https://doi.org/10.3311/PPch.22715
Creative Commons Attribution

Periodica Polytechnica Chemical Engineering, 68(1), pp. 61–71, 2024

Phytochemical Diversity and Related Bioactivity of Ethyl Acetate Fraction from Different Organs and Provenances of the Edible Halophyte *Crithmum Maritimum* L.

Inès Jallali[1,3], Mohamed Ali Benabderrahim[2*], Faten Medini[3], Hela Ben Ahmed[1], Chedly Abdelly[4], Riadh Ksouri[3]

[1] Laboratory of Plants Soil and Environment Interactions (LIPSE), Department of Biological Sciences, Faculty of Sciences of Tunis, University of Tunis El Manar, 20 Rue de Tolède, 2092 Tunis, Tunisia
[2] Arid and Oasis Cropping Laboratory (LACO, LR16IRA02), Arid Regions Institute, 4119 Medenine, Tunisia
[3] Laboratory of Aromatic and Medicinal Plants (LPAM), Biotechnology Center at the Technopark of Borj Cedria (CBBC), P.O.B. 901, 2050 Hammam-lif, Tunisia
[4] Laboratory of Extremophile Plants (LPE), Biotechnology Center at the Technopark of Borj Cedria (CBBC), P.O.B. 901, 2050 Hammam-lif, Tunisia
[*] Corresponding author, e-mail: medali_abderrahim@hotmail.com

Received: 04 June 2023, Accepted: 10 August 2023, Published online: 07 March 2024

Abstract

Sea fennel (*Crithmum maritimum* L.) is a facultative halophyte from the Apiaceae family. The variation of phenolic compounds, the antioxidant and antibacterial activities of different extracts of different organs of sea fennel were investigated. The roots, stems, leaves and seeds were collected from three bioclimatic stages of Tunisia. Solvent fractionation method (using aqueous acetone, petroleum ether and ethyl acetate) was adopted to separate the extract ingredient and to concentrate phenolics. Total phenolics, total flavonoids, and condensed tannins significantly varied between organs and localities. In leaves, the highest level of phenols was detected in the upper and sub-humid zones. However, in stems the high amounts of phenols were observed in semiarid climate. The antioxidant activity was evaluated through four different methods and the antimicrobial activity was assessed against five human pathogenic bacteria and a fungal strain. Multivariate analysis showed significant effect of organ and locality on the antioxidant properties. Results of the antibacterial activities showed significant differences among extracts and was reflected by a large spectrum of activity ranging from totally not active (Zones of inhibition (zi) < 1 mm) to very high activity (zi >16 mm). Combined statistical results allowed to select the more potent fraction to be analyzed using reversed-phase HPLC (RP-HPLC). The flavanol epigallocatechin was identified as a major phenolic compound which contributed greatly to the antioxidant capacity. The results established in this study highlighted the importance of this halophyte cash crop in the human diet as natural source of active components.

Keywords

Crithmum maritimum, biological activities, bioclimatic habitats, solvent fractionation, phenol characterization

1 Introduction

The global heating and the water deficiency were the most important environmental dangers threatening all communities over the world during the last decades, especially those populating the southern part of the globe. For this, researchers had diverted their efforts towards the investigation of new edible plants capable of replacing conventional ones in various zones such as the saline marginal lands. Among the proposed new cash crops, *Crithmum maritimum* L., was a facultative halophyte belonging to the Apiaceae family. It is known as rock samphire or sea fennel and it is widely thriving on Mediterranean shores, on southern and western coasts of Britain and Ireland and on the coast of the Black Sea [1]. It is the sole species of the genus *Crithmum*. This plant constituted a part of the human diet in the Mediterranean countries, where it was consumed pickled, as condiment or as salad ingredient [2], and oil extracted from its seeds had a fatty acid composition close to olive oil [3]. It was renowned since ancient time for its antiscorbutic effects, justifying its consumption by sailors to prevent scurvy [2]. It also had ethnopharmacological uses. Leaf infusion enjoyed aromatic, tonic, depurative, antiscorbutic and diuretic

Cite this article as: Jallali, I., Benabderrahim, M., A., Medini, F., Ahmed, H., B., Abdelly, C., Ksouri, R. "Phytochemical Diversity and Related Bioactivity of Ethyl Acetate Fraction from Different Organs and Provenances of the Edible Halophyte *Crithmum Maritimum* L.", Periodica Polytechnica Chemical Engineering, 68(1), pp. 61–71, 2024. https://doi.org/10.3311/PPch.22715

properties, as it improved digestion [2]. Previous studies reported the richness of this plant on vitamins, iodine, minerals, carotenoids, lipids and fatty acids, essential oil constituents, proteins and amino acids, and phenolic compounds [2–4] without detailed investigation on the chemical variation among their bioclimatic habitats and organs. One of the characteristics of phenolic compounds, which they generally share with all of the secondary metabolites, is to show unequal distribution between varieties and the physiological stages [5]. In fact, the distribution of phenolic compounds shows localized accumulations related to physiological functions and to the plant-environment interaction [6]. In this context, several studies have shown that the qualitative and quantitative profile of phenolic compounds varies considerably according to the environmental conditions such as temperature, rainfall, radiation exposure, soil salinity, texture and relative humidity, attacks by insects and herbivores, etc. [7, 8]. The environmental factors linked to the biotope of the plant, the genotype, the physiological stage of development and the plant part are intrinsic parameters at the origin of important variations in the intra-specific phenolic identity [8, 9]. In addition, technical factors such as harvesting, drying, and extraction conditions are crucial in determining the amount and the nature of investigated compounds. Thus, evaluating the molecules in plant matrices implies taking in consideration all the above-mentioned factors. In this context, this work focused on two main factors affecting the phenolic compounds:

1. the geographic origin counting all the edaphic and climatic parameters previously enumerated;
2. the plant organ which is an important physiological factor related to the position (aerial or in the soil), the texture constitution, the function (absorption, photosynthesis, reserve accumulation, etc.).

To the best of our knowledge, no study has been reported to elucidate the impact of the bioclimatic habitats and organs variation on the phenolic composition and the biological activities of the halophyte C. maritimum. Therefore, the present work aimed, in a first part, to study the effect of organs and locality variation on the phenolic compounds as well as their antioxidant and antimicrobial capacities in the fractionated extracts of the local halophyte C. maritimum. The second part focused the identification of the chemical phenolic profile of this species by reversed-phase HPLC (RP-HPLC).

2 Material and methods

2.1 Chemical and reagents

All organic solvents and pure phenolic standards used during the following experiments were HPLC grade, and they were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The used water was bi-distilled.

