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Study of the Efficacy of Two Extraction Techniques from *Crithmum maritimum* and *Salicornia europaea*

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Abstract The aim of this work was to study the characteristics of the extracts from two halophytes, sea fennel (*Crithmum maritimum* L.) and marsh samphire (*Salicornia europaea* L.) that naturally grow on the Mediterranean coast, by using ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE). Best conditions for UAE were found at temperature 50°C, 20 min extraction time, 1:30 ratio solid:solvent and 40% (v/v) ethanol concentration. For SFE best conditions were at 50°C and 300 bar pressures with 40% (v/v) ethanol concentration. Total extract yield, total phenolic content, total flavonoid content and antioxidant capacity of the extracts were measured. For both species the best results were obtained with UAE technique. Between the two halophytes, the *C. maritimum* extract was the richest one in antioxidant compounds (total phenol = 23.44 mg GAE_s g_{dw}^{-1}); under the best conditions the *S. europea* extract allowed recording only 9.31 mg GAE_s g_{dw}^{-1} of total phenol.

Keywords: *halophyte plant, extraction, SFE, UAE, bioactive compound, antioxidant compounds*

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1. Introduction

Halophytes are plants naturally adapted to saline conditions and represent 2% of terrestrial plants. As defensive strategy to the environmental stress this plant synthesizes secondary metabolites such as phenolic compounds with high antioxidant activity due to their redox properties [1,2]. The economic interest for halophytes plants is steadily increasing; these plants show potential application in food industry, medicine or cosmetic due to their richness in fibres, essential oils and phenolic compounds [1,3]. Different studies in the literature confirmed the health benefits of phyto-chemical compounds derived from halophytes [3,4].

Salicornia europaea and *Crithmum maritimum* are typical halophyte plants of Mediterranean coastline, Black Sea and Atlantic coasts growing up on maritime cliffs and sometimes in sand [3,5].

Sea fennel (*Crithmum maritimum* L.) is used in local cuisine as flavour enhancer of traditional food preparations. Renna et al. [6] optimize a dry system to preserve the qualitative features of sea fennel for food use. For its richness on carotenoids, iodine and phenolic compounds it is used in folk medicine as appetizer, antiscorbutic and diuretic disease [7]. Studies on acetone, chloroformic and methanolic extracts show that sea fennel leaves are rich in phenolic compounds, particularly in chlorogenic acid [5,8,9]. Moreover, the antibacterial activity of sea fennel essential oil against Gram-positive bacteria was reported by Senatore et al. [7].

Marsh samphire (*Salicornia europaea* or *Salicornia herbacea*) is the most widely distributed species in the *Salicornia* genus across the Mediterranean coastline. The genus includes also *Salicornia bigelovii* principally diffused on Korea coasts. *Salicornia* plants are rich in phytosterols, flavonoids, phenolic acids and dietary fibres [10,11,12]. This plant is very popular in folk medicine for anti-inflammatory property and it is usually used as an accompaniment to fish dishes [11]. The seeds oil was particularly high in polyunsaturated fatty acids, mostly linoleic acid. Kang et al. [10] demonstrated the antioxidant and cytotoxic effects against colon cancer cell of extract fractions from seeds of *Salicornia herbacea*. Moreover, Kim [12] studied the antioxidant activity of *Salicornia bigelovii* seed extracted with different solvents.

The most commonly used methods for obtaining antioxidant compounds from plants are the extractions with solvents characterized by different polarities; however, these methods (i.e., Soxhlet extraction, maceration and solvent extraction) require considerable volumes of solvent and are expensive and disadvantageous [13,14]. To considerably reduce solvent consumption and accelerate the extraction process, unconventional techniques have been recently investigated [13,15]. Studies on ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) for bioactive compounds extraction by different matrices have been developed in order to abbreviate the extraction time, increase the extraction yield and preclude

the degradation of phenolic compounds [15,16,17]. UAE is an efficient extraction method, which requires low energy and minimum consumption of solvent and has high reproducibility. Low molecular weight compounds, bioactive compounds, aromas, phenols, pigments and antioxidants from various sources have been extracted by this technique [17]. SFE is another fast, efficient and clean method for extraction of natural products from vegetable matrices [18]. To extract specific soluble components from raw materials SFE takes advantage by the gas properties, above their vapour–liquid critical points. Carbon dioxide (CO_2) is the most common gas used as supercritical fluid due to its moderate critical temperature (31.3°C) and pressure (72.9 atm) [16]. Due to the low polarity of supercritical CO_2 against more polar compounds from natural matrices, a co-solvent is also commonly used [16,18]. Various studies on the extraction of essential oils, tocotrienols, alkaloids, phenolic compounds, carotenoids and tocopherols from different food matrices were reported [16,19,20].

