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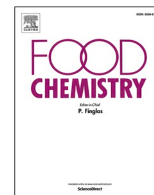

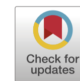

Insights into the phenolic composition and *in vitro* bioactivity of cardoon capitulum: A nutraceutical-oriented valorization study

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ABSTRACT

The capitulum constituents (stigma, corolla, bracts, pappus, and receptacle) of seven cardoon cultivars were studied for their polyphenolic composition and bioactive properties. Fifteen phenolic compounds were identified, secoiridoid, secoxyloganin, and apigenin-*O*-hexuronoside in higher concentrations. The bracts had the highest concentration of phenolic compounds, and the corolla had the greatest variety. The corolla of F4-1-4 and F1-34-1 cultivars, and bracts of F1-1-1, F1-19-4, and F4-37-1 cultivars revealed the greatest capacity to inhibit the thiobarbituric acid reactive substances formation (IC_{50} of 38 and 40.4 μg/mL, respectively). The corolla of F1-34-1 cultivar showed higher antihaemolytic activity than the positive control Trolox (IC_{50} 5.5 vs. 20 μg/mL). Bracts of the F4-25-2 cultivar showed higher cytotoxic activity. No hepatotoxicity or anti-inflammatory effects were presented by the studied samples. These bioactivities exhibit a significative correlation with phenolic compounds, in particular flavonoids. Antimicrobial capacity was also observed, with all samples presenting higher antifungal potential than positive controls.

1. Introduction

Cynara cardunculus L. is a plant belonging to the Asteraceae family, native to the Mediterranean basin, and commonly called cardoon. It is a species widely consumed in the Mediterranean diet due to its rich nutritional value and associated medicinal properties (e.g., antidiabetic, choleretic, cardiotonic, liver diseases treatment). This species is also used as a coagulant in the production of some protected origin (PDO) cheeses. Recently it has been proposed as a functional food due to its complex chemical composition (Conceição et al., 2018; Zayed et al., 2020). This species comprises three varieties: *var. scolymus* (L.) Fiori or artichoke, *var. altilis* DC or domesticated cardoon, and *var. sylvestris* (Lamk) Fiori or wild cardoon.

Cardoon is widely known for its varied and rich chemical composition. Nutritionally it is rich in fiber, vitamins, inulin, and minerals. It also has a wide variety of phenolic compounds, namely phenolic acids and flavonoids. Its bioactive potential has also been explored. Properties such as antioxidant, anti-diabetic, anti-proliferative, antimicrobial, anti-HIV, and anti-inflammatory activities have been demonstrated through different methodologies (Barracosa et al., 2019; Brás et al., 2020; Kuczmannová et al., 2016; Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022; Petropoulos, Fernandes, et al., 2019).

Due to the diversified industrial potential demonstrated, this species has been a target of great attention in the past decade. It is used in the production of paper pulp, biodiesel, food oil, lightwood panels, or animal feed, among others. Its rich composition in phenolic compounds has contributed to the exploitation of this species in the cosmetic industry (Ferraz et al., 2022; Marques et al., 2017; Neri et al., 2017; Yahya et al., 2018; Zayed et al., 2020). This potential is associated with the low costs of cultivation and high total income. Its noteworthy ability to withstand

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adverse environmental conditions, namely water stress, high levels of salinity, stony soils with variable pH, as well as the great temperature range, are characteristics that should favor the exploitation of this species in the future (Gominho et al., 2018; Pappalardo et al., 2020). Despite the wide variety of industrial applications of cardoon, about 60 to 85% of the plant tissues of this species are not properly used, contributing to the thousands of tons of waste generated worldwide. Producing countries such as Portugal, Spain, and Italy estimates that approximately 10 to 30 t/ha (62% corresponding to stems and leaves and 38% to heads) of dry biomass are generated depending on irrigation and fertilization conditions (Barracosa et al., 2019; Espada et al., 2021; Gominho et al., 2014; Neri et al., 2017; Raccuia & Melilli, 2007). As a result of the environmental impact associated with waste plant material, several efforts have been made to stimulate the circular economy and take advantage of the full potential of the species. The understanding of how factors such as the genetic background, state of maturation, plant tissue, etc., influence the species is crucial for their complete characterization and proper exploitation. These efforts are in line with the current worldwide concerns with a sustainable economy (Ferioli & D'Antuono, 2021; Francaviglia et al., 2016; Gominho et al., 2018; Pappalardo et al., 2020). This is essential for developing new applications and increasing the economic value of the species and their producing countries.

The present study aims to evaluate the phenolic composition and bioactive properties of different capitulum constituents (stigma, corolla, bracts, pappus, and receptacle) of seven selected cultivars of *Cynara cardunculus* L. The main objective is to determine the influence that cultivars' information can play on these characteristics and, in this way, select those that demonstrate the most promising potential. In this context, the increase in scientific knowledge and possible commercial interest associated with cardoon is of extreme importance for the proper utilization and economic valorization of this natural resource.

2. Material and methods

2.1. Plant material

Several inflorescences from seven cultivars of *Cynara cardunculus* L. var *altilis* were collected, individually, during July 2018 at the complete flowering stage in the region of Serra da Estrela (40°40′34″N; 7°42′25″W) (Penalva do Castelo, Portugal). The samples were identified by the initial collector, Professor Paulo Barracosa. A voucher for each cultivar was also prepared in the Herbarium of the School of Agriculture of Viseu. Each capitulum was separated into the different vegetable tissues: stigma (S), corolla (C), bracts (B), pappus (P), and receptacle (R) (Fig. 1). The samples were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA), grounded using a domestic blender to obtain a fine powder (~20 mesh), and stored under protection from the light until further analysis.

2.2. Extraction procedure

Each sample (approximately 1.5 g of fine powder) was extracted with a hydroethanolic solution (EtOH/H_2O, 80:20, *v/v*; 30 mL) for 1 h under vigorous stirring (150 rpm) and at room temperature. The supernatant was filtered through the Whatman paper No. 4 and the remaining residue was re-extracted under the conditions described above. The ethanolic phase was evaporated under reduced pressure (rotary evaporator

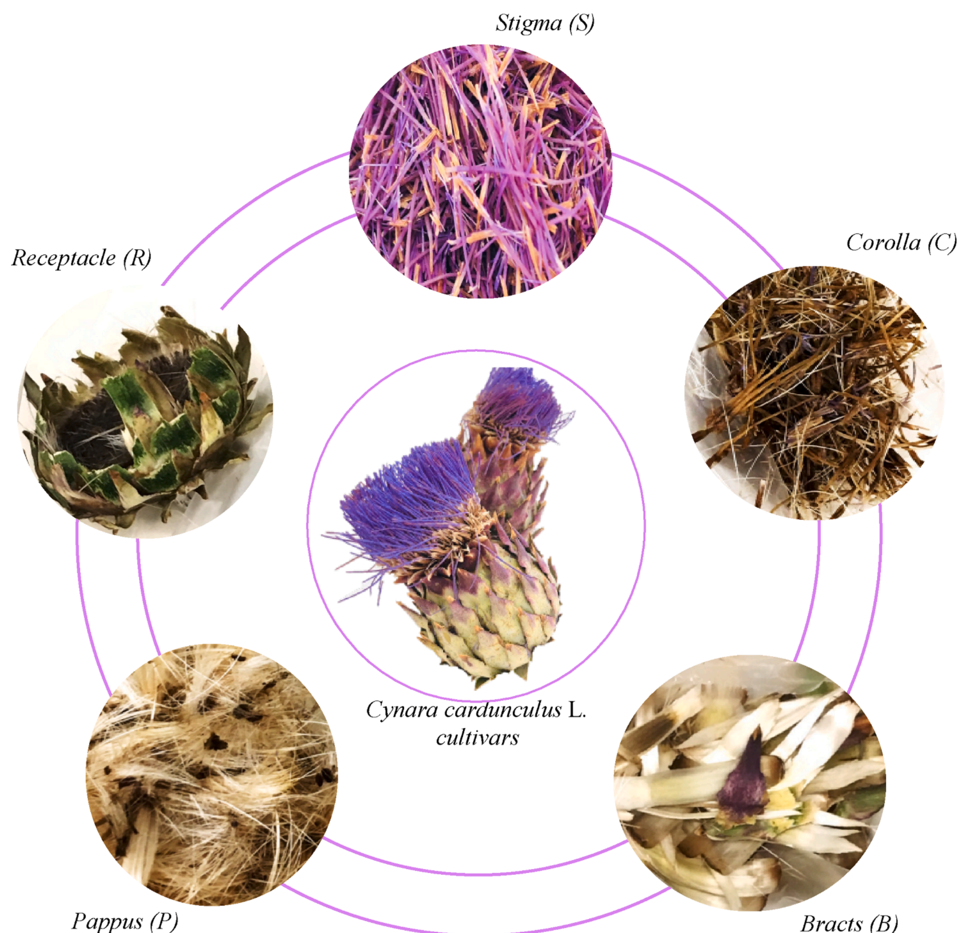

Fig. 1. Different plant tissues of the cardoon cultivars studied: stigma (S), corolla (C), bracts (B), pappus (P), and receptacle (R).

