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Vasiliki Zafeiropoulou
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece

Ekaterina-Michaela Tomou
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece

Ourania Ioannidou
Department of Pharmacognosy-Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece

Anastasia Karioti
Department of Pharmacognosy-Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece

Helen Skaltsa
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece

Corresponding Author:
Helen Skaltsa
Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece

Sea fennel: Phytochemical analysis of Greek wild and cultivated *Crithmum maritimum* L. populations, based on HPLC-PDA-MS and NMR methods

Vasiliki Zafeiropoulou, Ekaterina-Michaela Tomou, Ourania Ioannidou, Anastasia Karioti and Helen Skaltsa

Abstract
Sea fennel (*Crithmum maritimum* L.; Apiaceae) consists a major component of the Mediterranean diet and is considered as a functional food. Accumulating studies described its various pharmacological studies. In the last few years, there is an effort of cultivating this important medicinal species for uses in traditional medicine, in food and in cosmetic/pharmaceutic industries. Therefore, the identification of the chemical profile of the cultivated species compared to the wild populations is an urgent need, in order to standardize high quality end-products. As a result, the present study focuses on the analysis of the extracts from wild and cultivated *C. maritimum* populations, characterizing the extracts qualitatively and quantitatively by HPLC-PDA-MS. Based on NMR spectroscopy, the methanol and the cyclohexane extracts were further investigated, leading to the isolation and identification of ten compounds. Our results are congruous with previous studies, mentioning the presence of well-known antioxidants in plant foods of Mediterranean diet.

Keywords: Wild and cultivated *Crithmum maritimum*, sea fennel, falcarindiol, quinic acid derivatives, NMR

1. Introduction
Nowadays, the Mediterranean Diet (MD) is ubiquitously recognized as one of the best dietary patterns for health benefits, characterized by high consumption of natural products (i.e. vegetables, fruits, nuts and legumes) [1, 2]. Sea fennel (*Crithmum maritimum* L.; Apiaceae) is considered as a functional food and an important nutrient of the MD and Greek diet, being usually consumed fresh, pickled or as additive in salad [3, 4]. *C. maritimum* only species of the genus, commonly known as sea fennel or rock samphire, is a facultative halophyte growing on maritime cliffs and sometimes in sand along the Mediterranean and Black sea coasts [3]. Its organs (roots, leaves and fruits) are rich in several bioactive substances that could be used as aromatic, medicinal, antimicrobial and insecticide products and therefore it shows substantial economical and medicinal potentials [4]. Therefore, cultivation efforts have occurred around the world, focusing on increasing the nutritional value of the plant for uses in food, cosmetic and pharmaceutic industries [5-7]. Sea fennel's properties have already been known since antiquity. Precisely, its significant benefits have been mentioned in Plinius' Historia Naturalis and in Corpus Hippocraticum [3]. In late Byzantine era, *C. maritimum* was included in one recipe of 'Nikolaos Myrepsos' medical manuscript "Dynameron", under the name crithmon [8].
In the context of the Ethnopharmacology, *C. maritimum* has been used in various traditional medicines around the Mediterranean basin. The most interesting are considered the antiscorbutic, anti-inflammatory, diuretic, depurative, carminative, digestive properties and its use as therapeutic agent against common cold and wounds [10-14]. Previous few phytochemical studies have revealed the rich chemical profile of the specific halophyte, including vitamin C, fatty acids, carotenoids, polyacetylenes, flavonoids, tannins, phenolic acids and essential oil [4, 6, 11, 13, 15, 16-18]. Among them, many studies underlie the high amount of phenolic acids in this species, mainly of quinic acid derivatives, assigning the great antioxidant activity of *C. maritimum* [4, 18]. However, other additional pharmacological properties have been reported from different extracts of the plant, describing its antimicrobial, anti-inflammatory, vasodilatory and diuretic effects [4, 11-12, 15, 18-20]. In continuing our studies on Greek medicinal and aromatic plants, we now report on the investigation of *C. maritimum*. In particular, the present study focuses on the analysis of the extracts from wild and cultivated *C. maritimum* populations, aiming to characterize the extracts qualitatively and quantitatively.

Since the plant is used as food, the traditional food preparation (brine sea fennel) was also analyzed. The non-polar and polar extracts of wild population were studied by NMR spectroscopy, targeting the isolation of polyacetylenes and flavonoids.