While numerous studies on chemical composition of halophytes essential oil and on properties of their extracts obtained by toxic solvents (acetone, methanol and chloroformic) are available, [1,3,8,9], eco-friendly extraction approach like UAE or SFE that use only water or ethanol are not still applied. Therefore, the aim of this work was to compare the extracts of two halophytes (i.e. *Salicornia europaea* and *Crithmum maritimum*) obtained by UAE and SFE, optimizing the extraction conditions. In particular, total extract yield (TEY), total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity were measured by ABTS and FRAP assays to compare the efficacy of each technique and define the best extraction conditions.

2. Materials and Method

2.1. Raw Materials

Aerial parts of marsh samphire (*Salicornia europea* L.) were collected from many plants along the Apulia coast during the summer season. After harvesting the halophyte plants were immediately transported to the laboratory and processed. Plants were selected for absence of visual defects and uniform colour and washed in tap water to remove residuals, dipped for 1 min in chlorinated water (20 ml L^{-1}) and rinsed by immersion in tap water, then dried in vacuum oven (30°C). To obtain flours from dried plants a rotary blade homogenizer was used. To obtain freeze-dried sea fennel (*Crithmum maritimum* L.), the aerial part of plant was randomly collected from many plants along the shoreline in Mola di Bari (Bari, Italy). The fresh material was firstly frozen at -23°C and then it was freeze-dried for 72 hours with a condenser temperature of about -52°C. For freeze-drying (FD), a laboratory freeze dryer (LABCONCO FreeZone® Freeze Dry System, model 7754030, Kansas City, USA) equipped with a stoppering tray (LABCONCO FreeZone® Stoppering Tray Dryer, model 7948030, Kansas City, USA) was used. The dehydrated material was grinded (1 min; 0.78 G) in a blender (Sterilmixer lab, International PBI, Milan, Italy), to obtain the powder.

2.2. Supercritical Fluid Extraction (SFE)

Extractions were performed in triplicate using a laboratory-scale supercritical fluid extractor (Speed SFE-2, Applied Separation, Allentown, USA) and were conducted using the optimal extraction conditions described by Heffernan et al. [21] for brown macroalgae. Nevertheless, in order to optimize the extraction time, an overall extraction curve (OEC; extract yield versus time) was performed at 50°C, 300 bar with carbon dioxide flow of 2 L min^{-1} (4.5 purity degree; Sapio, Monza; Italy) and 10% of ethanol (v/v). In particular, the yield of *Crithmum maritimum* and *Salicornia europaea* extracts were quantified in grams at 10-minute intervals until reaching a balance and thus allowing for the optimum bioactive compound extraction. Furthermore, to improve SFE effectiveness, increased concentrations of ethanol (10, 20 or 40% v/v) were pumped. The obtained extracts were placed overnight in vacuum oven at 30°C. The dried residues were weighed to calculate the total extraction yield (TEY), then dissolved (protected from light) in 20 mL of absolute ethanol and filtered through a 0.45 µm syringe filter (OlimPeak Filters with Nylon Membrane, Teknokroma Analítica, SA. Sant Cugat del Vallés, Barcelona, Spain) before chemical analysis. Total phenolic content, total flavonoid content and antioxidant capacity measured by ABTS and FRAP assays were estimated for all the obtained extracts.

2.3. Ultrasound Assisted Extraction (UAE)

The ultrasound experiments were performed in triplicate using a laboratory-scale ultrasonic bath (CP104, C.E.I.A., Viciomaggio, Arezzo, Italy) according to the experimental conditions optimized by Topuz et al. [22] for red seaweed (*Laurencia obtuse*). At constant conditions of temperature (50°C), ethanol concentration (100%, v/v) and ratio solid:solvent (1:30, w/v), the effect of extraction time (20, 30 and 45 min) was investigated on concentration of bioactive compounds (total phenolics and flavonoids) and antioxidant activity (scavenging capacity against the radical ABTS and the ferric reducing/antioxidant power). Subsequently, the extractions were carried out at 50 °C adding different concentration of extracting solvent (0, 10, 20, 40, 80, 100 v/v ethanol) at a ratio of 1:30 (w/v). Finally, the resultant extracts were dried, recovered and analyzed.

