

Unraveling the nutraceutical potential of *Salicornia ramosissima* by-product – impact of gastrointestinal digestion and intestinal permeability on *in vitro* bioactivity

Diana Pinto^a, Inês Santos^a, Filipa Teixeira^a, Stefania Sut^b, Mónica Vieira^c, Miguel Salazar^d, Cristina Delerue-Matos^a, Stefano Dall'Acqua^{b,*}, Francisca Rodrigues^{a,*}

^a REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4249-015 Porto, Portugal

^b Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35121 Padova, Italy

^c RISE-Health, Center for Translational Health and Medical Biotechnology Research (TBIO), CQB, ESS, Polytechnic of Porto, R. Dr. António Bernardino de Almeida, 400, 4200-072 Porto, Portugal.

^d Agro-On/RiaFresh - Verduras da Ria Formosa, Sítio do Besouro, 8005-421 Faro, Portugal

ARTICLE INFO

Keywords:

Glasswort
Phytochemicals
Infogest protocol
Intestinal model
Radicals scavenging
Neuroprotective

ABSTRACT

Salicornia ramosissima by-product (SP) is an underexploited antioxidant-rich by-product. This study explored the phytochemical profile, bioaccessibility, and biological activity of SP before and after extraction (SBE and SAE, respectively) upon gastrointestinal simulated digestion and intestinal permeability. The phenolic and flavonoid concentrations increased during digestion, reaching bioaccessibility rates above 95 % for both SBE and SAE. Promising antioxidant/antiradical properties and neuroprotective effects were attested upon digestion. Regarding the phytochemical profile, 17 compounds were identified, including (di)caffeoylquinic acids, gallo-catechin, and triterpenoid saponins. The intestinal absorption of bioactive compounds from SAE and SBE intestinal digests was proven through a Caco-2/HT29-MTX cells co-culture model, with 4-caffeoylquinic acid (34.84 %) and 4,5-dicaffeoylquinic acid (26.73 %) reaching the highest permeation rates after 4 h, respectively, for SAE and SBE. These findings support the harnessing of SP as a promising functional and nutraceutical ingredient rich in pro-healthy compounds with proven bioactivity upon *in vitro* digestion and intestinal permeation.

1. Introduction

Phytochemicals are plant secondary metabolites abundantly produced to protect them against adverse environmental stressors (e.g., excessive light exposure, nutrient deficiency, low temperature, and pathogen infection) (Clifford et al., 2008; Rodrigues et al., 2018). In addition, these phytochemicals are valuable compounds endowed with human health benefits that are frequently incorporated in functional foods and dietary supplements (Cádiz Gurrea et al., 2019). Among them, phenolic compounds, carotenoids, glucosinolates, phytoestrogens, phytosterols, and organosulphur compounds stand out as the most commonly used owing to their capacity to exert long-term biological effects (Pinto, Silva, et al., 2023).

Halophytes embrace a wide variety of plants, including the *Salicornia*

genus that comprises up to 30 species, some with commercial value (Lopes et al., 2021). *Salicornia* L. (Amaranthaceae family) species are characterized by succulent stems and leaves, thriving in saline coastal and salt marshes with nutrient-poor soils (Pinto et al., 2021). Even though the halophytes consumption has long been documented in Asia, European countries have only recently demonstrated interest on them as conventional vegetables to supplement the diet, or as a seasoning (Lopes et al., 2021). Nonetheless, halophytes remain unknown as edible plants to many consumers and their potential as pro-healthy vegetables is still overlooked.

Halophytic plants, such as *S. ramosissima*, are garnering increased attention in food industry due to their nutritional benefits and adaptability to saline environments (Silva et al., 2007). In Portugal, food companies have demonstrated the economic viability of cultivating

* Correspondence to: REQUIMTE/LAQV - Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal.

** Correspondence to: Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35121 Padova, Italy.

E-mail addresses: stefano.dallacqua@unipd.it (S. Dall'Acqua), francisca.rodrigues@graq.isep.ipp.pt (F. Rodrigues).

Salicornia species on previously unexplored saline lands, leading to an average of 18 % increase in annual farm income and sparking interest among local farmers to repurpose abandoned lands using this halophyte (EU CAP Network Good Practice Report, 2021). Particularly, *S. ramosissima* holds great potential in food industry as a sustainable source of bioactive molecules, a natural salt substitute, and a functional ingredient in health-driven products, aligning with the growing market demand for nutritious and eco-friendly food alternatives (Pinto et al., 2021; Silva et al., 2024).

S. ramosissima J. Woods is a typical vegetation of salt marshes ecosystems in the Iberian Peninsula estuaries, encompassing a Portuguese native species (Lopes et al., 2021). Currently, this halophyte is also found along the European coast and inland regions of Western Europe (Lopes et al., 2021). *S. ramosissima*, usually known as glasswort, samphire, or sea asparagus, is a salt-tolerant plant that can endure severe droughts, short periods of flooding and resistant to diseases and pests, comprising a valuable food resource in case of future environmental threats (Lopes et al., 2021; Pinto et al., 2021). In Portugal, *S. ramosissima* is distributed in salt marshes on the Aveiro estuary, Algarve Coast Natural Park and Ria Formosa, being frequently used in local cuisine (as salt substitute) or trendy garnish in gourmet restaurants (Custódio, Lillebø, et al., 2021; Silva et al., 2007). Therefore, the phytochemical composition of glasswort is arousing interest within the scientific community, with studies demonstrating its vast potential owing to its anticancer, anti-inflammatory, antihyperglycemic, antihyperlipidemic, antimicrobial, antioxidant, and protective vessel properties (Cárdenas-Pérez et al., 2021; Nájara et al., 2023; Pinto et al., 2021). Recent studies described the richness of *S. ramosissima* in phenolic acids (gallic and caffeic acids derivatives), flavonoids (mainly flavonols), minerals (Na, K, Ca, and Mg), vitamins (B1, B6, and E) and carotenoids (Lima et al., 2020; Nájara et al., 2023; Silva et al., 2021). Moreover, some Portuguese start-up companies become pioneers in the cultivation, production, and incorporation of this halophyte into the nutrition and health system (Custódio, Rodrigues, et al., 2021). As an annual herbaceous plant, *S. ramosissima* produces the appreciated vegetable between March and September (Pinto et al., 2021). During the rest of the year, the leaves and shoots generated comprise an undervalued by-product that contains valuable antioxidants, such as polyphenols and natural pigments (namely chlorophylls and carotenoids) that, despite their richness, need to be repurposed (Marçal et al., 2023). Notwithstanding, there is only one study centered on the bioactivity and phytochemical composition of *S. ramosissima* by-product (Pinto et al., 2021).

The exploitation of halophytes as nutrients-rich foods emphasizes the importance of evaluating their digestibility and bioactivity during the gastrointestinal digestion using more realistic and useful models, including *in vitro* simulated digestion and cellular models that mimic the human digestion process and intestinal absorption (Pinto, Silva, et al., 2023). The digestibility of halophytes using *in vitro* models remains an unexplored research field, with no published studies regarding these edible plants.

In light of the biological potential of halophytes, this study aims to investigate the phytochemical profile, bioaccessibility, and bioactivity of *S. ramosissima* by-product before and after extraction (SBE and SAE, respectively) upon *in vitro* gastrointestinal digestion and intestinal permeability, considering its potential valorization as source of pro-healthy compounds for food and nutraceutical industries. In the digested by-product, interactions between phytochemicals and other components of the matrix, such as fibers or proteins, may occur, potentially altering their bioaccessibility, stability during digestion, and intestinal absorption (Pinto, Moreira, et al., 2023). Otherwise, the phytochemical compounds were isolated in the SAE, allowing to study directly their bioaccessibility in a concentrated form, without interactions within the food matrix (Danielski & Shahidi, 2025). As far as we know, this is the first time that the impact of gastrointestinal digestion and intestinal permeability was assessed on the phytonutrients' composition and bioactivity of *S. ramosissima* by-product.

2. Materials and methods

2.1. Sample

S. ramosissima by-product, composed of leaves and shoots that grow in salt marshes and coastal regions after collecting the edible halophyte between March and September, was generously provided by RiaFresh (Faro, Portugal) in March 2021. The by-product was collected from Ria Formosa (Faro, Portugal) in February 2021. After being dehydrated for 24 h at 41 °C, the samples were ground to 1 mm particle size in a grinder (Moulinex A320, Paris, France) and stored at room temperature ($\pm$ 20 °C) in the dark until further extraction. The composition and bioactivity of the *S. ramosissima* by-product were monitored after the sample pre-treatment (in March 2021) and before this study (in September 2023) by spectrophotometric assays to determine the TPC, TFC, and antioxidant properties, guaranteeing its stability during storage.

2.2. Extraction of *S. ramosissima* by-product

A conventional extraction was conducted at 80 °C for 10 min under constant agitation (200 RPM) using water as solvent and following the optimizing study performed by our research team (Pinto et al., 2021). Considering that the purpose of this study is to valorize *S. ramosissima* by-product for food and nutraceutical industry by applying green chemistry principles in compliance with the Sustainable Development Goals of the United Nations Agenda 2030 and the European Green Deal, water was selected as a GRAS (Generally Recognized as Safe) solvent. The extract obtained was filtered through Whatman n° 1 paper, frozen at −80 °C, lyophilized, and kept at 4 °C until subsequent assays.

2.3. Gastrointestinal simulated digestion

In vitro digestion was carried out using the INFOGEST protocol established by Brodkorb et al. (2019). The digestion was conducted on the powdered *S. ramosissima* by-product before and after extraction (corresponding to SBE and SAE, respectively). A blank sample (without extract) consisting of distilled water (the same solvent used to resuspend the lyophilized extracts) at an equal volume to the extracts was submitted to the same digestion procedure to eliminate any interferences from enzymes, solvents, and other reagents. The blank sample was also analyzed by HPLC and spectrophotometric assays for the correction of interferences from the digestive enzymes, salts, and buffers. The detailed procedure is described in the supplementary material. At the end of each digestion phase, aliquots were collected and centrifuged at 10,000 g for 10 min and stored at −80 °C. Three independent experiments were performed for each sample.

2.4. Solubilization of powdered dried by-product

Powdered dried SBE (1 g) was solubilized in 50 mL distilled water into a test tube and then placed for 10 min in a sonicator (Sonorex Super, Bandelin, Germany) and centrifuged at 10,000 g for 10 min (Irfan et al., 2022). After filtration, the liquid extract was stored at 4 °C.

2.5. Phenolic and flavonoid concentrations and antioxidant/antiradical activities

The TPC and TFC were determined by colorimetric assays applying the methods of Pinto, Silva, et al. (2023) and Pinto, Ferreira, et al. (2023). The antioxidant/antiradical assays, namely ABTS and DPPH radicals quenching and Ferric Reducing Antioxidant Power (FRAP), were conducted on digested and undigested extracts applying the methods of Pinto, Silva, et al. (2023). Detailed procedures were described in the supplementary material.

2.6. Counteracting activity against reactive oxygen species (ROS)

The ROS counteracting assays were performed on digested and undigested extracts by applying the methods of Gomes et al. (2007), as described in more detail in the supplementary material. The absorbance and fluorescence measurements were made on a microplate reader. The results were expressed as inhibition, in % or IC₅₀ (µg/mL), apart from ROO[•] (µg TE/mg dw).

2.7. Enzymes inhibitory assays

The activities of AChE and antioxidant enzymes, including SOD, CAT, and GSH-Px, were assessed in the presence of undigested and digested extracts using enzymatic kits. The LPO was assessed using a commercial kit by the formation of malondialdehyde (MDA) which is proportional to the fluorescence emitted. The AChE and SOD results were expressed as inhibition percentages. For CAT, GSH-Px, and LPO assays, the results were presented, respectively, as nmol/min/g dw, U/g dw, and nmol MDA/g dw.

