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A Year in the Life of Sea Fennel: Annual Phytochemical Variations of Major Bioactive Secondary Metabolites

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Abstract: Sea fennel (*Crithmum maritimum* L.) is one of the most abundant and widespread Mediterranean halophytes, traditionally harvested and used in the summer months. As the plant bioactive metabolites are strongly influenced by the plant vegetation period and environmental conditions, we investigated some of the main bioactive compounds from sea fennel leaves over a one-year period to gain a deeper insight into their annual changes. A comprehensive phytochemical analysis of the essential oils using GC-MS, as well as the major phenolic and carotenoid compounds using HPLC, was performed. The results showed a high positive correlation between temperature and all major bioactive compounds, especially phenolic acids, cryptochlorogenic acid, and chlorogenic acid (r = 0.887, p = 0.0001 and r = 0.794, p = 0.002, respectively), as well as the limonene content in the essential oil (r = 0.694, p = 0.012). PCA analysis clearly distinguishes the period from February to April from the rest of the year, which contained the least bioactive metabolites overall. The overall data analyzed show great variations in sea fennel phytochemicals during the period of a year, with β-carotene content being the least effected. Therefore, it can be concluded that the plant can be used as a functional food or in other industries, such as the cosmetic and/or pharmaceutic industries, beyond its typical harvest period (early to midsummer).

Keywords: sea fennel; essential oil; phenolics; carotenoids; phytochemical variations; meteorological conditions; Pearson correlation; principal component analysis; hierarchical cluster analysis

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1. Introduction

Sea fennel (*Crithmum maritimum* L.) is one of the most widespread facultative halophytes on the Croatian coast, growing mainly in large populations on rugged coastal areas, rocks, cliffs, and scrubland, where it is exposed to the mechanical influence of wind and waves and also to sea salt spray and, consequently, to high salinity, lack of soil, and significant temperature fluctuations [1,2]. Halophytes are plants that, despite the abiotic stress factors mentioned above, are also exposed to high salinity conditions during their lifecycle, so they have been forced to develop various adaptation mechanisms against these stressful environmental conditions [3–5]. To minimize the damage and cope with the negative effects of these factors and inhospitable growing conditions, plants have developed specific molecular and physiological responses that influence plant metabolism by inducing the synthesis, degradation, and accumulation of certain metabolites [6,7].

These plant adaptation mechanisms often result in increased production of beneficial phytochemicals, which have a valuable nutritional composition for human diet and are widely used in cuisine in various dishes, as well as in the food, pharmaceutical, and cosmetic industries [8–12]. The presence and content of these phytochemicals are influenced by

the type and level of stress to which the plant is exposed. Among the most known plant defense compounds from the group of secondary plant metabolites, which are primarily involved in the regulation of plant–environment interactions, are phenolic compounds, terpenes (terpenoids, phenylpropanoids, carotenoids), and nitrogen- or sulfur-containing metabolites (alkaloids, glucosinolates) [6,7,13,14].

The folk name for sea fennel is St. Peter's plant, and it is traditionally harvested at the beginning of summer, just around St. Peter's Day. As sea fennel is one of the most widespread Mediterranean halophytes and emerging crops of nowadays, this study aimed to investigate the influence of environmental factors on the content and profile of the plant's main secondary metabolites (namely essential oil components, phenolic compounds, and carotenoids) during the plant's one-year lifecycle, as well as to determine whether it remains viable as a functional food beyond its conventional harvest period.

2. Materials and Methods

2.1. Reagents

All chemicals and solvents were purchased from Honeywell Fluka (Charlotte, NC, USA), Sigma-Aldrich (St. Louis, MO, USA), and VWR (Radnor, PA, USA). The series of *n*-hydrocarbons C8–C40 used for the identification of the essential oil components were purchased from Supelco Inc. (Sigma Aldrich). Carotenoid standards: β-carotene was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), while neoxantin, violaxanthin and lutein, as well as phenolic compounds (neochlorogenic acid, chlorogenic acid, and criptochlorogenic acid) were purchased from Sigma-Aldrich.

2.2. Plant Material

Leaves of wild-grown sea fennel (*Crithmum maritimum* L.) were collected in Split, Croatia (43°50′10″ N, 16°48′67″ E) from October 2022 to September 2023. Morphological identification was carried out using a plant identification key by Dr. Tonka Ninčević Runjić. Part of the plant material predestined for essential oil isolation was air-dried at room temperature (15 days), while the plant material used for the extraction of phenolics and carotenoids was stored at −80 °C until lyophilized (Labconco, Kansas City, MO, USA).

2.3. Extractions of Essential Oils, Phenolics and Carotenoids

The essential oils (EO) were isolated from the sea fennel dry leaves using hydrodistillation in a Clevenger-type apparatus for 2.5 h according to Politeo et al. [15] with slight modification. A mixture of pentane:diethyl ether (3:1, v/v) was used as a trap for the essential oil. Prior to analysis, the EOs were dried over anhydrous sodium sulphate.

For phenolic and carotenoid extraction, the dry sea fennel leaves were homogenized to fine powder using an analytical mill (IKA-Werke GmbH & Co., Staufen, Germany). The homogenized samples for phenolic analysis were prepared following the protocol of Veršić Bratinčević et al. [16]; 0.5 g of homogenized plant material was extracted with 5 mL of methanol:H_2O (80:20, v/v), sonicated for 15 min at room temperature, and mixed for 3 h at room temperature in orbital shaker. The samples were then filtered with 0.45 µm PTFE syringe filters (Macherey-Nagel, Düren, Germany).