Büchi R-210, Flawil, Switzerland), and the remaining extract was frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) to perform the remaining analysis.

2.3. Phenolic composition identification and quantification

The obtained extracts (Section 2.2) were re-dissolved in EtOH/H_2O (20:80, *v/v*) at a final concentration of 10 mg/mL and filtered through a 0.22 μm nylon syringe filter. The phenolic compounds profile was analyzed by high-performance liquid chromatography coupled to a diode array detector and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS), a quaternary pump, an auto-sampler maintained at 5 °C, an automated thermostatted column slot, and a degasser, following the chromatographic conditions previously described by Mandim et al., 2021a. Briefly, the chromatographic separation was achieved using a Waters Spherisorb S3 ODS-2C18 column (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) maintained at 35 °C. The elution system was constituted by 0.1% formic acid in water (A) and acetonitrile (B), and the elution gradient was isocratic: 15% B (5 min), 15% B to 20% B (5 min), 20–25% B (10 min), 25–35% B (10 min), 35–50% B (10 min), and the columns was re-equilibrated through a flow rate of 0–5 mL/min. The detection was carried out using the DAD with 280 and 370 nm as selected wavelengths and in a mass spectrometer (MS) associated with the HPLC system via a DAD cell outlet. A Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source was used for MS detection in negative mode. The system was run using 50 psi of nitrogen as the sheath gas, a spray voltage of 5 kV, a source temperature of 325 °C, and a capillary voltage of 20 °V. The voltage for the tube lens offset was maintained at 66 V. The mass range of the entire scan was from *m/z* 100 to 1500. 35 arbitrary units was the collision energy employed. Data were acquired using the Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The tentative identification of the phenolic compounds was based on the comparison of the retention times and UV–Vis and mass spectra of the peaks with available commercial standards and the existing literature information. The quantification of the individual compounds was based on the peaks area obtained in the UV–Vis recorded at 280 or 370 nm, compared with calibration curves prepared for the similar commercial standards available (Extrasynthèse, Genay, France): compound number **1** was calibrated with chlorogenic acid (y = 168823x + 161172, r^2 = 0.9999; limit of detection (LOD) = 0.20 μg/mL; limit of quantification (LOQ) = 0.68 μg/mL); compounds number **2** and **3** with *p*-coumaric acid (y = 301950x + 6966.7, r^2 = 0.9999; LOD = 0.68 μg/mL; LOQ = 1.61 μg/mL); compound number **4** with oleuropein (y = 32226x + 12416, r^2 = 0.9997; LOD = 0.69 μg/mL; LOQ = 1.96 μg/mL); compounds number **5**, **7**, **10**, and **14** with quercetin-3-*O*-glucoside (y = 34843x − 160173, r^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL); compounds number **6** and **8** with naringenin (y = 18433x + 78903; r^2 = 0.9998; LOD = 0.17 μg/mL; LOQ = 0.81 μg/mL), and compounds number **9**, **11**, **12**, **13**, and **15** with apigenin 7-*O*-glucoside (y = 10683x − 45794; r^2 = 0.999; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL) (more information regarding the calibration curves is described in the Table S1 of the Supplementary Materials). In the cases where no standard of the same class was available, the quantification was performed using the calibration curve of the most similar phenolic compound. The results were expressed in mg per g of extract.

2.4. Bioactivities evaluation

Antioxidant, cytotoxic, anti-inflammatory, and antimicrobial activities were evaluated using the hydroethanolic extracts of the different cardoon capitulum constituents and cultivars. The analyzed extracts were obtained according to the procedure described in Section 2.2.

2.4.1. Antioxidant activity

The antioxidant potential of the extracts was evaluated through two different cell-based methodologies, i.e., the capacity to inhibit oxidative hemolysis (OxHLIA) and the formation of thiobarbituric acid reactive substances (TBARS). The synthetic antioxidant Trolox (Fisher Scientific, Lisbon, Portugal) was used as a positive control for both assays.

The OxHLIA assay evaluated the capacity to counteract the oxidative hemolysis in erythrocytes from sheep blood, according to the procedure described by (Lockowandt et al., 2019). The results were presented as the extracts' capacity to protect 50% of the erythrocyte population integrity (IC_{50} values μg/mL) after Δt 60 and 120 min of incubation. Phosphate-buffered saline (PBS; pH 7.4) was used as a negative control.

For the TBARS assay, the extracts were re-dissolved in water to obtain a solution of 5 mg/mL, which was further diluted to obtain the concentrations to be tested. The assay was performed as previously described (Lockowandt et al., 2019). The results were expressed as the concentration of the extract able to inhibit 50% of the lipid peroxidation process (IC_{50} values μg/mL). The results were considered extremely promising when the IC_{50} value was less than 50 μg/mL, high when it was between 50 and 100 μg/mL, average when it was between 100 and 150 μg/mL, and low when it was higher than 150 μg/mL.

2.4.2. Cytotoxic and hepatotoxic activity

The cytotoxic and hepatotoxic activities of the extracts were evaluated through the sulforhodamine B colorimetric assay, following the procedure previously described by Kessler et al. (2022). The extracts were tested against four tumor cell lines: cervical adenocarcinoma (HeLa), hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7), and non-small cell lung carcinoma (NCI-H460) (acquired from Leibniz-Institut DSMZ – German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany). The hepatotoxicity was evaluated using a non-tumor porcine liver primary culture (PLP2). The cytotoxic potential of extracts was classified according to the National Cancer Institute of the United States as highly cytotoxic when GI_{50} values are equal to or lower than 20 μg/mL, moderate activity with GI_{50} values between 21 and 200 μg/mL, weakly with values between 201 and 400 μg/mL, and with no activity with values higher than 400 μg/mL. Ellipticine (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control and the cells suspension without sample as the negative control. The results were expressed as extracts concentration able to inhibit 50% of cell proliferation (GI_{50}, μg/mL).

2.4.3. Anti-inflammatory activity

The anti-inflammatory capacity of the extracts was evaluated through the measurement of their capacity to inhibit the nitric oxide (NO) production induced by the lipopolysaccharide (LPS), in a murine macrophage cell line (RAW 264.7) and according to the procedure previously described (Mandim et al., 2019). The obtained extracts were re-dissolved in water at a final concentration of 8 mg/mL and the range of the concentration to be tested (6.25–400 μg/mL) was incubated with the murine macrophage cells. After 1 h the LPS (10 μL, 30 μg/mL in culture medium) was added, and 24 h afterward 100 μL of the supernatant was transferred to a new plate, the Griess reagent was added (100 μL) and the optical density (690 nm) was measured. The commercial anti-inflammatory dexamethasone (50 mM, Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control and the cells without LPS as a negative control. The results were expressed as the concentration of the extract that inhibited the production of the pro-inflammatory mediator (NO) in 50% (IC_{50} values, μg/mL).

2.4.4. Antimicrobial activity

The assay was carried out according to the procedure previously described by Añibarro-Ortega et al. (2020). The tested extracts were re-dissolved in 5% of dimethyl sulfoxide (DMSO) and diluted to obtain the range of the concentrations to be tested (between 8 and 0.0625 mg/mL). Six bacterial strains were used for the evaluation of the antibacterial potential, were three Gram-positive (*Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), and *Listeria monocytogenes* (NCTC

7973)) and three Gram-negative (*Escherichia coli* (ATCC 25922), *Salmonella enterica* serovar Typhimurium (ATCC 13311), and *Enterobacter cloacae* (ATCC 35030)). For the antifungal activity, the micromycetes *Aspergillus fumigatus* (ATCC), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC11730), *Penicillium funiculosum* (ATCC 36839), *Trichoderma viride* (IAM 5061), and *Penicillium aurantiogriseum* (*Penicillium verrucosum* var. *cyclopium*) (food isolate) were employed. All the bacteria and fungi species tested were acquired from the Mycological Laboratory of the Department of Plant Physiology at the Institute for Biological Research "Sinisa Stanković", University of Belgrade, Serbia. The positive controls used were sodium benzoate (E211) and potassium metabisulfite (E224). The results were expressed as the minimum inhibitory (MIC), bactericidal (MBC), or fungicidal (MFC) concentrations (mg/mL).

2.5. Statistical analysis

All the assays were carried out in triplicate. The results were expressed as the mean values ± standard deviation (except for the antimicrobial activity), both calculated using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The differences between samples were analyzed through a two-tailed paired Student's *t*-test at a 5% significance level, using the SPSS Statistics software (IBM SPSS Statistics for Mac OS, Version 26.0; IBM Corp., Armonk, NY, USA). Samples with significant differences were considered when the *p*-value was lower than 0.05. Moreover, Pearson's correlation analysis between the bioactivities and individual phenolic compounds and the sums of the different classes (total phenolic compounds, total phenolic acids, total flavonoids, and total iridoid glycoside) was performed with a 95% confidence level.