2. Materials and Methods

2.1. Plant materials

Aerial parts from wild *Crithmum maritimum* L. population were collected in June 2017 from maritime cliffs in a small coastal town, Parga (W. Greece-Ionian Sea).

Aerial parts from cultivated *Crithmum maritimum* L. population were collected in June 2017 from Thessaloniki (N. Greece). The plant materials were authenticated by Associate Prof. Th. Constantinidis (Department of Ecology & Systematics, Faculty of Biology, NKUA); a voucher specimen of the wild population was deposited to a personal Herbarium of the Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, NKUA, (Voucher Specimen Number: Zafeiropoulou & Skaltsa 001).

End product of boiled samphire deserved at low brine with a little vinegar (brined sea fennel) was provided by the market.

2.2. General experimental procedures of isolation and identification of the secondary metabolites

^1H, ^{13}C and 2D NMR spectra were recorded in $CDCl_3$ and CD_3OD on Bruker DRX 400 and Bruker AC 200 (50.3 MHz for ^{13}C NMR) instruments at 295 K. Chemical shifts are given in ppm (δ) and were referenced to the solvent signals at 7.24/3.31 and 77.0/49.0 ppm for ^1H and ^{13}C NMR, respectively. COSY (COrrelation Spectroscop Y), HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation) and NOESY (Nuclear Overhauser Effect Spectroscop Y) (mixing time 950 ms) experiments were performed using standard Bruker microprograms. The $[\alpha]_D$ values were obtained in $CHCl_3$ or CH_3OH on Perkin Elmer 341 Polarimeter. UV–Vis spectra were recorded on a Shimadzu UV-160A spectrophotometer, according to Mabry *et al*. (1970) [21]. VLC (Vacuum Liquid Chromatography): silica gel 60H (Merck, Art. 7736).22 CC (Column Chromatography): silica gel (Merck, Art. 9385), Sephadex LH 20 (Pharmacia). All the continuously fractionations were always monitored by TLC silica gel 60 F-254, (Merck, Art. 5554) and cellulose (Merck, Art. 5552) with visualization under UV (254 and 365 nm) and spraying with vanillin-sulphuric acid reagent and with Neu's reagent [23] for phenolic compounds. In the whole process, all the obtained fractions, and isolated compounds were evaporated to dryness in vacuum under low temperature and then were put in activated desiccators with P_2O_5 until their weights had stabilized, in order to eliminate the moisture from the samples that might influence pre-saturation performance and then lead to an intense water signal in the ^1H-NMR spectra, making it difficult to observe near signals.

2.3. Extraction and isolation

At a first step, air-dried powdered plant material (0.2 kg) from the wild population was extracted at room temperature with cyclohexane, dichloromethane, methanol and methanol: water (5:1), successively and concentrated to dryness to yield residues of 3.5 g, 1.0 g, 44.5 g and 21.9 g, respectively. Then, the same extraction procedure was followed for the air-dried powdered plant material (0.2 kg) from the cultivated population, affording residues of 4.0 g, 2.5 g, 35.0 g and 20.0 g, respectively.

The market end product of boiled samphire (brined sea fennel) was provided (Larissa, Greece) was filtered to eliminate the brine and the remained plant material was triturated and then extracted with n-butanol. Subsequently, the extract was concentrated to dryness to yield residue of 3.0 g.

Afterwards, a part of the cyclohexane extract (2.0 g) was submitted to CC over silica gel (CyHex:EtOAc:MeOH 100:0:0 to 0:0:100) and afforded 18 fractions. Fractions F (eluted with CyHex:EtOAc 90:10) and N (eluted with CyHex:EtOAc 70:30) were identified as compounds 2 (128.3 mg) and 3 (43.0 mg), respectively. Fraction D (165.0 mg) was further subjected to CC over silica gel (CyHex:DM:EtOAc 100:0:0 to 0:100:0) and yielded compound 1 (40.6 mg).

