

Preserving and hydrogel-matrixing the bioactive properties of aromatic medicinal halophytic herbs from the coastline of the Iberian Peninsula

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ABSTRACT

Edible wild plants are part of the ethnobotanic heritage of a certain geographical area and are important sources of essential oils, antioxidants, minerals, and special flavours. *Corema album* (Portuguese crowsberry), *Crithmum maritimum* (sea fennel), *Eryngium maritimum* (sea holly), *Helichrysum italicum* (curry plant) and *Otanthus maritimus* (cottonweed) wildy flourish along the sandy dunes of the coast of the Iberian Peninsula. These plants are locally known for their beneficial properties, with important value for food, cosmetics and/or medicinal applications. Hence, leaves of these endemic species were collected at four different locations and submitted to different preserving treatments (oven-drying, freezing, and freeze-drying). Acetonic extracts of the different plants submitted to the different post-harvesting treatments were analysed regarding their antioxidant capacities and phenolic contents. Plant extracts were also analysed by diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY-NMR). In general, freeze-drying was the best method of preserving plant minerals, antioxidants (~4 mgVCEAC/g fw) and polyphenols (~5 mgGAE/g fw). Minerals were quantified via energy-dispersive X-ray fluorescence spectrometry, and despite their location, all plants were rich in Ca, Cl, K, S and P. Hierarchical clustering and principal component analyses (PCA) pointed towards chemical/metabolic proximity between taxonomic families. Alginate hydrogels loaded with 0.1 % and 0.2 % (w/v) of extracts presented homogenous surface properties by scanning electron microscopy, good mechanical tensile strength (~30 MPa) and antibacterial activity against *S. aureus*. Edible alginate hydrogels enriched with plant extracts hold great nutraceutical potential to be used as natural preservatives for food coating and packaging or as sources of bioactive compounds for biomedical applications.

1. Introduction

Angiosperms of the Apiaceae, Asteraceae and Ericaceae families are

flowering plants widely spread and commonly used for ornamental purposes. That is the case of the salt-tolerant species *Corema album* (Ericaceae), *Crithmum maritimum* (Apiaceae), *Eryngium maritimum*

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(Apiaceae), *Helichrysum italicum* (Asteraceae) and *Othantus maritimus* (Asteraceae) that endemically inhabit the sandy dunes and rocky cliffs of the Atlantic coast of the Iberian Peninsula (Parracho, Vaz, Veríssimo, Ribeiro, 2021). These coastline xerophytes and facultative halophytes thrive in saline biotopes composed of dry, sandy, and rocky soils (Chen, Zhao, Yun, Yu, Zhou, Chen et al., 2023). In order to survive, these plants produce secondary metabolites, such as terpenoids and antioxidants, that inhibit the accumulation of reactive oxygen species and protect cellular structures and functions (Chakraborty, Biswas, Dey, Bhattacharjee & Chakraborty, 2023; Chen et al., 2023). Hence, it is essential to characterize these still poorly known salt-tolerant xerophytes and understand their resistance to abiotic stress (intense solar radiation, drought, and salinity), as a way to develop climate-resilient crops and overcome food scarcity (Grigore & Vicente, 2023).

Corema album (*C. album*) is a perennial highly branched shrub with a small pulpy berry (Portuguese crowberry) rich in minerals and antioxidants, such as polyphenols (e.g., phenolic acids, flavonoids, and anthocyanins) (Brito, Bertotti, Primitivo, Neves, Pires, Cruz et al., 2021; Marques, Martin, Amado, Lysenko, Osório, Batista de Carvalho et al., 2021). *C. album* leaves have shown to present beneficial properties by providing cyto-protection against oxidative stress (Martin, Marques, Amado, Barroca, Moreira da Silva, Batista de Carvalho et al., 2020; Silva, Santos, Pinto, Llorent-Martínez, Castilho, Batista de Carvalho et al., 2023). *Crithmum maritimum* (“sea fennel” or “rock samphire”) is an aromatic shrub with woody stems and salty succulent leaves, rich in minerals (Na, K and Ca), fibres, antioxidants, and essential oils (e.g., γ -terpinene, limonene, and sabinene) (Correia, Antunes, Tecelão, Neves, Pires, Cruz et al., 2024; Sunić, Ilić, Stanojević, Milenković, Lalević, Stanojević et al., 2024). *C. maritimum* has shown to have beneficial properties associated with antiscorbutic, digestive, carminative, diuretic, antibacterial, antioxidant, anti-inflammatory, purgative, insecticide and vermifuge effects (Lemoine, Rodrigues, Dauvergne, Cérantola, Custódio & Magné, 2024; Politeo, Ćurlin, Brzović, Auzende, Magné & Mekinić, 2024). *Eryngium maritimum* L. (“sea holly”) is a perennial subshrub rich in essential oils (sesquiterpenes) and antioxidants (Lajnef, Ferioli, Pasini, Politowicz, Khaldi, Filippo et al., 2018) that has been traditionally used as a diuretic, antimicrobial, anti-inflammatory, and antinociceptive herb (Beeby, Magalhães, Poças, Collins, Lemos, Barros et al., 2020). *Helichrysum italicum* is an aromatic shrub (“immortelle”, or “curry-herb”) used to prepare infusions, decoctions, and powders to treat dermatological, respiratory, and digestive problems, given the natural anti-inflammatory, antioxidant and antimicrobial properties presented by the plant material, rich in terpenoids, coumarins and phenolic compounds (Nebrigić, Kljakić, Zengin, Terzić, Mašković & Radojković, 2023; Oliva, Garzoli, Sabatino, Tadić, Costantini, Ragno et al., 2020; Primitivo, Neves, Pires, Cruz, Brito, Rodrigues et al., 2022). *Othantus maritimus* (“cotton weed”) is an aromatic perennial herb with flowers rich in monoterpenes, such as yomogi alcohol, camphor, artemisyl acetate and artemisia alcohol (Basile, Rigano, Sorbo, Conte, Rosselli, Bruno et al., 2013; Ruii, Anzani, Orrù, Floris, Caboni, Maccioni et al., 2013). *O. maritimus* has been traditionally used to treat toothaches, asthmatic bronchitis, and dysentery due to its anti-inflammatory and antimicrobial properties (Basile et al., 2013; Ruii et al., 2013). In addition, beyond producing phytochemicals, plants absorb essential minerals from the soil and concentrate them in their biomass (e.g., Ca, K and Mg). Thus, these plants are the source of important minerals, phytochemicals and bioactive molecules such as essential oils and polyphenols, with relevant properties to be applied in diets, cosmetics, and/or pharmaceuticals.

The beneficial use of plant materials in agrifood, cosmetics and/or pharmaceuticals depends on the ability to preserve and deliver whole products, extracts, nutrients or bioactive molecules. Several methods have been used to preserve and extend the shelf-life of plant materials, which include chemical (e.g., hyperosmotic), thermal (e.g., oven-drying) and/or non-thermal processes (e.g., freezing or freeze-drying). Freezing is considered a fast-preserving method, but beyond being

energetically consuming, food materials contain a high degree of moisture and not all plant materials can be frozen, since most of them suffer “enzymatic browning” (e.g., fruits, vegetables, cereals, aromatic herbs) (Tilley, McHenry, McHenry, Solah & Bayliss, 2023), which lowers their nutritive and organoleptic properties (texture, color, flavor, aroma, cell integrity, turgescence). Drying is one of the most employed methods for preserving medicinal plants, as a way of inhibiting microbial activity, reducing weight and facilitating handling, packaging, and transportation (Nakra, Tripathy & Srivastav, 2025). Heat-drying exposes plants to hot air at a set temperature in air-ventilated ovens, under uniform and controlled conditions, that can be easily monitored and adapted to each plant system. However, even if oven-drying (50–60 °C) and microwave drying (> 60 °C) can inactivate some undesirable enzymatic reactions and microorganisms, and improve textures, these processes can also lead to high degradation of phenolics, antioxidants or other heat-sensitive compounds (Oh, Siow, Lim, 2019). Thus, as a way to avoid excessive temperature, freeze-drying has been considered one of the best preserving methods of medicinal plants and aromatic herbs (Nakra et al., 2025). However, freeze-drying is energetically demanding, time-consuming, and imperfectly preserves certain volatiles and carotenoids (Liu, Zhang & Hu, 2022). Hence, the choice of an appropriate method of preservation depends not only on the plant material, but also on the final processing objectives and delivery systems. Regarding molecular delivery systems, natural polysaccharide-based matrices, such as alginate hydrogels, are outstanding delivery platforms due to their biocompatibility, accessibility, low price and tunable properties (Parracho, Vaz, Veríssimo & Ribeiro, 2023). Alginate-based matrices have been successfully used for a wide range of applications, that vary from food coating and packaging (Sun, Wang, Xiang, Ye & Wan, 2025) to smart materials/biosensing (Ren, Wang, Yang & Li, 2025; Viscusi & Gorrasí, 2024), bioremediation (He, Deng, Yuan, Li, Lin, Deng et al., 2025), drug delivery (Li, Zhang, Wang, Lu, Li, Zhang et al., 2025; Zhang, Zheng, Ma, Nan, Wu, Chu et al., 2025) and tissue engineering (Beaumont et al., 2021; Kaushal & Singh, 2023).