2.4. Chemical Characterization of the Extracts

The Folin-Ciocalteu assay was used to quantify the total phenol content (TPC) as milligrams of gallic acid equivalents (GAE) per gram of sample, using gallic acid calibration curve ($y=103.85x+1.7741$; $R^2=0.999$). For each sample, total phenols were estimated according to the colorimetric method explained by Spinelli et al. [20]. These authors also described the aluminium trichloride method for determining the total flavonoid content (TFC) expressed as milligrams of quercetin equivalents (QE) per gram of sample, calculated by standard curve of quercetin concentrations (400-5 ppm; $R^2=0.999$). A ferric reducing antioxidant power (FRAP) assay was employed to determine

the antioxidant activity of the extracts. The method, disclosed by Benzie and Strain [23], was slightly modified. The FRAP reagent was daily prepared, mixing at 37°C 100 mL acetate buffer (300 mM; pH 3.6), 10 mL TPTZ acid solution (10 mM of 2,4,6-tri[2-pyridyl]-s-triazine dissolved at 50 °C in 40 mM HCl) and 10 mL $FeCl_3$ aqueous solution (20 mM). Hence, after adding 200 µL of properly diluted extract to 3 mL of FRAP reagent and storage for 30 minutes at 37°C, the absorbance was read at 593 nm. Ferrous sulphate ($FeSO_4 \cdot 7\ H_2O$) was used as standard to quantify the antioxidant activity as µmol of $FeSO_4 \cdot 7\ H_2O$ per gram of sample (600-12.5 µM of; R^2=0.997). Finally, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay, as described by Marinelli et al. [24] was carried out to evaluate the scavenging capacity of extracts against ABTS radical. The results were expressed as milligrams of Trolox equivalents per gram of sample (600-5 ppm; R^2=0.999). All chemical data are reported as means ± SD for at least three replications.

2.5. Statistical Analysis

Statistica 7.1 for Windows (StatSoft Inc., Tulsa, USA) was used to evaluate statistically significant differences between samples. One-way ANOVA, followed by a post-hoc Fisher's test, was carried out.

3. Results and Discussion

3.1. Optimization of UAE Time

In order to select the most suitable extraction time, UAE was performed at 20, 30 and 45 min and at constant operating conditions of temperature (50°C), extraction solvent (ethanol 100%, v/v), and ratio solid:solvent (1:30, w/v). Pham et al. [25] reported that over 45 minutes, the extraction time had no significant influence on bioactive compound yields and antioxidant activities. The time was optimized in terms of total extraction yield (TEY), total phenolic and flavonoid contents (TPC and TFC), as well as antioxidant activity using $ABTS^+$ and FRAP assays of the *Crithmum maritimum* extracts (Table 1).

Table 1. Ultrasound-assisted extraction optimization time for *Crithmum maritimum* extract in terms of total extraction yield (TEY), total phenolic and flavonoid contents (TPC and TFC) and antioxidant activity (ABTS and FRAP) at constant conditions of temperature (50 °C) and extraction solvent (ethanol 100%; v/v)

	20 min	30 min	45 min
TEY (mg g_{dw}^{-1})	117.9±9.1^a	108.9±11.2^a	110.5±17.0^a
TPC (mg GAEs g_{dw}^{-1})	3.7±0.3^b	3.4±0.2^a	3.9±0.2^b
TFC (mg QEs g_{dw}^{-1})	3.7±0.5^b	3.1±0.2^a	4.0±0.2^b
ABTS (mg Tes g_{dw}^{-1})	10.2±1.0^a	10.8±1.5^a	10.6±1.6^a
FRAP (µmol$FeSO_4 g_{dw}^{-1}$)	75.1±5.4^b	63.7±2.7^a	73.9±4.8^b