2.8. Phytochemical composition by liquid chromatography coupled with mass spectrometry (LC-MS)

LC-MS was employed to assess the phytochemical composition of SBE and SAE and the respective digested fractions (Pinto et al., 2021). A 3.0 × 150 mm, 3.5 µm Agilent Eclipse XDB C-18 column was used for the stationary phase. The mobile phase was constituted by water with 1 % formic acid (A) and acetonitrile (B). The gradient starts at 90 % A isocratic for 2 min; in 10 min, 25 % A; and then in 15 min, 0 % A. The column temperature and flow rate were, respectively, defined at 30 °C and 0.4 mL/min. Two different MS platforms were used as detectors for qualitative purposes, including a quadrupole ion trap (QTrap) working in negative ion mode within a *m/z* range of 150 to 2000 and collecting data in the transdermal drug delivery system (TDDS) function. Compounds were identified based on their MS fragments and by comparison with the literature. For quantitative purposes, triple quadrupole MS 320 was used operating in negative mode. Transitions were obtained from the MS fragments observed in the ion trap and conditions for the triple quadrupole were optimized. After filtration through 0.45 µm nylon filters, 20 µL of samples were injected for the LC-MS measurements.

2.9. Intestinal cells viability

The intestinal cells viability was evaluated by MTT assay based on the uptake and metabolism of MTT reagent used as a vital mitochondrial dye (Pinto et al., 2021). The detailed procedure is described in the supplementary material. Extracts were tested at different concentrations between 31.25 and 1000 µg/mL.

2.10. Intestinal permeability assay

The intestinal digests were exposed to a Caco-2/HT29-MTX co-culture model cultivated for 21 days to simulate the intestinal mucosa (Araújo & Sarmiento, 2013). Aliquots were collected from the basolateral compartments at fixed times (0, 15, 30, 45, 60, 90, 120, 180, and 240 min). The procedure was conducted at 37 °C. The detailed procedure is described in the supplementary material. Permeability percentages of each phenolic compound were calculated at different times upon LC-MS analysis (Pinto, Silva, et al., 2023).

3. Results and discussion

3.1. Phenolic and flavonoid concentrations before and after gastrointestinal digestion

The TPC and TFC results of the undigested and digested SAE and SBE

are presented in Table 1.

As can be observed, the TPC raised for both SAE and SBE samples during the gastrointestinal digestion in the outlined sequence: oral < gastric < intestinal digested fractions, with statistical differences (*p* < 0.05) among the three digests. Additionally, the greatest value was determined for the SAE intestinal digest (2277.70 µg GAE/100 mg dw). Considering the undigested fractions, the TPC of SAE was 3-fold greater when compared to SBE. Undigested SAE and SBE showed lower TPC than their respective intestinal digested fractions.

The TFC results were also comparable to TPC, with flavonoid concentrations increasing during digestion. The highest TFC results were achieved for the undigested SAE (423.27 µg CE/100 mg dw) and SBE (413.90 µg CE/100 mg dw), being close (*p* > 0.05) to the respective intestinal digests (410.19 and 380.58 µg CE/100 mg dw, respectively).

Using the same technique, Pinto et al. (2021) optimized the extraction of *S. ramosissima* by-product at different temperatures (40–80 °C) and times (10–60 min). The TPC results were in line with the present study, varying between 480.35 and 905.16 µg GAE/100 mg dw. In another study, Silva et al. (2021) reported identical TPC (15.02 and 8.34 mg GAE/g DW, respectively) and TFC (8.44 and 8.41 mg CE/g DW) for conventional and microwave-assisted extracts (MAE) from *S. ramosissima*. Likewise, similar TPC (8.45–8.34 mg GAE/g dw) and TFC (0.37–0.27 mg CE/g dw) were achieved by Lima et al. (2020) for *S. ramosissima* cultivated at six different salinities (35–465 mM). The current outcomes are also in alignment with a study conducted by Oliveira-Alves et al. (2023) on different halophytes, namely *Crithmum maritimum*, *Disphyma crassifolium*, *Inula crithmoides*, *Mesembryanthemum crystallinum*, *Sarcocornia fruticosa*, and *S. ramosissima* (0.098–0.41 mg GAE/g on fresh weight, respectively, for *M. nodiflorum* and *S. ramosissima*).

In summary, the undigested SAE and its respective digests contained higher concentrations of polyphenols and flavonoids when compared to the undigested and digested SBE. Besides phenolic compounds, *S. ramosissima* by-product contains other macro and micronutrients, like fiber, lipids, minerals, proteins, and vitamins, which may interact with the polyphenols and prevent their release and bioaccessibility (Lima et al., 2020; Oliveira-Alves et al., 2023). Furthermore, the concentrations of polyphenols and flavonoids changed during the gastrointestinal simulated digestion, indicating that the digestion phase affects their contents as evidenced in previous studies for other food derivatives (Durán-Castañeda et al., 2023; Wu et al., 2022). This is primarily due to the low stability of phenolic compounds when submitted to digestive enzymes and pH changes, which promote their breakdown and metabolism into smaller compounds that become more bioavailable for gut absorption after digestion (Pinto, Silva, et al., 2023). Additionally, the highest phenolic and flavonoid concentrations were retained after intestinal digestion, sustaining that pancreatin and bile salts under neutral pH enable the release of these bioactives from *S. ramosissima* by-product commonly linked to glycosides, fibers, and proteins easily hydrolyzed by the digestive enzymes (Cheng et al., 2024; Li et al., 2023; Zhang et al., 2023).

3.2. Recovery of phenolic compounds and flavonoids

The phenolic recovery rates increased in the same order as TPC, reaching the highest percentages after intestinal digestion. SAE and SBE attained 37 % phenolic recovery after oral digestion, increasing to 56 % and 84 %, respectively, after gastric digestion. The bioaccessibility, corresponding to the phenolic recovery after intestinal digestion, achieved 120 % and 209 %, respectively, for SAE and SBE. These results indicate that the gut enzymes as well as the neutral pH, such as pancreatin and bile salts, allowed a greater release of phenolic compounds from SAE and SBE matrices into the digestion medium. These results were in line with Pinto, Ferreira, et al. (2023) who studied the effects of *in vitro* digestion in the phenolic concentration of chestnut shells. Upon intestinal digestion, the phenolic recovery rates exceeded

Table 1

TPC, TFC, phenolics and flavonoids recovery rates, and antioxidant/antiradical activities of undigested and digested SBE and SAE.

		TPC ($\mu\text{g GAE}/100$ mg dw)	Phenolics recovery upon digestion (%)	TFC ($\mu\text{g CE}/100$ mg dw)	Flavonoids recovery upon digestion (%)	ABTS ($\mu\text{g AAE}/100$ mg dw)	DPPH ($\mu\text{g TE}/100$ mg dw)	FRAP ($\mu\text{g FSE}/100$ mg dw)
SBE	In vitro digestion	Oral	233.99 $\pm$ 4.49 _{d,2}	37.52 $\pm$ 0.81 ^{c,1}	130.68 $\pm$ 19.41 ^{c,2}	31.42 $\pm$ 1.81 ^{a,2}	425.76 $\pm$ 22.02 ^{d,2}	156.70 $\pm$ 21.09 ^{c,2}
		Gastric	524.44 $\pm$ 17.91 ^{c,2}	84.08 $\pm$ 2.97 ^{b,1}	242.84 $\pm$ 37.29 ^{b,2}	58.32 $\pm$ 2.72 ^{b,2}	643.89 $\pm$ 52.60 ^{c,2}	272.69 $\pm$ 38.58 ^{b,2}
		Intestinal	1300.89 $\pm$ 35.17 ^{a,2}	208.57 $\pm$ 6.04 ^{a,1}	380.58 $\pm$ 29.14 ^{a,1}	92.37 $\pm$ 9.75 ^{c,1}	1313.40 $\pm$ 54.45 ^{a,2}	193.24 $\pm$ 26.74 ^{c,1}
	Undigested		623.76 $\pm$ 13.54 ^{b,2}	—	413.90 $\pm$ 66.02 ^{a,1}	—	762.36 $\pm$ 110.96 ^{b,2}	467.90 $\pm$ 53.70 ^{a,2}
		Oral	707.93 $\pm$ 3.99 _{d,1}	37.41 $\pm$ 1.30 ^{c,1}	328.39 $\pm$ 42.65 ^{b,1}	85.94 $\pm$ 10.93 ^{a,1}	835.35 $\pm$ 56.95 ^{c,1}	336.57 $\pm$ 51.61 ^{b,c,1}
		Gastric	1053.72 $\pm$ 45.47 ^{c,1}	55.70 $\pm$ 2.73 ^{b,2}	386.08 $\pm$ 46.21 ^{a,b,1}	91.21 $\pm$ 10.92 ^{a,1}	914.43 $\pm$ 82.88 ^{c,1}	421.20 $\pm$ 31.60 ^{b,1}
SAE	In vitro digestion	Intestinal	2277.70 $\pm$ 83.80 ^{a,1}	120.38 $\pm$ 4.36 ^{a,2}	410.19 $\pm$ 45.62 ^{a,1}	96.91 $\pm$ 10.78 ^{a,1}	4828.16 $\pm$ 190.97 ^{a,1}	220.12 $\pm$ 31.95 ^{a,1}
		Undigested	1892.25 $\pm$ 168.96 ^{b,1}	—	423.27 $\pm$ 25.62 ^{a,1}	—	1959.08 $\pm$ 97.36 ^{b,1}	1351.10 $\pm$ 228.23 ^{a,1}
								2990.61 $\pm$ 463.80 ^{a,1}
	Undigested							

AAE, ascorbic acid equivalents. CE, catechin equivalents. DW, dry weight. FSE, ferrous sulfate equivalents. FRAP, ferric reducing antioxidant power. GAE, gallic acid equivalents. TE, Trolox equivalents. TFC, total flavonoid content. TPC, total phenolic content. Different letters (a, b, c, and d) denote significant differences ($p < 0.05$) among digested and undigested fractions within the same sample. Different numbers (1 and 2) denote significant differences ($p < 0.05$) between SP and SE within the same digested fraction.

100 % probably due to the detection of non-phenolic reducing agents (such as ascorbic acid, sulfites, and some sugars), leading to over-estimation of the phenolic content (Pinto, Ferreira, et al., 2023). Additionally, the presence of phenolic glycosides in SBE and SAE may be metabolized into aglycones and glucose moieties under the gut environment owing to the effects of pancreatin and bile salts under neutral pH that promotes the release of aglycones (Pinto et al., 2024). Previous studies on sweet orange, jaboticaba peels and seeds, and functional cookies also reported recovery and bioaccessibility rates above 100 % for phenolic compounds (Inada et al., 2020; Peña-Vázquez et al., 2022; Pinto, Moreira, et al., 2023). Additionally, matrix interactions may also influence the recovery/bioaccessibility results, which emphasizes the importance of the HPLC quantification of the individual phenolic compounds to complement these spectrophotometric results and unveil if phenolic conjugates are hydrolyzed into aglycones during digestion that could lead to increased detection.

The flavonoid recovery percentages increased following the same pattern. A more pronounced increase was observed for SBE, with recovery rates of 31 %, 58 %, and 92 %, respectively, upon oral, gastric, and intestinal phases. Although SAE achieved identical flavonoid recovery percentages upon intestinal digestion (97 %), higher recovery rates were determined after oral and gastric phases (86 % and 91 %, respectively) when compared to SBE. These results were identical to the ones obtained for TFC, suggesting that flavonoids are more readily retrieved in the intestinal environment.

The bioaccessibility rates confirmed the positive influence of intestinal enzymes (namely pancreatin and bile salts) and neutral pH on the recovery of phenolic compounds, proposing that the intestinal conditions improved the polarity and solubility of these compounds, favoring their liberation from the extract and the subsequent biotransformation into simpler molecules. The solubilization of polyphenols in the digestive media also affects the TPC, TFC, and bioaccessibility results, emphasizing a higher solubility of these bioactive compounds in the intestinal digestive medium probably related to the effect of pancreatin and bile salts and neutral pH.

3.3. Antioxidant/antiradical properties before and after gastrointestinal digestion

The influence of simulated digestion on the antioxidant/antiradical activities of SBE and SAE is presented in Table 1.

