Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2024, Volume 15, Issue 8, Page 1201-1207

http://www.jmaterenvironsci.com

Impact of growing region on the phenolic compounds and the antioxidant activity of *Crithmum maritimum* L.

Mezni F.*, Slama A., Ayari F., Khaldi A.

[1]*Laboratory of Management and Valorization of forest Ressources, National Research Institute for Rural Engineering, Water and Forestry, University of Carthage, BP 10, 2080 Ariana, Tunisia*

**Corresponding author, Email address: faten-mez@hotmail.com*

Received *30 July 2024,*
Revised *26 Aug 2024,*
Accepted *31 Aug 2024*

Keywords:
- ✓ *Crithmum maritimum;*
- ✓ *Phenols;*
- ✓ *Flavonoids;*
- ✓ *Tannins;*
- ✓ *Antioxidants ;*
- ✓ *Growing area*

Citation*: Mezni F.., Slama A., Ayari F., Khaldi A. (2024) Impact of growing region on the phenolic compounds and the antioxidant activity of Crithmum maritimum L., J. Mater. Environ. Sci., 15(8), 1201-1207*

Abstract: The aim of this study was the determination of the effect of growing area on chemical properties and antioxidant activity of Sea Fennel growing wild in Tunisia. The work was conducted on methanol extract of plants collected from five different sites located in North of Tunisia. Total phenols, flavonoids and tannins content was conducted according to spectrophotometric methods. The antioxidant activity was test using DPPH assay. Results of polyphenolic, flavonoids, tannins and antioxidant activity showed significant differences between the sites studied. Total polyphenolic content ranged from 0.41 to 1.16 g GAE/ml. The values of flavonoids content varied between 0.59 and 2.03 mg RE/ml. The determination of tannins allowed to classify the sites in the following decreasing order: Monastir (2.59 mg EC/g DM), Cap Negro (1.81 mg EC/g DM), Bizerte (0.82 mg EC/g DM) Haouaria (0.77 mg EC/g DM) and Tabarka (0.7 mg EC/g DM). The site of Tabarka holds the most relevant antioxidant activity with a rate of 55.81%.

1. Introduction

In the plant world, phenolic compounds are secondary metabolites mainly known for their antioxidant properties (Nurzyńska-Wierdak, 2023; Fekkar *et al.* 2020; Elmsellem *et al.* 2019). They constitute a large group of more than 8,000 different compounds which are present in almost all plants, in various parts, such as the roots, the leaves, flowers, fruits and seeds, and they protect plants particularly against parasites and UV rays (Wang *et al.* 2016). Their distribution at the tissue level is not uniform. In addition, their concentration and the proportion of each of these polyphenols in plants are affected by multiple conditions (maturity at the time of harvest, soil type, sun exposure, temperature, humidity, etc.) (Baiano and Del Nobile, 2015). Several plants were reported to be important source of natural antioxidants (Elbouzidi *et al.*, 2024; Bouslamti *et al.* 2020; Lourenço *et al.* 2019).

Crithmum maritimum, commonly known as Sea Fennel, is one of these antioxidant rich plants. It is an edible halophyte with various economic interests because of its high secondary metabolite content. Numerous studies investigated the nutritional value of sea fennel as it is considered as a natural and health-promoting food ingredients (Kraouia *et al.* 2023; Pereira *et al.* 2017; Alema *et al.* 2018). Several studies reported that Sea fennel is rich in various bioactive compounds such as phenolic compounds (Houta et al., 2011; Mekinić et al. 2018; Politeo et al. 2023).

