castañeda-Loaiza et al., 2020; Lima et al., 2020; Martins-Noguerol et al., 2022), thus with a high potential for vitamin A and E activities, respectively. However, to the authors' knowledge, in-depth profiling of these compounds was still missing in *C. maritimum*. In addition, no published papers were available for the determination of sea fennel carotenoids and tocopherols with liquid chromatography.

Leaves and flowers of Italian wild and cultivated sea fennel clustered in two groups according to the score plot of the principal component analysis (Fig. 3), built on carotenoids and tocopherols. Leaves recorded higher concentrations of carotenoids and tocopherols than flowers, as it emerged from the boxplots (Fig. 3). Significant differences were found for neoxanthin with a mean value of 15.44 mg/kg DW of leaves *vs*. 1.63 mg/kg DW of flowers, lutein (152.73 *vs*. 41.59 mg/kg DW), β-carotene (76.29 *vs*. 17.12 mg/kg DW) and α-tocopherol (366.96 *vs*. 22.22 mg/kg DW). On the other hand, violaxanthin (25.43 *vs*. 2.81 mg/kg DW), zeaxanthin (3.64 *vs*. 2.69 mg/kg DW) and γ-tocopherol (23.96 *vs*. 17.12 mg/kg DW) did not display significant variations (Fig. 3).

In leaves (Table 1), neoxanthin, violaxanthin, lutein, and β-carotene were found at the lowest levels in the Sicilian wild population (SIC-L-WT), followed by sea fennel crop produced in the Marche Region (MAR-L-C), against the highest values found in the Sardinian wild population (SAR-L-WT).

Considering the main carotenoids in wild leaves, lutein and β-carotene ranged from 190.89 ± 12.19 to 106.41 ± 10.37 mg/kg DW and from 111.05 ± 14.04 to 44.37 ± 9.18 mg/kg DW, respectively. Leaves of sea fennel crop cultivated in the Marche region showed significantly lower levels of lutein (102.54 ± 6.22 mg/kg DW) and β-carotene (26.59 ± 1.61 mg/kg DW) than leaves of the two wild populations sampled in the same region (namely CON-L-WT sampled within the Conero Regional Park and MAR-L-WT sampled at Porto Potenza Picena, respectively) with ~0.7-fold and 3.2-fold difference, respectively. In cultivated sea fennel, calculated vitamin A (MAR-L-C, 0.44 mg/100 DW) was close to the daily recommended intake of 0.8 mg, while in all wild populations, it exceeded this value up to 1.85 mg/100 DW (Sardinian population, Fig. 4).

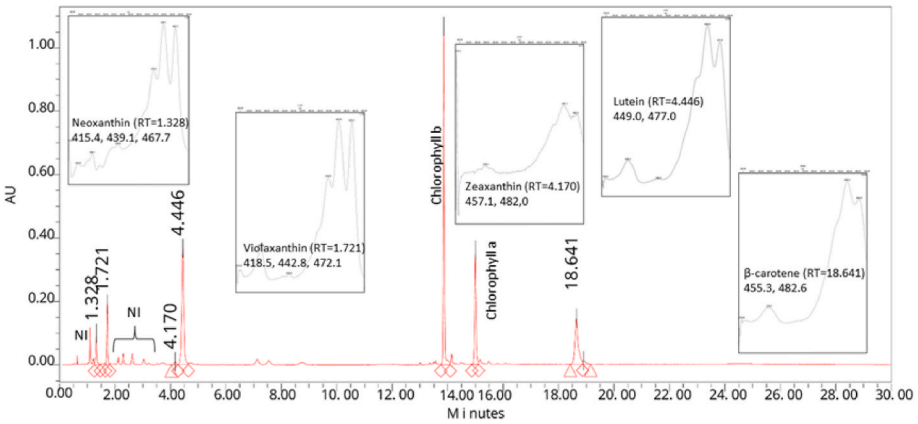

Fig. 1. UHPLC chromatogram of carotenoids acquired at 450 nm with the PDA detector. The peaks identified and quantified in the wild sea fennel leaves and flowers were: neoxanthin (retention time, RT = 1.328; Spectrum of absorbance, SA = 415.4, 439.1, 467.7); violaxanthin (RT = 1.721; SA = 418.5, 442.8, 472.1); zeaxanthin (RT = 4.170; SA: 457.1, 482.0); lutein (RT = 4.446; SA: 449.0, 477.0); β-carotene (RT = 18.641; SA: 455.3, 482.6). Chlorophyll *b* (RT = 13.861) and *a* (RT = 14.996) were identified but not quantified.

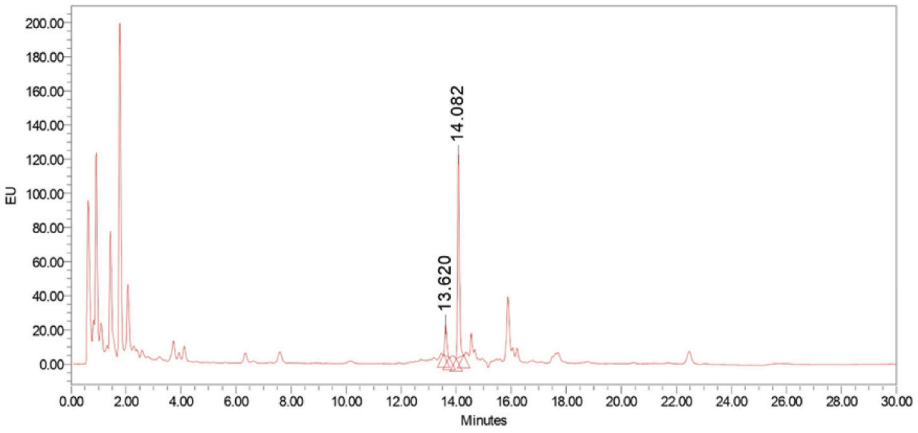

Fig. 2. UHPLC chromatogram of tocopherols acquired with the FLD. The peaks identified and quantified in the wild sea fennel leaves and flowers were: β + γ-tocopherol (retention time, RT = 13.620) and α-tocopherols (RT = 14.082).

