Research Article

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Exploring *Salicornia* species as potential health-promoting food: chemical fingerprinting and assessment of their bioactivities

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Abstract

BACKGROUND: Soil salinization is a growing environmental problem that compromises agricultural productivity, especially for salt-sensitive crops. In this context, halophytes, plants naturally adapted to saline environments, are gaining attention for their resilience and potential applications in sustainable agriculture, nutrition, and health. Among them, species of the genus *Salicornia*, commonly found in Mediterranean coastal areas, stand out for their ecological value and content of bioactive compounds. *Salicornia europaea*, known as 'sea asparagus', is marketed and consumed as a food, while *Salicornia fruticosa* and *Salicornia perennis* are less common and understudied. This study investigated the three *Salicornia* species with the aim of characterizing their chemical composition and evaluating their potential antioxidant and antidiabetic effects such as glucagon-like peptide 1 (GLP-1) secretion or glucose uptake inhibition in intestinal STC-1 cells.

RESULTS: By using ultrahigh-performance liquid chromatography diode array detector high-resolution Orbitrap electrospray ionization mass spectrometry (UHPLC-DAD-HR-Orbitrap-ESI-MS), a varied range of phenolic compounds, flavonoids, saponins, and fatty acids were identified in the phytocomplexes. *In vitro* assays showed that *S. europaea* extract is promising in inhibiting α-amylase enzyme and reducing the glucose uptake via sodium-glucose transporter 1 (SGLT1) in STC-1 cells.

CONCLUSION: The study highlighted the value of these *Salicornia* species as resilient plants with promising roles in the development of nutraceuticals and sustainable land use, particularly in salinity-affected environments.

Keywords: alternative crops; antidiabetic; antioxidant; chemical fingerprint; halophytes; *Salicornia*

INTRODUCTION

Soil salinization is a pressing environmental issue that includes the development of saline, sodic or alkaline soils, characterized by high salt concentrations, high levels of sodium cations (Na^+) and increased pH values, often linked to significant concentrations of carbonate ions (CO_3^{2-}) in the soil. Severe soil salinization alters the natural hydrological Earth cycles and degrades the soil's ability to provide essential resources, impacting agricultural yields, environmental health, and socio-economic security.[1] In this context, halophyte plants are gaining importance and are increasingly recognized for their resilience in harsh environments. Halophytes are a unique group of plants specially adapted to thrive in high salinity environments, such as coastal areas and salt marshes. The term 'halophyte' is derived from the Greek words *halos* (salt) and *phyton* (plant) and reflects their remarkable ability to survive and even thrive in conditions that would typically be inhospitable to most vegetation.[2,3] Their ability to grow in saline soils and under brackish water conditions makes them ideal candidates for expanding food production in regions where traditional crops fail due to salinization and water scarcity, addressing global challenges such as food security, nutritional deficiencies, and the need for alternative sustainable agricultural practices.[4,5]

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The Caryophyllales order contains the highest percentage of halophyte species (21% of the total). Within this order, the Amaranthaceae family comprises the highest number of halophyte genera, including *Salicornia* genus.[6] Traditionally, *Salicornia* species have been utilized by coastal communities for culinary, medicinal, domestic, handicraft, and other purposes. They can be used as a seasonal vegetable in salads, boiled (like spinach) especially as side dishes with fish or seafood or they can be dried as a salt substitute or preserved in oil and/or vinegar.[7-9] *Salicornia europaea* L. is included in the list of traditional Italian products (https://mipaaf.sian.it/) and the shoots, known as 'sea asparagus', are also marketed for culinary use. Among domestic/handicraft uses, it is known that the aerial parts of the *Salicornia* were dried and burned to produce the caustic soda used in the production of soap.[7,10] Furthermore, the *Salicornia* species have ethnomedicinal uses as a remedy for ailments such as inflammation, digestive disorders, skin conditions, and for depurative purposes. Ethnobotanical reports from Europe, North Africa, and parts of Asia highlight the role of *Salicornia* species in local diets and traditional medicine, reflecting a rich reservoir body of indigenous knowledge.[11] Scientific research over the past two decades has begun to validate some of these traditional uses. Phytochemical analyses have identified a variety of bioactive compounds in *Salicornia* species, including flavonoids, phenolic acids, phytosterols, saponins, and essential fatty acids, many of which exhibit antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective properties.[12-14] Extracts from *Salicornia* species have shown potential as antioxidant, anti-inflammatory, antimicrobial agents, and as functional ingredients in cosmetics and nutraceutical formulations.[15-18] Preliminary studies, supported by traditional knowledge, have suggested that certain *Salicornia* species may help reduce blood glucose levels through mechanisms such as inhibition of carbohydrate-digesting enzymes (α-amylase and α-glucosidase), and modulation of insulin sensitivity by *in vitro* assay and in animal models.[19-21] Given the global rise of type 2 diabetes mellitus and the limitations of current synthetic antidiabetic medications, including adverse side effects, there is an urgent need for safer and more sustainable alternatives. In this context, the investigation of *Salicornia* species as a natural source of antidiabetic agents has gained considerable importance.[20] Thus, three different *Salicornia* species have been selected: *S. europaea* L., *Salicornia fruticosa* (L.) L., and *Salicornia perennis* Mill.[22,23] *Salicornia europaea* is among the most extensively studied species within the genus, for its phytochemical composition and potential applications. Its richness in bioactive compounds, including phenolics and flavonoids, contributes to its nutritional and health value.[24] Instead, *S. fruticosa* and *S. perennis* remain relatively unexplored, with limited studies available on their phytochemistry, biological activities, and potential uses.[11] To fill this knowledge gap, the potential antioxidant and antidiabetic properties have been investigated and a comparative chemical analysis of the three phytocomplexes has been carried out, to promote these biomasses as high-value natural products, with health benefits. By using a combination of *in vitro* cell-free and cell-based assays, the study has explored key bioactivities, including enzyme inhibition, free radical scavenging, and insulin-sensitizing effects through glucagon-like peptide 1 (GLP-1) secretion.

MATERIALS AND METHODS

Chemicals and reagents

Ultrahigh-performance liquid chromatography (UHPLC) grade methanol, water, and formic acid, Folin–Ciocâlteu, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trolox, gallic acid, α-glucosidase enzyme from *Saccharomyces cerevisiae*, 4-*p*-nitrophenyl-α-D-glucopyranoside, α-amylase enzyme from porcine pancreas, starch, iodine, potassium iodide, sodium acetate anhydrous, 2,4,6-tripyridyl-s-triazine (TPTZ), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT), 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG) were obtained from Merck KGaA (Darmstadt, Germany). All analytical grade solvents were purchased from VWR (Milan, Italy). Rutin and pharbitoside A pure compounds were previously isolated in our laboratory and fully characterized using one-dimensional (1D)- and two-dimensional (2D)-nuclear magnetic resonance (NMR) techniques.

Plant material and extract preparation

The aerial parts of *S. europaea* and *S. perennis* Mill. were collected in July 2023 in Calambrone (Pisa, Tuscany, Italy) and the aerial parts of *S. fruticosa* were collected in September 2023 in Saline di Cervia (Ravenna, Emilia-Romagna, Italy) and identified by Prof. Fabiano Camangi.[22,23]

The raw material was oven-dried at 40 °C and then ground to maximize extraction efficiency. For each sample, 30 g of the powdered aerial parts were first defatted with 150 mL of *n*-hexane, then subjected to extraction with 150 mL of methanol (solid/liquid mg mL^{-1} ratio 1:5 *w/v*) by dynamic maceration (120 rpm) with a digital orbital shaker (IKA™ KS 501; Minerva S.r.l., Pisa, Italy) for three consecutive days at room temperature, renewing the solvent every 24 h. The solvent was removed under vacuum to obtain dry extracts. Finally, 1 g of each methanolic residue was partitioned with 10 mL of a mixture of *n*-butanol/water (1:1 *v/v*) to remove salts and sugars, obtaining dried *n*-butanol extracts, then properly injected into the liquid chromatography–mass spectrometry (LC–MS) system.

