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Unlocking the functional potential of *Mesembryanthemum nodiflorum*: Insights into digestibility, safety and intestinal uptake

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ABSTRACT

Mesembryanthemum nodiflorum, commonly known as slenderleaf iceplant, is an undervalued edible halophyte. The nutraceutical potential of *M. nodiflorum* was investigated in the current study by extracting bioactive compounds using ultrasound-assisted extraction (UAE) and assessing the influence of gastrointestinal digestion and intestinal permeability on their bioactive composition. The phenolic and flavonoid contents were the highest in the alcoholic extract, along with exceptional antioxidant/antiradical effects. The gastrointestinal digestion enhances the release and detectability of phenolic compounds and antioxidant activity, with bioaccessibility exceeding 100 %. Additionally, the extracts stimulated antioxidant enzymes and inhibited acetylcholinesterase (AChE), with alcoholic extract showing the highest inhibition (38 %). UPLC-QTOF-MS unveiled high concentrations of glycerophospholipids, tryptophan, and 5,5′-dihydroferulic acid. Ethanol was the most effective solvent for extracting bioactive compounds. Through an intestinal co-culture model, the intestinal digest from the alcoholic extract demonstrated notable permeation (66 % for rosmanol, 30 % for Lys-Asp-Tyr, and 15 % for tryptophan). These results highlight the bioactivity and intestinal absorption of *M. nodiflorum* extracts, repurposing this halophyte as a nutraceutical.

1. Introduction

Water scarcity and soil salinity are emerging challenges that affect different regions around the world and worsen every year, triggering interest in halophytes as alternative solutions (Oliveira-Alves et al., 2023). These plants grow in saline environments with limited water availability and poor soil quality, offering the potential for human consumption due to pharmacological properties and unique sensory characteristics (Lima et al., 2021; Lopes et al., 2023). Food industry is directing its efforts towards developing innovative and more nutritious functional foods effective in preventing chronic diseases (Diana Pinto et al., 2023) and halophytes arise as excellent option. These plans are subject to multiple abiotic (*e.g.*, inadequate nutrition, pollutants, soil acidification, and ultraviolet rays) and biotic (*e.g.*, competition and pathogen attacks) stressors (Lima et al., 2021; Lopes et al., 2023), synthesizing secondary metabolites, including phenolics, carotenoids, saponins, and alkaloids, to combat saline stress and protect cellular structures from the harmful effects of oxidation, without promoting

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toxic heavy metal accumulation (Arena et al., 2020; Lima et al., 2021; Lopes et al., 2023). Moreover, halophytes are interesting sources of minerals, fiber, proteins, and lipids, being appreciated as a complement to salads or as a condiment, beyond their therapeutic potential, particularly in bacterial infections (Lopes et al., 2023).

The species of *Mesembryanthemum* genus (~ 70) are facultative halophytes, capable of thriving in both saline and low-salt environments, being native to the Mediterranean, Saudi Arabia, South Africa, Southern Australia, and California (Arena et al., 2020). Nevertheless, there are only three edible species with commercial value, namely *M. crystallinum, M. edule,* and *M. nodiflorum* (Arena et al., 2020). *M. nodiflorum* is an annual halophytic plant comprising long, highly branched stems (up to 30 cm) and semi-circular leaves. The tender and succulent leaves are used in salads due to their salty and acidic taste (Castañeda-Loaiza et al., 2020). This halophyte demonstrated beneficial effects in combating ocular infections, with leaf extracts utilized in the production of soaps and antiseptic poultices to treat burns and wounds (Arena et al., 2020). Moreover, *M. nodiflorum* exhibited bactericidal and antitumor activities, making it an excellent candidate for extracting bioactive compounds with potential applications in pharmaceutical or cosmetic products (Arena et al., 2020). The content of bioactive compounds in the edible halophyte *M. nodiflorum* and its potential application in developing healthier foods have, in recent years, increased the scientific and industrial interest in this species, suggesting new avenues for its use.

In the declaration of the 23rd Hohenheim Consensus Meeting, bioactive compounds were defined as "*essential and non-essential compounds (e.g. vitamins or polyphenols) from natural sources and are part of the food chain, exerting positive effects on human health*" (Johnson et al., 2023). Various classes of bioactive compounds (including polyphenols, flavonoids, carotenoids, and alkaloids) exerting interesting biological properties (such as anticancer, antidepressant, antidiabetic, anti-inflammatory, antilipidemic, and antioxidant activities) were identified in plant-derived matrices, proposing diverse functions in food, cosmetic, and nutraceutical industries and multiple benefits for society (Lopes et al., 2023; D. Pinto, A. M. Silva et al., 2023). These bioactive molecules may be recovered from halophytes using eco-innovative obtaining safe and sustainable extracts (Lopes et al., 2023). Nonetheless, most of the studies focused on the biological properties of these bioactive compounds in extracts, without evaluating the effects of gastrointestinal digestion (Pinto, López-Yerena et al., 2024; D. Pinto, A. M. Silva et al., 2023). As a multifaceted process, the bioaccessibility and bioavailability of nutrients depend on their release from the food matrix, digestive stability, and passage through the intestinal epithelium (Farré et al., 2020). Gastrointestinal digestion provides insights into the interactions of nutrients within the digestive tract, evaluating the digestibility and bioaccessibility of essential nutrients and bioactive molecules (D. Pinto, A. S. Ferreira et al., 2023). Additionally, *in vitro* cellular models (*e.g.,* Caco-2/HT29-MTX co-culture) simulate the intestinal mucosa, being a valuable tool to estimate the intestinal absorption rates of digested nutrients (D. Pinto, A. M. Silva et al., 2023). HT29-MTX represent the goblet cells from the human intestine to assess the mucosal adhesion of transporter carriers, while Caco-2 cells mimic the colon epithelium owing to the enzymes, microvilli, nuclear receptors, tight junctions, and transporters present on them (Pinto, López-Yerena et al., 2024). Therefore, these models contribute to the interpretation of these intricate and dynamic mechanisms.

The present study attempts, for the first time, to valorize the halophytic plant *M. nodiflorum* for nutraceutical applications offering a complete and detailed view of the potentially useful constituents. Detailed chemical analysis of the secondary metabolites that can be extracted is obtained and used to evaluate the impact of the simulated gastrointestinal digestion and intestinal permeation on the bioaccessibility and bioavailability of the phytochemicals recovered by UAE. Furthermore, the extracts after simulated gastrointestinal digestion were studied for their bioactivity on acetylcholinesterase (AChE), antioxidant enzymes, and radicals scavenging. Noteworthy, digestibility, intestinal absorption, and bioactivity have not yet been investigated for the phytonutrient-rich *M. nodiflorum* extracts.

2. Materials and methods

2.1. Sample

M. nodiflorum samples were gently provided by RiaFresh (Faro, Portugal) in September 2023. After washing and dehydrating at 41 °C for 36 h, samples were milled to 1 mm of particle size in a Moulinex A320 grinder (France) and, subsequently, kept at 4 °C.

2.2. Ultrasound-assisted extraction (UAE) of M. nodiflorum bioactive compounds

The extraction was conducted in a 13 mm-diameter ultrasonic probe from Sonic Vibracell (model VCX50, Newtown, CT, USA; No. 630–0219) at 30 W/m^2 of intensity during 31 min, according to the experimental model validated by Silva, Pinto, *et al.* (Silva, Pinto et al., 2022). The extractor solvents selected were water and ethanol and the solid/liquid ratio applied corresponded to 10 % (*w/v*). Three extracts, namely aqueous, hydroalcoholic 50:50 (*v/v*), and alcoholic, were prepared. After centrifuging at 5000 RPM for 30 min and filtering through Whatman n° 1 paper, the extracts were lyophilized upon freezing at −80 °C.

2.3. Simulated gastrointestinal digestion

In vitro digestion was conducted, in triplicate, on lyophilized extracts resuspended in distilled water (50 mg/mL) according to the INFOGEST protocol (Brodkorb et al., 2019). Simultaneously, a blank sample containing only distilled water was subjected to the digestion experiment. A detailed description of the methodology is available in the supplementary material.

2.4. Quantification of phenolic and flavonoid concentrations and assessing antioxidant and radical scavenging properties

The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant/antiradical activity by ABTS and FRAP assays were performed on undigested and digested fractions following the procedures described by Pinto, López-Yerena, *et al.* (Pinto, López-Yerena et al., 2024). The scavenging potential against reactive oxygen species (ROS) was evaluated by applying the procedures of Pinto, Silva, et al. (D. Pinto, A. M. Silva et al., 2023). A detailed description of the methodologies is available in the supplementary material.

2.5. Enzymatic inhibitory activities

The biological activities of undigested and digested samples in AChE, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were analyzed using commercial enzymatic kits (Sigma-Aldrich, Steinheim, Germany). Lipid peroxidation (LPO) assay was carried out in a Merck kit (Darmstadt, Germany).

2.6. ***Phenolic profile by*** *UPLC-QTOF-MS*

M. nodiflorum extracts were analyzed using a High-Resolution Mass spectrometer composed of an Agilent 1290 UPLC system connected to 1290 series Diode Array and Quadrupole Time of Flight mass spectrometer. A detailed description of the methodology is available in the supplementary material.

2.7. Intestinal cells viability

The cells viability of *M. nodiflorum* extracts was assessed on two intestinal lines, namely Caco-2 and HT29-MTX, through MTT assay following the detailed methodology available in the supplementary material.