A part of the methanol extract (6.0 g) was pre-fractionated by VLC over silica gel (10.0 x 3.0 cm), using as eluent mixtures of increasing polarity (dichloromethane: methanol: water) to yield finally six fractions of 500 mL (A-F). Fraction C (227.4 mg; eluted with DM:MeOH:H_2O 70:30:3) was subjected to CC Sephadex LH 20 (MeOH) and yielded a mixture of compounds 4 and 5 (1.4 mg). Fraction D (1.8 g; eluted with DM:MeOH:H_2O 50:50:5) was further fractionated by VLC over silica gel (10.0 x 3.0 cm), using as eluent mixtures of increasing polarity (Dichloromethane: methanol: water) to yield finally eleven fractions of 500 mL (A-K). Sub-fraction F (198.7 mg; eluted with (DM:MeOH:H_2O 70:30:3) was subjected to CC Sephadex LH 20 (MeOH) and gave compounds 7 (3.8 mg) and 10 (4.2 mg). Combined sub-fractions F to N (45.3 mg; eluted with 100% MeOH) were submitted to CC over silica gel (DM:MeOH:H_2O 100:0: to 0:100:0) and afforded compounds 6 (25.0 mg) and 10 (4.7 mg). Sub-fraction G was subjected to CC Sephadex LH 20 (MeOH) and was further purified by CC over silica gel (DM:MeOH:H_2O 100:0: to 0:100:0) and gave compounds 9 (22.1 mg) and 8 (5.1 mg).

2.4. HPLC-PDA-MS analyses

2.4.1. Chemicals

All solvents used were HPLC grade; CH_3CN, MeOH and formic acid (85% $_{V/V}$) for HPLC were purchased from Sigma Aldrich. Water was purified by a Milli-Qplus system from Millipore (Milford, MA, USA). All laboratory chemicals used in this study were of reagent grade.

2.4.2. Standards

For the quantitative analysis, chlorogenic acid was purchased from Extrasynthèse. For the qualitative analysis, rutin was purchased from Sigma Aldrich, while 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic esters were previously isolated from *Stachys recta* [24].

2.4.3. HPLC-PDA-MS apparatus

ESIMS and UV (PDA) spectra were recorded on an LC-DAD-MS Thermo Finnigan system (LC Pump Plus, Autosampler, Surveyor PDA Plus Detector) interfaced with an ESI MSQ Plus (Thermo Finnigan) and equipped with an Xcalibur software. The same column, time period and flow rate were used during the HPLC–MS analyses. The mass spectrometer operated in both negative and positive ionization modes, scan spectra were from m/z 100 to 800, gas temperature was at 350 ºC, nitrogen flow rate at 10 L/min, and capillary voltage 3500V. For negative ion mode the cone voltage was 100 V, whereas in positive 80V. The column was a Zorbax SB-Aq RP-C_{18} column (150mm x 3mm) with a particle size of 5 μm (Agilent) maintained at 30 °C. The eluents were H_2O at pH 2.8 by formic acid (0.05% v/v) (A)

and acetonitrile (B) and with a flow rate of 0.4 mL/min. Samples were analyzed using a gradient program as follows: 0-5 min, 90% to 85% A; 5-13 min, 85-22%A; 13-18min 22%A; 18-20, 22-25%A; 20-23 min, 25%A; 23-27 min, 25-40%A; 27-30 min, 40-10%A; 30-35 min, 10%A. Injected volume of the samples was 5 µL of solution. The UV–vis spectra were recorded between 220 and 600 nm and the chromatographic profiles were registered at 250, 280 and 330 nm.

2.4.4. Identification of peaks and peak purity

Identification of all constituents was performed by HPLC–PDA and MS analysis by comparing the retention time, the UV and MS spectra of the peaks in the samples with those of authentic reference samples or isolated compounds and in some cases, data reported in the literature. The purity of the peaks was checked by a Photodiode Array Detector coupled to the HPLC system, comparing the UV spectra of each peak with those of authentic references samples and/or by examination of the MS spectra.

2.4.5. Quantitative determination of constituents

The method of external standard was applied to quantify the caffeoylquinic acids. Quantification was performed using a regression curve, each point in triplicate. Measurements were performed at 330 nm and the linearity range of responses of the standard was determined on five concentration levels with three injections for each level. Calibration graphs for HPLC were recorded with sample amounts ranging from 9 x 10-3 µg and 0.14 µg: stock solutions of the standards were prepared at different concentrations ranging from 0.0046 mg/ml to 0.023 mg/ml and injected into HPLC (injection volumes varying from 2 to 10 µl).