3. Results and discussion

3.1. Composition in phenolic compounds

The results regarding the phenolic composition of the studied cardoon cultivars, peak characteristics, and tentative identification are presented in Table 1, and the quantification of each compound is shown in Tables 2 and 3. HPLC chromatograms with the phenolic profiles of each plant tissue studied recorded at 280 and 370 nm are presented in the Supplementary Materials (Table S1). The tentative identification of the compounds was based on their retention times (Rt), the wavelength of maximum absorption in the UV–vis spectra (λmax), deprotonated ion ([M-H]$^-$), and fragmentation pattern (MS^2). In total fifteen compounds were identified, of which one secoiridoid monoterpene (peak **4**), eleven flavonoid glycosides (peaks **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, and **15**), and three phenolic acids (peaks **1**, **2**, and **3**).

Compound **1**, 5-*O*-caffeoylquinic acid was positively identified by comparison with the commercial standard; it was previously reported in cardoon tissues, namely in the bracts (Mandim et al., 2021b), seeds (Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022), and heads (Mandim, Petropoulos, et al., 2020). Similarly, peak **9** (apigenin-7-*O*-rutinoside) was also previously identified by comparison with the commercial standard; and found in the inflorescences, stigma, and corolla of cardoon (Mandim, Petropoulos, et al., 2020).

Peaks **2** (3-*p*-coumarouylquinic acid) and **7** (luteolin-*O*-hexuronuside) were previously identified in inflorescences, stigma, and corolla from distinct cardoon genotypes (Dias et al., 2018) and petioles (Mandim et al., 2021a). The tentative identification of peak **2** was based on the hierarchical keys developed by Clifford et al. (2007, 2008) for the identification of chlorogenic acid derivatives; and for peak **7**, that presented a deprotonated ion [M-H]$^-$ at *m/z* 461 and a unique MS^2 fragment at *m/z* 285 (a luteolin derivative indicated by the UV spectra) that corresponded to the loss of a hexuronoside unit (176 u).

The *p*-coumaroyl acid hexoside (peak **3**) and the apigenin-*O*-malonyl-hexoside (peak **15**) were reported in cardoon heads and bracts (Mandim, Petropoulos, et al., 2020; Mandim et al., 2021b), while for peak **14** (luteolin-*O*-malonyl-hexoside) was previously described in several vegetable tissues of cardoon, namely bracts, blades, and petioles (Mandim et al., 2021a,b; Mandim, Mandim, Petropoulos, Pinela, Dias, Kostic, et al., 2022).

The chromatographic confirmation of these peaks was confirmed based on their UV and mass spectra. Peak **3**, presented MS^2 (*m/z* 163 and 119) and a characteristic UV spectrum of chlorogenic acids derivatives, that clearly indicate the presence of a *p*-coumaric acid derivative. The difference between the presented deprotonated ion at *m/z* 325 and the 163 MS^2 fragment, resulted in the loss of the hexose unit, being tentatively identified as *p*-coumaroyl acid hexoside. As for peaks **15** ([M-H]$^-$ at *m/z* 517) and **14** ([M-H]$^-$ at *m/z* 533), presented the same loss pattern in MS^2, namely [M-H-44-204(42 + 162)]$^-$, with the fragments at *m/z* 489/285 and 473/269, respectively, that corresponded to the loss of a the malonyl unit (44 + 42) flowed by the hexosyl unit (162).

The secoiridoid secoxyloganin (peak **4**) was tentatively identified according to the chromatographic and spectral data described by Jiménez-López et al. (2017); while for luteolin-*O*-deoxyhexosyl-hexoside (peak **5**) was based on the described by Petropoulos, Fernandes, Tzortzakis, et al. (2019). To the best of our knowledge, these compounds have not been previously reported in cardoon plant tissues.

Peak **6** with a pseudomolecular ion [M-H]$^-$ at *m/z* 579 ([M-H-308]$^-$, corresponding to the loss of a deoxyhexosyl-hexoside moiety, was tentatively identified as naringenin-*O*-deoxyhexosyl-hexoside, and peak **8**, with a pseudomolecular ion [M – H]$^-$ at *m/z* 433 ([M-H-162]$^-$, loss of a hexoside), as naringenin-*O*-hexoside. Peak **10** ([M-H]$^-$ at *m/z* 577) produced a fragment at *m/z* 315 (-86 u + -176 u, associated with

Table 1
Phenolic compounds tentatively identified in the hydroethanolic extracts of *C. cardunculus* L. cultivars' different parts.

Peak	Rt (min)	λ_{max} (nm)	[M-H]$^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	S	C	B	P	R
1	6.7	326	353,22	191,03(100);179,34(10);161,13(5);135,02(5)	5-*O*-Caffeoylquinic acid		✓	✓		✓
2	10.88	306	337,26	191,06(100);173,99(5);163,00(12)	3-*p*-Coumarouylquinic acid	✓				
3	11.40	323	325,24	163,01(100);119,07(31)	*p*-Coumaroyl acid hexoside				✓	
4	14.45	307	403,28	371,54(5);223,13(100);179,08(32);121,34(16)	Secoxyloganin		✓			✓
5	17.44	342	593,37	285,15(100)	Luteolin-*O*-deoxyhexosyl-hexoside		✓		✓	
6	17.99	283	579,37	271,02(100)	Naringenin-*O*-deoxyhexosyl-hexoside		✓	✓		
7	18.34	345	461,35	285,10(100)	Luteolin-*O*- hexuronoside		✓	✓	✓	✓
8	19.61	283	433,19	271,16(100)	Naringenin-*O*-hexoside		✓			
9	20.79	338	577,33	269,24(100)	Apigenin-7-*O*-rutinoside	✓				
10	20.89	338	577,38	315,13(100)	Isorhamnetin-*O*-malonyl-glucuronide		✓	✓	✓	
11	22.30	337	431,43	269,03(100)	Apigenin-*O*-hexoside			✓		
12	22.45	337	445,24	269,07(100)	Apigenin-*O*- hexuronoside	✓	✓		✓	✓
13	23.11	337	431,42	269,02(100)	Apigenin-*O*-hexoside			✓		
14	23.25	338	533,34	489,15(100);285,05(80)	Luteolin-*O*-malonyl-hexoside		✓		✓	
15	27.57	347	517,12	473,26(25);269,05(100)	Apigenin-*O*-malonyl-hexoside		✓	✓	✓	✓

Rt – retention times; λ_{max} – wavelength of maximum absorption; [M-H]$^-$ – deprotonated ion; MS^2 – fragmentation pattern; S – stigma; C – corolla; B – bracts; P – pappus; R – receptacle.

Table 2
Phenolic compounds quantification (mg/g extract) in the hydroethanolic extracts from different parts of distinct *C. cardunculus* L. cultivars.

