

Review

Environmental eco-physiology and economical potential of the halophyte *Crithmum maritimum* L. (Apiaceae)

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The present contribution reviews information on *Crithmum maritimum* L., a facultative halophyte belonging to the Apiaceae family and typical of coastal ecosystems. It grows wild on maritime rocks, piers, breakwaters and sandy beaches along the Mediterranean, Pacific and Atlantic coasts. Its propagation by germination, vegetative multiplication and the *in vitro* culture techniques is quite easy. Salinities exceeding 50 mM NaCl inhibit seed germination but seem without impact on seed viability. At the vegetative stage, growth was stimulated by low salinity but was markedly reduced at high levels of salt without any symptoms of toxicity. *C. maritimum* L. has been largely used for nutritional and medicinal purposes. The plant is also edible and it is consumed in the traditional diet of the first European farmers, as a potent source of minerals, vitamin C, essential oils and other biomolecules. The fruit of *C. maritimum* L. is rich in lipids (about 44% on dry weight basis) with oleic acid as major component (78.6% of the total fatty acids). All these features make this species one of the most promising halophytes in the context of biosaline agriculture.

Key words: *Crithmum maritimum*, salinity, nutritional and medicinal uses, biosaline agriculture.

INTRODUCTION

Crithmum maritimum L. (Apiaceae) is a halophyte also known as crest marine, marine fennel, sea fennel, sampier and rock samphire (Atia et al., 2009a). It is typical of rocky coastal ecosystems, since it grows wild on maritime rocks, piers and breakwaters and sandy beaches. This aromatic plant grows wild in rock crevices, rocky shores and shingle beaches along the Mediterranean and Black sea coasts, as well as along the Atlantic coast of Portugal and of south and south-west England, Wales and Southern Ireland. This plant also occurs along the coasts of other countries (e.g. Canada) as a naturalized species (Ozcan, 2000; Ben Amor et al.,

2005; Cornara et al., 2009). *C. maritimum* L. shows substantial economical and medicinal potentials: it is edible aromatic and has a powerful scent. Its organs (roots, leaves and fruits) are rich in several bioactive substances that could be used as aromatic, medicinal, antimicrobial and insecticide (Atia et al., 2009a; Meot-Duros and Magné 2009; Meot-Duros et al., 2010). In temperate climates, the plant is used for ornamental decoration in rock gardens along the sea (Franke, 1981). It is also cultivated in many areas across Europe for several economic and industrial purposes (Franke, 1981; Atia et al., 2010a; Meot-Duros et al., 2008).

In the recent years, products of halophytes are more and more produced and sold in the markets through the world (Geissler et al., 2009; Koyro and Lieth, 2008; Koyro et al., 2008). The benefit of herbal drugs has been considered since ancient times (Grabley and Thiericke, 1999; Cornara et al., 2009), and even today many halophytes are investigated for the development of new phytocompounds. The demand from the medicinal and cosmetic industry for essential oils or other products of *C. maritimum* is rapidly increasing (Grigoriadou and

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ABBREVIATIONS: ABA, abscisic acid; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); BA, 6-Benzyladenine; B5, gamborg medium; GA₃, gibberellic acid 3; IBA, indole-3-butyric acid; MS, murashige and skoog medium; MDA, malondialdehyde.

Maloupa, 2008). The objective of this review is to go over and to update our knowledge about the medicinal halophyte *C. maritimum* with a particular emphasis on its eco-physiological responses to saline environment, its economic importance and its potential as a promising candidate for bio-saline agriculture.

CLASSIFICATION

Taxonomy

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class	Magnoliopsida
Subclass:	Rosidae
Order:	Apiales
Family:	Apiaceae
Genus:	Crithmum
Species:	<i>Crithmum maritimum</i> L.

Crithmum: from Greek krite: barley, from resemblance of fruit to barleycorn.

Maritimum: of the sea.