The total carotenoid content was as follows: SAR-L-WT > TUS-L-WT > APU-L-WT > MAR-L-WT > CON-L-WT > CAL-L-WT > SIC-L-WT > MAR-L-C. In flowers of wild populations, the total carotenoid content was MAR > TUS > SAR > APU > CAL > SIC. Lutein and β-carotene ranged from 48.25 ± 4.12 to 32.59 ± 2.84 mg/kg DW and from 20.39 ± 5.44 to 10.29 ± 0.88 mg/kg DW, respectively (Table 1).

Spectrophotometric methods to date applied to sea fennel revealed a wide range of total carotenoid content from a maximum of 470 mg/kg DW of the whole plant (Sousa et al., 2022), 338 mg/kg of edible leaves (Guil-Guerrero & Rodríguez-García, 1999), 62.2 mg/kg DW of aerial parts (Nabet et al., 2017), and a minimum of 2.43–4.25 mg/kg DW of leaf tissue of salt-stressed hydroponically grown sea fennel (Labiad et al., 2021). Other *Apiaceae* species, namely *Hydrocotyle asiatica, Daucus carota,* and *Coriandrum sativum* displayed 90, 121, and 675 mg β-carotene/kg DW, respectively (Raju et al., 2007).

Leaves of *C. maritimum* can be considered a good source of lutein, with a content of up to ~190 mg/kg DW, similar to other halophytes such as *Mesembryanthemum nodiflorum, Suaeda maritima*, and *Sarcocornia fruticose* (88.9–197.0 mg/kg DW) and a better source than green leafy vegetables known as a source of lutein (i.e., Romaine lettuce, 33.19 mg/kg) (Castañeda-Loaiza et al., 2020). Lutein is an antioxidant and anti-inflammatory compound involved in eye function, and a daily intake of 5–12 mg of lutein is recommended. It possesses promising effects against neurological disorders, eye diseases, microbial infections, and skin irritation (Mitra et al., 2021).

Regarding leaves of wild populations (Table 2), the highest value of α-tocopherol was found in the wild population sampled in Calabria (CAL-L-WT, 528.12 ± 51.37 mg/kg DW) against the lowest value recorded in CON-L-WT (270.58 ± 15.26 mg/kg DW). Moreover, CON-L-WT also showed the lowest value of γ-tocopherol contrary to the wild population sampled in Tuscany which displayed the highest value (31.62 ± 7.38 mg/kg DW). For total tocopherol content, the sample order was CAL-L-WT > TUS-L-WT > APU-L-WT > SIC-L-WT > MAR-L-WT > SAR-L-WT > CON-L-WT > MAR-L-C. In the flowers of wild populations, total tocopherol content was TUS > APU > SAR > CAL > MAR > SIC. In detail, α- and γ-tocopherols ranged from 6.18 ± 0.00 to 33.17 ± 2.28 mg/kg DW and from 17.36 ± 1.30 to 36.73 ± 2.55 mg/kg DW, respectively (Table 2).

Leaves of *C. maritimum* are an excellent source of α-tocopherol (namely vitamin E) (Fig. 4), and for the first time, a notably high amount of this compound was detected in this halophyte species (528.12 mg/kg DW).

With regards to other halophytes, *A. macrostachyum* displayed 87.4 mg/kg DW (Barreira et al., 2017) whereas values up to 176 mg/kg DW were found in *Salicorniaceae* (Castañeda-Loaiza et al., 2020; Lima et al., 2020). All wild and cultivated dried leaves herein assayed can be considered as a high source of vitamin E with a content exceeding 3.6 mg/100 g (Reg. EU 1169/2011) (Fig. 4). Tocopherols provide vitamin E activity under different forms (α, β, γ, δ), but according to the European Food Safety Authority (EFSA) guidelines, only α-tocopherol can be used