3. Results and discussion

3.1. HPLC-PDA-MS apparatus

The constituents were identified by UV and MS spectral data. The qualitative profiles of the samples were quite similar (Table 2). In Figure 1 are presented the HPLC-PDA chromatogram of the hydroalcoholic extract of *C. maritimum* from Parga. Data concerning identification of the peaks are shown in Table 1, where the retention time, UV-vis absorptions and electrospray ionization mass spectrometry in both positive and negative ion mode of the compounds detected are reported. The developed analytical system led to the separation and identification of the constituents. Overall, 12 compounds were identified, belonging mainly to quinic acid derivatives. A few flavonoids were also detected. Negative ionization mode at voltage of 100 eV gave the best results, for the identification of both the acylated quinic acid derivatives and flavonoid glycosides, while positive ionization at 80 eV led to the formation of the $[M+Na]^+$ pseudomolecular ion in most of the cases.

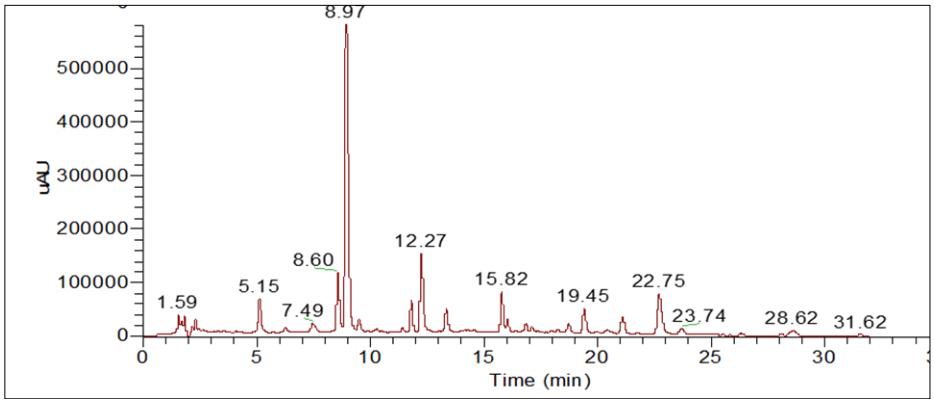

Fig 1: Example of an HPLC-PDA chromatogram of the *Crithmum maritimum* (CRIPMW) hydroalcoholic extract.

Table 1: Positive and negative MS fragmentation and Uv-vis absorption data of the compounds detected in the extracts of *Crithmum maritimum*.

No	Rt	UV (nm)	Negative	Positive	Identification
1	5.2	297, 325	178.9, 190.9, 353.1 $[M-H]^-$	163.0, 354.9 $[M+H]^+$, 376.9 $[M+Na]^+$	1- or 3-Caffeoylquinic acid
2	8.6	298, 325	173.1, 191.1, 353.1 $[M-H]^-$	163.1, 354.9 $[M+H]^+$, 377.1 $[M+Na]^+$	4-Caffeoylquinic acid
3	9.0	298, 325	191.1, 353.1 $[M-H]^-$ 706.9 $[2M-H]^-$	355.0 $[M+H]^+$, 377.1 $[M+Na]^+$	chlorogenic acid (5CQA)
4	9.4	298, 318	179.1, 191.1, 353.1 $[M-H]^-$	-	cis-chlorogenic acid
5	11.9	270, 334	353.1, 472.9, 593.0 $[M-H]^-$	595.1 $[M+H]^+$, 617.1 $[M+Na]^+$	Vicenin-2
6	12.3	311	191.1, 337.0 $[M-H]^-$, 675.1 $[2M-H]^-$	338.9 $[M+H]^+$, 361.1 $[M+Na]^+$, 699.1 $[2M+Na]^+$	p-coumaroyl quinic acid
7	13.3	297, 325	191.1, 367.1 $[M-H]^-$	369.1 $[M+H]^+$, 391.1 $[M+Na]^+$, 759.1 $[2M+Na]^+$	Feruloyl-quinic acid
8	15.8	255, 265sh, 353	300.9, 608.9 $[M-H]^-$	303.1 $[M+H]^+$, 611.1 $[M+Na]^+$, 633.1 $[2M+Na]^+$	quercetin-3-O-rutinoside
9	15.9	255, 265sh, 350	300.9, 608.9 $[M-H]^-$	303.1 $[M+H]^+$, 611.1 $[M+Na]^+$, 633.1 $[2M+Na]^+$	quercetin-3-O-robinoside
10	18.8	255, 265sh, 346	301.1, 463.0 $[M-H]^-$	303.1 $[M+H]^+$, 487.1 $[M+Na]^+$	quercetin-3-O-glucoside
11	19.5	297, 325	172.9, 179.1, 353.1, 514.9 $[M-H]^-$	163.1, 499.0 $[M-H_2O+H]^+$, 517.1 $[M+H]^+$, 539.1 $[M+Na]^+$	3,4-dicaffeoyl quinic acid
12	21.2	297, 327	179.1, 191.0, 353.1, 515.1 $[M-H]^-$	163.1, 499.0 $[M-H_2O+H]^+$, 517.1 $[M+H]^+$, 538.9 $[M+Na]^+$	3,5-dicaffeoyl quinic acid
13	22.8	297, 327	161.1, 173.1, 179.1, 191.0, 353.1, 515.1 $[M-H]^-$	163.1, 499.0 $[M-H_2O+H]^+$, 517.0 $[M+H]^+$, 538.9 $[M+Na]^+$	4,5-dicaffeoyl quinic acid
14	23.7	255, 266, 296sh, 332	300.9 $[A-H]^-$, 463 [M-coumaroyl-$H]^-$, 625 $[M-H]^-$	303.1 $[A+H]^+$, 324.9 $[A+Na]^+$, 627.1 $[M+H]^+$, 648.9 $[M+Na]^+$	Quercetin-3-O-p-coumaroylhexoside, tentatively