Hence, since different methods must be chosen according to the crops and products to preserve and deliver, the objectives of this work were to: I) evaluate the influence of geospatial factors and of post-harvesting treatments on the mineral profile, phenolic content and antioxidant capacity of leaf extracts of the five xerophytes studied herein, and II) develop and characterize the mechanical and antibacterial properties of alginate-based hydrogels enriched with the herbal extracts. To do so, we have submitted the leaves of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimus* (collected from different dune systems along the Portuguese west coast) to three different post-harvesting treatments (60 °C oven-drying, -20 °C freezing and -50 °C freeze-drying) and analysed the resulting plant materials and extracts in terms of antioxidant capacity (AC), total phenolic content (TPC) and mineral profile in Ca, Cl, Cu, Fe, K, Mn, P, S and Zn. Hierarchical clustering algorithms and principal component analyses (PCA) were used to correlate differences in minerals, antioxidants and polyphenols between plants, harvesting locations, and post-harvesting treatments. Plant extracts were also characterized by diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY-NMR) and described in terms of their antibacterial and antifungal activity. Alginate-based edible hydrogels enriched with herbal extracts, were also produced. The hydrogels prepared were characterized in terms of mechanical tensile strength, analysed by scanning electron microscopy (SEM) and tested against Gram (+) pathogenic bacteria, such as the pathobiont *Staphylococcus aureus*, responsible for various human/animal infections and food poisoning.

2. Materials and methods

2.1. Plant material

Leaves of *Corema album*, *Crithmum maritimum*, *Eryngium maritimum*,

Helichrysum italicum and *Otanthus maritimus* (Fig. 1) were hand-collected from different beach dunes (soil pH 4.5 to 5.5) of the district of Leiria, Portugal, during September 2021 (average temperature: $\sim 20^\circ\text{C}$ and humidity: $\sim 55\%$): Pedrógão Sul ($39^\circ 54' 43.8''\text{N}$ $8^\circ 57' 16.4''\text{W}$), Pedras Negras ($39^\circ 47' 01.1''\text{N}$ $9^\circ 01' 10.1''\text{W}$), Salgado ($39^\circ 32' 42.7''\text{N}$ $9^\circ 06' 42.7''\text{W}$) and Salir do Porto ($39^\circ 30' 05.8''\text{N}$ $9^\circ 08' 55.9''\text{W}$). Once harvested and taxonomically identified, leaves were cleansed with ultrapure Milli-Q water and submitted to three different preserving treatments: I) oven-drying at 60°C for 48 h, followed by storage (dark room, 20°C ; 50 % humidity), II) freeze-drying at -50°C for 48 h, followed by storage (dark room, 20°C ; 50 % humidity), and III) freezing/storage at -20°C .

2.2. Chemicals

All chemicals were of analytical grade or higher. ABTS ([2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]-diammonium salt), gallic acid, vitamin C and acetone were obtained from Sigma-Aldrich (Merck KGaA, Germany). Folin-Ciocalteu reagent was purchased from Biochem Chemopharma (Nevers, France), while sodium carbonate was obtained from Scharlau (Scharlab, Spain). Isotope-labelled compounds were acquired from Cambridge Isotope Laboratories Inc. (Massachusetts, USA). Ultrapure Milli-Q (Millipore Sigma, Germany) Type 1 water ($< 0.060\ \mu\text{S}/\text{cm}$) was used in all experiments. Sodium alginate (average molecular weight $\sim 200,000\ \text{g}/\text{mol}$, food grade) was purchased from Sosa Ingredients (Barcelona, Spain).

2.3. Micro energy-dispersive X-ray fluorescence

Mineral profiles were determined by micro energy-dispersive X-ray fluorometry ($\mu\text{-EDXRF}$), by making use of a Bruker M4 Tornado

spectrometer equipped with a Rh target X-ray tube anode and Zirconium collimators, as described in previous work (Ribeiro, Cruz, Almeida, Silva, Silva, Silva et al., 2025). Freeze-dried samples were previously reduced to powder in a mortar and converted into pellets (2 cm diameter x 1 mm thickness). Detection was performed with a XFlash® SDD detector with energy resolutions above 145 eV. Absolute quantification of the various elements was performed by making use of the respective method. Determinations were validated through comparison with standards for organic matrices: orchard NIST-SRM1571, poplar leaves GBW07604 and bush branches GBW07603. Results were obtained in $\mu\text{g}\cdot\text{g}^{-1}$ for P, S, Fe, Zn, Mn and Cu and in $\text{mg}\cdot\text{g}^{-1}$ for Ca, Cl and K. Samples were analysed in triplicate of three independent samples.

2.4. Total phenolic content

Acetonic extracts of the various leaves were prepared and tested for the presence of phenolic compounds by the Folin-Ciocalteu assay, as described previously (Pinteus, Neves, Tecelão, Silva, Cruz, Bernardino et al., 2024). Given its efficiency to extract both polar and apolar antioxidants and phenolic compounds, acetone was the solvent chosen to produce the extracts (Primitivo et al., 2022). Samples were obtained after manual crushing in a mortar. All extractions were carried out in a 1:10 ratio (in 70 % v/v acetone) for 2 h under magnetic stirring, at 9000 rpm, 20°C . Samples were filtered through $11\text{-}\mu\text{m}$ -pore qualitative filters (Whatman, UK). Supernatants were used for testing. Small aliquots (250 μL) of plant extracts and standard solutions of gallic acid (0.05, 0.10, 0.20, 0.40 and 0.60 g/L) were mixed with the Folin-Ciocalteu reagent. Sodium carbonate was added to the mixture and allowed to react for 60 min, at 20°C . Absorbances were measured at 750 nm (Varian Cary 50 Scan UV Visible spectrophotometer). Total phenolic content was expressed as milligrams of gallic acid equivalents per g of sample in fresh

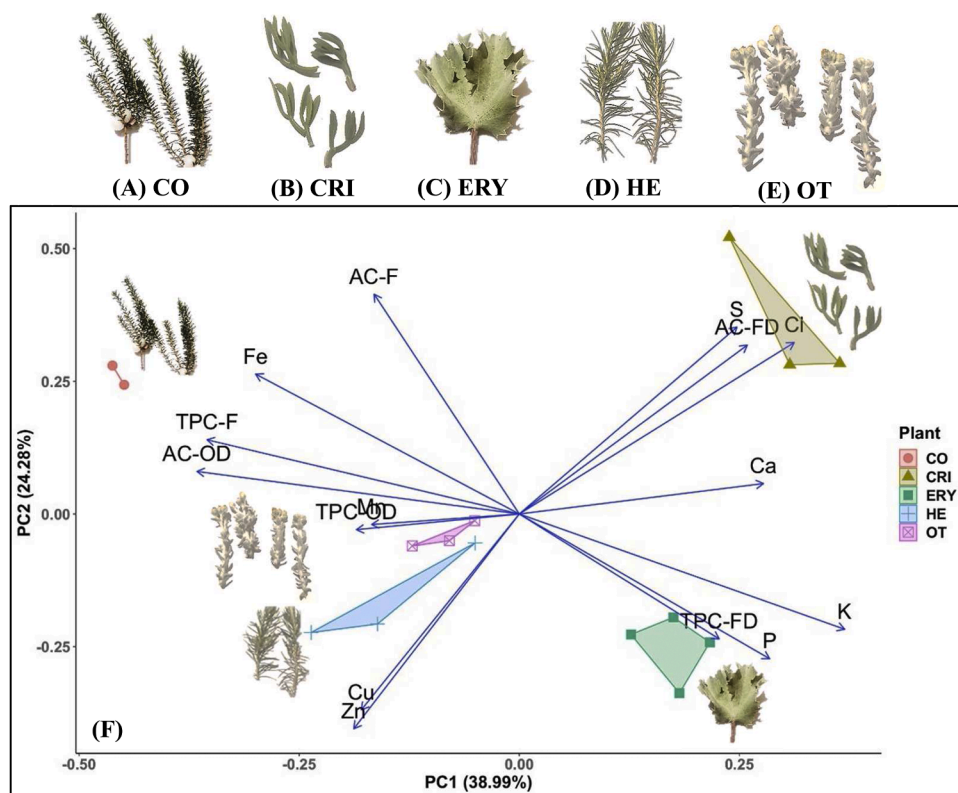


Fig. 1. Leaves of (A) *Corema album* (CO), (B) *Crithmum maritimum* (CRI), (C) *Eryngium maritimum* (ERY), (D) *Helichrysum italicum* (HE) and (E) *Otanthus maritimus* (OT). (F) Principal component analysis (PCA) biplot for the two main components PC1 (38.99 %) and PC2 (24.28 %) of differences in minerals (Ca, Cl, K, P, S, Cu, Fe, Mn, and Zn), antioxidants (AC) and polyphenols (TPC), between plants, harvesting locations, and post-harvesting treatments (freeze-drying – FD, oven-drying – OD and freezing – F).

weight (mg GAE/g fw), by making use of the calibration curve. Samples were analysed in triplicate of three independent samples.