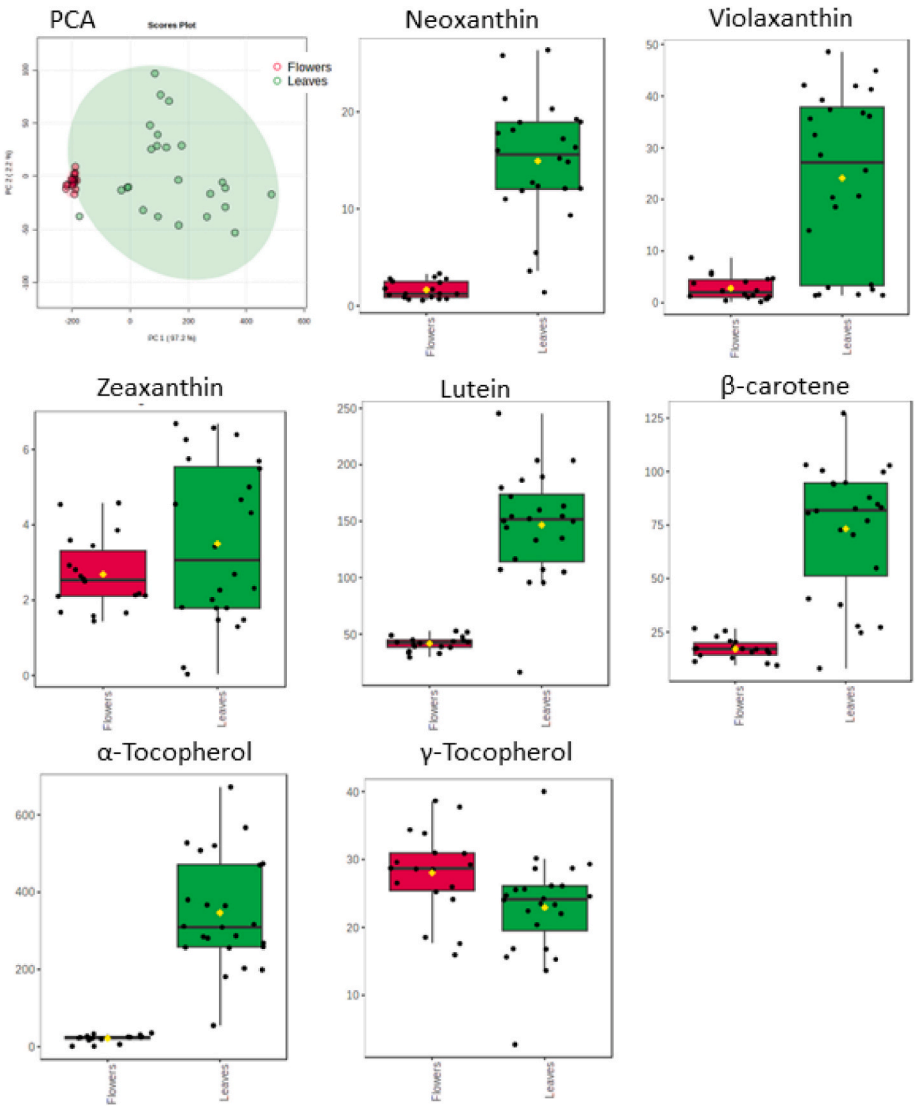

Fig. 3. Score plot of the principal component analysis (PCA) of flowers (wild types) and leaves (wild and cultivated types) using carotenoids and tocopherols as variables. Boxplots of flowers (wild types) and leaves (wild and cultivated types) of carotenoids and tocopherols, reported as concentrations, mg/kg DW.

to calculate vitamin E (EFSA NDA Panel, 2015a). However, γ-tocopherol was found to be superior in antioxidant, anticancer, and anti-inflammatory effects in cells and animal models. Thus, its presence in *C. maritimum* is potentially relevant (Jiang et al., 2022).

The difference observed for the contents of either carotenoids or tocopherols could be attributed to the ecotype of populations, to the vegetation stage variation, caused mainly by the geographical origin of samples. Accordingly, the phytochemical profile of sea fennel has been already found to be habitat dependent (Martins-Noguerol et al., 2022; Meot-Duros & Magné, 2009) and vegetation stage dependent (Generalić Mekinić et al., 2018; Jallali et al., 2012).

3.2. Major caffeoylquinic acids in flowers and leaves

To date, the polyphenol profile of sea fennel growing in different Mediterranean regions has been investigated in depth. Available data confirm it as an excellent source of chlorogenic acid (3-O-caffeoylquinic acid) and its isomers, namely cryptochlorogenic (4-O-caffeoylquinic acid) and neochlorogenic (5-O-caffeoylquinic acid) acids, as well as of flavonoids such as rutin and quercetin (Alves-Silva et al., 2020; Kadoglidou et al., 2022; Piatti et al., 2023).

The major hydroxycinnamic acids found in the leaves and flowers of the Italian sea fennel populations herein assayed are reported in Table 3. In general, flowers of wild populations contained a higher amount of hydroxycinnamic acids than leaves, in agreement with previous studies (Pereira et al., 2017; Politeo et al., 2023; Souid et al., 2021). In leaves, chlorogenic acid was the most abundant compound, with a content ranging from 8.41 to 65.07 mg/g DW, which is moderately higher than that reported in previous studies, attesting at 7.07–16.28 (Generalić Mekinić et al., 2018), 1.4–4.8 (Martins-Noguerol et al., 2022), ~3 (Castillo et al., 2022), or ~28 mg/g DW (Meot-Duros & Magné, 2009).

In flowers, 3,5-di-O-caffeoylquinic acid was the predominant compound ranging from 41.00 to 57.73 mg/g DW, followed by chlorogenic acid, ranging from 34.19 to 53.63 mg/g DW. High levels of 3,5-di-O-caffeoylquinic acids were also found by Piatti et al. (2023) and Zafeiropoulou et al. (2020) in Italian and Greek sea fennel, respectively.

Regarding leaves, the wild population from Apulia displayed the highest value of caffeoylquinic acids, while for flowers, the highest abundance of these compounds was found in the wild population from Sicily. Notably, leaves of sea fennel cultivated in Marche region showed more chlorogenic acids than wild populations sampled in the same region (MAR-L-C, 38.93 mg/g DW *vs.* 8.41 mg/g DW in MAR-L-WT and 28.23 mg/g DW in CON-L-WT).

