Food Bioscience 50 (2022) 102131

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Effects of the seasonal variation in the phytochemical composition and bioactivities of the wild halophyte *Suaeda fruticosa*☆

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ARTICLE INFO

Keywords:
Halophyte
Suaeda fruticosa
HPLC-DAD/ESI-MS
O-glycosylated and acylated flavonoids
Betalains
Bioactivities

ABSTRACT

Suaeda fruticosa is an edible medicinal species belonging to the Amaranthaceae (Chenopodiaceae) family widely distributed in the Mediterranean regions, especially in Tunisia. This species shoots change their colour from green to red-violet along the different seasons, being an interesting matrix to study for its chemical composition. Therefore, in a ground-breaking study, the hydromethanolic extracts of green and red-violet Tunisian *S. fruticosa* shoots were profiled for their individual phenolic compounds and betalains by High Performance Liquid Chromatography coupled with a Diode-Array Detector and Mass Spectrometry by Electrospray Ionization (HPLC-DAD/ESI-MS), and the *in vitro* antioxidant, antibacterial, and hepatotoxic properties were also assessed. The obtained results revealed that the hydromethanolic extracts contain interesting levels of phenolic compounds especially *O*-glycosylated and acylated flavonoids, while betacyanins were the main betalain compounds found. As expected, red-violet shoots were richer in phenolic compounds (45.5 ± 0.2 mg/g extract) and betalains (33.3 ± 0.2 mg/g extract) than greener shoots. Flavonoid/betalains rich extracts presented lower EC_{50} values for $ABTS^{+}$, DPPH, and TBARS; as also lower MIC values against Gram-positive bacteria. This study showed that the wild halophyte growing in a hard environment, were drought and salinity are dominant, is an important source of flavonoids and betanin, important molecules that can find application in different industries, given the constant demands for natural bioactives and colouring compounds. Overall, these natural compounds may be valorised and further investigated to validate their potential as functional ingredients.

1. Introduction

Suaeda fruticosa (Forssk. ex J.F.Gmel.) is an edible medicinal species belonging to the Amaranthaceae (Chenopodiaceae) family and Caryophyllales order. It is a halophyte species widely distributed in the Mediterranean region especially in Tunisia. The shoots of this halophyte change their colour between seasons, namely from green in spring to red-violet in the summer. Phenolic compounds and pigments are natural antioxidants, highly demanded for the pharmaceutical, cosmetics and nutraceutical industries as functional ingredients. Several halophytes' species were investigated in traditional medicine for the treatment of many health problems and diseases (Ksouri et al., 2012). In Tunisia, for example, *Suaeda* is one of the most investigated halophytes genera. Hence, *Suaeda mollis* was used in the south of Tunisia to treat wounds, and its ashes were added to the chewing tobacco composition (Chaieb, M., & Boukhris, 1998). Another Sueda species, *Suaeda maritima,* was traditionally consumed in salads and used for diabetes treatment (Oueslati et al., 2012). In addition, some scientific researchers reported that *Suaeda* genera is characterised for having an interesting antioxidant capacity, besides other biological potentialities, highlighting the antiviral activity against hepatitis (Agoramoorthy, Chen, & Hsu, 2008; Barreira et al., 2017). It was also used for smoothing and toning the skin

☆ Influence of seasonal variation in *Suaeda fruticosa* chemical and bioactive profile.