UHPLC-DAD-HR-Orbitrap-ESI-MS qualitative-quantitative analyses of *Salicornia* species extracts

Qualitative-quantitative chemical analyses of *Salicornia* species extracts (2 mg mL^{-1}, methanolic solutions) were performed by UHPLC (Vanquish Flex Binary pump) coupled with a diode array detector (DAD) and high-resolution (HR) Q Exactive Plus mass spectrometry (MS), based on Orbitrap technology, equipped with an electrospray ionization (ESI) source (Thermo Fischer Scientific Inc., Bremem, Germany).

The chromatographic runs were performed by using a Kinetex® Biphenyl C-18 column (2.1 mm × 100 mm, 2.6 µm) equipped with a Security Guard™ Ultra Cartridge (Phenomenex, Bologna, Italy) at a flow rate of 0.5 mL min^{-1} with a splitting system of 1:1 to MS and DAD-ultraviolet (UV) detector, respectively. The autosampler and the column oven were maintained at a temperature of 4 and 35 °C, respectively, and the injection volume was set at 5 µL. The elution was performed using a mixture of methanol/formic acid 0.1% (solvent B) and water/formic acid 0.1% v/v (solvent A) and a linear gradient 5% to 100% (B) within 35 min was chosen. ESI-HRMS was recorded in the negative and positive ion modes. DAD data were registered in a 200–600 nm range using three preferential channels at 254, 280, and 325 nm for phenols. Nebulization voltage of 3500 V, capillary temperature of 300 °C, sheath gas (nitrogen) 20 arbitrary units, auxiliary gas (nitrogen) three arbitrary units, HCD (higher-energy C-trap dissociation) of 18 eV were applied as ionization settings.[25] For the analysis of specialized metabolites in *Salicornia* extracts, a scan range of

m/z 135–2000 was applied, recording MS both in full (70 000 resolution, 220 ms maximum injection time) and data dependent-MS^2 scan (17 500 resolution, 60 ms maximum injection time).

The tentatively annotated specialized metabolites were quantified by using two calibration curves. Rutin for flavonoids and pharbitoside A for saponins were chosen as pure external standards. Triplicate solutions of both standards were prepared using stock methanol solutions with a concentration of 1 mg mL^{-1}. For both standards, a good linearity was displayed in a concentration range of 0.98–125 μg mL^{-1}, with a correlation coefficient (R^2) equal to 0.987 in the case of rutin, and 0.974 for pharbitoside A. Xcalibur 4.1 software and Microsoft® Office Excel program were used for data visualization and data processing. Quantitative results were expressed as milligrams per 100 grams of fresh plant weight (mg 100 g^{-1} fw) ± standard deviation (SD).

Total polyphenol content by Folin–Ciocâlteu method

The total polyphenol content (TPC) of *Salicornia n*-butanol fractions was determined by using Folin–Ciocâlteu reagent,[26] by using gallic acid as the standard. *Salicornia n*-butanol fractions (75 μL) at different concentration were mixed with 500 μL of Folin–Ciocâlteu reagent and 500 μL of sodium carbonate (10% *w/v*). Distilled water was added to reach the final volume of 1.5 mL. After 1 h of incubation in the dark, the absorbance of the reaction mixture was measured at 723 nm against a blank (solvent instead of the plant extract). The results were expressed as milligrams of gallic acid equivalent per gram of dried weight (mg GAE g^{-1}). All measurements were conducted in triplicate and the spectrophotometric measurements were done in 96-well microplates by using an UV-visible spectrophotometer (SPECTROstarNano BMG Labtech, Ortenberg, Germany).

Determination of antioxidant activity

The *Salicornia n*-butanol fractions were tested for their antioxidant activity by three *in vitro* tests: DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays.

DPPH radical scavenging activity

For DPPH method, different concentrations of extracts (0.016–1.00 mg mL^{-1}, 50 μL) were mixed with the radical DPPH (200 μL). The absorbance was monitored by a spectrophotometer at 515 nm after 30 min and results were expressed as milligrams of Trolox equivalents per gram of dried sample (mg TE g^{-1}).[26]

ABTS radical scavenging assay

For the ABTS assay, each fraction, or blank or standard (15 μL) was added to 235 μL of $ABTS^{\cdot+}$ solution and, after 2 h of incubation in the dark, the absorbance was read at 734 nm on a UV-visible spectrophotometer (SPECTROstarNano BMG Labtech). All solutions were freshly prepared for the analysis. Results were expressed as milligrams of Trolox equivalents per gram of dried sample (mg TE g^{-1}).[27]

Ferric reducing antioxidant power (FRAP)

The reduction of the iron(III) (Fe^{3+}) complex of tripyridyltriazine $[Fe(III)(TPTZ)_2]^{3+}$ to Fe^{2+} complex $[Fe(II)(TPTZ)_2]^{2+}$ was determined by the FRAP method. The FRAP reagent was prepared by mixing 38 mmol L^{-1} of sodium acetate anhydrous buffer in distilled water, pH 3.6, with 20 mmol L^{-1} $FeCl_3{\cdot}6H_2O$ in distilled water and 10 mmol L^{-1} of TPTZ in 40 mmol L^{-1} hydrochloric acid (HCl, 10:1:1). FRAP reagent (225 μL) and 25 μL of *Salicornia* fractions, or blank or standard, were mixed in a 96-well plate and incubated for 40 min at 37 °C in darkness. Absorbance was measured at 593 nm.[26]

Inhibition of the carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase

The potential antidiabetic activity of the *Salicornia* extracts was evaluated by assessing their ability to inhibit key enzymes involved in carbohydrate metabolism, namely α-amylase and α-glucosidase.

α-Amylase inhibition

The ability of the *Salicornia* extracts to inhibit the α-amylase enzyme was determined as previously reported.[28] Briefly, 50 μL of α-amylase enzyme solution (5 U mL^{-1}) was mixed with 25 μL of different concentrations (0.016–1.00 mg mL^{-1}) of each fraction and incubated at 37 °C for 10 min. Then, 100 μL of 1% starch solution was added, and the reaction was incubated for an additional 10 min at 37 °C. The reaction was then stopped by adding 25 μL of 0.1 M HCl. To measure the absorbance, 50 μL of iodine–potassium iodide solution (1:5) was added to each well and the absorbance was measured at 630 nm after incubating the plate at 37 °C for 10 min. Acarbose was used as positive control. Results were expressed as half-maximal inhibitory concentration (IC_{50}, in μg mL^{-1}).

Glucosidase inhibition

The inhibitory activity of the fractions on the α-glucosidase enzyme was assessed as previously described.[29] Different concentrations of each sample (20 μL) were mixed with 50 μL of buffer and 40 μL of α-glucosidase enzyme solution (0.1 U mL^{-1}). The reaction mixture was pre-incubated at 37 °C for 10 min, followed by the addition of 40 μL of the substrate, 4-*p*-nitrophenyl-α-D-glucopyranoside (2.5 mmol L^{-1}). The plate was then incubated at 37 °C for 15 min. After incubation, 100 μL of 0.2 mol L^{-1} sodium carbonate was added, and the absorbance was measured at 405 nm. Acarbose was used as positive control. Results were expressed as IC_{50} (in μg mL^{-1}).