Phenolic Compounds Quantification (mg/g extract)							
Peak number	F1-1-1	F4-1-4	F1-19-4	F4-25-2	F1-33-3	F1-34-1	F4-37-1
Stigma							
2	0.0288 ± 0.0003^{f}	0.102 ± 0.004 ^{a}	0.0456 ± 0.0004^{c}	0.069 ± 0.001^{b}	0.042 ± 0.001 ^{d}	0.040 ± 0.002 ^{e}	0.0140 ± 0.0004 ^{g}
9	9.1 ± 0.1 ^{a}	6.5 ± 0.1^{b}	7.8 ± 0.1 ^{ab}	5.55 ± 0.01 ^{ab}	6.40 ± 0.02 ^{ab}	5.1 ± 0.1^{b}	5.06 ± 0.02^{b}
12	11.8 ± 0.3 ^{a}	7.6 ± 0.1^{b}	6.6 ± 0.1^{b}	5.3 ± 0.1^{b}	3.9 ± 0.1^{b}	10.5 ± 0.2 ^{a}	14.17 ± 0.02 ^{a}
Corolla							
1	n.d.	n.d.	n.d.	n.d.	2.0 ± 0.1 ^{a}	n.d.	n.d.
4	5.8 ± 0.2 ^{d}	12.7 ± 0.2 ^{a}	11.1 ± 0.2^{b}	11.2 ± 0.2^{b}	n.d.	7.2 ± 0.1^{c}	2.10 ± 0.02 ^{e}
5	0.5074 ± 0.0004 ^{e}	0.529 ± 0.003 ^{d}	0.487 ± 0.001^{f}	0.5492 ± 0.0003^{c}	n.d.	0.621 ± 0.001 ^{a}	0.567 ± 0.002^{b}
6	n.d.	n.d.	n.d.	n.d.	7.91 ± 0.02 ^{a}	n.d.	n.d.
7	0.508 ± 0.003^{f}	0.634 ± 0.002 ^{d}	0.661 ± 0.001^{c}	0.580 ± 0.002 ^{e}	n.d.	1.09 ± 0.01^{b}	1.63 ± 0.02 ^{a}
8	n.d.	n.d.	n.d.	n.d.	2.73 ± 0.03 ^{a}	n.d.	n.d.
10	0.58 ± 0.01 ^{e}	0.706 ± 0.003^{b}	0.624 ± 0.002 ^{d}	0.689 ± 0.003^{c}	1.99 ± 0.01 ^{a}	0.687 ± 0.005^{c}	0.619 ± 0.001 ^{d}
12	1.08 ± 0.02^{f}	2.32 ± 0.01^{b}	1.70 ± 0.02 ^{d}	1.27 ± 0.01 ^{e}	2.5 ± 0.1 ^{a}	1.13 ± 0.01^{f}	1.8 ± 0.1^{c}
14	0.503 ± 0.002 ^{e}	0.526 ± 0.003^{c}	0.5148 ± 0.0004 ^{d}	0.528 ± 0.002^{c}	n.d.	0.578 ± 0.003^{b}	0.90 ± 0.01 ^{a}
15	0.90 ± 0.01 ^{d}	1.02 ± 0.02 ^{a}	0.983 ± 0.002^{b}	0.86 ± 0.01 ^{e}	0.524 ± 0.001 ^{g}	0.93 ± 0.01^{c}	0.835 ± 0.001^{f}
Bracts							
1	4.52 ± 0.02^{b}	4.33 ± 0.03^{b}	2.47 ± 0.04^{c}	0.51 ± 0.04 ^{d}	tr	12.3 ± 0.4 ^{a}	0.35 ± 0.01 ^{d}
6	13.2 ± 0.2 ^{a}	8.95 ± 0.001^{c}	9.4 ± 0.2^{b}	3.0 ± 0.1 ^{d}	tr	9.5 ± 0.1^{b}	1.63 ± 0.01 ^{e}
7	2.27 ± 0.03^{c}	2.12 ± 0.01 ^{d}	4.4 ± 0.1^{b}	0.87 ± 0.01^{f}	0.546 ± 0.002 ^{g}	6.1 ± 0.2 ^{a}	1.61 ± 0.01 ^{e}
10	2.45 ± 0.03 ^{d}	2.24 ± 0.03 ^{e}	2.84 ± 0.02 ^{a}	2.72 ± 0.01^{b}	0.702 ± 0.004 ^{g}	2.491 ± 0.002^{c}	1.55 ± 0.01^{f}
11	2.64 ± 0.04 ^{d}	2.4 ± 0.1 ^{e}	6.6 ± 0.1 ^{a}	2.9 ± 0.2^{c}	0.859 ± 0.003^{f}	5.31 ± 0.04^{b}	2.95 ± 0.04^{c}
13	1.01 ± 0.01 ^{e}	1.097 ± 0.003 ^{d}	1.62 ± 0.04 ^{a}	1.25 ± 0.01^{c}	0.596 ± 0.004 ^{h}	1.41 ± 0.02^{b}	0.88 ± 0.02^{f}
15	0.61 ± 0.01 ^{e}	0.60 ± 0.01 ^{e}	1.35 ± 0.02 ^{a}	0.649 ± 0.003 ^{d}	0.448 ± 0.001^{f}	0.90 ± 0.01^{b}	0.71 ± 0.01^{c}
Pappus							
3	0.040 ± 0.001^{b}	0.052 ± 0.003 ^{a}	0.0035 ± 0.0005 ^{g}	0.0239 ± 0.0003 ^{d}	0.007 ± 0.001^{f}	0.0323 ± 0.0001^{c}	0,0145 ± 0.0003 ^{e}
5	0.491 ± 0.002^{c}	0.4828 ± 0.0002 ^{e}	0.4802 ± 0.0003 ^{e}	0.4873 ± 0.0002 ^{d}	0.722 ± 0.001 ^{a}	0.502 ± 0.003^{b}	0.481 ± 0.003 ^{e}
7	0.6320 ± 0.0005 ^{e}	0.873 ± 0.004^{c}	0.5562 ± 0.0002^{f}	0.86 ± 0.02^{c}	0.811 ± 0.001 ^{d}	1.44 ± 0.01 ^{a}	1.21 ± 0.02^{b}
10	0.515 ± 0.001 ^{e}	0.5902 ± 0.0005^{b}	0.471 ± 0.002^{f}	0.542 ± 0.001^{c}	0.850 ± 0.002 ^{a}	0.59 ± 0.01^{b}	0.535 ± 0.001 ^{d}
12	2.0 ± 0.1 ^{d}	1.86 ± 0.02 ^{e}	2.773 ± 0.001^{b}	1.41 ± 0.02^{f}	1.854 ± 0.002 ^{e}	2.52 ± 0.05^{c}	3.1 ± 0.1 ^{a}
14	0.5066 ± 0.0005 ^{d}	0.507 ± 0.001 ^{d}	0.484 ± 0.002 ^{e}	0.513 ± 0.002^{c}	0.7408 ± 0.0004 ^{a}	0.547 ± 0.004^{b}	0.550 ± 0.004^{b}
15	0.468 ± 0.001^{f}	0.469 ± 0.001^{f}	0.66 ± 0.01^{b}	0.485 ± 0.001 ^{e}	0.703 ± 0.002 ^{a}	0.585 ± 0.005^{c}	0.547 ± 0.002 ^{d}
Receptacle							
4	2.76 ± 0.04 ^{d}	8.7 ± 0.2 ^{a}	3.06 ± 0.05^{c}	1.01 ± 0.05 ^{e}	0.295 ± 0.002 ^{g}	0.404 ± 0.001^{f}	3.53 ± 0.03^{b}
7	0.720 ± 0.004 ^{a}	0.543 ± 0.001^{c}	0.682 ± 0.004^{b}	0.480 ± 0.001 ^{e}	0.5134 ± 0.0005 ^{d}	0.4752 ± 0.0003^{f}	0.4768 ± 0.0002 ^{ef}
12	1.7496 ± 0.0004^{b}	1.24 ± 0.01 ^{d}	4.79 ± 0.04 ^{a}	0.91 ± 0.01 ^{e}	1.69 ± 0.02^{c}	0.791 ± 0.001^{f}	0.578 ± 0.005 ^{g}
15	0.686 ± 0.001 ^{a}	0.493 ± 0.001 ^{d}	0.65 ± 0.01^{b}	0.4473 ± 0.0003^{f}	0.55 ± 0.01^{c}	0.4763 ± 0.0004 ^{e}	0.4514 ± 0.0002^{f}

Results are presented as the mean ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). n.d. – not detected; tr – traces; S – stigma; C – corolla; B – bracts; P – pappus; R – receptacle. Calibration curves: compound **1** – chlorogenic acid ($y = 168823x + 161172$, $r^2 = 0.9999$); compound **2** and **3***p*-coumaric acid ($y = 301950x + 6966.7$, $r^2 = 0.9999$); compound **4** oleuropein ($y = 32226x + 12416$, $r^2 = 0.9997$); compounds **5**, **7**, **10**, and **14** quercetin-3-*O*-glucoside ($y = 34843x - 160173$, $r^2 = 0.9998$); compounds **6** and **8** naringenin ($y = 18433x + 78903$; $r^2 = 0.9998$); compounds **9**, **11**, **12**, **13**, and **15** apigenin 7-*O*-glucoside ($y = 10683x - 45794$; $r^2 = 0.999$).

malonyl and glucuronyl moieties, respectively) and based on its UV and mass fragmentation, it was tentatively identified as isorhamnetin-*O*-malonyl-glucuronide, taking into account that distinct isorhamnetin derivatives have been previously described in cardoon cultivars. Peaks **11** and **13**, with a pseudomolecular ion $[M-H]^-$ at *m/z* 431, revealed fragmentation patterns ($[M-H-162]^-$) coherent with apigenin *O*-hexosides. In turn, peak **12**, with a pseudomolecular ion $[M-H]^-$ at *m/z* 445 ($[M-H-176]^-$), was tentatively assigned as apigenin-*O*-hexuronoside.

The chemical composition of the different studied plant tissues varies. The corolla was the part with higher variability of phenolic compounds, having ten of the fifteen polyphenols identified in the different cultivars studied. Apigenin-*O*-hexoside was the compound present in higher concentrations in stigma (7.6–14.17 mg/g extract), except in cultivars F1-19-4, F4-25-2, and F1-33-3, in which apigenin-7-*O*-rutinoside stood out (5.55–7.8 mg/g extract). In corolla, secoxyloganin was the most abundant one (2.10–12.7 mg/g extract) in all cultivars except F1-33-3, in which it was not detected. Secoxyloganin also stood out in receptacles (1.01–8.7 mg/g extract). Although only four phenolic compounds were detected, secoxyloganin showed greater abundance in all cultivars studied, except F1-19-4, F1-33-3, and F1-34-1. In these, apigenin-*O*- hexuronoside had a higher concentration (0.791–4.79 mg/g extract) than the other identified compounds. In bracts, naringenin-*O*-deoxyhexosyl-hexose was the most prominent phenolic compound (3.0–13.2 mg/g extract), except in cultivars F1-33-3 and F4-37-1. Seven phenolic compounds were identified in pappus, apigenin-*O*-hexuronoside exhibit a higher abundance (1.41–2.773 mg/g extract) in all cardoon cultivars studied.