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https://doi.org/10.1016/j.fbio.2022.102131
Received 20 May 2022; Received in revised form 13 October 2022; Accepted 19 October 2022
Available online 27 October 2022
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in cosmetics as a slimming cream helping to dissolve localized fat (Oueslati et al., 2012). In particular, the species *Suaeda fruticosa*, was extensively used as a replacement of soap, and the black seeds were traditionally known for their tonicardiac, anti-infectious, and healing effects (Ayaz et al., 2022; Chaieb, M., & Boukhris, 1998; Oueslati et al., 2012; Saleem et al., 2021) Moreover, it was reported that *S. fruticosa* exhibits hypoglycaemic and hypolipidemic activities (Ahmad et al., 2021). Trendy, it was confirmed that halophytes species can be used as functional foods for human consumption due to their richness in minerals, fibres, polyunsaturated fatty acids (mainly omega-3 and -6), with low caloric index (Barreira et al., 2017; Maatallah Zaier et al., 2020). Nowadays, consumers are becoming more observant of the quality of food, especially regarding food quality and safety, preferring foods richer in natural additives, that are associated with low/none harmful effects, providing health benefits. These natural ingredients can be polyphenols, vitamins, among others, that exert different biological effects and/or can even add colour to food products, as in the case of carotenoids, anthocyanins, and betalains. These later, betalains, for example, are antioxidant pigments (Chhikara, Kushwaha, Sharma, Gat, & Panghal, 2019) due to their ability to scavenge free radicals (De Mejia, Zhang, Penta, Eroglu, & Lila, 2020; Esatbeyoglu, Wagner, Schini-Kerth, & Rimbach, 2015), that also present a wide range of biological activities (Chhikara et al., 2019; De Mejia et al., 2020) such as antiviral and antimicrobial (Delgado-Vargas, Jiménez, Paredes-López, & Francis, 2000; Vulić et al., 2012; Čanadanović-Brunet et al., 2011), as also gastroprotective effect (Karampour, Arzi, Rezaie, Pashmforosh, & Rad, 2019), intrinsic activity (Gandía-Herrero & García-Carmona, 2013), cytotoxic activity (Khan, Sri Harsha, Giridhar, & Ravishankar, 2012; Vulić et al., 2012), and even cancer prevention (Gandía-Herrero, Escribano, & García-Carmona, 2016). Bearing in mind the potentiality of *S. fruticosa* in terms of biological activities and the colour change of this specie between seasons, this ground-breaking study aimed to assess the differences in the chemical composition and bioactivities of the green and red-violet shoots along, by profiling the phenolic and betalain composition by chromatographic methodologies; as also study the antioxidant, hepatotoxic, and antimicrobial capacities of the phenolic-betalain-enriched extracts. This study will contribute to deep the knowledge regarding the phytochemical profile and bioactive potential of the hydromethanolic extracts of green and red-violet phenotypes of *S. fruticosa* shoots.

2. Material and methods

2.1. Samples

The wild Tunisian halophyte, *Suaeda fruticosa* (Fig. 1), of two phenotypic stages (green and red-violet) were collected from the salt marshes (Sebkha of Sidi el Heni) located in the southwest of Sousse (Tunisia) in an area of about 5 m^2. Green shoots were collected in March and red-violet shoots in August 2019. The taxonomic identification was performed by Dr Abderzzak Smaoui at the Laboratory of Extremophile Plants (Center of biotechnology Borj Cedria Tunisia). The collected fresh material was lyophilized (Büchi R-20, Flawil, Switzerland), grounded for the obtaining of a fine dried powder (20 mesh), and stored at −20 °C until further analysis.

2.2. Extracts preparation

The hydromethanolic extracts (methanol:water, 80:20, *v/v*) of green and red-violet shoots dried powder were prepared by maceration, as previously described by Melgar et al. (2017). Briefly, 1 g of the dried samples was mixed with 30 mL of solvent and was stirred for 1 h at room temperature (25 °C). After filtration using a Whatman no.4 filter paper, the residue was re-extracted using the same volume of solvent in the same conditions. The combined extracts were evaporated under vacuum at 40 °C to remove the methanol, using a rotary evaporator (Büchi, 3000 series, Flawil, Switzerland), and the aqueous phase was frozen and further lyophilized. The resulting lyophilized extract was stored at room temperature (25 °C) under vacuum until further assays. Red-violet samples containers were covered with aluminium paper in each step of the extraction procedure to protect the colouring compounds from degradation.

Fig. 1. Specimen of *Suaeda fruticose*, wild Tunisian halophyte, collected the salt marshes (Sebkha of Sidi el Heni), Tunisia.