STC-1 cell culture condition

STC-1 cells were chosen among intestinal cell models because they are a well-established enteroendocrine system in which sodium-glucose transporter 1 (SGLT1)-dependent glucose uptake is functionally coupled to GLP-1 secretion. STC-1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), streptomycin (100 μg mL^{-1}), penicillin (100 units mL^{-1}), and 2 mmol L^{-1} glutamine and maintained in a humidified atmosphere with 5% carbon dioxide (CO_2) at 37 °C. *Salicornia europaea* extract was dissolved in dimethyl sulphoxide (DMSO) and different concentrations were tested. DMSO-treated cells (0.8% *v/v*) were used as control (CTRL) in all experiments.

Cell viability

Cell viability was tested on STC-1 cells using the MTT assay.[30] STC-1 cells were cultivated in a 96-well plate (1.5×10^4 cells per well), incubated during the night, and treated with the extract at several concentrations (10–400 μg mL^{-1}) for 2 and 24 h. The concentration range tested (10–400 μg mL^{-1}) was selected based on extract solubility, and commonly used ranges for *in vitro* assessment of bioactive extracts. After medium remotion, phosphate-buffered saline (PBS) was used to wash cells which were subsequently incubated for 4 h with MTT solution (0.75 mg mL^{-1}) in PBS. Then, the

solution was removed, and a solubilization solution (1:1 DMSO/isopropanol) was added to the cells. The solubilized formazan product was quantified at 560 nm using a UV-visible spectrophotometer (SPECTROstarNano BMG Labtech). Each extract was tested in triplicate.

Intestinal glucose uptake

STC-1 cells were seeded in 96-well black plate with clear bottom at the density of 1.5×10^4 cells per well. After 48 h of incubation, cells were treated for 2 h with different concentrations of *S. europaea* extracts (50–400 μg mL^{-1}) or apigenin (50 μmol L^{-1}) in glucose-free media. At the end of the treatment, the media was replaced with 100 μL of glucose-free media containing 100 μmol L^{-1} of the fluorescent glucose derivative 2-NBDG (λ ex 485 nm, λ em 535 nm). Glucose uptake was stopped by washing twice with ice-cold PBS. The fluorescent intensity was measured using Varioskan™ LUX Multimode Microplate Reader (ThermoFisher Scientific, Waltham, MA, USA). The results were expressed as the percentage of glucose uptake relative to the untreated cells.[31]

Glucagon-like peptide 1 (GLP-1) secretion

STC-1 cells (2×10^5 cells per well) were seeded into 12-well plates for 48 h. The cells were washed three times with PBS and incubated with 100, 200, and 400 μg mL^{-1} of *S. europaea* fraction for 120 min. At the end of the treatment, the medium was collected and centrifuged at 12 000 × *g* for 5 min to remove cellular debris.[30] GLP-1 secretion was evaluated by GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen BMS2194; Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions.

Western blot analysis

The total content of proteins extracted from STC-1 cells was quantified by Bradford assay.[32] For each sample, about 25 μg of protein was subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) *w/v*, and protein bands were transferred to nitrocellulose membranes. For the detection of target proteins, membranes were incubated overnight with specific mouse or rabbit antibodies at 4 °C: anti-β-actin (1:5000) from Sigma-Aldrich, Merck (Milan, Italy) and SGLT-1 (1:500) from antibodies. After incubation with suitable secondary antibodies, the bands were visualized with chemiluminescence reagents by iBright 1500 Imaging system (ThermoFisher Scientific, Milan, Italy). Densitometric analysis was performed by using ImageJ software (version 1.53k; National Institutes of Health, Bethesda, MD, USA) and results were expressed as percentage of the value of untreated control sample (100%).

Statistical analysis

All experiments were conducted in triplicate and results were reported as mean ± SD. One-way analysis of variance (ANOVA) were performed using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA) software to process the chemical data and GraphPad Prism 8 (Software GraphPad, San Diego, CA, USA) for the biological ones. The *P* values ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical profiles of *Salicornia* species extracts

The chemical fingerprint of *n*-butanol residues of the three *Salicornia* species extracts was assessed by comparing retention times, UV absorption, HR full mass spectra, and fragmentation patterns with data reported in previous works and considering a mass error < 5 ppm on the experimental molecular mass. A total of 31 compounds, including an organic acid (**1**), phenolic acid derivatives (**2**, **4**–**6**, **12**, **18**, **19**, **21**), a sulphate derivative (**3**), amino acid derivatives (**7**, **8**), flavonoids (**9**, **10**, **11**, **13**–**17**, **20**, **22**), fatty acids (**23**, **24**, **31**), and saponins (**25**–**30**), were tentatively identified (Table 1 and Fig. 1) in accordance with previous data reported in the literature.[11,24,33,34] The chemical profile of the three *Salicornia* species showed the occurrence of the same metabolites, except for few substances, while major differences can be observed in their amount among the samples.

Compound **1**, annotated as quinic acid,[34] was detected in all *Salicornia* samples. In addition, quinic acid derivatives were found in the three species, such as two caffeoylquinic acids (isomers **2** and **4**), two dicaffeoylquinic acids (isomers **12** and **18**), a *p*-coumaroylquinic acid (compound **6**), a caffeoyl dihydrocaffeoylquinic acid (compound **19**), and a caffeoylferuloylquinic acid (compound **21**). The identification of these components was based on the presence on the MS^2 fragmentation pattern of diagnostic ion products at *m/z* 191.06 corresponding to quinic acid, generated by the cleavage of an ester bond with a caffeoyl moiety (−162 u) or a *p*-coumaroyl portion (−146 u).[25,35,36] Compounds **2** and **6** were not found in *S. perennis*, while the other hydroxycinnamic acids are distributed in all three species with compounds **18** and **19** highly expressed in *S. europaea*.

For compound **3**, the presence of diagnostic product ions at *m/z* 96.96 and 79.96, corresponding to a sulphate group (HSO_4^- and SO_3^-, respectively), and the neutral loss of 151 u, suggested the annotation of homovanillyl alcohol sulphate,[37] more abundant in *S. perennis* but not found in *S. europaea*.

Among amino acid derivatives, *N*-acetylphenylalanine (**7**) and *N*-acetyltryptophan (**8**), showing the loss of an acetyl portion (−42 u), were detected in all samples.[38] Among flavonoids, quercetin, isorhamnetin, and kaempferol derivatives were found in the three *Salicornia* extracts. Compounds **9**–**11** were all annotated as quercetin glycosides, due to the presence in the fragmentation pathway of the product ion at *m/z* 301.03 attributed to the aglycone portion of quercetin (**20**), thus tentatively identified as quercetin pentosylhexoside (**9**), quercetin rutinoside (**10**), and quercetin hexoside (**11**).[39] Compounds **13**, **14**, **16**, and **17** were all attributed to isorhamnetin derivatives, as deduced by the presence in the MS^2 spectra of the diagnostic product ion at *m/z* 315.05 (aglycone portion, compound **22**),[40,41] while compound **15** was annotated as kaempferol hexoside. Compounds **13** and **14** were detected only in *S. perennis* extract, whereas compound **9** only in *S. fruticosa* and *S. perennis* extracts. Compounds **23**, **24**, and **31** were identified as trihydroxyoctadecadienoic acid, trihydroxyoctadecenoic acid, and hydroxylinolenic acid, respectively, belonging to fatty acid compound class and showing the consecutive neutral loss of one/two molecules of water.[42] Finally, among triterpenoid saponins, oleanolic acid derivatives were found, showing the loss of pentosyl (−132 u), hexosyl (−162 u) and/or glucuronyl (−176 u) moieties, thus tentatively annotated as oleanolic acid glucuronide hexoside (**27**), oleanolic acid glucuronide dihexoside (**28**), and oleanolic acid glucuronide pentoside (**29**). Compounds **25**, **26**, and **30** were not fully identified, but were recognized as belonging to triterpenoid saponins due to their MS^2 fragmentation patterns.[43,44]