Table 2: Amounts of chlorogenic acid and total quinic acid derivatives found in the extracts of *Crithmum maritimum.*

Sample	% chlorogenic acid	% total quinic acid derivatives
cricM	3.947 ± 0.030	8.100 ± 0.049
cricMW	4.361 ± 0.022	8.358 ± 0.056
cripM	4.860 ± 0.017	10.988 ± 0.085
cripMW	5.802 ± 0.010	11.236 ± 0.007
Brine Sea fennel	4.639 ± 0.075	7.639 ± 0.038

Mean of three measurements; cricM & cricMW: methanol & methanol -water extracts from cultivated *C.maritimum*, respectively; cripM & cripMW: methanol & methanol -water extracts from wild *C.maritimum*, respectively.

3.2. Identification of constituents by HPLC-PDA-MS

All extracts had similar content and contained mainly acylated quinic acid derivatives with main representative chlorogenic acid (5-caffeoyl quinic acid), which was the predominant phenolic acid. This peak appeared at 9.0 min and was identified by use of the reference standard and of course from the data of phytochemical analyses. The peak at 9.4 min having absorption maxima at only 318 nm was tentatively identified as a cis isomer of chlorogenic acid. Peak 6 at 12.3 min showing pseudomolecular ion $[M-H]^-$ at m/z = 337 and UV maximum at 311 nm was attributed to a coumaroyl quinic acid, without however being able to assign the exact position of the acylation. Similarly, peak 7 at 13.3 min having UV maxima at 297 and 325 nm and quasi-molecular ions $[M-H]^-$ at m/z 367.1 was attributed to a feruloyl quinic acid derivative.

Two further peaks at 19.5 min, 21.2 min and 22.8 min showing quasi-molecular ions $[M-H]^-$ at m/z 515 were assigned to dicaffeoylquinic acid derivatives. Their fragmentation patterns were of diagnostic importance [25]. Apart from typical fragments at m/z 353, 191 and 179, which are common for all quinic derivatives, acylation at position 4 of the quinic acid gives rise to a characteristic signal at m/z 173 $[\text{quinic acid}–H–H_2O]^-$ which is not observed for the other isomers [26]. Therefore, peak 12 was assigned to caffeoylquinic acid with possible substitution on positions [1, 3, 5], whereas peaks 11 and 13 were assigned to dicaffeoyl quinics acid who carry one caffeoyl unit on position 4. Co-chromatography with 3,5- and 4,5- dicaffeoylquinic acids, previously isolated [24] confirmed the identification of peaks 12 and 13 as 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, respectively.