2.3. Phytochemical composition: phenolic and betalains profile

The lyophilized extracts were re-dissolved in a methanol:water solution (20:80, *v/v*). To determine the phenolic profiles a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) was used, as previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Detection was carried out with a diode array detector (DAD) using 280 nm and 370 nm as the preferred wavelengths and connected in line with a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source and working in negative mode. For betalains determination, the same equipment was used, but following the protocol previously described by Roriz et al. (2017), 530 nm was used as the preferred wavelength, and the mass spectrometer an ESI source worked in positive mode. For both family of compounds, the data acquisition was carried out with the Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The phenolic and betalain compounds were identified through the available standard compounds and by using literature information regarding the fragmentation pattern. Quantification was performed using 7-level calibration curves obtained from commercial standard compounds: apigenin-7-*O*-glucoside ($y = 10.683x - 45.794$, $R^2 = 0.996$, Limit of Detection (LOD) = 0.10 μg/mL; Limit of Quantification (LOQ) = 0.53 μg/mL); gomphrenin III ($y = 14670\,x - 19725$, $R^2 = 0.9997$, LOD = 0.78 μg/mL; LOQ = 1.97 μg/mL), and quercetin-3-*O*-glucoside ($y = 34.843x - 160.173$, $R^2 = 0.9998$, LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL); The results were expressed in mg per g of extract.

2.4. Bioactivities of the hydromethanolic extracts

2.4.1. Antioxidant activity

The lyophilized hydromethanolic extracts of green and red-violet samples were re-dissolved in methanol:water (80:20, *v/v*) and water, respectively, to obtain stock solutions of 5 mg/mL, which were further diluted to obtain a range of six concentrations below the stock solution. The antioxidant activity was evaluated through the total antioxidant activity using ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) test, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging

activity, reducing power, and lipid peroxidation inhibition in porcine brain homogenates by using the TBARS (thiobarbituric acid reactive substances) assay (Sarmento, Barros, Fernandes, Carvalho, & Ferreira, 2015). The results were expressed as EC_{50} values (concentration able to exert 50% of antioxidant activity) and expressed in μg/mL. Trolox was used as the positive control.

2.4.2. Antibacterial activity

The antibacterial activity was tested against clinical isolated bacterial strains, three Gram-positive: *Enterococcus faecalis*, *Listeria monocytogenes*, MRSA (Methicillin-resistant *Staphylococcus aureus*)) and six Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. The minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) were measured by the method previously described by Svobodova et al. (2017), based on the microdilution method using de *p*-iodonitrotetrazolium chloride (INT) (Panreac Applichem-Barcelona, Spain) as a viability marker, with the viable microorganisms reducing the yellow dye to a characteristic pink colour. The extracts were analysed in triplicate and diluted in MHB in a concentration's range of 0.015–20 mg/mL. The dilutions were added to each well (96-well microplate) and inoculated with each inoculum (1×10^8 cfu/mL) studied. Two negative controls were prepared, one with MHB and the other with the extract; and one positive control was also prepared, with MHB and the inoculum.

Ampicillin and imipenem were used as positive controls for the Gram-negative bacteria, while ampicillin and vancomycin were used for Gram-positive bacteria. The results were expressed in mg/mL.

2.4.3. Hepatotoxicity

The lyophilized hydromethanolic extracts were re-dissolved in water, to obtain a stock solution of 8 mg/mL, which was further diluted to obtain a range of five concentrations below the stock solution (8–0.063 mg/mL). Non-tumour cells were prepared from a freshly harvested porcine liver obtained from a local slaughterhouse (named PLP2, porcine liver primary cells) and tested (Abreu et al., 2011) through the Sulforhodamine B assay. Ellipticine was used as the positive control and the results were expressed in GI_{50} values (sample concentration achieving 50% of growth inhibition in liver primary cells PLP2).

2.5. Statistical analysis

Three replicates of each plant part and all the assays described above were independently analysed in triplicate ($n = 9$). For phenolic compounds quantification and comparison between phenotypes, a Student's *t*-test was used to determine the significant difference between the two different phenotypes, with $p = 0.05$.