Successively, among these annotated specialized metabolites, flavonoids and saponins were quantified (Table 2). *Salicornia fruticosa* resulted in the species with the highest amount of identified

Table 1. Ultrahigh-performance liquid chromatography high-resolution Orbitrap electrospray ionization tandem mass spectrometry (UHPLC-HR-Orbitrap-ESI-MS^2) data of the tentatively annotated compounds, detected in *Salicornia europaea* (SE), *Salicornia fruticosa* (SF), and *Salicornia perennis* (SP) extracts

Compound number	Compound	t_R (min)	$[M-H]^-$	Formula	Error (ppm)	ESI-MS^2 (*m/z*)[a]	Extract
Organic acid							
1	Quinic acid	0.64	191.0559	$C_7H_{12}O_6$	−1.05	173.05, 146.94, 133.01, **102.95**	SE, SF, SP
Phenolic acids							
2	Caffeoylquinic acid I	3.22	353.0880	$C_{16}H_{18}O_9$	0.57	**191.06**, 179.03, 173.05, 135.04	SE, SF
4	Caffeoylquinic acid II	5.25	353.0880	$C_{16}H_{18}O_9$	0.57	**191.06**, 179.03, 173.05, 135.04	SE, SF, SP
5	*p*-Coumaric acid	7.42	163.0396	$C_9H_8O_3$	−3.07	**119.05**, 93.03	SE, SF, SP
6	*p*-Coumaroylquinic acid	7.54	337.0931	$C_{16}H_{18}O_8$	0.59	**191.06**; 173.04; 163.04; 119.05	SE, SF
12	Dicaffeoylquinic acid I	12.02	515.1195	$C_{25}H_{24}O_{12}$	0	**353.09**, 191.06, 179.03, 173.04	SE, SF, SP
18	Dicaffeoylquinic acid II	13.79	515.1195	$C_{25}H_{24}O_{12}$	0	**353.09**, 191.06, 179.03, 173.04	SE, SF, SP
19	Caffeoyl dihydrocaffeoylquinic acid	14.40	517.1344	$C_{25}H_{26}O_{12}$	−1.35	**355.10**, 337.09, 191.06, 179.03	SE, SF, SP
21	Caffeoylferuloylquinic acid	16.06	529.1355	$C_{26}H_{26}O_{12}$	0.76	367.10, 193.05, **173.04**, 161.02	SE, SF, SP
Sulphate derivative							
3	Homovanillyl alcohol sulphate	3.95	247.0281	$C_9H_{12}O_6S$	−0.40	202.98, **96.96**, 79.96, 69.06	SF, SP
Amino acid derivatives							
7	*N*-Acetylphenylalanine	8.08	206.0821	$C_{11}H_{13}NO_3$	−0.97	**164.07**, 147.04	SE, SF, SP
8	*N*-Acetyltryptophan	9.22	245.0933	$C_{13}H_{14}N_2O_3$	0.41	226.88, 203.08, 142.07, 116.05	SE, SF, SP
Flavonoids							
9	Quercetin pentosylhexoside	10.51	595.1309	$C_{26}H_{28}O_{16}$	0.67	301.03, **300.03**, 271.02, 255.03	SF, SP
10	Quercetin rutinoside	11.25	609.1465	$C_{27}H_{30}O_{16}$	0.66	301.03, **300.03**	SE, SF
11	Quercetin hexoside	11.37	463.0884	$C_{21}H_{20}O_{12}$	0.43	301.03, **300.03**, 271.02, 255.03	SE, SF, SP
13	Isorhamnetin deoxyhexosylhexoside I	12.25	623.1620	$C_{28}H_{32}O_{16}$	0.32	315.05, **314.04**, 299.02, 271.02	SP
14	Isorhamnetin pentosylhexoside	12.77	609.1465	$C_{27}H_{30}O_{16}$	0.66	**315.05**, 300.03, 271.02	SP
15	Kaempferol hexoside	12.95	447.0936	$C_{21}H_{20}O_{11}$	0.67	285.04, **284.03**, 255.03, 227.03	SE, SF, SP
16	Isorhamnetin deoxyhexosylhexoside II	13.45	623.1620	$C_{28}H_{32}O_{16}$	0.32	315.05, **314.04**, 299.02, 271.02	SE, SF, SP
17	Isorhamnetin hexoside	13.56	477.1038	$C_{22}H_{22}O_{12}$	0	315.05, **314.04**, 299.02, 285.04	SE, SF, SP
20	Quercetin	14.91	301.0354	$C_{15}H_{10}O_7$	0	284.03, 179.00, **151.02**, 121.03	SE, SF, SP
22	Isorhamnetin	18.07	315.0511	$C_{16}H_{12}O_7$	0.32	301.03, **300.03**, 271.02, 255.03	SE, SF, SP
Fatty acids							
23	Trihydroxyoctadecadienoic acid	18.30	327.2178	$C_{18}H_{32}O_5$	0.31	309.21, 291.20, 269.18, 251.16	SE, SF, SP
24	Trihydroxyoctadecenoic acid	19.23	329.2333	$C_{18}H_{34}O_5$	0	311.22, 293.21, 249.22, 229.14	SE, SF, SP
31	Hydroxylinolenic acid	24.93	293.2122	$C_{18}H_{30}O_3$	0	275.20, 274.88, 265.22, 235.17	SE, SF, SP
Saponins							
25	Triterpenoid saponin	21.54	807.3812	—	—	631.35, 469.29, 336.18, 303.27	SE, SF, SP
26	Triterpenoid saponin	23.18	777.4073	—	—	615.35, 439.32, 307.17	SE, SF, SP
27	Oleanolic acid glucuronide hexoside	23.85	793.4395	$C_{42}H_{66}O_{14}$	1.89	631.38, 569.38, 455.35	SE, SF, SP
28	Oleanolic acid glucuronide dihexoside	23.87	955.4537	$C_{47}H_{72}O_{20}$	−0.73	793.44, 631.38, 569.38, 455.35	SE, SF, SP
29	Oleanolic acid glucuronide pentoside	24.04	925.4434	$C_{46}H_{70}O_{19}$	−0.54	793.44, 631.38, 569.38, 455.35	SE, SF, SP
30	Triterpenoid saponin	24.35	645.3281	—	—	469.29, 214.21, 193.03	SF, SP

Note: The molecular formula and the calculated mass errors were also reported.
[a] The base ion peaks are indicated in bold.