2.5. Antioxidant activity

The antioxidant capacity of the various leaf extracts was evaluated by the ABTS⁺ radical scavenging assay, as described previously (Sebastião, Vaz, Pires, Cruz, Moreno, Brito et al., 2024). Leaf extracts were prepared as described in the preceding section (see 2.4 Total phenolic content). Supernatants were used for testing. Samples (plant extracts and standard solutions) were added to the ABTS solution. After a 6 min-reaction period, absorbances were read at 734 nm (Varian Cary 50 Scan UV Visible spectrophotometer). Ascorbic acid was used as the standard compound for the calibration curve (0.03, 0.05, 0.10, 0.15, 0.20 and 0.25 g/L of Vitamin C). Results report on antioxidant activity expressed in milligrams of VCEAC (Vitamin C Equivalent Antioxidant Capacity) per g of sample in fresh weight (mg VCEAC)/g fw, by making use of the calibration curve. Samples were analysed in triplicate of three independent samples.

2.6. NMR experiments

NMR solutions were prepared by adding freeze-dried plant material to deuterated acetone- d_6 70 % (v/v) followed by overnight extraction and centrifugation at $4000 \times g$ (Heraeus, Waltham, USA). NMR experiments were conducted in a 9.4 T Bruker Avance III 400 spectrometer (Bruker, Billerica, USA) operating at 400.13 (1H) MHz, equipped with a 5 mm high power diffusion BBI probe Bruker DiffBB probe. Pseudo-2D 1H DOSY-NMR experiments were acquired with the longitudinal-eddy-current-delay (LED) sequence and according to the diffusion bipolar pulse pair stimulated echo method (Wu, Chen & Johnson, 1995), with 32 k complex points, 16 scans per increment, and a spectral width of 4562.04 Hz, at 25 °C. Data was obtained with a diffusion delay of 50 ms and 4 ms small delta, linear gradient pulse amplitudes ranging from 2 to 95 % during 2 ms, with a total of 16 steps, and an eddy current delay of 5 ms. Temperature calibration was performed using standards. Data was processed and analyzed with Topspin v4.0 (Bruker) and MestReNova 9.1 (Mestrelab). Chemical shifts were reported in ppm, relative to the methyl group $-CH_3$ 1H (δ = 2.05 ppm) of acetone.

2.7. Antibacterial activity

The antibacterial activity of extracts of *C. album* leaves was evaluated via two-fold broth microdilution assays, as previously described (Correia et al., 2024). Given the broad application of these aromatic and medicinal herbs in diets, cosmetics, and/or pharmaceuticals, four clinically relevant Gram (-) (*Escherichia coli* ATCC 25,922 and *Klebsiella pneumoniae* ATCC 27,799) and Gram (+) (*Staphylococcus aureus* ATCC 29,213 and *Staphylococcus epidermidis* ATCC 12,228) bacteria, associated with human/animal microbiota were chosen as model bacteria for the antibacterial assays. Plant extracts were obtained in solvents with different hydrophobicity, such as petroleum ether, acetone, ethanol and 1:1 ethanol/water, as a way to obtain different families of compounds. Extracts were evaporated and added to Mueller–Hinton broth (MHB) medium at concentrations ranging from 2.00 to 600 $\mu g/mL$. Plates were incubated for 24 h, 37 °C, in the absence and presence of the extracts. Sterility (MHB without inoculation), growth (MHB with inoculation) and quality controls (antibiotic cefotaxime) were also performed. Minimal inhibitory concentration (MIC) was defined as the concentration leading to no microbial growth.

2.8. Antifungal activity

The antifungal activity of extracts of *C. album* leaves was evaluated via two-fold broth microdilution assays. Given the broad application of these aromatic and medicinal herbs in diets, cosmetics, and/or

pharmaceuticals, various clinically relevant fungi (*Candida albicans* ATCC 10,259, *Candida parapsilosis* ATCC 22,019 and *Candida auris* ATCC IEC-01), as well as fungi relevant for the food industry (*Saccharomyces cerevisiae* ATCC 18,824 and a wild strain of *Komagataella phaffii*) were chosen as model fungi for the antifungal assays. *K. phaffii* was obtained from the National Collection of Pathogenic Fungi (NCPF, UK), as described previously (Primitivo et al., 2022). RPMI 1640 synthetic medium, at 2 % (w/v) of glucose, glutamine-supplemented was used for fungal growth at 37 °C (*Candida* strains) and at 28 °C (*K. phaffii* and *S. cerevisiae*), for 24 h. Extracts were obtained and prepared at the same concentrations as described in the previous section (see 2.7 Antibacterial activity). Sterility, growth and quality controls (antifungal fluconazole) were also included. Minimal fungal concentrations (MFC) were considered the concentrations leading to no fungal growth.

2.9. Hydrogel preparation

Alginate-based hydrogels were prepared as described previously (Pereira, Carvalho, Vaz, Gil, Mendes & Bártolo, 2013, 2011). Solutions of edible sodium alginate (1.0 % w/v) were prepared under continuous magnetic stirring (600 rpm). In the case of the alginate-plant enriched hydrogels, acetonic extracts of leaves of *C. album*, *C. maritimum*, *E. maritimum* and *H. italicum* were obtained, completely dried under rotary-evaporation for 12 h and added, as powder, to the alginate solutions, at 0.2 % (w/v). Films were casted into small hexagonal dishes (ϕ = 3.0 cm) and left to dry for 48 h, at 20 °C, 50 % humidity. Dried films were immersed in 5 mL of 2.0 % (w/v) $CaCl_2$ for 5 min, for Ca-reticulation. Calcium cross-linked hydrogels were rinsed with milli-Q water and dried at room temperature, for 48 h.

2.10. Hydrogel characterization

Hydrogel surface properties were analysed by scanning electron microscopy (SEM) using a Tescan, Vega 3. Samples were sputter-coated with gold/palladium for 45 s and visualised with an electron beam of 5 kV at three magnifications (500x, 1000x and 3000x). Hydrogels were subjected to tensile mechanical tests using a texturometer (TA.XTplusC, Stable Micro Systems, UK). Tests were carried out using a 30 kg ($\approx$ 294.20 N) load cell at a speed of 1 mm/min. Samples were prepared in a rectangular shape, 10 mm wide, 40 mm long, with an average thickness of 0.03 mm (N = 3), measured with a digital micrometer (Würth, Germany). The Young modulus was obtained from the elastic region of the graph.

The disk diffusion method was used to test the antimicrobial susceptibility of the hydrogels enriched with the plant extracts. The antimicrobial susceptibility of each alginate extract-enriched hydrogel was tested for each plant separately, as well as a mixture (by joining the various extracts) to reproduce the effect of the consumption/application of herbal mixtures of the aromatic medicinal herbs here under study. Fresh Petri dishes (90 mm diameter) were prepared with autoclaved agar MHB medium. Bacterial suspensions of *Staphylococcus aureus* ATCC 6538 grown in MHB medium were used for plate inoculation (at an optical density of 0.5, according to the Mcfarland scale - 1.5×10^8 bacteria/mL) by means of a sterile swab. Hydrogels were placed on the inoculated agar Petri dishes. Alginate hydrogels enriched with the antibiotic ciprofloxacin (5 $\mu g/mL$) were used as an antibacterial positive control. Non-enriched hydrogels were used as a negative control. Petri dishes were incubated at 37 °C, for 24 h. Microbial growth inhibition was determined by measuring inhibition halos around the discs. All samples and controls were tested in triplicate. Additional antibacterial tests against *S. aureus* in liquid media were also carried out with alginate hydrogels encapsulating the mixture of herbal extracts (of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum*, and *O. maritimum* leaves, each contributing 1:5 to total weight) at three concentrations (0.10 %, 0.15 % and 0.20 % w/v). Microvolumes (100 μL) of each hydrogel were cast into the wells of a 96-well plate and left to dry for 48 h. Dried gels were

crosslinked with 2.0 % (w/v) CaCl_2 , rinsed with milli-Q water and dried again for 48 h. Negative controls (hydrogels with no herbal extract) and positive controls (hydrogels with 5 $\mu\text{g/mL}$ ciprofloxacin) were also included. Each condition was tested in quadruplicate. A small volume (200 μL) of a *S. aureus* culture (Optical density OD_{600} 0.1) was added to each well and incubated for 5 h, at 37 °C. ODs were measured at 600 nm. Results were plotted and normalized relatively to the *S. aureus* control (alginate hydrogel with no herbal extract).

2.11. Statistical analysis

Statistical analyses were performed with the R software. Quantitative variables were analyzed via means and standard deviations. A two-way ANOVA was applied to analyze differences between plants, locations, and post-harvesting treatments, with significant differences $p < 0.01$. Pearson's correlation was used to correlate the antioxidant capacity (ABTS assay) present in the leaf extracts with their total phenolic content (Folin-Ciocalteu assay). A principal component analysis (PCA) was used to relate the tested variables (harvesting location and preservation method) with the mineral profile and content in polyphenols and antioxidants presented by each plant. Hierarchical clustering was carried out to obtain dendrograms based on squared Euclidean distances.