3. Results and discussion

3.1. Phytochemical composition

3.1.1. Phenolic profile

The chromatographic data, retention time, λ_{max}, deprotonated ion, fragmentation pattern, tentative identification, and quantification of the phenolic composition present in the hydromethanolic extracts of green and red-violet shoots of *S. fruticosa* are presented in (Table 1). Eight phenolic compounds were found, all being *O*-glycosylated flavonoids, and its tentative identification was performed by comparison with data already existing in the literature. Peak 1 presented a deprotonated ion $[M-H]^-$ at *m/z* 637 and one major MS^2 fragments at *m/z* 285 (luteolin aglycone), corresponding to the loss of two hexuronosyl units (176 u + 176 u), being tentatively identified as luteolin-*O*- dihexuronoside, as previously reported by (Algamdi, Mullen, & Crozier, 2011). A similar behaviour was observed for peak 3 ($[M-H]^-$ at *m/z* 621) presenting the apigenin aglycone (*m/z* at 269), being tentatively identified as apigenin-*O*-dihexuronoside (Živković et al., 2014). Peak 5 ($[M-H]^-$ at *m/z* 461) presented one major MS^2 fragments at *m/z* 285 (luteolin aglycone), corresponding to the loss of one hexuronosyl unit, being tentatively identified as luteolin-*O*-hexuronoside (Živković et al., 2014). Finally, peaks **2** ($[M-H]^-$ at *m/z* 959), **4** ($[M-H]^-$ at *m/z* 651), **6** ($[M-H]^-$ at *m/z* 813), **7** ($[M-H]^-$ at *m/z* 797), and **8** ($[M-H]^-$ at *m/z* 827) were tentatively identified as apigenin 7-*O*-{2′-*O*-feruloyl [glucopyranosyl-(1–3′)]-*O*-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside}, chrysoeriol 7-*O*-glucurono-pyranosyl-(1–2)-*O*-glucuronopyranoside, luteolin 7-*O*-[2′-*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside], apigenin 7-*O*-[2' - *O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucurono-pyranoside], and chrysoeriol-7-*O*-[2′-*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside], respectively, as previously described by Marczak, Znajdek-Awizeń, and Bylka (2016) in *Axyris amaranthoides*.

The highest total proportions were quantified in the red-violet

Table 1
Retention time (Rt), wavelengths of maximum absorption (λ_{max}), mass spectral data, tentative identification, and quantification (mg/g of extract) of the phenolic compounds present in the hydromethanolic extracts of *S. fruticosa*. (Mean ± SD).

Peak	Rt (min)	Λmax (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg/g extract)		*t*-Student Test *p*-Value
						Green	Red-violet	
1	13.49	338	637	285(100)	Luteolin-*O*-dihexuronoside	Nd	0.71 ± 0.01	–
2	14.79	324	959	527 (100),269(5)	Apigenin 7-*O*-{2′-*O*-feruloyl-[glucopyranosyl-(1–3′)]-*O*-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside}	Nd	1.44 ± 0.01	–
3	16.06	334	621	269(5)	Apigenin-*O*-dihexuronoside	6.19 ± 0.03	2.94 ± 0.01	<0.001
4	17.13	346	651	351(100)285 (5)	Chrysoeriol 7-*O*-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside	7.5 ± 0.1	10.29 ± 0.04	<0.001
5	18.35	347	461	285(100)	Luteolin-*O*-hexuronoside	2.3 ± 0.1	3.6 ± 0.1	<0.001
6	21.49	334	813	285(100)	Luteolin 7-*O*-[2' -*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside]	Nd	0.92 ± 0.01	–
7	22.84	332	797	527 (100),269(5)	Apigenin 7-*O*-[2' -*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside]	10.4 ± 0.01	5.8 ± 0.2	<0.001
8	23.94	337	827	527 (100),369(5)	Chrysoeriol 7-*O*-[2' -*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucuronopyranoside]	13 ± 1	19 ± 1	<0.001
					Total O-glycosylated flavonoids	**39 ± 1**	**45.5 ± 0.2**	<0.001

nd- not detected. Standard calibrations curves used for quantification: apigenin-7-*O*-glucoside (y = 10,683x – 45,794, LOD = 0.10 μg/mL and LOQ = 0.53 μg/mL, peaks 2, 4, and 8) and quercetin-3-*O*-glucoside ($y = 34843x - 160173$, LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL, peaks 1, 5, 7, and 9).