Using a slightly modified protocol from Nartea et al. [17], carotenoid extraction of homogenized dry plant material was performed. The samples (0.5 g) were extracted with 2.5 mL of acetone, vortexed, stored at 4 °C for 15 min, and centrifuged at 1370 rpm for 10 min, and then the procedure was repeated one more time. The prepared samples were filtered using 0.45 µm PVDF syringe filters (Macherey-Nagel, Düren, Germany) and injected into the UHPLC system for the determination of β-carotene, while the samples for the determination of lutein, neoxanthin, and violaxanthin were filtered, evaporated, and resuspended in 80% methanol. The solvent was evaporated using a rotary evaporator (Thermo Scientific, Waltham, MA, USA).

2.4. Gas Chromatography–Mass Spectrometry

EO analysis was performed using a gas chromatograph (model 8890 GC) equipped with an automatic liquid injector (model 7693A) and a tandem mass spectrometer (MS/MS) (model 7000D GC/TQ), all from Agilent Inc. (Santa Clara, CA, USA) according to Politeo et al. [18]. The EO compounds were separated on the HP-5MS UI column (30 m × 0.25 mm × 0.25 µm, Agilent Inc.), with the temperature program set for the first 3 min at 60 °C followed by heating to 246 °C at 3 °C/min and isothermal maintenance for 25 min. The inlet temperature was set to 250 °C, the sample injection volume was 1 µL, and helium was used as the carrier gas. The ionization energy was set to 70 eV, and the temperature of the ion source was 200 °C, while the temperature of the quadrupoles was 150 °C. Individual compounds were identified by comparing their retention indices with series of *n*-alkanes and by comparing their mass spectra with the Wiley 7 MS library (Wiley, NY, USA) and the NIST02 (Gaithersburg, MD, USA) mass spectral database, as well as with the literature data [19]. All samples were injected in duplicate, and the results were expressed as the % of chromatogram peak areas.

2.5. Ultra High-Performance Liquid Chromatography

2.5.1. Phenolics

The phenolic compounds were separated and quantified according to the protocol previously described by Veršić Bratinčević et al. [16] using Shimadzu HPLC Nexera LC-40 (Shimadzu, Kyoto, Japan) equipped with a UV-VIS detector. A Phenomenex C18 reverse-phase column (250 mm × 4.6 mm × 5 µm; Torrance, CA, USA) was used, with a flow rate of 1 mL/min and temperature of 35 °C. Ultra-pure water/85% *o*-phosphoric acid (99.8/0.2, *v*/*v*) was used for mobile phase A, while mobile phase B was methanol/acetonitrile (1/1, *v*/*v*). The concentration gradient was set at an initial 4% of B followed by 15% B at 16 min, 35% B at 50 min, and 4% B at 62 min, with a final hold to 65 min. The phenolic compounds were identified by comparing their retention times and absorption maximums at 220 and 320 nm with acquired corresponding standards and quantified with external standard calibration curves. The concentrations are expressed as mean ± standard deviation in mg of phenolic compound per gram of dry plant (mg/g).

2.5.2. Carotenoids

Carotenoid compounds were separated and quantified using ultra high-performance liquid chromatography (UHPLC) coupled with a diode array detector (DAD) (Ultimate 3000RS, Thermo Fisher Scientific, Waltham, MA, USA) using Chromeleon™ Chromatography Data System Software version 7.2.9 (Thermo Fisher Scientific, Waltham, MA, USA) according to Generalić Mekinić et al. [20]. For separation, a reversed phase column (Halo C30; 150 mm × 3 mm, 2.7 µm; Advanced Materials Technology, Wilmington, DE, USA) was used, with a gradient elution of solvent A (1% acetic acid in water), solvent B (methanol), and solvent C (acetonitrile). The concentration gradient was as follows: 70% C (30% A) isocratically for 0.2 min; 0.2–8 min to 100% C; 8–9 min to 30% B and 70% C; and 9–17 min 30% B and 70% C, with a total analysis time of 22 min. The flow rate was set to 1 mL/min and 10 µL of the sample was injected into the system. Carotenoids peaks were identified by comparing their retention times with those of authentic standards at 415, 425, and 450 nm, as well as with spiked samples using standard solutions. Calibration curves were constructed with 6 calibration points in the range of 0.05–20 µg/mL. Lutein, neoxanthin, and violaxanthin were diluted in methanol/water (80:20, *v*/*v*), while *β*-carotene was diluted in acetone. Chromeleon™ Chromatography Data System Software version 7.2.9 (Thermo Fisher Scientific, Waltham, MA, USA) was used to collect, record, process, and integrate the data. Analyses were performed in duplicate, and concentrations are expressed as mean ± standard deviation in mg of carotenoid compound per gram of dry plant (mg/g).

2.6. Statistical Analyses

All statistical analyses were performed using SPSS software, version 25.0 (IBM Corporation, New York, NY, USA).

2.6.1. Analysis of Variance

A one-way analysis of variance (ANOVA) was performed to determine the differences in bioactive compounds from sea fennel EOs, phenolics, and carotenoids throughout a 12-month period. The data were tested for normal distribution and log-transformed afterward, which was sufficient to fulfil the homogeneity requirements. One-way ANOVA was performed, and if there were statistically significant difference, Tuckey's honestly significant test for equal variances was performed at a significance level of $p \leq 0.05$.