3. Results and discussion

3.1. Geographic location and mineral profile

Plant material (Fig. 1) was harvested from four locations spread along 50 km of the Leiria district coastline (20 to 50 m into the sandy dunes). Locations differed in terms of soil granularity, dryness, altitude, surrounding vegetation, variety of species and number of specimens. Given that plant metabolism depends on P, K, S and Ca as macronutrients ($> 0.1 \text{ mg.g}^{-1}$ dry weight (dw)), and on trace metals such as Cu, Fe, Mn and Zn, as micronutrients ($< 0.1 \text{ mg.g}^{-1}$ dw) (Maathuis, 2009), the mineral profile of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum* leaves was obtained by $\mu\text{-EDXRF}$. Results are shown in Table 1.

C. album (Portuguese crowberry) specimens were found at two locations (Pedrogão Sul and Pedras Negras). *C. album* leaves presented similar amounts of K, P and S within standard deviation, but different amounts of Ca, Fe, Zn and Mn, that could be related to variations in soil composition and levels of abiotic and/or biotic stress. Nonetheless, the mineral profile presented by the leaves is close to the profile previously reported for *C. album* berries which are rich in Ca, Fe and Zn (Brito et al.,

2021). Given the salt-tolerance ability of this halophyte plant, high levels of chloride (Cl) were also detected in *C. album* leaves.

C. maritimum (sea fennel) specimens were found at three locations (Pedrogão Sul, Pedras Negras and Salgado) and varied in terms of mineral profile (Table 1). Likewise, as a salt-tolerant species, *C. maritimum* presented high levels of Cl, but varied in the contents of micro- and macro-minerals. Depending on location, the edible spongy leaves of sea fennel presented different levels of P, S, K and Ca, but also of Fe, Zn and Mn. Since the west coast of Leiria's region is a calcareous Mesozoic basin composed of Fe-podzols sandy soils, different levels of dryness and composition in P, K, Ca, Fe, Zn and Mn will influence the chemical profile of these endemic plants. Yet, despite the edaphoclimatic differences, *C. maritimum* leaves presented a similar profile to the mineral composition of *C. maritimum* aerial parts previously reported (Correia et al., 2024), which are rich in Ca, K, P and S.

E. maritimum (sea holly) is one of the most widely spread halophytes of the sandy dunes of the Iberian Peninsula (Clausing et al., 2000). Sea holly was found at the four harvesting locations (Pedrogão Sul, Pedras Negras, Salgado and Salir do Porto) and regardless of location, the plant material showed a similar profile in macro- and micro-minerals (Table 1), pointing towards a conserved metabolism. Moreover, the mineral profile of *E. maritimum* leaves, here firstly described, is similar to the profile presented by the leaves of *C. maritimum* and *H. italicum*, composed by high amounts of P, S, K, Ca and Cl.

H. italicum (curry plant) specimens were found at three locations (Pedras Negras, Salgado and Salir do Porto) and did not vary greatly in terms of mineral profile (Table 1), except for the plant material collected at a more sloughy sandy soil (Salir do Porto - HE-04). Leaves collected from a waterier soil presented lower levels of P, S, K and Cl, but higher levels of Ca, Fe, Mn and Cu. Even so, the different amounts of minerals found fit within the range of values previously reported for the flowers (Primitivo et al., 2022) and aerial parts (Bianchini, Santoni, Paolini, Bernardini, Mouillot & Costa, 2009) of *H. italicum*.

O. maritimum (cotton weed plant) specimens were found at three locations (Pedrogão Sul, Pedras Negras and Salgado). The mineral profile presented by the *O. maritimum* leaves (here firstly described) is identical to the mineral profile detected for *H. italicum* (also a member of the Asteraceae family), with similar amounts of Ca, Cl, Fe and Zn but with lower amounts of K, P, S, Cu and Mn. Despite the different edaphoclimatic conditions experienced by the plants, these variations may be pointing towards different mineral accumulation propensities exhibited by the two plant species.

Overall, as salt-tolerant species, all plant leaves presented high levels of Cl. Chloride (Cl^-) enters plants through the roots and is mobile within

Table 1
Mineral profile of plant leaves determined by $\mu\text{-EDXRF}$.

Plant	P	S	K*	Ca*	Fe	Zn	Cl*	Mn	Cu
CO-01	114±23.8	356±26.9	0.37±0.005	1.06±0.006	180±2.37	11.7 ± 0.39	0.22±0.005	19.7 ± 0.83	3.7 ± 0.24
CO-02	137±26.0	309±25.0	0.42±0.005	0.49±0.004	125±1.88	9.00±0.33	0.20±0.005	41.6 ± 1.15	2.9 ± 0.20
CRI-01	360±42.4	704±38.3	1.74±0.012	0.91±0.006	46.7 ± 1.50	4.20±0.28	2.42±0.018	20.6 ± 0.98	1.4 ± 0.17
CRI-02	371±43.3	1440±55.2	1.15±0.010	1.12±0.007	64.5 ± 1.72	5.90±0.34	4.00±0.024	12.3 ± 0.80	2.5 ± 0.24
CRI-03	275±49.0	805±54.2	1.90±0.017	1.51±0.010	20.5 ± 1.28	4.96±0.42	2.52±0.025	17.1 ± 1.24	—
ERY-01	551±69.0	455±40.4	1.75±0.015	1.36±0.010	32.3 ± 1.53	12.3 ± 0.63	1.24±0.018	6.37±0.72	4.2 ± 0.40
ERY-02	417±45.6	478±31.4	1.80±0.012	0.95±0.006	31.3 ± 1.12	11.7 ± 0.45	1.36±0.014	5.22±0.48	4.6 ± 0.31
ERY-03	596±71.8	432±39.4	2.20±0.018	1.27±0.010	26.7 ± 1.40	16.8 ± 0.74	0.87±0.015	20.1 ± 1.29	4.4 ± 0.41
ERY-04	500±65.5	349±35.2	1.83±0.015	1.11±0.009	28.9 ± 1.40	10.9 ± 0.57	0.59±0.012	32.5 ± 1.57	4.0 ± 0.37
HE-02	437±61.0	417±38.3	1.37±0.013	0.41±0.005	47.9 ± 1.65	11.2 ± 0.53	0.49±0.011	13.3 ± 0.93	3.4 ± 0.31
HE-03	336±53.8	326±34.1	1.26±0.012	0.50±0.005	31.6 ± 1.34	20.3 ± 0.80	0.41±0.010	27.8 ± 1.34	5.5 ± 0.40
HE-04	286±50.1	293±32.6	1.10±0.011	0.60±0.006	57.2 ± 1.81	23.0 ± 0.75	0.25±0.007	52.8 ± 1.85	7.7 ± 0.47
OT-01	242±45.4	305±32.7	1.04±0.011	0.55±0.006	46.0 ± 1.60	12.0 ± 0.54	0.40±0.010	1.74±0.33	2.9 ± 0.20
OT-02	197±41.0	196±26.3	0.93±0.011	0.58±0.006	38.4 ± 1.45	13.1 ± 0.56	0.34±0.009	2.27±0.38	2.5 ± 0.26
OT-03	164±37.4	168±24.3	0.87±0.010	0.61±0.006	22.6 ± 1.10	12.3 ± 0.54	0.30±0.008	5.31±0.57	4.2 ± 0.34

Values in mean ± standard deviation in $\mu\text{g.g}^{-1}$ for P, S, Fe, Zn, Mn and Cu and mg.g^{-1} (*) for Ca, Cl and K ($n = 3$) in dry weight (dw). CO, *Corema album* leaf extracts, CRI, *C. maritimum*; ERY, *E. maritimum* leaf extracts; HE, *H. italicum* leaf extracts; OT, *Othantus maritimus*. 01- Pedrogão Sul beach dunes; 02- Pedras Negras beach dunes; 03- Salgado beach dunes; 04-Salir do Porto beach dunes.

the plant (White & Broadley, 2001). Active transport dominates Cl⁻ influx at low concentrations, but at high saline concentrations passive Cl⁻ influx also occurs (White & Broadley, 2001), explaining the high amounts of Cl observed for all plants. Moreover, while *E. maritimum* (found at four locations) was the most consistent species in terms of mineral composition regardless of location; *C. maritimum* (found at three locations) was the most variable species, showing different patterns of macro- and microminerals, depending on harvesting location.