samples with a major abundance of chrysoeriol 7-*O*-[2′-*O*-feruloyl-glucuronopyranosyl-(1–2)-*O*-glucurono-pyranoside containing 10.29 mg/g extract. The hydromethanolic extracts of red-violet shoots revealed the highest levels of phenolic compounds 24.93 mg/g extract against 7.04 mg/g in the green phenotype. In conclusion, the phenolic profile of *Suaeda fruticosa* is marked by a wide richness in phenolic compounds mainly in red-violet phenotypes compared to green phenotypes. Halophytes are species that can live in hard environments where drought soil salinity accumulations are major abiotic stress (Maatallah, Talbi Zribi, Salhi, Abdelly, & Barhoumi, 2021). To survive in hard environment halophytes developed efficient biochemical strategies to manage excessive intracellular salt concentration. Therefore, extremophile species are an important natural source of secondary metabolites potentially accumulated in response to oxidative damage caused under environmental stress (high salinity accumulation, lack of water, temperature, UV exposure …) (Petropoulos, Karkanis, Martins, & Ferreira, 2018). These secondary metabolites are mainly phenolic compounds, particularly flavonoids and derivatives (Ksouri et al., 2012; Liu et al., 2010; Oueslati et al., 2012). In general, flavonoids have been detected in halophytes from the Amaranthaceae family (Chenopodiaceae) which is exactly the case in the present study.

The phenolic profile of *Suaeda fruticosa* is distinguished by the marked presence of *O*-glycosylated and acylated flavonoids. Oueslati et al. (2012), confirmed the flavonoids richness of *Suaeda fruticosa* green shoots, as also identified a new isorhamnetin glycoside, with high antioxidant potential. However, the phytochemical composition of red-violet hydromethanolic extracts of *Suaeda fruticosa* was not previously studied, as far as the authors knowledge, so it was not possible to make comparisons with previously described results.

Noori, Talebi, and Nasiri (2015) suggested that coloured plants (green-red, green-pink, green-purple, pink, red, or purple) are directly or indirectly correlated with phenolic compounds especially flavonoids: rutin, isorhamnetin, quercetin, and kaempferol; all detected in the studied species. Zhao, Tan, Liu, Li, and Chen (2009) reported that several regulatory and structural genes are dissimilarly influenced by developmental signals and environmental stress which altered flavonoids biosynthetic pathways. This may explain quantities difference of the phenolic content between green and red-violet phenotypes. Our results are also in agreement with previous studies reporting the seasonal variation of proteins, lipids, carbohydrates, minerals, vitamins, and tocopherols, among others, quantities in the same plant (Maatallah Zaier et al., 2020). A similar study was performed in *S. fruticosa* tocopherols content, one of the richest halophytes species, observing that the red-violet shoots presented the highest quantities of total tocopherols (13.66 ± 0.19 mg/100g fresh weight - fw) than the greener shoots (2.91 ± 0.13 mg/100g fw) with a major abundance of the α-tocopherol isomer (11.42 ± 0.14 mg/100g fw) in the red-violet shoots (Maatallah Zaier et al., 2020). These results with tocopherols content confirmed that the seasonal variation is associated with the control of primary and secondary metabolites. And thus, confirmed the effect of the environmental conditions on antioxidants biomolecules accumulation as a strategy of plants adaptation (Jallali et al., 2012; Ksouri et al., 2008).

3.1.2. Betalains profile

Concerning betalains profile in the hydromethanolic extracts of red-violet of *Suaeda fruticosa* shoots, the results are presented in Table 2. The results revealed that the hydromethanolic extracts contain purely betacyanins with a total absence of betaxanthins **(Table 2).** Four major peaks were detected, peak **1** ($[H]^+$ at *m/z* 551), **2** ($[H]^+$ at *m/z* 813), and **3/4** ($[H]^+$ at *m/z* 903), tentatively identified as betanidin-5-*O*-β-glucoside (Betanin) according to (Lee et al., 2014), 6′-*O*-malonyl-amaranthin (celoscristatin) according to (Lystvan, Kumorkiewicz, Szneler, & Wybraniec, 2018), and according to (Cai, Sun, & Corke, 2001; Khan & Giridhar, 2015) celosianine II/Isocelosianine II, respectively.

