ORIGINAL PAPER

Effect of in vitro digestion of *Cudrania cochinchinensis* root extracts on phenolic compounds, bioactivity, bioaccessibility and cytotoxicity on HepG2 cells

Wenran Tian[1,2,3] · Ding Cheng[1,2,3] · Xianghui Yan[1,2,3] · Guohua Zhang[1,2,4] · Guibing Zeng[1,2,3] · Xianliang Bao[1,2,3] · Zheling Zeng[1,2,3] 🄴 · Ping Yu[1,2,3] · Deming Gong[1,2,5]

Abstract

The study aimed to investigate the bioaccessibility and the stability of purified ethanol extracts (PEE) from *Cudrania cochinchinensis* roots, and the changes in antioxidant activity and enzyme inhibition activity and cytotoxicity by in vitro digestion. No compounds were lost during the gastrointestinal digestion (GID) process, 15 compounds were tentatively identified using UPLC-TOF-MS/MS technology, including five phenolic and ten flavonoid compounds. After the gastric digestion (GD), the bioaccessibility of phenolic and flavonoid compounds was 55.66% and 52.44%, respectively. After the intestinal digestion (ID), the bioaccessibility of phenolic and flavonoid compounds reduced to 39.70% and 33.36%, respectively. The gastrointestinal digestion (GID) decreased the bioaccessibility of phenolic compounds. All compounds were affected throughout the gastrointestinal digestion (GID), among which the more affected compounds were 4-hydroxybenzoic acid, eriodictyol-7-O-glucoside, resveratrol and 3,6,3',4'-tetrahydroxyflavone (compared with the peak intensity of the undigested PEE). Furthermore, there was a reduction in the antioxidant activity, inhibition activity against α-glucosidase and tyrosinase, and cytotoxicity toward HepG2 cells of the PEE after GID. There was a significantly positive correlation between the bioactivity and the total phenolic/flavonoid contents. The results showed that in vitro digestion had significant effects on the phenolic content and bioactivity. Although phenolic contents were decreased during GID, *C. cochinchinensis* roots is still a good source of bioactive compounds for the development of functional foods with health benefits.

Keywords Gastrointestinal digestion · Bioaccessibility · Antioxidant activity · Enzyme inhibition activity · Cytotoxicity · UPLC-TOF-MS/MS

✉ Zheling Zeng
  zlzengjx@hotmail.com; zlzengjx@163.com

✉ Ping Yu
  cpu_yuping@126.com

1 State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

2 Jiangxi Province Key Laboratory of Edible and Medicinal Resources Exploitation, Nanchang University, Nanchang 330031, China

3 School of Resource and Environmental and Chemical Engineering, Nanchang University, Nanchang 330031, China

4 School of Food Science and Technology, Nanchang University, Nanchang 330031, China

5 New Zealand Institute of Natural Medicine Research, 8 Ha Crescent, Auckland 2104, New Zealand

Abbreviations

ABTS	2,2'-Azino-bis-(3-ethylbenzothia-zoline-6-sulfonic acid)
C. cochinchinensis	*Cudrania cochinchinensis*
CEE	Crude ethanol extracts
CUPRAC	Cupric reducing antioxidant capacity
DPPH	1,1-Diphenyl-2-picrylhydrazyl
ESI	Electrospray ionization
FRAP	Ferric reducing antioxidant power
GID	Gastrointestinal digestion
GD-PEE	Gastric digestion-purified ethanol extracts
ID-PEE	Intestinal digestion-purified ethanol extracts
PEE	Purified ethanol extracts
TAC	Total antioxidant capacity

| TIC | Total ion chromatogram |
| UPLC-TOF-MS/MS | Ultra performance liquid chromatography-time of flight-mass spectrometry/mass spectrometry |

Introduction

Vegetables and fruits are considered to be good sources of phenolic compounds. In the past decades, phenolic compounds, especially phenolic acids, flavonoids, and anthocyanins, have been demonstrated to be important health-promoting dietary phytochemicals. Evidence has confirmed that phenolic compounds delayed the onset of some chronic diseases, such as inflammation, hypertension, arteriosclerosis and cardiovascular disease [1–3].