MORPHOLOGICAL DESCRIPTION

C. maritimum L. (Apiaceae) is a highly branched perennial herb of up to 30 to 60 cm in height (Cornara et al., 2009). The root is a strong, thick and gnarled (Franke, 1981). The leaves are fleshy and succulent Figure 1. They extend radially forming a rosette. They have a sheath-like base with the short petiole ending in a pinnate compound blade, which is usually divided into 3 leaflets, each again pinnate. The leaflets are 2 to 5 cm long, 0.6 cm wide and linear to lanceolate with a conical, sometimes spiny tip. From the end of July to mid-August, a stalk up to 30 cm high with 2 or 3 smaller leaves develops from the terminal bud and ends in a compound umbel with 10 to 20 rays, bearing an involucre and involucels, each one consisting of several leaflets.

The species flowers between June and September and the fruit begins to mature in November-December. The flowers are of yellowish or greenish-white colour and produce ovoid fruits which consist of two mericarps each of them subdivided in 5 ribs. The Fruit are 5 to 6 mm long, 1.5 to 2.5 mm large, ovoid-oblong, not compressed, corky and olive-green to purple (Figure 1). At maturation stage, in each groove of the spongy mesocarp, several brown lines are visible. They represent the vittae. The carpophore is also present (Franke, 1981; Atia et al., 2010b).

Cultivation and multiplication aptitude

This species produces high number of viable fruits that

permit their multiplication by germination without any inconvenience. The germination of *C. maritimum* fruit has been reported to be maximal in distilled water (Atia et al., 2009a). The reed light, the nitrate and other nitrogen compounds significantly promote and accelerate germination (Atia et al., 2009b).

Although *C. maritimum* provide a limited number of cuttings, the propagation by softwood cuttings is also possible without any inconvenient. When the cuttings produce sufficient roots, they can be easily transplanted to the main field.

In vitro propagation is a useful technique for mass multiplication and germplasm conservation of any plant species (Kavitha et al., 2010). The multiplication of the *C. maritimum* L. via *in vitro* culture technique has been recently reported (Grigoriadou and Maloupa, 2008). Different culture media have been used for *in vitro* culture of other species of the family of Apiaceae (Hirai et al., 1997). Shoot production of *C. maritimum* was significantly stimulated when shoot tip explants were cultured in MS medium. MS seems to be the most effective of the basal media tested for *in vitro* cultivation of *C. maritimum* as it leads to a significantly increase of number of new microshoots produced / explant (3.4) and enhances shoot height (3.1 cm).

According to Grigoriadou and Maloupa (2008), the B5 medium favours rooting (92.5% of the microshoots develop roots) with and an average of 5.8 roots/explant and 0.6 cm length. When added at 2.5 to 10 μ M, BA significantly increased shoot proliferation. Further, the combination of different IBA concentrations with 2.5 μ M BA significantly improved the rooted microshoots, the number of roots and root length.

ECO-PHYSIOLOGICAL RESPONSES TO SALT STRESS

Germination stage

Despite *C. maritimum* L. usually grows in the vicinity of seawater, salinities exceeding 50 mM NaCl were found to inhibit its germination (Atia et al., 2006; Atia et al., 2009a). In the natural conditions of *C. maritimum* L., fruits are continuously exposed to various ions including; Na⁺, Mg²⁺, Ca²⁺, Cl⁻, and SO₄²⁻. In a recent report, we show that the salt-induced inhibition of seed germination was salt-specific and could be classified in the following decreasing order: MgCl₂, MgSO₄, Na₂SO₄, NaCl. Magnesium salts, that is, MgCl₂ and MgSO₄, restrict germination via their osmotic and ionic effects. At very low osmotic potentials, Mg²⁺ exerted a strong toxic effect that may be explained by the high loss of nutrients from seeds, especially phosphorus, nitrate, sulphate and calcium.

Sodium salts, that is, Na₂SO₄ and NaCl, adversely affected germination mainly via an osmotic effect, since high germination recovery could be observed after seed



Figure 1. General aspect of *C. maritimum*: (A) Aspect of cultivated plant, (B) detailed view of leaves. Note the succulence aspect, (C) inflorescence aspect at the flowering stage, (D) inflorescence view after fruit formation, (E) inflorescence aspect after fruit maturation, (F) and (G) detailed view of the fruit during pre-maturation stage, (H) details view of fruit at maturation.

transfer in distilled water. This suggests that in natural conditions, the plant produces seed banks consisting in seeds that remain viable and germinate after the winter rains, so that the plant can successfully establish (Atia et al., 2011).