S. fruticosa red-violet shoots contain 33.3 ± 0.2 mg/g extract with a major abundance of isocelosianine II in the order of 18.04 ± 0.04 mg/g extract followed by celosianine II. Betanidin-5-*O*-β-glucoside (Betanin) and 6′-*O*-malonyl-amaranthin (celoscristatin) in the order of 6.7 ± 0.1, respectively; 4.4 ± 0.2 and 4.12 ± 0.2 mg/g extract.

Commonly, betalains extraction from plant tissues is carried out with aqueous methanol solution due to their high polarity. Betacyanins are not flavonoids, but they contain a partly glycosylated phenolic group which confers their specific range of bioactivities different from phenolic acids or flavonoids (Strack, Vogt, & Schliemann, 2003). There for, betacyanins stands out for their importance as bioactive compounds with high antioxidant power. Meanwhile, betalain compounds are not found in the green phenotypes.

The most outstanding result is the betanin concentration (4.4 ± 0.2 mg/g extract), which revealed to be 7.5-fold times higher than the concentration found Roriz et al. (2017) in *Gomphrena globosa* L. (0.5861 mg/g extract considering that the moisture of that plant was 5%). It should be noted that *G. globosa* is considered one of the biggest sources of betanin.

Hayakawa and Agarie (2010) reported that betacyanin production is induced by abiotic and biotic stresses, which potentially cause ROS production in *Suaeda japonica* Makino. Hence, betacyanin accumulated in *Suaeda japonica* when the temperature dropped significantly in February to March and November to December (Hayakawa & Agarie, 2010) which is in agreement with the results presented herein. Strack et al. (2003) reported that the halophytes species *Mesembryanthemum crystallinum* was used as a model system to analyse UV-light induced betacyanin formation and simultaneous formation of flavonols conjugates (Ibdah et al., 2002). Interestingly, our results revealed that *Suaeda fruticosa* red-violet shoots contain a mix of four different types of betacyanins (betanin, malonyl-amaranthin, celosianin II, and isocelosianin II). Strack et al. (2003) reported that mixed betacyanins have been accumulated after light stress in epidermal layers (Vogt et al., 1999). Hence, we suggest that mixing different types of betacyanins in red-violet shoots acts as an adaptation biochemical strategy under light stress.

3.2. Bioactivity evaluation

The results for the antioxidant properties of the hydromethanolic extract of *S. fruticosa* are shown in Table 3. There is variability obtained from the two phenotypes that revealed significant differences in the scavenging of free radicals (Table 3). The hydromethanolic extracts from

Table 2
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification, and quantification (mg/g of extract) of betalains in the hydromethanolic extracts of *S. fruticosa* red-violet shoots. (Mean ± SD).

Peak	Rt	Λmax (nm)	$[H]^+$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification mg/g extract
1	16,71	533	551	389(100), 345(5),150(5)	Betanidin-5-*O*-β-glucoside (Betanin)	4.4 ± 0.2
2	18,64	534	813	727(71), 551(5), 389(60)	6′-*O*-malonyl-amaranthin (celoscristatin)	4.2 ± 0,.2
4	19,63	540	903	551(25), 389(100)	Celosianin II	6.7 ± 0.1
5	20,78	540	903	551(30), 389(100)	Isocelosianin IIt	18.0 ± 0.0
					Total	**33.3 ± 0.2**

Standard calibrations curve used for quantification: Gomphrenin III ($y = 14670x - 19725$, $R^2 = 0.9997$).

Table 3
Antioxidant and hepatotoxic activity of the hydromethanolic extracts of red-violet and green shoots of *S. fruticosa* (mean ± SD).

