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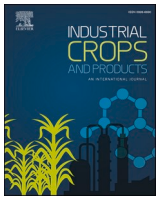

Phenolic composition and *in vitro* bioactive and enzyme inhibitory properties of bell pepper (*Capsicum annuum* L.) plant extracts

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ABSTRACT

The phenolic composition and bioactive properties of Holland and Italian bell pepper plant (*Capsicum annuum* L.) hydroethanolic extracts were investigated by HPLC-DAD-ESI/MS and *in vitro* cell- and enzyme-based assays, respectively. *O*-Glycosylated luteolin derivatives were identified as the major compounds, representing ~60% of the phenolic fraction. Both extracts displayed remarkable antioxidant activity and ability to inhibit α-glucosidase and the formation of advanced glycation end products (AGEs) compared to the positive controls, which yielded statistically higher or equal IC_{50} values. Notably, the extracts were up to 57 times more effective than aminoguanidine in inhibiting AGEs. The lipase enzyme involved in fat metabolism and the pro-inflammatory mediator nitric oxide were also inhibited in some extent, with orlistat and dexamethasone being 10–13.4-fold and ~17-fold more effective than the extracts, respectively. A notable cytotoxicity occurred on the tested human tumor cell lines, mainly on Caco-2 with GI_{50} values ranging from 18 to 65 μg/mL. Furthermore, activity greater than that of food preservatives was observed against some foodborne fungi and bacteria. These results highlight bell pepper plant by-products as a renewable source of functional ingredients that could be utilized in food, nutraceutical, and medicinal formulations to manage type 2 diabetes, obesity, and other oxidative stress-mediated conditions.

1. Introduction

Oxidative stress stands out as a crucial contributor to the pathogenesis of several chronic and metabolic disorders such as diabetes mellitus, cardiovascular and neurodegenerative diseases, obesity, and cancer (Fatima et al., 2023). It arises when the production of reactive oxygen species overwhelms the cell antioxidant defenses, leading to lipid, protein, and DNA damage and disruption of vital cellular processes (Fatima et al., 2023). However, the oxidative stress-related damage can be prevented or slowed down by antioxidants such as naturally occurring phenolic compounds that are widely found in fruits, vegetables, and herbs (Fatima et al., 2023; Rudrapal et al., 2022). These secondary metabolites can neutralize different reactive species and protect against cellular oxidative stress and lipid peroxidation, which is why they have been used as bioactive ingredients in functional foods, food supplements, and nutraceuticals, among other health formulation (Añibarro-Ortega et al., 2022; Rudrapal et al., 2022). Therefore, the consumption of polyphenol-rich foods has been strongly encouraged and

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associated with a decrease in various chronic diseases, while the search for natural antioxidant compounds and extracts has been in the research spotlight.

Nowadays, the growing interest in natural bioactive compounds is increasingly aligned with principles of sustainability, resource-use efficiency, and circular bioeconomy, which has promoted their recovery from agri-food waste and by-products (Añibarro-Ortega et al., 2020; Chel-Guerrero et al., 2022; Herrera-Pool et al., 2021). Furthermore, the increase in the world population has promoted the need to intensify the production of horticultural crops to meet the growing demands for plant-based foods. However, economic and ecological issues have arisen due to the generation of enormous quantities of undervalued plant by-products, which may cause severe environmental problems if not properly handled. Therefore, future-oriented approaches aligned with the United Nations' 2030 Agenda for Sustainable Development and biorefinery concept must be explored and applied to promote sustainable production and consumption patterns.

Among the most important food crops, the Solanaceae family comprises many economically important species worldwide, which also stand as valuable sources of pharmacologically active compounds. *Capsicum* spp. (sweet bell peppers and some chili pepper varieties) are good examples, among which *C. annuum* L. stands as the most common and widely cultivated of the five domesticated species of this genus (Añibarro-Ortega et al., 2022). In 2022, the world production of peppers and chilies reached almost 37 million tons in a crop-growing surface area of 2 million ha (FAOSTAT, 2023). In addition to pepper fruits, this annual crop generates tons of plant biomass which, at the end of the fruit production cycle, is composed into organic fertilizer, disposed of in landfills, or incinerated with no economic return to the farmer. However, while pepper fruits are widely studied and well-known for their nutritional value and variety of carotenoids (*e.g.*, capsanthin, capsorubin, lutein, β-carotene), steroidal saponins (*e.g.*, capsicoside), and phenolic alkaloids (*e.g.*, capsaicin), the crop remaining have not received much attention from the industrial sector or the scientific community (Añibarro-Ortega et al., 2022; Kim et al., 2014). Nevertheless, these plant by-products could be renewable sources of valuable secondary metabolites with the potential to be exploited as active ingredients in diverse drug and nutraceutical products for the management of oxidative stress-related diseases and complications, among other consumer goods.

Therefore, this work was caried out to valorize Holland (HBPP) and Italian (IBPP) bell pepper plants at the post-harvesting stage through the characterization of the phenolic composition of hydroethanolic extracts prepared from this plant biomass and the evaluation of a wide range of biological properties, namely *in vitro* antioxidant, antidiabetic, anti-obesity, anti-inflammatory, antimicrobial, and cytotoxic activities.