2.2 Plant sampling

According to the availability of this species throughout Tunisia, we tried to vary the collection sites according to the bioclimatic stages, based on the rainfall coefficient of Emberger [10]. *C. maritimum* was harvested in its native salty ecosystems from Tabarka (P1) (upper humide, 1029 mm/year), Kelibia (P2) (sub-humide, 710 mm/year), and Monastir (P3) (lower semi-arid, 350 mm/year). *C. maritimum* was harvested at the end of September when umbels were in full fructification stage. The harvested plants were identified by Professor Smaoui Abderrazek, and a voucher specimen [PLM80] was deposited at the Herbarium of the laboratory at the Biotechnology Center at Technopark of Borj-Cédria. Morphological differences were noted between provenances. An increasing gradient in plant size and shape was seen in *C. maritimum* going from the North to the South regions. In addition, P3 had narrower and longer leaves with a lighter green color compared to the other two provenances. A slight shift in the synchronization of physiological stages was also distinguished. The formation of umbels was early in P3. It therefore became essential to respect this gap by collecting plants reaching almost the same level of physiological differentiation.

2.3 Preparation of plant extracts

Samples of each organ (root, stem, leaf, and seed) were air-dried in shadow at room temperature and grinded to a fine powder. The main steps involved in acquiring quality bioactive molecule are the selection of a suitable solvent and fractionation method. The organ powders were differentially extracted in steps as resumed in Fig. 1. The extraction consisted in a separation of the active compounds according to their affinity to different solvents. The first step consisted in extracting the maximum of phenolics having different molecular weights by using a mixture of acetone (60%) and water (40%). The same dry matter was exhaustively extracted by 300 mL of the aqueous actone for 3 times. The obtained extracts were combined (300 mL × 3). The organic part was then evaporated, and the residual aqueous fraction was mesured and extracted by an equivalent volume of petroleum ether for 3 successive times

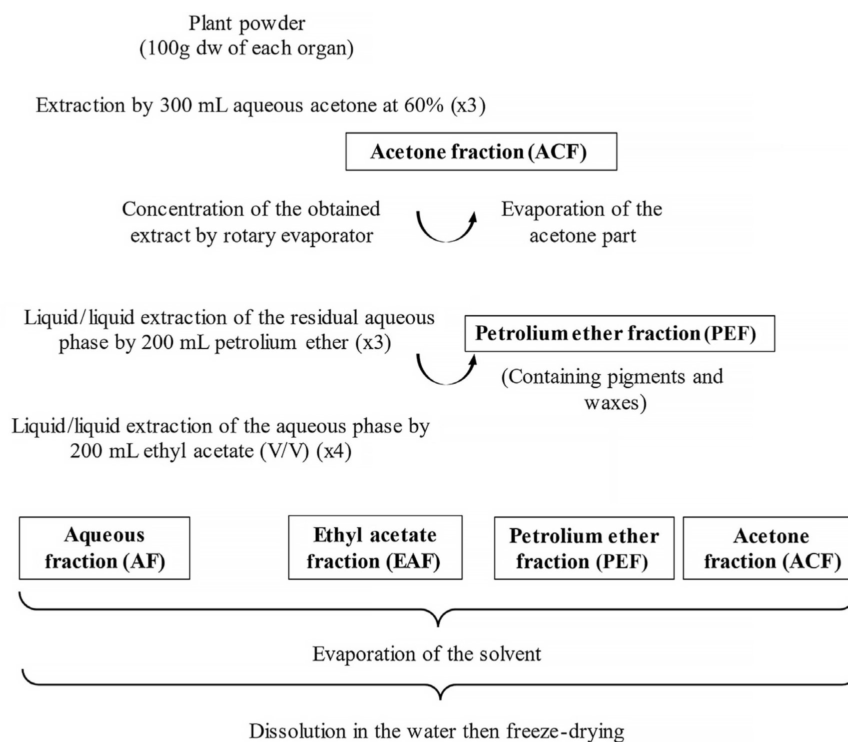

Fig. 1 Explanatory drawing of solvent fractionation of C. *maritimum* phenolic compounds from different plant organs (root, stem, leaf and seed) collected at three different geographic locations

to eliminate pigments and waxes. The volume of the cleaned aqueous phase (V = 200 mL) was added to an equal volume of ethyl acetate (V = 200 mL), then subjected to a liquid/liquid extraction (V/V). This application was repeated 4 times on the same aqueous phase for a maximum recovery of phenolic compounds. The obtained ethyl acetate extracts (4 × 200 mL) were combined and evaporated under vacuum". The choice of the ethyl acetate as an extracting solvent was based on our previous work reporting the specific affinity of this solvent to phenolic acids and flavonoids existing in *C. maritimum* [3]. The preliminary analyses comparing the four fractions (acetone fraction: ACF, petroleum ether fraction: PEF, ethyl acetate fraction: EAF, aqueous fraction: AF) (Fig. 1) showed that ethyl acetate fraction (EAF) expressed the highest amounts of phenolic compounds and antioxidant activities (data not shown). Accordingly, EAF of the different organs and provenances were retained for analysis in this study. They were evaporated under vacuum at 35 °C until complete dryness, dissolved in water and freeze dried then kept at 4 °C until use.

2.4 Total polyphenol (TPC), total flavonoid (TFC) and condensed tannins content (CTC) contents

The TPC and TFC in different *C. maritimum* extracts were quantified by the colorimetric methods described by Dewanto et al. [11]. The calibration curve was established using gallic acid and the TPC were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE g^{-1} dw). For the TFC the unit was expressed as mg catechin equivalent per gram of dw (mg CE g^{-1} dw). A slightly modified vanillin method of Sun et al. [12] was used to measure the amounts of Proanthocyanidins. The amount of CTC was expressed as mg (+)-catechin equivalent per gram of dw (mg CE g^{-1} dw).

2.5 Analysis of phenolic compounds by reverse phase hight performance liquid chromatography (RP-HPLC)

RP-HPLC analysis of *C. maritimum* EAF was carried out using an Agilent 1100 series HPLC system equipped with diode array detector (G 1315A), on-line degasser (G 1322A), quaternary pump (G 1311A), a thermostatic autosampler (G 1313A), column heater (G 1316A) and controlled by HP ChemStation rev. 10.0 software (Agilent). The RP-HPLC was performed on ODS C18 column (4 µm, 250 × 4.6 mm, Hypersil), used as stationary phase at ambient temperature. Elution program using of acetonitrile (solvent A) and water sulphuric acid (0.2%) (solvent B) was as follows: 10% A/90% B 0–10 min, 30% A/70% B 10–15 min, 70% A/30% B 15–20 min, 85% A/15% B 20–22 min, 90% A/10% B 22–26 min,

100% A 26–30 min. The flow rate was kept at 0.5 mL min^{-1}. Sample solution was prepared in 50% methanol at 5 mg mL^{-1} then filtered with Agilent 0.45 µm filter. Twenty microliters of samples were injected and peaks were detected at 280 nm. Peak identification was obtained by comparing the retention time and the UV spectra of *C. maritimum* phenolics chromatograms with those of pure standards under the same chromatographic conditions. Quantitative determination was done by preparing calibration curves of standards diluted in the same solvent as samples. Integration was done manually.