In general, phenolic compounds possess strong biological activity

Table 1
Carotenoids, expressed as mg/g dry weight (DW), of leaves (L) and flowers (F) of sea fennel wild (WT) and cultivated (C) populations sampled in different Italian regions. CAL = Calabria; CON = Marche, Conero Park; MAR = Marche; APU = Apulia; SAR = Sardinia; SIC = Sicily; TUS = Tuscany.

mg/kg DW	Neoxanthin			Violaxanthin			Zeaxanthin			Lutein			β-carotene			Total carotenoids		
CAL-L-WT	12.86	±	0.86	bcd	29.55	±	4.52	ab	1.88	±	0.16	e	152.10	±	8.91	abc	82.37	±	10.76	b	278.76	±	19.30	b
CON-L-WT	14.95	±	2.98	abc	37.88	±	3.01	a	2.43	±	0.23	de	140.07	±	10.43	bcd	85.50	±	8.95	b	280.83	±	18.47	b
MAR-L-WT	17.96	±	1.68	abc	41.75	±	6.01	a	4.94	±	0.59	b	156.16	±	3.34	ab	88.43	±	10.42	ab	309.24	±	20.90	ab
APU-L-WT	19.57	±	1.64	ab	18.33	±	3.78	b	1.65	±	0.16	e	187.31	±	15.96	ab	87.69	±	10.53	ab	314.01	±	17.99	ab
SAR-L-WT	21.29	±	4.66	a	41.04	±	4.48	a	4.21	±	0.69	bc	190.89	±	12.19	a	111.05	±	14.04	a	368.49	±	12.60	a
SIC-L-WT	6.12	±	2,93	de	2.35	±	0.78	c	6.33	±	0.50	a	106.41	±	10.37	cd	44.37	±	9.18	c	165.58	±	23.17	c
TUS-L-WT	18,99	±	5,95	abc	30.98	±	11.78	ab	1.60	±	0.37	e	186.32	±	51.64	ab	84.28	±	12.40	b	322.18	±	74.86	ab
MAR-L-C	11,80	±	0,72	cd	1.53	±	0.09	c	6.11	±	0.37	a	102.54	±	6.22	d	26.59	±	1.61	cd	148.57	±	9.02	cd
CAL-F-WT	1,45	±	1,17	e	2.41	±	3.07	c	1.99	±	0.27	e	37.93	±	4.93	e	17.02	±	5.26	d	60.80	±	14.39	e
MAR-F-WT	1.41	±	1.19	e	3.80	±	4.25	c	2.26	±	0.26	de	48.25	±	4.12	e	20.39	±	5.44	cd	76.11	±	15.02	de
APU-F-WT	2,27	±	1,11	e	4.14	±	1.64	c	1.57	±	0.10	e	41.65	±	2.51	e	15.42	±	1.20	d	65.04	±	6.54	e
SAR-F-WT	1,40	±	0,32	e	1.85	±	1.96	c	2.69	±	0.21	de	41.87	±	2.98	e	19.86	±	4.89	d	67.68	±	10.13	e
SIC–F-WT	1,00	±	0,58	e	1.04	±	0.56	c	4.32	±	0.41	bc	32.59	±	2.84	e	10.29	±	0.88	d	49.25	±	4.95	e
TUS-F-WT	2,23	±	0,89	e	3.61	±	1.19	c	3.28	±	0.41	cd	47.07	±	5.76	e	19.73	±	1.47	d	75.93	±	8.17	de

Data are reported as the mean of three analytical replicates ± standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

such as antioxidant, anti-inflammatory, and antiproliferative activities (Giordano et al., 2021). Being secondary metabolites, many factors could have generated the variation in their contents here reported such as geographical origin, adverse climate conditions, soil salinity, nutrient deficiency, etc. (Kraouia et al., 2023). Raw material standardization plays a pivotal role in defining the final *in vitro* activity of the designed extracts (Nartea et al., 2022). Here, cultivated sea fennel was compared with wild plants of different geographical origins to provide some reference contents of the variation of the main caffeoylquinic acids present in the flowers and leaves of Italian wild and cultivated sea fennel.

3.3. Aqueous extracts: chemical characterization, antioxidant potential, lipase, and carbohydrate-hydrolyzing enzymes inhibition

Aqueous extracts were produced by choosing leaves and flowers with the highest TPC (Fig. 5a). Later, the quantification of TFC and TCC was done as well in extracts (Fig. 5b and c). Phenols represented the dominant compounds with a prominent role due to their health properties (Rahman et al., 2022). In general, a certain variability in TPC was observed. Extracts of leaves from Apulian population (APU-L-WT) and of flowers from Sicilian population (SIC–F-WT) were characterized by the highest content, with values attesting at 55.62 and 55.89 mg GAE/g DW of plant material, respectively (Fig. 5a).

The lowest value (34.02 mg GAE/g DW of plant material) was found in the extract of leaves from Marche region population (CON-L-WT) (Fig. 5a). A similar trend was also observed in TFC (Fig. 5b). APU-L-WT and SIC–F-WT exhibited the highest values attesting at 23.96 and 23.84 mg QE/g DW, respectively.