3.2. Post-harvesting treatments and antioxidant capacity

In addition to the mineral profile, all plants were evaluated in terms of total phenolic content (TPC) and of antioxidant capacity (AC), upon submission to three different post-harvesting treatments (oven-drying, freeze-drying and freezing). Results are shown in Table 2 and Figure S1. Freeze-drying has shown to be the best method to preserve the antioxidant capacity (AC) and total phenolic contents (TPCs) of *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum* leaves, followed by oven-drying and freezing (Table 2, Figure S1). Although less sustainable, freeze-drying is usually the most effective method of preserving polyphenols and other antioxidants of aromatic and medicinal leaves, as also seen for chia (Dziadek, Kopeć, Dziadek, Sadowska & Cholewa-Kowalska, 2022) and lemon myrtle (Saifullah, McCullum, McCluskey & Vuong, 2019). Freeze-drying promotes the rapid transition from a fresh hydrated state to a vacuum-dehydrated state with minimal water presence, effectively slowing down compound degradation, both

via enzymatic and non-enzymatic reactions (Nakra et al., 2025). Moreover, freeze-drying is also known to increase compound extraction efficiency since it maintains most of the porous structure of plant tissues, easing solvent penetration and the release of bioactive compounds (Nakra et al., 2025).

However, in what concerns herbs and spices, oven-drying can also be an adequate practice. In the case of *C. album* leaves, oven-drying was the post-harvesting treatment leading to higher contents of antioxidants and polyphenols (Table 2 and Figure S1). *C. album* leaves (Fig. 1.A) have thicker cuticles that may require some heat to soften cell walls and promote content release. Moreover, *C. album* can also present more heat-labile compounds such as the terpenoids that compose the plant's essential oils (e.g., limonene and eucalyptol) (Mahanta, Bora, Kemprai, Borah, Lal & Haldar, 2021). Hence, oven-drying at 50/60 °C can increase microbiological safety, inactivate oxidative enzymes, and promote phytochemical bioaccessibility (Nourzad, Naghdi, Kalateh, Mehrafarin & Saeidi-Sar, 2024), without considerably interfering with the bioactive properties of the plant material, as previously seen for the AC and TPCs of oven-dried basil (Sęczyk, Ozdemir & Kołodziej, 2022) or for the volatile compounds and odour of hot air-dried ginger (Yu, Guo & Wang, 2022). Leaves of oven-dried (50 °C, 24 h) *Bambusa vulgaris* also presented higher phytochemical content and antioxidant activity, when compared to other types of bamboo leaves (such as *Bambusa multiplex*, *Bambusa tuldoidea*, *Dinorchloa sublaevigata*, *Gigantochloa levis*, or *Schizostachyum brachycladum*) that were better preserved by freeze-drying (Benjamin, Ng, Saikim & Rusdi, 2022). In what regards freezing (which always maintains a high degree of moisture), even if it can reduce the activity of oxidases and be combined with other methodologies (ultrasounds, high pressure, antifreeze proteins) (Pérez-Bermúdez, Guerra-Valle, Beldarrain-Iznaga, Opazo-Navarrete, Orellana-Palma & Petzold, 2025) it can also induce cell damage due to the formation of ice crystals and to the release and oxidation of polyphenols and antioxidants (Parandi, Pero & Kiani, 2022; Zhao & Takhar, 2017), justifying the lower AC and TPC observed for *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum* leaves. Moreover, the AC and TPC values obtained per plant, harvesting location and post-harvesting treatment presented a positive correlation among them (Pearson's correlation ~0.4), indicating that polyphenols are greatly contributing to the antioxidant capacity of the extracts, as corroborated by the DOSY NMR experiments carried out.

¹H DOSY-NMR spectra were collected for deuterated acetonic (*d*₆-acetone) extracts of the freeze-dried leaves of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum*. A DOSY analysis was carried out to generate pseudo-2D plots of chemical shifts versus diffusion coefficients (Figure S2). Table S1 shows the molecular weights estimated from the diffusion coefficients, according to the Stokes-Einstein Gierer-Wirtz method (SEGWE) for dilute solutions and/or pure solvents (Evans, Dal Poggetto, Nilsson & Morris, 2018). Molecular weight estimations by the SEGWE method are more accurate for small molecules (<1000 g/mol), which typically show diffusion coefficients in the range of 1 × 10⁻¹⁰ to 1 × 10⁻⁸ m².s⁻¹, as seen for the diffusion coefficients presented here (Table S1). The plots confirm the presence of polyphenols and terpenoids as the main compounds present in the acetonic extracts. The NMR signals of the pseudo-2D plots can be assigned to aromatic, hydroxyl and aliphatic protons, associated with diffusion coefficients of low molecular mass compounds/moieties such as isoprene (68.12 g/mol), hydroxybenzene (94.11 g/mol), benzyl alcohol (108.14 g/mol), monoterpenes and sesquiterpenes (130 a 220 g/mol), diterpenes (~300 g/mol), triterpenes (400 to 600 g/mol), as well as of polyphenolic acids, flavanols and flavonols (100 to 400 g/mol). The acetonic extracts of *C. album* leaves were able to extract the aromatic alcohols, hydroxycinnamic acids and sesquiterpenes present in the plant (Brito et al., 2021; Silva et al., 2023), while in the case of *C. maritimum* the extract reported the presence of monoterpenes (e.g., limonene, sabinene and terpinene), polyphenolic acids and flavonoids (Correia et al., 2024; Šunić et al., 2024), and in the case of *H. italicum*, the presence of

Table 2
Antioxidant capacity and total phenolic content per plant, location, and post-harvesting treatment.

Plant	Antioxidant capacity – AC (mgVCEAC/g fw)			Total phenolic content - TPC (mgGAE/g fw)		
	oven-dried	freeze-dried	frozen	oven-dried	freeze-dried	frozen
CO-01	3.58 ± 0.08 ^a	2.26 ± 0.14 ^b	1.01 ± 0.04 ^c	5.91 ± 0.37 ^a	2.81 ± 0.31 ^b	3.66 ± 0.35 ^c
CO-02	3.05 ± 0.10 ^a	1.91 ± 0.07 ^b	1.35 ± 0.16 ^c	5.16 ± 0.01 ^a	2.54 ± 0.40 ^b	3.75 ± 0.01 ^c
CRI-01	0.80 ± 0.04 ^d	8.39 ± 1.29 ^e	0.72 ± 0.24 ^f	0.75 ± 0.03 ^d	3.83 ± 0.28 ^e	0.77 ± 0.01 ^f
CRI-02	1.99 ± 0.24 ^d	6.24 ± 1.13 ^e	1.44 ± 0.07 ^f	3.99 ± 0.73 ^d	4.76 ± 0.46 ^e	0.16 ± 0.01 ^f
CRI-03	0.66 ± 0.06 ^d	5.33 ± 1.10 ^e	0.90 ± 0.13 ^f	0.68 ± 0.02 ^d	4.19 ± 0.29 ^e	0.30 ± 0.01 ^f
ERY-01	0.88 ± 0.07 ^g	2.47 ± 0.08 ^h	0.50 ± 0.01 ⁱ	3.74 ± 1.12 ^g	7.18 ± 0.44 ^h	0.87 ± 0.11 ⁱ
ERY-02	1.33 ± 0.25 ^g	3.33 ± 0.16 ^h	0.74 ± 0.09 ⁱ	4.72 ± 0.31 ^g	9.97 ± 0.85 ^h	0.66 ± 0.06 ⁱ
ERY-03	0.98 ± 0.28 ^g	2.33 ± 0.06 ^h	0.55 ± 0.05 ⁱ	4.66 ± 0.38 ^g	6.30 ± 0.13 ^h	0.68 ± 0.10 ⁱ
ERY-04	1.09 ± 0.19 ^g	2.73 ± 0.09 ^h	0.54 ± 0.19 ⁱ	3.76 ± 0.33 ^g	5.31 ± 0.07 ^h	0.53 ± 0.08 ⁱ
HE-02	3.21 ± 0.09 ^j	3.27 ± 0.13 ^j	0.76 ± 0.24 ^m	1.94 ± 0.25 ^j	3.99 ± 0.08 ^j	0.94 ± 0.10 ^m
HE-03	3.00 ± 0.23 ^j	2.32 ± 0.15 ^j	0.78 ± 0.10 ^m	1.84 ± 0.14 ^j	2.89 ± 0.26 ^j	0.96 ± 0.11 ^m
HE-04	2.32 ± 0.07 ^j	3.29 ± 0.13 ^j	0.96 ± 0.05 ^m	2.20 ± 0.62 ^j	3.60 ± 0.12 ^j	1.20 ± 0.10 ^m
OT-01	1.28 ± 0.39 ⁿ	2.56 ± 1.10 ^o	0.83 ± 0.03 ^p	2.60 ± 0.46 ⁿ	2.87 ± 0.50 ^o	0.73 ± 0.05 ^p
OT-02	2.44 ± 0.15 ⁿ	3.50 ± 1.00 ^o	0.68 ± 0.03 ^p	3.64 ± 0.02 ⁿ	4.92 ± 1.10 ^o	0.73 ± 0.03 ^p
OT-03	2.36 ± 0.08 ⁿ	3.26 ± 0.99 ^o	0.90 ± 0.13 ^p	3.80 ± 0.34 ⁿ	5.54 ± 1.11 ^o	1.16 ± 0.01 ^p

Values in mean ± standard deviation (n = 3). Different letters mean significant differences (p < 0.05). CO, *Corema album* leaf extracts; CRI, *C. maritimum*; ERY, *E. maritimum* leaf extracts; HE, *H. italicum* leaf extracts; OT, *Othantus maritimus*. 01- Pedrógão Sul beach dunes; 02- Pedras Negras beach dunes; 03-Salgado beach dunes; 04-Salir do Porto beach dunes. fw, fresh weight.

terpenoids, curcumins and hydroxybenzoic acids, as described previously (Primitivo et al., 2022). Regarding *E. maritimum* leaves, as expected, the extracts presented monoterpenes, sesquiterpenes and diterpenes (Lajnef et al., 2018; Kowalczyk et al., 2014), whereas the extracts of *O. maritimum* leaves presented terpenoids and hydroxybenzoic acids (Basile et al., 2013; Ruii et al., 2013) (Figure S2).