2.6 Antioxidant capacity

2.6.1 Total antioxidant capacity

Information describing the assessment of the total antioxidant activity was detailed in Jallali et al. [13]. The principle of this test consisted on reducing molybdate ions by the antioxidant molecules present in the sample to test. The efficiency of the extract was reflected by the number of green phosphate/Mo(V) complexes formed and was positively correlated with the color intensity of the solution, quantified at 695 nm and expressed as mg GAE g^{-1} dw.

2.6.2 Radical-scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Hatano et al. [14] was applied to assess the antiradical activity of *C. maritimum* extracts. Results were expressed as IC_{50} (mg mL^{-1}), the concentration needed to inhibit 50% of the DPPH radical present in the solution.

2.6.3 *β*-Carotene bleaching test (BCBT)

The antioxidant effectiveness of the ethyl acetate fractions in lipidic system was assessed by the slightly modified protocol of BCBT reported by Koleva et al. [15].

2.6.4 Reducing power

The experimental proceeding of the iron (III) reducing activity of the extract fractions was extensively detailed in Jallali et al. [11]. Effective concentration (EC_{50} in mg mL^{-1}) corresponded to 0.5 absorbance, obtained from linear regression analysis.

2.7 Antimicrobial activities

2.7.1 Strains

The antibacterial activity was carried out against five human pathogenic bacteria and one fungal strain. They were including Gram-positive (*Gram*⁺): *Staphylococcus aureus* ATCC 25923, and Gram-negative: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 *Salmonella typhimirium* LT2 DT104, *Enterococcus faecalis* ATCC 29212. The fungal strain was *Candida albicans* 2091. These strains are usually used in antimicrobial tests as they are known to be multidrug resistant germs and common to food environments. Besides, they are among the most frequent species implicated in many human diseases. But above all, the choice of strains was imposed by their availability in the laboratory during experiments.

2.7.2 Antimicrobial experiments

The antimicrobial effects were assessed by the disc diffusion method extensively described in Hajlaoui et al. [16]. Samples of bacterial stocks were grown on Mueller-Hinton broth and were incubated at 37 °C for 18–24 h. As for *C. albicans*, the fungal culture was grown on potato dextrose agar (PDA) broth at 25 °C for 7 days.

The optical density of both bacterial and fungal cultures obtained was adjusted to McFarland turbidity standards of 0.5. Petri plates were prepared by pouring 20 mL of Mueller Hinton agar (MH) for all bacteria. The inocula were spread on the top of the solidified media then allowed to dry. Soft agar (5 mL), pre-inoculated with 100 µL fungal suspensions, was used to uniformly overlay the PDA agar plates, ensuring the fungal seedling. Sterile discs of 6 mm were sacked by 10 µL of extracts diluted at 100 mg mL^{-1} and applied the on inoculated agar (PDA and MH).

Negative controls were prepared using the same solvents employed to dissolve the plants extracts. Gentamycin (10 µg/disc) and amphotericin B (100 µg/disc) were used as positive controls. The dishes were incubated at 37 °C for 18–24 h for bacterial strains, and at 25 °C for 72 h for fungi isolates. The diameters of the inhibition halos were evaluated in millimeters.

2.8 Statistical analyses

All analyses were done at least in triplicate and data were expressed as mean ± SD (standard deviation). One and two-way ANOVA were performed followed by Newman-Keuls multiple comparison test ($P < 0.05$) to analyze the effects of the provenance (P) and the organs (O) as factors, as well as their interactions (P × O) on the studied parameters.

Principal component analysis (PCA) was used to obtain information on the interrelations between the studied parameters (plant organs and geographic origins). Analyses were computed on the data using XLSTAT 2021.5 trial version [17]. The freely available Heatmapper web server [18, 19] was used to generate

a heat map to interactively visualize the studied data including polyphenolic compounds, antioxidant, and antibacterial activities of organs from studied provenances.

3 Results and discussion

3.1 Phenolic compound contents

The variance analysis showed significant effect of provenance, organ as well as their interaction (Table 1). There was no specific order of organ richness on TPC and TFC among the three studied locations. The TPC varied between 7.7 and 45 GAE g^{-1} dw. The variation was more important in TFC that changing from 6.9 to 53.7 mg CE g^{-1} dw. Amounts in leaf and seed extracts from humid regions (P1 and P2) were distinguished and statically higher than the other ones. Leaf 2 extract came in the second range while stem and leaf extract enclosed the highest concentrations of polyphenols among the four organs of the semi-arid region (P3). When comparing the three provenances, plants belonging to the upper humid were the richest one on phenolic compounds. Concerning the condensed tannins contents, concentration repartition was different from that of total polyphenols and flavonoids, with distinguished high amounts recorded in Leaf 3 extract. The condensed tannin contents did not exceeded 14.6 mg CE g^{-1} dw. Moreover, factors such as stage phenology and variation in environmental conditions, which impact the phenolic's biosynthetic pathways, influence the quantity, quality, and biological activities associated with polyphenols [20].

3.2 Antioxidant activities

Total antioxidant activity of *C. maritimum* differed statistically between studied extracts depending on the plant part and the locality (Table 2). Results displayed that, for both provenances P1 and P2, leaf and seed extracts expressed the highest antioxidant activities. However, stem extract was the most potent among P3 organ extracts, followed by the leaf one. As for the antiradical activity, IC_{50} values ranged between 18 and 122 µg mL^{-1} (Table 2). Leaf 1 and 2 extracts divulged the highest antiradical activity, followed by stem 3, thus reproducing the same tendency of total antioxidant activity (TAA). However, the DPPH test highlighted the richness of Root 1 extract on antiradical compounds reflected by the important IC_{50} value. The quantification was based on the abundance of the compound and not on its efficiency. Hence, we concluded that root extract contained low amounts of a very potent antiradical molecule or only a specific antioxidant compound. This point was largely discussed above, and many authors demonstrated that the antioxidant activity of phenolic

Table 1 Phenolic compound contents in the *C. maritimum* ethyl acetate fractions of the different organs from the three provenances

Provenance	Organ	TPC (mg GAE g^{-1} dw)	TFC (mg CE g^{-1} dw)	CTC (mg CE g^{-1} dw)
Tabarka (P1)	Root 1	23.4 ± 1.6^b	25.4 ± 1.8^b	2.1 ± 0.1^c
	Stem 1	7.7 ± 0.7^c	9.4 ± 0.4^c	3.2 ± 0.3^b
	Leaf 1	46.6 ± 0.8^a	53.7 ± 0.8^a	6.5 ± 0.7^a
	Seed 1	45.0 ± 3.5^a	53.2 ± 0.2^a	5.2 ± 0.4^a
Kelibia (P2)	Root 2	11.8 ± 0.9^c	10.8 ± 0.2^d	2.4 ± 0.1^c
	Stem 2	11.3 ± 1.1^c	13.2 ± 0.3^c	5.6 ± 0.2^b
	Leaf 2	34.5 ± 1.8^a	48.5 ± 1.0^a	6.9 ± 0.8^a
	Seed 2	14.5 ± 1.7^b	34.0 ± 1.5^b	7.6 ± 0.5^a
Monastir (P3)	Root 3	8.6 ± 0.7^d	6.9 ± 0.4^d	3.2 ± 0.2^c
	Stem 3	26.1 ± 1.2^a	24.9 ± 1.4^a	8.0 ± 0.9^b
	Leaf 3	23.0 ± 1.2^b	18.3 ± 1.4^b	14.6 ± 0.7^a
	Seed 3	16.3 ± 1.2^c	10.9 ± 1.4^c	3.1 ± 0.2^c
Provenance (P)	F	315.630	1371.800	130.622
	p	< 0.0001	< 0.0001	< 0.0001
Organ (O)	F	420.720	1627.383	346.994
	p	< 0.0001	P < 0.0001	< 0.0001
P × O	F	195.272	633.092	114.747
	p	< 0.0001	< 0.0001	< 0.0001