Antioxidant activity (EC_{50}, mg/mL)	Phenotypes		*t*-Student Test *p*-Value
	Green	Red-violet	
ABTS+	1.47 ± 0.07	1.19 ± 0.02	<0.001
DPPH	0.32 ± 0.00	0.30 ± 0.00	>0.001
Reducing power	1.06 ± 0.00	2.14 ± 0.30	<0.001
TBARS	1.26 ± 0.00	0.45 ± 0.00	>0.001
Hepatotoxic activity (IC_{50}, µg/mL)			
PLP2	>400	>400	–

EC_{50} values correspond to the extract concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. Trolox EC_{50} values: 44.82 ± 1.59 µg/mL (DDPH), 28.71 ± 1.21 µg/mL (reducing power) and 6.76 ± 0.3 µg/mL (TBARS inhibition). GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2. Different letters mean significant differences ($p < 0.005$).

shoots of *S. fruticosa* showed a strong scavenging capacity, in ABTS + assay, with an EC_{50} value equal to 0.30 and 0.32 mg/mL for the red -violet and green phenotypes, respectively. The estimated reducing ferric power capacity is shown in Table 2. The results revealed an EC_{50} value equal to 1.06 mg/mL for green phenotypes and 2.14 mg/mL for red-violet shoots. Regarding the lipid peroxidation method of the two halophyte species (green and red-violet phenotype) are represented in Table 3. Hydromethanolic extracts registered EC_{50} equal to 1.26 mg/mL (green shoots) 0.45 mg/mL (red-violet shoots). Independently of the phenotype, all areal parts inhibit lipid peroxidation with a high inhibition rate. The red-violet phenotypes showed a better EC_{50} compared to the green phenotype. Overall, independently of the used test, *S. fruticosa* shoots presents an interesting antioxidant potentiality. The obtained results are consistent with previous work on seasonal variation in phenolic contents and antioxidant activities (Jallali et al., 2012; Ksouri et al., 2008). None of the extracts were able to cause the death of the analysed bacterial strain.

In *Suaeda japonica*, the total radical scavenging capacity evaluated by DPPH didn't decrease in the red leaves (Hayakawa & Agarie, 2010), which is totally in agreement with the results presented herein. In addition, the lipid peroxidation evaluated by MDA content was almost the same in leaves containing different levels of betacyanins. These results suggested that betacyanin that are mainly present in the vacuole acts as a ROS scavenger (Tanaka, Sasaki, & Ohmiya, 2008). Betacyanin accumulation in red-violet shoots of *S. fruticosa* seems to be important for the amelioration of ROS induced photoinhibition and related photo-oxidative stresses. For TBARS analysis, the exhibited results are consistent with those found by Chekroun-Bechlaghem et al. (2019) with an $EC_{50} = 0.44$ mg/mL. Concerning the evaluation of the antibacterial effect of the hydromethanolic extracts on the Gram-negative and Gram-positive bacteria (Table 4), the hydromethanolic extracts of green and red-violet phenotypes of *S. fruticosa* revealed equal MIC values (20 mg/mL) for *E. coli,* and *K. pneumoniae.* The green extracts revealed a lowest value than red-violet extracts, showing a stronger potential in inhibiting the tested bacterial strains. Regarding the Gram-positive bacteria, Red-violet extracts seem more efficient against *E. faecalis* and MRSA with lower MIC values (10 mg/mL), whereas green extracts revealed MIC values of 20 mg/mL. It seems like the synergy of flavonoids and betalains in red-violet shoots ameliorated the antibacterial power. To the best of the author's knowledge, the antibacterial activity of the hydromethanolic betalains rich extracts derived from red-violet aerial parts of *S. fruticosa* have not been reported. However, few previous studies were related regarding the evaluation of the antibacterial activity of green phenotypes. Rashid, Iftikhar, Arshad, and Iqbal (2000) reported a good antibacterial effect of *S. fruticosa* ethanolic extract against *E. coli.* Bilal and Hossain (2019) reported that the methanolic extract of *S. maritima* green shoots showed moderate to high inhibition against all Gram-positive and Gram-negative bacterial strains for example MIC value obtained by methanolic extract against *E. coli* is 252.44 µg/mL, *K. pneumonia* 226.16 µg/mL, and *S. aureus* 188.32 µg/mL. Furthermore, the antibacterial activity is highly dependent of the solvent type, compounds containing and the location of plant collection which can highly influence the phytochemical composition (Bilal & Hossain, 2019).