2.6.2. Pearson Correlation Coefficient

To determine whether there is a correlation between the climatic parameters (temperature, relative humidity, rainfall, wind strength and cloud abundance) and the phytochemicals from sea fennel EOs, phenolics, and carotenoids over a 12-month period, an analysis of Pearson's correlation coefficient was performed. The correlation was considered significant at the level of $p \leq 0.05$. The data on climatic parameters were taken from the Croatian Meteorological and Hydrological Service weather station (coordinates: 43.5167° N, 16.4167° E).

2.6.3. Principal Component Analysis

Principal component analysis (PCA) was performed to investigate and summarize the phytochemical data obtained from the sea fennel harvest period of 12 months (data for EO was calculated only for the compounds > 1%). A two-dimensional score plot was created to determine whether the factors could allocate phytochemicals from different months to clusters.

2.6.4. Hierarchical Cluster Analysis

Hierarchical clustering analysis using Ward's minimum variance method was used to measure the distance between each pair of phytochemicals obtained from sea fennel in the 12-month harvest period (data for EO was calculated only for compounds in the amount > 1%). Additionally, a cluster analysis dendrogram was used to visualize connections between phytochemicals.

3. Results and Discussion

The EOs were isolated using hydrodistillation and analyzed using GC-MS. Twenty-eight compounds were identified in the samples, accounting for 96.60% (December) to 99.49% (April) of the EOs (Table 1 and Table S1). The greatest amount of identified compounds was categorized into two chemical groups, including terpenes and saturated aliphatic compounds. The proportion of terpenes was highest in September and lowest in April, while the distribution of saturated aliphatic compounds was inversely proportional.

The majority of terpenes were monoterpenes, with limonene dominating in all samples (Table 1, Figure 1).

Table 1. Chemical composition of volatile compounds in the amount >1% identified in sea fennel leaf essential oils throughout a 1-year period.