The ferric-reducing potential of SAE and SBE increased significantly

($p < 0.05$) throughout the gastrointestinal digestion, reaching the maximum results after the intestinal phase (2594.22 and 1817.02 $\mu\text{g FSE}/100$ mg dw, respectively), which were similar ($p > 0.05$) to the undigested samples (2990.61 and 1813.89 $\mu\text{g FSE}/100$ mg dw). The FRAP results indicated that the undigested SAE and its digests had almost 2-fold higher antioxidant activity by ferric reduction than SBE and its digests.

Likewise, the antiradical activity of SAE and SBE by ABTS and DPPH radicals scavenging improved significantly ($p < 0.05$) throughout the gastrointestinal digestion. The SAE intestinal digest (4828.16 $\mu\text{g AAE}/100$ mg dw) was the best ABTS quencher, followed by the undigested SAE (1959.08 $\mu\text{g AAE}/100$ mg dw). Lower results were attained for SBE, with the highest value being determined after the intestinal phase (1313.40 $\mu\text{g AAE}/100$ mg dw), succeeding the undigested fraction (762.36 $\mu\text{g AAE}/100$ mg dw). Therefore, the ABTS response of the intestinal fractions were 72 % and 146 % higher, respectively, for SBE and SAE when compared to their undigested samples. Statistical differences ($p < 0.05$) were obtained when compared all digested and undigested fractions, apart from oral and gastric SAE digested samples ($p > 0.05$). In the DPPH assay, a distinct pattern was observed, with the highest values being obtained for the undigested SAE and SBE samples (1351.10 and 467.90 $\mu\text{g TE}/100$ mg dw, respectively), followed by their gastric digests (421.20 and 272.69 $\mu\text{g TE}/100$ mg dw). The DPPH scavenging response increased from the oral to gastric phases, but dropped by half in the intestinal phase for both samples. These results can be attributed to the chemical structures of phenolics, which may enhance their ability to scavenge free radicals (Rice-Evans & Miller, 1996). The molecules' racemization may be influenced by the pH, resulting in the formation of chiral enantiomers with distinct biological reactivities, explaining the discrepancies observed. Therefore, antioxidant molecules may be more reactive during digestion, especially at an acidic pH (gastric medium) as opposed to a neutral one (intestinal medium) (Rice-Evans & Miller, 1996).

In fact, gastrointestinal fluids can influence the TPC, TFC, and antioxidant activity results determined by spectrophotometric assays (Sejbuk et al., 2024). The overestimated results may occur due to interactions between digestive enzymes, bile salts, and other components present in digestive fluids or food matrix (e.g., phytochemicals, fatty acids, sugars, minerals, etc.), which can affect the solubility and stability of polyphenols (Jakobek, 2015; Sejbuk et al., 2024). For instance, bile salts can form micelles that enhance solubility and interfere with spectrophotometric detection (Mosele et al., 2015). Additionally, the

presence of proteins and peptides released during digestion can react with polyphenols, forming complexes that can increase the TPC and antioxidant activity (Jakobek, 2015; Tagliazucchi et al., 2012). Bile acids and digestive enzymes also exhibit redox properties that may interfere with DPPH and ABTS assays, leading to an overestimation of antioxidant capacity (Mosele et al., 2015). Moreover, the enzymatic and pH effects may also lead to overestimated TPC and TFC results. Particularly, the alkaline environment of the intestinal phase can promote phenolic ionization, altering their reactivity with Folin-Ciocalteu and aluminum chloride reagents (Sejbuk et al., 2024). To minimize the overestimation, complementary methods (such as LC-MS) should be used for an accurate quantification of phenolic compounds. Additionally, the combination of *in vitro* digestion models with cellular assays can provide better insights into the bioaccessible fraction rather than depending only on spectrophotometric methods (Tagliazucchi et al., 2012).

Therefore, the antioxidant/antiradical results emphasize the significant influence of gastrointestinal conditions, including digestive enzymes and pH alterations, reinforcing the remarkable role of polyphenols and flavonoids to the antioxidant and radicals counteracting properties. Additionally, identical findings were attested in other food matrices, namely coffee (*Coffea arabica* L.) silverskin, Himalayan wild cherries (*Prinsepia utilis*), and chestnut (*Castanea sativa*) shells, proving an improvement of antioxidant and antiradical effects upon gastrointestinal digestion (Castaldo et al., 2020; Liu, Cheng, et al., 2021; Pinto, Ferreira, et al., 2023; Pinto, Silva, et al., 2023).

3.4. Counteracting activity against ROS

The results of the scavenging capacity against pro-oxidant reactive species of undigested and digested SBE and SAE are presented in Table 2.

Even though the counteracting potential showed a distinct pattern for each reactive species tested, SAE and its digests were better ROS scavengers when compared to the SBE and its digests. Comparing all the ROS studied, the highest scavenging ability was achieved against HOCl, followed by $O_2^{\bullet -}$.

Regarding the $O_2^{\bullet -}$ quenching assay, identical inhibitory rates were attained for all three SAE digests ($p > 0.05$), with percentages above 90 %. Otherwise, the SBE intestinal digest was the least efficient in capturing $O_2^{\bullet -}$, with 53 % inhibition, despite being still significantly higher ($p < 0.05$) than the undigested SBE (with 38 % inhibition). Among the undigested samples, SAE presented a stronger counteracting

potential than the SBE, requiring 85 $\mu\text{g/mL}$ to neutralize 50 % of the $O_2^{\bullet -}$ produced in the tested medium. The undigested SBE inhibited only 38 % of the $O_2^{\bullet -}$ generated.

Considering the H_2O_2 assay, the quenching ability of SAE enhanced in the outlined sequence: undigested sample < gastric < oral < intestinal digests, with similar outcomes ($p > 0.05$) for gastric and undigested fractions. The SAE intestinal digest revealed the highest H_2O_2 scavenging power (79 % inhibition) among digests. SBE oral and gastric digests did not present activity against this reactive species, while the intestinal digest displayed 64 % inhibition, suggesting that the polyphenols retrieved in the intestinal medium are better H_2O_2 scavengers. Within the undigested samples, the best quencher was SAE (47 % inhibition), while SBE inhibited 43 % of the H_2O_2 produced. However, both undigested fractions showed significantly lower H_2O_2 counteracting ability ($p < 0.05$) than the digested fractions, corroborating that the gastrointestinal digestion had a positive impact on the scavenging response of SAE and SBE.

The HOCl quenching capacity improved as follows: gastric < intestinal < oral digests < undigested samples. Significantly different results ($p < 0.05$) were determined for the SAE digests, while similar inhibitory rates ($p > 0.05$) were achieved for the SBE digested fractions. Regarding the undigested samples, the most effective scavenger was SAE, exhibiting the lowest IC_{50} value (10.59 $\mu\text{g/mL}$) followed by SBE ($IC_{50} = 37.63$ $\mu\text{g/mL}$).

The $ROO^{\bullet}$ counteracting capacity was estimated through the ORAC method, evaluating the protective benefits against LPO damage that improved during the gastrointestinal digestion. Among digested fractions, the highest value was determined for the SAE intestinal digest (32.14 $\mu\text{g TE/mg dw}$). Likewise, the undigested SAE showed a higher $ROO^{\bullet}$ quenching capacity (38.03 $\mu\text{g TE/mg dw}$) than the undigested SBE (4.41 $\mu\text{g TE/mg dw}$). Significantly different results ($p < 0.05$) were obtained for the undigested SAE and its three digested samples. Oppositely, SBE gastric and intestinal digested fractions exhibited similar $ROO^{\bullet}$ counteracting power ($p > 0.05$).

Overall, the promising findings obtained on the ROS scavenging capacity highlight the potential contribution of phenolic compounds and saponins (identified by chromatographic analysis) to the bioactivity of digested and undigested SBE and SAE, which have shown effective radicals counteracting effects in recent studies (Pinto et al., 2021; Richards & Chaurasia, 2022; Silva et al., 2021).

Table 2
Scavenging potential of undigested and digested SBE and SAE against ROS.

		$O_2^{\bullet -}$		H_2O_2		HOCl		$ROO^{\bullet}$
		% Inhibition	IC_{50} ($\mu\text{g/mL}$)	% Inhibition	IC_{50} ($\mu\text{g/mL}$)	% Inhibition	IC_{50} ($\mu\text{g/mL}$)	$\mu\text{g TE/mg DW}$
SBE	In vitro digestion	Oral	98.38 $\pm$ 0.09 ^{a,2}	—	n.d.	—	—	0.60 $\pm$ 0.08 ^{c,2}
		Gastric	90.74 $\pm$ 0.10 ^{b,2}	—	n.d.	—	—	12.14 $\pm$ 1.70 ^{a,1}
		Intestinal	52.78 $\pm$ 2.42 ^{c,2}	—	63.98 $\pm$ 0.51 ^{a,2}	—	—	12.33 $\pm$ 2.03 ^{a,2}
	Undigested		37.76 $\pm$ 4.06 ^d	—	42.72 $\pm$ 2.55 ^{b,2}	—	37.63 $\pm$ 0.87 ¹	4.41 $\pm$ 0.29 ^{b,3}
		Oral	99.95 $\pm$ 0.05 ^{a,1}	—	65.49 $\pm$ 4.32 ^b	—	48.71 $\pm$ 2.82 ^{a,1}	1.18 $\pm$ 0.07 ^{d,1}
		Gastric	100.19 $\pm$ 0.04 ^{a,1}	—	52.67 $\pm$ 5.63 ^c	—	19.43 $\pm$ 0.17 ^{c,2}	13.12 $\pm$ 0.49 ^{c,1}
SAE	In vitro digestion	Intestinal	90.95 $\pm$ 9.20 ^{a,1}	—	79.74 $\pm$ 1.59 ^{a,1}	—	33.50 $\pm$ 3.36 ^{b,1}	32.14 $\pm$ 0.93 ^{b,1}
	Undigested		—	85.45 $\pm$ 1.62 ¹	47.02 $\pm$ 0.26 ^{c,1}	—	10.59 $\pm$ 0.47 ²	38.03 $\pm$ 0.53 ^{a,2}
		Gallic acid	—	23.84 $\pm$ 0.32 ²	—	400.71 $\pm$ 5.68	3.67 $\pm$ 0.04 ³	764.14 $\pm$ 0.00 ¹

IC_{50} corresponds to the concentration required to scavenge 50 % of the reactive species in a tested medium (mean $\pm$ SEM). Different letters (a, b, c, and d) indicate significant differences ($p < 0.05$) between digested and undigested fractions within the same sample. Different numbers (1, 2, and 3) indicate significant differences ($p < 0.05$) between SP and SE within the same digested fraction and positive control.

3.5. Antioxidant enzymes functions and protection against lipid peroxidation

Besides the non-enzymatic antioxidants (e.g., phenolic compounds and saponins), the antioxidant enzymes also exert a key role in the mitigation of oxidative stress consequences by scavenging free radicals and, consequently, prevent their deleterious effects on biomolecules (Cecerska-Heryć et al., 2022). The results of antioxidant enzymes activities and lipid peroxidation assay in the presence of undigested and digested SAE and SBE are displayed in Table 3.

The SOD catalyzes the dismutation of the O_2^- into O_2 and H_2O_2 , resulting in the decrease of the oxidative stress in cells (Pinto, Ferreira, et al., 2023). Therefore, greater inhibition of this enzyme leads to higher oxidative stress. The highest inhibition percentages were determined after gastric digestion for both SAE and SBE (80 % and 65 % SOD inhibition, respectively). Concerning SAE, the lowest result was obtained upon intestinal digestion (25 %), indicating that the intestinal fraction enables a more protective effect against oxidative stress, as would be predicted given the increased concentration of bioactive compounds. Regarding SBE, the SOD activity was the highest in the presence of the undigested fraction (33 % inhibition), followed by the oral digest (40 % inhibition), increasing after gastric digestion (65 % inhibition) and decreasing after intestinal digestion (58 % inhibition). Additionally, statistical differences ($p < 0.05$) were attained between undigested and digested fractions for SBE and SAE.