Statistical analyses, such as a cluster analysis and a principal component analysis (PCA), were conducted to correlate differences in minerals, antioxidants and polyphenols between plants, harvesting locations, and post-harvesting treatments. The PCA scree plot indicates that >75 % of the variance is explained by three main components (Figure S3.A), which validates the adequacy of the PCA. In addition, Table S3 presents the PCA matrix, and Fig. 1 displays the PCA biplot for the two main components (63.3 % of the variance). Despite of harvesting location, the PCA found similarities between the chemical composition of *H. italicum* (Asteraceae) and *O. maritimum* (Asteraceae) leaves, as well as between *C. maritimum* (Apiaceae) and *E. maritimum* (Apiaceae) leaves, pointing towards the metabolic/genetic proximity among taxonomical families, which also supports the apart positioning of *C. album* (Ericaceae) in the top left quadrant. The chemical resemblance between plants of the same family is also pointed out by the hierarchical clustering carried out, which organized the data into two main groups (*C. maritimum* and *E. maritimum* leaves and *H. italicum* and *O. maritimum* leaves) with shorter squared Euclidean distance in the dendrogram (Figure S3). In addition, the minerals present in higher amounts Ca, Cl, K, P and S (right side of the PCA bi-plot) relate to the higher amounts of AC and TPC found in the freeze-dried (FD) plants (reinforcing the preserving efficiency of the freeze-drying method), while the amount of microminerals Cu, Fe, Mn and Zn better relate to the lower quantities of TPC and AC found in oven-dried (OD) and frozen (F) plants.

3.3. Anti-microbial activity of leaf extracts

The antimicrobial activity of extracts of *C. album* leaves was evaluated via antibacterial and antifungal assays. The leaf extracts were obtained by making use of four extracting solvents/solutions: petrol ether (EP), acetone (Ac), ethanol (EtOH) and ethanol:water 50 % (EtOH50). Antibacterial activity was tested against Gram (-) (*E. coli* and *K. pneumoniae*) and Gram (+) (*S. aureus* and *S. epidermidis*) bacteria. Antifungal activity was tested against pathogenic (*C. albicans*, *C. parapsilosis*, *C. auris*) and non-pathogenic (*K. phaffii* and *S. cerevisiae*) fungi. Minimal inhibitory concentrations (MIB) were determined in µg/mL and are displayed in Table S2.

Ethanol (100 % and 50 % ethanol) extracts of *C. album* leaves (after total evaporation and resuspension in MHB medium) were not efficient in inhibiting the growth of the bacteria tested, even at concentrations of 600 µg/mL of extract (Table S2). Conversely, non-polar extracts obtained with petrol ether (EP) and acetone (Ac) were able to inhibit the growth of *Staphylococcus* bacteria, specifically *S. aureus*, (MIC of 150 µg/mL - Ac extract) and *S. epidermidis*, (MIC of 300 µg/mL - Ac and EP extracts). Regarding antifungal activity, some inhibitory properties were observed for the extracts obtained in EP, Ac and EtOH (Table S2), with Ac extracts showing the highest antifungal activity. EP extracts were active against *K. phaffii* (MIC of 150 µg/mL), while EtOH extracts were active against *C. parapsilosis* (MIC, 300 µg/mL), *C. auris* (MIC, 300 µg/mL), *K. phaffii* (MIC, 150 µg/mL) and *S. cerevisiae* (MIC, 300 µg/mL), and Ac extracts were active against *C. albicans* (MIC, 150 µg/mL) *C. parapsilosis* (MIC, 75 µg/mL), *C. auris* (MIC, 75 µg/mL), *K. phaffii* (MIC, 38 µg/mL) and *S. cerevisiae* (MIC, 300 µg/mL).

Regarding *C. maritimum* (sea fennel) leaves, we have previously shown that non-polar extracts obtained in petrol ether inhibited the bacterial growth of *S. aureus* (MIC, 75 µg/mL) and *S. epidermidis* (MIC, 150 µg/mL), as well as the fungal growth of *C. albicans* (MIC, 150 µg/mL), *C. parapsilosis* (MIC, 300 µg/mL), and *K. phaffii* (MIC, 150 µg/mL) (Correia et al., 2024). In addition, other authors reported antibacterial activity of chloroform extracts of *C. maritimum* leaves against

Pseudomonas strains (MIC, 100 µg/mL) (Meot-Duros, Le Floch & Magné, 2008) and of acetonitrile extracts against *E. coli* and *S. aureus* (Jallali, Zaouali, Missaoui, Smeoui, Abdelly & Ksouri, 2014), also pointing towards antimicrobial activity against both Gram (+) and Gram (-) bacteria.

Extracts of the aerial parts of *E. maritimum* have also been previously tested against several bacteria (Meot-Duros et al., 2008; Traversier, Gaslonde, Lecso, Michel & Delannay, 2020). Ethanol (80 % v/v) extracts have shown to be active against Gram (+) bacteria such as *S. aureus* and the *Streptococcus* genus (*Streptococcus bovis*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae*, *Streptococcus pneumoniae*) (Traversier et al., 2020), whereas chloroformic extracts were active against Gram (-) bacteria of the *Pseudomonas* (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*) and *Salmonella* (*Salmonella arizonae*) genera (Meot-Duros et al., 2008).

Concerning *H. italicum* (curry plant), we have previously shown that non-polar and medium-polar extracts of *H. italicum* flowers presented antibacterial activity against Gram (-) (*K. pneumoniae*) and Gram (+) (*S. aureus*) bacteria, along with antifungal activity against *S. cerevisiae* and *K. phaffii* (Primitivo et al., 2022). Additionally, other authors have shown that essential oils and extracts of *H. italicum* leaves showed antibacterial properties against *Bacillus subtilis*, *Micrococcus luteus*, *S. epidermidis*, and *S. mutants* (with MICs varying between 5 and 500 µg/mL), as well as antifungal activity against *C. albicans* and antiviral activity against Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV) (D'Abrosca, Buommino, Caputo, Scognamiglio, Chambery, Donnarumma et al., 2016; Viegas, Palmeira-de-Oliveira, Salgueiro, Martinez-de-Oliveira & Palmeira-de-Oliveira, 2014), pointing towards the extensive and valuable antimicrobial activity presented by the *H. italicum* plant material.

Leaf extracts and essential oils of *O. maritimum* have also shown antibacterial and antifungal properties. Antibacterial activities were detected against Gram (+) species (*Enterococcus faecalis*, *S. aureus*), and Gram (-) species (*Campylobacter jejuni*) (Basile et al., 2013; Muselli, Rossi, Desjobert, Bernardini, Berti, Costa et al., 2007). In turn, antifungal properties were described against *C. albicans* and filamentous phytopathogenic fungi (*Botrytis cinerea* and *Rhizoctonia solani*) (Basile et al., 2013), along with insecticidal and larvicidal activity (Christodouloupoulou, Tsoukatou, Tziveleka, Vagias, Petrakis & Roussis, 2005; Pavela, 2008).

Hence, given the attested antimicrobial properties presented by the aerial parts/leaves of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum*, these plant materials hold great potential to be used as natural preservatives for the food industry or as natural sources of bioactive compounds for cosmetics and/or pharmaceutical applications.

3.4. Biomechanical properties of alginate-enriched hydrogels

Herbs are often used for flavoring and seasoning, while providing color and aroma, and enhancing the bioactive properties of food dishes. Hence, leaves of *C. album*, *C. maritimum*, *E. maritimum*, *H. italicum* and *O. maritimum* could be used, either separately or as an herbal mixture, to enrich alginate-based hydrogels to be applied in the agri-food and/or pharmaceutical sectors. Alginate films can work not only as a support/vehicle to preserve and deliver herbal mixtures/extracts, but also to protect from compound oxidation/degradation, while enriching the nutraceutical properties and extending the shelf-life of food products (Nath, Sharma, Debnath, Sharma, Inbaraj, Dikkala et al., 2023). Thus, we have prepared several alginate hydrogels, loaded with extracts at 0.2 % (w/v) of the different plant leaves studied herein. The hydrogels exhibited thickness (~30 µm), flexibility and transparency levels similar to alginate hydrogels enriched with *Aloe vera* extracts, as previously reported by Pereira et al. (Pereira et al., 2011, 2013). Given that the texture and mechanical properties held by the hydrogels are relevant for their applicability, we have analysed their surface properties and mechanical strength by scanning electron microscopy (SEM) and tensile

tests (Fig. 2), respectively.