Compound	RI	RI_L	January	February	March	April	May	June	July	August	September	October	November	December
α-Pinene	927	932	8.14 ± 0.16 a	3.8 ± 0.10 b	7.68 ± 0.00 a	3.15 ± 0.02 b,c	2.89 ± 0.17 c	1.57 ± 0.00 d	0.48 ± 0.03 e	0.61 ± 0.00 f	0.56 ± 0.01 e,f	1.34 ± 0.10 d	1.07 ± 0.01 g	0.82 ± 0.04 h
Sabinene	966	969	6.81 ± 0.15 a	0.28 ± 0.00 b	0.30 ± 0.01 b	0.15 ± 0.00 c	0.59 ± 0.05 d	0.97 ± 0.00 e	1.75 ± 0.03 f	4.19 ± 0.04 g	11.38 ± 0.24 h	8.83 ± 0.58 i	2.24 ± 0.04 j	1.82 ± 0.11 f,j
β-Myrcene	985	988	1.33 ± 0.03 a	0.56 ± 0.02 b,c	0.56 ± 0.01 b,c	0.41 ± 0.01 d	0.26 ± 0.01 e	0.47 ± 0.01 b,d	0.43 ± 0.02 d	0.66 ± 0.01 c,e	1.01 ± 0.05 f	1.55 ± 0.15 a	0.72 ± 0.02 e	0.91 ± 0.04 f
α-Terpinene	1011	1014	1.06 ± 0.03 a	0.05 ± 0.02 b,c	0.04 ± 0.00 b,d	0.04 ± 0.02 b	0.11 ± 0.00 b,e,f	0.23 ± 0.01 e,g	0.19 ± 0.04 e,g	0.50 ± 0.02 a,g	0.51 ± 0.00 a,g	0.35 ± 0.04 a,f,g	0.14 ± 0.01 c,d,f,g	0.16 ± 0.02 c,d,f,g
Limonene	1023	1024	58.74 ± 0.85 a	71.00 ± 1.53 b	74.19 ± 0.06 b	73.72 ± 0.09 b	81.79 ± 0.72 c,d	83.52 ± 0.23 c,d	82.13 ± 0.59 c,d	84.53 ± 0.50 c,d	76.31 ± 0.49 b,c	72.72 ± 2.62 b	82.98 ± 0.87 d	72.46 ± 1.33 b
(Z)- β -Ocimene	1032	1032	11.65 ± 0.06 a	4.86 ± 0.16 b	6.48 ± 0.07 c	6.44 ± 0.04 c	3.17 ± 0.15 d	4.70 ± 0.06 b	3.83 ± 0.03 d,e	4.10 ± 0.10 b,e	4.72 ± 0.16 b	7.39 ± 0.62 c	6.40 ± 0.16 c	4.84 ± 0.23 b
γ-Terpinene	1052	1054	2.28 ± 0.05 a	0.18 ± 0.01 b,c	0.17 ± 0.01 b,c	tr.	0.87 ± 0.03 d,e	0.89 ± 0.02 d,e	0.48 ± 0.02 f	0.98 ± 0.04 d,g	1.04 ± 0.03 d	0.83 ± 0.09 e,g	0.33 ± 0.01 c,f	0.42 ± 0.03 f
(Z)-*p*-Mentha-2,8-dien-1-ol	1115	1133	0.33 ± 0.01 a	1.45 ± 0.06 b	0.90 ± 0.03 c	1.30 ± 0.01 b	0.75 ± 0.00 c,d	0.37 ± 0.02 a,e	0.56 ± 0.11 d,e	tr.	tr.	0.31 ± 0.03 a	0.24 ± 0.04 a	0.84 ± 0.04 c
Terpinen-4-ol	1171	1174	0.90 ± 0.05 a,h	0.38 ± 0.01 b,c	0.24 ± 0.02 b	tr.	0.68 ± 0.01 a,c,d	0.47 ± 0.05 b,d,e	1.47 ± 0.03 f,g	1.25 ± 0.08 f,h	1.72 ± 0.05 g	0.59 ± 0.09 a,b	0.64 ± 0.18 a,c,e	0.68 ± 0.04 a,c,e
α-Terpineol	1184	1186	0.30 ± 0.02 a	0.93 ± 0.09 b	tr.	n.d.	n.d.	0.19 ± 0.01 a	0.66 ± 0.06 b,c	0.29 ± 0.02 a	0.21 ± 0.02 a	0.25 ± 0.04 a	0.43 ± 0.05 a,c	2.23 ± 0.012 d
(*E*)-Carveol	1213	1215	0.29 ± 0.01 a	1.18 ± 0.04 b	0.58 ± 0.00 c	0.58 ± 0.06 c	1.07 ± 0.02 b,d,f	0.99 ± 0.04 b,e,f	0.91 ± 0.09 d,e,f	tr.	0.19 ± 0.01 a	0.25 ± 0.04 a	0.29 ± 0.02 a	0.85 ± 0.03 f
Limonene-1,2-diol	1322	1343	0.17 ± 0.01 a	0.63 ± 0.04 b	0.31 ± 0.01 c	0.25 ± 0.03 a,c	1.01 ± 0.05 d	0.33 ± 0.00 c	0.49 ± 0.02 e	tr.	tr.	tr.	n.d.	tr.
Tetradecane	1400	1400	0.68 ± 0.1 a,b	3.19 ± 0.02 c	1.57 ± 0.04 d	4.22 ± 0.24 e	0.87 ± 0.06 a,e	tr.	1.19 ± 0.10 a,d	0.39 ± 0.04 b,e	0.23 ± 0.02 b,e	0.96 ± 0.15 a	0.37 ± 0.09 b,e	3.38 ± 0.03 c
Germacrene B	1548	1559	1.51 ± 0.06 a	1.28 ± 0.10 a	1.34 ± 0.06 a	0.62 ± 0.02 b	tr.	tr.	n.d.	n.d.	n.d.	tr.	0.29 ± 0.13 b,c	0.12 ± 0.12 c
Hexadecane	1600	1600	0.72 ± 0.00 a	3.10 ± 0.34 b	1.47 ± 0.1 c	4.04 ± 0.09 d	0.50 ± 0.1 a	tr.	0.69 ± 0.07 a	0.28 ± 0.01 a	tr.	0.68 ± 0.13 a	0.17 ± 0.03 a	3.00 ± 0.16 b
Octadecane	1800	1800	0.33 ± 0.03 a,b,c	1.57 ± 0.25 d	0.72 ± 0.01 a,e	2.06 ± 0.04 f	tr.	n.d.	0.13 ± 0.01 b,g	tr.	tr.	0.38 ± 0.07 c,h	n.d.	1.75 ± 0.37 i
Monoterpenes			92.70	81.20	89.92	84.31	90.43	93.24	89.66	96.26	96.55	94.59	94.42	82.67
Monoterpenoides			2.47	6.10	3.12	3.68	5.52	2.94	5.11	1.72	2.33	1.56	1.91	5.20
Sesquiterpenes			1.51	1.28	1.34	0.62	0.00	0.00	0.00	0.00	0.00	2.03	0.55	8.13
Aldehydes			0.52	0.91	0.56	0.48	0.45	0.58	0.28	0.15	0.19	0.38	0.17	0.48
Cyclic ketones			0.00	0.63	0.00	0.08	1.23	1.55	0.87	0.00	0.00	0.00	0.00	0.00
Saturated hydrocarbons			1.73	7.86	3.76	10.33	1.37	0.19	2.02	0.67	0.23	2.03	0.55	8.13
Total chromatogram identified (%)			98.93	97.98	98.70	99.49	98.99	98.50	97.94	98.80	99.30	98.57	97.35	96.60

RI—retention indices on the SH-5MS column, RI_L—retention indices from the literature, n.d.—not detected, tr.—traces (<0.10%). Identified compounds (in the amount > 1% of chromatogram peak area for at least one month; entire data can be accessed in Table S1) are expressed as mean ± SD. Different letters (a–j) denote a statistically significant difference ($p < 0.05$).

Figure 1. Graphical presentation of changes in (**a**) the most abundant volatile compounds from sea fennel leaves essential oil (**b**) the most abundant phenolic and carotenoid compounds from sea fennel leaves during a 1-year period in dependence of weather condition.

This cyclic monoterpene was most abundant in August (84.53%) and least abundant in January (58.74%), which was confirmed by a statistically significant difference (Table 1). January was the only month that showed a significant difference compared to all other months. The amount of limonene correlated with the average temperature level (r = 0.694, $p = 0.025$), as did the amount of α-pinene (r = 0.641, $p = 0.025$) and terpinen-4-ol (r = 0.647, $p = 0.023$), while germacrene B (r = −0.770, $p = 0.004$), hexadecane (r = −0.602, $p = 0.038$), and octadecane (r = −0.627, $p = 0.029$) were also correlated, but negatively (Table 2). Limonene

is one of the most common terpenes found in nature, with a pleasant lemon-like odor and is therefore often used as a flavoring and fragrance in food and cosmetics. Apart from its flavoring properties, it has been extensively studied for its other beneficial biological properties, namely its anti-inflammatory, antioxidant, antiviral anticancer, antidiabetic, and gastroprotective effects [21].