Souid et al. (2020) evaluated the total phytochemical content of *C. maritimum* leaves methanolic extract and found values of 26 mg GAE/g DW and 12 mg QE/g DW for TPC and TFC, respectively. The analysis of eighteen *C. maritimum* genotypes from different regions of Greece showed TPC values in the range 2.55–10.84 mg GAE/g of DW and TFC values in the range 2.25–15.08 mg of catechin equivalents (CE)/g DW (Kadoglidou et al., 2022). More recently, TPC values of 31.7 mg GAE/g DW and 23–33 mg GAE/g DW depending on the season (spring-summer) were found (Meot-Duros & Magné, 2009; Souid et al., 2021).

Variability in TPC and TFC was also observed when different plant organs were investigated. TPC values of 11.52 and 9.43 mg GAE/g extract were recorded with extracts of Tunisian sea fennel leaves and flowers, respectively. TFC ranged from 3.93 to 3.71 mg CE/g DW (Houta et al., 2013).

A TCC ranging from 0.43 to 0.80 mg βC/g DW of plant material was recorded for the extract of leaves from sea fennel crop (MAR-L-C) and that of flowers from the wild Sicilian population (SIC–F-WT), respectively (Fig. 5c). A significant TCC was also found in APU-L-WT (0.78 mg βC/g DW). Our data agree well with those found by Nabet et al. (2017), whereas they significantly differ from those reported by Sousa et al. (2022), who found a lower content of total carotenoids. The variability of TCC that emerges from the available literature might be attributed to the type of solvent used for the extraction.

The major hydroxycinnamic acids were assessed in the freeze-dried extracts (Table 4). The chlorogenic acid reached the highest value in the extract of leaves of the Sicilian population (89.03 mg/g DW extract), followed by that of Sicilian flowers (62.41 mg/g DW extract) and cultivated leaves from Marche (54.14 mg/g DW extract). Notably, the extract of flowers from the Sicilian population was also the richest in 3,5-di-O-caffeoylquinic acid. Accordingly, Alemán et al. (2019) prepared an aqueous extract with 42.61 mg chlorogenic acid/g DW extract and ethanolic at 40% with 58.48 mg chlorogenic acid/g DW extract from the whole plant of sea fennel. The authors chose to entrap the extract in soy phosphatidylcholine, recommending the use of an aqueous extract than an ethanolic one for better efficacy of entrapment (Alemán et al., 2019).

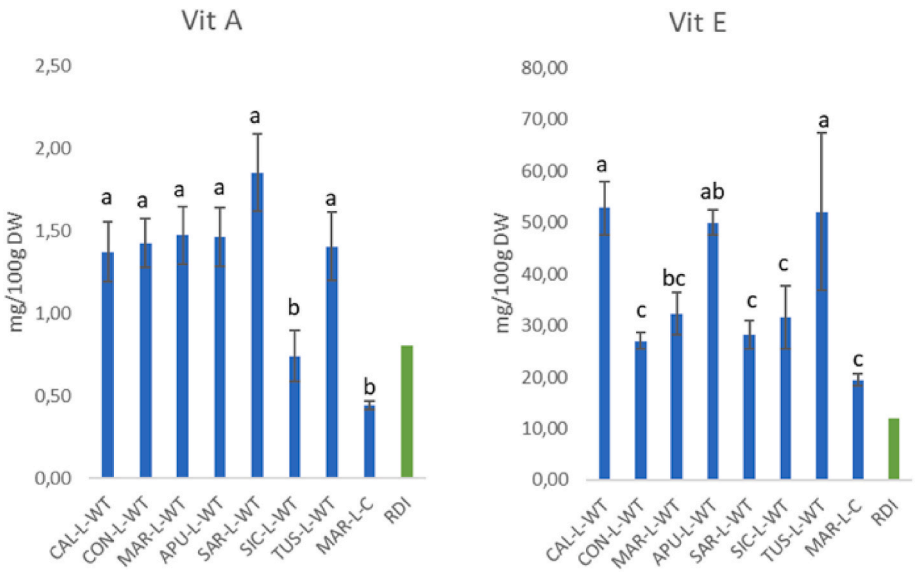

Fig. 4. Retinol activity equivalent (RAE) was calculated considering 1 μg retinol for each 6 μg of β-carotene (EFSA NDA Panel, 2015b). Vitamin E was calculated considering only α-tocopherol as proposed by the EFSA NDA Panel (EFSA NDA Panel, 2015a). Recommended daily intake of 0.8 mg per day of Vitamin A and 12 mg of Vitamin E. Data are reported as the mean of three analytical replicates ± standard deviation. Different letters mean statistical difference ($p < 0.05$) after one-way Anova Tukey test. DW, dry weight.

Table 2
Tocopherols, expressed as mg/g dry weight (DW) of leaves (L) and flowers (F) from sea fennel cultivated (C) and wild (WT) populations sampled in different Italian regions. CAL = Calabria; CON = Marche, Conero Regional Park; MAR = Marche; APU = Apulia; SAR = Sardinia; SIC = Sicily; TUS = Tuscany.