The bioclimatic stages were defined according to the rainfall coefficient of Emberger [10] as followed: $Q_2 = 2000\ P/(M^2–m^2)$, with P: annual precipitations (mm), M: average maxima of the hottest month (K), m: average minima of the coldest month (K), Different letters in the same column of each provenance showed significant difference test at $p < 0.05$; Means (means of three replicates) followed by the same letter in column of each provenance are not significantly different at $p < 0.05$ using Newman-Keuls.; TPC: total polyphenols content; TFC: total flavonoid content; CTC: condensed tannins content; CE: g^{-1} catechin equivalent; GAE: gallic acid equivalent. F: F-value of Fisher-Snedecor test.

Table 2 Antioxidant activities of *C. maritimum* ethyl acetate fractions of four organs from different provenances.

Provenance	Organ	TAA (mg GAE g^{-1} dw)	DPPH (IC_{50}: µg mL^{-1})	FRAP (EC_{50}: µg mL^{-1})	BCBT (IC_{50}: µ mL^{-1})
Tabarka (P1)	Root 1	50.0 ± 1[c]	23.0 ± 1.4[c]	445 ± 6[d]	72 ± 2.7[b]
	Stem 1	38.7 ± 2[d]	71.3 ± 1.8[a]	1400 ± 12[a]	175 ± 4[a]
	Leaf 1	130.3 ± 4[a]	24.7 ± 0.9[c]	580 ± 6[c]	60 ± 4[c]
	Seed 1	115.0 ± 6[b]	32.2 ± 1.9[b]	800 ± 4[b]	52 ± 3[d]
Kelibia (P2)	Root 2	51.7 ± 3[c]	37.1 ± 0.8[c]	1120 ± 4[c]	265 ± 7[b]
	Stem 2	49.7 ± 0.3[c]	59 ± 0.8[b]	1250 ± 12[b]	140 ± 4[c]
	Leaf 2	131.3 ± 5[a]	18 ± 0.4[d]	445 ± 6[d]	82 ± 4[d]
	Seed 2	79.7 ± 6[b]	122 ± 1.5[a]	2130 ± 7[a]	290 ± 5[a]
Monastir (P3)	Root 3	48.7 ± 2[d]	116 ± 1.1[a]	2290 ± 9[a]	942.7 ± 9[a]
	Stem 3	131 ± 3[a]	70 ± 1.2[d]	745 ± 6[d]	61 ± 4[d]
	Leaf 3	108 ± 2[b]	85 ± 0.9[c]	1340 ± 13[c]	180 ± 7[c]
	Seed 3	78.3 ± 4[c]	96 ± 1.3[b]	1580 ± 12[b]	550 ± 8[b]
Positive control	BHT	–	11.5 ± 0.4	–	75 ± 0.1
	BHA	–	6.1 ± 0.3	–	48 ± 0.2
	AsA	–	–	37.3 ± 0.1	–
Provenance (P)	F	80.220	7578.276	23749.446	12847.229
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Organ (O)	F	6770.109	2223.503	13526.143	7123.150
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001
P × O	F	211.609	2649.336	19816.898	5328.235
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Different letters in the same column of each provenance showed significant difference test at $p < 0.05$; Means (means of three replicates) using Newman-Keuls. Means followed by the same letter in the same column of each provenance are not significantly different at $p < 0.05$. (means of three replicates). BHT: Butylated hydroxtoluene; BHA: Butylated hydroxyanisole; AsA: Ascorbic acid; Provenance (P): the effect of provenance variability; F: F-value of Fisher-Snedecor test.

compounds is not only dependent on their amounts but essentially on their structure and interactions [3, 21]. These findings were confirmed by ferric reducing antioxidant power (FRAP) test results having the same inclination but expressing a modest to low antioxidant potential (Table 2). Concerning the β-carotene bleaching test, results were interesting with a maximum capacity of inhibition of linoleic acid oxidation recorded in Seed 1 extract (IC_{50} = 52 µg mL^{-1}). It even exceeded the antioxidant potential of the positive controls Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA). Stem 1 and 2 extracts expressed high antioxidant potential as compared to other plant parts. The two-way analysis confirmed these results showing significant effect of origin (P), organ (O) and their interactions (P × O) on antioxidant activities (Table 2). The effect of the intrinsic and extrinsic factors on the amounts and bioactivity of secondary metabolites was described in previous works [1, 3, 4, 8, 9, 20]. Jallali et al. [3, 4] showed that *C. maritimum* phenolic contents, antioxidant and antimicrobial activities varied significantly according to the development stage, the extraction method and the geographic origin. This variability is due to the inference of these secondary metabolites in the metabolism functions and the interaction of the plants with their environment. Polyphenols, representing one of the most important phytochemical groups in plants, are of considerable importance for the morphology and physiology of plants. They are produced by them during normal development or in response to biotic or abiotic stresses such as infection, injury, UV irradiation, exposure to ozone, pollutants and other hostile conditions [3, 5, 6, 21]. Phenolic pigments (anthocyanins and flavonols), for example, contribute to growth and reproduction since they participate in the coloring of plant organs and play an important role in the pollination of colored flowers or the dissemination of fruits by attracting insects and birds [21]. In addition, certain phenolic compounds are only accumulated in well-defined organs. Thus, anthocyanins were abundant in ripe fruit (apple, red grape, strawberry) whereas they exceptionally appeared in the other organs of the plant [5]. Generally, the antioxidant properties of phenolic compounds maintain regular physiological status in tissues directly or

indirectly affected by biotic and abiotic stresses [6]. Besides, results displayed that phenolic contents were positively concomitant with the assessed antioxidant tests. This fact implied that phenolic compounds were probably the major contributors to the antioxidant activities of *C. maritimum* extracts as reported previously by many researches which demonstrated the direct implication of phenolic compounds in the antioxidant response of plants to different constraints, particularly within halophytic species [3, 22].