Table 2. The correlation between climatic parameters and major bioactive compounds from wild-grown sea fennel leaves.

Climatic Parameter		Average Temperature (°C)	Relative Humidity (%)	Average Wind Strength	Relative Cloud Cover (1/10)
			Essential oils		
Compound	α-Pinene	**r = −0.641** **p= 0.025**	r = 0.097 p = 0.765	r = 0.508 p = 0.092	r = 0.255 p = 0.425
	Limonene	**r = 0.694** **p = 0.012**	r = −0.318 p = 0.314	r = −0.299 p = 0.346	r = −0.242 p = 0.448
	Terpinen-4-ol	**r = 0.647** **p = 0.023**	r = −0.300 p = 0.344	r = −0.150 p = 0.642	r = −0.390 p = 0.210
	α-Terpineol	r = −0.277 p = 0.384	**r = 0.623** **p = 0.030**	r = −0.135 p = 0.677	r = 0.191 p = 0.553
	Germacrene B	**r = −0.770** **p = 0.004**	r = 0.060 p = 0.854	**r = 0.593** **p = 0.042**	r = 0.097 p = 0.765
	Hexadecane	**r = −0.602** **p = 0.038**	r = 0.240 p = 0.453	r = 0.195 p = 0.543	r = 0.199 p = 0.536
	Octadecane	**r = −0.627** **p = 0.029**	r = 0.312 p = 0.323	r = 0.179 p = 0.578	r = 0.240 p = 0.453
			Phenolics		
	Chlorogenic acid	**r = 0.794** **p = 0.002**	r = −0.497 p = 0.101	r = −0.523 p = 0.081	r = −0.330 p = 0.296
	Criptochlorogenic	**r = 0.887** **p = 0.0001**	**r = −0.623** **p = 0.030**	r = −0.388 p = 0.212	**r = −0.617** **p = 0.032**
			Carotenoids		
	Lutein	r = 0.249 p = 0.436	**r = 0.588** **p = 0.044**	r = −0.306 p = 0.333	r = 0.375 p = 0.229

r = Pearson's correlation coefficient. Correlation was considered significant at level of $p \leq 0.05$ (in bold) and presented only if at least one tested parameter was significantly correlated (entire data can be accessed in Table S2). Data for EO were calculated only for compounds in the amount greater than 1%.

(*Z*)-β-Ocimene (11.65%) and γ-terpinene (2.28%) also differed significantly from all other months in January, while they were the most abundant compared to the other months. Sabinene (11.38%, September) and α-pinene (8.14%, January), in addition to the others mentioned above, contributed significantly to the high percentage of monoterpenes, which was highest in October and lowest in February (Figure 1, Table 1). Limonene (58.37%), sabinene (26.46%), terpinen-4-ol (5.59%), and γ-terpinene (2.81%) dominated in the EO samples of the aerial parts of sea fennel collected in central Dalmatia (Split) in June 2006 [22]. Politeo et al. [15] analyzed EO samples of sea fennel leaves from the same location collected in September 2022, and the results showed differences compared to the abovementioned study by Kulišić-Bilušić [22]. Sabinene (51.47%) was the most abundant compound followed by limonene (36.28%), terpinene-4-ol (5.35%), and γ-terpinene (3.49%). Since there was no influence of geographical origin, these differences in chemical profile were likely due to climatic differences. Ozcan et al. [23] compared EO of sea fennel from different locations in Turkey collected at different times. Sabinene, limonene, (*Z*)-β-ocimene, γ-terpinene, and *p*-cymene were the most abundant terpenes in the EOs they analyzed, but they found

variations due to location and climatic factors. Pavela et al. [24] also found differences in the chemical composition of the EO of sea fennel when they compared the samples from central and southern Italy. Limonene and γ-terpinene dominated in the sample from central Italy, while thymol methyl ether and γ-terpinene dominated in the sample from Sicily, suggesting that geographical origin and climatic differences could influence the differences in chemotype.

Germacrene B was the only sesquiterpene identified and was detected only in trace amounts or not at all in the period from May to October (Table 1), which was confirmed by negative correlations with average temperature levels (Table 2). Similarly, the only positive correlation with the average wind strength was found for germacrene B level (r = 0.593, $p = 0.042$).

In addition to GC-MS EO analysis, HPLC-UV/Vis analysis of phenolic acids present in the sea fennel leaf samples collected from the same area at various phenophases in a period of 12 months were performed (Table 3).

Our focus was on the quantitative analysis of major phenolic acids present in sea fennel, as follows: chlorogenic acid, neochlorogenic acid, and criptochlorogenic acid to study differences in the vegetation stages of sea fennel and the accumulation of biologically active phenolics in the plants. These phenolic acids were analyzed due to their highest concentrations in Croatian sea fennel samples, as previously reported in our studies [15,16,18,20]. Furthermore, the level of total phenolics, flavonoids, and tannins was also previously determined, as well as the assessment of antioxidant activity using FRAP and DPPH assays. The results indicate that sea fennel leaves represent a promising reservoir of phenolic antioxidant compounds [16,20]. The chlorogenic acid was the dominant phenolic acid in all 12 months, followed by criptochlorogenic acid and neochlorogenic acid.