In terms of surface, the enriched hydrogels presented a similar homogenous pattern to simple alginate hydrogels, as judged by the SEM images obtained at different magnifications (3000x, 1000x and 500x) (Fig. 2). Alginate hydrogels, with no extracts, showed a smooth, non-porous surface, with few defects, while extract-loaded hydrogels showed a more irregular surface with some particles. These particles could be undissolved material or result from extract aggregation upon drying, as previously seen for alginate hydrogels containing *Betula utilis* (Ishfaq, Khan, Khalid & Asghar, 2023), and *Hibiscus sabdariffa* L. extracts (Aydin & Zorlu, 2022).

Regarding mechanical strength, the dried hydrogels enriched with the herbal extracts showed good tensile mechanical properties (Fig. 2), with a breakage resistance of 27.8 ± 3.5 MPa, higher than the control (21.4 ± 3.5 MPa) due to their higher density. The plant material is not cross-linked to the hydrogel's network, so the extracts can work as plasticizing material, explaining the lower elasticity(E)/Young's modulus of 1.61 ± 0.08 GPa obtained, compared to control (2.09 ± 0.19 GPa). Nonetheless, these elasticity and tensile strength (TS) values are comparable to those previously reported for alginate films (TS, 35 MPa; E 1.9 GPa) of similar thicknesses (~ 20 μ m) (Ureña, Carullo, Phung, Fournier, Farris & Lagorce, 2024), as well as for antibacterial polyvinyl alcohol films (TS, ~ 40 MPa; E, ~ 2.3 GPa; thickness, 120 μ m) (Sapalidis, Sideratou, Panagiotaki, Sakellis, Kouvelos, Papageorgiou et al., 2018), developed as coating agents for food packaging, and polyethylene glycol (PEG)-bovine serum albumin (BSA) hydrogels (E, 2.2 GPa; thickness, 160 μ m) developed for biomedical applications (Coelho, Paiva, Almeida, Jesus, Marteleira, Ramos et al., 2025).

Hence, the mechanical properties (tensile strength and Young's modulus) shown by the hydrogels reported herein are ideal for applications that require flexibility and/or mechanical durability. The tensile strengths presented by the dried hydrogels, not only fit within the expected range for alginate-based matrices (Ji, Park, Oh, Nguyen, Shin, Kim et al., 2022; Pereira et al., 2013) but are also adequate to produce a brittle thin edible barrier (~ 30 μ m) of biocompatible and biodegradable material for food coating or packaging. Dried alginate-based edible films incorporating various functional compounds have already been successfully applied in the coating of fresh-cut fruits, mushrooms, cheese, fish, poultry, pork and beef, by delaying deterioration and extending the shelf-life of these perishable products (Li, Wang, Zhu & Li, 2024).

3.5. Anti-bacterial properties of alginate-enriched hydrogels

S. aureus was chosen as a bacterium of reference to test the antimicrobial capacity of the hydrogels, given the antibacterial activity

presented by all extracts against this pathogen (Table 3). Hydrogels were tested according to the disk diffusion method. Fig. 3 shows the various Petri dishes of *S. aureus*, cultured for 24 h in the absence and presence of the various alginate-enriched hydrogels. Hydrogels carrying the broad-spectrum fluoroquinolone antibiotic ciprofloxacin at 5.0 μ g/mL were used as positive controls of bacterial inhibition (Fig. 3C). Normal widespread *S. aureus* growth was detected on the agar plates with no hydrogels (Fig. 3A), as well as on the plates with alginate hydrogels solely (Fig. 3B), with no extracts. In turn, bacterial growth inhibition was observed for hydrogels loaded at 0.2 % (w/v) with leaf extracts of *C. album* (Fig. 3D), *C. maritimum* (Fig. 3E), *E. maritimum* (Fig. 3F), *H. italicum* (Fig. 3G) and *O. maritimum* (Fig. 3H), when compared with the alginate hydrogel carrying no extract (Fig. 3B). In addition, hydrogels loaded with an herbal mixture of the various plant extracts (Fig. 3I) presented higher levels of bacterial inhibition (inhibition halo – 116 mm), than those observed for ciprofloxacin (inhibition halo – 88 mm) (Fig. 3J). Moreover, additional antimicrobial assays performed on 96-well plates casted with alginate hydrogels encapsulating the mixture of herbal extracts at three concentrations of 0.10 %, 0.15 % and 0.20 % (w/v) were also carried out. Results are shown in Fig. 3K. An inhibition effect of ~ 75 % was observed against *S. aureus*, in the presence of hydrogels encapsulating the herbal extracts. The three concentrations tested produced similar inhibition effects, since the release of compounds was limited to the diffusion rate controlled by the alginate matrix. Interestingly, the lowest concentration of 0.1 % (w/v) was enough to produce an evident inhibition effect in the first 5 h of incubation. This inhibition effect was higher than the inhibitory effect produced by the antibiotic (ciprofloxacin), also corroborating the results obtained with the disk diffusion method. The data indicates that these alginate-enriched films present great potential as new coating systems for food packaging, wound dressing or scaffolds for tissue engineering with antimicrobial and antioxidant properties. Several naturally derived antimicrobial polysaccharide-based hydrogels with activity against Gram (-) (e.g., *E. coli*) and Gram (+) (e.g., *S. aureus*) bacteria have already been developed to be applied in food safety, wound dressing and coating of medical devices (Lam, Clairoux & Udenigwe, 2024; Saqib, Khaled, Liu & Zhong, 2022).

Regarding food technology, various alginate films loaded with herbal extracts have already been developed for compound encapsulation, intelligent packaging, browning control or for carrying bioactive molecules as “functional hydrogels” (Li et al., 2024; Wu, Zhang, Fan, 2024). Edible coatings of alginate-enriched hydrogels with barberry (*Berberis vulgaris* L.) extract have shown to improve the microbial, chemical, and sensory properties of chicken meat (Valizadeh, Aliakbarlu, Kaboudari & Ghorbani, 2023) while alginate films enriched with beetroot (*Beta*

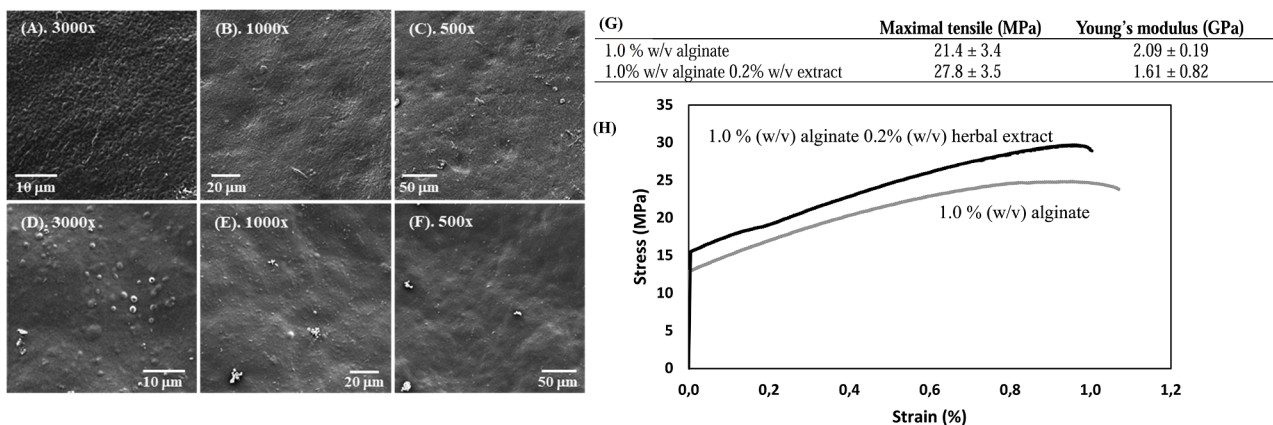


Fig. 2. Biomechanical properties of alginate hydrogels. (A-F) Scanning electron microscopy images at three magnifications (3000x, 1000x and 500x) of 1.0 % (w/v) alginate hydrogels (A-C) and of 1.0 % (w/v) alginate hydrogels enriched with 0.2 % (w/v) plant extract (D-F). (G) Maximal tensile strengths and elasticity modulus obtained from the stress-strain curves. Values in mean $\pm$ standard deviation ($n = 3$). (H) Stress-strain curves of the hydrogels subjected to tensile tests: 1.0 % (w/v) alginate and 1.0 % (w/v) alginate with 0.2 % (w/v) herbal extract.

Table 3

Antibacterial capacity of camarinha, sea fennel, sea holly, curry plant and cottonweed leaves.

Bacteria	<i>C. album</i>	<i>C. maritimum</i>	<i>E. maritimum</i>	<i>H. italicum</i>	<i>O. maritimum</i>
<i>E. coli</i>	–	–	–	–	–
<i>K. pneumoniae</i>	–	–	–	+	–
<i>S. aureus</i>	+	+	+	+	+
<i>S. epidermidis</i>	+	+	n.d.	n.d.	n.d.