2.8. *In vitro intestinal permeability on Caco-2/HT29-MTX co-culture model*

The extract digests obtained after the intestinal phase were exposed to a co-culture model composed of Caco-2 and HT29-MTX cells to mimic the intestinal mucosa (Antunes et al., 2013). The integrity of cellular monolayers was assessed by measuring the transepithelial electrical resistance (TEER). UPLC-QTOF-MS was used to analyze the permeates following the method previously described. A detailed description of the methodology is available in the supplementary material.

2.9. *Statistical analysis*

The statistical analysis was conducted on IBM SPSS Statistics v29 software (Chicago, IL, USA). One-way ANOVA and Tukey's HSD test were used to compare results between the digested and undigested fractions, while a *t*-test was applied to compare results between the extract and by-product for each digested fraction. Significant differences were denoted for a $p < 0.05$. Three independent experiments ($n = 3$) were performed for each assay. The ROS scavenging calculations were done in GraphPad Prism v9 software (La Jolla, *CA*, USA).

3. Results and discussion

3.1. *Extraction yields*

The extraction yields of *M. nodiflorum* extracts are summarized in Supplementary Table 1. The greatest extraction yield was determined for the aqueous extract (21.04 %), followed by hydroalcoholic (20.98 %) and alcoholic (20.49 %) extracts, without statistical differences between the three extracts ($p > 0.05$). Arena *et al.* (Arena et al., 2020) extracted antioxidant compounds from *M. nodiflorum* collected along the Sicilian coasts, reporting lower yields when compared to the present study (0.79, 0.43, 12.30, and 18.39 g/100 g, respectively, for supercritical fluid, *n*-hexane, acetone, and ethanol extracts), except for the aqueous extract that showed a higher extraction yield (33.32 g/100 g), aligning with the findings of this study. Similar outcomes were described by Correia *et al.* (Correia et al., 2022) and Silva *et al.* (Silva et al., 2021) for extracts from another halophyte, *Salicornia ramosissima*, prepared by Subcritical Water Extraction (SWE) and Microwave-Assisted Extraction (MAE), respectively. Correia *et al.* (Correia et al., 2022) obtained extraction yields ranging between 21.27 % and 21.65 % for different temperatures (110–180 °C), while Silva *et al.* (Silva et al., 2021) achieved an extraction yield of 26.10 %.

3.2. *Quantification of phenolic and flavonoid concentrations*

The TPC and TFC were determined for undigested and digested *M. nodiflorum* extracts prepared by UAE. Table 1 presents the results.

Considering the extraction solvent, the TPC and TFC increased significantly ($p < 0.05$) in the listed sequence: aqueous < hydroalcoholic < alcoholic extracts, except for TFC between the aqueous and hydroalcoholic extracts ($p > 0.05$). Comparing with other studies conducted on *M. nodiflorum* using different extraction techniques, the UAE allowed to obtain better results as reported by other authors (Arena et al., 2020; Lima et al., 2021; Silva et al., 2021, 2024). For example,. Arena *et al.* (Arena et al., 2020) obtained a TPC of approximately 4, 0.75, and 0.25 mg GAE/g DW, respectively, for alcoholic, aqueous, and Supercritical Fluid Extraction (SFE) extracts, whereas Lima *et al.* (Lima et al., 2021) achieved TPC ranging between 2.09 and 4.39 mg GAE/g DW for *M. nodiflorum* extracts prepared by ultrasonic bath and cultivated under different salinities (35–465 mmol/L). These outcomes are also in line with a study conducted on the halophyte *Disphyma crassifolium*, using the same extraction technique and conditions (TPC: 8.49 and 53.13 mg GAE/g DW, respectively, for aqueous and alcoholic extracts; TFC: 8.02 and 18.98 mg CE/g DW, respectively) (Silva et al., 2024). The authors demonstrated that ethanol allows a higher solubility of phenolic compounds from this halophyte. Furthermore, Silva *et al.* (Silva et al., 2021) reported identical outcomes for *S. ramosissima* extracts obtained through conventional extraction and MAE (TPC: 15.02 and 8.34 mg GAE/g DW,

Table 1
TPC, TFC, phenolics and flavonoids recovery rates, antioxidant/antiradical activities of *M. nodiflorum* extracts prepared by UAE before and after *in vitro* digestion.

			TPC (mg GAE /g DW)	Phenolics recovery upon digestion (%)	TFC (mg CE/ g DW)	Flavonoids recovery upon digestion (%)	ABTS (mg AAE/g DW)	FRAP (mg FSE/g DW)
Aqueous extract	*In vitro* digestion	Oral	4.05 ± 0.07^{c}	97.76 ± 1.65^{c}	0.67 ± 0.00^{c}	8.70 ± 0.06^{b}	4.19 ± 0.20^{c}	3.67 ± 0.01^{c}
		Gastric	8.24 ± 0.12^{b}	198.97 ± 2.89^{b}	0.76 ± 0.00^{c}	9.82 ± 0.01^{b}	4.86 ± 0.23^{c}	8.45 ± 0.12^{b}
		Intestinal	15.96 ± 0.16^{a}	385.23 ± 3.76^{a}	3.98 ± 0.07^{b}	51.31 ± 0.85^{a}	25.11 ± 0.21^{a}	14.25 ± 0.24^{a}
	Undigested extract		$4.14 \pm 0.57^{3, c}$	–	$7.75 \pm 0.17^{2, a}$	–	$6.92 \pm 0.96^{3, b}$	$8.72 \pm 1.19^{3, b}$
Hydroalcoholic extract	*In vitro* digestion	Oral	4.89 ± 0.01^{c}	78.18 ± 0.10^{c}	0.69 ± 0.00^{c}	8.56 ± 0.05^{c}	4.93 ± 0.04^{c}	6.09 ± 0.00^{d}
		Gastric	7.59 ± 0.11^{b}	121.33 ± 1.82^{b}	$1.60 \pm 0{,}00^{c}$	19.92 ± 0.03^{b}	7.23 ± 0.35^{c}	10.95 ± 0.04^{c}
		Intestinal	16.38 ± 0.06^{a}	261.79 ± 0.95^{a}	4.63 ± 0.14^{b}	57.80 ± 1.76^{a}	27.14 ± 0.11^{a}	19.21 ± 0.47^{a}
	Undigested extract		$6.26 \pm 0.81^{2, c}$	–	$8.02 \pm 0.50^{2, a}$	–	$9.62 \pm 1.19^{1, b}$	$14.51 \pm 1.13^{2, b}$
Alcoholic extract	*In vitro* digestion	Oral	7.41 ± 0.25^{c}	54.97 ± 1.88^{c}	1.03 ± 0.07^{c}	8.23 ± 0.56^{b}	3.72 ± 0.04^{c}	13.19 ± 0.40^{b}
		Gastric	10.20 ± 0.14^{b}	75.59 ± 1.06^{b}	1.84 ± 0.06^{c}	14.78 ± 0.46^{b}	5.03 ± 0.15^{c}	18.70 ± 0.74^{b}
		Intestinal	21.25 ± 0.58^{a}	157.53 ± 4.31^{a}	6.56 ± 0.41^{b}	52.58 ± 3.28^{a}	23.72 ± 0.67^{a}	33.84 ± 0.02^{a}
	Undigested extract		$13.49 \pm 1.96^{1, b}$	–	$12.48 \pm 1.87^{1, a}$	–	$8.12 \pm 0.67^{2, b}$	$30.98 \pm 3.44^{1, a}$

AAE, ascorbic acid equivalents. CE, catechin equivalents. DW, dry weight. FSE, ferrous sulfate equivalents. FRAP, ferric reducing antioxidant power. GAE, gallic acid equivalents. TFC, total flavonoid content. TPC, total phenolic content. Different letters (a, b, c, and d) denote significant differences ($p < 0.05$) between digested and undigested fractions within the same extract. Different numbers at superscript (1, 2, and 3) denote significant differences ($p < 0.05$) between the three extracts.

respectively; TFC: 8.44 and 8.41 mg CE/g DW).

Regarding the digested fractions, TPC and TFC improved during the digestion for the three extracts in the outlined sequence: oral < gastric < intestinal. This trend was also suggested by different authors for other food matrices, namely walnuts, almonds, cashew nuts, and chestnut shells (M. Li et al., 2023; D. Pinto, A. S. Ferreira et al., 2023; Pinto, López-Yerena et al., 2024, 2023). The TPC increased 93.7 % and 103.5 % after the intestinal and gastric phases, respectively, for the aqueous extract; 115.8 % and 55.2 %, respectively, for the hydroalcoholic extract; and 108.3 % and 37.7 %, respectively, for the alcoholic extract. Comparing with the undigested extracts, the TPC was 1.6, 2.6, and 3.9-fold higher, respectively, after the intestinal digestion of alcoholic, hydroalcoholic, and aqueous extracts, with significant differences ($p < 0.05$). The alcoholic extract accomplished the best result after intestinal digestion (21.25 mg GAE/g DW). Statistically significant outcomes ($p < 0.05$) were outlined for TPC between the digests for the three extracts. Additionally, the TFC increase corresponded to 13.4 % and 423.7 % upon gastric and intestinal phases, respectively, for the aqueous extract; 131.9 % and 189.4 %, respectively, for the hydroalcoholic extract; and 78.6 % and 256.5 %, respectively, for the alcoholic extract. Only the intestinal digests exhibited significantly different TFC ($p < 0.05$) for the three extracts.