2. Material and methods

2.1. Plant material, chemicals, and biological material

Fresh Holland (HBPP) and Italian (IBPP) bell pepper (*Capsicum annuum* L.) plants at the end of the fruit production cycle were provided by farmers from Bragança, Portugal, in October 2019. The plant material, consisting of aerial parts (leaves and branches) without the main stem, was rinsed in abundant tap water, drained, freeze-dried, finely powdered using a household grinder, and finally vacuum-sealed for storage at −20 ºC until analysis. These plants produce distinctly shaped fruits. Holland bell peppers are medium to large in size, with a rounded, square shape with four lobes. Their skin is initially pale green, firm, and glossy, gradually becoming bright red when mature. In turn, Italian bell peppers are elongated and pointed fruits with thin pulp and skin, and a bright green color that changes to red when fully ripe.

The chemicals and biological material used in this study and their suppliers are listed in **Table A.1**.

2.2. Obtention of hydroethanolic extracts

Bell pepper plant samples (~1 g) underwent an extraction procedure detailed by Añibarro-Ortega et al. (2020). This extraction involved stirring the plant material in 80% ethanol for 1 h, filtering and collecting the upper phase, re-extracting the sediment, and again collecting the upper phase. Subsequently, ethanol was eliminated from the extracts and water underwent freeze-drying to yield dry extracts for subsequent analysis.

2.3. HPLC-DAD-ESI/MS analysis of phenolic compounds

The extracts, redissolved in 20% ethanol at 5 mg/mL, underwent filtration before being injected into the liquid chromatograph. The separation and detection of compounds were conducted using the equipment and conditions detailed by Añibarro-Ortega et al. (2020). Compound annotation relied on chromatographic data comparations with available standards; otherwise, data were cross-referenced against literature. Calibration curves (see supplementary **Table A.2**) were used for quantification of the identified compounds. Results were given as mg equivalents per g of extract.

2.4. Evaluation of bioactive properties

2.4.1. Antioxidant activity

The antioxidant activity was assessed *in vitro* through inhibition of the AAPH-induced oxidative hemolysis of sheep red cells (OxHLIA) and the Fe^{2+}-ascorbic acid-stimulated formation of reactive lipid peroxidation by-products such as malondialdehyde (MDA) in pig brain cells. The procedures outlined by Lockowandt et al. (2019) and Pinela et al. (2012) were follow. In the OxHLIA assay, IC_{50} values (μg/mL) were determined at time intervals of 60, 120 and 180 min using GraphPad Prism® 8. The MDA assay, focused on tracking the formation of pink-colored 1:2 malondialdehyde-thiobarbituric acid adducts in the reaction system, yielded results reported as EC_{50} values (μg/mL).

2.4.2. α-Glucosidase and advanced glycation end products (AGEs) formation inhibition capacities

The extracts capacity to inhibit yeast α-glucosidase and the formation of advanced glycation end products (AGEs) was evaluated following the procedures outlined by Millán-Laleona et al. (2023), using acarbose and aminoguanidine as positive controls, respectively. Results were given as IC_{50} (μg/mL).

2.4.3. Pancreatic lipase inhibition capacity

The lipase inhibition assay was employed as described by Millán-Laleona et al. (2023), using orlistat as the positive control. Results were given as IC_{50} (μg/mL).

2.4.4. Nitric oxide production inhibition capacity

The inhibitory effect of the extracts (0.125–8 mg/mL) on nitric oxide (NO) production by RAW 264.7 macrophage cells stimulated by lipopolysaccharides from *Escherichia coli* O111:B4 was evaluated as described by Sobral et al. (2016), using dexamethasone and samples lacking LPS as positive and negative controls, respectively. Results were given as EC_{50} (μg/mL).

2.4.5. Antiproliferative activity

The extracts (0.125–8 mg/mL) were studied for their ability to inhibit cell proliferation in human gastric (AGS), epithelial colorectal (Caco-2), breast (MCF-7), non-small cell lung (NCI-H460) cancer cell lines, along with a non-tumor porcine liver primary cell culture (PLP2). The sulforhodamine B assay was employed as detailed by Sobral et al. (2016), using ellipticine and cell suspension without extract as positive and negative controls, respectively. Results were given as GI_{50} (μg/mL).

Table 1
Phenolic compounds annotated and quantified in bell pepper plant extracts. The retention time (Rt), wavelengths of maximum absorption (λ_{max}) in the UV-Vis region, and pseudomolecular and MS^2 fragment ions (with relative abundance) are presented.