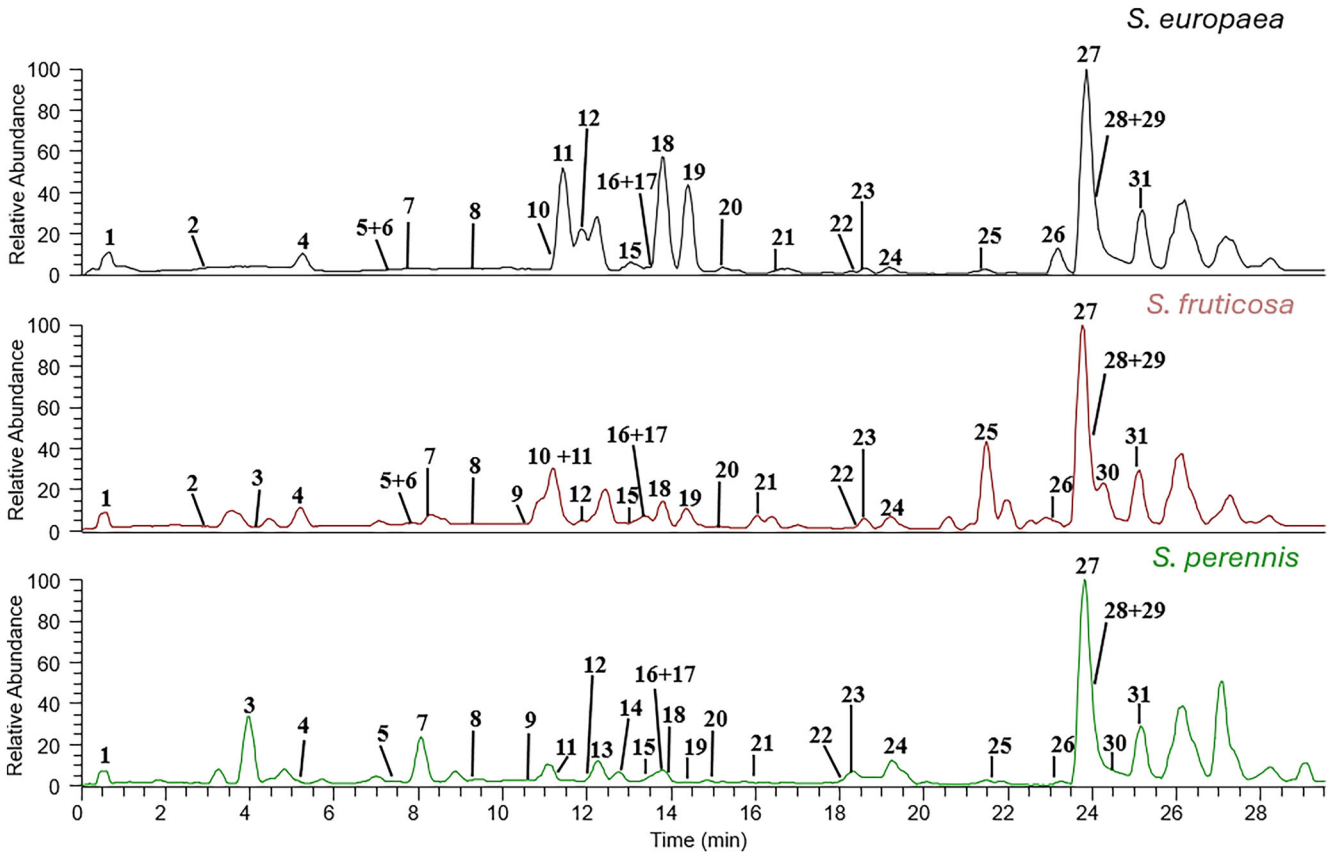

Figure 1. Comparison of LC–MS qualitative profiles of all studied *Salicornia* species *n*-butanol extracts, obtained in the negative ion mode. Each number corresponds to compounds listed in Table 1.

flavonoids (91 ± 7 mg 100 g^{-1} fw ± SD) and saponins (437 ± 29 mg 100 g^{-1} fw ± SD), while *S. europaea* and *S. perennis* showed a very similar amount. *Salicornia fruticosa* species resulted particularly rich in quercetin rutinoside (48 ± 4 mg 100 g^{-1} fw ± SD), and an isomer of isorhamnetin deoxyhexosylhexoside II (16 ± 1 mg 100 g^{-1} fw ± SD), while *S. europaea* in quercetin hexoside (20 ± 1 mg 100 g^{-1} fw ± SD). Among the annotated saponins, oleanolic acid glucuronide hexoside was the most abundant in *S. fruticosa* extract (38 ± 3 mg 100 g^{-1} fw ± SD) but found in high amount also in *S. perennis* (25 ± 1 mg 100 g^{-1} fw ± SD).

Determination of total polyphenol content and antioxidant activity

The polyphenol content, determined by Folin–Ciocâlteu reagent, showed notable variation among the *Salicornia* species, with *S. europaea* exhibiting the highest level (159.99 ± 8.11 mg GAE g^{-1}), followed by *S. fruticosa* and *S. perennis* (Table 3). TPC is widely recognized for its strong correlation with antioxidant activity, as polyphenols play a key role in neutralizing free radicals. Consistent with this evidence, *S. europaea* demonstrated the highest antioxidant capacity among the three species, which aligns with its elevated polyphenol content (Table 3). To provide a comparative assessment across different antioxidant assays, the Relative Antioxidant Capacity Index (RACI) was calculated. RACI offers a standardized metric to rank samples based on their antioxidant potential, where higher scores indicate stronger activity. In agreement with the polyphenol results, *S. europaea* achieved the highest RACI score, followed by *S. fruticosa* and *S. perennis* (Table 3). Notably, the polyphenol content and antioxidant activity observed in this study exceeded those reported in previous investigations.[45-47]

This enhanced activity may be partially attributed to the partitioning process applied during extract preparation, which likely removed salts and sugars that could interfere with antioxidant function, as previously demonstrated by Kim *et al.*[48]

Effect on the activity of carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase

The investigation of the antidiabetic potential of *Salicornia* species was conducted by a multifaceted mechanism involving inhibition of carbohydrate-hydrolysing enzymes, modulation of intestinal glucose transporter, and GLP-1 secretion. The inhibition of α-amylase and α-glucosidase enzymes is significant in the context of managing diabetes and controlling blood sugar levels. Both enzymes play crucial roles in carbohydrate digestion and glucose absorption, and their inhibition can delay the breakdown of starches and sugars, thereby preventing rapid increases in blood glucose levels. The enzyme α-amylase catalyses the breakdown of starch into maltose and dextrin in the digestive system. By inhibiting α-amylase, starch digestion is delayed, resulting in slower release of glucose into the bloodstream. This can be beneficial for controlling postprandial (after-meal) blood glucose spikes. α-Glucosidase enzyme breaks down oligosaccharides and disaccharides into glucose in the small intestine. It acts on the final steps of carbohydrate digestion, hydrolysing the bonds in

Table 2. Quantitative results of flavonoids and saponins tentatively identified in *Salicornia* species *n*-butanol extracts