In general, pappus and bracts of cultivars F1-1-1, F4-1-4, and F1-19-4 are the plant tissues with the lowest (4.7, 4.83, and 5.429 mg/g extract, respectively), and the highest content (26.7, 21.8, and 28.8 mg/g of extract, respectively) in total phenolic compounds (TPC). For the remaining cultivars, the receptacle showed the lowest content of phenolic compounds (TPC between 2.14 and 5.03 mg/g extract). In the cases of F4-25-2 and F1-33-3 cultivars, corolla presented higher concentrations (15.6 and 17.6 mg/g of extract, respectively) than the remaining tissues. For the F1-34-1 cultivar, the bracts stood out with the highest concentration (38.1 mg/g of extract), while for the F4-37-1, it was the stigma (49.24 mg/g of extract). Although the chemical composition of cardoon has been previously described, some of the identified phenolic compounds had not yet been reported. This fact

Table 3
Total contents (mg/g extract) of phenolic acids, flavonoids, iridoids, and total phenolic compounds in the extracts of different *C. cardunculus* cultivars.

Phenolic Compounds Quantification (mg/g extract)							
	F1-1-1	F4-1-4	F1-19-4	F4-25-2	F1-33-3	F1-34-1	F4-37-1
Stigma							
TPC	20.9 ± 0.4^{a}	14.2 ± 0.2^{d}	14.4 ± 0.1^{d}	10.9 ± 0.1^{e}	10.3 ± 0.1^{f}	15.6 ± 0.1^{c}	19.24 ± 0.01^{b}
TPA	0.0288 ± 0.0003^{f}	0.102 ± 0.004^{a}	0.0456 ± 0.0004^{c}	0.069 ± 0.001^{b}	0.042 ± 0.001^{d}	0.040 ± 0.002^{e}	0.0140 ± 0.0004^{g}
TF	20.9 ± 0.4^{a}	14.1 ± 0.2^{d}	14.3 ± 0.1^{d}	10.9 ± 0.1^{e}	10.3 ± 0.1^{f}	15.6 ± 0.1^{c}	19.22 ± 0.01^{b}
Corolla							
TPC	9.9 ± 0.2^{f}	18.4 ± 0.3^{a}	16.1 ± 0.2^{c}	15.6 ± 0.2^{d}	17.6 ± 0.1^{b}	12.3 ± 0.1^{e}	8.5 ± 0.1^{g}
TPA	n.d.	n.d.	n.d.	n.d.	2.0 ± 0.1	n.d.	n.d.
TIG	5.8 ± 0.2^{d}	12.7 ± 0.2^{a}	11.1 ± 0.2^{b}	11.2 ± 0.2^{b}	n.d.	7.2 ± 0.1^{c}	2.10 ± 0.02^{e}
TF	4.08 ± 0.03^{f}	5.74 ± 0.03^{c}	4.97 ± 0.02^{d}	4.475 ± 0.004^{e}	15.63 ± 0.05^{a}	5.05 ± 0.04^{d}	6.4 ± 0.1^{b}
Bracts							
TPC	26.7 ± 0.4^{c}	21.8 ± 0.1^{d}	28.8 ± 0.2^{b}	11.88 ± 0.04^{e}	3.15 ± 0.01^{g}	38.1 ± 0.5^{a}	9.7 ± 0.1^{f}
TPA	4.52 ± 0.04^{b}	4.33 ± 0.03^{b}	2.47 ± 0.04^{c}	0.51 ± 0.04^{d}	tr	12.3 ± 0.4^{a}	0.35 ± 0.01^{d}
TF	22.2 ± 0.3^{c}	17.4 ± 0.1^{d}	26.35 ± 0.20^{a}	11.372 ± 0.003^{e}	3.15 ± 0.01^{g}	25.8 ± 0.1^{b}	9.3 ± 0.1^{f}
Pappus							
TPC	4.7 ± 0.1^{f}	4.83 ± 0.02^{e}	5.429 ± 0.005^{d}	4.32 ± 0.03^{g}	5.697 ± 0.001^{c}	6.2 ± 0.1^{b}	6.5 ± 0.1^{a}
TPA	0.040 ± 0.001^{b}	0.052 ± 0.003^{a}	0.0035 ± 0.0005^{g}	0.0239 ± 0.0003^{d}	0.007 ± 0.001^{f}	0.0323 ± 0.0001^{c}	0.0145 ± 0.0003^{e}
TF	4.6 ± 0.1^{f}	4.78 ± 0.02^{e}	5.426 ± 0.004^{d}	4.30 ± 0.03^{g}	5.690 ± 0.001^{c}	6.2 ± 0.1^{b}	6.4 ± 0.1^{a}
Receptacle							
TPC	5.92 ± 0.04^{c}	11.0 ± 0.1^{a}	9.19 ± 0.02^{b}	2.8 ± 0.1^{f}	3.05 ± 0.01^{e}	2.147 ± 0.002^{g}	5.03 ± 0.04^{d}
TiG	2.76 ± 0.04^{c}	8.7 ± 0.2^{a}	3.06 ± 0.05^{c}	1.01 ± 0.05^{e}	0.295 ± 0.002^{g}	0.404 ± 0.001^{f}	3.53 ± 0.03^{b}
TF	5.92 ± 0.04^{c}	11.0 ± 0.1^{a}	9.19 ± 0.02^{b}	2.8 ± 0.1^{f}	3.05 ± 0.01^{e}	2.147 ± 0.002^{g}	5.03 ± 0.04^{d}

Results are presented as the mean ± standard deviation. Different letters in the same row correspond to significant differences ($p < 0.05$). n.d. – not detected; tr – traces; S – stigma; C - corolla; B – bracts; P – pappus; R – receptacle; TPC – total phenolic compounds; TPA – total phenolic acids; TF – total flavonoids; TIG – total iridoid glycoside (i.e., secoxyloganin).

might be explained by the cultivars' information influence, as well as the geographic location of the plant (Chihoub et al., 2019; Dias et al., 2018; Mandim, Dias, et al., 2020), highlighting the importance of considering the different conditions for a complete characterization of the product.

3.2. Bioactive properties

3.2.1. Antioxidant activity

The antioxidant potential of cardoon hydroethanolic extracts was tested through two cell-based assays – TBARS and OxHLIA. The obtained results are presented in Table 4. The lower IC_{50} values correspond to better antioxidant potential. The IC_{50} value for the TBARS assay represents the extract amount that inhibits lipid peroxidation by 50%. While for the OxHLIA assay, it's related to the concentration necessary to delay 50% of the red blood cells lysis by AAPH-derived free radicals during a certain time. All the samples studied showed the ability to inhibit the oxidative process in both assays used, except the sample F4-1-4C in the case of the OxHLIA assay at Δt 120 min. Nevertheless, there were differences in the antioxidant power among the distinct cultivars tested, as well as with the type of plant tissue.

Regarding the TBARS assay, the lowest IC_{50} values, and consequently a higher antioxidant potential, were demonstrated by the F1-34-1 cultivar (IC_{50} between 40.4 and 221 μg/mL). Bracts (B) showed high potential in three of the seven cultivars studied (F1-1-1, F1-19-4, and F4-37-1, IC_{50} between 82 and 95 μg/mL), although the lowest IC_{50} values were observed for the corolla of cultivars F1-34-1 and F4-1-4 (IC_{50} of 40.4 and 38 μg/mL, respectively). Receptacles presented the highest IC_{50} values, therefore the lowest antioxidant activity, particularly for F1-1-1, F1-34-1, and F4-25-2 cultivars (IC_{50} between 221 and 1879 μg/mL). None of the studied extracts showed higher antioxidant activity than the commercial antioxidant Trolox (IC_{50} of 5.4 μg/mL).

The results also vary among cultivars and plant tissues in the OxHLIA assay. Pappus gave the lowest IC_{50} values in four of the seven cultivars studied when using an incubation time (Δt) of 60 min, i.e., F1-33–3, F1-19-4, F4-25-2, and F4-37-1 (IC_{50} between 81 and 181 μg/ml). Also, at Δt 120 min, pappus extract has lower IC_{50} values than other plant tissues, particularly for cultivars F1-19-4, F4-25-2, and F4-37-1 (IC_{50} between 180 and 471 μg/mL). However, the lowest IC_{50} was found for the corolla of the F1-34-1 cultivar, with values that were approximately four times lower than those of the commercial antioxidant Trolox, used as a positive control, for a delay in hemolysis of Δt 60 min (IC_{50} of 5.5 and 20 μg/mL, respectively), and three times for a Δt of 120 min (IC_{50} of 14 and 41 μg /mL, respectively).