Chromatographic data					Annotation	Content (mg/g extract)	
Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)		HBPP	IBPP
1	6.25	321	353	191(100), 179(5), 135(<5)	*cis*-3-*O*-Caffeoylquinic acid	6.66 ± 0.09	9.5 ± 0.1
2	7.48	321	353	191(100), 179(5), 135(<5)	*trans*-3-*O*-Caffeoylquinic acid	1.77 ± 0.03	1.55 ± 0.03
3	8.85	321	353	191(100)	5-*O*-Caffeoylquinic acid	0.80 ± 0.02	1.05 ± 0.02
4	14.74	280	449	287(100)	Eriodictyol-*O*-hexoside	traces	traces
5	17.39	347	579	285(100), 447(85), 327(10), 561(10), 459(5)	Luteolin-*O*-hexosyl-*O*-pentoside	2.30 ± 0.03	3.15 ± 0.06
6	18.59	345	579	285(100), 447(85), 327(10), 459(5), 561(<5)	Luteolin-*O*-hexosyl-*O*-pentoside	1.39 ± 0.01	1.72 ± 0.05
7	19.55	346	579	285(100), 447(80), 327(12), 459(5)	Luteolin-*O*-hexosyl-*O*-pentoside	1.55 ± 0.02	1.84 ± 0.04
8	20.58	348	665	621(100), 489(15), 285(12)	Luteolin-*O*-malonyl-pentosyl-hexoside	2.43 ± 0.03	2.63 ± 0.05
9	21.84	348	665	621(100), 489(31), 285(64)	Luteolin-*O*-malonyl-pentosyl-hexoside	10.1 ± 0.1	10.9 ± 0.2
10	22.99	348	665	621(100), 489(26), 285(64)	Luteolin-*O*-malonyl-pentosyl-hexoside	2.36 ± 0.03	2.64 ± 0.09
11	24.13	348	665	621(100), 489(23), 285(67)	Luteolin-*O*-malonyl-pentosyl-hexoside	2.90 ± 0.04	3.67 ± 0.06
12	25.20	348	665	621(100), 489(19), 285(67)	Luteolin-*O*-malonyl-pentosyl-hexoside	2.03 ± 0.03	2.58 ± 0.07
13	25.86	349	649	605(100), 269(12)	Apigenin-*O*-malonyl-pentosyl-hexoside	2.45 ± 0.04	3.70 ± 0.09
14	28.05	349	649	605(100), 269(10)	Apigenin-*O*-malonyl-pentosyl-hexoside	2.49 ± 0.06	2.72 ± 0.06
15	28.89	349	649	605(100), 269(13)	Apigenin-*O*-malonyl-pentosyl-hexoside	2.04 ± 0.04	2.28 ± 0.05
					Σ Phenolic acids	9.2 ± 0.2	12.1 ± 0.2
					Σ Flavonoids	32.1 ± 0.4	37.8 ± 0.2
					Σ Phenolic compounds	41.3 ± 0.6	50.0 ± 0.4

Compounds 1–3, 5–12, and 13–15 were quantified using the chlorogenic acid, quercetin-3-*O*-glucoside, and apigenin-7-*O*-glucoside standards, respectively. The results are presented as mean ± SD. All quantified compounds had statistically significant differences ($p \leq 0.008$) between samples according to a Student's t-test.