A useful approach to overcome the salt-induced seed dormancy observed in halophytes consists in the exogenous application of germination-promoting substances. In this way, nitrate, ammonium, and GA_3 proved to significantly enhance seed germination of *C. maritimum* L. under salinities (Atia et al., 2009a). Interestingly, red light application was also efficient for seed germination induction under salinity (Atia et al., 2009c). It seems

that salt inhibits germination partly by increasing ABA content in seeds. This was confirmed by the fact that the germination was inhibited by exogenous ABA addition in the imbibition medium. This inhibition was alleviated by nitrate (Figure 2). Fluridone, an inhibitor of ABA synthesis, alleviated the salt-induced restriction of germination too (Atia et al., 2009b).

In *C. maritimum* L. fruit, NaCl is mainly accumulated in the external envelopes, that is, the spongy coat, the secretory envelope and the endocarp layer (Atia et al., 2010b). This phenomenon is of vital eco-physiological significance for this halophyte, since it preserves the embryo viability even if the salinity increases in the

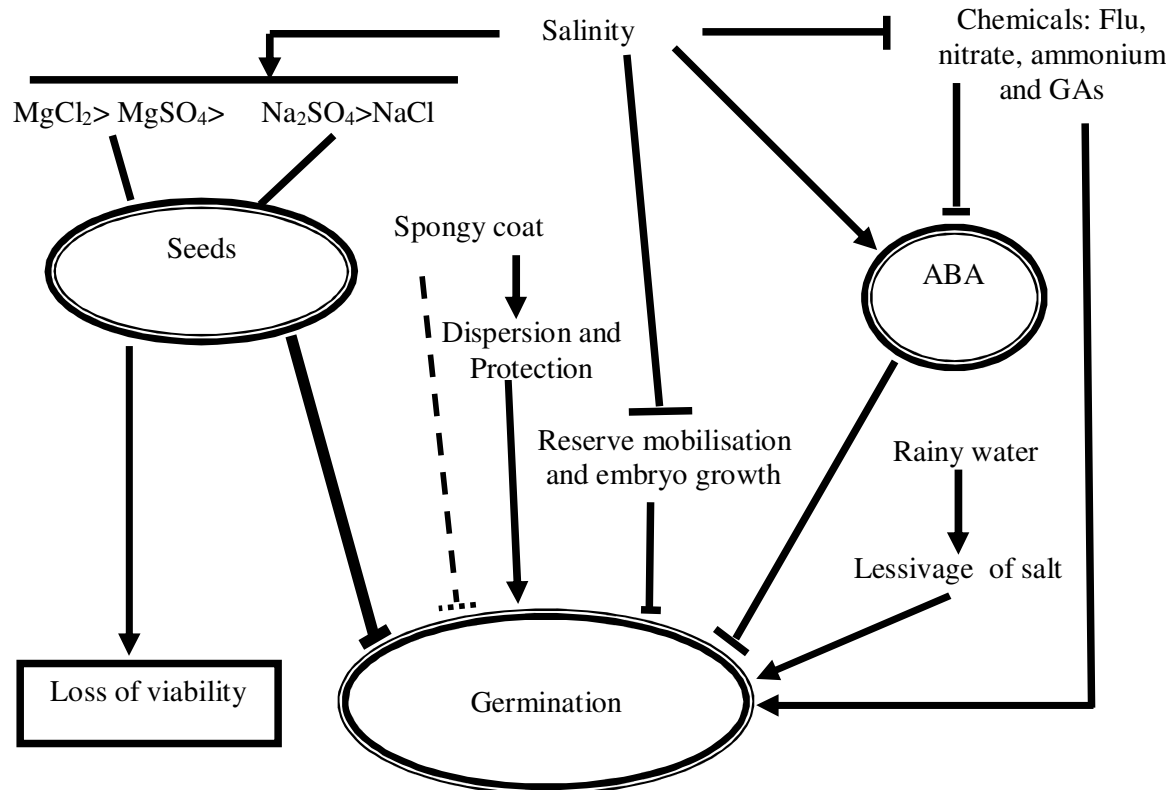


Figure 2. A working model explaining the eco-physiological responses of *C. maritimum* to salinity during germination stage.

medium (Figure 3). Upon salt leaching by winter rains, water imbibition starts and seed germination can take place allowing *C. maritimum* L. to establish in the saline biotopes.