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

As can be seen in the table, when the extraction time varied from 20 to 45 min, little differences were observed among extracts. In particular, when UAE was carried out for 20 and 45 min the bioactive compounds content and the antioxidant capacity of the extracts did not statistically differ. However, it is noteworthy that with an extraction time of 30 min, their values decreased and then re-increased. In effect, Tao et al. [26] found that in the first 10-20 min of extraction the dissolution of the soluble components on the surfaces of the matrix occurs and it is at this stage that up to 90% of the recovery of the total content of the phenolic compounds can be achieved, thus indicating a considerable rapid extraction rate. Probably, the exposure of an ultrasonic irradiation of 30 min caused negative effects like oxidation, degradation, and/or polymerization of the phenolic and flavonoid compounds [27,28,29]. As consequence, the derived secondary compounds could not be estimated by Folin-Ciocalteu reagent assay that, according to several authors, is not specific only for phenols [30,31,32]. Hence, based on the results and previous considerations, 20 min was selected as the extraction time for the subsequent UAE experiments.

3.2. Chemical Characterization of Extracts by UAE

In Table 2 are reported TEY, TPC, TFC, ABTS and FRAP of *Crithmum maritimum* and *Salicornia europaea* extracts obtained by UAE. Extracts were obtained at different ethanol concentrations (0, 10, 20, 40, 80, 100 v/v), and at constant operating conditions of temperature (50°C), extraction time (20 min) and ratio solid:solvent (1:30, w/v). As can be seen from the data listed in the Table 2, for both *Salicornia europaea* and *Crithmum maritimum,* the lowest efficiency of extraction was obtained with the use of pure ethanol. This could be due to the fact that ethanol has higher viscosity than water (0.684 and 0.5465 mPa·s at 50 °C, respectively), which allows not only diffusing with major difficultly into the matrix but also reducing the force of the implosion of the cavitation bubbles [33]. Furthermore, several studies agree that using highly pure organic solvents can lead to the dehydration and collapse of plant cells, as well as denaturation of the proteins of the cell wall, thus making further difficult the extraction of bioactive compounds [34]. Besides, Trabelsi et al. [35] found that the phenolic extraction by aqueous mixtures of ethanol was superior by 14-folds as compared to the same pure solvents. This result was in agreement with the data summarized in Table 2, according to which it is necessary to use an ethanol/water binary mixture in order to improve the extraction of bioactive compounds and to increase the antioxidant activity. The extraction efficiency increased by increasing the percentage of ethanol up to 40% for *Crithmum maritimum* and between 40-80% for *Salicornia europaea*. Rostagno et al. [36] and Li et al. [37] argued that for UAE the presence of water, in an ethanol-water mixture, caused a reduction of solution viscosity and an increase of plant swelling, thus increasing the surface area for solute-solvent contact and mass transfer.

Data listed in Table 2 show that the maximum amount of TEY (391.93-416.10 mg g_{dw}^{-1}) was obtained for *Salicornia europaea* using ethanol at a concentration between 10 and 40%. Similarly, for *Crithmum maritimum* the highest yield value was achieved with 10-40% ethanol (360.20-378.57 mg g_{dw}^{-1}). It is also noted that when UAE was carried out with 40% of ethanol, the *Crithmum*

maritimum extract (Cri_UAE/40%) showed the highest values of TPC (23.44±1.08 mg GAEs g_{dw}^{-1}) and TFC (16.63±0.60 mg QEs g_{dw}^{-1}). As long as *Salicornia europaea* is concerned, data suggest that among marsh samphire extracts, Sal_UAE/80% appeared to be the sample with the highest values (9.31±0.45 mg GAEs g_{dw}^{-1} and 8.72±0.24 mg QEs g_{dw}^{-1}), followed by Sal_UAE/40% (9.31±0.32 mg GAEs g_{dw}^{-1} and 7.84±0.15 mg QEs g_{dw}^{-1}). Data listed in Table 2 also highlight that the antioxidant activity of plant extracts was closely associated to their phenolic content: the extract with the highest antioxidant activity was consistently the extract with the greatest phenolic content. In effect, Cri_UAE/40% showed the highest ability to scavenge ABTS (59.76±3.39 mg Tes g_{dw}^{-1}) and FRAP (453.60±22.47 µmol $FeSO_4$ g_{dw}^{-1}). This correlation is confirmed by numerous previous studies [4,38,39].

Data listed in the table also show that a reduction of bioactive compound concentrations (TPC and TFC) and scavenging capacities (ABTS and FRAP) of extracts was observed with amounts of water greater than 60% (therefore 20% and 10% ethanol) for both halophytes tested in this work. This decrease could be due to the production of free radicals derived from the ultrasound dissociation of water. In effect, Rostagno et al. [36] claimed that due to the induction of oxidative reactions, these species could compete with the target compounds available in the food matrix for extraction, consequently decreasing the extraction efficiency.