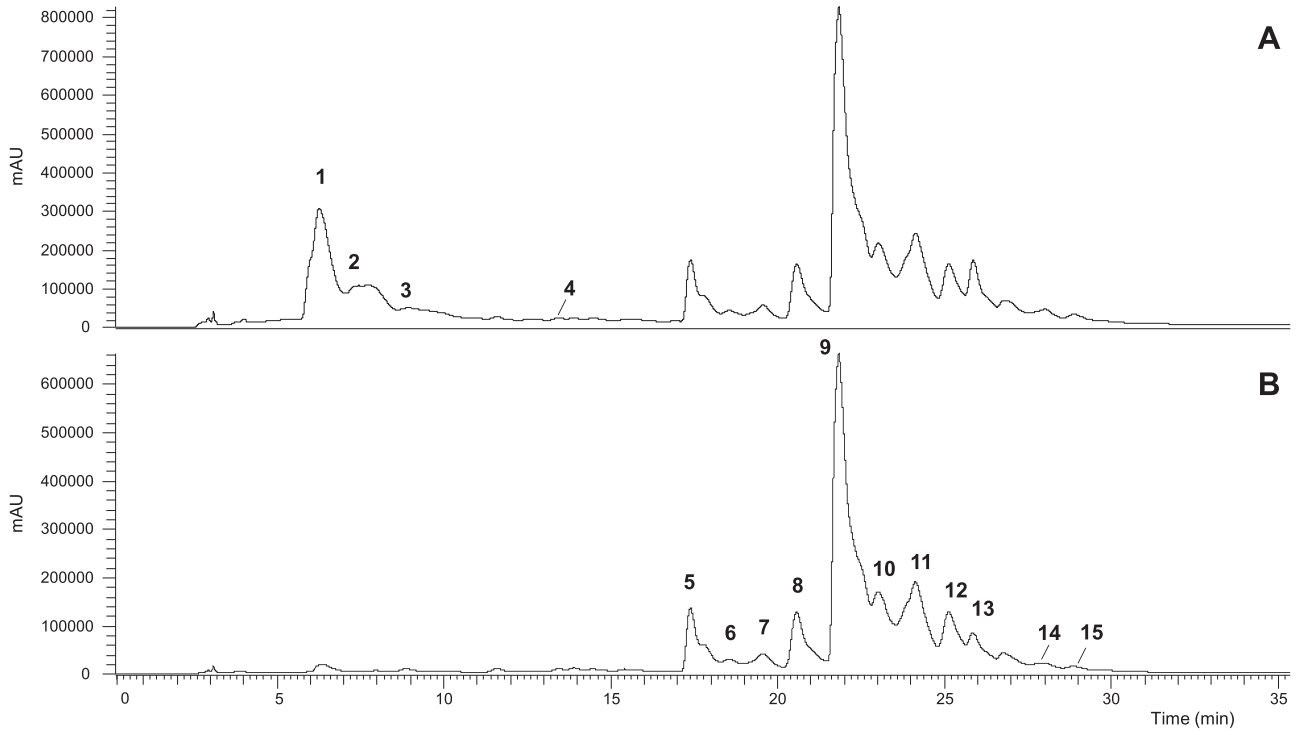

Fig. 1. HPLC-chromatographic profile of phenolic compounds in HBPP extract recorded at (A) 330 nm and (B) 370 nm. See Table 1 for compound annotation.

2.4.6. Antimicrobial activity

The extracts in 30% ethanol underwent testing against Gram-negative bacteria and Gram-positive bacteria, as well as micromycetes (see supplementary **Table S.1**). Minimum inhibitory and bactericidal or fungicidal concentrations (MIC, MBC, MFC, respectively, mg/mL) were determined as described by Soković et al. (2010) and Soković and van Griensven (2006). Positive controls comprised sodium benzoate (E211) and potassium metabisulfite (E224), while 30% ethanol served as the negative control.

2.5. Statistical analysis

The experiments were performed in triplicate and results were given as mean ± SD (excluding antimicrobial activity). The uncertain digit's decimal place in the mean was determined by rounding the SD to one significant figure. All statistical tests were caried out at a 5% significance level using SPSS Statistics software version 22.0. Two-sample comparisons employed a two-tailed paired Student's t-test, while *in vitro* assay results underwent one-way ANOVA with Post-Hoc Tukey's HSD test for multiple comparisons.

3. Results and discussion

3.1. Phenolic composition of bell pepper plant extracts

Table 1 provides chromatographic data utilized for the annotation of phenolic compounds in bell pepper plant (BPP) extracts. The table also highlights an identical phenolic profile in both HBPP and IBPP extracts, consisting of three phenolic acids and twelve flavonoids. The fingerprint chromatogram of the HBPP extract at 330 and 370 nm is shown in Fig. 1.

Compounds **1** and **2** (pseudomolecular ion $[M-H]^-$ at *m/z* 353, a main fragment ion at *m/z* 191, from quinic acid, and less abundant fragments at *m/z* 179 and *m/z* 135) were annotated as *cis*- and *trans*-3-*O*-caffeoylquinic acids, while compound **3** ($[M-H]^-$ at *m/z* 353, with main and only fragment ion at *m/z* 191) was annotated as 5-*O*-caffeoylquinic acid according to Clifford et al. (2006, 2003). Compound **4** had its deprotonated ion $[M-H]^-$ at *m/z* 449, with the main fragment ion at *m/z* 287 (eriodictyol aglycone) resulting from the loss of a sugar moiety, thus being annotated as eriodictyol-*O*-hexoside based on MS/DAD data. Compounds **5**, **6**, and **7** were annotated as luteolin-*O*-hexosyl-*O*-pentoside due to their pseudomolecular ion $[M-H]^-$ at *m/z* 579 and main fragment ions at *m/z* 285 (luteolin aglycone), *m/z* 447, and *m/z* 327 (two sugar moieties), and λ_{max} at 346 nm. Compounds **8**, **9**, **10**, **11**, and **12** had their pseudomolecular ion $[M-H]^-$ at *m/z* 665, with main fragment ions at *m/z* 621, *m/z* 489, and *m/z* 285, and λ_{max} at 347 nm. In addition to two sugar moieties, the loss of a malonyl group was detected (*m/z* 44 and *m/z* 42), leading the authors to annotate these compounds as luteolin-*O*-malonyl-pentosyl-hexoside. Compounds **13**, **14**, and **15** were annotated as apigenin-*O*-malonyl-hexosyl-pentoside due to their deprotonated ion $[M-H]^-$ at *m/z* 649, with main fragment ions at *m/z* 605, *m/z* 269 (apigenin aglycone), which show the loss of two sugar moieties and a malonyl group.

In quantitative terms, luteolin-*O*-malonyl-pentosyl-hexoside (19.83–22.41 mg/g extract) was identified as the most abundant phenolic constituent in both HBPP and IBPP extracts, followed by *cis*-3-*O*-caffeoylquinic acid (6.66–9.5 mg/g extract), apigenin-*O*-malonyl-pentosyl-hexoside (6.98–8.70 mg/g extract), and luteolin-*O*-hexosyl-*O*-pentoside (5.25–6.71 mg/g extract) (Table 1). These compounds were found in higher quantities in the IBPP extract, whose phenolic fraction was made up of 24.2% phenolic acids and 75.6% flavonoids. It is also noteworthy that *cis*-3-*O*-caffeoylquinic acid was the predominant isomer in both BPP extracts, which possibly resulted from the isomerization of the naturally occurring *trans* isomer upon strong sunlight exposure before harvesting the plant material (Clifford et al., 2017).