CAT is involved in the detoxification of H_2O_2 , protecting against oxidative stress (Pinto, Silva, et al., 2023). Concerning SBE, the CAT activity improved as follows: oral < undigested < gastric < intestinal digested samples, achieving statistical differences ($p < 0.05$) among all fractions. Concerning SAE, the highest value was achieved after gastric digestion (186.84 nmol/min/g dw), while the undigested fraction and its oral and intestinal digests yielded similar outcomes (30.04, 37.41, and 43.48 nmol/min/g dw, respectively, with $p > 0.05$).

GSH-Px is an antioxidant enzyme that defenses cells from free radicals injuries (Pinto, Ferreira, et al., 2023). The GSH-Px activity of SBE and SAE enhanced during the gastrointestinal digestion in the outlined sequence: oral < gastric < intestinal fractions. Concerning the undigested samples, SBE displayed the lowest value (18.01 U/g dw), while the undigested SAE displayed the greatest value (186.34 U/g dw).

A distinct pattern was noticed for the LPO assay, which was evaluated by quantifying MDA concentrations in the presence of undigested and digested SAE and SBE, to estimate the oxidative stress status. The results indicated a rise in the MDA concentrations during digestion, with the highest values attributed to the intestinal fractions (1637.63 and 2163.90 nmol MDA/g dw, respectively, for SBE and SAE) and the lowest result being ascribed to the undigested SBE (480.21 nmol MDA/g dw). Conversely, the undigested SAE did not indicate any effect. Statistical differences ($p < 0.05$) were determined between the digested samples for SAE and SBE, while the undigested SBE disclosed similar results ($p > 0.05$) to its gastric fraction.

Table 3

Effects of undigested and digested SBE and SAE on AChE, antioxidant enzymes activities and lipid peroxidation.

			AChE	SOD	CAT	GSH-Px	LPO
			Inhibition (%)		CAT activity (nmol/min/g dw)	GSH-Px activity (U/g dw)	MDA concentration (nmol MDA/g dw)
SBE	In vitro digestion	Oral	34.67 ± 2.63 ^{a,1}	39.61 ± 4.10 ^{c,2}	18.78 ± 0.00 ^{d,2}	7.96 ± 1.07 ^{c,2}	194.73 ± 7.75 ^{c,1}
		Gastric	24.58 ± 1.96 ^{b,1}	65.34 ± 1.20 ^{a,2}	41.15 ± 6.64 ^{b,2}	22.11 ± 3.14 ^{b,2}	550.76 ± 36.09 ^{b,1}
		Intestinal	31.18 ± 0.86 ^{a,1}	57.49 ± 0.34 ^{b,1}	52.89 ± 8.48 ^{a,1}	53.20 ± 7.77 ^{a,2}	1637.63 ± 107.58 ^{a,2}
	Undigested		33.08 ± 3.66 ^{a,1}	32.97 ± 0.17 ^{d,2}	30.02 ± 0.56 ^{c,1}	18.01 ± 4.27 ^{b,2}	480.21 ± 11.20 ^b
SAE	In vitro digestion	Oral	33.61 ± 3.41 ^{a,1}	68.84 ± 1.37 ^{b,1}	37.41 ± 5.84 ^{b,1}	46.17 ± 0.91 ^{c,1}	174.07 ± 6.89 ^{c,2}
		Gastric	20.41 ± 0.13 ^{b,2}	80.44 ± 0.34 ^{a,1}	186.84 ± 18.28 ^{a,1}	81.32 ± 7.54 ^{b,c,1}	338.85 ± 12.47 ^{b,2}
		Intestinal	21.55 ± 0.92 ^{b,2}	25.85 ± 3.76 ^{d,2}	43.48 ± 2.05 ^{b,2}	124.75 ± 27.77 ^{b,1}	2163.90 ± 103.20 ^{a,1}
	Undigested		16.92 ± 0.23 ^{b,2}	40.10 ± 4.78 ^{c,1}	30.04 ± 3.75 ^{b,1}	186.34 ± 59.33 ^{a,1}	n.d.

Different letters (a, b, c, and d) indicate significant differences ($p < 0.05$) between digested and undigested fractions within the same sample. Different numbers (1 and 2) indicate significant differences ($p < 0.05$) between SP and SE within the same digested fraction.

In summary, these findings underline the beneficial effects of *S. ramosissima* by-product in human health even after gastrointestinal digestion, reducing the oxidative stress, stimulating the antioxidant enzymes activities, preventing the lipid peroxidation, and potentially protecting from many chronic diseases. Furthermore, these findings are supported by the TPC and antioxidant/antiradical assays, emphasizing the benefits of incorporating this plant into diet. In a study performed by Homayouni et al. (2024) on three different *Salicornia* species (*S. europaea*, *S. bigelovii*, and *S. persica*) exposed to different NaCl concentrations (0–500 mM), the SOD activity values ranged between 10 and 50 U/mg protein, while the CAT activity results varied between 3.5 and 9.5 U/mg protein. Additionally, the protective role of *Salicornia* species against LPO had a more significant impact at lower salt concentrations, with results varying between 0.5 and 3.5 μ mol MDA/g dw (Homayouni et al., 2024).

3.6. Neuroprotection by AChE inhibition

The anti-cholinergic activity of SBE and SAE before and after gastrointestinal digestion was assessed using an enzymatic kit. The results are presented in Table 3.

The undigested SBE inhibited the AChE activity twice as effectively as the undigested SAE, with inhibition rates of 33 % and 17 %, respectively. For SAE, the highest AChE inhibition was observed after oral digestion (34 %), while identical outcomes ($p > 0.05$) were obtained after gastric and intestinal digestions (20 % and 22 %, respectively). Conversely, the undigested SBE and its oral and intestinal digests showed identical AChE inhibitory potential (33 %, 35 %, and 31 %, respectively), without statistical differences ($p > 0.05$). Additionally, a substantially lower enzymatic inhibitory capacity ($p < 0.05$) was noticed for SAE after gastric digestion (25 %). In summary, the undigested and digested SBE fractions revealed stronger neuroprotective effects when compared to the undigested and digested SAE, as indicated by higher AChE inhibition rates. These results align with the TPC and TFC, suggesting that the neuroprotective potential of *S. ramosissima* by-product upon digestion may be attributed to the gradual release of bioactive molecules (mainly polyphenols and saponins), which raise the acetylcholine levels and improve the neurological function (Pinto, Silva, et al., 2023). Other studies attested the neuroprotection of these compounds by attenuating the central nervous system pathologies, like stroke, Alzheimer's, Huntington's, and Parkinson's (Jiang et al., 2017; Sun et al., 2015).

In addition, it is important to compare the results with studies on standard AChE inhibitors, namely galantamine and donepezil, to assess their anticholinergic efficacy. The inhibitory capacity of *M. nodiflorum* extracts and digested fractions were lower than those reported for galantamine and donepezil, which inhibited 50 % AChE activity at 0.31 μ g/mL and 1.30 μ g/mL, respectively (Hv et al., 2020; Inthachai et al., 2024).

Furthermore, a similar trend was identified in other food matrices

during *in vitro* digestion studies, such as chestnut shells, insects-containing beef burgers, and phenolics-rich functional cookies (Pinto, Moreira, et al., 2023; Pinto, Silva, et al., 2023; Rocchetti et al., 2024). For instance, identical anti-cholinergic activity was reported for chestnut shells extracts after intestinal digestion (16.44 %–45.19 % AChE inhibition for extracts obtained by different eco-friendly techniques) (Pinto et al., 2024; Pinto, Silva, et al., 2023). Regarding halophytes studies, a similar AChE inhibitory potential was reported for an aqueous extract from *S. ramosissima* by-product, with inhibition percentages varying between 24 % and 32 %, respectively, at 250 and 1000 µg/mL (Pinto et al., 2021).

3.7. Phytochemical profiling by LC-MS

During digestion, phytochemicals undergo metabolic transformations that impact their bioaccessibility, absorption, and health effects within the human body (Pinto, Silva, et al., 2023). Given the biological relevance of these phytochemicals, this study aimed to evaluate the bioactive composition of *S. ramosissima* by-product upon gastrointestinal digestion. Table 4 presents the bioactive molecules identified in the undigested SAE and SBE. Fig. 1 presents the LC-MS chromatograms of the undigested SAE and its oral, gastric, and intestinal digested fractions.

The SAE showed three main peaks, revealing an ultraviolet (UV) maximum at 330 nm and some minor peaks with a UV maximum at 280 nm. Bioactive compounds were identified by observing the MSⁿ fragments and comparing them with the respective standards. In the first part of the chromatogram, a peak with a molecular ion [M-H][−] at *m/z* 191 and MS² fragments at 85 and 111 was assigned to quinic acid, which was confirmed by comparison with the reference compound. A quinic acid metabolite, namely quinic acid sulfate, was also detected by the molecular ion [M-H][−] at *m/z* 271, indicating the loss of sulfate moiety (−80) and remaining the quinic acid residue at *m/z* 191. Additionally, three isobaric species, corresponding to the three intense peaks with a UV maximum at 330 nm, were assigned to the isomers 3-caffeoylquinic acid (3-CQA) or neochlorogenic acid, with an intense fragment at *m/z* 191 and a minor fragment at *m/z* 179, 5-caffeoylquinic acid (5-CQA) or chlorogenic acid with a base peak at *m/z* 191, and 4-caffeoylquinic acid (4-CQA) or cryptochlorogenic acid with MS fragments at *m/z* 191 and 173, which are in agreement with the literature (Clifford et al., 2003;

Clifford et al., 2008). Another derivative, 3-feruloylquinic acid (3-FQA), was identified by a molecular ion [M-H][−] at *m/z* 367 and an intense fragment at *m/z* 191, corresponding to quinic acid [145].

Three other peaks, displaying a UV maximum at 330 nm and a molecular ion [M-H][−] at *m/z* 515 in negative ion mode, were attributed to three isomers of dicaffeoylquinic acid (diCQA) derivatives, namely 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA (Clifford et al., 2003). Each diCQA derivative exhibits diagnostic MSⁿ behavior, with a base peak ion at *m/z* 353 for all derivatives. Additionally, significant peaks were observed at *m/z* 191 and 179 for 3,5-diCQA, at *m/z* 335 and 173 for 3,4-diCQA, and at *m/z* 191 and 179 for 4,5-diCQA. Minor peaks at *m/z* 353 and 367 indicated the presence of caffeoylquinic and feruloylquinic acids (CQAs and FQAs, respectively) (Clifford et al., 2008). CQAs offer numerous health benefits associated with their biological activities, including antioxidant properties by scavenging free radicals and reducing oxidative stress, protecting cells against damage, and lowering the risk of chronic diseases such as cancer and cardiovascular pathologies (Lu et al., 2020). Moreover, the anti-inflammatory and antimicrobial effects make them potential candidates for food preservation and therapeutic formulations (Clifford, 1999; dos Santos et al., 2006; Manach et al., 2004).

Among flavonoids, gallicocatechin was identified based on the molecular ion [M-H][−] at *m/z* 305 and the MS fragment at *m/z* 225, which was confirmed by comparison with the reference standard.

Furthermore, at longer retention times, some peaks with non-significant UV absorption were detected by mass spectrometry, suggesting the presence of saponins in *S. ramosissima* by-product, in line with other studies (Mroczek, 2015). Peaks with a molecular ion [M-H][−] at *m/z* 793 and relevant fragments at *m/z* 673, 631, 613, 569, and 455 were consistent with the spectral data reported for chikusetsusaponin IV A (also known as calendulose G) formed by an oleanolic acid aglycone, one hexose (Hex), and one hexuronic acid (HexUA) moiety (Liu, Shi, et al., 2021). Considering this, two isomeric saponins with the same *m/z* value were detected. Other two isomeric saponins with a molecular ion [M-H][−] at *m/z* 809 were tentatively identified as hederagenin derivatives linked with Hex and HexUA based on the MS fragments 647 and 471 (Mroczek, 2015). Additionally, a peak with a molecular ion [M-H][−] at *m/z* 823 was attributed to acetylsaikosaponin D considering the MS fragmentation patterns (Liu, Shi, et al., 2021). Another oleanolic saponin derivative was observed, with molecular ion

Table 4
Phytochemical composition of SBE and SAE identified by LC/DAD-ESI-MS.