Antioxidant activity is a widely studied property that has been associated with the modulation of several physiopathological processes in the organism. It is also one of the most studied bioactive properties in cardoon. Many studies highlight the antioxidant potential demonstrated by this species and the influence that different variables (e.g., plant material, state of maturation, geographic location, viability, genetic background, etc.) may have on this activity (Mandim et al., 2021a; Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022). Statistically significant differences in the antioxidant activity evaluated through different assays (i.e., DPPH, reducing power, β-carotene, and TBARS) among different genotypes of several Italian cardoon samples were shown by Petropoulos et al. (2018). Dias et al. (2018) found that the corolla has a greater DPPH scavenging activity and reducing power than other plant tissues. Although different antioxidant assays have been used in the present work, this observation is in agreement with the results herein obtained, corroborating the relevant antioxidant potential associated with the corolla of this species. As for seeds, higher antioxidant capacity was demonstrated for viable than for non-viable ones (Mandim, Petropoulos, et al., 2020).

Several studies associate the content of phenolic compounds with the antioxidant activity of the species, due to the ability of these compounds to scavenge reactive nitrogen and oxygen molecules, thus interrupting the radical chain, as well as inhibiting enzymatic activities leading to the

Table 4
Antioxidant, cytotoxic, and anti-inflammatory activities of the hydroethanolic extracts obtained from the different cardoon samples studied.

Sample		TBARS (IC_{50}, μg/mL)	OxHLIA (IC_{50}, μg/mL)		Cytotoxic activity (GI_{50}, μg/mL)					RAW 264.7 (IC_{50}, μg/mL)
			$\Delta t = 60$ min	$\Delta t = 120$ min	HeLa	MCF-7	NCI-H460	HepG2	PLP2	
F1-1-1	S	202 ± 6^{r}	168 ± 12^{klmn}	427 ± 17^{ijklm}	>400	253 ± 8^{d}	343 ± 11^{b}	>400	>400	>400
	C	142 ± 1^{u}	179 ± 10^{jklm}	378 ± 12^{klmno}	>400	367 ± 12^{a}	>400	>400	>400	>400
	B	86 ± 3^{wx}	209 ± 12^{hijk}	394 ± 22^{jklmn}	374 ± 19^{a}	271 ± 26^{c}	>400	>400	>400	>400
	P	256 ± 4^{p}	266 ± 16^{gh}	486 ± 19^{hijk}	>400	>400	>400	>400	>400	>400
	R	325 ± 7^{n}	149 ± 11^{klmno}	435 ± 19^{ijkl}	313 ± 17^{b}	293.0 ± 21.5^{b}	204 ± 15^{d}	259 ± 14^{c}	>400	>400
F4-1-4	S	1463 ± 13^{e}	286 ± 22^{gh}	1056 ± 83^{cd}	>400	>400	>400	>400	>400	>400
	C	38 ± 1^{y}	871 ± 41^{a}	na	>400	>400	>400	>400	>400	>400
	B	824 ± 3^{g}	288 ± 15^{g}	624 ± 17^{fg}	>400	>400	>400	>400	>400	>400
	P	1635 ± 8^{c}	103 ± 7^{mno}	333 ± 17^{lmno}	>400	>400	>400	>400	>400	>400
	R	1058 ± 16^{f}	71 ± 5^{op}	279 ± 16^{nop}	305 ± 19^{b}	>400	>400	>400	>400	>400
F1-19-4	S	223 ± 5^{q}	648 ± 40^{c}	1811 ± 32^{a}	366 ± 13^{a}	>400	>400	>400	>400	>400
	C	390 ± 2^{l}	835 ± 22^{ab}	1484 ± 31^{b}	>400	>400	>400	>400	>400	>400
	B	95 ± 1^{w}	389 ± 24^{e}	774 ± 32^{e}	>400	>400	>400	>400	>400	>400
	P	187 ± 1^{s}	81 ± 4^{op}	471 ± 22^{hijk}	>400	>400	>400	>400	>400	>400
	R	118 ± 1^{v}	375 ± 21^{ef}	714 ± 37^{ef}	>400	>400	>400	>400	>400	>400
F4-25-2	S	371 ± 3^{m}	838 ± 22^{ab}	1411 ± 31^{b}	254 ± 23^{d}	>400	>400	>400	>400	>400
	C	602 ± 4^{i}	261 ± 14^{ghi}	476 ± 22^{hijk}	>400	>400	>400	>400	>400	>400
	B	2263 ± 3^{a}	253 ± 13^{ghij}	583 ± 29^{gh}	153 ± 7^{e}	>400	289 ± 14^{c}	292 ± 11^{b}	>400	>400
	P	1596 ± 10^{d}	93 ± 3no	180 ± 8^{p}	>400	>400	>400	>400	>400	>400
	R	1879 ± 16^{b}	271 ± 8^{gh}	508 ± 13^{ghij}	>400	>400	>400	>400	>400	>400
F1-33-3	S	166 ± 2^{t}	450 ± 26^{de}	977 ± 45^{d}	>400	>400	>400	>400	>400	>400
	C	386 ± 4^{l}	791 ± 54^{b}	1757 ± 88^{a}	>400	>400	>400	>400	>400	>400
	B	451 ± 8^{j}	116 ± 6^{lmno}	308 ± 11^{lmnop}	>400	>400	>400	>400	>400	>400
	P	82 ± 2^{x}	106 ± 7^{lmno}	320 ± 17^{lmno}	>400	>400	>400	>400	>400	>400
	R	309 ± 9^{o}	116 ± 3^{lmno}	299 ± 10^{mnop}	>400	>400	>400	>400	>400	>400
F1-34-1	S	196 ± 1^{rs}	310 ± 10^{fg}	540 ± 12^{ghi}	365 ± 22^{a}	>400	>400	>400	>400	>400
	C	40.4 ± 0.1^{y}	5.5 ± 0.3^{p}	14 ± 1^{q}	>400	>400	>400	>400	>400	>400
	B	133 ± 2^{u}	114 ± 5^{lmno}	252 ± 9^{op}	>400	>400	371 ± 19^{a}	>400	>400	>400
	P	121 ± 2^{v}	596 ± 28^{c}	1030 ± 42^{cd}	242 ± 7^{d}	>400	300 ± 16^{c}	318 ± 8^{a}	>400	>400
	R	221 ± 1^{q}	503 ± 27^{d}	834 ± 47^{e}	>400	>400	>400	>400	>400	>400
F4-37-1	S	120 ± 1^{v}	432 ± 24^{de}	1148 ± 24^{c}	>400	>400	>400	>400	>400	>400
	C	114 ± 1^{v}	590 ± 28^{c}	1134 ± 37^{c}	>400	>400	>400	>400	>400	>400
	B	82 ± 1^{x}	184 ± 7^{ijkl}	342 ± 9^{lmno}	>400	>400	>400	>400	>400	>400
	P	715 ± 6^{h}	181 ± 12^{jklm}	360 ± 29^{klmno}	>400	>400	>400	>400	>400	>400
	R	421 ± 1^{k}	598 ± 27^{c}	986 ± 63^{d}	>400	>400	>400	>400	>400	>400
Positive control		5.4 ± 0.3	20 ± 1	41 ± 2	1.0 ± 1	1.21 ± 0.02	0.9 ± 0.1	1.1 ± 0.1	2.3 ± 0.2	16 ± 1

Results were expressed as the mean values ± standard deviation. S – stigma; C – corolla; B – bracts; P – pappus; R – receptacle. Different letters correspond to significant differences (p less than 0.05). Statistical differences obtained by Student's t-test. na – no activity. Positive control for antioxidant activity – Trolox (IC_{50} values in μg/mL); for cytotoxic activity – Ellipticine (GI_{50} values in μg/mL); for anti-inflammatory activity – Dexamethasone (IC_{50} values in μg/mL).

formation of radicals, to chelate metal ions, and to act synergistically with other compounds with antioxidant capacity (Petropoulos et al., 2018).

The results obtained seem to support a relation between the content of phenolic compounds and the antioxidant potential. Thus, the samples with a higher ability to inhibit the formation of thiobarbituric acid reactive species are also the ones with the highest levels of phenolic compounds. For example, bracts of the cultivar F1-34-1 showed the lowest IC_{50} values for the TBARS assay and were the plant tissue with the highest content of phenolic compounds (TPC = 38.1 mg/g dw). The high concentration of phenolic compounds such as secoxyloganin, apigenin-*O*-hexoside, and apigenin-*O*-hexurunoside in those samples could be related to the demonstrated activity. This relation was not observed for the results obtained with the OxHLIA assay, suggesting that other classes of bioactive compounds could also be involved in the antioxidant capacity of cardoon. More studies are, therefore, needed to better understand which bioactive compounds are related to this activity.