Cudrania cochinchinensis, a perennial undershrub of Moraceae family, is widely distributed in Southern China, Japan and Korea. In China, the roots of *C. cochinchinensis* have been used as a traditional medicine to treat humid jaundice, hepatoprotective, gonorrhea and bruising [4]. It was found that phenolic and flavonoid compounds in the extracts of *C. cochinchinensis* roots possessed strong tyrosinase inhibitory activities [5]. The extract of *C. cochinchinensis* roots exhibited strong antioxidant activities [6, 7]. While these studies offer valuable information on the chemical identity and bioactivity of the plant constituents, their stability and bioaccessibility in the gut must be taken into account. It has been reported that gastrointestinal conditions, such as temperature, pH value and related enzymes have significant effects on the stability and bioaccessibility of the phenolic compounds [8, 9]. For example, Correa-Betanzo et al. [10] found that anthocyanins have low bioavailability due to their low stability under the alkaline conditions of the small intestine. Goulas et al. [11] reported that flavonoids from carob fruit had low release efficiencies than phenolic acids during in vitro digestion.

In vitro simulated GID models have been widely used for investigating the changes in the stability, bioaccessibility, and biological activity of phytochemicals. Compared to the human clinical trials, in vitro approaches have the advantages of being more straightforward, efficient and cheaper. The study aimed to evaluate the effects of ethanol extracts from *C. cochinchinensis* roots under simulated GID conditions on the bioaccessibility, antioxidant activity and inhibition activities against α-glucosidase and tyrosinase, as well as cytotoxicity on HepG2 cells. Moreover, the phenolic profile changes during GID were assessed by UPLC-TOF-MS/MS.

Materials and methods

Chemicals and reagents

Gallic acid, Folin–Ciocalteu reagent (2 N), rutin, DPPH, ABTS, tripyridyltriazine (TPTZ), α-glucosidase, tyrosinase, pepsin, pancreatin, bile salts* and trolox were purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). AB-8 macroporous resins were purchased from Donghong Chemical Co. Ltd. (Xian, China), and HPLC-grade formic acid and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, USA). Cell Counting Kit-8 and HepG2 human liver cancer cells were obtained from the Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The deionized water used in the experiment was procured using a Milli-Q system (Merck Millipore, USA). All other chemicals and reagents used in this study were of analytical grade.

Plant material and sample preparation

The *C. cochinchinensis* roots used in this study were collected from the Ta-pieh Mountains in LiuAn City, Anhui Province, China in September 2019. The *C. cochinchinensis* roots were air dried and then pulverized into a homogenate by a pulverizer (FW100, 107 Tianjin Taistite Instrument Co., Ltd, Tianjin, China) and then passed through 30–40 mesh. Samples were placed in aluminum bags, evacuated and stored at 4 °C until further used.

Preparation of the crude ethanol extracts

The flour of *C. Cochinchinensis* roots was extracted with 80% ethanol solution (v/v) to solvent ratio of 1:20 (w/v) with continuous stirring at room temperature for 2 h. The mixture was centrifuged at 4000 rpm, 25 °C for 15 min, and the residue was re-extracted twice with 80% ethanol as described above. After removal of ethanol with rotary evaporator under vacuum conditions at 50 °C, the crude ethanol extracts (CEE) was obtained, freeze-dried and stored at − 20 °C until use.