In summary, these results suggested that digestive fluids, enzymes, and pH changes exert a key role in the release of phenolic compounds from the halophyte matrix and their conversion into smaller molecules available for intestinal uptake. The intestinal digests retained the highest concentrations of polyphenols and flavonoids, indicating that the action of pancreatin and bile under neutral pH favors the delivery of these bioactive molecules from *M. nodiflorum* extracts. For instance, phenolic glycosides probably present in the *M. nodiflorum* extracts (and not detected by spectrophotometric assays due to interferences with the methods) may undergo hydrolysis and originate aglycones (easily detected by spectrophotometry) and glucose moieties, thereby increasing the TPC during digestion (Cheng et al., 2024). Moreover, polyphenols from *M. nodiflorum* can be bound to plant fibers through glycosidic and ester bonds (which are difficult to separate and extract), being released during digestion by acid hydrolysis, alkaline hydrolysis, and enzymatic action (H. Li et al., 2023). Pepsin promotes the hydrolysis of chemical bonds (*e.g.*, covalent and hydrogen bonds), leading to the release of polyphenols linked to macromolecules (*i.e.*, proteins and carbohydrates), weakening the ester linkages where phenolic acids interact with the cell wall and, consequently, releasing phenolic acids (Cheng et al., 2024).

3.3. *Bioaccessibility*

The recovery of phenolic and flavonoid compounds upon *in vitro* digestion increased as follows: oral < gastric < intestinal. As displayed in Table 1, oral digests retained the lowest percentages of polyphenols, increasing after gastric digestion and reaching the highest recovery rates upon intestinal phase, with statistical differences ($p < 0.05$) between digested fractions for the three extracts. The phenolic recovery rates exceeded 100 % for gastric and intestinal digests, indicating that some polyphenols present in their glycosidic form in the *M. nodiflorum* extracts, as proven in previous studies (Cheng et al., 2024; Falleh et al., 2011), were likely hydrolyzed into aglycones under gastric and intestinal environments, releasing the aglycone and glucose moieties. However, the phenolic glycosides are usually not detected by spectrophotometric techniques, acting as interferents in these assays.

The same pattern was observed for flavonoids. The oral digests retained the lowest flavonoid recovery rates, which increased after gastric digestion, without statistically different results ($p > 0.05$) except for the hydroalcoholic extract. The greatest flavonoid recovery rates were achieved upon intestinal digestion, showing statistical differences ($p < 0.05$) between the digested fractions of the three extracts.

These results reinforce the significant influence of pH neutralization and intestinal enzymes (pancreatin and bile) on polyphenols and flavonoids recovered comparing to gastric and oral phases. In addition, the high recovery rates after intestinal digestion could be attributed to the higher solubility and polarity of polyphenols within this digestive medium, enabling their release and subsequent metabolization into less complex molecules.

3.4. *Antioxidant/antiradical activities upon simulated digestion*

The improvement of antioxidant/antiradical activities of the three extracts was attested upon digestion following this sequence: oral < gastric < undigested extract < intestinal fractions, as shown in Table 1. The higher activity of the undigested extract compared to the oral and gastric phases can be attributed to its original, unaltered phytochemical profile, which still contains intact phenolic and flavonoid compounds that may be partially degraded, diluted, or transformed by enzymes and pH changes during the initial digestive stages.

The ABTS radical scavenging response increased by 16 %, 46.5 %, and 35.3 %, respectively, from the oral to the gastric phase for the aqueous, hydroalcoholic, and alcoholic extracts; however, these changes were not statistically significant ($p > 0.05$) between the digestion phases for each extract. Nevertheless, a significant increase was achieved after intestinal digestion, corresponding to 275.7 %, 371.9 %, and 416.7 %, respectively, for the hydroalcoholic, alcoholic, and aqueous extracts. Among the undigested extracts, the hydroalcoholic extract showed the highest ABTS scavenging potential, followed by alcoholic and aqueous extracts, with statistical results ($p < 0.05$).

The FRAP assay followed a similar pattern to ABTS with some discrepancies. Regarding the aqueous extract, the results indicated a 130 % increase in the FRAP response between oral and gastric digestion, while an increase of 68.7 % was obtained after the intestinal digestion. Concerning the hydroalcoholic extract, the increase was 75.5 % and 79.8 %, respectively, after the intestinal and gastric phases, while the digestion of the alcoholic extract led to an increase of 41.7 % and 63.3 %, respectively. Comparing the undigested extracts, the antioxidant activity of the alcoholic extract was 2.1 and 3.6-fold higher than the hydroalcoholic and aqueous extracts, respectively, with statistical differences ($p < 0.05$).

In summary, the results indicate that the gastrointestinal digestion conditions (namely enzymes and pH changes) influence the antioxidant/antiradical properties of *M. nodiflorum* extracts, showing consistent findings with the TPC and TFC and attesting the outstanding contribution of polyphenols and flavonoids to the antioxidant/antiradical effects. The better antioxidant responses achieved after intestinal digestion could be ascribed to the increasing number of hydroxyl groups of polyphenols, being released from monomers or aglycones within the intestinal medium (D. Pinto, A. S. Ferreira et al., 2023). Furthermore, the release and biotransformation of other food nutrients during digestion, mediated by enzymes and pH changes, such as fatty acids, minerals, and vitamins, may provide a greater potential for free radical scavenging (ABTS) and ferric ion reduction (FRAP) (Pinto, López-Yerena et al., 2024). Previous studies on other foods and by-products, namely chestnut shells, also demonstrated an improvement in antioxidant/antiradical activities upon digestion (D. Pinto, A. S. Ferreira et al., 2023; Pinto, López-Yerena et al., 2024, 2023).

3.5. *ROS counteracting efficiency*

The results of the ROS counteracting efficiency of *M. nodiflorum* extracts are displayed in Table 2.

The ROS scavenging potential enhanced during digestion as follows: oral < gastric < intestinal phases, except for $O_2^{\bullet-}$. Comparing the three undigested extracts, the alcoholic extract was the best $O_2^{\bullet-}$ and $ROO^{\bullet}$ scavenger, while the hydroalcoholic extract was the best H_2O_2 quencher and the aqueous extract unveiled better HOCl scavenging ability. Among all ROS tested, the highest counteracting efficiency after digestion was

Table 2
ROS scavenging potential of undigested and digested *M. nodiflorum* extracts prepared by UAE.

			$O_2^{\bullet -}$		H_2O_2		HOCl		ROO$^{\bullet}$
			% Inhibition	IC$_{50}$ (µg/mL)	% Inhibition	IC$_{50}$ (µg/mL)	% Inhibition	IC$_{50}$ (µg/mL)	µg TE/mg DW
Aqueous extract	*In vitro* digestion	Oral	91.52 ± 5.30 ^{a}	–	42.01 ± 0.44 ^{a}	–	73.98 ± 1.64 ^{c}	–	2.45 ± 0.02 ^{d}
		Gastric	96.92 ± 0.21 ^{a}	–	41.61 ± 1.91 ^{a}	–	81.46 ± 1.42 ^{b}	–	17.13 ± 1.51 ^{c}
		Intestinal	7.95 ± 0.40 ^{b}	–	54.97 ± 4.88 ^{a}	–	89.29 ± 0.13 ^{a}	–	35.89 ± 4.95 ^{b}
	Undigested extract		–	337.40 ± 5.03 ^{2}	38.25 ± 3.56 $^{1, a}$	–	–	78.42 ± 3.08 ^{2}	198.99 ± 25.04 $^{2, a}$
Hydroalcoholic extract	*In vitro* digestion	Oral	97.95 ± 0.02 ^{a}	–	46.31 ± 0.22 ^{b}	–	72.08 ± 0.57 ^{b}	–	1.43 ± 0.21 ^{d}
		Gastric	88.11 ± 1.62 ^{b}	–	37.52 ± 0.67 ^{c}	–	86.84 ± 1.95 ^{a}	–	10.99 ± 1.66 ^{c}
		Intestinal	23.73 ± 0.68 ^{c}	–	54.66 ± 1.11 ^{a}	–	86.63 ± 0.61 ^{a}	–	26.58 ± 1.01 ^{b}
	Undigested extract		–	477.27 ± 20.62 ^{1}	43.46 ± 2.34 $^{1, b}$	–	–	85.86 ± 3.11 ^{2}	218.59 ± 34.90 $^{2, a}$
Alcoholic extract	*In vitro* digestion	Oral	5.57 ± 0.02 ^{c}	–	50.00 ± 1.50 ^{b}	–	27.92 ± 3.33 ^{c}	–	2.97 ± 0.41 ^{d}
		Gastric	62.39 ± 2.74 ^{a}	–	44.37 ± 1.31 ^{b}	–	63.95 ± 3.30 ^{b}	–	21.33 ± 3.30 ^{c}
		Intestinal	49.13 ± 2.56 ^{b}	–	54.41 ± 0.60 ^{a}	–	95.18 ± 0.22 ^{a}	–	53.53 ± 7.79 ^{b}
	Undigested extract		–	32.12 ± 0.40 ^{3}	16.88 ± 0.56 $^{2, c}$	–	–	179.91 ± 16.34 ^{1}	230.58 ± 26.01 $^{2, a}$
Positive control	Gallic acid		–	23.84 ± 0.32 ^{3}	–	400.71 ± 5.68	–	3.67 ± 0.04 ^{3}	764.14 ± 0.00 ^{1}

IC$_{50}$ corresponds to the concentration required to scavenge 50 % of the reactive species in a tested medium (mean ± SEM). Different letters (a, b, and c) indicate significant differences between undigested and digested fractions within the same extract ($p < 0.05$). Different numbers (1, 2, and 3) indicate significant differences ($p < 0.05$) between the undigested extracts and the positive control.

reached for HOCl.