Apigenin and luteolin glycoside derivatives have been described in pepper leaves (Assefa et al., 2021; Herrera-Pool et al., 2021; Kim et al., 2014; León-Chan et al., 2017) and fruits (Kim et al., 2023), as well as 5-*O*-caffeoylquinic acid (Assefa et al., 2021; Kim et al., 2014). Kim et al. (2011) quantified higher luteolin concentrations in paprika (var. Special) leaves (14.34 μg/g dry weight) than in fruits (0.36–0.64 μg/g dry weight), in which capsaicin predominated (4.59–6.41 μg/g dry weight). According to León-Chan et al. (2017), a higher content of luteolin glycosides relative to apigenin glycosides in bell pepper leaves indicates a need to quench reactive oxygen species generated by abiotic stress conditions, because luteolin has greater antioxidant activity than apigenin. Comparted to tomato plant leaf extracts, another economically important Solanaceae species, both HBPP and IBPP extracts come out with about three times more phenolic acids and twice as more flavonoids (Añibarro-Ortega et al., 2020).

3.2. Bioactive properties of bell pepper plant extracts

3.2.1. Antioxidant activity

The antioxidant activity of HBPP and IBPP hydroethanolic extracts was evaluated using the OxHLIA and MDA bioassays. OxHLIA monitored the antioxidant behavior over time to assess both short-term and long-term antioxidative protection, as the extract effectiveness is influenced by reaction kinetics, as well as the rate at which antioxidant compounds engage with specific radicals, among other factors. It is noteworthy that certain antioxidants may exhibit a rapid reaction, depleting quickly in the system, whereas provide longer-lasting antioxidant protection (Antolovich et al., 2002). As shown in Table 2, while both extracts had the same efficacy in protecting red blood cells against lysis induced by the physiologically relevant AAPH-derived free radicals for a 60 min Δt ($IC_{50} \approx 17$ μg/mL), the IBPP extract displayed greater efficacy for the longer time intervals assessed than the HBPP extract. Interestingly, the antihemolytic activity of the BPP extracts was greater than that of Trolox, the synthetic α-tocopherol analog used as a positive control. In general, the IBPP extract was the one that stood out the most in this bioassay, but also for its ability to inhibit the formation of the highly reactive MDA, which result from the *in vitro* oxidation of the brain tissue used as lipid substrate. This result may be related to the higher content of phenolic compounds in this extract (Table 1).

Table 2
Antioxidant, enzyme inhibitory, anti-inflammatory, and cytotoxic activities of bell pepper plant extracts and positive controls.

		HBPP	IBPP	Positive control
Oxidative hemolysis inhibition				Trolox
IC_{50} values (μg/mL)	Δt 60 min	16.6 ± 0.5^{a}	17.3 ± 0.5^{a}	19.6 ± 0.7^{b}
	Δt 120 min	39 ± 1^{b}	33 ± 1^{a}	41 ± 1^{b}
	Δt 180 min	61 ± 2^{b}	48 ± 2^{a}	63 ± 1^{b}
MDA formation inhibition				Trolox
EC_{50} values (μg/mL)		30.7 ± 0.5^{c}	19.2 ± 0.3^{b}	5.4 ± 0.3^{a}
α-Glucosidase inhibition				Acarbose
IC_{50} values (μg/mL)		147 ± 55^{a}	233 ± 98^{a,b}	380 ± 19^{b}
AGEs formation inhibition				Aminoguanidine
IC_{50} values (μg/mL)		45 ± 3^{b}	1.3 ± 0.2^{a}	74 ± 16^{c}
Lipase inhibition				Orlistat
IC_{50} values (μg/mL)		403 ± 33^{b}	539 ± 112^{b}	40 ± 10^{a}
NO production inhibition				Dexamethasone
EC_{50} values (μg/mL)		272 ± 11^{b}	269 ± 10^{b}	16 ± 1^{a}
Cell growth inhibition				Ellipticine
GI_{50} values (μg/mL)	AGS	126 ± 7^{c}	61 ± 4^{b}	1.23 ± 0.03^{a}
	CaCo-2	18 ± 2^{b}	65 ± 1^{c}	1.21 ± 0.02^{a}
	MCF-7	133 ± 1^{b}	159 ± 13^{c}	1.02 ± 0.02^{a}
	NCI-H460	142 ± 2^{c}	22.6 ± 0.3^{b}	1.02 ± 0.01^{a}
	PLP2	310 ± 21^{b}	325 ± 7^{b}	2.3 ± 0.2^{a}

The results are presented as mean ± SD. In each line, different letters (a-c) indicate statistically significant differences ($p < 0.05$) between samples according to the one-way ANOVA.