Plants: *Corema album* (camarinha), *C. maritimum* (sea fennel), *E. maritimum* (sea holly), *H. italicum* (curry plant), *O. maritimum* (cottonweed). +, susceptible - microbial inhibition observed, –, not susceptible, microbial inhibition not observed, n.d., not determined.

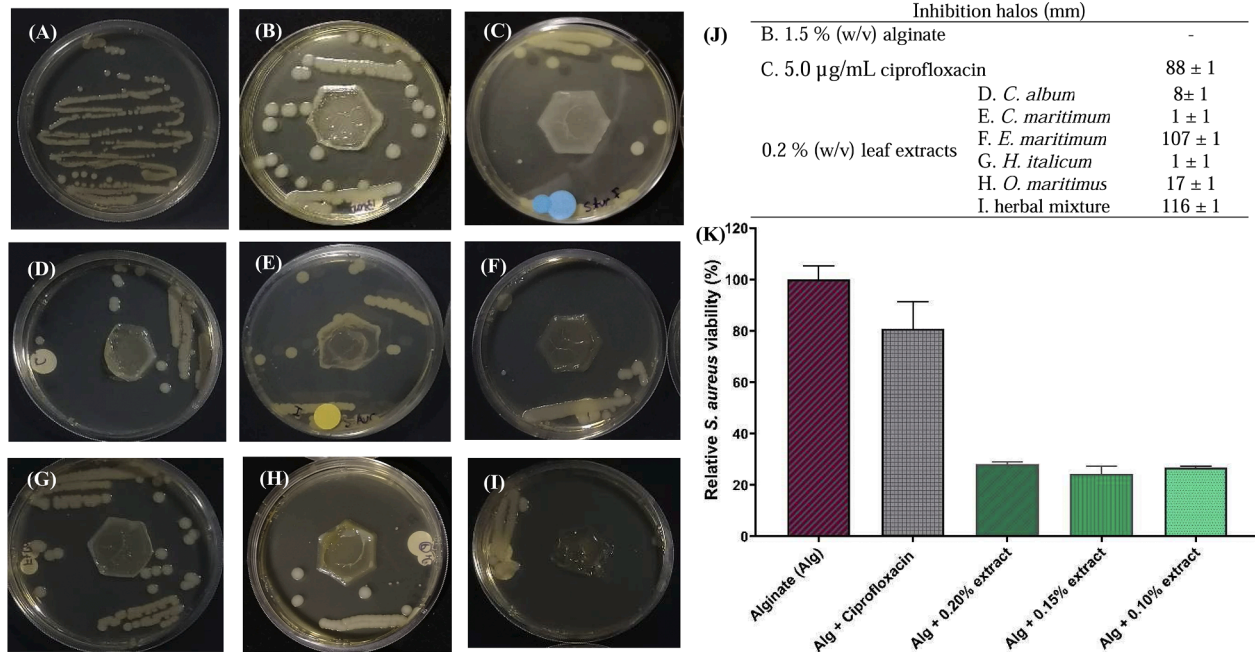


Fig. 3. Antibacterial susceptibility tests against *S. aureus*. Disk-diffusion antibacterial susceptibility tests – 24 h incubation (A–I): *S. aureus* culture (A) with 1.0 % (w/v) alginate hydrogels with no extracts (B); with ciprofloxacin 5.0 µg/mL (C); with 0.2 % (w/v) of extracts of *C. album* leaves (D); with 0.2 % (w/v) extracts of *C. maritimum* leaves (E); with 0.2 % (w/v) extracts of *E. maritimum* leaves (F); with 0.2 % (w/v) extracts of *H. italicum* leaves (G); with 0.2 % (w/v) extracts of *O. maritimum* leaves (H), and with 0.2 % (w/v) extracts of an herbal mixture of the various leaves (I). Inhibition halos obtained from the disk-diffusion antibacterial susceptibility tests (J). Values in mean ± standard deviation ($n = 3$). (K) Results of antimicrobial assays performed on 96-well plates casted with alginate hydrogels with herbal extracts at 0.10 %, 0.15 % and 0.20 % (w/v). Values in mean ± standard deviation ($n = 4$).

vulgaris) extract (Guo, Yuan, He, Zhang, Li, Zhang et al., 2023) presented improved mechanical properties and higher antioxidant capacity, when monitoring pork meat spoilage during storage. In addition, herbal extracts of *Panax notoginseng*, *Bletilla striata* and *Dendrobium officinale* loaded into alginate-based hydrogels were used to effectively treat gastric ulcers (Di, Zhou, Zhou, Di, Wang & Di, 2023), and *Amaranthus spinosus* and *Rubia cordifolia* extracts added to alginate/carrageenan/cellulose hydrogels were used to inhibit bacterial growth (Li, Man, Wang & Zhang, 2023). Also, alginate beads encapsulating *Mentha aquatica* extracts were used for eradicating bacteria and promoting fast regeneration in a murine model of *Pseudomonas aeruginosa* burn infection (Solhtalab, Nikokar, Mojtahedi, Shokri, Karimian, Mahdavi et al., 2024), whereas alginate-hydrogels loaded with oregano oil were used as antibacterial and antioxidant membranes for wound-healing (Iqbal, Ashraf, Nazir, Alshawwa, Iqbal & Ahmad, 2023), and alginate hydrogels loaded with *Scutellaria baicalensis* radix extract (rich in flavonoids such as baicalin, baicalein, and wogonin) were used to treat periodontal diseases in a porcine model (Chanaj-Kaczmarek, Osmalek, Szymańska, Winnicka, Karpiński, Dyba et al., 2021).

4. Conclusions

Salt-tolerant plants endemic to the coastal areas of the Iberian

Peninsula, such as *C. album* (crowberry), *C. maritimum* (sea fennel), *E. maritimum* (sea holly), *H. italicum* (curry plant), and *O. maritimum* (cottonweed), are naturally rich in minerals and bioactive compounds with antioxidant and antimicrobial properties. All these plants present high amounts of Ca, Cl, K, S and P and are rich in terpenoids and antioxidants, such as polyphenols. Freeze-drying and oven-drying have shown to be good methods of preserving the antioxidant/polyphenolic content of these plants/leaves, when compared to freezing. Consistently, according to a PCA analysis, the amounts of minerals present in higher quantities (Ca, Cl, K, P and S) relate to the higher amounts of antioxidants and polyphenols found in freeze-dried plants, while the amounts of microminerals (Cu, Fe, Mn and Zn) relate to the lower amounts of antioxidants and polyphenols found in frozen plants. The antimicrobial properties of the extracts can be transferred into alginate hydrogels, as a way to preserve natural compounds, and transpose antimicrobial properties and antioxidant capacity to other products. Even if these halophytes are not largely cultivated as crops but grow wildly in certain areas of the European coastlines (under variable conditions and soils) their aerial parts can be easily preserved and hold great potential as a source of nutraceuticals. Hence, controlled release studies as well as studies on the shelf life and durability of these hydrogels will need to be carried out in the future, in order to develop coating agents, for intelligent packaging, wound dressing or tissue engineering.

Author's agreement and ethical statement

All authors have seen and approved the final version of the manuscript. The article reports original work developed by the authors, hasn't received prior publication and isn't under consideration for publication elsewhere. This work did not involve the use of animal or human subjects.

CRediT authorship contribution statement

Tiago Parracho: Investigation. **Pedro F. Cruz:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Cláudia C. Peralta:** Investigation, Data curation. **Cândida G. Silva:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Maria Jorge Campos:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Marta Neves:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Rachel Cordeiro:** Writing – review & editing, Methodology, Investigation. **Daniela Trindade:** Writing – review & editing, Methodology, Investigation. **Carla Moura:** Investigation. **Zaida L. Almeida:** Investigation. **Cidália D. Pereira:** Writing – review & editing, Formal analysis, Data curation. **Carla Guimarães:** Writing – review & editing, Formal analysis, Data curation. **Rui M.M. Brito:** Resources, Methodology, Funding acquisition. **Mauro Guerra:** Methodology, Investigation, Formal analysis, Data curation. **Fernando Reboredo:** Writing – review & editing, Methodology, Investigation. **Paula Veríssimo:** Writing – review & editing, Supervision, Investigation. **Vânia Ribeiro:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Daniela C. Vaz:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.fhfh.2025.100239](https://doi.org/10.1016/j.fhfh.2025.100239).

Figure S1. Antioxidant capacity and total phenolic content of acetonic extracts of the plant leaves. Figure S2. Pseudo 2D-DOSY NMR spectra of the acetonic extracts of the plant leaves. Table S1. DOSY-NMR molecular weight estimations of compounds in the acetonic plant extracts. Table S2. Minimal inhibitory concentration of non-polar extracts of *C. album* leaves against bacteria (*E. coli*, *K. pneumoniae*, *S. aureus*, and *S. epidermidis*) and fungi (*C. albicans*, *C. parapsilosis*, *C. auris*, *K. phaffii*, *S. cerevisiae*). Figure S3. Hierarchical clustering and principal component analysis (PCA) matrix between plants, harvesting locations, post-harvesting treatments, minerals, antioxidants and polyphenols.

Data availability

Data will be made available on request.

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