The $O_2^{\bullet -}$ scavenging capacity varied among undigested extracts, being the best result obtained for the alcoholic extract (with the lowest IC$_{50}$ value), followed by aqueous and hydroalcoholic extracts, with 10 to 15-fold lower quenching ability. The three extracts disclosed statistically different outcomes ($p < 0.05$). Additionally, the alcoholic extract showed a similar $O_2^{\bullet -}$ scavenging ability to gallic acid ($p > 0.05$). Different patterns were highlighted for the digested samples of the three extracts. In the hydroalcoholic extract, the highest inhibition percentage was obtained after the oral phase (97.95 %), while the lowest inhibition was attained after the intestinal phase (23.73 %). Regarding the aqueous extract, the highest inhibition percentages were obtained after the gastric (96.92 %) and oral (91.52 %) phases, with a significantly lower $O_2^{\bullet -}$ inhibition after the intestinal phase (7.95 %). Concerning the alcoholic extract, the gastric digest was the best $O_2^{\bullet -}$ scavenger (62.39 %), followed by the intestinal (49.13 %) and oral (5.57 %) digests.

Regarding H_2O_2 scavenging assay, the hydroalcoholic extract showed the best results (43.46 %), whereas the alcoholic extract displayed the lowest counteracting potential (16.88 %). The intestinal digests were the most efficient in eliminating this species, inhibiting 54.41 %, 54.66 %, and 54.97 % of the H_2O_2 generated, respectively, by the alcoholic, hydroalcoholic, and aqueous extracts. Nevertheless, the gastric digests were the least efficient, scavenging between 37.52 % and 44.37 % of the H_2O_2 produced. Therefore, the three extracts revealed an increase in the H_2O_2 neutralizing potential after digestion.

Considering the HOCl assay, the best scavenging responses among undigested extracts were attained for aqueous and hydroalcoholic extracts, showing no statistical differences ($p > 0.05$). Similar to the H_2O_2 assay, intestinal digestion led to the best HOCl-eliminating capacity (89.29 %, 86.63 %, and 95.18 %, respectively, for aqueous, hydroalcoholic, and alcoholic extracts). The lowest inhibition percentages were determined after oral digestion. Significant differences ($p < 0.05$) were determined among the digested fractions for the three extracts, except for the gastric and intestinal digests of the hydroalcoholic extract.

The ROO$^{\bullet}$ inhibiting potential was higher for the undigested extracts. Concerning the digested fractions, the intestinal fractions showed the highest inhibiting potential for this radical, thereafter gastric and oral phases, displaying statistical differences ($p < 0.05$) among digested fractions for the three extracts.

The results regarding ROS scavenging efficiency may be supported by the phenolic profiling of *M. nodiflorum* extracts and respective digested fractions, mainly tryptophan, 5–5′dihydroferulic acid, coniferyl alcohol, loliolide, rosmanol, dihydroactinidiolide, and glycerophospholipids, whose eliminating ability was proven in latest studies (Pinto et al., 2021). These findings are consistent with those described for *S. ramosissima* extracts obtained by conventional extraction and MAE (Pinto et al., 2021; Silva et al., 2021).

3.6. Antioxidant enzymes responses and protection from lipid peroxidation

Oxidative stress is mitigated by antioxidant enzymes and non-enzymatic antioxidants, reducing the toxic effects of pro-oxidant species (Pinto, López-Yerena et al., 2024). Table 3 summarizes the results of enzymatic inhibitory assays.

The CAT activity in the presence of alcoholic extract was 7.3-fold and 18.3-fold higher than the hydroalcoholic and aqueous extracts. After *in vitro* digestion of alcoholic extract, a significant drop in the CAT activity was observed, with lower results determined after the oral phase, reducing even more in the gastric digest and increasing upon intestinal phase. The aqueous extract showed the lowest CAT response for the undigested and digested fractions. Regarding the hydroalcoholic extract, the CAT response enhanced through digestion in the outlined sequence: oral < gastric < intestinal, with a 5.3-fold higher CAT activity upon the intestinal phase in contrast to the undigested fraction.

The GSH-Px activity improved in all three extracts after the digestion, reaching 1.7-fold higher results after the intestinal phase compared to the undigested fractions. The aqueous extract achieved the best GSH-

Table 3

Effects of undigested and digested *M. nodiflorum* extracts prepared by UAE on antioxidant enzymes activities and lipid peroxidation.

			CAT (nmol/min/g DW)	GSH-Px (µmol/min/g DW)	SOD (µmol/min/g DW)	LPO (nmol MDA/mg DW)
Aqueous extract	*In vitro* digestion	Oral	33.28 ± 4.50^{a}	52.48 ± 8.00^{b}	63.29 ± 1.02^{b}	198.43 ± 20.67^{c}
		Gastric	31.85 ± 2.86^{a}	93.33 ± 2.31^{b}	32.25 ± 0.17^{d}	612.39 ± 81.88^{b}
		Intestinal	32.40 ± 1.75^{a}	276.23 ± 74.25^{a}	70.77 ± 1.02^{a}	3938.27 ± 67.73^{a}
	Undigested extract		$24.38 \pm 2.25^{a,2}$	$154.34 \pm 44.56^{a,1}$	$43.36 \pm 2.22^{1, c}$	$228.09 \pm 34.45^{c, 2}$
Hydroalcoholic extract	*In vitro* digestion	Oral	73.01 ± 6.74^{b}	47.46 ± 8.55^{b}	46.86 ± 2.73^{b}	427.44 ± 27.56^{b}
		Gastric	98.16 ± 8.52^{b}	153.99 ± 10.25^{a}	37.20 ± 1.02^{c}	332.08 ± 55.13^{b}
		Intestinal	323.45 ± 24.30^{a}	171.61 ± 13.20^{a}	66.18 ± 1.71^{a}	1984.83 ± 116.55^{a}
	Undigested extract		$60.94 \pm 4.50^{b,2}$	$98.39 \pm 23.65^{b, 1}$	$32.97 \pm 1.20^{1, c}$	$380.35 \pm 43.07^{b, 2}$
Alcoholic extract	*In vitro* digestion	Oral	92.09 ± 9.44^{b}	64.69 ± 13.64^{c}	41.79 ± 2.73^{b}	383.58 ± 0.00^{c}
		Gastric	53.73 ± 4.81^{b}	134.23 ± 2.63^{b}	50.97 ± 1.71^{b}	368.20 ± 58.37^{c}
		Intestinal	130.46 ± 16.80^{b}	178.46 ± 7.67^{a}	65.82 ± 0.17^{a}	3178.72 ± 133.09^{b}
	Undigested extract		$447.18 \pm 62.94^{a,1}$	$104.50 \pm 9.55^{b,1}$	$22.10 \pm 4.61^{2, c}$	$3748.45 \pm 223.95^{a,1}$

Different letters (a, b, and c) indicate significant differences between undigested and digested fractions within the same extract ($p < 0.05$). Different numbers (1, 2, and 3) indicate significant differences ($p < 0.05$) between the undigested extracts.

Px responses before and after the digestion. Comparing the three undigested extracts, the GSH-Px activity enhanced in the following order: hydroalcoholic < alcoholic < aqueous extracts, without statistically significant differences ($p > 0.05$).

An identical response was obtained for the SOD activity, with 1.6, 2, and 2.9-fold higher results after intestinal digestion, respectively, for aqueous, hydroalcoholic, and alcoholic extracts when compared to the respective undigested extracts, with significant differences ($p < 0.05$).

LPO assay measures the production of malondialdehyde (MDA) in response to oxidative stress. In contrast to antioxidant enzymes, there was a loss of protection against LPO after the digestion of aqueous and hydroalcoholic extracts. The protective response of alcoholic extract against LPO improved significantly ($p < 0.05$) after the three digestive phases when compared to the undigested extract. Among undigested extracts, the best results were obtained for aqueous and hydroalcoholic extracts (228.09 and 380.35 nmol MDA/g, respectively), followed by the alcoholic extract (3748.45 nmol MDA/g).

Overall, these outcomes reinforce an upmodulating response of antioxidant enzymes and protective effect from LPO before and after the digestion, corroborating the phenolic concentrations and antioxidant/antiradical results.

3.7. *Inhibition of acetylcholinesterase activity*

The AChE inhibition by *M. nodiflorum* extracts before and after simulated digestion was also assessed (Fig. 1).

The undigested extracts, tested at 1000 µg/mL, achieved an inhibition of 31.17 %, 28.31 %, and 27.38 %, respectively, for alcoholic, hydroalcoholic, and aqueous extracts. Only the alcoholic extract showed statistical differences ($p < 0.05$) among the undigested extracts.