Studies on the antioxidant activity of pepper leaves are scarce in the literature. Kim et al. (2014) reported potent DPPH and ABTS radical-scavenging activity for ethanolic extracts prepared from full-grown red pepper leaves. The authors also conducted an online HPLC–$ABTS^+$ screening assay by post-column mixing of separated analytes with radical solution to identify active compounds. *N*-caffeoylputrescine, a polyamine phenolic conjugate, and a delphinidin anthocyanin provided the best free radical-scavenging capacity and HepG2 cell protection effect against oxidative stress. By applying the same radical-scavenging methods, Kim et al. (2011) observed that the antioxidant activity of paprika (var. Special) leaf extract was greater than that of β-carotene (due to the combined effects of different phytochemicals, especially lutein), but lower than that of red paprika fruit extracts.

3.2.2. α-Glucosidase and AGEs formation inhibition capacities

α-Glucosidase inhibitors function as antidiabetic drugs by slowing down carbohydrate digestion and glucose absorption in the small

intestine due to their ability to diminish the hydrolytic cleavage of non-reducing ends of dietary oligosaccharides, thereby reducing the release of α-glucose (Hossain et al., 2020). While inhibitors such as acarbose, emiglitate, miglitol, and voglibose have been employed to manage postprandial hyperglycemia and prevent type 2 diabetes, recent attention has shifted towards discovering novel bioactive substances with potent inhibitory effects and minimal side effects (Hossain et al., 2020). As shown in Table 2, both BPP extracts were more effective in inhibiting α-glucosidase than acarbose, the most prescribed α-glucosidase inhibitor, and the HBPP extract was 2.6 times more active than this non-insulinotropic oral antidiabetic drug with an IC_{50} value of 147 μg/mL. The α-glucosidase inhibition percentage caused by the extracts is illustrated in **Fig. A.1**.

According to the investigation conducted by Park et al. (2016), the aqueous extract of pepper (cv. Dangjo) leaves and the isolated luteolin 7-*O*-glucoside display notable α-glucosidase inhibitory activity *in vitro*. Moreover, when orally administered to streptozocin-induced diabetic mice challenged with sucrose, both the extract and the isolated compound notably reduced blood glucose levels. α-Glucosidase inhibition was thus proposed as a mechanism behind the observed hypoglycemic effect, suggesting that pepper leaves could be a promising sustainable candidate for the management of type 2 diabetes. In turn, Assefa et al. (2021) reported caffeoyl-putrescine as a strong α-glucosidase inhibitory compared to acarbose and suggested that the antidiabetic effect of pepper leaves may be due to a possible synergetic action between caffeoyl putrescine and apigenin and luteolin aglycones against α-glucosidase. However, no pepper leaf compound other than luteolin 7-*O*-glucoside and caffeoyl-putrescine is known to be implied in the inhibition of this enzyme. In a different study, Park et al. (2020) identified quantitative trait loci controlling α-glucosidase inhibitory activity in pepper leaves and fruits, which varied according to plant age and organ. Furthermore, although at some stages the inhibitory activity in leaves was comparable to that in fruits, the range of activity in leaves was broader than that in fruits. This information could contribute to the development of pepper varieties with greater potential to inhibit this digestive enzyme.

Diabetic complications can also be prevented by inhibiting the formation of AGEs, which are actively produced and accumulated during chronic hyperglycemia through non-enzymatic reactions between amino groups of proteins and carbonyl of reducing sugars. These heterogeneous metabolites are potent inducers of oxidative stress and inflammation and play a key role in the progression of vascular problems (Vijaykrishnaraj and Wang, 2021). Therefore, the discovery of anti-glycation agents with reduced toxicity is necessary to prevent and control diabetic complications. As shown in Table 2, the HBPP and IBPP extracts were respectively 1.6 and 56.9 times more effective in inhibiting AGEs formation than aminoguanidine, a scavenger of reactive carbonyl groups used as positive control. The AGEs formation inhibition obtained with different concentrations of the two extracts is shown in **Fig. A.1** in Supplementary Material. The greater ability of the IBPP extract to inhibit the formation of AGEs compared to HBPP may be attributed to its significantly higher content of luteolin and apigenin derivatives (Table 1), which are compounds described as potent inhibitors of AGE formation (Qin et al., 2021). To the best of the authors' knowledge, this is the first study describing the AGEs formation inhibition capacity of bell pepper plant extracts. For bell pepper fruits, Shukla et al. (2016) found that green and red pepper hydromethanolic extracts inhibit vesperlysine-type AGEs more effectively than those of yellow pepper (which displayed greater radical scavenging and α-glucosidase inhibition activities), while pentosidine-type AGEs were similarly inhibited by these bell pepper extracts. However, the bioactive compounds behind these effects were not identified. Overall, BPP extracts characterized in this work appeared as promising natural α-glucosidase and AGEs inhibitors and could be used for formulating functional food ingredients or bio-based medications for type 2 diabetes.