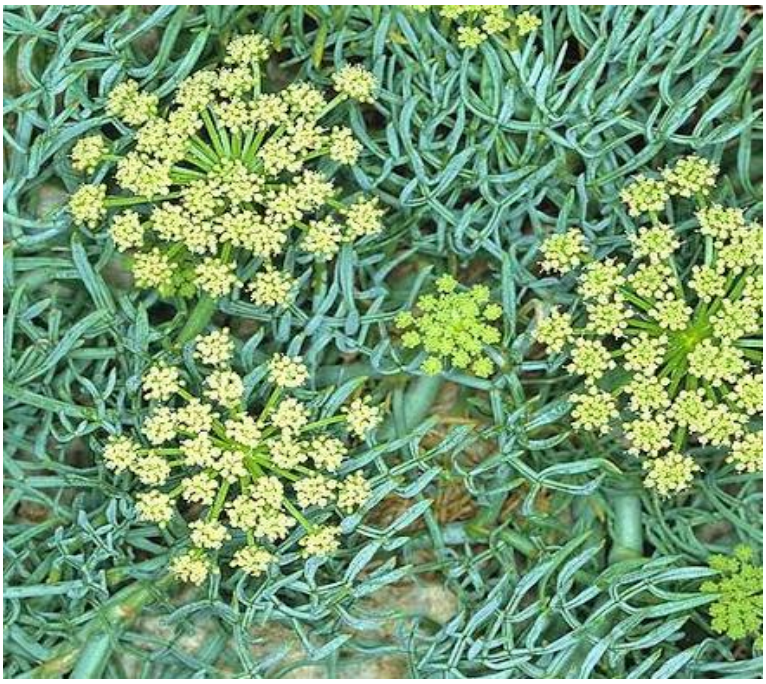

Photo : *Crithmum maritimum*

The biological properties of this plant were also studied. Sea Fennel was reported to have antioxidant (Wang *et al.* 2016; Baiano and Del Nobile, 2015), antibacterial (Amoruso, *et al.* 2022; Souid *et al.* 2022), antifungal (Nabet *et al.* 2016), cytotoxic (Aleman et al. 2018), anticancer (Chen *et al.* 2021), anti-inflammatory (Aleman *et al.* 2018) and antimutagenic (Souid *et al.* 2022), properties.

In this study, we investigated the effect of growing area on chemical properties and antioxidant activity of Sea Fennel growing wild in Tunisia.

2. Methodology

2.1 Plant material and extract preparation

Plants of *Crithmum maritimum* were collected from five different sites located in North of Tunisia during October 2022 (**Table 1**). Whole plants were dried at 40°C and then ground. 5 g of the obtained powder was soaked in 20 ml of solvent (methanol 80%) for 24 hours with intermittent shaking. The extracts were filtered through Whatman filter paper into pill vials. The obtained filtrates were used for the experiments.

2.2 Determination of total polyphenolic compounds

The total phenols content was determined by Folin Ciocalteu method (Singleton and Rossi, 1965). Briefly, 2.5 ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) was added to 2 ml of 7.5% sodium carbonate and 0.5 ml of Sea Fennel extracts. The mixtures were allowed to stand for 30 min

and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25 and 0.5 g/L solutions of gallic acid. The content of phenolics was expressed in mg of gallic acid per mL of extract (mg GAE/g DM).

Table 1. Geographic characterization of the sites of *Crithmum maritimum* samples

Site	Latitude (N)	Longitude (E)	Altitude (m)
Bizerte	37.2744	9.8738	9
Tabarka	36.9644	8.7619	2
Cap Negro	37,1039	8,9819	0
Monastir	35.6667	10.8333	20
Haouaria	37.0534	11.0117	28

2.3 Determination of flavonoids content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method (Quettier Deleu *et al.* 2000). 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg of rutin equivalent per mL of extract (mg RE/g DM).

2.4 Determination of condensed tannins

The method described by Broadhurst and Jones (1978) was used to determine the total condensed tannin content in Sea Fennel plants. 0.5 ml of the extract was mixed with 3 ml of vanillin (4% in methanol) and 1.5 ml of Hydrochloric acid. After incubation for 15 min at 20°C in the dark, the absorbance was read at 500 nm. The condensed tannin content was calculated from a calibration curve prepared with a solution of catechin (30 ppm). The results were expressed in mg of catechin equivalent per mL of juice (mg CE/g DM).

2.5 Free radical scavenging activity

The antioxidant capacity of the studied samples was determined applying the DPPH assay. The DPPH radical scavenging capacity was measured according to by Brand-Williams *et al.* (1995). 5 µl of the extracts was mixed with 5 ml of DPPH solution (0.004%, in ethanol). The reaction mixture was incubated for 30 min at room temperature and the absorbance was read at 517 nm against a blank. The radical scavenging activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = ((1\text{-DO sample})/\text{DO control})) \times 100$$

2.6 Statistical analysis

The statistical analysis was done with the GLM procedure (General Linear Models) of the SAS (9.0) program. Correlations were performed by SPSS.20 program.

3. Results and Discussion

Results of polyphenolic, flavonoids, tannins and antioxidant activity are summarized in **Table 2**. Statistical analysis showed significant differences between the sites studied. Total polyphenolic content

ranged from 0.41 to 1.16 mg GAE/g DM. The highest amount of polyphenols was recorded by plants harvested from Monastir site although the lowest value was determined for Tabarka site. Concerning flavonoids, the values vary between 0.59 and 2.03 mg RE/g DM. Cap Negro is in the lead with a value of 2.03 mg RE/g DM and Tabarka holds the lowest value. The determination of tannins allowed to classify the sites in the following decreasing order: Monastir (2.59 mg EC/g DM), Cap Negro (1.81 mg EC/g DM), Bizerte (0.82 mg EC/g DM) Haouaria (0.77 mg EC/g DM) and Tabarka (0.7 mg EC/g DM). Concerning the antioxidant activity, the site of Tabarka holds the most relevant result with a rate of 55.81%. For the remaining sites, the values are: 51.8% for Cap Negro, 51.03% for Bizerte, 34.62% for Haouaria and 28.55% for Monastir.