According to the obtained results, higher concentrations of chlorogenic acid were found in the warmer months, from March to October, peaking in June (60.59 mg/g), with a statistically significant difference from all the other months. Conversely, lower concentrations were noted in colder months (late autumn and winter), starting in November, when the lowest concentration was observed (0.46 mg/g), which was also statistically different from all other months. The results of the Pearson correlation statistics (Table 2) support this finding; chlorogenic acid was positively correlated with the average temperature (r = 0.794, $p = 0.002$).

Chlorogenic acid, a phenolic compound found in various plant species, is known for its multiple positive biological activities and health-beneficial effects, which is why it has significant potential for a variety of industrial applications [25,26]. Chlorogenic acid, together with its isomers and higher derivatives, has also been identified as the predominant class of phenolics in sea fennel infusions [27]. Meot and Magne [26] conducted a one-year study on *Crithmum maritum* (L.), in which they quantified chlorogenic acid in sea fennel leaves from four different vegetation periods at two sampling sites. They demonstrated that chlorogenic acid accumulation was greater in samples collected under cliffs than in samples collected on sandhills, suggesting that the specimens are exposed to greater stress at these sites. The results of our recently published study on sea fennel collected along the coast showed slightly higher concentrations of chlorogenic acid compared to the results obtained at both locations in the study by Meot and Magne [20,26]. The results of our study are consistent with the findings of several authors and with the available data on the cultivation of sea fennel in various geographical regions. For example, concentrations of chlorogenic acid in leaves sampled from different areas were as follows: in Algeria, 0.64 g/100 g DW; in Croatia, ranging from 0.56 to 1.63 g/100 g DW; in France, 0.73 g/100 g DW; and in Italy, ranging from 0.81 to 1.19 g/100 g DW [28–31].

Table 3. Concentrations of phenolic and carotenoid compounds in sea fennel leaves throughout a 1-year period.

Compound	January	February	March	April	May	June	July	August	September	October	November	December
Phenolics (mg/g)												
Neochlorogenic acid	0.5 ± 0.00 a	0.05 ± 0.00 b	0.23 ± 0.00 c	0.05 ± 0.04 b	0.25 ± 0.00 c	0.98 ± 0.00 d	2.84 ± 0.00 e	3.52 ± 0.01 f	5.05 ± 0.00 g	1.33 ± 0.01 h	0.05 ± 0.00 a	0.23 ± 0.00 c
Chlorogenic acid	0.51 ± 0.01 a	1.8 ± 0.02 b	28.89 ± 0.02 c	16.68 ± 0.03 d	52.13 ± 0.04 e	60.59 ± 0.09 f	49.66 ± 0.02 e	33.84 ± 0.06 f	30.43 ± 0.05 c	20.94 ± 0.00 g	0.46 ± 0.02 h	3.74 ± 0.01 i
Criptochlorogenic acid	0.06 ± 0.00 a	0.10 ± 0.00 b	0.72 ± 0.00 c	0.22 ± 0.01 d	0.67 ± 0.06 c	2.52 ± 0.04 e	5.14 ± 0.00 f,g	4.74 ± 0.07 f	5.90 ± 0.00 g	1.86 ± 0.06 h	0.09 ± 0.00 b	0.37 ± 0.00 i
Carotenoids (mg/kg)												
Neoxantin	8.72 ± 0.14 a	n.d.	n.d.	n.d.	4.15 ± 0.18 b	1.95 ± 0.14 c,d	1.88 ± 0.02 c,e	2.18 ± 0.09 d,e	7.63 ± 0.05 a	5.64 ± 0.05 f	8.27 ± 0.01 a	n.d.
Violaxanthin	4.22 ± 0.06 a	13.64 ± 0.05 b	14.30 ± 0.82 b	n.d.	n.d.	4.85 ± 0.03 a	n.d.	n.d.	n.d.	15.07 ± 0.04 b	5.44 ± 0.88 a	5.17 ± 0.16 a
Lutein	90.77 ± 2.00 a,b,c	n.d.	3.13 ± 0.12 d	5.65 ± 0.35 e	125.63 ± 0.01 f	85.90 ± 0.07 a	55.60 ± 1.12 g	108.54 ± 0.54 h	98.45 ± 0.56 b,h	99.52 ± 1.23 c,h	74.58 ± 0.20 i	182.48 ± 0.13 j
β-Carotene	52.89 ± 1.33 a	24.76 ± 1.29 b	72.51 ± 0.62 c	103.03 ± 0.29 d	111.01 ± 0.41 d,e	88.62 ± 2.395 f	34.87 ± 0.53 g	173.79 ± 1.99 h	118.00 ± 2.41 e	67.17 ± 0.21 c	76.94 ± 2.02 c,i	85.59 ± 1.21 f,i

Concentrations were expressed as mean ± SD in mg of compound per g or kg of dry plant. Different letters (a–j) denote statistically significant difference ($p < 0.05$).

The highest concentrations of neochlorogenic acid and criptochlorogenic acid were recorded in September, reaching 5.05 mg/g and 5.9 mg/g, respectively. The level of neochlorogenic acid from September was statistically different from all other months. Criptochlorogenic acid also differed significantly from all other months, with the exception of July. It also showed a high positive Pearson correlation with average temperature and a negative correlation with relative humidity and relative cloud cover ($r = 0.887$, $p = 0.0001$; $r = -0.623$, $p = 0.030$ and $r = -0.617$, $p = 0.032$, respectively), while neochlorogenic acid did not correlate with any of the weather parameters tested.