Finally, four more minor peaks at 11.9, 15.8, 15.9 and 19.6 min were attributed to the flavonoids vicenin-2, quercetin-3-O-rutinoside (rutin), quercetin-3-O-robinoside and quercetin-3-O-glucoside on the basis of UV absorption, phytochemical isolations and co-chromatography with reference standards.

3.3. Quantitative analysis

Caffeoyl quinic acids are expressed as chlorogenic acid equivalents and a weight correction factor was used. Results are presented in detail in Table 2. Samples from Parga contained higher amounts of chlorogenic acids and its derivatives.

In all the extracts the main caffeoylquinic acid was chlorogenic acid, whereas the amounts of flavonoids are significantly lower. Therefore, the biological activity documented for the plant should be attributed to chlorogenic acid and its derivatives. What is most important that the traditional food preparation brined sea fennel maintains the qualitative and quantitative characteristics of the fresh plant.

3.4. Structure elucidation of extracts by NMR spectroscopy

Given that the extracts of the wild *C. maritimum* were proven slightly more abundant in total quinic acid derivatives than the samples from cultivated population, they were chosen for further chemical investigation. In total, ten compounds were isolated from the cyclohexane and methanol extract (Figure 2). Based on the literature, falcarindiol consists a major secondary metabolite of the family Apiaceae [27-28]. In order to track the specific compound in our extracts, we applied 1D-NMR spectroscopy in the non-polar extracts of the wild *C. maritimum*. The ^{1}H-NMR of the cyclohexane extract illustrated the proton signals of falcarindiol (Figure 3). Hence, we analysed the aforementioned extract and overall, three non-polar compounds were isolated and identified, corresponding to one fatty aldehyde; octadecanal (1) [29], one polyacetylene; falcarindiol (2) [27] and one monoterpene; O-geranylvanillin (3) [11]. The ^{1}H and ^{13}C-NMR data of falcarindiol are presented (Supplementary data; Table S1 & Figures S1-S5).

Furthermore, to obtain a preliminary phytochemical profile, focusing on the isolation of flavonoids, 1D-NMR was utilized as a rapid method to analyze directly the plant polar extracts. Among them, the methanol extract showed characteristic signals of the groups of flavonoids. Therefore, it was chosen for further fractionations by CC techniques.

As a result, the chemical investigation on the methanol extract of wild *C. maritimum* aerial parts revealed in total seven compounds, including one phenolic acid; E-caffeic acid (4) [30], two quinic acid derivatives; chlorogenic acid (5) [31] and 3,4-dicaffeoyl quinic acid (6) [32] and four flavonoids; quercetin-3-O-glucoside (7) [33], quercetin-3-O-galactoside (8) [33], quercetin-3-O-rutinoside (9) [34], quercetin-3-O-robinoside (10) [35]. The structure elucidation of all the isolated compounds was undertaken by NMR spectroscopy and their spectroscopic data were compared to those previously formerly published.

Although compound 1 belongs to the fatty acid derivatives and it is a common metabolite of many plants, it has not been isolated from *C. maritimum*, previously. As it has been already mentioned, compound 2 is a characteristic component of the plants of Apiaceae family and our study confirmed its occurrence in the specific species [11]. It is well known that polyacetylenes exhibit a wide range of pharmacological activities, including antimicrobial, anti-inflammatory and anticancer activity [36-38]. Indeed, Wang and colleagues (2017) reported that falcarindiol also showed great anti-atherosclerotic property. Compound 3 has been mentioned in the species *C. maritimum* in previous studies [11, 13, 39].

Phenolic acids and mainly quinic acid derivatives are presented in abundance in the polar extracts of *C. maritimum*, determining the significant antioxidant activity of the plant. However, compound 4 has not been reported previously. Caffeic acid is a predominant secondary metabolite in plant's kingdom. It is also a constituent of fruits, legumes, coffee and tea [40, 41]. Accumulating pharmacological studies exhibited its remarkable pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, antiproliferative, antigen toxic and hepatoprotective activity [42-44]. Compounds 5 and 6 categorize into the quinic acid derivatives and are main components of natural products. A series of health benefits have been associated with the specific compounds. Thus, we could assume that the presence of caffeic acid with the combination of chlorogenic acid and 3,4-dicaffeoyl quinic

acid might act as synergetic agents, enhancing the antioxidant activity and improving the nutrition value of *C. maritimum*.