The AChE inhibition by the aqueous and hydroalcoholic extracts decreased during simulated digestion as follows: oral (17.25 % and 23.96 %, respectively) > gastric (14.86 % and 18.05 %) > intestinal (14.06 % and 17.57 %). Nevertheless, no statistical results ($p > 0.05$) were observed between the three digested fractions for the hydroalcoholic extract, emphasizing that all digested fractions led to similar AChE inhibition. Additionally, the gastric and intestinal digests of the aqueous extract showed no statistically different results ($p > 0.05$).

Oppositely, the AChE inhibition by the alcoholic extract decreased after gastric and oral digestion (21.09 % and 30.99 %, respectively), increasing considerably ($p < 0.05$) upon the intestinal phase (37.70 %). Noteworthy, the intestinal digest achieved a higher AChE inhibition than the undigested extract (31.17 %). The same increasing trend was observed in the TPC, suggesting that the digestive process led to an increase in phenolic compounds that have been associated with neuroprotective properties, explaining these findings (D. Pinto, A. S. Ferreira

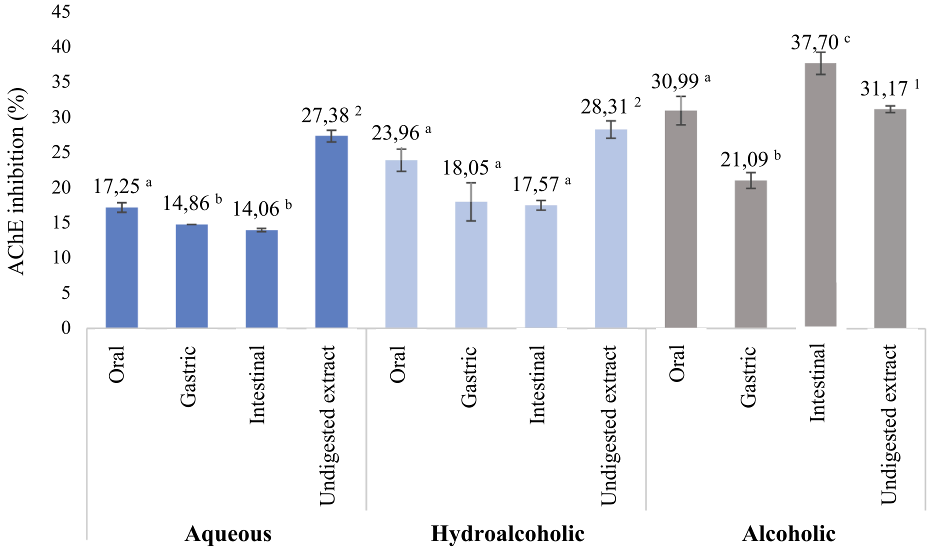

Fig. 1. Inhibition of AChE activity in the presence of undigested and digested *M. nodiflorum* extracts prepared by UAE. Different letters (a, b, and c) indicate significant differences between digested fractions within the same extract ($p < 0.05$). Different numbers (1, 2, and 3) indicate significant differences ($p < 0.05$) between the undigested extracts.

et al., 2023; Pinto, López-Yerena et al., 2024, 2023). Regarding halophytes, Pinto *et al.* (Pinto et al., 2021) studied the anticholinergic activity of *S. ramosissima* by-product aqueous extract, reporting inhibition percentages up to 32.34 % (at 1000 μg/mL).

3.8. Phytochemical profile by UPLC-QTOF-MS

The phytochemical profile of aqueous, hydroalcoholic, and alcoholic *M. nodiflorum* extracts was analysed by UPLC-QTOF-MS. Fig. 2 presents the UPLC chromatograms of *M. nodiflorum* extracts, while Table 4 presents the main phytochemicals identified in the three extracts and their respective concentrations.

The total concentration of bioactive compounds identified in the three *M. nodiflorum* extracts increased in the following order: hydroalcoholic (138.44 μg/mL) < aqueous (295.41 μg/mL) < alcoholic (380.41 μg/mL). In the aqueous extract, 10 compounds were detected based on the most intense signals and mass spectrometry data, including 2 hydroxycinnamic acids, 2 benzofurans, 1 diterpene, 1 phenylpropanoid, 1 lipid derivative, 1 amino acid, 1 tripeptide, and 1 dipeptide. Moreover, 15 compounds were identified in both hydroalcoholic and alcoholic extracts, including the same compounds identified in the aqueous extract together with 2 dihydrochalcone derivatives, 1 stilbene, 1 anthocyanin derivative, and 1 flavonoid glycoside. As far as we know, studies on the phytochemical profile of *M. nodiflorum* halophyte are limited (Oliveira-Alves et al., 2023). Nevertheless, previous research on *Mesembryanthemum* species has identified a wide variety of secondary metabolite classes (Oh et al., 2024).

The aqueous extract presented a limited number of peaks that have significant UV absorption and a relatively low number of intense peaks. The main peak at m/z 274 was assigned to a glycerophospholipid (157.32 μg/mL), followed by tryptophan (78.63 μg/mL) and 5,5′-dihydroferulic acid (13.10 and 12.30 μg/mL for both isomers). Lys-Asp-Tyr, dihydroactinidiolide, loliolide, rosmanol, and coniferyl alcohol were quantified in considerable amounts (5.83–8.39 μg/mL), while residual levels of threonyl threonine and coumaroyl tyramine were also detected (<1.5 μg/mL).

Comparing the hydroalcoholic extract with alcoholic extract, a similar phytochemical profile was shown with a larger number of identified compounds and some differences in the concentrations of the compounds extracted. These results highlight the more efficient extraction by ethanol, corroborating the outcomes of the spectrophotometric assays. The glycerophospholipid was identified as the major compound in both extracts (57.07 and 242.03 μg/mL, respectively, in hydroalcoholic and alcoholic extracts), followed by 5,5′-dihydroferulic acid isomers (16.74–18.55 and 16.95–18.67 μg/mL) and tryptophan (12.99 and 49.88 μg/mL). Large amounts of dihydroactinidiolide (7.67 and 6.05 μg/mL), loliolide (6.66 μg/mL for both extracts), and phlorin (8.10 and 7.01 μg/mL) were also quantified in hydroalcoholic and alcoholic extracts, while phlorin was not detected in the aqueous extract. Scirpusin A and 3-hydroxyphloretin-2′-*O*-xylosylglucoside were quantified with a 3-fold higher concentration in hydroalcoholic extract when compared to the alcoholic extract. Likewise, the amounts of cyanidin-3-*O*-rutinoside and luteolin-glucoside were almost 2-fold higher in hydroalcoholic extract. Conversely, Lys-Asp-Tyr, coniferyl alcohol, and rosmanol concentrations in the alcoholic extract were, respectively, 2, 8, and 11-fold higher when compared to the hydroalcoholic extract. Additionally, similar levels of coumaroyl tyramine and threonyl threonine were detected with <1.34 μg/mL.

Therefore, lipid derivatives are the major compounds class in the three extracts with 53 %, 55 %, and 64 % of the total content, respectively, in aqueous, hydroalcoholic, and alcoholic extracts. In the aqueous extract, peptides and amino acids represent the second most abundant class (30 %), followed by phenolic compounds and derivatives (13 %). Otherwise, a similar prevalence of both peptides/amino acids and phenolic compounds was detected in the hydroalcoholic extract (16 % each), while the alcoholic extract contained a higher phenolics rate (18 %) than peptides (15 %). The highest percentage of terpenoids was found in the hydroalcoholic extract (14 %), followed by residual amounts of aqueous (4 %) and alcoholic (3 %) extracts. For instance, Oh *et al.* (Oh et al., 2024) investigated the phytochemical composition of *M. crystallinum* by LC-QTOF/MS, reporting that almost 70 % of the compounds identified were lipids, alongside residual amounts of organic acids, benzenoids, phenylpropanoids/polyketides, and nucleosides. In another study, Kang and Joo (Kang & Joo, 2023) attested the presence of hydroxybenzoic acids and derivatives, hydroxycinnamic acids and derivatives, flavanols, flavonols, proanthocyanidins, sesquiterpenoids, coumarins, and chalcones in different organs of *M. crystallinum*, namely cotyledon, shoot, and stem.

In addition, ethanol was more efficient than water in recovering a wide variety of phytochemicals (*i.e.*, phenolic compounds, lipids, terpenoids, peptides, and amino acids) from *M. nodiflorum* due to its chemical and solubility properties. Particularly, the mixed polarity of ethanol – arising from the polar hydroxyl group and non-polar hydrocarbon tail – allows it to solvate both polar and non-polar compounds, disrupting cell membranes, breaking hydrogen bonds, and further enhancing the extraction of the bioactive molecules. The alcoholic extract proved to be the most promising, displaying the highest total concentration of phytochemicals, along with significant amounts of lipids, phenolic compounds, and peptides, alongside making it suitable for food and nutraceutical applications since ethanol is considered a GRAS (generally recognized as safe) solvent.

3.9. Effects of M. nodiflorum extracts on Caco-2 and HT29-MTX cells

The bioactive molecules' absorption mainly occurs in the small intestine, sustaining the selection of Caco-2 and HT29-MTX cells to assess the potential impact of *M. nodiflorum* extracts on intestinal mucosa (Pinto, López-Yerena et al., 2024). Caco-2 cells resemble human enterocytes forming a monolayer with tight junctions and microvilli, although they lack mucus production (Pinto et al., 2020). Oppositely, this drawback is addressed by HT29-MTX cells that produce mucin, a crucial factor influencing compounds' transport through the intestinal tract (Pinto et al., 2020). The Caco-2 and HT29-MTX viability was evaluated through MTT reduction by mitochondrial dehydrogenase of living cells, indicating the physiological state of the cells and their proliferation rate. The results are shown in Fig. 3.