3.3 Antimicrobial activities

Results of the antibacterial activities of *C. maritimum* extracts were represented in Table 3. Significant differences were observed and showed a large spectrum of activity ranging from totally not active (zi < 1 mm) to very high activity (zi > 16 mm) [23]. All extracts had medium to high antibacterial activity against S. aureus, while *E. coli* and *P. aeruginosa* were the most resistant strains. In addition, all extracts exhibited low to high antibacterial activity on *E. faecalis* and *S. typhimirium*. It seems that *C. maritimum* extracts were more potent on *Gram*$^{+}$ bacteria than on *Gram*$^{-}$ ones. Concerning the antifungal activity, *C. albicans* growth was affected by only some of *C. maritimum* extracts. Similar to the antioxidant activities, there was no defined order of potency between plant organs. In each geographic region, there was a specific order of organs. Stem and seed extracts from P3 and P1, respectively, divulged the most important antimicrobial effects, thus emphasizing the hypothesis that geographic variation was the major factor affecting the phenolic composition. The resistance of *Gram*$^{-}$ strains could be due to their specific anatomy characterized by the presence of a unique outer membrane. This kind of cell wall structure play an important role in preventing antibiotics from penetrating the bacterial cell, and partially explains the fact that *Gram*$^{-}$ bacteria are generally more resistant to antibiotics than *Gram*$^{+}$ ones [24] In addition, the low efficiency of *C. maritimum* extracts on *Gram*$^{-}$ bacteria may be explained by two other hypotheses. From one hand, extracts were obtained by differential solvent pre-purification, meaning that the obtained extracts were mixtures of molecules (pigments, waxes, and phenolics) sharing the same affinity to the used solvent. From the other hand structure-activity relationship of phenolic compounds was a key factor defining the specific mechanism of toxicity against strains, neither by enzyme inhibition and substrate deprivation on membranes, nor by metal ions deprivation [4].

3.4 Multivariate analyses

PCA and Heatmap analyses were conducted using all studied parameters including TPC, TFC, CTC, antioxidant, and antimicrobial activities. The two first PCA axes explained 64% of total inertia. The axis 1 explained 42.26% of total inertia and was positively correlated to phenolic compounds

Table 3 Antibacterial and antifungal activities of *C. maritimum* extracts expressed as diameter of the inhibition zone (mm)

Provenance	Organ		Bacterial strains			Fungal strain	
			Gram^{+}			*Gram*$^{-}$	
		S. aureus	*E. coli*	*E. faecalis*	*S. typhimirium*	*P. aeruginosa*	*C. albicans*
Tabarka (P1)	Root 1	12.0 ± 0.0^{b}	0.0 ± 0.0^{b}	10.7 ± 0.7^{b}	10.3 ± 0.7^{a}	0.0 ± 0.0^{b}	7.3 ± 0.7^{d}
	Stem 1	10.7 ± 0.6^{c}	0.0 ± 0.0^{b}	10.7 ± 0.7^{b}	10.7 ± 0.7^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{e}
	Leaf 1	11.7 ± 0.6^{b}	8.3 ± 0.6^{a}	13.3 ± 0.7^{a}	7.7 ± 0.7^{b}	8.3 ± 0.7^{a}	8.3 ± 0.7^{c}
	Seed 1	14.3 ± 0.6^{a}	7.7 ± 0.6^{a}	13.7 ± 0.7^{a}	11.3 ± 1.3^{a}	7.3 ± 0.7^{a}	8.3 ± 0.7^{c}
Kelibia (P2)	Root 2	12.7 ± 0.6^{b}	0.0 ± 0.0^{b}	11.3 ± 1.3^{a}	12.3 ± 0.7^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{e}
	Stem 2	13.3 ± 0.6^{b}	0.0 ± 0.0^{b}	7.7 ± 0.7^{b}	10.3 ± 0.7^{b}	0.0 ± 0.0^{b}	8.3 ± 0.0^{c}
	Leaf 2	15.3 ± 0.6^{a}	0.0 ± 0.0^{b}	10.3 ± 0^{a}	7.7 ± 0.7^{c}	0.0 ± 0.0^{b}	0.0 ± 0.0^{e}
	Seed 2	10.3 ± 0.6^{c}	7.3 ± 0.6^{a}	11.7 ± 0.7^{a}	9.7 ± 0.7^{b}	7.0 ± 0.0^{a}	7.7 ± 0.7^{d}
Monastir (P3)	Root 3	12.3 ± 0.6^{b}	0.0 ± 0.0^{b}	12.7 ± 0.7^{ab}	9.7 ± 0.7^{b}	7.3 ± 0.7^{a}	9.3 ± 0.0^{b}
	Stem 3	16.33 ± 0.6^{a}	7.3 ± 0.6^{a}	12.7 ± 0.7^{ab}	10.3 ± 0.7^{ab}	0.0 ± 0.0^{b}	9.3 ± 0.7^{b}
	Leaf 3	11.3 ± 1.15^{b}	7.3 ± 0.6^{a}	11.7 ± 0.7^{b}	11.3 ± 1.3^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{e}
	Seed 3	12.3 ± 0.6^{b}	0.0 ± 0.0^{b}	13.3 ± 0.7^{a}	9.7 ± 0.7^{b}	7.3 ± 0.6^{a}	10.7 ± 0.7^{a}
Positive controls							
Gentamicine (10 µg/disc)	32.5 ± 0.6	28 ± 0.3	26.3 ± 0.6	20 ± 0.5	29 ± 0.7	–	
Amphotericine B (20 µg/disc)	–	–	–	–	–	14.6 ± 0.5	

Means followed by the same letter in the same column are not significantly different at $p < 0.05$ (means of three replicates).
The diameter of the disks used is 6 mm. Zone of inhibition (zi) < 1mm: not active; zi = 1 mm: weak activity; 2 < zi < 3 mm: medium activity; 4 < zi < 5 mm: quite high activity; 6 < zi < 9 mm: high activity; zi > 9 mm: very high activity [23].

(TPC, TFC, CTC), TAA and antibacterial activities (against *S. aureus*, *P. aeruginosa* and *E. coli*). It's negatively correlated to FRAP, BCBT activities and *S. typhimirium* growth inhibition. The axis 2 explained 21.74% of inertia and was positively correlated with DPPH and antimicrobial activities against *E. faecalis* and *C. albicans* (Table 3). Fig. 2 illustrated the PCA plots of the studied variables and organs. The stem and root of P1 and P2 constituted a group characterized by low TPC, TFC and CTC and high antibacterial effect on *S. typhimirium*. The P3 root and seed were characterized by an important DPPH, FRAP and BCBT activities. PCA showed an important potentiality of P1 leaf and seed having crucial antimicrobial activities. This structuration was not correlated with provenances and organs. The Heatmap analysis was conducted using all studied variables (Fig. 3) and showed two different groups (G1 and G2). The first one (G1) was divided into two sub-groups (SG1–1 and SG1–2). The first one (SG1–1) grouped Stem 3, Leaf 3, Root 1, and Seed 2. It was characterized by high DPPH, BCBT and FRAP activities and was less rich on phenolic compounds compared to G2. Stem 1, Stem 2, Root 2 and Seed 3 were gathered on the SG1–2 which was distinguished by its TPC and TFC and lower CTC contents as compared to SG1–1. This sub-group was highlighted by its distinct antioxidant activities. However, the second group (G2) grouped Leaf 1, Seed 1 and Leaf 2 (Fig. 3), and confirmed by PCA (Fig. 2). It was marked by its richness on phenolic compounds (TPC, TFC, and CTC) and its antioxidant and antimicrobial activities. Results of these three latter extracts were the most interesting among all studied ones. Among these three extracts, Leaf 1 was the most correlated with phenolic compound contents and the antioxidant activities. For that reason, the Leaf 1 EAF was selected to be analyzed by reversed-phase HPLC (RP-HPLC).