3.2.2. *Cytotoxic activity*

The results obtained for the cytotoxic activity in the studied samples are in Table 4, expressed as the concentration of the extract able to inhibit cell proliferation by 50% (GI_{50} values, μg/mL). As can be observed, only some of the studied cultivars revealed the ability to inhibit the proliferation of the assayed cell lines. None of the extracts of F1-33-3 and F4-37-1 cultivars showed cytotoxic activity ($GI_{50} > 400$ μg/mL), while for F1-19-4 and F4-1-4, only the stigma and receptacle, respectively, were able to inhibit HeLa proliferation (GI_{50} values of 366 and 305 μg/mL, respectively). Among the remaining samples, the F1-1-1 exhibited the most significant cytotoxic activity, as its tissues, except pappus, showed the ability to inhibit any of the different tumor lines tested (GI_{50} between 204 and 367 μg/mL), especially the extract of the receptacle. Also, extracts from samples F1-34-1P (GI_{50} between 242 and 318 μg/mL) and F4-25-2B (GI_{50} between 153 and 292 μg/mL) showed the ability to inhibit the proliferation of HeLa cell lines, NCI-H460, and HepG2 with GI_{50} values lower than those obtained for the remaining samples tested. These observations suggest that these extracts could be

possible sources of compounds with promising anti-tumor power. In general, samples that do not exhibit antiproliferative activity, do not generally present interesting antioxidant activity, especially in the TBARS assay. This fact may be related to the increased concentration of reactive species (e.g., ROS and RNS) that can cause a cellular redox disturbance and oxidative damage to the lipid membrane or other cellular constituents (do Carmo et al., 2018).

Although the antiproliferative activity of distinct cardoon extracts has been previously described, most of the published studies evaluated the influence that parameters such as tissue viability, growth cycle, and plant tissue have on the demonstrated potential (Mandim et al., 2021a; Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022). Bracts and heads in younger maturation stages stand out with higher cytotoxic activity (Mandim et al., 2021b; Mandim, Petropoulos, et al., 2020). Velez et al. (2012) reported that the extract of wild cardoon leaf inhibits the main key stages of carcinogenesis (namely initiation, proliferative, and progression phases).

3.2.3. Hepatotoxic activity

The results for the activity of cardoon extracts against a primary culture of non-tumor cells PLP2 are also shown in Table 4. None of the tested extracts showed hepatotoxicity (GI_{50} values >400 μg/mL), suggesting that this species is safe as the different plant tissues studied do not influence the proliferation of non-tumor cells. This is in agreement with what was found in previous studies (Mandim, Dias, et al., 2020).

3.2.4. Anti-inflammatory activity

The results of the anti-inflammatory assessment of the studied *C. cardunculus* cultivars are in Table 4. This activity was determined by the ability of the extracts to inhibit the production of NO, a chemical mediator of the inflammatory process, from mouse macrophage cells (RAW 264.7) upon stimulation with LPS. The inflammatory response is a complex process that occurs at the cellular and molecular levels. The results were expressed as the concentration of extract responsible for the inhibition of NO production at 50%. As can be seen in the Table, none of the studied extracts showed NO radical scavenging activity through the cell-based assay performed (IC_{50} > 400 μg/mL). The anti-inflammatory activity of this species has been recently explored using different approaches. Thus, *C. cardunculus* L. leaves demonstrated the ability to inhibit TNF-α-induced inflammation via the NF-kB pathway in CaCo2 cells (Speciale et al., 2022). The influence of the growth cycle of different cardoon tissues was evaluated using the same *in vitro* assay used in the present work. Samples of floral capitula and bracts in younger maturation stages showed higher activity, contrary to what was observed for petioles and seeds that did not show the ability to inhibit NO production (Mandim et al., 2021a; Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022). The influence of the growth cycle of different cardoon tissues was evaluated using the same *in vitro* assay used in the present work. Samples of floral capitula and bracts in younger maturation stages showed higher activity, contrary to what was observed for petioles and seeds that did not show the ability to inhibit NO production (Salem et al., 2017). The authors found that those extracts were able to inhibit the production of chemical mediators involved in the inflammatory process, such as prostaglandins, serotonin, and histamine (Salem et al., 2017). As above indicated, none of the samples tested in the present study showed the capacity to inhibit NO formation. The present study was performed using a cellular model that is unable to specify the mechanisms of the demonstrated anti-inflammatory potential. In this sense, the performance of additional and more precise molecular methodologies that evaluate other parameters related to the inflammatory process would be an asset, and consequently, identifying the mechanisms and the class of compounds involved in the proven activity.

3.2.5. Antimicrobial activity

The antibacterial potential of the plant tissues of the studied cardoon cultivars is presented in Table 5. The results are expressed as minimum inhibitory (MIC) and bactericidal (MBC) concentrations. All extracts analyzed could inhibit bacterial growth. Negative control 5% DMSO did not interfere with microbial growth. The Gram-positive *Bacillus cereus* showed was the most sensitive to the studied extracts with the lowest MIC (values between 0.5 and 2 mg/mL). Especially the receptacle and the corolla of F1-33-3 and F1-19-4 cultivars (MIC = 0.5 mg/mL). All extracts tested showed equal or greater activity than that observed for the E211 positive control against the Gram-negative *Enterobacter cloacae*. In particular, the stigma and receptacle of the F4-25-2 cultivar showed lower MIC values (MIC = 0.5 and 1 mg/mL, respectively) than the positive controls used. Previous studies with various plant tissues harvested at different stages of the growth cycle also proved the antibacterial potential of this species. In particular, the petioles showed higher potential than other tissues analyzed (Mandim et al., 2021a). By contrast, Dias et al. (2018) found that the corolla of samples with different genetic information possessed the greatest antibacterial potential, while Petropoulos, Fernandes, et al. (2019), demonstrated significant antibacterial activity for seeds of different cultivars of cardoon. The results obtained in the present work prove the influence of both plant tissue and cultivars background on the antibacterial potential of this species.

The antifungal activity of the cultivars under study was also analyzed. The obtained results are presented in Table 5 expressed as minimum inhibitory (MIC) and fungicidal (MFC) concentrations. All the samples tested showed higher antifungal potential (lower MIC values) than the positive controls used (E211 – sodium benzoate and E224 – potassium metabisulfite). Bracts of the F1-1-1 cultivar demonstrated higher antifungal potential, with lower MIC and MFC values against all tested fungi (MIC values between 0.12 and 0.5 mg/mL), except for the *Aspergillus niger* and *A. versicolor*. Pappus of the F4-25-2 and F4-37-1 cultivars stood out (MIC = 0.06 mg/mL) against *A. niger*, and pappus and bracts samples of F1-33-3 and F1-19-4 against *A. versicolor*, respectively (MIC = 0.12 mg/mL). The antifungal potential of the different vegetable tissues of cardoon had been previously proven, as well as the influence of the growth cycle (Mandim et al., 2021a; Mandim, Petropoulos, Pinela, Dias, Giannoulis, et al., 2022; Scavo et al., 2019), and the species genetic information (Dias et al., 2018; Petropoulos, Fernandes, et al., 2019).

3.2.6. Correlation between phenolic composition and the studied bioactivities

A Pearson's correlation analysis was performed, and the obtained correlation coefficients (*R*) are presented in Table S3 of the Supplementary Materials. Most of the phenolic compounds identified in the extracts of the cardoon cultivars show negative correlation values with the assays used to evaluate the antioxidant activity, particularly for the TBARS assay. The negative correlation values are related to the antioxidant potential results being exhibited in IC_{50} values, and therefore lower values are associated with higher antioxidant potential. The total flavonoid content is the class of phenolic compounds that exhibits a more significant correlation (R of −0.299 for TBARS and −0.354 for OxHLIA, $\Delta t = 60$ min, and −0.251 for OxHLIA, $\Delta t = 60$ min) compared to the other classes of compounds. Noteworthy that the presence of *p*-coumaroyl acid hexoside exhibits a strong positive correlation with the TBARS assay and consequently indicates that its presence contributes negatively to the observed antioxidant capacity. On the other hand, apigenin-7-*O*-rutinoside and apigenin-*O*-hexuronoside are the compounds with the highest correlation for both assays used.

Regarding the antiproliferative capacity of the extracts, also in this case lower GI_{50} values indicate a more interesting activity. In agreement with what was observed for antioxidant potential, also in the case of cytotoxic activity most of the phenolic compounds identified exhibited negative correlation values with the different cell lines tested. The compounds secoxyloganin, apigenin-*O*-malonyl-hexoside, 5-*O*-caffeoylquinic acid, and apigenin-*O*-hexoside showed the highest correlation

Table 5
Antibacterial and antifungal potential of the hydroethanolic extracts obtained from the different cardoon samples studied.