mg/kg DW	γ-T				α-T				Total tocopherols			
CAL-L-WT	26.20	±	1.05	b	528.12	±	51.37	a	554.32	±	51.77	a
CON-L-WT	14.85	±	1.06	d	270.58	±	15.26	b	285.43	±	16.00	b
MAR-L-WT	18.00	±	2.06	cd	322.72	±	41.54	b	340.72	±	43.44	b
APU-L-WT	23.81	±	0.66	bc	500.25	±	24.19	a	524.07	±	24.29	a
SAR-L-WT	28.31	±	2.05	b	282.60	±	27.94	b	310.91	±	29.96	b
SIC-L-WT	25.26	±	3.60	bc	315.87	±	60.97	b	341.13	±	64.50	b
TUS-L-WT	31.62	±	7.38	ab	521.26	±	153.40	a	552.87	±	160.27	a
MAR-L-C	23.65	±	1.44	bc	194.29	±	11.79	b	217.94	±	13.23	b
CAL-F-WT	17.36	±	1.30	cd	33.17	±	2.28	c	50.54	±	3.58	c
MAR-F-WT	25.11	±	0.93	bc	23.42	±	3.33	c	48.53	±	3.37	c
APU-F-WT	30.36	±	0.98	ab	24.80	±	2.55	c	55.15	±	2.79	c
SAR-F-WT	28.23	±	1.57	b	23.27	±	1.58	c	51.51	±	2.33	c
SIC–F-WT	30.52	±	3.33	ab	6.18	±	0.00	c	32.58	±	3.33	c
TUS-F-WT	36.73	±	2.55	a	22.47	±	4.73	c	59.20	±	7.28	c

Data are reported as the mean of three analytical replicates ± standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

Table 3
Hydroxycinnamic acids, expressed as mg/g dry weight (DW) of leaves (L) and flowers (F) of cultivated (C) and wild (WT) sea fennel populations sampled in different Italian regions. CAL = Calabria; CON = Marche, Conero Regional Park; MAR = Marche; APU = Apulia; SAR = Sardinia; SIC = Sicily; TUS = Tuscany.

mg/g DW	Neochlorogenic acid				Chlorogenic acid				Cryptochlorogenic acid				Total chlorogenic acids				3,5-di-O-caffeoylquinic acid			
CAL-L-WT	3.78	±	0.04	cd	20.76	±	0.21	f	4.72	±	0.05	c	29.27	±	0.30	e	6.54	±	0.07	cf
CON-L-WT	3.31	±	0.10	d	28.23	±	0.82	ef	3.84	±	0.11	c	35.38	±	1.02	de	2.95	±	0.09	cf
MAR-L-WT	1.76	±	0.07	ef	8.41	±	0.35	g	1.88	±	0.08	d	12.05	±	0.50	f	1.86	±	0.08	f
APU-L-WT	6.96	±	0.59	a	65.07	±	5.52	a	8.53	±	0.72	a	80.57	±	6.83	a	9.14	±	0.78	e
SAR-L-WT	5.57	±	0.44	b	46.06	±	3.64	bc	6.46	±	0.51	b	58.09	±	4.59	b	7.78	±	0.61	ef
SIC-L-WT	5.24	±	0.48	b	33.24	±	3.04	de	6.57	±	0.60	b	45.04	±	4.12	cd	5.46	±	0.50	cf
TUS-L-WT	4.17	±	0.34	e	26.95	±	2.22	ef	4.39	±	0.36	c	35.51	±	2.93	de	5.39	±	0.44	cf
MAR-L-C	1.68	±	0.14	ef	38.93	±	3.33	cd	1.04	±	0.09	d	41.65	±	3.56	cd	47.97	±	4.10	bc
CAL-F-WT	1.62	±	0.05	ef	38.45	±	1.25	cd	1.76	±	0.06	d	41.83	±	1.36	cd	41.60	±	1.36	cd
MAR-F-WT	1.45	±	0.16	f	39.36	±	4.39	cd	1.17	±	0.13	d	41.98	±	4.69	cd	48.28	±	5.39	b
APU-F-WT	2.01	±	0.06	ef	44.45	±	1.27	c	1.29	±	0.04	d	47.75	±	1.37	c	52.08	±	1.49	ab
SAR-F-WT	2.02	±	0.15	ef	44.68	±	3.26	c	1.83	±	0.13	d	48.53	±	3.54	bc	46.98	±	3.43	bcd
SIC–F-WT	2.30	±	0.07	e	53.63	±	1.58	b	1.77	±	0.05	d	57.70	±	1.69	b	57.73	±	1.70	a
TUS-F-WT	1.50	±	0.05	f	34.19	±	1.07	de	1.24	±	0.04	d	36.93	±	1.15	de	41.00	±	1.28	d

Data are reported as the mean of three analytical replicates ± standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