The results of total polyphenols, flavonoids and tannins content found in our study are different from those determined, in other studies, for Tunisian Sea Fennel. Houta *et al.* (2015) demonstrated that Sea Fennel extracts contained higher amount of phenols and similar amounts of flavonoids and tannins when compared with our findings. Houta *et al.* (2015) worked on cultivated Sea Fennel plants, while our study was carried out on wild Sea Fennel plants. Several studies highlighted that the total polyphenols and antioxidant activities are higher in the wild plants compared to the cultivated plants (Disciglio *et al.*, 2017; Goławska *et al.*, 2023). It is well known that phenol content varies widely in plants depending on several factors such as the species, the physiological stage, and the growing area (the soil composition and the growth environment). It is also known that plants synthesize a greater amount of these secondary compounds when they are exposed to several types of stress (water, saline, thermal, and nutritional) (European Commission, 2002), and wild plants would generally be subjected to more stress than cultivated plants.

Table 2. Total phenols, flavonoids, tannins and antioxidant activity of Sea Fennel extracts

Sites	Polyphenols (mg GAE/g DM)	Flavonoids (mg RE/g DM)	Tannins (mg CE/g DM)	Antioxidant Activity (%)
Bizerte	0.44	0.77	0.82	51.03
Tabarka	0.41	0.59	0.7	55.81
Cap Negro	0.68	2.03	0.77	51.8
Monastir	1.16	1.82	2.59	28.55
Haouaria	0.56	1.29	0.77	34.62

Sea Fennel extracts exhibited high antioxidant activity. These findings are in accordance with those reported by Pedreiro *et al.* (2023) and by Meot-Duros and Magne (2009).

The role of antioxidants is widely shown in protection against certain diseases due to their possible interaction with many enzymes and their antioxidant properties. Specifically, flavonoids are attributed varied properties: anti-tumor, anti-inflammatory, analgesic, anti-allergic, antispasmodic, antibacterial, hepatoprotective, estrogenic and/or anti-estrogenic. They are also known to modulate the activity of several enzymes or cellular receptors. Flavonoids promote vascular relaxation and prevent the clumping of blood platelets. Therefore, they reduce the clotting of the blood and making it more fluid. They limit the oxidation of blood lipids and contribute to the fight against atherosclerotic plaques. They are also anxiolytic and protect our arteries against atherosclerosis and reduces thrombosis (Sannomiya *et al.* 2005; Ito *et al.* 2005; De Barros *et al.* 2008; Win *et al.* 2008; Gurbuz *et al.* 2009).

Significant differences in the chemical profile were recorded between the studied sites. The variations observed are probably due to numerous factors, notably the climatic and environmental factors

(temperature, altitude, sunshine and precipitation), the geographical area, drought and diseases (Ebrahimi *et al.*, 2008; Andarwulan *et al.*, 2010), the period of samples collection and the stage of development of the plant (Miliauskas *et al.*, 2004; Laita *et al.*, 2024).

Conclusion

The methanol extract of *C. maritimum* showed important amounts of phenolic compounds. The percentage of the different compounds differs from one collection site to another. The antioxidant test revealed that the extracts from the five studied sites exhibit significant anti-radical activity. Therefore, other studies are needed to identify the phenolic compounds responsible of the antioxidant activity of the Sea Fennel, and evaluate how phenolic substances contribute to this activity.

Acknowledgement, this work was carried out with the support of the Ministry of Higher Education and Scientific Research –Tunisia- within the framework of the PRIMA program supported by the European Union. Project title: "Innovative sustainable organic sea fennel (*Crithmum maritimum* L.)-based cropping systems to boost agrobiodiversity, profitability, circularity, and resilience to climate changes in Mediterranean small farms" (acronym: SEAFENNEL4MED) (https://seafennel4med.com/ (accessed on 15 December 2022)).

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References

Aleman A., Marín D., Taladrid D., Montero P., Gómez-Guillén M.C. (2018) Encapsulation of antioxidant sea fennel (*Crithmum maritimum*) aqueous and ethanolic extracts in freeze-dried soy phosphatidylcholine liposomes. *Food Res. Int*., 119, 665–674.