The findings of this work suggest that *Crithmum maritimum* extracts were richer in valuable components than *Salicornia europea* extracts when the same extraction conditions are used. It is known that content of polyphenols and other bioactive compounds in halophytes vary widely according to species, location, growing conditions and harvest season of the plants [40,41]. Boestfleisch et al. [42] also demonstrated that altering the saline growing environment it is possible to increase the concentration of valuable secondary metabolites of halophytes, so as to behave like authentic large-scale natural reactors.

Table 2. Total extract yield (TEY), total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity measured by ABTS and FRAP assays for *Crithmum maritimum* (Cri) and *Salicornia europaea* (Sal) extracts by ultrasound-assisted extraction (UAE) at different ethanol concentrations (0, 10, 20, 40, 80 and 100%; v/v).

	TEY	TPC	TFC	ABTS	FRAP
	(mg g_{dw}^{-1})	(mg GAEs g_{dw}^{-1})	(mg QEs g_{dw}^{-1})	(mg Tes g_{dw}^{-1})	(µmol $FeSO_4$ g_{dw}^{-1})
Cri_UAE/0%	324.9±13.8^{c}	15.9±0.4^{f}	10.7±0.2^{h}	39.8±1.6^{e}	267.6±9.7^{g}
Cri_UAE/10%	362.4±8.7^{d}	16.5±0.6^{f}	10.7±0.3^{h}	48.5±1.7^{g}	303.3±4.5^{h}
Cri_UAE/20%	360.2±8.3^{d}	18.7±0.7^{g}	12.7±0.4^{i}	52.9±4.7^{h}	359.0±8.6^{i}
Cri_UAE/40%	378.6±28.6^{d,e}	23.4±1.1^{i}	16.6±0.6^{m}	59.8±3.4^{i}	453.6±22.5^{l}
Cri_UAE/80%	332.4±6.0^{c}	19.2±1.2^{h}	15.8±1.3^{l}	42.7±3.7^{f}	368.4±17.7^{i}
Cri_UAE/100%	117.9±9.2^{b}	3.7±0.3^{b}	3.7±0.5^{c}	10.2±1.0^{b}	75.1±5.4^{c}
Sal_UAE/0%	378.7±12.1^{d,e}	4.9±0.1^{c}	2.7±0.1^{b}	15.1±1.0^{c}	58.7±1.6^{b}
Sal_UAE/10%	416.1±19.2^{f}	5.6±0.3^{c}	4.3±0.2^{d}	16.5±1.3^{c}	76.9±4.9^{c}
Sal_UAE/20%	391.9±5.3^{e,f}	7.7±0.4^{d}	6.1±0.4^{e}	21.6±0.8^{d}	124.6±6.6^{d}
Sal_UAE/40%	405.8±11.9^{f}	9.3±0.3^{e}	7.8±0.2^{f}	22.8±1.2^{d}	143.2±4.0^{e}
Sal_UAE/80%	332.3±12.0^{c}	9.3±0.5^{e}	8.7±0.2^{g}	21.4±1.1^{d}	180.2±9.7^{f}
Sal_UAE/100%	55.4±3.0^{a}	1.3±0.1^{a}	1.2±0.1^{a}	4.5±0.3^{a}	27.4±0.8^{a}

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

3.3. Optimization of SFE time

To optimize the supercritical extraction time, an overall extraction curve (OEC) was performed for *Crithmum maritimum* (Figure 1) and *Salicornia europaea* (data not shown).

In particular, a yield curve in function of the cycle number (1 cycle included 10 min of static condition and 10 min of dynamic phase) was constructed operating at 50 °C, 300 bar and CO2+10% of ethanol (v/v). It was observed that yield increased with the growing time; moreover, for a number of cycles bigger that 8 the yield remained constant. The final yields of *Crithmum maritimum* were ranging between 0.458 and 0.468 mg gdw-1. The same trend was found for *Salicornia europaea*. On the basis of these results, it was established that 8 cycles are the more profitable extraction condition to be used for the other experimental steps.