Compound number	Compounds	*Salicornia* species extracts (mg 100 g^{-1} fw ± SD)		
		Salicornia europaea	*Salicornia fruticosa*	*Salicornia perennis*
Flavonoids				
9	Quercetin pentosylhexoside	Not detected^{B}	4.3 ± 0.5^{A}	0.37 ± 0.004^{B}
10	Quercetin rutinoside	0.24 ± 0.01^{B}	48 ± 4^{A}	Not detected^{B}
11	Quercetin hexoside	20 ± 1^{A}	14 ± 1^{B}	0.77 ± 0.02^{C}
13	Isorhamnetin deoxyhexosylhexoside I	Not detected^{B}	Not detected^{B}	6.4 ± 0.2^{A}
14	Isorhamnetin pentosylhexoside	Not detected^{B}	Not detected^{B}	4.4 ± 0.04^{A}
15	Kaempferol hexoside	1.5 ± 0.1^{A}	0.64 ± 0.06^{B}	0.065 ± 0.003^{C}
16	Isorhamnetin deoxyhexosylhexoside II	0.022 ± 0.002^{B}	16 ± 1^{A}	1.4 ± 0.007^{B}
17	Isorhamnetin hexoside	1.2 ± 0.1^{B}	4.2 ± 0.3^{A}	4.4 ± 0.1^{A}
20	Quercetin	0.39 ± 0.01^{B}	2.3 ± 0.3^{A}	0.25 ± 0.01^{B}
22	Isorhamnetin	0.014 ± 0.001^{C}	1.1 ± 0.1^{B}	2.4 ± 0.03^{A}
Saponins				
25	Triterpenoid saponin	0.027 ± 0.005^{B}	225 ± 15^{A}	0.23 ± 0.02^{B}
26	Triterpenoid saponin	15 ± 1^{B}	26 ± 2^{A}	3.8 ± 0.2^{C}
27	Oleanolic acid glucuronide hexoside	11 ± 1^{C}	38 ± 3^{A}	25 ± 1^{B}
28	Oleanolic acid glucuronide dihexoside	16 ± 1^{A}	8.5 ± 0.6^{C}	12 ± 0.3^{B}
29	Oleanolic acid glucuronide pentoside	12 ± 1^{A}	12 ± 1^{A}	2.7 ± 0.1^{B}
30	Triterpenoid saponin	Not detected^{B}	127 ± 7^{A}	0.27 ± 0.01^{B}
	Total flavonoids	23 ± 1	91 ± 7	20 ± 0.4
	Total saponins	54 ± 4	437 ± 29	44 ± 2

Note: The superscript uppercase letters indicate statistically significant differences among the samples; fw, fresh weight; SD, standard deviation.

disaccharides like sucrose and maltose, and oligosaccharides into glucose.[49]

The results of the α-glucosidase inhibition assay revealed that none of the *Salicornia* species showed significant activity at the tested concentrations. In contrast, *Salicornia* species demonstrated notable inhibitory effects on the α-amylase enzyme, with *S. europaea* showing the lowest IC_{50} value (0.43 ± 0.046 mg mL^{-1}), followed by *S. perennis* (0.64 ± 0.08 mg mL^{-1}) and *S. fruticosa* (0.96 ± 0.14 mg mL^{-1}) (Fig. 2). These results indicate that *S. europaea* is the most potent at inhibiting α-amylase among the tested species, with its inhibitory effect almost twice as strong as that of *S. fruticosa*. Results were compared with acarbose, a well-known α-amylase inhibitor, which exhibited an IC_{50} of 0.033 ± 0.003 mg mL^{-1}. Previous studies have shown that water and 70% ethanol extracts from the fiber fraction of *Salicornia ramosissima*[50] and from the aerial parts of *S. europaea*[34] exhibit only weak inhibitory activity against carbohydrate-hydrolysing enzymes. Additionally, the 70% ethanol extract of *Salicornia herbacea* showed weak inhibition of α-glucosidase, with an inhibition rate of 10.2 ± 0.5% at a concentration of 0.5 mg mL^{-1}.[51] Similarly, seed oil from *Salicornia brachiata* demonstrated inhibition rates of 16.80%, 19.00%, and 30.00% at concentrations of 16, 32, and 48 mg mL^{-1}, respectively.[52] Thus, the observed inhibitory activity of these species against α-amylase suggests that *Salicornia* could be considered a potential candidate for managing blood glucose levels, particularly in terms of slowing starch digestion.

Antidiabetic activity of *Salicornia* species on STC-1

STC-1 viability

The impact of *Salicornia* extracts on cell viability was assessed in intestinal STC-1 cells using the MTT assay, a standard method for evaluating cell metabolic activity and viability. The study involved treatments with various concentrations (10–400 μg mL^{-1}) of *Salicornia* species after 2 and 24 h. At all tested concentrations and time points, *S. europaea* had no significant effect on the metabolic activity of STC-1 cells compared to the control group (CTRL). In contrast, *S. fruticosa* induced a reduction in STC-1 cell viability at the higher concentrations (100–400 μg mL^{-1}) after 24 h (IC_{50} = 262.11 ± 8.4 μg mL^{-1}) of treatment. *Salicornia perennis* also decreased cell viability across the

Table 3. Total polyphenol content and antioxidant activity (mean ± standard deviation) of *Salicornia* species *n*-butanol extracts

Species	TPC (mg GAE g^{-1})	ABTS (mg TE g^{-1})	FRAP (mg TE g^{-1})	DPPH (mg TE g^{-1})	RACI
Salicornia europaea	159.99 ± 8.11^{A}	587.03 ± 27.22^{A}	789.73 ± 37.15^{A}	230.59 ± 21.22^{A}	1.08
Salicornia fruticosa	113.58 ± 2.64^{B}	517.81 ± 12.58^{B}	274.08 ± 24.14^{B}	123.42 ± 9.92^{B}	−0.29
Salicornia perennis	97.05 ± 5.72^{C}	405.60 ± 5.55^{C}	240.61 ± 19.92^{B}	112.14 ± 9.45^{B}	−0.79

Note: The superscript uppercase letters indicate statistically significant differences among the samples; TPC, total polyphenol content; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; TE, Trolox equivalent; RACI, Relative Antioxidant Capacity Index.

same concentration range (100–400 μg mL^{-1}) with effects observed as early as 2 h post-treatment (Fig. 3). Cytotoxic concentrations were excluded from the study in subsequent experiments. Despite similar LC–MS profiles among the three *Salicornia* species, the observed differences in cytotoxicity are likely derived from a combination of factors, including undetected or unannotated bioactive compounds, variations in compound abundance and potency, matrix effects, and differing biological interactions. It is plausible that some biologically active compounds were present at relevant concentrations in one extract but absent or below detection limits in others. Additional contributing factors may include differences in saponin isomer composition, synergistic or antagonistic interactions among components, extract stability, and differential cellular uptake or stress responses.

Glucose uptake and SGLT-1 protein expression

Modulating glucose uptake in the small intestine is a key strategy for maintaining energy homeostasis and managing metabolic disorders such as type 2 diabetes (T2D). The small intestine plays a central role in nutrient absorption, and its regulation directly influences systemic glucose levels and overall metabolic health. Excessive glucose absorption in the small intestine is associated with hyperglycemia and the progression of T2D. Therefore, targeting intestinal glucose uptake, particularly by inhibiting SGLT1, the primary mediator of glucose absorption, is a promising therapeutic approach to reduce postprandial blood glucose levels.[53]

In this study, the effects of the different *Salicornia* species on glucose uptake were investigated using the fluorescent glucose analogue 2-NBDG. Treatment with *S. europaea* significantly reduced glucose uptake dose-dependently, while *S. fruticosa* showed no significant effect, as illustrated in Fig. 4. Although *S. perennis* exhibited cytotoxic effects at higher concentrations, no reduction in glucose uptake was observed at non-toxic doses (10 and 50 μg mL^{-1}, data not shown). Glucose uptake in the small intestine is predominantly facilitated by glucose transporters such as SGLT1, and modulation of these transporters represents a key mechanism for regulating intestinal glucose absorption and maintaining systemic glucose homeostasis.[54] As shown in Fig. 5, *S. europaea* treatment led to a dose-dependent down-regulation of SGLT1 protein expression, supporting its potential role in reducing intestinal glucose absorption and managing T2D. In the context of metabolic diseases such as T2D, SGLT1 plays a pivotal role in regulating postprandial blood glucose levels. Enhanced or dysregulated activity of SGLT1 can lead to increased glucose absorption, contributing to hyperglycemia and exacerbating insulin resistance.[55] Consequently, SGLT1 has emerged as a promising therapeutic target; inhibiting its activity can reduce glucose uptake from the intestine, thereby lowering postprandial glucose spikes and improving glycemic control.