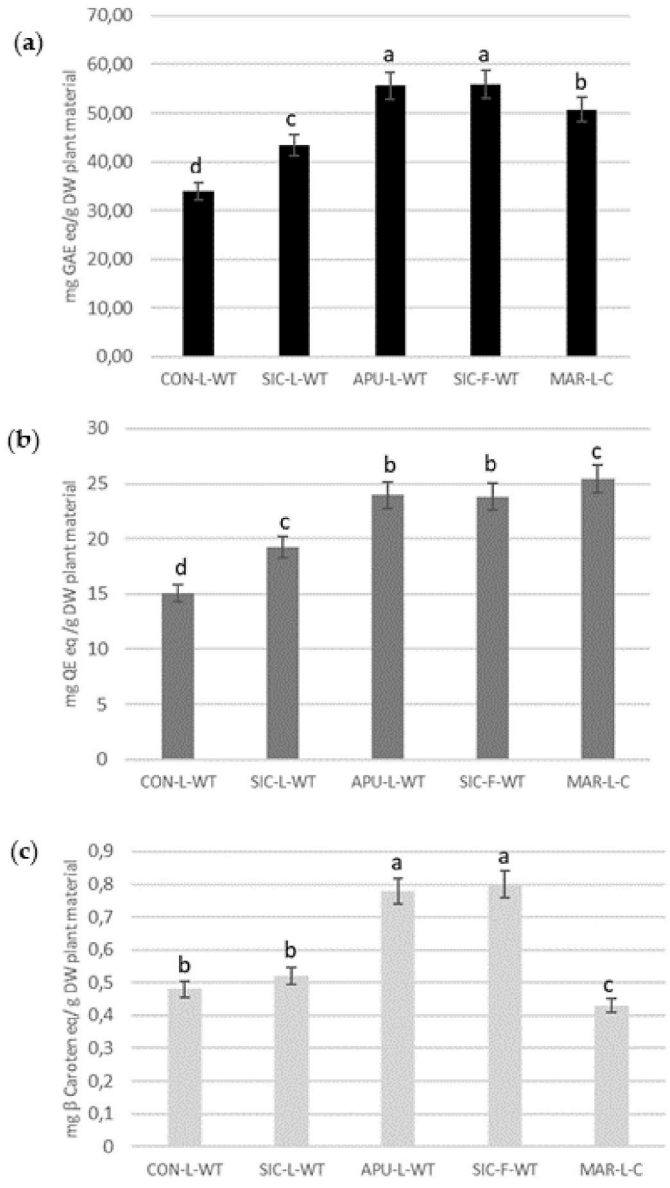

Fig. 5. (**a**) TPC (total phenols content), (**b**) TFC (total flavonoids content) and (**c**) TCC (total carotenoids content) in *C. maritimum* aqueous extracts. DW, dry weight.

The antioxidant potential of the assayed Italian sea fennel populations is reported in Table 5. Despite a low TPC content, CON-L-WT displayed a promising radical scavenging activity against $ABTS^{+\cdot}$ radical with an IC_{50} value of 3.83 μg/mL. The other populations exhibited IC_{50} values ranging from 5.15 to 6.97 μg/mL for APU-L-WT and MARC-L-C, respectively. A positive correlation was observed between ABTS data with TPC ($r = 0.73$) and TFC ($r = 0.83$). However, a different behaviour was observed when the DPPH test was assessed. In this case, the radical scavenging potential expressed as IC_{50} value ranged from 148.00 (extract from SIC-L-WT) to 326.53 μg/mL (extract from MARC-L-C), respectively. Pearson's correlation coefficient evidenced that DPPH data were positively correlated with neochlorogenic and cryptogenic acid ($r = 0.79$) and total chlorogenic acids ($r = 0.63$).

Two of the five tested populations showed FRAP values close to or higher than the BHT used as a positive control. SIC–F-WT exhibited a FRAP value of 70.22 μM Fe (II)/g at 2.5 mg/mL. A radical scavenging activity ranging from 83.5%-88.0% to 92.7%–92.9% against DPPH and ABTS radicals was detected in *C. maritimum* infusion from Portugal, as well as a promising FRAP value (Pereira et al., 2017). Similarly, for sea fennel methanolic extracts, Houta et al. (2013) found the following rank of potency as radical scavengers towards DPPH: leaves > flowers > stems.

Previously, Jallali et al. (2012) demonstrated that in sea fennel aerial parts, the reproductive stage rather than the harvesting period influenced the accumulation of bioactive compounds and bioactivity, with plants harvested in summer exhibiting higher antioxidant activity. Moreover, the comparison of different extraction methods showed that the extract obtained by maceration contained the most significant amounts of phenolic compounds. In contrast, the application of the Soxhlet apparatus led to an extract characterized by a high radical scavenging activity and reducing power effect.

The positive correlation between TPC and TFC contents and antioxidant activities suggests that these compounds may be the main contributors to the antioxidant activities of *C. maritimum* extracts.

In recent years, the interest in herbs and spices capable of counteracting/preventing obesity and related pathologies increased. A perusal analysis of the literature revealed that no previous studies were carried out on *C. maritimum* carbohydrate-hydrolyzing enzymes and lipase inhibitory properties.

A moderate lipase inhibitory activity was observed with IC_{50} values in the range of 141.98–438.78 μg/mL for SIC-L-WT and MARC-L-C, respectively. A positive correlation was found between pancreatic lipase inhibitory activity and chlorogenic acid ($r = 0.58$). As reported in

Table 5
Evaluation of *C. maritimum* samples antioxidant potential through a multi-target approach.

Sample	DPPH test	ABTS test	FRAP test
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	μM Fe (II)/g
CON-L-WT	298.26 ± 5.71^{c}	3.83 ± 0.61^{a}	56.65 ± 1.86^{c}
SIC-L-WT	148.00 ± 3.22^{a}	5.59 ± 0.77^{b}	55.29 ± 1.44^{c}
APU-L-WT	197.66 ± 3.86^{b}	5.15 ± 0.56^{b}	67.23 ± 2.75^{b}
SIC–F-WT	310.86 ± 6.09^{d}	6.88 ± 0.87^{c}	70.22 ± 2.88^{a}
MAR-L-C	297.84 ± 5.21^{c}	6.97 ± 0.93^{c}	48.38 ± 1.44^{d}
Sign.	**	**	**

Data are reported to mean ± Standard Deviation ($n = 3$). ^: [2.5 mg/mL]. Ascorbic acid and BHT were used as positive control in antioxidant tests. Ascorbic acid: IC_{50} of 5.04 ± 0.81 and 1.72 ± 0.14 μg/mL in DPPH and ABTS test, respectively; BHT 63.26 ± 2.31 μM Fe (II)/g in FRAP test. Differences within and between groups were evaluated by one-way ANOVA followed by Tukey's multiple range test. Results followed by different letters in the same column are significantly different at $^{**}p < 0.01$.