Regarding the aqueous extract, the cell viability was the highest at 500 μg/mL in both cell lines (94 % and 90 %, respectively, in Caco-2 and HT29-MTX cells), without statistical differences ($p > 0.05$) comparing to the control. Likewise, in the hydroalcoholic extract, the cell viability was the highest at 500 μg/mL (104 % and 69 %, respectively). However, statistically different results ($p < 0.05$) were attested in HT29-MTX cells when compared to the control. Additionally, the alcoholic extract led to the highest decrease in viability for both cell lines. In Caco-2 cells, the viability percentages varied between 85.55 % and 58.78 % (at 31.25 and 1000 μg/mL) in the presence of the alcoholic extract. Concerning HT29-MTX cells, the viability was the highest at 31.25 μg/mL (79.06 %), while the lowest viability was attained at 1000 μg/mL (53.97 %). Considering all results, the optimal non-cytotoxic concentration was 500 μg/mL for the three *M. nodiflorum* extracts, with viability rates above 60 % for both cells.

Arena *et al.* (Arena et al., 2020) evaluated the cytotoxicity of *M. nodiflorum* extract prepared by SFE on HS-68 fibroblasts. After 48 h, the extract did not induce toxicity when compared to the control used, concluding that the *M. nodiflorum* extract protects the cells against oxidative stress-induced mortality (Arena et al., 2020). Furthermore, *S. ramosissima* extracts obtained by eco-friendly techniques did not cause toxic effects on both cell lines up to 1000 μg/mL (Silva et al., 2021).

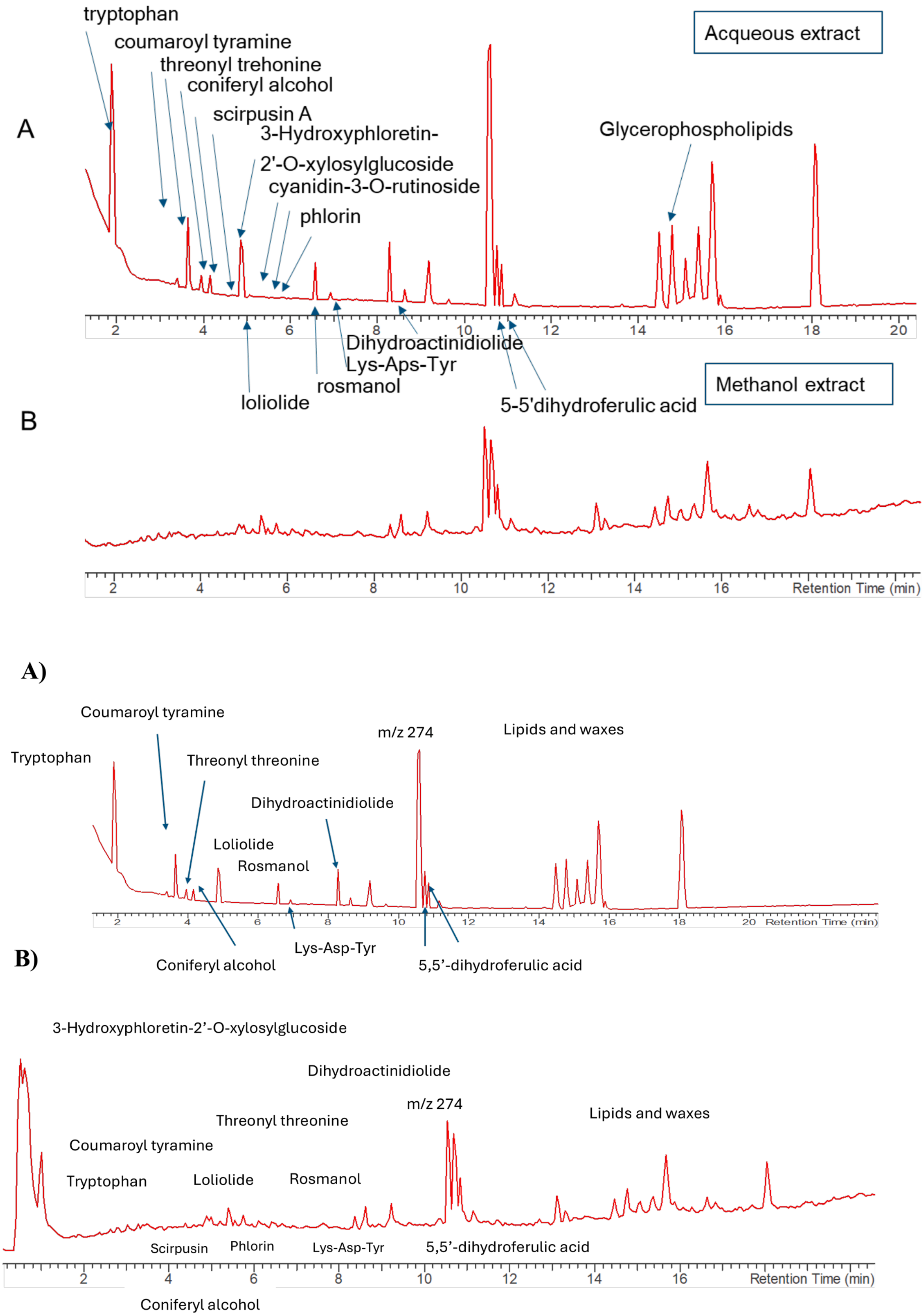

Fig. 2. UPLC-QTOF-MS chromatographs of the main phytochemical compounds identified in aqueous (A) and alcoholic (B) extracts.

Table 4
Phytochemical profile of *M. nodiflorum* aqueous, hydroalcoholic, and alcoholic extracts by UPLC-QTOF-MS.

Retention time (min)	Accurate MS	Formula	Compound identification	Amount (µg/mL)		
				AQ	HA	ALC
1.98	205.0975	$C_{11}H_{12}N_2O_2$	Tryptophan	78.63	12.99	49.88
3.65	284.1037	$C_{17}H_{17}NO_3$	Coumaroyl tyramine	0.23	0.26	0.23
3.97	221.11665	$C_8H_{16}N_2O_5$	Threonyl threonine	1.11	0.24	1.34
4.14	181.08564	$C_{10}H_{12}O_3$	Coniferyl alcohol	5.83	1.46	16.83
4.38	471.14569	$C_{28}H_{22}O_7$	Scirpusin A	n.d.	2.40	0.65
4.75	585.18838	$C_{26}H_{32}O_{15}$	3-Hydroxyphloretin-2′-*O*-xylosylglucoside	n.d.	0.32	0.10
4.90	197.11724	$C_{11}H_{16}O_3$	Loliolide	6.25	6.66	6.66
5.00	595.16668	$C_{27}H_{31}O_{15}$	Cyanidin-3-*O*-rutinoside	n.d.	1.29	0.64
5.41	309.0945	$C_{12}H_{16}O_8Na$	Phlorin	n.d.	8.10	7.01
5.67	449.1086	$C_{21}H_{20}O_{11}$	Luteolin-glucoside	n.d.	0.65	0.35
6.26	331.20923	$C_{20}H_{26}O_4$	Rosmanol	6.00	0.84	7.00
6.56	425.19225	$C_{19}H_{28}N_4O_7$	Lys-Asp-Tyr	8.39	3.20	6.02
8.28	181.1224	$C_{11}H_{16}O_2$	Dihydroactinidiolide	6.25	7.67	6.05
10.40	391.13625	$C_{20}H_{22}O_8$	5–5′-Dihydroferulic acid	12.30	18.55	18.67
10.56	391.13625	$C_{20}H_{22}O_8$	5–5′-Dihydroferulic acid isomer	13.10	16.74	16.95
14.50	274.2766	$C_{40}H_{74}NO_{11}P$	Glycerophospholipids	157.32	57.07	242.03
Total concentration				295.41	138.44	380.41

n.d., not detected. AQ, aqueous extract. HA, hydroalcoholic extract. ALC, alcoholic extract.

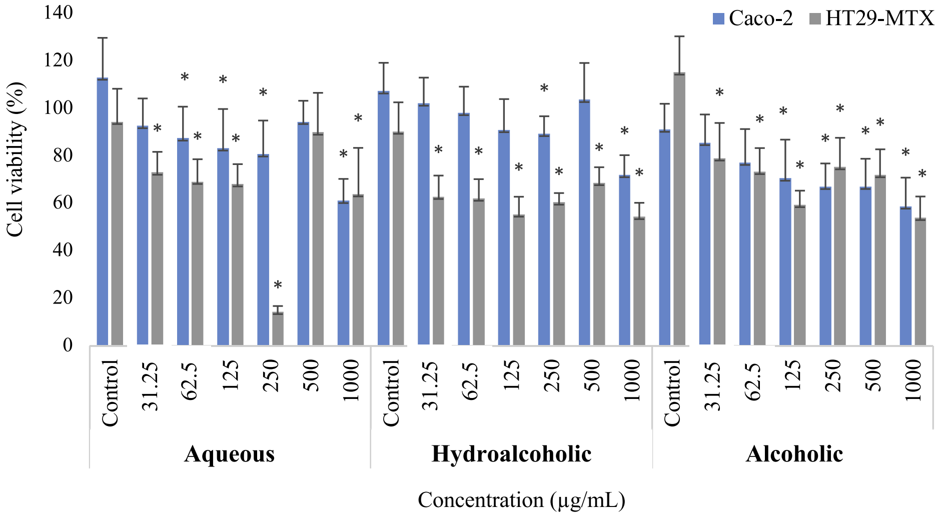

Fig. 3. Effects of different concentrations of *M. nodiflorum* extracts prepared by UAE on intestinal cells viability (Caco-2 and HT29-MTX) by MTT assay. Values are expressed as mean ± standard deviation. * $p < 0.05$ *versus* control.