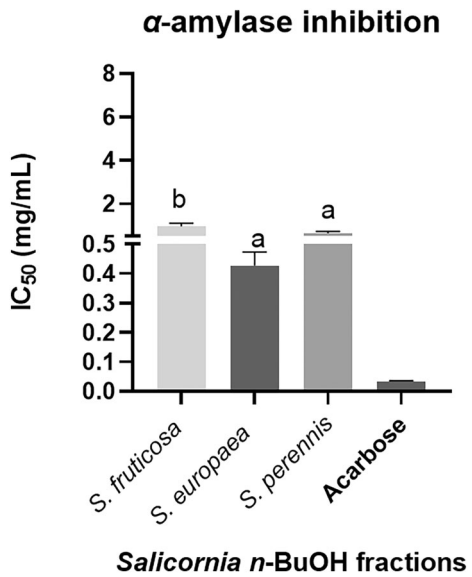

Figure 2. Inhibitory effect of *Salicornia* species *n*-butanol extracts on α-amylase enzyme.

Phytochemicals such as flavonoids, saponins, and dicaffeoylquinic acids are abundant in plant-based foods. Growing evidence suggests that dietary phytochemicals can modulate carbohydrate metabolism at multiple levels. Their effects on intestinal glucose transporters have been extensively studied *in vitro* using models such as intestinal brush border membrane vesicles, everted intestinal sacs, and Caco-2 cell monolayers; several flavonoids and phenolic acids have been shown to inhibit glucose transport.[56] Additionally, the antidiabetic activity of dicaffeoylquinic acids as phenolic acids is associated to the modulation of gut microbiota and bile acid metabolism,[51] whereas the saponins, a class of glycosylated triterpenoids, exert hypoglycemic effects by stimulating insulin release, improving β-cell survival, and inhibiting intestinal glucose absorption.[57] Supporting our data, a previous study demonstrated that administration of *S. herbacea* powder, either alone or combined with exercise (swimming), in diabetic rats, significantly increased the expression of glucose transporters GLUT-4 and GLUT-2 in the liver and muscle.[58] This was further supported by elevated glycogen levels in both tissues, suggesting that *S. herbacea* holds promise as a natural therapeutic agent for diabetes management. Moreover, the phytoconstituents in *Salicornia* species, like laminarin isolated from *S. herbacea*, stimulate glucose uptake via AMPK-p38 MAPK pathway in L6 muscle cells.[24] While the observed SGLT1 modulatory effects can be correlated with the overall extract composition and supported by literature on *Salicornia* phytoconstituents, the specific compounds responsible for these effects remain unidentified. Elucidating the active constituents will require targeted approaches, such as bioactivity-guided fractionation and detailed chemical characterization, to establish structure–activity relationships and clarify the mechanisms underlying the observed effects. However, to date, no studies have investigated the effects of *Salicornia* species on glucose transport or absorption in intestinal cells.

Glucagon-like peptide 1 (GLP-1) secretion

Inhibition of SGLT1 may additionally modulate the secretion of incretin hormones, including GLP-1. In healthy humans, GLP-1 is secreted after eating, lowering glucose concentrations by promoting insulin secretion and suppressing glucagon release. GLP-1 stimulates insulin secretion via the GLP-1 receptor on pancreatic β-cell. In T2D patients, GLP-1 release seems to be down-regulated.[59] In this study, the secretion of GLP-1 was evaluated testing the supernatant of STC-1 after *S. europaea* treatment (2 h) and the highest concentrations (200 and 400 μg mL^{-1}) significantly increased the GLP-1 secretion dose-dependently (Fig. 6). Combining increased GLP-1 secretion with glucose uptake inhibition through the SGLT-1 reduction in the small intestine represents a promising dual approach to managing T2D. This strategy targets both the enhancement of insulin secretion and the reduction of postprandial glucose absorption,

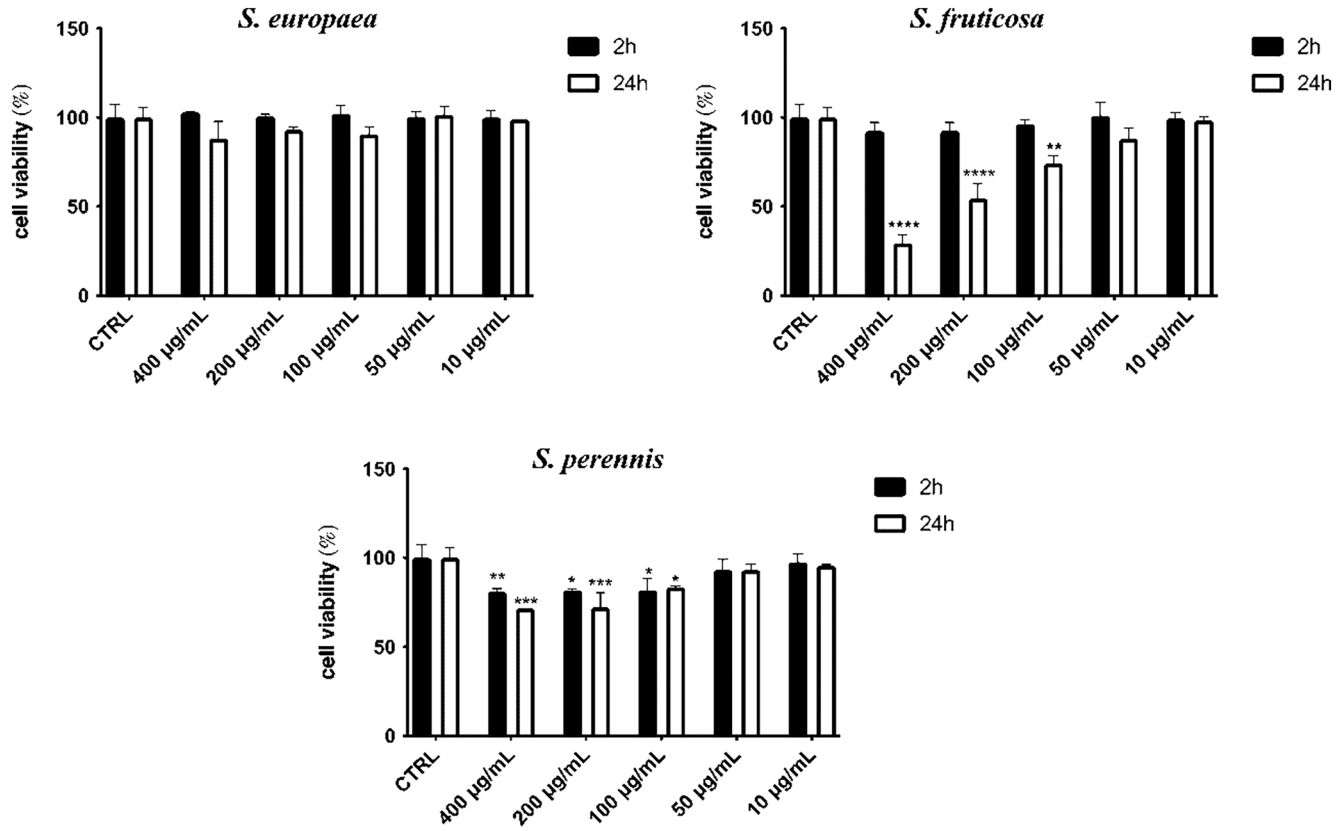

Figure 3. Cytotoxicity analysis by MTT assay in STC-1 cells treated for 2 and 24 h with different concentrations (10–400 µg mL^{-1}) of *Salicornia* species *n*-butanol extracts. Data are represented as the mean ± standard deviation of three independent replicates ($n = 3$). ****$P < 0.001$; ***$P < 0.005$, ** $P < 0.01$, * $P < 0.05$ *versus* control cells (CTRL).