3.5 Chromatographic analysis of the selected EAFs

Eight phenolic compounds were identified by RP-HPLC from the Leaf 1 EAF, two flavonoids and six phenolic acids (Table 4). Epigallocatechin, a flavanol, was the major compound representing the half of identified phenolic amounts (50.66%), followed by gallic and chlorogenic acids (13.51 and 10.41%, respectively). The remaining flavonoid (quercetin-3-galactoside) and phenolic acids (vanillic, rosmarinic, *p*-coumaric and trans-2-hydroxycinnamic acids) were present in lower proportions. Previous works reported the chemical composition of the whole aerial part of this plant collected from Tunisia and described a similar composition with some differences [3, 25].

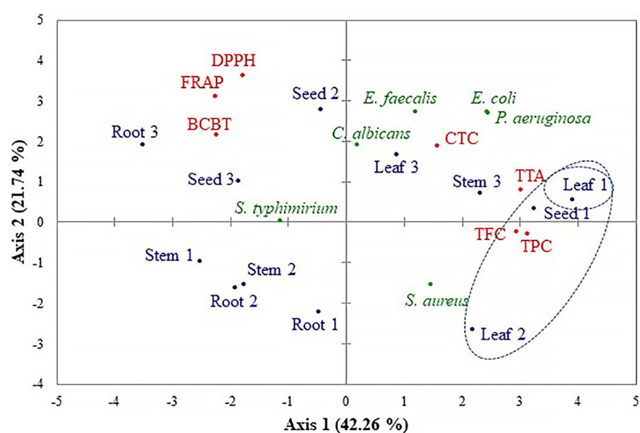

Fig. 2 Principal Component Analysis plot of phenolic compounds, antioxidant and antimicrobial activities of organs from *C. maritimum* provenances. TPC: Total Polyphenols Content; TFC: Total Flavonoid Content; CTC: Condensed Tannins Content; TAA: Total Antioxidant Activity; DPPH test: 2,2-Diphenyl-1-picrylhydrazyl test; FRAP test: Ferric Reducing Antioxidant Power; BCBT: *β*-carotene Bleaching Test. The big and small circles show the highest extracts on total phenolic compounds and biological activities and the selected extract for RP-HPLC analysis, respectively

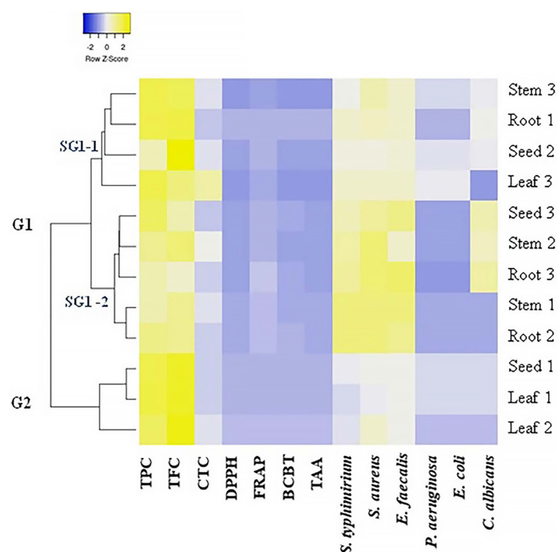

Fig. 3 Heatmap of polyphenols contents, antioxidants and antimicrobial activities of the organ extracts. The mean values refer to colors from the minimum displayed in blue to the maximum represented with yellow color. TPC: Total Polyphenols Content; TFC: Total Flavonoid Content; CTC: Condensed Tannins Content; TAA: Total Antioxidant Activity; DPPH test: 2,2-Diphenyl-1-picrylhydrazyl test; FRAP test: Ferric Reducing Antioxidant Power; BCBT: *β*-Carotene Bleaching Test

They confirmed that almost of bioactive compounds belonged to these two chemical classes. In fact, the differential extraction adopted in this work aimed to concentrate bioactive phenolics in one fraction, based on the affinity of the used solvent to these molecules. The ethyl acetate as solvent was well studied based on its physico-chemical

Table 4 Percentages of RP-HPLC identified phenolic compounds from the ethyl acetate fraction of *C. maritimum* leaf extract

N°	Identified phenolic compounds	RT (min)	%
1	Gallic acid	5.19	13.51 ± 1.2
2	Epigallocatechin	6.35	50.66 ± 2.4
3	Chlorogenic acid	8.29	10.41 ± 1.4
4	Vanillic acid	10.64	4.27 ± 0.5
5	Rosmarinic acid	11.05	4.72 ± 0.7
6	Quercetin-3-galactoside	13.09	2.47 ± 0.5
7	*p*-Coumaric acid	13.9	4.95 ± 0.7
8	Trans-2-hydroxycinnamic acid	14.5	2.25 ± 0.3

RT: Retention time, %: percentage of the identified compound in the ethyl acetate fraction of *C. maritimum*.

properties. Chemical structure of this solvent presents polar carbon-oxygen bonds, conferring it a polar character. However, it has also non polar alkyl groups, making it capable of dissolving non polar molecules. The presence of both polar and nonpolar moieties in its structure explains its affinity to a wide range of phenols of low to medium weight, especially flavonoids and some phenolic acids [26]. A previous study [27] adopted another differential extraction technic using the polar solvent *n*-butanol for the same species growing in Algeria. As a result, they identified different composition with the predominance of chlorogenic acid, followed by rutin. Many other studies reported the chlorogenic acid as a major phenol of the aerial parts of *C. maritimum* [28, 29]. The difference observed in this study with regard to previous ones could be explained by:

1. the extraction conditions specific to each method (affinity of the solvent used, time of extraction, heating, etc.);
2. the biotic (physiological stage, plant organ, genetic variability) and/or abiotic (edapho-climatic conditions) factors.