N°	Rt (min)	HR-MS [M – H] [−]	Ppm calculated vs measured	MS ⁿ fragments	Chemical Compound Formula	Compounds
1	1.8	191.0555	15	85, 111.1	C ₇ H ₁₁ O ₆	Quinic acid*
2	4.3	353.0875	10	191.1, 179.1	C ₁₆ H ₁₇ O ₉	3-CQA
3	6.7	353.0875	6	191.1	C ₁₆ H ₁₇ O ₉	5-CQA*
4	7.26	271.2223	9	191.1	C ₁₆ H ₁₇ O ₉	Quinic acid sulfate
5	7.3	353.0876	14	173.1, 191	C ₇ H ₁₂ O ₉ S	4-CQA*
6	7.34	367.1029	10	191.1	C ₁₇ H ₁₉ O ₉	3-FQA*
7	7.5	305.0661	2	225	C ₁₅ H ₁₃ O ₇	Gallicocatechin*
8	8.3	515.1190	4	353.1, 335, 173	C ₂₅ H ₂₃ O ₁₂	3,4-diCQA
9	8.75	515.1190	7	353.2, 191.5, 179	C ₂₅ H ₂₃ O ₁₂	3,5-diCQA*
10	8.82	515.1190	9	353, 191, 179, 673, 631, 613,	C ₂₅ H ₂₃ O ₁₂	4,5-diCQA*
11	10.9	793.4374	4	569, 455	C ₄₂ H ₆₅ O ₁₄	Chikusetsusaponin IV A/ calendulose G isomer 1
12	11.5	809.4324	2	647, 471	C ₄₂ H ₆₆ O ₁₅	Hex + HexUA + hederagenin isomer 1
13	12.4	809.4324	3	647, 471	C ₄₂ H ₆₆ O ₁₅	Hex + HexUA + hederagenin isomer 2
14	13.5	793.4374	6	673, 631, 613, 569, 455	C ₄₂ H ₆₅ O ₁₄	Chikusetsusaponin IV A/ calendulose G isomer 2
15	14.2	821.46873	4	673, 631, 613, 569, 455	C ₄₄ H ₆₉ O ₁₄	Acetylsaikosaponin D isomer 1
16	15.2	821.46872	1	673, 631, 613, 569, 455	C ₄₄ H ₆₉ O ₁₄	Acetylsaikosaponin D isomer 2
17	15.4	777.4449	6	615, 539, 469, 437, 407	C ₄₂ H ₆₆ O ₁₃	23-Hydroxy-13β,28β-epoxyolean-11-ene-16-one 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside

CQA, caffeoylquinic acid. diCQA, dicaffeoylquinic acid. FQA, feruloylquinic acid. Hex, hexose. HexUA, hexuronic acid. * compounds identified with the respective commercial standard.

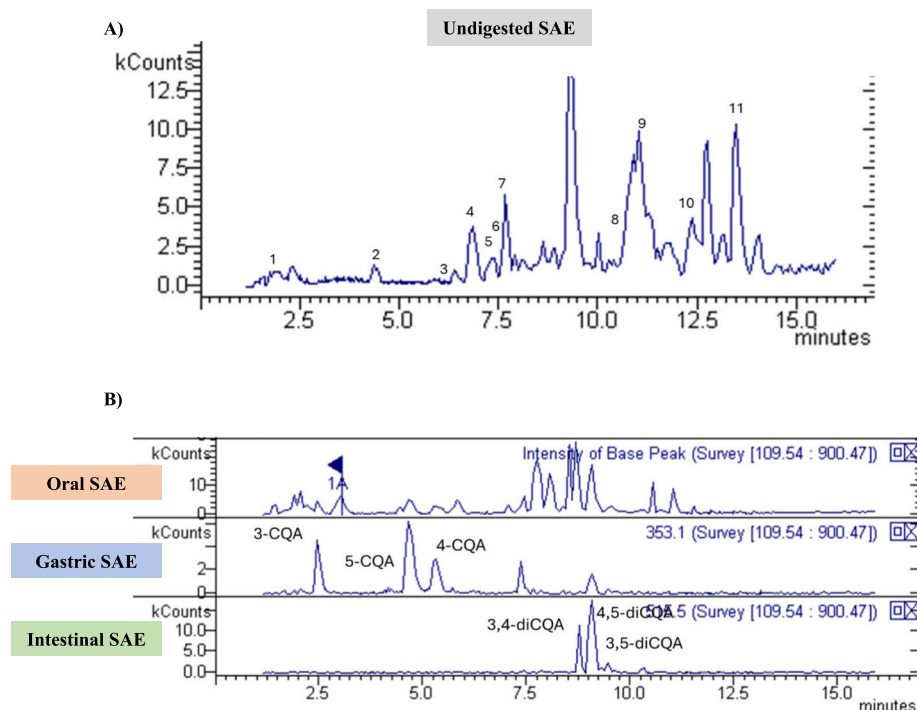


Fig. 1. LC-MS chromatograms of the phytochemical analysis of undigested SAE (A) and its oral, gastric, and intestinal digests (B). The undigested and digested samples were identified in the charts. Peak identification as follows: (1) quinic acid; (2) 3-CQA; (3) 5-CQA; (4) Quinic acid sulfate; (5) 4-CQA; (6) 3-FQA; (7) gallicocatechin; (8) 3,4-diCQA; (9) 3,5-diCQA; (10) 4,5-diCQA; and (11) chikusetsusaponin IV A/ calendulose G isomer.

[M-H]⁻ at m/z 777 and MS fragments at 615, 539, 469, 437, and 407 (Liu, Cheng, et al., 2021). These compounds are recognized for their antioxidant, hepatoprotective, and cholesterol-lowering effects (Marrelli et al., 2016; Qu et al., 2012; Sharma et al., 2023; Vinarova et al., 2015). Noteworthy, a similar composition was observed for SAE and SBE. Nonetheless, SAE was richer in phenolic compounds, mainly CQAs and derivatives and flavan-3-ols, when compared to SBE. The saponins profile and concentrations were identical for SAE and SBE.

Regarding digestion, SAE and SBE showed no significant compositional changes after oral digestion. However, the gastric fractions were marked by notable alterations in the chromatographic profile, with the most prominent change being an increase in the intensity of peaks corresponding to monocatefeylquinic acids and a relative decrease in diCQA derivatives. This suggests the partial cleavage of the ester linkages, resulting in the formation of monocatefeylquinic acids from the identified diCQA. Regarding saponins, no significant changes were observed, probably due to the greater resistance of their ether linkages.

The SAE, after oral and intestinal digestion, displayed several peaks with significant absorption in the diode array detector. These peaks were characterized by typical UV maxima at 330 nm, indicative of hydroxycinnamic acids, and at 280 nm, suggesting the presence of flavan-3-ols. Furthermore, the *in vitro* digestion did not alter the saponin profile but affected significantly the CQAs. These findings reinforce alterations in the phenolic profile of SBE and SAE during digestion, indicating the influence of the digestive enzymes and pH on the polyphenols' retention in each phase. DiCQAs were partially hydrolyzed into CQAs, which was particularly evident in SBE where CQA peaks were prominent after digestion but almost undetectable before digestion.

Noteworthy, future studies should identify bound phenolic compounds in undigested samples to assess their influence on phytochemical composition and bioactivity. Additionally, advanced chromatographic and mass spectrometry techniques should be used to explore the metabolic profile and track digestion-induced biotransformation of individual phytochemicals, providing a deeper understanding of their stability, metabolism, and bioactivity.

3.8. Intestinal cells viability after exposure to *S. ramosissima* by-product

The potential effects of SAE on the intestinal mucosa were assessed using Caco-2 and HT29-MTX cells that mimic the small intestine where bioactive compounds are primarily absorbed. The intestinal cells viability assessed by MTT assay is presented in Fig. 2.

Regarding HT29-MTX cells, the highest viability rates were observed following the exposure to SAE at concentrations between 31.25 and 500 $\mu\text{g/mL}$, with percentages exceeding 100 %. Nevertheless, HT29-MTX viability decreased significantly ($p < 0.05$) to 76 % after exposure to 1000 $\mu\text{g/mL}$ of SAE extract. In addition, Caco-2 viability varied between 86 % and 62 %, respectively, at 31.25 and 250 $\mu\text{g/mL}$, decreasing significantly ($p < 0.05$) to 21 % after exposure to 500 and 1000 $\mu\text{g/mL}$. Taken together, these results suggest that the optimal non-cytotoxic concentration of SAE extract was 250 $\mu\text{g/mL}$, with viability percentages of at least 60 % for both cell lines, guaranteeing the extract safety on intestinal cells.

For instance, Silva et al. (2021) assessed the viability of *S. ramosissima* extracts obtained by MAE and conventional extraction on the same two cell lines, reporting viability rates higher than 80 % for both cells at all tested concentrations (0.1–1000 $\mu\text{g/mL}$). The intestinal cell effects were also evaluated after exposure to *Disphyma crassifolium* halophyte (Silva et al., 2024). The authors selected the concentration of 10 $\mu\text{g/mL}$ as the optimal non-cytotoxic one, reporting lower viability rates on Caco-2 and HT29-MTX cells for extract concentrations of 1000 $\mu\text{g/mL}$.

3.9. *In vitro* intestinal permeability assay

After gastrointestinal digestion, bioactive compounds and metabolites may be absorbed through the intestinal mucosa. The intestinal digested fractions of SAE and SBE were tested on an *in vitro* intestinal model formed by a Caco-2/HT29-MTX co-culture. Tables 5 and 6 summarize the permeation rates of the phytochemicals present in the intestinal digests of SAE and SBE, respectively, at each time point.

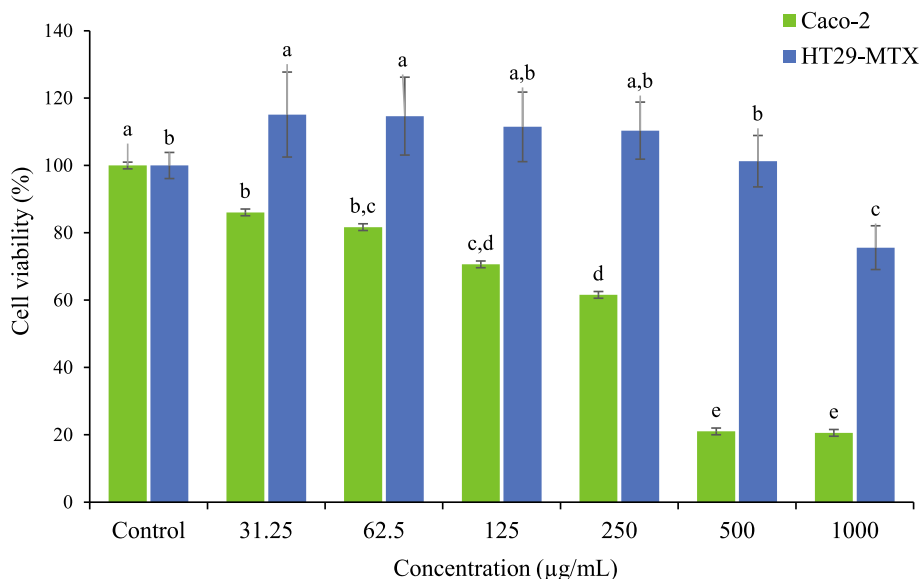


Fig. 2. Effects of *S. ramosissima* by-product after extraction (SAE) on intestinal cells viability (Caco-2 and HT29-MTX) by MTT assay. Different letters (a–e) indicate significant differences ($p < 0.05$) between extract concentrations and control for the same cell line.

Table 5

Permeation of phytochemical compounds from SAE intestinal digest across the intestinal co-culture model.