Vegetative stage

Several authors showed that *C. maritimum* L. is a facultative halophyte (Ben Hamed et al., 2004; Ben Amor et al., 2005). According to Ben Hamed et al. (2007), leaf growth was stimulated by 50 mM NaCl, unaffected at 100 mM NaCl and was significantly decreased at 300 mM NaCl but without any toxicity symptoms. Root growth was significantly reduced at 100 and 300 mM NaCl (Ben Hamed et al., 2007) Table 1.

In *C. maritimum* L., accumulating high amounts of Na⁺ and Cl⁻ in leaves had no severe impact on their water status, which is indicative of includer behaviour (Ben Hamed et al., 2004; Ben Amor et al., 2005, 2006). By opposition to excluders halophyte which secrete the salt by specific leaf structure like glands or trichomes, includer halophytes sequester these toxic ions in their vacuoles. Ben Amor et al. (2005) showed that salt treatment reaching 200 mM NaCl did not induce membrane lipid peroxidation since MDA values in both roots and shoots remained close to those control. This

was concomitant with the stimulation of activities of the protective antioxidant enzymes, namely superoxide dismutase (SOD), catalase and peroxidase. Thus, the antioxidative system was efficient to protect the plant tissues against the toxic ions like Na⁺ and Cl⁻ (Ben Hamed et al., 2007) Table 1.

Salt tolerance aptitude of *C. maritimum* L. was also attributed to its ability to maintain potassium supply, a convenient water supply and/or highly capacity to conserve tissue hydration and to exhibit an efficient antioxidant system (Ben hamed et al., 2004). This performance is also related to a set of morphological adaptations including leaf succulence, abundance of palisade parenchyma, and aquifer parenchyma, a thick cuticle layer and a low number of stomata avoiding water loss.

ETHNOBOTANICAL AND MEDICINAL USES

C. maritimum L. has been largely used for nutritional and medicinal purposes. The plant is edible; it was consumed in the traditional diet of the first European farmers, as it is a significant source of minerals. It was cultivated in gardens and was sold on London streets as 'Crest Marine' (Guil-Guerrero et al., 1998). The use of Samphire as a condiment and pickle or as an ingredient in a salad