In the colder months, from November to April, a notable decrease in the concentrations of these compounds is observed, demonstrating the influence of seasonal variations in the metabolite abundance. Sea fennel contains isomers of chlorogenic acid (CGA), among which chlorogenic, cryptochlorogenic, and neochlorogenic acids stand out as prominent compounds [12], which is consistent with the results of our study. However, in Greek sea fennel genotypes, the CGA isomer 5-coumaroyl-quinic acid surpasses chlorogenic acid in frequency [32]. Such results point us to botanical diversity, which enriches our understanding of the chemical composition of sea fennel and highlights the importance of geographical factors in shaping plant metabolite profiles. Lower concentrations of phenolics can also be explained by the fact that plants undergo a period of dormancy or reduced growth during a period of late autumn and winter (colder) months. Additionally, halophytes can synthesize secondary metabolites in response to oxidative damage caused by various stressors, primarily due to their antioxidant properties, which are highly valued in human nutrition [33]. During warm weather, halophytes may encounter multiple stressors, such as salinity, temperature fluctuations, and variations in water availability. These stressors, which are prevalent in spring and summer periods, collectively stimulate the biosynthesis of phenolic compounds in halophytes, contributing to the heightened phenolic content observed during these seasons [34].

Three xanthophylls (neoxanthin, violaxanthin, and lutein) and one carotene (β-carotene) were quantified in sea fennel leaf samples collected throughout the year using UHPLC-UV/Vis-DAD. In all samples analyzed, the predominant compound was lutein, followed by β-carotene. Violaxanthin and neoxanthin were also detected, but in significantly lower concentrations, or they were absent in some of them. According to our results, the highest concentrations of the measured carotenoids were observed from August to January. Specifically, the maximum concentration of lutein was recorded in December (182.48 mg/kg), β-carotene in August (173.79 mg/kg), violaxanthin in October (15.07 mg/kg), and neoxanthin in January (8.72 mg/kg). Lutein and β-carotene were also statistically different from all the other months analyzed in the given period. Carotenoid levels did not correlate with temperature for several EO compounds and phenolics. The only mild correlation was observed for relative humidity and lutein levels. Violaxanthin was not detected between April and October (except in June), with a decrease in concentration observed in November and December, followed by irregular growth. Neoxanthin was also not detected between February and April, as well as in December. In December, lutein reaches its maximum concentration, which then decreases drastically during winter months, with the minimum concentration measured being 3.13 mg/kg. β-Carotene peaks in August, following a significant drop in concentration in February (24.76 mg/kg).

To our knowledge, information on the concentration of individual carotenoids is quite limited. Nartea et al. [35] reported on the carotenoid contents in Italian wild and cultivated sea fennel leaf samples, in which the concentration of neoxanthin ranged from 6.12 to 21.29 mg/kg, violaxanthin from 2.35 to 41.04 mg/kg, lutein from 102.54 to 190.89 mg/kg, and β-carotene from 26.59 to 111.05 mg/kg, which is consistent with our findings.

Carotenoids not only play a crucial role in the physiological processes of plants and the functional dissipation of excess solar energy for photosynthesis but also contribute significantly to the overall appeal of edible fruit and vegetables. They are responsible for imparting the vibrant colors of these foods and serve as precursors of many important volatile flavor compounds [36]. In addition, carotenoids possess antioxidant properties,

and some of them (such as β-carotene) are provitamin A components, so their consumption reduces the risk of various cancer types, as well as ophthalmological or cardiovascular diseases [37].

Neoxanthin, lutein, violaxanthin, zeaxanthin, and antheraxanthin are amongst the plant carotenoids that are ubiquitously present in various plants. Their biosynthesis is dynamically regulated throughout the entire lifecycle of the plant and subject to control from various aspects, including developmental stages, environmental influences, and epigenetic regulation [38]. The changes in the carotenoid composition of sea fennel throughout the year can be explained by variations in environmental conditions and by different developmental stages of sea fennel. Specifically, light conditions, including radiation levels and photoperiod, vary between the different monthly harvest periods; spring and summer months are characterized by longer photoperiods and higher light intensity, while autumn and winter months are characterized by shorter photoperiods and lower light intensity. Considering the functional role of carotenoids in plants, the increased content observed in our study could also be due potential stress, especially environmental conditions, in which various stressor for the plants occur.

Principal component analysis (Figure 2) was performed to describe and summarize the variations between the different compound classes (EO compounds in the amount > 1%), phenolics and carotenoids over a 12-month period.