As it is well known, flavonoids could be found in many natural sources in human diet. The beneficial effects on human health of flavonoids are widely recognized, including the antioxidant, anti-inflammatory, anticancer, neuroprotective and cardiovascular properties [45].

Furthermore, it is also proven that they prevent some chronic diseases and cancer [45-46]. Flavonols are one of the most common sub-class of flavonoids, which are presented in plant foods [45]. Precisely, quercetin is considered as the most abundant antioxidant in nutrition and recently, it is also consumed as a dietary supplement [46-47]. Considering the flavonoid load, our data revealed four flavonols, corresponding to quercetin derivatives (compounds 7-10). Compounds 7 and 9 have been isolated previously from the specific species, while compounds 8 and 10 were isolated for the first time from *C. maritimum*. It is important to point out that quercetin-3-O-robinoside (compound 10) consists a rare secondary metabolite of plants.

Over the last years, several studies mention the rich amounts of flavonoids and phenolic acids in MD and in Greek diet.[48-49] Many plants which are used in these diets contain various polyphenols. Our results are congruous with these studies, confirming the presence of well-known antioxidants (flavonols and quinic acid derivatives).

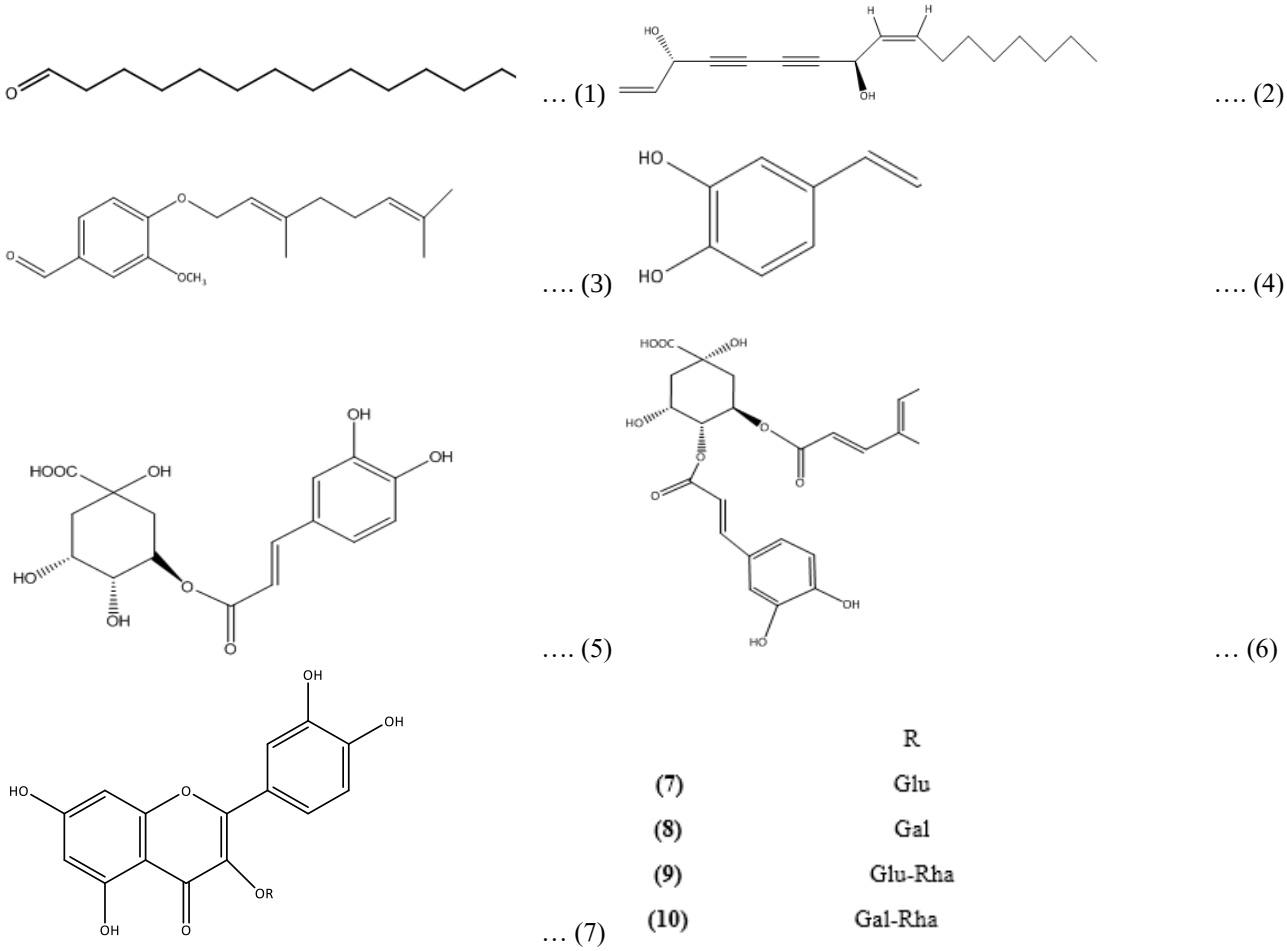

Fig 2: Structures of compound 1-10.

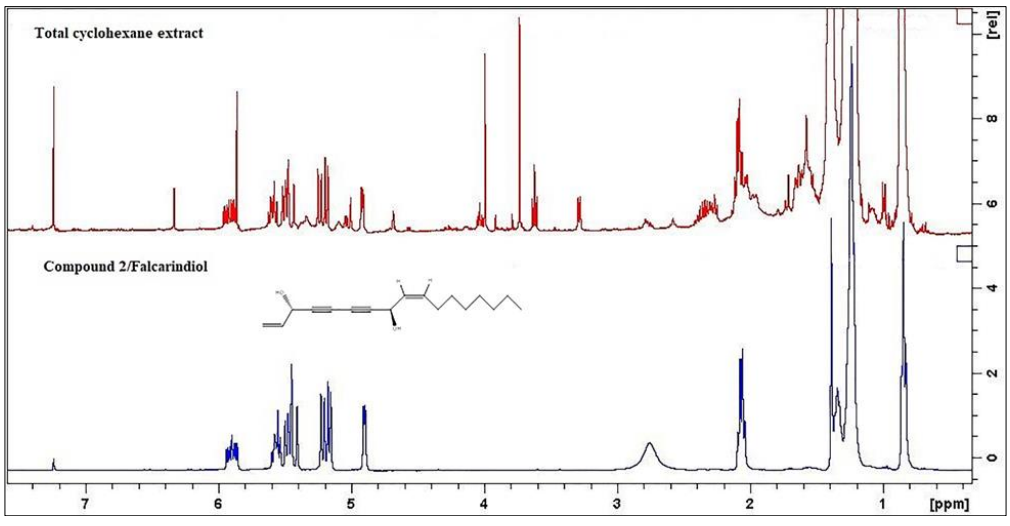

Fig 3: [1]H-NMR spectra of total extract and compound 2.

4. Conclusions