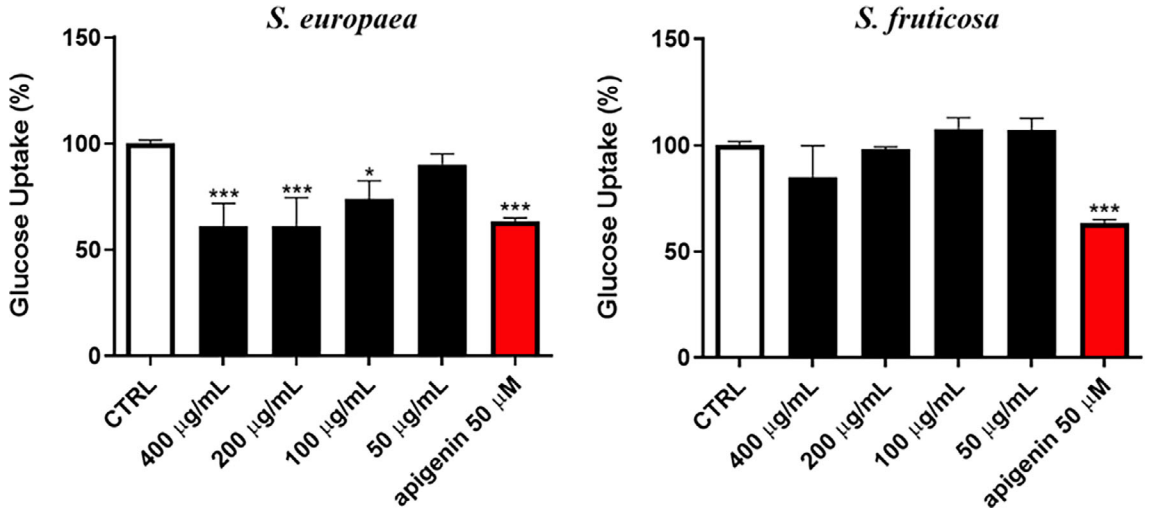

Figure 4. Inhibition of glucose uptake measured by 2-NBDG, after treatment with different concentrations of *Salicornia europaea* and *Salicornia fruticosa* extracts for 2 h. Apigenin was used as positive control. Data are represented as the mean ± standard deviation of three independent replicates ($n = 3$). ***$P < 0.005$, * $P < 0.05$ *versus* control cells (CTRL).

addressing key aspects of glucose homeostasis. Nutritional factors can modulate GLP-1 secretion and glucose transporter activity contributing to decreased glucose uptake. For instance, dietary components such as dietary fibres and certain polyphenols have been shown to stimulate GLP-1 release and reduce the glucose uptake.[60,61] In particular, quercetin, has shown potential in regulating glucose homeostasis by promoting GLP-1 secretion,[62] and certain quercetin glucosides have been shown to inhibit the sodium-dependent glucose transporter SGLT-1 in intestinal epithelial cells, thereby reducing dietary glucose absorption and potentially mimicking the effect of pharmaceutical SGLT inhibitors.[63]

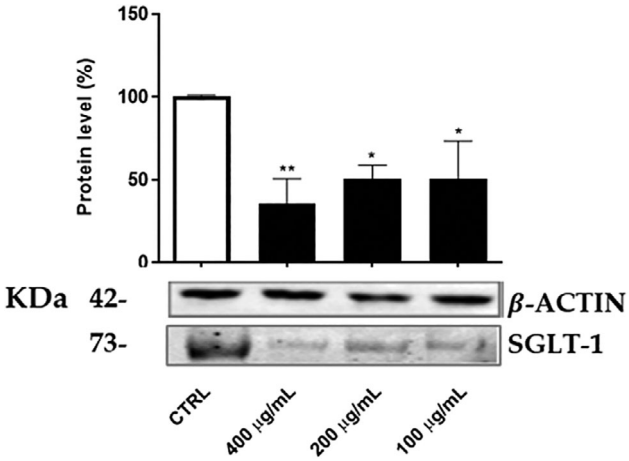

Figure 5. Effect of *Salicornia europaea* extract on sodium-glucose transporter 1 (SGLT-1) protein expression in STC-1 cells after 2 h of treatment. Densitometric analysis of the immunoreactive bands are expressed as the mean ± standard deviation of three independent experiments ($n = 3$). The protein levels were normalized with β-actin content. Data were normalized to control cells set to 100%. $^{**}P < 0.01$, $^{*}P < 0.05$ *versus* untreated cells (CTRL).

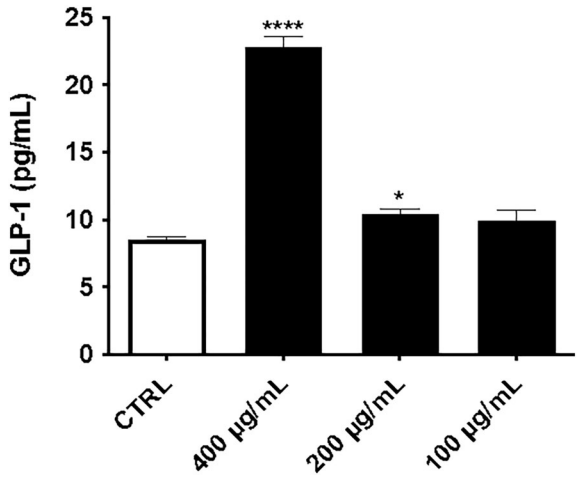

Figure 6. GLP-1 secretion measured by ELISA kit of *Salicornia europaea* extract. Data are represented as the mean ± standard deviation of three independent replicates ($n = 3$). $^{****}P < 0.001$, $^{*}P < 0.05$ *versus* control cells (CTRL).

Collectively, these phytochemicals represent promising multifunctional agents for type 2 diabetes mellitus management. Their diverse mechanisms, ranging from intestinal glucose absorption inhibition (SGLT-1, carbohydrate-digesting enzymes) to incretin enhancement (GLP-1) highlight their therapeutic potential as adjuncts to existing pharmacotherapies.

CONCLUSIONS

The phytochemical investigation of the three *Salicornia* species extracts revealed a rich and varied chemical profile. The presence of many phenolic acid derivatives and flavonoids highlighted the potential health value of these halophyte plants. Through a comparative chemical and biological approach, this study provides new insights into the antidiabetic potential of both the well-studied *S. europaea* and the comparatively understudied species *S. fruticosa* and *S. perennis*. Particularly, this work provides the first evidence that *Salicornia* extracts can modulate intestinal glucose uptake through the regulation of the sodium–glucose cotransporter SGLT1. The evaluation of the antidiabetic potential indicated a multifactorial mode of action, including mild inhibition of α-amylase activity, down-regulation of SGLT1, and stimulation of GLP-1 secretion. Among the extracts, the *n*-butanol fraction of *S. europaea* demonstrated the most pronounced α-amylase inhibitory effect, suggesting a potential role in modulating postprandial hyperglycemia and warranting further investigation. Notably, treatment with *S. europaea* extract significantly downregulated SGLT1 protein expression in STC-1 cells, which was associated with a reduction in glucose uptake. These findings suggest that bioactive compounds within the *S. europaea* phytocomplex may modulate intestinal glucose absorption by interfering with SGLT1 expression or function. This dual mechanism – targeting both enzymatic digestion and glucose transport – highlights the therapeutic potential of *S. europaea* in glycemic control and supports its further exploration as a functional food or phytotherapeutic agent in the management of T2D.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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