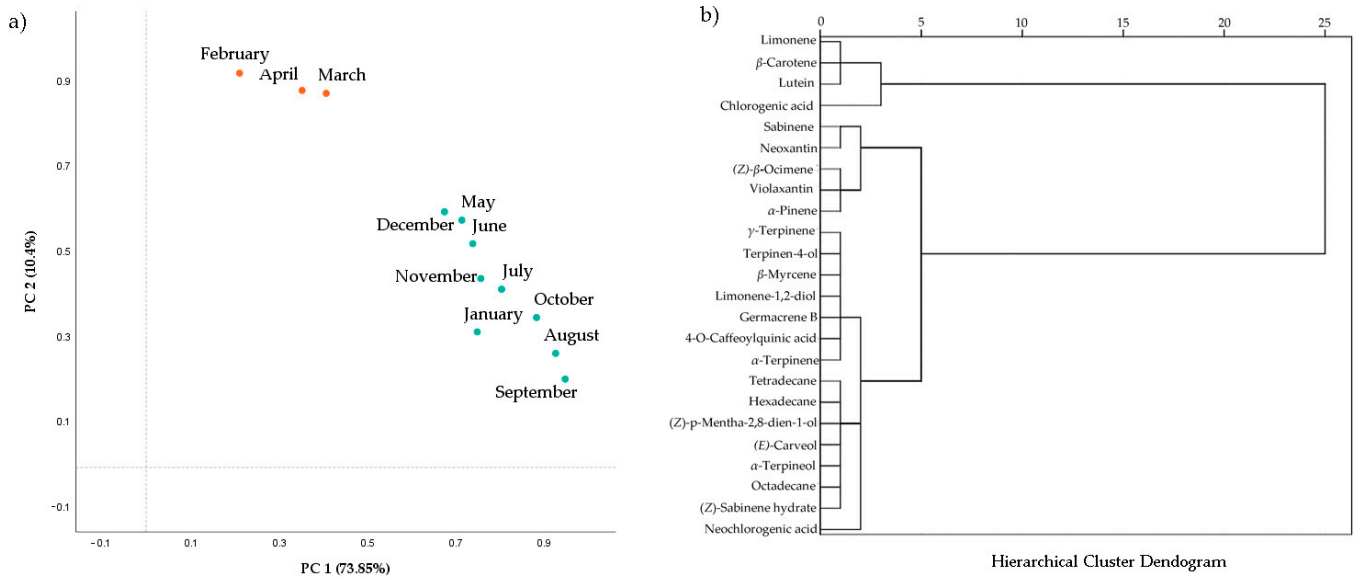

Figure 2. Principal component analysis (**a**) and hierarchical cluster analysis (**b**) of the most abundant compounds from sea fennel leaves during a year period.

The first two PCAs describe 84.25% of the variance and differentiate sea fennel samples from February to April in one and all other months (May–January) in the other. The most dominant sea fennel compounds were also determined as factors with the largest contribution to the differentiation of these groups. Limonene from the EO compounds, as well as lutein and β-carotene from carotenoids, had the largest contribution to variance. Limonene levels were quite constant from February to April (71.00–74.19%) and differed significantly from most other months (May–August, November, and January). β-Carotene levels were highly variable in each of these months (from 24.76 in February to 103.03 mg/kg in April) but differed significantly from most other sampling periods in a year. Lutein levels were lowest in the given months; in February, lutein was not even detected, while in March and April, it was found in very low amounts (3.13 and 5.56 mg/kg, respectively) when compared to all other months (from 55.60 in July until 182.48 mg/kg in December).

Hierarchical cluster analysis revealed two major clusters. The first cluster was composed of limonene, β-carotene, lutein, and chlorogenic acid, which are the most abundant

compounds overall in sea fennel leaves throughout a period of a year. The second major cluster was consistent with two smaller clusters, with sabinene, neoxanthin, (*Z*)-β-ocimene, violaxanthin, and α-pinene contributing to first one and 15 remaining compounds, with the smallest abundance amongst the entire year, contributing the second cluster.

Whereas most of the major bioactive compounds are drastically reduced in winter/early springtime period, β-carotene levels, excluding the months of February and July (when the flowering stage of sea fennel begins), remain mostly unaffected by the vegetation phase and weather conditions, making sea fennel a valuable nutritional source, even in its phytochemical least abundant period.

4. Conclusions

The observation of the major bioactive sea fennel phytochemicals over a period of 12 months from the wild-grown population revealed great variability between the compounds studied. Variations were noted in both the differences in essential oil profiles, as well as in the levels of phenolics and carotenoids. The highest accumulation of most bioactive compounds was observed in early summer before the plant reached its flowering stage. The PCA analysis distinguished the period from February to April, which contained the least bioactive phytochemicals overall. Furthermore, the analysis indicated a significant influence of temperature on several major bioactive metabolites.

Our findings show that certain amounts of bioactive phytochemicals can be accessed through the entire year, with vegetation and environmental conditions having the least effect on β-carotene level. From this, we conclude that the plant can be used as a source of valuable and functional components beyond its typical harvest period (early to midsummer).

The amount of different beneficial phytochemicals in sea fennel implies that the crop has great potential for use in various industries, especially in the food, cosmetic, or pharmaceutical industries. The fact that sea fennel is an emerging crop suggests that the plant will be increasingly used in its natural habitat, which could lead to a disruption of natural homeostasis. Therefore, the sustainable agricultural production of this crop is a good way to take advantage of its potential.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app14083440/s1: Table S1: Chemical composition of volatile compounds identified in sea fennel leaf essential oils throughout a 1-year period, Table S2: Pearson correlations for weather conditions and sea fennel phytochemicals, Figure S1: Representative chromatograms of sea fennel leaves (a) GC-MS total ion chromatogram of essential oils, (b) HPLC chromatogram of phenolics at 220 nm (upper) and 320 nm (lower) line, (c) UHPLC chromatogram of carotenoids at lutein, neoxanthin, and violaxanthin at 415, 425, and 450 nm and β-carotene at 450 nm.

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