Andarwulan N., Batar, R., Sandrosari D. A., Bolling B., Wijaya H. (2010) Flavonoid content and antioxidant activity of vegetable from Indonesian. *Food chem*., 121, 1231-1235.

Amoruso F., Signore A., Gómez P.A., Martínez-Ballesta M.D.C., Giménez A., Franco J.A., Fernández J.A., Egea-Gilabert C. (2022) Effect of Saline-Nutrient Solution on Yield, Quality, and Shelf-Life of Sea Fennel (*Crithmum maritimum* L.) Plants. *Horticulturae.*, 8, 127.

Baiano A., del Nobile M.A. (2015) Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. *Crit. Rev. Food Sci. Nutr*., 56, 2053–2068. doi: 10.1080/10408398.2013.812059.

Bouslamti, M., Loukili, E.H. ; Elrherabi, A., et al. (2023) Phenolic Profile, Inhibition of α-Amylase and α-Glucosidase Enzymes, and Antioxidant Properties of Solanum elaeagnifolium Cav. (Solanaceae): In Vitro and In Silico Investigations. *Processes*, 11, 1384. https://doi.org/10.3390/pr11051384

Brand-Williams W., Cuvelier M.E., Berset C. (1995) Use of a free radical method to evaluate antioxidant activity, *Food. Sci. Tech*., 28, 25-30.

Broadhurst R.B., Jones W.T. (1978) Analysis of condensed tannins using acidified vanillin. J. Scien. *Food Agricult*., 29, 788–794.

Chen C.Y., Liu C.M., Yeh H.C., Wu H.M., Li W.J., Li H.T. (2021) Flavonoids of *Crithmum maritimum*. *Chem. Nat. Compd*., 57, 917–920.

De Barros M.P., Lemos M., Maistro E. L., Leite M. F., Soosa J. P. B., Bastos J. K., de Andradede SF. (2008) Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. *J. Ethnopharmacol*. 120, 372-377.

Disciglio G., Tarantino A., Frabboni L., Gagliardi A., Michela Giuliani M., Tarantino E., Gatta G. (2017) Qualitative characterisation of cultivated and wild edible plants: Mineral elements, phenols content and antioxidant capacity. *It. J. Agro*., 12, 1036.

Ebrahimi N.S., Hadian, J., Mirjalili, M.H., Sonboli, A. et Yousefzadi, M. (2008) Essential oil composition and antimibacterial activity of *Thymus caramanicus* at different phonological stages. *Food chem*., 110, 927-931.

Elbouzidi A., Taibi M., Laaraj S., *et al.*, (2024) Chemical profiling of volatile compounds of the essential oil of grey-leaved rockrose (Cistus albidus L.) and its antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer activity in vitro and in silico, *Frontiers in Chemistry* 12, 1334028, https://doi.org/10.3389/fchem.2024.1334028

Elmsellem H., El Ouadi Y., Mokhtari M., *et al*. (2019), A natural antioxidant and an environmentally friendly inhibitor of mild steel corrosion: a commercial oil of basil (ocimum basilicum l.), *Journal of Chemical Technology and Metallurgy*, 54, 4, 742-749

European Commission, 2002. Directive of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, 2002/46/EC. In: *Official Journal*, L 183, 12.7.2002.

Fekkar G., Yousfi F., Elmsellem H., *et al.* (2020), Eco-friendly Chamaerops humilis L. fruit extract corrosion inhibitor for mild steel in 1 M HCl, *Int. J. Corros. Scale Inhib.*, 9(2), 446-459, https://doi.org/10.17675/2305-6894-2020-9-2-4

Goławska S., Łukasik I., Chojnacki A.A., Chrzanowski G. (2023) Flavonoids and Phenolic Acids Content in Cultivation and Wild Collection of European Cranberry Bush *Viburnum opulus* L. *Molecules.*, 28(5),2285. https://doi.org/10.3390/molecules28052285

Gurbuz I., Yesilada E., Ito S. (2009). An anti-ulcerogenic flavonol diglucoside from *Equisetum palustre* L. *J. Ethnopharmacol*., 121, 360 -365.

Houta O., Akrout A., Neffati M., Amri H. (2011) Phenolic Contents, Antioxidant and Antimicrobial Potentials of *Crithmum maritimum* Cultivated in Tunisia Arid Zones, *J. Biol. Act. Prod. Nat*., 1, 138-143, DOI: 10.1080/22311866.2011.10719081

Ito C., Itoigawa M., Onoda S., Hosokawa A., Ruabgrungsi N., Okuda T., Tokuda H., Nishino H. Furukawa H. (2005) Chemical constituents of *Murraya siamensis*: three coumarins and their anti- tumor promoting effect. *Phytochem*., 66, 567 -572.