SAE intestinal digest									
Compounds	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min	Apical 240 min
Quinic acid*	0	0	0	1.40 ± 0.07 ^d	2.23 ± 0.11 ^c	3.00 ± 0.15 ^b	3.70 ± 0.19 ^a	5.11 ± 0.26	5.25 ± 0.26
3-CQA	0	0	0	0	0	2.93 ± 0.15 ^b	4.14 ± 0.21 ^a	5.86 ± 0.29	4.70 ± 0.24 ^a
5-CQA*	0	0	0	0	0	20.28 ± 1.01 ^a	9.98 ± 0.50 ^b	31.93 ± 1.60	4.09 ± 0.20 ^c
4-CQA*	0	0	0	0	0	17.50 ± 0.88 ^b	77.90 ± 3.90	34.84 ± 1.74 ^a	5.90 ± 0.30 ^c
3-FQA*	0	0	0	0	0	0	0	0	0
Gallocatechin*	0	0	0	0	1.17 ± 0.06 ^d	1.89 ± 0.10 ^c	3.35 ± 0.17 ^b	4.00 ± 0.20 ^a	5.08 ± 0.25
3,4-diCQA	0	0	0	0	2.28 ± 0.12 ^c	7.88 ± 0.39 ^b	9.65 ± 0.49 ^a	14.01 ± 0.70	8.46 ± 0.43 ^{a,b}
3,5-diCQA*	0	0	0	0	2.04 ± 0.10 ^c	7.04 ± 0.35 ^b	8.63 ± 0.43 ^a	12.52 ± 0.63	8.28 ± 0.42 ^a
4,5-diCQA*	0	0	0	0	2.04 ± 0.10 ^c	7.04 ± 0.35 ^b	8.63 ± 0.43 ^a	12.52 ± 0.63	8.93 ± 0.45 ^a
Chikusetsusaponin IV A/ calendulose G isomer 1	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	3.09 ± 0.15 ^a	3.63 ± 0.19
Hex + HexUA + hederagenin isomer 1	0	0	0.22 ± 0.02 ^c	0.24 ± 0.02 ^c	1.14 ± 0.06 ^d	5.39 ± 0.27 ^b	7.50 ± 0.38 ^a	8.80 ± 0.44	4.42 ± 0.23 ^c
Hex + HexUA + hederagenin isomer 2	0	0	0	0	0	0	0	0	2.50 ± 0.13
Chikusetsusaponin IV A/ calendulose G isomer 2	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.03 ± 0.01 ^b	0.03 ± 0.00 ^b	0.03 ± 0.01 ^b	0.04 ± 0.00 ^b	0.05 ± 0.01 ^b	6.60 ± 0.33 ^a	9.69 ± 0.49
Acetylsaikosaponin D isomer 1	0	0	0.70 ± 0.04 ^d	1.06 ± 0.05 ^d	2.06 ± 0.10 ^c	3.52 ± 0.18 ^b	6.67 ± 0.33	7.21 ± 0.36	4.44 ± 0.22 ^a
Acetylsaikosaponin D isomer 2	0	0	0	0	0	0	0	0	1.56 ± 0.08
23-Hydroxy-13β,28β-epoxyolean-11-ene-16-one 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside	0	0	0	0	1.11 ± 0.06 ^d	1.71 ± 0.09 ^c	2.71 ± 0.14 ^b	3.45 ± 0.17 ^a	5.92 ± 0.30

CQA, caffeoylquinic acid. diCQA, dicaffeoylquinic acid. FQA, feruloylquinic acid. Hex, hexose. HexUA, hexuronic acid. Different letters (a–e) denote significant differences ($p < 0.05$) between fixed times. * compounds identified with the respective commercial standard.

The main phytochemicals that penetrated across the intestinal barrier include 8 CQAs and derivatives (with 3-FQA only identified in SAE samples), 1 flavanol, 3 triterpenoid saponins, 1 saponin derivative, and 1 fatty acid (only present in SBE samples).

Considering the SAE intestinal digest, the permeation of bioactive compounds enhanced in the following order: chikusetsusaponin IV A

isomer 1 < 23-hydroxy-13β,28β-epoxyolean-11-ene-16-one 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside < gallocatechin < quinic acid < 3-CQA < chikusetsusaponin IV A isomer 2 < acetylsaikosaponin D isomer 1 < Hex + HexUA + hederagenin isomer 1 < 3,5-diCQA = 4,5-diCQA < 3,4-diCQA < 5-CQA < 4-CQA, with rising percentages up to 240 min. The highest rates were reached for 4-CQA and 5-CQA, with

Table 6

Permeation of phytochemical compounds from SBE intestinal digest across the intestinal co-culture model.

SBE intestinal digest									
Compounds	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min	Apical 240 min
Quinic acid*	0	2.61 ± 0.13 ^e	4.04 ± 0.20 ^e	6.74 ± 0.34 ^d	8.09 ± 0.41 ^d	14.84 ± 0.74 ^b	22.27 ± 1.11 ^a	25.98 ± 1.30	11.49 ± 0.58 ^c
3-CQA	0	0	0	0	0	1.67 ± 0.09 ^c	6.95 ± 0.35 ^a	12.11 ± 0.61	5.00 ± 0.25 ^b
5-CQA*	0	0	0	0	0	2.24 ± 0.12 ^b	3.80 ± 0.19 ^a	4.88 ± 0.25	2.24 ± 0.12 ^b
4-CQA*	0	0	0	0	0	1.00 ± 0.05 ^c	4.45 ± 0.23 ^a	8.50 ± 0.43	3.30 ± 0.17 ^b
Gallocatechin*	0	0	0	0	0.33 ± 0.02 ^d	0.52 ± 0.03 ^c	0.93 ± 0.05 ^b	1.11 ± 0.06 ^a	1.41 ± 0.07
3,4-diCQA	0	0	0	0	1.79 ± 0.09 ^g	9.23 ± 0.47 ^b	12.55 ± 0.63 ^a	16.43 ± 0.82	9.88 ± 0.50 ^b
3,5-diCQA*	0	0	0	0	7.88 ± 0.40 ^b	9.65 ± 0.49 ^b	15.42 ± 0.77 ^a	22.16 ± 1.11	9.03 ± 0.45 ^b
4,5-diCQA*	0	0	0	0	7.94 ± 0.40 ^c	13.82 ± 0.69 ^b	18.63 ± 0.93 ^a	26.73 ± 1.34	7.50 ± 0.38 ^c
Chikusetsusaponin IV A/ calendulose G isomer 1	29.14 ± 1.46	4.86 ± 0.25 ^e	5.67 ± 0.29 ^e	6.61 ± 0.33 ^{d,e}	7.71 ± 0.39 ^{c,d}	9.00 ± 0.45 ^{b,c}	10.50 ± 0.53 ^b	12.39 ± 0.62 ^a	9.71 ± 0.49 ^b
Hex + HexUA + hederagenin isomer 1	0	0	0	0.02 ± 0.01 ^d	0.09 ± 0.01 ^c	0.31 ± 0.02 ^b	0.46 ± 0.03 ^a	0.69 ± 0.04	0.42 ± 0.02 ^a
Hex + HexUA + hederagenin isomer 2	0	0	0.02 ± 0.00 ^e	0.02 ± 0.01 ^e	0.11 ± 0.01 ^d	0.54 ± 0.03 ^b	0.75 ± 0.04 ^a	0.88 ± 0.04	0.44 ± 0.02 ^c
Chikusetsusaponin IV A/ calendulose G isomer 2	0	0	0	0	0	0	0	0.14 ± 0.01 ^a	10.24 ± 0.51
Acetylsaikosaponin D isomer 1	0.45 ± 0.03 ^f	0.90 ± 0.05 ^{e,f}	1.43 ± 0.08 ^e	2.86 ± 0.15 ^d	4.05 ± 0.21 ^c	6.94 ± 0.35 ^b	9.15 ± 0.46 ^a	11.57 ± 0.58	7.00 ± 0.35 ^b
Acetylsaikosaponin D isomer 2	0.47 ± 0.02 ^f	0.70 ± 0.04 ^f	1.18 ± 0.06 ^f	1.97 ± 0.10 ^e	3.88 ± 0.20 ^d	4.67 ± 0.24 ^c	7.57 ± 0.38 ^a	9.92 ± 0.50	6.25 ± 0.31 ^b
23-Hydroxy-13β,28β-epoxyolean-11-ene-16-one 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside	0	0	0	0	1.11 ± 0.06 ^d	1.71 ± 0.09 ^c	2.71 ± 0.14 ^b	3.45 ± 0.17 ^a	5.92 ± 0.30
Oleic acid	0	0	0	0	1.10 ± 0.06 ^c	3.11 ± 0.16 ^b	3.81 ± 0.19 ^a	5.51 ± 0.28	0.04 ± 0.01 ^d

CQA, caffeoylquinic acid. diCQA, dicaffeoylquinic acid. FQA, feruloylquinic acid. Hex, hexose. HexUA, hexuronic acid. Different letters (a–f) denote significant differences ($p < 0.05$) between fixed times. * compounds identified with the respective commercial standard.

more than 30 % permeation after 4 h, while the three diCQAs identified achieved almost 15 % of permeation. Noteworthy, it was not possible to quantify 3-FQA since its concentration was lower than the limit of quantification of the chromatographic method. In general, the lowest permeation percentages were attained for saponins (3.09–8.80 % at 4 h) and gallocatechin (4.00 % at 4 h).

Regarding the SBE intestinal digested fraction, the bioactive molecules permeation improved as follows: chikusetsusaponin IV A isomer 2 < Hex + HexUA + hederagenin isomer 1 < Hex + HexUA + hederagenin isomer 2 < gallocatechin < 23-hydroxy-13β,28β-epoxyolean-11-ene-16-one 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside < 5-CQA < oleic acid < 4-CQA < acetylsaikosaponin D isomer 2 < acetylsaikosaponin D isomer 1 < 3-CQA < chikusetsusaponin IV A isomer 1 < 3,4-diCQA < 3,5-diCQA < quinic acid < 4,5-diCQA, with increasing percentages from 15 to 240 min. The highest permeation rates were obtained for 4,5 diCQA, quinic acid, and 3,5-diCQA, achieving maximum permeability percentages of 27 %, 26 %, and 22 %, respectively, after 4 h. Two CQA derivatives (3,4-diCQA and 3-CQA) and two triterpenoid saponins (chikusetsusaponin IV A isomer 1 and acetylsaikosaponin D isomer 1) achieved permeation rates up to 16 %. Oleic acid, a saponin derivative, gallocatechin, and 5-CQA also permeated the intestinal barrier with percentages varying between 1 % and 6 % after 4 h. Moreover, chikusetsusaponin IV A isomer 2 and both isomers of Hex + HexUA + hederagenin had the lowest permeation, attaining rates lower than 1 %.

In general, the highest permeation rates were obtained for the SAE intestinal digest (up to 35 %), especially for 5-CQA, 4-CQA, Hex + HexUA + hederagenin isomer 1, and chikusetsusaponin IV A isomer 2. Nevertheless, the intestinal fraction of SBE reached higher permeation percentages (up to 27 %) for quinic acid, 3-CQA, 3,4-diCQA, 3,5-diCQA,

4,5-diCQA, chikusetsusaponin IV A isomer 1, and acetylsaikosaponin D isomers 1 and 2.

Additionally, a substantial proportion of bioactive compounds, characterized by limited intestinal permeability (<35 % and < 27 %, respectively, for SAE and SBE), are likely retained within the intestinal barrier (Muhammad Bilal et al., 2019), while a small fraction of these molecules were recovered in the apical compartment (<10 % and < 12 %, respectively, for SAE and SBE). This retention can be ascribed to the complex chemical structures of these compounds and/or their biotransformation into metabolites that were not detected in this analysis due to the lack of standards, limitations of the chromatographic technique, and potential interferences from cellular constituents that may not allow the transport of these compounds from the apical to the basolateral side of the intestinal epithelium (Muhammad Bilal et al., 2019). Despite this, the bioactive compounds retained in the cell barrier may still provide health benefits, such as cytoprotective effects (Toschi et al., 2022).