The favorable benefits of MD on human health has been well established. Polyphenols are main constituents in foods with flavonoids and phenolic acids being the most widespread antioxidants in plant foods. Sea fennel consists one of the basic food components in MD and in Greek diet. The current study presents the phytochemical investigation of wild and cultivated *C. maritimum* populations. Both samples exhibited high content of quinic acid derivatives, mainly chlorogenic acid, with wild population being slightly more abundant. Further chemical study of the methanol extract revealed the isolation of four quercetin derivatives. We could assume that the high phenolic cargo and the presence of flavonols, could enhance the beneficial effects of the investigated species. There is an urgent need for characterization of the phytochemicals and standardization of traditional foods, in order to meet the contemporary perceptions of high quality and safety. We believe that our results could help to identify the antioxidants in the MD and Greek Diet, revealing the rich phytochemical profile of this important ethno pharmacological plant.

4.1 Supporting Information: Table S1. ^{1}H-NMR and ^{13}C-NMR spectra of the compound 2 ($CDCl_3$), Figure S1. ^{1}H-NMR spectrum of compound 2 ($CDCl_3$), Figure S2. COSY spectrum of compound 2 ($CDCl_3$), Figure S3. HSQC spectrum of compound 2 ($CDCl_3$), Figure S4. HMBC spectrum of compound 2 ($CDCl_3$), Figure S5. ^{13}C-NMR spectrum of compound 2 ($CDCl_3$).

5. Acknowledgements

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6. Conflicts of interest

The authors declare no conflict of interest.

7. References

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