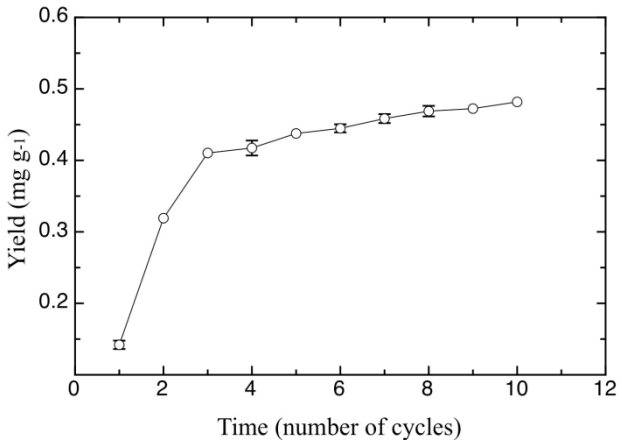

Figure 1. Overall extraction curves of SFE employed at 50°C and at 300 bar with ethanol 10% (v/v) for *Crithmum maritimum*. Data are the obtained yield as function of time (number of cycles)

Table 3. Total extract yield (TEY), total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity measured by ABTS and FRAP assays for *Crithmum maritimum* (Cri) and *Salicornia europea* (Sal) extract by supercritical fluid extraction (SFE) with different ethanol concentrations (10, 20 and 40%; v/v).

	TEY (mg g_{dw}^{-1})	TPC (mg GAEs g_{dw}^{-1})	TFC (mg QEs g_{dw}^{-1})	ABTS (mg TEs g_{dw}^{-1})	FRAP (µmol $FeSO_4$ g_{dw}^{-1})
Cri_SFE/10%	23.36±0.03^{a}	0.26±0.03^{a}	0.12±0.01^{a}	0.82±0.12^{a}	8.09±0.17^{a}
Cri_SFE/20%	26.26±0.57^{b}	0.28±0.02^{b,c}	0.17±0.01^{c}	1.16±0.02^{b}	9.13±0.37^{d}
Cri_SFE/40%	44.87±0.53^{c}	0.78±0.02^{c}	0.49±0.02^{b,c}	2.07±0.19^{c}	14.67±1.06^{d}
Sal_SFE/10%	11.01±0.71^{d}	0.15±0.01^{b}	0.09±0.01^{b}	0.71±0.10^{a}	5.22±0.13^{b}
Sal_SFE/20%	16.30±0.01^{e}	0.29±0.01^{b,c}	0.16±0.01^{c,d}	1.16±0.14^{b}	10.22±0.60^{c}
Sal_SFE/40%	18.92±1.05^{f}	0.29±0.03^{d}	0.18±0.01^{e}	1.33±0.10^{d}	10.38±0.60^{e}

Values are means of three replications ± standard deviation. Values in the same column followed by different superscript letters differ significantly ($P < 0.05$).

3.4. Chemical Characterization of Extracts by SFE

The SFE working conditions for both *Crithmum maritimum* and *Salicornia europaea* were characterized by constant extraction temperature (50 °C) and pressure (300 bar) and different ethanol concentrations (10, 20 or 40% v/v, respectively). Ethanol concentrations over 40% were not tested. In fact, the high flow rate decreases the residence time of solvent inside the extraction vessel, then, the system exits from thermodynamic equilibrium and, consequently, the solvent leaves the extractor unsaturated. According to Kumoro and Hasan [43], the solvent flow rate is related to the mass transfer resistance and to thermodynamic equilibrium. In Table 3 are reported TEY, TPC, TFC, ABTS and FRAP of *Crithmum maritimum* and *Salicornia europaea* extracts. As can be observed for both of them the yield was directly influenced by the ethanol percentage used in the SFE technique. As confirmed by Spinelli et al. [20] the increase of the polarity of the supercritical fluid by adding a polar modifier such as ethanol is the variable that significantly interfered on the extraction. Ethanol addition promotes the rupture in solute-matrix interactions and substitutes with co-solvent molecules in solid active sites [20,44]. Between the halophytes tested, a greater yield was obtained for the sea fennel extract with the highest ethanol concentration (Cri_SFE/40%).