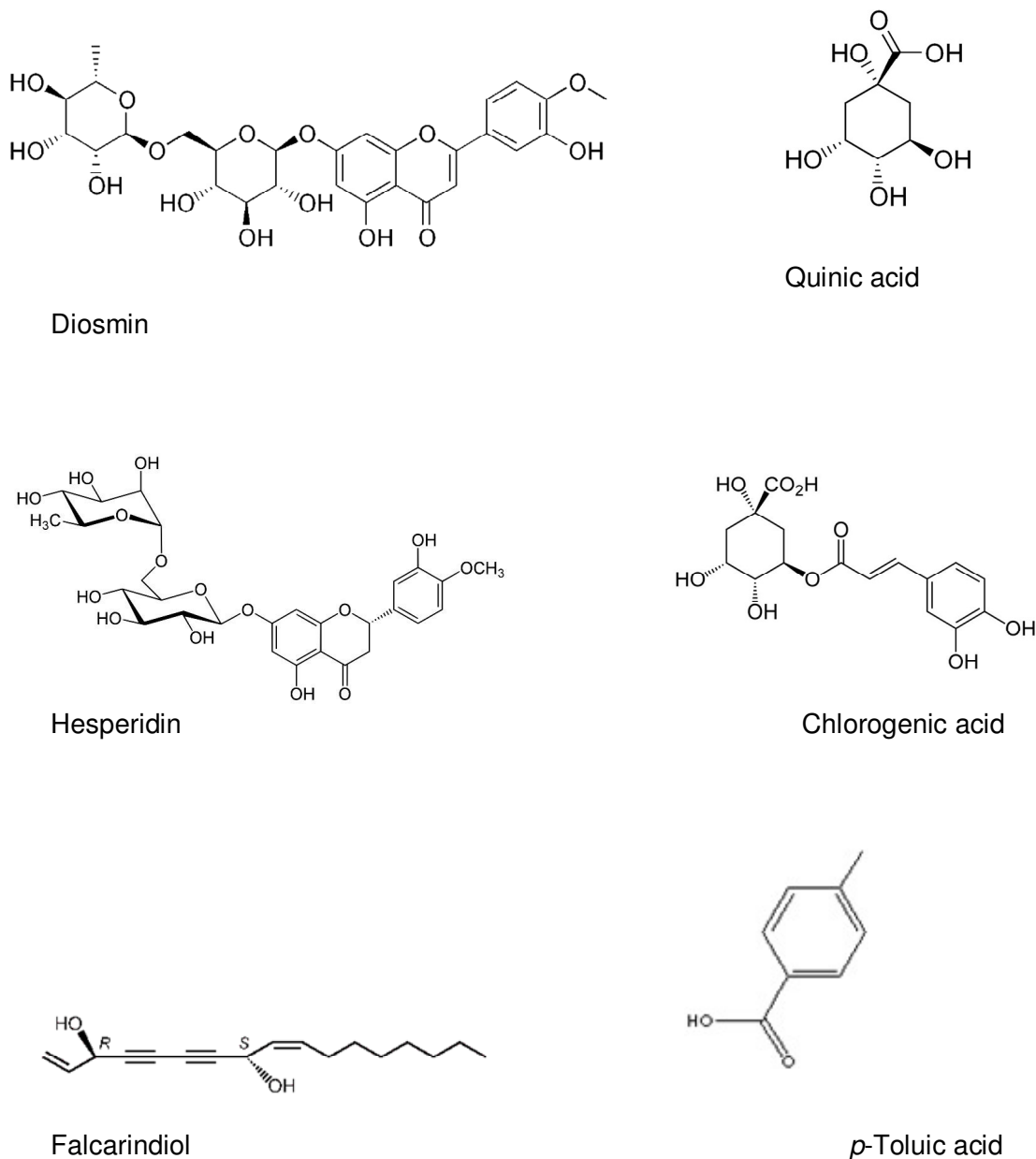


Figure 3. Chemical structure of some biological active compounds found in *C. maritimum* L.

is well known (Atia et al., 2006; Meot-Duros et al., 2009). For instance, in many European countries, the leaves are washed, cut into small pieces and prepared for salads by mixed juice and olive oil. In British Isles, the leaves were formerly pickled and kept like capers in vinegar. Rock Samphire Hash is a traditional British recipe. This was prepared by mixing stems and leaves of *C. maritimum* L. with a pickled cucumber and caper which cooked in stock. Then this was bound with an egg yolk (<http://www.celt.net.org.uk/recipes/ancient/wild-food/entry.php?term>). In a Greek legend, it is even mentioned as a vegetable served to Theseus by Hekate.

Cornara et al. (2009) reported that *C. maritimum* L. is

used in folk medicine as appetizer, tonic, carminative, diuretic and vermifuge. Sailors used to consume food preparations based on *C. maritimum* L. or eat leaves as protection against scurvy (Cunsolo et al., 1993). When going on fishing trips, sailors took fresh leaves with them. But on longer voyages the leaves were apparently kept pickled in vinegar for better preservation. In Italy, the decoction of shoots harvested before fructification were used against inflammations of the urinary tract and prostate and colics. It has tonic and purgative action while the infusion of leaves has been largely used for the digestive diseases and for renal therapy (Franke, 1981; Cunsolo et al., 1993; Guil-Guerrero and Rodriguez-

Table 1. Summary of the eco-physiological salt-responses during the vegetative stage of *C. maritimum* L.