3.2.3. Pancreatic lipase inhibition capacity

Pancreatic lipase is the key enzyme for the digestion and absorption of dietary lipids in the gastrointestinal tract and its inhibition emerges as an effective approach to prevent the progression of obesity (Liu et al., 2020). This epidemic health problem is also a risk factor for many other conditions, such as type 2 diabetes, hypertension, and cardiovascular diseases. In this work, the lipase inhibition capacity of the BPP extracts was studied and the results are shown in Table 2 and **Fig. A.1**. The inhibitory effect achieved with the extracts was not as high as that of the positive control orlistat. Orlistat is presently the only lipase inhibitor diet medication approved for the treatment of obesity through fat absorption inhibition (Liu et al., 2020). However, the obtained IC_{50} values of 403–539 μg/mL highlight the studied plant extracts as potential sources of natural anti-obesity agents that deserve future attention to increase the range of available medications.

Studies exploring the potential of pepper extracts to inhibit pancreatic lipase are scarce. Marrelli et al. (2016) reported that hot pepper flower extract significantly inhibits pancreatic lipase compared to orlistat. In turn, Shukla et al. (2016) obtained greater pancreatic lipase inhibition with yellow and red pepper extracts than with green pepper extract, as well as higher lipid peroxidation inhibition, free radicals scavenging activity, and total polyphenol content. In a different work with aqueous red pepper leaf extract, Oh et al. (2023) observed that it inhibits fat accumulation in high-fat diet-fed mice via adipogenesis and lipogenesis suppression, suggesting its potential in preventing obesity. Based on another *in vivo* study, Kim et al. (2020) suggested that red pepper seed extract enhances glycemic control by inhibiting hepatic gluconeogenesis in obese diabetic mice. On the other hand, it is also known that capsaicin, the major active compound from chili peppers, is involved in weight loss in obese individuals (Zheng et al., 2017).

3.2.4. Nitric oxide production inhibition capacity

Nitric oxide (NO) is a signaling molecule that plays a pivotal role in the pathogenesis of inflammation. According to the data presented in Table 2, the BPP extracts had capacity to inhibit the NO production by LPS-stimulated murine macrophages, exhibiting EC_{50} values of 269–273 μg/mL. Even though these values are higher than those obtained with the corticosteroid dexamethasone, these concentrations are relatively low for natural crude extracts. According to Cho et al. (2020), hydroethanolic extracts from immature pepper parts ameliorate the LPS-stimulated inflammatory response by decreasing the NO production and levels of interleukin-6 and TNF-α in RAW 264.7 cells, with leaf extracts showing greater activity and content of luteolin glycosides than fruit extracts. The mechanism underlying the anti-inflammatory effect of bell pepper leaf aqueous extract on concanavalin A-stimulated mouse spleen cells was previously explored by Hazekawa et al. (2017). They observed inhibition in inflammatory cytokine production and cell proliferation without inducing cytotoxicity, along with suppression of the expression of inflammatory proteins. This immunosuppressive effect occurred via T-cell activation inhibition through the NF-κB pathway. Marrelli et al. (2016) also described hot pepper flower hydroethanolic extract as anti-inflammatory, reporting an EC_{50} value of 264 μg/mL for the inhibition of NO production by LPS-stimulated RAW 264.7 cells without inducing cytotoxic effect. This result is comparable to that obtained in this study for the BPP extracts.

3.2.5. Antiproliferative activity

The antiproliferative activity of the extracts prepared from bell pepper plants at the end of the fruit production cycle was assessed on human tumoral cell lines and the results are expressed as GI_{50} values in Table 2. In all cases, cell growth inhibition occurred with GI_{50} values below 160 μg/mL. Both extracts showed notable cytotoxicity particularly on Caco-2 cells ($GI_{50} \leq 65$ μg/mL), while the IBPP extract displayed remarkable efficacy on NCI-H460 and AGS cells, the most common types of stomach and lung cancers, with GI_{50} values of 22.6 and 61 μg/mL, respectively. These findings suggest that there is a need to promote

Table 3
Antibacterial activity of bell pepper plant extracts and positive controls.

Bacterial strains	HBPP		IBPP		E211		E224	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	3.07	6.14	1.52	3.04	4.00	4.00	1.00	1.00
Bacillus cereus	1.54	3.07	1.52	3.04	0.50	0.50	2.00	4.00
Listeria monocytogenes	1.54	3.07	1.52	3.04	1.00	2.00	0.50	1.00
Salmonella enterica subsp. *enterica* serovar Typhimurium	3.07	6.14	1.52	3.04	1.00	2.00	1.0	1.00
Escherichia coli	1.54	3.07	1.52	3.04	1.00	2.00	0.50	1.00
Enterobacter cloacae	0.77	1.54	0.76	1.52	2.00	4.00	0.50	0.50

Table 4
Antifungal activity of bell pepper plant extracts and positive controls.

Fungal strains	HBPP		IBPP		E211		E224	
	MIC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MFC (mg/mL)	MIC (mg/mL)	MFC (mg/mL)
Aspergillus fumigatus	1.54	3.07	1.52	3.04	1.00	2.00	1.00	1.00
Aspergillus versicolor	0.77	1.54	0.38	0.76	2.00	4.00	1.00	1.00
Aspergillus niger	0.77	1.54	0.76	1.52	1.00	2.00	1.00	1.00
Penicillium funiculosum	0.77	1.54	0.76	1.52	1.00	2.00	0.50	0.50
Penicillium verrucosum var. *cyclopium*	0.77	1.54	0.76	1.52	2.00	4.00	1.00	1.00
Trichoderma viride	0.77	1.54	0.76	1.52	1.00	2.00	0.50	0.50

further studies to elucidate the mechanisms underlying the observed antiproliferative effects.