Finally, concerning the hepatotoxicity **(Table 3)**, the extracts revealed no toxicity at the maximum tested concentration of 400 µg/mL. The need to perform this type of *in vitro* hepatotoxicity tests relies on the potential application of these extracts into food products, which means a first *in vitro* screening about the safety of these extracts for the final consumer (ANS) 2012); because even though it was never associated a putative pathogenicity of this plant, for a future application of these extracts, the confirmation of its non-toxicity is extremely important. Other potential bioactivities of *S. fruticosa*, it's the hypoglycemic effect of the aqueous extracts described by Benwahhoud, Jouad, Eddouks, and Lyoussi (2001), that is of potential interest for further investigations, namely to correlate with the presence of the identified phenolic and betalain compounds.

4. Conclusion

Overall, the studied shoots of the wild Tunisian halophyte *Suaeda fruticosa* (green and red-violet) revealed high contents in important molecules, namely phenolic compounds and betalains. The coexistence

Table 4
Antibacterial activity of the hydromethanolic extracts obtained by maceration of red-violet and green shoots of *S. fruticosa* (mean ± SD).

	S. fruticosa				Antibiotics					
	Green		Red-violet		Ampicillin (20 mg/mL)		Imipenem (1 mg/mL)		Vancomycin (1 mg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Escherichia coli	20	>20	20	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Klebsiella pneumoniae	20	>20	20	>20	10	20	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	20	>20	>20	>20	20	>20	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	>20	>20	>20	>20	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	>20	>20	>20	>20	>20	>20	0.5	1	n.t.	n.t.
Gram-positive bacteria										
Enterococcus faecalis	20	>20	10	>20	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
Listeria monocytogenes	>20	>20	>20	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	20	>20	10	>20	<0.15	<0.15	n.t.	n.t.	0.25	0.5

MRSA - methicillin resistant *S. aureus*; MSSA - methicillin MIC values correspond to the minimal extract concentration that inhibited the bacterial growth. MBC values correspond to the minimal extract concentration that kills bacteria; n.t. - not tested.

of flavonoids and betacyanins presented an important antioxidant power for the two phenotypes, and also the exhibited antibacterial effect can be correlated with the presence of flavonoids. This study showed that *S. fruticosa* red-violet extracts are very rich in betalain (even more than other betalain-rich plant matrix), that could be applied in several industrial fields as high-added value compound. Additionally, it can also confer functional properties, to novel food developed with this plant matrix, beneficial for human health and wellbeing.

Author statement

Mariem Maatallah Zaier: Sample collecting, Investigation, Writing - original draft. Maria Inês Dias: HPLC investigation, supervision, & review. Filipa Mandim: TBARS investigation. Ricardo C. Calhelha: Cytotoxicity investigation. Sandrina A. Heleno: Antibacterial investigation, edit and review. Isabel C.F.R. Ferreira: Project administration, Resources. Lotfi Achour: Project administration, Resources. Lillian Barros: Conceptualization, supervision, methodology & review. Adnen Kacem: Conceptualization, Supervision & review.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

Acknowledgements

This work was done under the Bilateral Tunisian-Portuguese scientific research: Red-violet halophytes as a new source of biomolecules and their applications in medical, nutraceutical and food industries.

The authors are grateful to The Tunisian Ministry of High Education and research for financial support through national funds to Laboratory of Bioressources Integrative Biology and valorizatin. The authors are grateful the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020) and SusTEC (LA/P/0007/2021); for the F. Mandim PhD grant (SFRH/BD/146614/2019), and L. Barros, M.I. Dias, and R. Calhelha thank the national funding by FCT, P.I., through the institutional and S.A. Heleno to the individual scientific employment program-contract, for their contracts.

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