From another side, the concentration of phenolic compounds in the leaves can be related to the biological roles performed by these molecules within the plant such as flower and fruit pigmentation, UV protection, plant defense against pathogens, plant fertility and pollen germination, and as signal molecules in plant-microbe interactions [30]. In fact, phenolic acids and flavonoids are accumulated in leaves to cope with the deleterious effects of reactive oxygen species generated during the photosynthetic process [31]. Particularly, epigallocatechin ($C_{15}H_{14}O_7$), identified as major phenolic compound, is a monomer of prodelphinidins having 306.27 g/mol molecular weight and having a great antioxidant activity [32]. It is one of the predominant compounds of green tea extracts representing about 2.34 to 47.68% of total catechins [33, 34]. As well, it has antiviral, antibacterial, anticancer and anti-diabetic capacities [33, 34]. It was also demonstrated that epigallocatechin is able to ameliorate the symptoms of Alzheimer disease by reducing the toxicity of Aβ oligomers and fibrils [35]. Gallic acid, identified as well in good proportions, is considered as a powerful antioxidant phenolic compound disclosing high scavenging activity [36]. Rosmarinic acid shows a strong scavenging ability, three folds greater than that of torolox [36]. Hence, phenolic compounds identified in *C. maritimum* EAF have strong antioxidant capacities, related to numerous biological properties such as anticarcinogenic, antimutagenic and anti-HIV activities (inhibition of HIV replication) [3], antitumor, antimicrobial, anti-inflammatory, and antihepatitic activity as well as liver protection [37]. They also, inhibit blood coagulation and the oxidation of low density lipoproteins (LDLs) involved in the development of atherosclerosis [37].

4 Conclusion

Results obtained in this study showed that *C. maritimum* was a food plant rich in phenolic compounds with important antioxidant and antimicrobial properties, tightly related to the plant organ and geographic origin. Thus, these results highlighted the importance of evaluating the effect of the interfering intra- and extrinsic factors on the quantitative and qualitative values of bioactive compounds, especially within food plants because of their direct implication in human dietary and health. In this context, *C. maritimum* was considered as a promoting cash crop due to its richness on phenolic acid and flavonoids, essentially the epigallocatechin known for its potent biological effects and benefits on human health. This species may be considered as a candidate for the future as it was harnessing edible, medicinal, aromatic and economic potentialities, besides its halophytic trait. This important latter characteristic allowed it to colonize salty unexploited marginal zones and substitute conventional plants in lands affected by drought. Taking in consideration the information afforded by this study, these results could be helpful to agricultural, food processing and pharmaceutical industries for a better exploitation of bioactive molecules from this species.

Acknowledgement

The project presented in this article is supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02).

References

[1] Maleš, Ž., Žuntar, I., Nigović, B., Plazibat, M., Vundać, V. B. "Quantitative analysis of the polyphenols of the aerial parts of rock samphire *Crithmum maritimum* L.", Acta Pharmaceutica, 53(2), pp. 139–144, 2003.

[2] Bartnik, M., Wierzchowska-Renke, K., Głowniak, P., Głowniak, K. "Phenolic acids in *Crithmum maritimum* L. (Apiaceae) after Tytanit fertilization", Acta Societatis Botanicorum Poloniae, 86(3), 3560, 2017. https://doi.org/10.5586/asbp.3560

[3] Jallali, I., Megdiche, W., M'Hamdi, B., Oueslati, S., Smaoui, A., Abdelly, C., Ksouri, R. "Changes in phenolic composition and antioxidant activities of the edible halophyte *Crithmum maritimum* L. with physiological stage and extraction method", Acta Physiologiae Plantarum, 34(4), pp. 1451–1459, 2012. https://doi.org/10.1007/s11738-012-0943-9

[4] Jallali, I., Zaouali, Y., Missaoui, I., Smeoui, A., Abdelly, C., Ksouri, R. "Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L.", Food Chemistry, 145, pp. 1031–1038, 2014. https://doi.org/10.1016/j.foodchem.2013.09.034

[5] Macheix, J. J., Fleuriet, A., Jay-Allemand, C. "Les composés phénoliques des végétaux: Un exemple de métabolites secondaires d'importance économique", (Plant phenolic compounds: An example of economic importance), Presses Polytechniques et Universitaires Romandes, 2005 (in French) ISBN 2-88074-625-6

[6] Ksouri, R., Megdiche Ksouri, W., Jallali, I., Debez, A., Magné, C., Hiroko, I., Abdelly, C. "Medicinal halophytes: Potent source of health promoting biomolecules with medical, nutraceutical and food applications", Critical Reviews in Biotechnology, 32(4), pp. 289–326, 2012. https://doi.org/10.3109/07388551.2011.630647

[7] Bose, J., Rodrigo-Moreno, A., Shabala, S. "ROS homeostasis in halophytes in the context of salinity stress tolerance", Journal of Experimental Botany, 65(5), pp. 1241–1257, 2014. https://doi.org/10.1093/jxb/ert430

[8] Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulâaba, M., Smaoui, A., Abdelly, C. "Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes", Comptes Rendus Biologies, 331(11), pp. 865–873, 2008. https://doi.org/10.1016/j.crvi.2008.07.024

[9] Chetoui, I., Messaoud, C., Boussaid, M., Zaouali, Y. "Antioxidant activity, total phenolic and flavonoid content variation among Tunisian natural populations of *Rhus tripartita* (Ucria) Grande and Rhus pentaphylla Desf.", Industrial Crops and Products, 51, pp. 171–177, 2013. https://doi.org/10.1016/j.indcrop.2013.09.002

[10] Emberger, L. "Une classification biogéographique des climats", (A biogeographic classification of climates), Recueil des Travaux des Laboratoires de Botanique, Géologie et Zoologie de la Faculté des Sciences de l'Université de Montpellier, Série Botanique, 7, pp. 3–43, 1955. (in French)

[11] Dewanto, V., Wu, X., Adom, K. K., Liu, R. H. "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity", Journal of Agricultural and Food Chemistry, 50(10), pp. 3010–3014, 2002. https://doi.org/10.1021/jf0115589

[12] Sun, B., Richardo-da-Silvia, J. M., Spranger, I. "Critical factors of vanillin assay for catechins and proanthocyanidins", Journal of Agricultural and Food Chemistry, 46(10), pp. 4267–4274, 1998. https://doi.org/10.1021/jf980366j

[13] Jallali, I., Waffo Téguo, P., Smaoui, A., Mérillon, J.-M., Abdelly, C., Ksouri, R. "Bio-guided fractionation and characterization of powerful antioxidant compounds from the halophyte *Inula crithmoïdes*", Arabian Journal of Chemistry, 13(1), pp. 2680–2688, 2020. https://doi.org/10.1016/j.arabjc.2018.06.020

[14] Hatano, T., Kagawa, H., Yasuhara, T., Okuda, T. "Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects", Chemical and Pharmaceutical Bulletin, 36(6), pp. 2090–2097, 1988. https://doi.org/10.1248/cpb.36.2090

[15] Koleva, I. I., Teris, A. B., Jozef, P. H., Linssen, A. G., Lyuba, N. E. "Screening of plant extracts for antioxidant activity: A comparative study on three testing methods", Phytochemical Analysis, 13(1), pp. 8–17, 2002. https://doi.org/10.1002/pca.611