Physiological parameter	Low salinity	Moderate salinity	High salinity	References
Growth	Stimulated	Not affected	Reduced	Ben Amor et al. (2005)
Tissue hydratation	Not affected	Not affected	reduced	Grigoriadou and Maloupa (2008)
Toxic ion accumulation	Accumulated	Accumulated	Highly accumulated	Ben Hamed et al. (2004)
Potassium uptake	Maintained	Maintained	Maintained	Ben Amor et al. (2005)
Photosynthetic activity	Maintained	Maintained	Maintained	Ben Hamed et al. (2004)
Antioxidant systems	Stimulated	Stimulated	Reduced	Ben Amor et al. (2005) Ben Hamed et al. (2007)
Toxicity or tolerance	Tolerance	Tolerance	Tolerance	Ben Amor et al. (2006)

Table 2. Geographical variability in the major volatile compounds from *C. maritimum* L. (adapted from Pateira et al. 1999).

Compounds	Portugal	France	Italy
Sabinene	24.4	-	0.7
α -Pinen	0.2	-	0.1
Cis- β -Ocimen	3.9	-	-
γ -Terpinene	35.0	1%	22.9
4-allylanisol	-	25%	-
Terpinen-4-ol	4.9	-	0.2
Tymol methyl ether	15	-	25.5
Dillapiol	1.5	25%	0.1

Garcia, 1999). In Folk veterinary, the aerial parts were used as food integrator for rabbits and leaves as galactagogue (Cornara et al., 2009). *C. maritimum* L. leaves are rich in several compounds such as vitamin C, caratenoids, flavonoids as well as bioactive substances that could be used for aromatic, medicinal, antimicrobial and insecticide. The oils extracted from leaves showed the presence of high concentrations of fatty acids of the ω -3 and ω -6 series. These fatty acids play an important role in modulating human metabolism and have beneficial effects against coronary heart diseases (Guil-Guerrero and Rodriguez-Garcia, 1999). Recent works by Meot-Duros et al. (2008) revealed that *C. maritimum* L. leaves extract exhibited high phenol content and high ABTS radical scavenging activity. The *C. maritimum* apolar extract had strong antimicrobial activity against *Micrococcus luteus*, *Salmonella arizonae*, *Erwinia carotovora*, *Pseudomonas fluorescens*, *P. aeruginosa*, *P. marginalis*, *Bacillus cereus* and *Candida albicans* (Meot-Duros et al., 2008). Furthermore, *C. maritimum* L. essential oil was studied, both for its antioxidant (Ozcan, 2000) and its antibacterial properties (Ruberto et al., 2000). Essential oils had strong antibacterial action against a large panel of human pathogenic bacteria (Roosi et al., 2007). This is the case of the Gram-positive

bacteria *B. cereus* and *M. luteus* (Glowniak et al., 2006).

PHYTOCHEMISTRY

Volatile compounds

Both shoots and the fruit of *C. maritimum* L. are rich in volatile compounds (Atia et al., 2009). The volatile oil yield reaches about 0.8% in fruits and to 0.15 to 0.3% in leaves (Franke, 1981). The major volatile oils identified consist of sabinene, dillapiole, α -pinen, γ -terpinene (crithmen), p-cymol, apiole, cis- β -Ocimene, thymol and terpinen-4-ol (Table 2). Other less abundant volatile compounds were also found in *C. maritimum* L. such as: α -Thujene, Camphene, α -Phellandrene, Limonene, Cineole, trans- β -Ocimene, trans-2-Ovten-1-ol, Terpinolene, Linalool, trans-*p*-Menthen-1-ol, and Myristicin. In leaves, the percentage of the major volatile oils is highly variable: e.g. α -pinen (0.8 to 1.2%), sabinene (33 to 40%), myrcene (1.6 to 1.8%), α -terpinene (1.1 to 2.2%), p-cymene (3.7 to 9.3%), cis- β -Ocimene (2 to 2.7%), γ -terpinene (22.3 to 28%), terpinen-4-ol (5 to 7.3%), the thymolmethylether (12.9 to 15.5%) and dillapiole (1.1 to 3.1%) (Pateira et al., 1999). Concerning

the fruits, it has been reported that they contain approximately 8 to 40% dillapiole, 12% α -pinen and up to 48% γ -terpinene (Franke, 1981).

Antioxidants, polyphenols and flavonoids

C. maritimum L leaves are rich in several compounds such as vitamin C, caratenoids, and flavonoids (Guil-Guerrero and Rodriguez-Garcia, 1999). Quantitative analyses of the content of flavonoids, tannins and total polyphenols in the aerial parts of *C. maritimum* L., showed that the content of flavonoids was 0.08 to 0.42%.