The obtained results for TPC highlight that the SFE extraction with 10 and 20% of ethanol (v/v) for *Crithmum maritimum* give a phenolic content (0.26 and 0.28 mg GAEs g_{dw}^{-1}, respectively) comparable to the TPC obtained for *Salicornia europaea* at 20 and 40% of ethanol (both 0.29 mg GAEs g_{dw}^{-1}). Differently, extraction with 40% of ethanol more than doubles the total phenolic content of *Crithmum maritimum* extract (0.78 ± 0.02 mg GAEs g_{dw}^{-1}) compared to the other extraction conditions. Similar trend was observed also for FRAP. In particular, the lowest values of antioxidant capacity, in terms of FRAP, were obtained for Sal_SFE/10% (5.22±0.13 µmol $FeSO_4$ g_{dw}^{-1}), whereas, in the case of *Crithmum maritimum* extract the lowest value was obtained at 10 and 20% of ethanol. Contrarily, the best results were obtained for Cri_SFE/40% characterized by 14.67±1.06 µmol $FeSO_4$ g_{dw}^{-1}. A strong correlation between TPC and FRAP assay was confirmed also by literature data [20,45]. It was successfully shown that samples with high level of phenolic content also contain flavonoids in great amount [46]. In particular, a TFC under 0.2 mg QEs g_{dw}^{-1} was reported for all tested samples except for Cri_SFE/40% that shows a TFC content of 0.49 ± 0.02 mg QEs g_{dw}^{-1}. As can be observed in Table 3 for both tested plants the ABTS was strictly related to the ethanol concentration used in the SFE. In particular, comparable results for each tested concentration were obtained for *Crithmum maritimum and Salicornia europaea* extract with 10 and 20% of ethanol, unlike the SFE extraction with 40% of ethanol that allowed to get higher values only for sea fennel. Data highlight that the best results in SFE extraction were obtained for *Crithmum maritimum* extract with 40% of ethanol. The richness in antioxidant compounds of sea fennel among different halophytic species were also demonstrated by Meot-Duros et al. [8] that examine the antioxidants of extracts with methanol. Moreover, other works in the literature report that both sea fennel leaves and *Salicornia herbacea* seed extracted with water/methanol or acetone respectively, are full in phenolic compounds [5,12,47]. Many factors influence the halophytes secondary metabolites and their chemical profile [9]. However, on the basis of our knowledge no studies have been carried out on supercritical fluid extraction of halophytes for a possible comparison.

3.5. Comparison of Two Extraction Methods

In Figure 2 (a, b) are reported the TEY and TFC for *Crithmum maritimum and Salicornia europaea* extracts obtained with UAE and SFE. As can be observed, the yield and TFC values were significantly lower in SFE extracts as compared to UAE extracts. According to other works in the literature, UAE significantly increases the extraction yield and the phenolic compounds of many natural matrices, reducing the extraction time compared to alternative extraction methods as SFE [13,33].

The production of cavitation bubbles in the solvent in the UAE extraction promotes better extraction yield and antioxidant activity [33]. Also TFC of extracts by SFE were significantly lower than the antioxidant compounds of extract obtained by UAE for both plants. UAE increased the total flavonoid content more than ten times compared to SFE. Similar trend were also observed for TPC. Antioxidant activity of extracts obtained by SFE and UAE, measured by ABTS and FRAP assays, showed statistically significant differences (Table 2 and Table 3). ABTS values of the halophyte extracts by using UAE were significantly higher (i.e. at the best tested conditions 59.76±3.39 and 21.44±1.07 mg Tes g_{dw}^{-1}

for *Crithmum maritimum and Salicornia europaea*, respectively) than that obtained by SFE (2.07±0.19 and 1.33±0.10 mg Tes g_{dw}^{-1}, respectively). Also for FRAP the best results were obtained for *Crithmum maritimum* extract with UAE method (453.60±22.47 μmol $FeSO_4$ g_{dw}^{-1}). Among the tested halophytes, *Crithmum maritimum* extract resulted always richer in antioxidant compounds compared to *Salicornia europaea* extract obtained under the same conditions. These results are related to the different environmental factors that can influence the secondary metabolites and the chemical profile of halophytes [9].