[16] Hajlaoui, H., Trabelsi, N., Noumi, E., Snoussi, M., Fallah, H., Ksouri, R., Bakhrouf, A. "Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and Mentha pulegium) used in the Tunisian folkloric medicine", World Journal of Microbiology and Biotechnology, 25(12), pp. 2227–2238, 2009. https://doi.org/10.1007/s11274-009-0130-3

[17] ADDINSOFT "XLSTAT (2021.5)", [computer program] Availabe at: https://www.xlstat.com [Accessed: 02 Janurary 2022]

[18] Heatmapper "Heatmapper (online free version)", [computer program] Available at: http://www.heatmapper.ca [Accessed: 26 Janurary 2022]

[19] Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., Wishart, D. S. "Heatmapper: Web-enabled heat mapping for all", Nucleic Acids Research, 44(W1), pp. W147–W153, 2016. https://doi.org/10.1093/nar/gkw419

[20] Maina, S., Ryu, D. H., Bakari, G., Misinzo, G., Nho, C. W., Kim, H. Y. "Variation in phenolic compounds and antioxidant activity of various organs of African cabbage (*Cleome gynandra* L.) accessions at different growth stages", Antioxidants, 10(12), 1952, 2021. https://doi.org/10.3390/antiox10121952

[21] Naczk, M., Shahidi, F. "Extraction and analysis of phenolics in food", Journal of Chromatography A, 1054(1-2), pp. 95–111, 2004. https://doi.org/10.1016/S0021-9673(04)01409-8

[22] Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., Legault, J. "Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk", Food Chemistry, 132(2), pp. 943–947, 2012. https://doi.org/10.1016/j.foodchem.2011.11.072

[23] Rodriguez Vaquero, M. J., Alberto, M. R., Manca de Nadra, M. C. "Antibacterial effect of phenolic compounds from different wines", Food Control, 18(2), pp. 93–101, 2007. https://doi.org/10.1016/j.foodcont.2005.08.010

[24] Głoniak, P., Łoś, R., Skalicka-Woźniak, K., Widelski, J., Burczyk, J., Malm, A. "Activity of *Crithmum maritimum* L. (Apiaceae) against Gram-positive bacteria", Annales Universitatis Mariae Curie - Sklodowska, 19, pp. 123–127, 2006.

[25] Najjaa, H., Ben Arfa, A., Doria, E., Boubakri, A., Trabelsi, N., Falleh, H., Tlili, H., Neffati, M. "Phenolic composition of some Tunisian medicinal plants associated with anti-proliferative effect on human breast cancer MCF-7 cells", The EuroBiotech Journal, 4(2), pp. 104–112, 2020. https://doi.org/10.2478/ebtj-2020-0012

[26] Trabelsi, N., Oueslati, S., Falleh, H., Waffo-Téguo, P., Papastamoulis, Y., Mérillon, J. M., Abdelly, C., Ksouri, R. "Isolation of powerful antioxidants from the medicinal halophyte *Limoniastrum guyonianum*", Food Chemistry, 135(3), pp. 1419–1424, 2012. https://doi.org/10.1016/j.foodchem.2012.05.120

[27] Boutellaa, S., Zellagui, A., Öztürk, M., Bensouici, C., Tokul Ölmez, Ö., Menakh, M., Duru, M. E. "HPLC-DAD profiling and antioxidant activity of the n-butanol extract from aerial parts of Algerian *Crithmum maritimum* L.", Acta Scientifica Naturalis, 6(1), pp. 8–16, 2019. https://doi.org/10.2478/asn-2019-0002

[28] Nabet, N., Boudries, H., Chougui, N., Loupassaki, S., Souagui, S., Burló, F., Hernández, F., Carbonell-Barrachina, Á. A., Madani, K., Larbat, R. "Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts", International Journal of Food Properties, 20(8), pp. 1843–1855, 2017. https://doi.org/10.1080/10942912.2016.1222541

[29] Zafeiropoulou, V., Tomou, E. M., Ioannidou, O., Karioti, A., Skaltsa, H. "Sea fennel: Phytochemical analysis of Greek wild and cultivated *Crithmum maritimum* L. populations, based on HPLC-PDA-MS and NMR methods", Journal of Pharmacognosy and Phytochemistry, 9(6), pp. 998–1004, 2020.

[30] Schijlen, E. G. W. M., de Vos, C. H. R., van Tunen, A. J., Bovy, A. G. "Modification of flavonoid biosynthesis in crop plants", Phytochemistry, 65(19), pp. 2631–2648, 2004. https://doi.org/10.1016/j.phytochem.2004.07.028

[31] Jallali, I., Zaouali, Y., Mkadmini, K., Smaoui, A., Abdelly, C., Ksouri, R. "Phytochemistry and antioxidant activities of *Rhus tripartitum* (Ucria) grande leaf and fruit phenolics, essential oils, and fatty acids", Natural Product Communications, 17(4), 1934578X221089110, 2022. https://doi.org/10.1177/1934578X221089110

[32] Dobashi, Y., Hirano, T., Hirano, M., Ohkatsu, Y. "Antioxidant and photo-antioxidant abilities of catechins", Journal of Photochemistry and Photobiology A: Chemistry, 197(2–3), pp. 141–148, 2008. https://doi.org/10.1016/j.jphotochem.2007.12.019

[33] Zhang, Y. M., Rock, C. O. "Evaluation of epigallocatechin gallate and related plant polyphenols as inhibitors of the FabG and FabI reductases of bacterial type II fatty-acid synthase", Journal of Biological Chemistry, 279(30), pp. 30994–31001, 2004. https://doi.org/10.1074/jbc.M403697200

[34] Xu, Y.-Q., Gao, Y., Granato, D. "Effects of epigallocatechin gallate, epigallocatechin and epicatechin gallate on the chemical and cell-based antioxidant activity, sensory properties, and cytotoxicity of a catechin-free model beverage", Food Chemistry, 339, 128060, 2021. https://doi.org/10.1016/j.foodchem.2020.128060

[35] Chen, T., Yang, Y., Zhu, S., Lu, Y., Zhu, L., Wang, Y., Wang, X. "Inhibition of Aβ aggregates in Alzheimer's disease by epigallocatechin and epicatechin-3-gallate from green tea", Bioorganic Chemistry, 105, 104382, 2020. https://doi.org/10.1016/j.bioorg.2020.104382

[36] Lo Scalzo, R. "Measurement of free radical scavenging activity of gallic acid and unusual antioxidants as sugars and hydroxyacids", Electronic Journal of Environmental, Agricultural and Food Chemistry, 9(8), pp. 1360–1371, 2010.

[37] Santhiago, M., Peralta, R. A., Neves, A., Micke, G. A., Vieira, I. C. "Rosmarinic acid determination using biomimetic sensor based on purple acid phosphatase mimetic", Analytica Chimica Acta, 613(1), pp. 91–97, 2008. https://doi.org/10.1016/j.aca.2008.02.050