Kraouia M, Nartea A, Maoloni A, Osimani A, Garofalo C, Fanesi B, Ismaiel L, Aquilanti L, Pacetti D. (2023) Sea Fennel (Crithmum maritimum L.) as an Emerging Crop for the Manufacturing of Innovative Foods and Nutraceuticals. Molecules. 28(12), 4741. https://doi.org/10.3390/molecules28124741

Laita M., Sabbahi R., Elbouzidi A., *et al*. (2024) Effects of Sustained Deficit Irrigation on Vegetative Growth and Yield of Plum Trees Under the Semi-Arid Conditions: Experiments and Review with Bibliometric Analysis, *ASEAN Journal of Science and Engineering*, 4(2), 167-190

Lourenço S.C., Moldão-Martins M., Alves V.D. (2019) Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules.* 24(22), 4132. doi: 10.3390/molecules24224132

Meot-Duros L., Magne C. (2009) Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant. Physiol. Biochem*., 47, 37–41

Miliauskas G., Venskutonis P.R., Van Beek T.A. (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extract. *Food chem*., 85, 231-23.

Mekinić I.G., Šimat V., Ljubenkov I., Burčul F., Grga M., Mihajlovski M., Lončar R., Katalinić V., Skroza D. (2018) Influence of the vegetation period on sea fennel, *Crithmum maritimum* L. (*Apiaceae*), phenolic composition, antioxidant and anticholinesterase activities. *Ind. Crop. Prod.*, 124, 947–953.

Nabet N., Boudries H., Chougui N., Loupassaki S., Souagui S., Burló F., Hernández F., Carbonell-Barrachina A., Madani K., Larbat R. (2016) Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts. *Int. J. Food Prop*., 20, 1843–1855.

Nurzyńska-Wierdak, R. (2023). "Phenolic Compounds from New Natural Sources—Plant Genotype and Ontogenetic Variation" *Molecules*, 28, no. 4, 1731. https://doi.org/10.3390/molecules28041731

Pereira C.G., Barreira L., Neng N.D.R., Nogueira J.M.F., Marques C., Santos T.F., Varela J., Custódio L. (2017) Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of *Crithmum maritimum* L. *Food Chem. Toxicol*., 107, 581–589.

Politeo O., Popović M., Veršić Bratinčević M., Kovačević K., Urlić B., Generalić Mekinić I. (2023) Chemical Profiling of Sea Fennel (*Crithmum maritimum* L., *Apiaceae*) Essential Oils and Their Isolation Residual Waste-Waters. *Plants*., 12, 214.

Pedreiro S., Figueirinha A., Cavaleiro C., Cardoso O., Donato M.M., Salgueiro L., Ramos F. (2023) Exploiting the *Crithmum maritimum* L. Aqueous Extracts and Essential Oil as Potential Preservatives in Food, Feed, Pharmaceutical and Cosmetic Industries. *Antioxidants* (Basel).,12(2),252. doi: 10.3390/antiox12020252. PMID: 36829810; PMCID: PMC9952163.

Quettier-Deleu C., Gressier B., Vasseur J., Dine T., Brunet J., Luyck M., Cazin M., Cazin J.C., Bailleul F., Trotin F. (2000) Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol*., 72,35-40.

Souid A.; Della Croce C.M., Frassinetti S., Gabriele M., Pozzo L., Ciardi M., Abdelly C., Ben Hamed K., Magné C., Longo V. (2021) Nutraceutical Potential of Leaf Hydro-Ethanolic Extract of the Edible Halophyte *Crithmum maritimum* L. *Molecules.*, 26, 5380.

Singleton V.L., Rossi J.A. (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Enol. Vitic*., 16, 144-158.

Sannomiya M., Fonseca V.B., Da silva M.A., Rocha L.R.M. Dos Santos L.C., Hiruma-Lima C.A., Britoc A.R.M.S., Vilegas W. (2005) Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. *J. Ethnopharmacol*., 97, 1- 6.

Win N.N., Awale S., Esumi H., Tezuka Y., Kadota S. (2008) Novel anticancer agents, kayeassamins C-I from the flower of *Kayea assamica* of Myanmar. *Bioorg. Med. Chem*., 16, 8653 - 8660.

Wang F., Li Y., Zhang Y.J., Zhou Y., Li S., Li H.B. (2016) Natural products for the prevention and treatment of hangover and alcohol use disorder. *Molecules*., 21, 64. Doi: 10.3390/molecules21010064.