Pepper fruit extracts have been explored for their antitumor potential and reported to be able to inhibit cell growth and induce apoptosis through caspase-3 expression on T47D breast cancer cells (Kurnijasanti and Fadholly, 2021) and to have a cytotoxic effect on PC-3 prostate tumor cells (Chilczuk et al., 2021). In a different experiment, habanero pepper (*C. chinense* J.) leaf and stem extracts were reported to potentiate the cytotoxic effect of vinblastine against parental breast cancer cells (MCF–7/Sens) (Chel-Guerrero et al., 2022), thus highlighting the antitumor potential of *C. chinense* by-products.

The toxicity of both HBPP and IBPP extracts was also examined on porcine liver primary culture (Table 2). Interestingly, the GI_{50} values obtained for the PLP2 cell line ($\geq$ 310 μg/mL) were higher compared to those obtained for the tumor cell lines. Therefore, it was possible to achieve an antiproliferative effect on the tumor cell lines without reaching the inhibitory concentration that affects these nontumor cells.

3.2.6. Antimicrobial activity

The BPP extracts were tested against food-borne microorganisms and the results are presented in Table 3. The extracts displayed varying degrees of antibacterial activity against all tested bacterial strains. *Enterobacter cloacae* seemed to be the most sensitive bacteria to the tested extracts, with MIC and MBC values 2.6 times lower than those of sodium benzoate (E211). Higher concentrations of this food preservative were also required against *S. aureus*. Furthermore, the extracts demonstrated superior inhibitory and bactericidal effects against *B. cereus* compared to potassium metabisulfite (E224), a food additive with antimicrobial and antioxidant properties. Curiously, the IBPP extract was found to be twice as effective as the HBPP extract against *S. aureus* and *S.* Typhimurium.

Antibacterial properties were previously attributed to bell pepper leaf and fruit ethanolic extracts against *S. aureus* and *E. coli* (Gahongayire et al., 2018) and to chili pepper (*C. annuum*) extracts against *L. monocytogenes*, *B. cereus*, *S. aureus*, and *S.* Typhimurium (Dorantes et al., 2000). The effect of an aqueous peptide-enriched fraction isolated from bell pepper leaves on the growth inhibition of *Ralstonia solanacearum*, a soil bacterium with devastating effects on Solanaceae crops, and *Clavibacter michiganensis* ssp. *michiganensis*, the bacterium responsible for bacterial canker or bacterial leaf spot disease of tomatoes, was also described (Games et al., 2013). The same authors isolated a Hevein-like antimicrobial peptide from bell pepper leaves and attributed the antimicrobial effects to its capacity to bind to chitin and reduce or stop the bacterial cell wall elongation (Games et al., 2016).

In general, the BPP extracts demonstrated remarkable antifungal activity compared to the tested positive controls (Table 4). The extract concentrations required to inhibit and kill *A. versicolor*, *P. verrucosum* var. *cyclopium*, *A. niger*, *P. funiculosum*, and *T. viride* were lower than those of E211. Moreover, the extract exhibited better inhibitory effects against *A. versicolor*, *A. niger*, and *P. verrucosum* var. *cyclopium* compared to E224. Furthermore, the IBPP extract was twice as effective as the HPPP extract against *A. versicolor*, an important food spoilage and sterigmatocystin producing species. In a different study, antimicrobial activity was obtained with *n*-butanol and ethyl acetate extracted fractions from pepper leaves and fruits, and the ethyl acetate fraction was particularly effective against *Candida albicans* (Bakht et al., 2020).

4. Conclusions

This study allowed identifying *O*-glycosylated luteolin derivatives as the most abundant phenolic compounds in Holland and Italian bell pepper plant hydroethanolic extracts, followed by *cis*-3-*O*-caffeoylquinic acid and *O*-glycosylated apigenin. The qualitative phenolic profile of the two BPP was similar, with a predominance of flavonoids over phenolic acids. Compared to the positive controls, both hydroethanolic extracts displayed remarkable antioxidant activity *via* lipid peroxidation and oxidative hemolysis inhibition and the ability to inhibit the enzyme α-glucosidase and the formation of AGEs. The lipase enzyme involved in fat metabolism and the pro-inflammatory mediator NO were also inhibited in some extent. Notable cytotoxic effects occurred on the tested human tumor cell lines, mainly on Caco-2. Furthermore, the extracts were more active against some foodborne microorganisms than the food preservatives E211 and E224. Overall, these results highlight BPP by-products as a renewable feedstock that could be used to produce functional ingredients for food, nutraceutical, and pharmaceutical formulations to manage type 2 diabetes mellitus, obesity, and other oxidative stress-mediated conditions. Further studies will be relevant to identify other active constituents, namely steroidal compounds, and the mechanisms of action underlying the bioactivities, as well as *in vivo* experiments.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.118546.

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