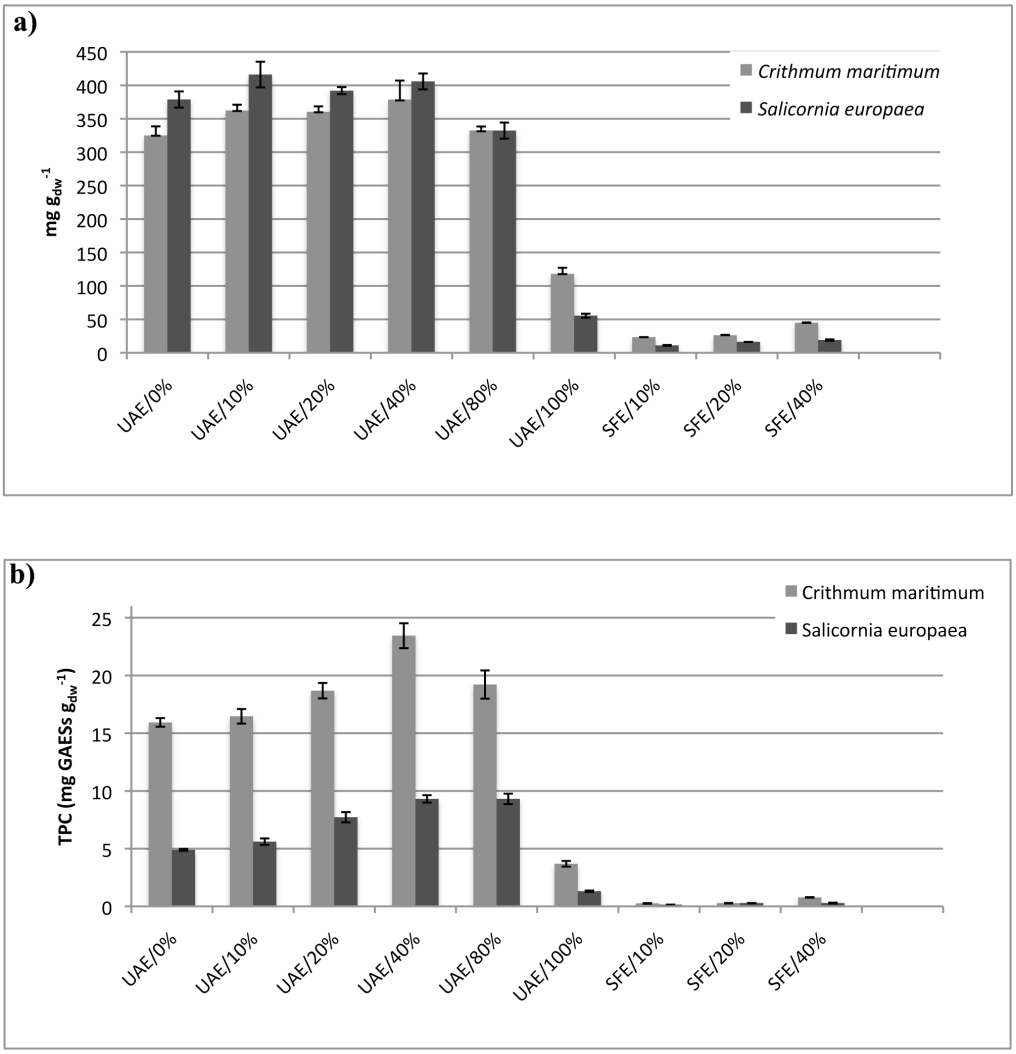

Figure 2. Total extract yield (a), total polyphenol content (b) for *Crithmum maritimum and Salicornia europaea* extracts with ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) at different ethanol concentrations

3. Conclusion

In this work the characteristics of *Crithmum maritimum* and *Salicornia europaea* extracts were studied. In particular, the most profitable extraction conditions were optimized for ultrasound-assisted extraction and supercritical fluid extraction in terms of ethanol concentration, ad related to total extract yield, total phenolic content, total flavonoid content and antioxidant activity measured by ABTS and FRAP assays.

The obtained data showed that for both *Crithmum maritimum* and *Salicornia europaea* the best results were obtained with ultrasound-assisted extraction that allowed to obtain an increase of the antioxidant compounds of about ten times higher compared to SFE. Between the two tested halophytes, *Crithmum maritimum* resulted the richest one. In particular, the UAE method gave the highest total phenol and flavonoid contents (23.44 mg GAE_s g_{dw}^{-1} and 16.63 mg QE_s g_{dw}^{-1}, respectively) for *Crithmum maritimum* extract with 40% of ethanol; differently, lower values were obtained for *Salicornia europaea* extract in the same conditions (9.31 mg GAE_s g_{dw}^{-1} and 7.84 mg QE_s g_{dw}^{-1}, respectively).

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