The tannin content ranged from 0.10 to 2.65%, while the content of total polyphenols varied from 4.72 to 9.48%. The highest contents of flavonoids, tannins and total polyphenols were found in the samples collected before flowering and at the beginning of flowering (Males et al., 2003). The fruit of *C. maritimum* L. was by high accumulation of polyphenols. For instance, the endocarp layer accumulated *O*-dihydroxyphenols (Atia et al., 2009 b).

The chlorogenic acid, a phenolic compound with high radical-scavenging activity, was also found in *C. maritimum* aerial parts (Meot-Duros and Magné, 2009) Figure 3. The same authors identified the quinic acid, another important phenolic compound. Quinic acid is a cyclic polyol. It is produced synthetically by hydrolysis of chlorogenic acid. This acid is used for the fabrication of Tamiflu, a medicament for the treatment of influenza A and B strains (<http://www.quinine-buchler.com/quinicacid.htm>). The aromatic ether *O*-geranylvanillin 3 was also isolated from *C. maritimum* L. (Cunsolu et al., 1993). There is also evidence of some crithmic acid (*p*-toluyl acid). In addition, polyacetylene compounds such as falcarinon have been found in *C. maritimum* L. (Hegnauer, 1973; Franke, 1981).

Recently, the occurrence of two bioactive flavonoids was reported in *C. maritimum* L. Diosmin (3', 5, 7-trihydroxy-4'-methoxyflavone 7-rutinoside) and hesperidin (3', 5, 7-trihydroxy-4'-ethoxyflavanone 7-rhamnoglucoside) (Figure 2a and b) (Cornara et al., 2009). Meot-Duros et al. (2010) purified the falcarindiol from the leaf apolar extract of *C. maritimum* L. Falcarindiol is an antibacterial and cytotoxic compound with multiple biological activities, such as anti-inflammatory, antiplatelet-aggregatory and antimutagenic properties (Miyazawa et al., 1996; Christensen and Brandt, 2006). Falcarindiol extracted from *C. maritimum* L. strongly inhibited the growth of *M. luteus* and *B. cereus*. Moreover, this compound showed cytotoxicity against IEC-6 cells (Meot-Duros et al., 2010).

Lipids

The oils extracted from *C. maritimum* L. leaves showed the presence of high concentrations of fatty acids of the ω -3 and ω -6 series (Guil-Guerrero and Rodriguez-

Garcia, 1999). On the dry weight basis, their percentage reaches 2.02% for neutral lipids, 0.57% for the glycolipids and 0.26% for the phospholipids. In the fruit, the percentage of lipids reaches 44.4% on the dry weight basis. *C. maritimum* L. fruit oil was also rich with oleic acid (78.6%), low level of palmitic acid (4.8%) and non negligible amount of linoleic acid (15.4%) (Atia et al., 2010a). This composition is similar to olive oil and canola oil. These results confirm the good quality of *C. maritimum* L. oil.

Others

Several water-soluble compounds were observed in *C. maritimum*. Among these solutes, the carbohydrates namely sucrose and glucose, followed by organic acids like malate and quinate (Meot-Duros and Magné, 2009). *C. maritimum* L. leaves is a significant source of minerals; hydrochlorates, sulphates, carbonates, potash, acetate, iodine, and bromide (<http://www.aromalves.com>). Other forms of minerals were also found in *C. maritimum* L. fruit like phosphates, calcium, sulphur and sulphured amino acids (Atia et al., 2010b).

CONCLUSION

In this review, we tried to present an updated overview about the halophyte *C. maritimum* L. This plant has been receiving the interest of the scientific community due to its economical and fundamental interests: significant salt tolerance in conjunction with potent medicinal and economic importance. This species can grow in saline land and irrigated with diluted sea water or diluted brackish water. However, during germination and the early growth stages, irrigating with non saline water is needed. It is possible to successfully cultivate this species in saline environments.

By studying the traditional uses and the phytochemistry, we consider that *C. maritimum* L. is a promising halophyte for biosaline agriculture and could be proposed as a new industrial cash crop halophyte.

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