		F1-1-1					F4-1-4					F1-19-4					F4-25-2				
		S	C	B	P	R	S	C	B	P	R	S	C	B	P	R	S	C	B	P	R
Antimicrobial activity (mg/mL)																					
Staphylococcus aureus	MIC	4	2	4	2	4	4	2	4	2	4	2	2	4	2	2	2	4	4	2	4
	MBC	8	4	8	4	8	8	4	8	4	8	4	4	8	4	4	4	8	8	4	8
Bacillus cereus	MIC	1	2	2	1	1	2	1	1	1	1	1	0.5	1	1	1	1	2	1	1	1
	MBC	2	4	4	2	3	4	2	2	2	2	2	1	2	2	2	2	4	2	2	2
Listeria monocytogenes	MIC	2	2	2	2	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
	MBC	4	4	4	4	4	2	2	2	2	2	2	2	2	2	4	2	2	2	2	2
Escherichia coli	MIC	2	2	2	2	2	2	1	1	2	2	2	2	4	2	4	2	2	2	2	1
	MBC	4	4	4	4	4	4	2	2	4	4	4	4	8	4	8	4	4	4	4	2
Salmonella typhimurium	MIC	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	0.5
	MBC	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	2	1
Enterobacter cloacae	MIC	0.5	1	1	2	1	1	2	1	0.5	1	1	1	2	1	2	0.5	1	1	1	1
	MBC	1	2	2	4	2	2	4	2	1	2	2	2	4	2	4	1	2	2	2	2
Antifungal activity (mg/mL)																					
Aspergillus fumigatus	MIC	0.5	0.5	0.12	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.5
	MFC	1	1	0.25	1	1	1	1	1	1	1	1	1	0.5	1	0.5	1	1	1	1	1
Aspergillus niger	MIC	0.25	0.5	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.25	0.25	0.06	0.12
	MFC	1	1	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.12	0.25
Aspergillus versicolor	MIC	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	MFC	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Penicillium funiculosum	MIC	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	MFC	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Trichoderma viride	MIC	0.5	0.5	0.12	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.5
	MFC	1	1	0.25	1	1	1	1	1	1	1	1	1	0.5	1	0.5	1	1	1	1	1
Penicillium verrucosum var. *cyclopium*	MIC	0.25	0.25	0.25	0.5	0.5	0.12	0.25	0.12	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.12	0.25	0.06	0.25	0.12
	MFC	0.5	0.5	0.5	1	1	0.25	0.5	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.12	0.5	0.25

		F1-33-3					F1-34-1					F4-37-1					Positive Control	
		S	C	B	P	R	S	C	B	P	R	S	C	B	P	R	E211	E224
Antimicrobial activity (mg/mL)																		
Staphylococcus aureus	MIC	4	4	4	4	4	4	2	4	2	4	4	4	2	4	4	4	1
	MBC	8	8	8	8	8	8	4	8	4	8	8	8	4	8	8	4	1
Bacillus cereus	MIC	1	1	1	2	0.5	1	1	2	1	1	1	2	1	2	1	0.5	2
	MBC	2	2	2	4	1	2	2	4	2	2	2	4	2	4	2	0.5	4
Listeria monocytogenes	MIC	1	1	2	2	2	1	2	1	1	1	1	1	1	1	1	1	0.5
	MBC	2	2	4	4	4	2	4	2	2	2	2	2	2	2	2	2	1
Escherichia coli	MIC	2	2	2	2	2	2	2	2	2	2	8	2	2	2	2	1	0.5
	MBC	4	4	4	4	4	4	4	4	4	4	>8	4	4	4	4	2	1
Salmonella typhimurium	MIC	2	2	2	2	2	2	2	2	1	2	2	2	1	2	2	1	1
	MBC	4	4	4	4	4	4	4	4	2	4	4	4	2	4	4	2	1
Enterobacter cloacae	MIC	1	1	2	2	1	1	1	1	1	1	1	1	0.5	1	1	2	0.5
	MBC	2	2	4	4	2	2	2	2	2	2	2	2	1	2	2	4	0.5
Antifungal activity (mg/mL)																		
Aspergillus fumigatus	MIC	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	1	1
	MFC	1	0.5	1	0.5	1	1	1	1	0.5	1	1	1	1	1	1	2	1
Aspergillus niger	MIC	0.25	0.5	0.12	0.12	0.25	0.5	0.25	0.25	0.25	0.25	0.12	0.12	0.06	0.12	0.25	1	1
	MFC	0.5	1	0.25	0.25	0.5	1	0.5	0.5	0.5	0.5	0.25	0.25	0.12	0.25	0.5	2	1

(continued on next page)

Table 5 (*continued*)

		F1-1-1					F4-1-4					F1-19-4					F4-25-2				
		S	C	B	P	R	S	C	B	P	R	S	C	B	P	R	S	C	B	P	R
Aspergillus versicolor	MIC	0.25	0.5	0.5	0.12	0.5	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25				2	1
	MFC	0.5	1	1	0.25	1	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5				2	1
Penicillium funiculosum	MIC	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.5	0.25	0.25	0.25	0.25	0.25				1	0.5
	MFC	1	1	1	1	1	1	0.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5				2	0.5
Trichoderma viride	MIC	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.12	0.12	0.12	0.5				1	1
	MFC	1	0.5	1	0.5	1	1	1	1	0.5	1	0.5	0.25	0.25	0.25	1				2	1
Penicillium verrucosum var. *cyclopium*	MIC	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.5	0.25				2	1
	MFC	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	1	0.5	1	0.5				4	1

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration; MFC – minimal fungicidal concentration. S - stigma; C – corolla; B – bracts; P – pappus; R – receptacle. Positive controls: E211 - sodium benzoate and E224 - potassium metabisulfite. Negative control 5% DMSO did not interfere with microbial growth.

values with HeLa, MCF-7, NCI-H460, and HepG2 cell lines, respectively.

These results are in agreement with the described by several studies that observed a higher correlation between the flavonoids and the bioactive potential, namely luteolin and apigenin derivates (Petropoulos et al., 2018; Shang et al., 2022). Flavonoids' chemical structure, including the degree of hydrolyzation and their positions, and the glycosylation grade, affects the scavenging reactive oxygen species capacity and their interaction with other molecules. Further studies to isolate these compounds and evaluate their bioactive potential would be essential to prove their potential and verify whether the other constituents of the plant have synergistic or antagonistic effects.

4. Conclusion

The results obtained in this study prove the influence that both plant tissues and cultivars' backgrounds have on the biochemical potential of cardoon. A very limited number of studies have explored all the constituents of *C. cardunculus* capitulum and the influence of the cultivars to properly characterize the spe be aware of its full potential. A total of fifteen phenolic compounds were identified, with varied distribution and abundance among the cultivars and tissues studied. The corolla presented a greater variety of phenolic compounds, while the bracts showed higher concentrations. The corolla revealed a higher antioxidant potential, particularly cultivars F4-1-4, and F1-34-1, regarding inhibition in the formation of thiobarbituric acid reactive substances (TBARS), and cultivar F1-34-1 to inhibit the oxidative hemolysis (OxHLIA assay), being in this latter case higher than the commercial antioxidant Trolox. The bracts of the F4-25-2 cultivar stood out with greater antiproliferative capacity against all the tumor cell lines tested. None of the assayed extracts demonstrated hepatotoxic and anti-inflammatory activities. The influence of cultivars and plant tissue on antibacterial and antifungal activities was also proven. The obtained results boost the potential associated with cardoon. It proves to be a rich source of phenolic compounds, and it also exhibits a higher antioxidant and antifungal potential than the positive controls used, without presenting any toxic effects. Nevertheless, correlation studies regarding the compounds responsible for the exhibited bioactive properties are necessary. The results that have been disseminated support its promising exploitation as a nutraceutical ingredient. Cardoon exhibits a promising future as a substitute for some artificial additives, such as food preservatives. However, given the influence demonstrated by factors such as growth cycle, genetic information, cultivar, and geographic location, among others, intensive study and thorough characterization of these variables are needed. They are essential to determine the ideal characteristics that enhance these final applications. Although cardoon has a special importance in the diet of the Mediterranean basin countries, its consumption is not very frequent in the rest of the world. More complete knowledge regarding its rich biochemical composition may be a significant incentive for its more frequent and widespread consumption.

CRediT authorship contribution statement

Filipa Mandim: Methodology, Investigation, Formal analysis, Writing – original draft. **José Pinela:** Methodology, Writing – review & editing. **Sandra Marcelino:** Methodology. **Maria Inês Dias:** Methodology, Writing – review & editing. **Paulo Barracosa:** Methodology, Investigation, Writing – review & editing. **Marija Ivanov:** . **Marina Soković:** Methodology, Resources. **Celestino Santos-Buelga:** Conceptualization, Methodology, Writing – review & editing. **Lillian Barros:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137480.

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