In addition, the integrity of tight junction dynamics in cellular models is measured as transepithelial electrical resistance (TEER) (Srinivasan et al., 2015). Supplementary Fig. 1 presents the TEER values measured during the 21 days of cultivation as well as the 4 h of permeability assay. The TEER values decreased until the 11th day (271–285 Ω/cm²), stabilized until the 18th day (215–267 Ω/cm²), and then slightly decreased up to the 21st day (217–227 Ω/cm²) (Supplementary Fig. 1 A). These results suggested a stable cell growth rate. During the permeability assay, the TEER values remained almost constant for SAE and SBE (Supplementary Fig. 1B), varying between 145 Ω/cm² (at 120 min) and 202 Ω/cm² (at 15 min) for SAE; and between 143 Ω/cm² (180 min) and 203 Ω/cm² (240 min) for SBE, guaranteeing the integrity of the cell barrier.

4. Conclusion

This is the first study that assessed the bioaccessibility, bioavailability, and bioactivity of phytochemicals from powdered and liquid extract of *S. ramosissima* by-product after gastrointestinal simulated digestion and intestinal permeability. The results revealed dynamic changes in phenolic and flavonoid contents of SAE and SBE through *in vitro* gastrointestinal digestion, with phenolic compounds found in higher concentrations than flavonoids. Digestion enhanced bioaccessibility and bioactivity with stronger antioxidant/antiradical and neuroprotective properties observed in SAE. Chromatographic analysis identified a richer profile in SAE, including CQAs, saponins, and one flavanol. While digestion significantly affected CQAs, partially hydrolyzing diCQAs to CQAs, the saponin profile remained unchanged. The intestinal absorption of phenolic compounds and saponins was demonstrated, with 4-CQA and 4,5-diCQA showing the highest permeation rates, respectively, in SAE and SBE (35 % and 27 %). These findings highlight *S. ramosissima* by-product as a promising source of bioactive compounds for functional foods and nutraceuticals. Nevertheless, the study acknowledges the limitations of the static *in vitro* digestion model using INFOGEST protocol, which does not entirely simulate the human digestion. Therefore, future research should incorporate dynamic digestion models and colonic fermentation assays that represent more accurately the complexity of gastrointestinal digestion. This study provides a step forward in our project goal to valorize *S. ramosissima* by-product as an active ingredient with pro-healthy effects for the food and nutraceutical industry.

CRediT authorship contribution statement

Diana Pinto: Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis. **Inês Santos:** Methodology, Investigation. **Filipa Teixeira:** Methodology, Investigation. **Stefania Sut:** Validation, Software, Methodology, Investigation. **Mónica Vieira:** Supervision. **Miguel Salazar:** Investigation. **Cristina Delerue-Matos:** Resources, Investigation. **Stefano Dall’Acqua:** Writing – review & editing, Validation, Methodology, Investigation. **Francisca Rodrigues:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

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

Acknowledgments

The authors kindly thank Riafresh (Faro, Portugal) for the samples. This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Filipa Teixeira (2024.01202.BD) is thankful for her Ph.D. grant financed by POPH-QREN and subsidized by the European Science Foundation and Ministério da Ciência, Tecnologia e Ensino Superior. Francisca Rodrigues thanks to the scientific contract (2023.06819.CEECIND/CP2842/CT0008) financed by FCT/MCTES - CEEC Individual Program Contracts.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.144665>.

Data availability

Data will be made available on request.

References

- Araújo, F., & Sarmento, B. (2013). Towards the characterization of an *in vitro* triple culture intestine cell model for permeability studies. *International Journal of Pharmaceutics*, 458(1), 128–134. <https://doi.org/10.1016/j.ijpharm.2013.10.003>
- Brodtkorb, A., Egger, L., Alming, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourliou-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., ... Recio, I. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols*, 14(4), 991–1014. <https://doi.org/10.1038/s41596-018-0119-1>
- Cádiz Gurra, M. D. L., Soares, S., Jiménez, F. J. L., Ochoa, Á. F., Pinto, D., Delerue-Matos, C., ... Rodrigues, F. (2019). Chapter 4 - effects of nutritional supplements on human health. In C. M. Galanakis (Ed.), *Nutraceuticals and natural product pharmaceuticals* (pp. 105–140). Academic Press. <https://doi.org/10.1016/B978-0-12-816450-1.00004-0>
- Cárdenas-Pérez, S., Piernik, A., Chanona-Pérez, J. J., Grigore, M. N., & Perea-Flores, M. J. (2021). An overview of the emerging trends of the *Salicornia* L. genus as a sustainable crop. *Environmental and Experimental Botany*, 191, Article 104606. <https://doi.org/10.1016/j.envexpbot.2021.104606>
- Castaldo, L., Narváez, A., Izzo, L., Graziani, G., & Riti, A. (2020). *In vitro* bioaccessibility and antioxidant activity of coffee silverskin polyphenolic extract and characterization of bioactive compounds using UHPLC-Q-Orbitrap HRMS. *Molecules*, 25(9), 2132. <https://doi.org/10.3390/molecules25092132>
- Cecerska-Heryć, E., Krauze, K., Szczęśniak, A., Goryniak-Mikołajczyk, A., Serwin, N., Śleboda-Taront, D., Jacek, R., Heryć, R., Michalczyk, A., & Dolegowska, B. (2022). Activity of erythrocyte antioxidant enzymes in healthy women depends on age, BMI, physical activity, and diet. *Journal of Health, Population and Nutrition*, 41(1), 35. <https://doi.org/10.1186/s41043-022-00311-z>
- Cheng, M., He, J., Gu, Y., Wu, G., Tan, L., Li, C., ... Zhu, K. (2024). Changes in phenolic compounds and antioxidant capacity of *Artocarpus heterophyllus* lam. (jackfruit) pulp during *in vitro* gastrointestinal digestion. *Antioxidants*, 13(1) pp. 37–49. <https://doi.org/10.3390/antiox13010037>
- Clifford, M. N. (1999). Chlorogenic acids and other cinnamates – Nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, 79(3), 362–372. [https://doi.org/10.1002/\(SICI\)1097-0010\(19990301\)79:3](https://doi.org/10.1002/(SICI)1097-0010(19990301)79:3)
- Clifford, M. N., Johnston, K. L., Knight, S., & Kuhnert, N. (2003). Hierarchical scheme for LC-MSn identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry*, 51(10), 2900–2911. <https://doi.org/10.1021/jf026187q>
- Clifford, M. N., Kirkpatrick, J., Kuhnert, N., Roozendaal, H., & Salgado, P. R. (2008). LC-MSⁿ analysis of the *cis* isomers of chlorogenic acids. *Food Chemistry*, 106(1), 379–385. <https://doi.org/10.1016/j.foodchem.2007.05.081>
- Custódio, L., Rodrigues, M. J., Pereira, C. G., Castañeda-Loaiza, V., Fernandes, E., Standing, D., ... Sagi, M. (2021). A review on *Sarcocornia* species: Ethnopharmacology, nutritional properties, phytochemistry, biological activities and propagation. *Foods*, 10(11), 2778. <https://doi.org/10.3390/foods10112778>
- Custódio, M., Lillebø, A. L., Calado, R., & Villasante, S. (2021). Halophytes as novel marine products – A consumers’ perspective in Portugal and policy implications. *Marine Policy*, 133, Article 104731. <https://doi.org/10.1016/j.marpol.2021.104731>
- Danielski, R., & Shahidi, F. (2025). Effect of simulated gastrointestinal digestion on the phenolic composition and biological activities of guava pulp and processing by-products. *Food Chemistry*, 465, Article 142080. <https://doi.org/10.1016/j.foodchem.2024.142080>
- Durán-Castañeda, A. C., Cardenas-Castro, A. P., Pérez-Jiménez, J., Pérez-Carvajal, A. M., Sánchez-Burgos, J. A., Mateos, R., & Sáysago-Ayerdi, S. G. (2023). Bioaccessibility of phenolic compounds in *Psidium guajava* L. varieties and *P. Friedrichsthalianum* Nied. After gastrointestinal digestion. *Food Chemistry*, 400, Article 134046. <https://doi.org/10.1016/j.foodchem.2022.134046>
- EU CAP Network Good Practice Report. (2021). Salivatae – Salicornia Production Unit. Tackling the issue of soil salinity in an economically, socially and environmentally sustainable manner. Available online https://eu-cap-network.ec.europa.eu/good-practice/salivatae-salicornia-production-unit_en#section-resources;tab_id=overview accessed on 29/03/2025.
- Gomes, A., Fernandes, E., Silva, A. M. S., Santos, C. M. M., Pinto, D. C. G. A., Cavaleiro, J. A. S., & Lima, J. L. F. C. (2007). 2-Styrylchromones: Novel strong scavengers of reactive oxygen and nitrogen species. *Bioorganic & Medicinal Chemistry*, 15(18), 6027–6036. <https://doi.org/10.1016/j.bmc.2007.06.046>
- Homayouni, H., Razi, H., Izadi, M., Alemzadeh, A., Kazemini, S. A., Niazi, A., & Vicente, O. (2024). Temporal changes in biochemical responses to salt stress in three *Salicornia* species. *Plants*, 13(7), 979. <https://doi.org/10.3390/plants13070979>
- Hv, S., Raj, A., Gouthamchandra, K., Chandrappa, S., & Shyamprasad, K. (2020). Kinetics and computational analysis of cholinesterase inhibition by REVERC3, a bisdemethoxycurcumin-rich *Curcuma longa* extract: Relevance to the treatment of Alzheimer’s disease. *SAGE Open Medicine*, 8. <https://doi.org/10.1177/2050312120973499>
- Inada, K. O. P., Silva, T. B. R., Lobo, L. A., Domingues, R. M. C. P., Perrone, D., & Monteiro, M. (2020). Bioaccessibility of phenolic compounds of Jaboticaba (*Plinia Jaboticaba*) peel and seed after simulated gastrointestinal digestion and gut microbiota fermentation. *Journal of Functional Foods*, 67, Article 103851. <https://doi.org/10.1016/j.jff.2020.103851>