Table 4
Hydroxycinnamic acids, expressed as mg/g dry weight (DW), in freeze-dried aqueous extracts of leaves (L) and flowers (F) of cultivated (C) and wild (WT) sea fennel populations sampled in different Italian regions. CON = Marche, Conero Regional Park; MAR = Marche; APU = Apulia; SIC = Sicily.

mg/g DW of extract	Neochlorogenic acid	Chlorogenic acid	Cryptogenic acid	Total chlorogenic acids	3,5-di-O-caffeoylquinic acid
CON-L-WT	0.50	3.76	0.56	4.82	0.16
SIC-L-WT	14.70	89.03	19.26	122.99	7.38
APU-L-WT	5.52	47.88	6.97	60.37	2.75
SIC–F-WT	2.14	62.41	2.41	66.96	39.80
MAR-L-C	7.30	54.14	9.12	70.56	5.47

Data are reported as single replica.

Table 6, SIC-L-WT resulted in the most active against α-amylase (IC_{50} value of 103.77 μg/mL), followed by CON-L-WT (IC_{50} value of 122.34 μg/mL). Interestingly, cultivated sea fennel leaves exhibited the lowest activity. A positive correlation was also recorded between α-amylase inhibitory activity and TFC ($r = 0.66$). The following rank of potency in α-glucosidase inhibitory effect was observed: SIC–F-WT > SIC-L-WT > MARC-L-C > CON-L-WT > APU-L-WT with IC_{50} values in the range 128.55–180.60 μg/mL. The only positive correlation with α-glucosidase inhibitory activity was with TFC ($r = 0.75$).

4. Conclusions

The results herein collected suggest that leaves of wild and cultivated sea fennel populations can be considered an excellent source of vitamins A and E as well as lutein, γ-tocopherol, and chlorogenic acid. Great variability was seen in the phytochemical profile of the assayed populations and in the *in vitro* bioactivity of the extracts, both depending on the region of growth (for wild ecotypes) and the plant organ.

Thus, the cultivation of this halophyte represents an opportunity to standardize its nutritional and functional traits, in view of its further exploitation by the food and nutraceutical industries.

Among the assayed populations, the water extract from wild Sicilian sea fennel leaves was the most active against pancreatic lipase, and hence the most promising in terms of inhibition of key enzymes in the treatment of metabolic disorders. This activity was partially correlated with chlorogenic acid, whereas α-amylase and α-glucosidase inhibitory activities were likely correlated to total flavonoids. Thus, a deeper characterization of flavonoids seems interesting to better understand and improve those bioactivities in sea fennel crop. Moreover, new nutraceuticals might be formulated with sea fennel extracts for the prevention of diseases associated with oxidative stress and hyperglycemic condition.

In the next future, further apolar extracts rich in carotenoids and tocopherols from the leaves of wild and cultivated sea fennel populations will be tested for anti-aging and skin protection properties in view of the potential formulation of nutricosmetics.

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CRediT authorship contribution statement

Ancuta Nartea: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Oghenetega Lois Orhotohwo:** Formal analysis, Writing – review & editing. **Benedetta Fanesi:** Software, Visualization. **Paolo Lucci:** Writing – review & editing. **Monica Rosa Loizzo:** Data curation, Methodology, Writing – original draft. **Rosa Tundis:** Formal analysis, Writing – review & editing. **Lucia Aquilanti:** Funding acquisition, Project administration. **Simona Casavecchia:** Methodology. **Giacomo Quattrini:** Methodology. **Deborah Pacetti:** Conceptualization, Project administration, Resources.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lucia Aquilanti reports financial support was provided by European Union.

Table 6
Lipase, α-amylase, and α-glucosidase inhibitory activity of *C. maritimum* extracts [IC_{50} (μg/mL)].

Sample	Lipase	α-Amylase	α-Glucosidase
CON-L-WT	184.43 ± 3.70^{c}	122.34 ± 2.68^{b}	173.89 ± 3.45^{c}
SIC-L-WT	141.98 ± 3.56^{a}	103.77 ± 2.89^{a}	146.71 ± 2.84^{b}
APU-L-WT	175.88 ± 3.87^{b}	161.89 ± 2.85^{c}	180.60 ± 2.81^{d}
SIC–F-WT	412.89 ± 5.48^{d}	205.76 ± 3.14^{d}	128.55 ± 2.48^{a}
MAR-L-C	438.78 ± 5.77^{d}	321.89 ± 3.89^{e}	170.61 ± 2.75^{c}
Sign.	**		**

Data are reported to mean ± Standard Deviation (SD) ($n = 3$). Orlistat was used as a positive control in lipase test (IC_{50} value of 37.4 ± 1.0 μg/mL) whereas acarbose was the positive control in α-amylase and α-glucosidase tests (IC_{50} values of 50.12 ± 1.13 and 35.5 ± 1.10 μg/mL, respectively). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey's multiple range test. Results followed by different letters in the same column are significantly different at $^{**}p < 0.01$.

Data availability

Data will be made available on request.

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