3.10. Intestinal permeability of intestinal digests from *M. nodiflorum* extracts

In vitro cellular models that simulate the human intestine are also used to predict the nutrient absorption from digests collected after *in vitro* gastrointestinal digestion. Several factors can affect intestinal permeation, including the compound concentration, molecular size, structure, and hydrophilicity. Fig. 4 presents the bioactive compounds' permeation percentages at different time points for the three *M. nodiflorum* extracts.

As shown, 10 compounds from the aqueous extract and 15 compounds from hydroalcoholic and alcoholic extracts permeated the intestinal barrier, with permeation rates up to 66 %.

Rosmanol was the main compound permeating across the intestinal barrier for the three extracts, reaching the highest permeation rate after 4 h (65.10 % for the aqueous extract and 66.32 % for hydroalcoholic and alcoholic extracts). Among the benefits, rosmanol exerts anticancer, anti-inflammatory, antimicrobial, antioxidant, and neuroprotective effects, offering a promising candidate for dietary supplements or nutraceuticals (Aziz et al., 2022).

Lys-Asp-Tyr, a tripeptide composed of lysine, aspartate, and tyrosine, reached 26.50 % of permeation for the aqueous extract and 30.12 % for the hydroalcoholic and alcoholic extracts upon 4 h. These amino acids are crucial for various physiological processes, emphasizing their importance in nutrition and potential therapeutic applications (Farré et al., 2020). Particularly, lysine is an essential amino acid involved in protein synthesis and immune function, while aspartate plays a key role in cellular energy production and nitrogen metabolism (Farré et al., 2020). Tyrosine acts as a neurotransmitter precursor (*e.g.*, dopamine) involved in various metabolic pathways and functions related to stress and mood regulation (Farré et al., 2020).

Tryptophan is another essential amino acid obtained only through diet, exerting a fundamental role in protein biosynthesis and physiological processes, such as metabolism, inflammation, oxidative stress, neuronal function, intestinal homeostasis, and immune response (Rutherfurd & Moughan, 2012). This amino acid permeated the intestinal barrier, achieving 15.10 %, 14.20 %, and 14.58 % permeation after 4 h, respectively, for aqueous, hydroalcoholic, and alcoholic extracts and probably serving as a precursor for bioactive compounds.

3-Hydroxyphloretin-2′-*O*-xylosylglucoside was the second most abundant compound in the permeated samples of hydroalcoholic extract, with 64 % permeation after 4 h. However, this compound was

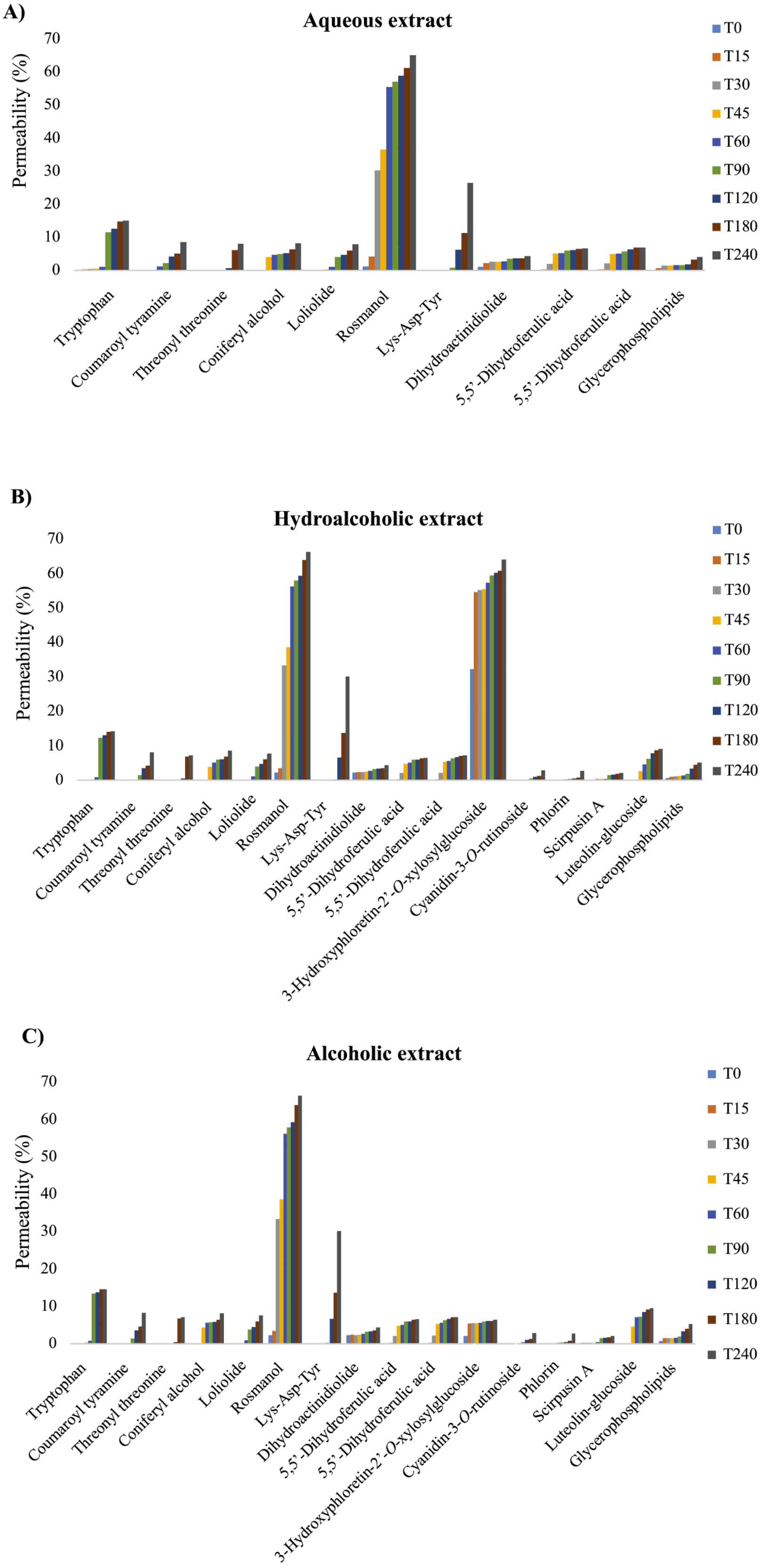

Fig. 4. Intestinal permeability of intestinal digests of *M. nodiflorum* extracts prepared by UAE, namely aqueous (A), hydroalcoholic (B), and alcoholic extracts (C). Results are expressed as mean ± standard deviation ($n = 3$).

not detected in the permeates of aqueous extract, while only 6.4 % permeation was obtained for the alcoholic extract. Although limited data is available on 3-hydroxyphloretin-2′-*O*-xylosylglucoside, previous studies on phloretin derivatives revealed their health benefits in diabetes, reducing oxidative stress, and supporting immune function closely ascribed to anti-inflammatory, antioxidant, and glucose transport inhibitory properties (Trifan & Luca, 2023).

Luteolin-glucoside, a glycosylated form of the flavonoid luteolin, was only identified in the permeates of hydroalcoholic and alcoholic extracts, with permeation rates above 9 %. This luteolin derivative has demonstrated potent antioxidant and anti-inflammatory properties, protecting against various chronic diseases (*e.g.*, cancer, cardiovascular and neurodegenerative pathologies) and potentially aiding in the treatment of inflammatory conditions and age-related illnesses (Caporali et al., 2022; Luo et al., 2017), which highlights its potential use in nutraceuticals aimed at mitigating chronic disorders. These results corroborate the findings reported in the literature proving the low bioavailability and intestinal absorption of luteolin-glucoside (Luo et al., 2017).

5,5′-Dihydroferulic acid, coumaroyl tyramine, threonyl threonine, coniferyl alcohol, and loliolide were also transported through the intestinal barrier, achieving permeation rates between 6.47 % and 8.61 % after 4 h, with similar results obtained for the three extracts. For instance, 5,5′-dihydroferulic acid has been linked to systemic antioxidant effects, potentially modulating inflammation and oxidative stress and improving gut health (Pinto, Lozano-Castellón et al., 2024). This metabolite was also identified in another study regarding the intestinal permeation of chestnut shells extract (Pinto, Lozano-Castellón et al., 2024). Coumaroyl tyramine, a phenolic amide, exhibits neuroprotective or cognitive-enhancing properties (Balaraju & Salimath, 2017), while the dipeptide threonyl threonine may share related bioactivities to amino acid threonine, maintaining the adequate fat metabolism in liver, reducing the fat accumulation, and promoting the efficient digestive and intestinal functions (Rutherfurd & Moughan, 2012). Loliolide, a monoterpenoid lactone, was described as a therapeutic agent for diabetes and depression, alongside exerting antioxidant, antifungal, antibacterial, and anticancer effects (Dias et al., 2020). Furthermore, coniferyl alcohol was also suggested as a valuable candidate for nutraceuticals, pharmaceuticals, and functional foods, acting as a precursor in the biosynthesis of lignin and other bioactive compounds (Qiang et al., 2022).