- Inthachai, W., Chantong, B., Pitchakarn, P., Takoon, C., Karinchai, J., Suttisansanee, U., & Temviriyankul, P. (2024). Enhancing therapeutic efficacy of donepezil, an Alzheimer's disease drug, by *Diplazium esculentum* (Retz.) Sw. And its phytochemicals. *Pharmaceuticals*, 17(3), 341. <https://doi.org/10.3390/ph17030341>
- Irfan, S., Ranjha, M. M. A. N., Nadeem, M., Safdar, M. N., Jabbar, S., Mahmood, S., ... Ibrahim, S. A. (2022). Antioxidant activity and phenolic content of sonication- and maceration-assisted ethanol and acetone extracts of *Cymbopogon citratus* leaves. *Separations*, 9(9), 244. <https://doi.org/10.3390/separations9090244>
- Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry*, 175, 556–567. <https://doi.org/10.1016/j.foodchem.2014.12.013>
- Jiang, X. W., Bai, J. P., Zhang, Q., Hu, X. L., Tian, X., Zhu, J., ... Zhao, Q. C. (2017). Caffeoylquinic acid derivatives protect SH-SY5Y neuroblastoma cells from hydrogen peroxide-induced injury through modulating oxidative status. *Cellular and Molecular Neurobiology*, 37(3), 499–509. <https://doi.org/10.1007/s10571-016-0387-7>
- Li, H., Liu, T., Li, F., Wu, X., & Wu, W. (2023). Effects of rice bran rancidity on the release of phenolics and antioxidative properties of rice bran dietary fiber *in vitro* gastrointestinal digestion products. *Food Research International*, 173, Article 113483. <https://doi.org/10.1016/j.foodres.2023.113483>
- Lima, A. R., Castañeda-Loaiza, V., Salazar, M., Nunes, C., Quintas, C., Gama, F., ... Barreira, L. (2020). Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of *Salicornia ramosissima* commercially produced in soilless systems. *Food Chemistry*, 333, Article 127525. <https://doi.org/10.1016/j.foodchem.2020.127525>
- Liu, W., Cheng, X., Kang, R., Wang, Y., Guo, X., Jing, W., Wei, F., & Ma, S. (2021). Systematic characterization and identification of saikosaponins in extracts from *Bupleurum marginatum* var. *stenophyllum* using UPLC-PDA-Q/TOF-MS. *Frontiers in Chemistry*, 9, Article 747987. <https://doi.org/10.3389/fchem.2021.747987>
- Liu, X., Shi, J., Yi, J., Zhang, X., Ma, Q., & Cai, S. (2021). The effect of *in vitro* simulated gastrointestinal digestion on phenolic bioaccessibility and bioactivities of *Prinsepia utilis* Royle fruits. *LWT*, 138, Article 110782. <https://doi.org/10.1016/j.lwt.2020.110782>
- Lopes, M., Roque, M. J., Cavaleiro, C., & Ramos, F. (2021). Nutrient value of *Salicornia ramosissima*—A green extraction process for mineral analysis. *Journal of Food Composition and Analysis*, 104, Article 104135. <https://doi.org/10.1016/j.jfca.2021.104135>
- Lu, H., Tian, Z., Cui, Y., Liu, Z., & Ma, X. (2020). Chlorogenic acid: A comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions. *Comprehensive Reviews in Food Science and Food Safety*, 19(6), 3130–3158. <https://doi.org/10.1111/1541-4337.12620>
- Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79(5), 727–747. <https://doi.org/10.1093/ajcn/79.5.727>
- Marçal, R., Sousa, P., Marques, A., Pereira, V., Guilherme, S., Barreto, A., ... Pacheco, M. (2023). Exploring the antioxidant and genoprotective potential of *Salicornia ramosissima* incorporation in the diet of the european seabass (*Dicentrarchus labrax*). *Animals*, 14(1), 93. <https://doi.org/10.3390/ani14010093>
- Marrelli, M., Conforti, F., Araniti, F., & Statti, G. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules*, 21, 1404. <https://doi.org/10.3390/molecules21101404>
- Mosele, J. I., Macià, A., Romero, M.-P., Motilva, M.-J., & Rubió, L. (2015). Application of *in vitro* gastrointestinal digestion and colonic fermentation models to pomegranate products (juice, pulp and peel extract) to study the stability and catabolism of phenolic compounds. *Journal of Functional Foods*, 14, 529–540. <https://doi.org/10.1016/j.jff.2015.02.026>
- Mroczek, A. (2015). Phytochemistry and bioactivity of triterpene saponins from *Amaranthaceae* family. *Phytochemistry Reviews*, 14(4), 577–605. <https://doi.org/10.1007/s11101-015-9394-4>
- Muhammad Bilal, H., Sadia, H., Marwa, W., Ahsan, J., Muhammad Adil, F., & Ali, T. (2019). Bioavailability and metabolic pathway of phenolic compounds. Eds. In S.-H. Marcos, G.-M. Rosario, & P.-T. Mariana (Eds.), *Plant physiological aspects of phenolic compounds* (pp. Ch. 5). <https://doi.org/10.5772/intechopen.84745>. IntechOpen.
- Nájar, A. M., Romero-Bernal, M., del Río, C., & Montaner, J. (2023). A review on polyphenols in *Salicornia ramosissima* with special emphasis on their beneficial effects on brain ischemia. *Nutrients*, 15(3), 793. <https://doi.org/10.3390/nu15030793>
- Oliveira-Alves, S. C., Andrade, F., Sousa, J., Bento-Silva, A., Duarte, B., Caçador, I., ... Bronze, M. R. (2023). Soilless cultivated halophyte plants: Volatile, nutritional, phytochemical, and biological differences. *Antioxidants*, 12(6), 1161. <https://doi.org/10.3390/antiox12061161>
- Peña-Vázquez, G. I., Domínguez-Fernández, M. T., Camacho-Zamora, B. D., Hernandez-Salazar, M., Urías-Orona, V., De Peña, M.-P., & de la Garza, A. L. (2022). *In vitro* simulated gastrointestinal digestion impacts bioaccessibility and bioactivity of sweet orange (*Citrus sinensis*) phenolic compounds. *Journal of Functional Foods*, 88, Article 104891. <https://doi.org/10.1016/j.jff.2021.104891>
- Pinto, D., Ferreira, A. S., Lozano-Castellón, J., Laveriano-Santos, E. P., Lamuela-Raventós, R. M., Vallverdú-Queralt, A., ... Rodrigues, F. (2023). Exploring the impact of *in vitro* gastrointestinal digestion in the bioaccessibility of phenolic-rich chestnut shells: A preliminary study. *Separations*, 10(9), 471. <https://doi.org/10.3390/separations10090471>
- Pinto, D., Moreira, M. M., Švarc-Gajić, J., Vallverdú-Queralt, A., Brezo-Borjan, T., Delerue-Matos, C., & Rodrigues, F. (2023). *In-vitro* gastrointestinal digestion of functional cookies enriched with chestnut shells extract: Effects on phenolic composition, bioaccessibility, bioactivity, and α -amylase inhibition. *Food Bioscience*, 53, Article 102766. <https://doi.org/10.1016/j.fbio.2023.102766>
- Pinto, D., Moreira, M. M., Vallverdú-Queralt, A., Delerue-Matos, C., & Rodrigues, F. (2024). Insights into the digestion of chestnut (*Castanea sativa*) shells bioactive extracts—Ultrasound vs. microwave-assisted extraction. *International Journal of Food Science & Technology*, 59(7), 5128–5142. <https://doi.org/10.1111/ijfs.17253>
- Pinto, D., Reis, J., Silva, A. M., Salazar, M., Dall'Acqua, S., Delerue-Matos, C., & Rodrigues, F. (2021). Valorisation of *Salicornia ramosissima* biowaste by a green approach – An optimizing study using response surface methodology. *Sustainable Chemistry and Pharmacy*, 24, Article 100548. <https://doi.org/10.1016/j.scp.2021.100548>
- Pinto, D., Silva, A. M., Dall'Acqua, S., Sut, S., Vallverdú-Queralt, A., Delerue-Matos, C., & Rodrigues, F. (2023). Simulated gastrointestinal digestion of chestnut (*Castanea sativa* mill.) shell extract prepared by subcritical water extraction: Bioaccessibility, bioactivity, and intestinal permeability *in vitro* assays. *Antioxidants*, 12(7), 1414. <https://doi.org/10.3390/antiox12071414>
- Qu, L., Xin, H., Zheng, G., Su, Y., & Ling, C. (2012). Hepatoprotective activity of the total saponins from *Actinidia valvata* Dunn root against carbon tetrachloride-induced liver damage in mice. *Evidence-Based Complementary and Alternative Medicine*, 2012, Article 216061. <https://doi.org/10.1155/2012/216061>
- Rice-Evans, C. A., & Miller, N. J. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Society Transactions*, 24(3), 790–795. <https://doi.org/10.1042/bst0240790>
- Richards, A., & Chaurasia, S. (2022). Antioxidant activity and reactive oxygen species (ROS) scavenging mechanism of *Eriodictyon californicum*, an edible herb of North America. *Journal of Chemistry*, 2022(1), Article 6980121. <https://doi.org/10.1155/2022/6980121>
- Rocchetti, G., Zengin, G., Giuberti, G., Cervini, M., & Lucini, L. (2024). Impact of *in vitro* gastrointestinal digestion on the phenolic bioaccessibility and bioactive properties of insect-containing beef burgers. *Antioxidants*, 13(3), 365. <https://doi.org/10.3390/antiox13030365>
- Rodrigues, F., Cádiz-Gurrea, M. D. L. L., Nunes, M. A., Pinto, D., Vinha, A. F., Linares, I. B., ... Carretero, A. S. (2018). Chapter 12 - cosmetics. In C. M. Galanakis (Ed.), *Polyphenols: Properties, recovery, and applications* (pp. 393–427). Woodhead Publishing. <https://doi.org/10.1016/B978-0-12-813572-3.00012-9>
- dos Santos, M. D., Almeida, M. C., Lopes, N. P., & de Souza, G. E. (2006). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biological and Pharmaceutical Bulletin*, 29(11), 2236–2240. <https://doi.org/10.1248/bpb.29.2236>
- Sejebuk, M., Mironczuk-Chodakowska, I., Karav, S., & Witkowska, A. M. (2024). Dietary polyphenols, food processing and gut microbiome: Recent findings on bioavailability, bioactivity, and gut microbiome interplay. *Antioxidants*, 13(10), 1220. <https://doi.org/10.3390/antiox13101220>
- Sharma, K., Kaur, R., Kumar, S., Saini, R. K., Sharma, S., Pawde, S. V., & Kumar, V. (2023). Saponins: A concise review on food related aspects, applications and health implications. *Food Chemistry Advances*, 2, Article 100191. <https://doi.org/10.1016/j.focha.2023.100191>
- Silva, A. M., Lago, J. P., Pinto, D., Moreira, M. M., Grosso, C., Cruz Fernandes, V., ... Rodrigues, F. (2021). *Salicornia ramosissima* bioactive composition and safety: Eco-friendly extractions approach (microwave-assisted extraction vs. conventional maceration). *Applied Sciences*, 11(11), 4744. <https://doi.org/10.3390/app11114744>
- Silva, A. M., Moreira, M. M., Teixeira, F., Ferraz, R., Salazar, M., Delerue-Matos, C., & Rodrigues, F. (2024). Insights into the bioactive composition, antioxidant properties and *in vitro* cell effects of *Disphyma crassifolium*. *Foods*, 13(8), 1219. <https://doi.org/10.3390/foods13081219>
- Silva, H., Caldeira, G., & Freitas, H. (2007). *Salicornia ramosissima* population dynamics and tolerance of salinity. *Ecological Research*, 22(1), 125–134. <https://doi.org/10.1007/s11284-006-0008-x>
- Srinivasan, B., Kolli, A. R., Esch, M. B., Abaci, H. E., Shuler, M. L., & Hickman, J. J. (2015). TEER measurement techniques for *in vitro* barrier model systems. *Journal of Laboratory Automation*, 20(2), 107–126. <https://doi.org/10.1177/2211068214561025>
- Sun, A., Xu, X., Lin, J., Cui, X., & Xu, R. (2015). Neuroprotection by saponins. *Phytotherapy Research*, 29(2), 187–200. <https://doi.org/10.1002/ptr.5246>
- Tagliazucchi, D., Helal, A., Verzelloni, E., & Conte, A. (2012). The type and concentration of milk increase the *in vitro* bioaccessibility of coffee chlorogenic acids. *Journal of Agricultural and Food Chemistry*, 60(44), 11056–11064. <https://doi.org/10.1021/jf302694a>
- Toschi, A., Piva, A., & Grilli, E. (2022). Phenol-rich botanicals modulate oxidative stress and epithelial integrity in intestinal epithelial cells. *Animals*, 12(17), 2188. <https://doi.org/10.3390/ani12172188>
- Vinarova, L., Vinarov, Z., Atanasov, V., Pantcheva, I., Tcholakova, S., Denkov, N., & Stoyanov, S. (2015). Lowering of cholesterol bioaccessibility and serum concentrations by saponins: *In vitro* and *in vivo* studies. *Food & Function*, 6(2), 501–512. <https://doi.org/10.1039/c4fo00785a>
- Wu, H., Liu, Z., Lu, P., Barrow, C., Dunshea, F. R., & Suleria, H. A. R. (2022). Bioaccessibility and bioactivities of phenolic compounds from roasted coffee beans during *in vitro* digestion and colonic fermentation. *Food Chemistry*, 386, Article 132794. <https://doi.org/10.1016/j.foodchem.2022.132794>
- Zhang, L., Wu, T., Zhang, Y., Chen, Y., Ge, X., Sui, W., Zhu, Q., Geng, J., & Zhang, M. (2023). Release of bound polyphenols from wheat bran soluble dietary fiber during simulated gastrointestinal digestion and colonic fermentation *in vitro*. *Food Chemistry*, 402, Article 134111. <https://doi.org/10.1016/j.foodchem.2022.134111>