Trace rates (4–5 % after 4 h) of glycerophospholipids and sesquiterpenoid dihydroactinidiolide were also quantified in the permeates of the three extracts, while the phenolic compounds cyanidin-3-*O*-rutinoside, phlorin, and scirpusin A from hydroalcoholic and alcoholic extracts were detected at lowest permeation rates (<3 %). The lowest permeability rates may be explained by the bioconversion into metabolites alongside their poor solubility in aqueous solvents, affecting gut absorption (D. Pinto, A. M. Silva et al., 2023). Additionally, some compounds may have been preserved in the intestinal barrier, also providing health effects, for example, cellular cytoprotection by alleviating oxidative stress injuries (Pinto, López-Yerena et al., 2024).

The TEER was measured during the co-culture and permeability

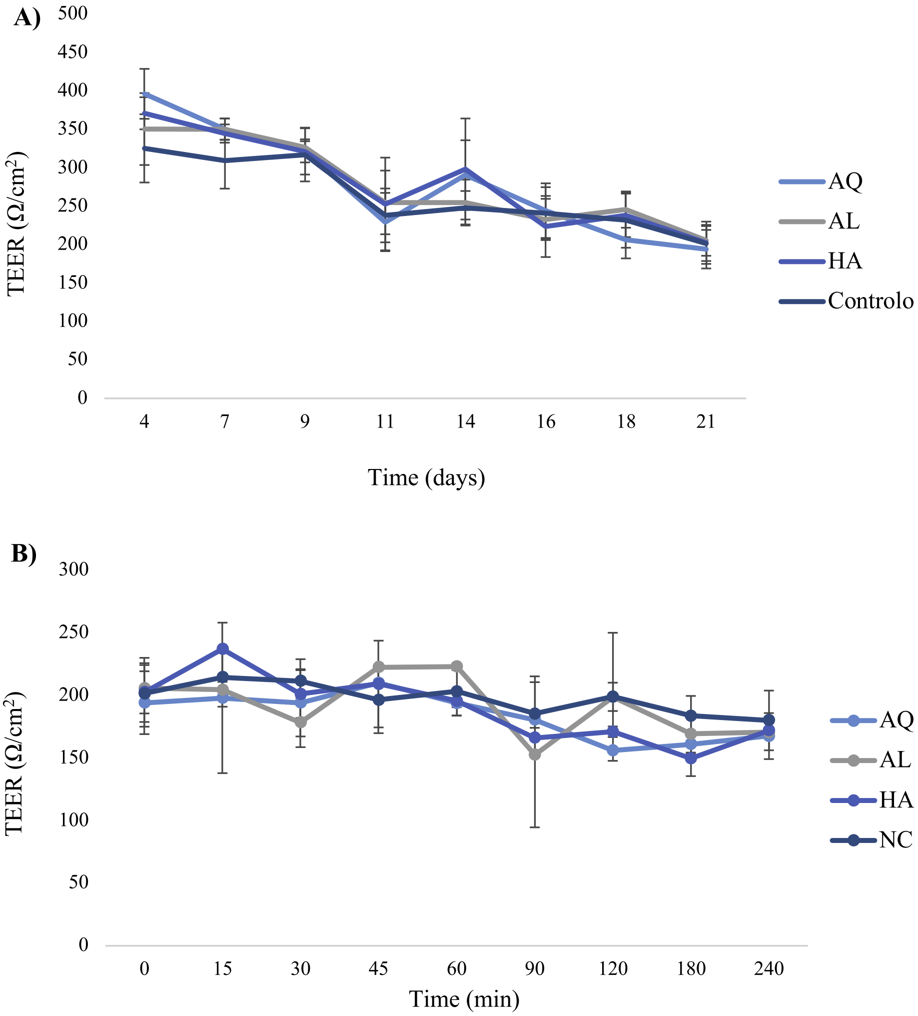

Fig. 5. TEER measurements during 21 days of Caco-2/HT29-MTX co-culture model (A) and 240 minutes of permeability assay (B). Results are expressed as mean ± standard deviation (n=3).

assay to monitor the integrity of living cells. A reduction in TEER values may indicate a decrease in membrane integrity. The TEER results are shown in Supplementary Figure 1. During the 21 days of co-culture (Fig. 5A), the TEER values showed a gradual decrease until the 11th day. On the 14th day, there was a slight increase in TEER, reaching the lowest levels on the 21st day. The TEER varied from 202 Ω/cm^2 (at 21st day) to 396 Ω/cm^2 (at 4th day). During the 240-min assay (Figure 5B), the TEER remained almost unchanged, varying between 156 Ω/cm^2 (at 120 min) and 210 Ω/cm^2 (at 45 min) for the aqueous digest; 153 Ω/cm^2 (at 90 min) and 223 Ω/cm^2 (at 45 min) for the alcoholic digest; 150 Ω/cm^2 (at 180 min) and 237 Ω/cm^2 (at 15 min) for the hydroalcoholic digest; and 214 Ω/cm^2 (at 15 min) and 180 Ω/cm^2 (at 240 min) for the negative control. These results were in agreement with those reported in the permeation of baby kiwi leaves and chestnut shells extracts (Pinto, Lozano-Castellón et al., 2024; Silva, Almeida et al., 2022), attesting the integrity of the cell barrier during the permeability assay.

The outcomes of this work offer valuable insights for food industry and food engineering sectors. The extraction and bioaccessibility data obtained for *M. nodiflorum* highlight its potential as a novel ingredient for functional foods, contributing to product diversification and formulation of nutraceuticals. From a manufacturing perspective, UAE demonstrated to be an efficient and scalable green technology that can be adapted to industrial processes, reducing the extraction time, solvent use, and energy consumption. Additionally, understanding the digestive stability and intestinal permeability of its bioactive compounds provides critical information for designing functional products with improved bioavailability, supporting production optimization and innovation within the food sector

4. Conclusion

M. nodiflorum demonstrated to be a rich source of bioactive compounds with promising health benefits and potential to protect from oxidative stress-mediated pathologies and neurodegenerative disorders. Notwithstanding, no previous studies have investigated the bioaccessibility and intestinal absorption of *M. nodiflorum* extracts by coupling accurate chemical composition, *in vitro* simulated digestion and an intestinal permeability model. The results disclosed an increase in TPC, TFC, bioaccessibility, and antioxidant/antiradical properties after gastrointestinal digestion, emphasizing distinct bioaccessibility and intestinal absorption rates for the three extracts. Neuroprotective properties were also noticed before and after digestion, with the best anticholinergic response being achieved for the intestinal digest of alcoholic extract. Taken together, the most promising outcomes were attained for the alcoholic extract, exhibiting an interesting phytochemical profile rich in glycerophospholipids, tryptophan, 5,5′-dihydroferulic acid, and coniferyl alcohol. Additionally, the intestinal permeability assay pointed out rosmanol as the compound with the highest permeation rates in the three extracts (65 % for aqueous extract and 66 % for both hydroalcoholic and alcoholic extracts after 4 h). Therefore, the repurposing of slenderleaf iceplant for food and nutraceutical uses reinforces the need to address the impact of *in vitro* simulated digestion and intestinal permeation models on bioavailability, bioaccessibility, and intestinal absorption of phytochemical compounds. Although this study provides novel insights into the digestibility, bioaccessibility, and intestinal absorption of *M. nodiflorum* extracts, several limitations should be considered. The phytochemical composition of this halophyte can vary depending on the growing region and climatic conditions, which may influence its bioactive profile. In addition, the use of *M. nodiflorum* extracts in nutraceutical formulations or as food ingredients introduces further variability, as different doses, processing methods, and the presence of other food components can alter the bioaccessibility and stability of its secondary metabolites. Moreover, when incorporated into foods, this plant may be consumed raw or cooked, and cooking or processing could modify its phytochemical composition. Finally, the present work was conducted entirely *in vitro*, using static digestion models and a co-culture intestinal cell system, which do not fully replicate the complexity of human gastrointestinal physiology and metabolism. Future research should therefore include *in vivo* studies to confirm these findings, assess the impact of different extraction and processing strategies, evaluate long-term safety and efficacy, and investigate interactions with gut microbiota and other food matrices to fully support the use of *M. nodiflorum* as a functional food or nutraceutical ingredient. In summary, this study provided novel findings that could be used to predict the digestibility and bioactivity of *M. nodiflorum* extracts, supporting their use in the design of healthy nutraceuticals and functional foods.

Ethical guidelines

Ethics approval was not required for this research.

Etical

The authors declare that no animal experiments have been conducted in this study.

CRediT authorship contribution statement

Diana Pinto: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Raquel Vieira:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Filipa Teixeira:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Stefania Sut:** Methodology, Investigation, Formal analysis. **Mónica Vieira:** Methodology, Investigation, Formal analysis, Data curation. **Miguel Salazar:** Methodology, Investigation. **Cristina Delerue-Matos:** Methodology, Formal analysis, Data curation. **Stefano Dall'Acqua:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Francisca Rodrigues:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101315.

Data availability

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

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