Purification of CEE

CEE was further purified to obtain the purified ethanol extracts (PEE) by the AB-8 macroporous adsorption resin according to the method described by Zhu et al. [12] with a slight modification. Briefly, the bioactive components

of CEE were separated and enriched on a glass column (16 × 300 mm) wet-packed with 50 g AB-8 macroporous adsorption resin. The CEE was appropriately diluted with distilled water and loaded onto the column at a constant flow rate of 1.0 mL/min. After reaching equilibration, the column was washed with 100 ml distilled water to remove impurities and eluted with 70% ethanol at the flow velocity of 2 mL/min. At last, all the eluates were concentrated in a vacuum rotary evaporator at 50 °C and then freeze-dried under vacuum conditions. The dry powder of PEE was stored at − 20 °C in the dark for subsequent analysis.

Simulated in vitro gastrointestinal digestion

The in vitro digestion of PEE was carried out according to the method reported by Guimarães Drummond et al. [13], with slight modifications. Briefly, in gastric digestion (GD) step, PEE samples (500 mg) were dispersed in 5 mL of deionized water containing 35 mM NaCl at the ratio of 1:10 (w/v) to obtain 100 mg/mL PEE solution, the pH value was adjusted to 2.0 with 1 M HCl, and the mixtures were incubated in a shaker water bath at 37 °C for 5 min. Then, pepsin (25 mg) was added (E:S 1:20 w/w), and pH value was again adjusted to 2.0. The digestions were shaken at 150 rpm, 37 °C for 90 min. At the end of gastric phase, an aliquot of the supernatant was taken and freeze-dried to yield the gastric digestion-purified ethanol extracts (GD-PEE). Then, the pH value of mixtures was adjusted to 7.0 with 1 M NaHCO$_3$. 2 mg of CaCl$_2$, 45 mg of bile salt and 50 mg of pancreatin (E:S 1:10 w/w) were added, and the pH value of the mixtures from last step was again adjusted to 7.0 with 1 M NaHCO$_3$ to simulate intestinal digestion. After 120 min of intestinal digestion at 37 °C, the mixture was centrifuged at 12,000 rpm for 15 min to remove impurities. The supernatants were freeze-dried to yield the intestinal digested-purified ethanol extracts (ID-PEE) and kept at − 20 °C until further used.

Analyses of total phenolic and total flavonoid

Determination of total phenolic content (TPC)

The total phenolic content was measured using the Folin–Ciocalteu spectrophotometric method [14]. Briefly, 200 μL of sample solution (0.5 mg/mL) diluted with deionized water was mixed with 100 μL of Folin–Ciocalteu reagent (2 N). The mixture was allowed to react for 6 min at ambient temperature in the dark. Then, 1 mL of 7% sodium carbonate solution (Na$_2$CO$_3$) was added to neutralize the reaction. The green color developed after 1 h and then absorbance value at 760 nm measured using a TU-1950 UV–vis spectrophotometer (Purkinje General Instrument Co., Beijing, China). Gallic acid was used as a reference

to obtain the content of phenolic, and the results were expressed as milligram of gallic acid equivalents per gram of dry weight of sample (mg GAE/g dw).

Determination of total flavonoid content (TFC)

The content of total flavonoid in the extract was determined by using the method [15] reported previously, with minor modifications. Briefly, 2.5 mL of sample (0.25 mg/mL) diluted with deionized water and 300 μL of NaNO$_2$ (5%) were mixed for 6 min; then 300 μL of AlCl$_3$ (10%) was added and allowed to react at room temperature for 6 min, followed by addition of 4% sodium hydroxide (4.4 mL), and incubation for 10 min. The absorbance of the mixture was determined at 510 nm using a spectrophotometer. Rutin was used as a reference to obtain the content of total flavonoids, and the results were expressed as milligram of rutin equivalents per gram of dry weight of sample (mg RE/g dw).

Bioaccessibility of phenolic and flavonoid after simulated digestion

The bioaccessibility through an in vitro gastrointestinal digestion was calculated using the following equation:

Bioaccessibility index (%)

$$= (\text{final concentration/initial concentration}) \times 100,$$

where the final concentration is the TPC/TFC at the end of gastric and intestinal digestion, and initial concentration is the TPC/TFC in the undigested purified ethanol extracts (PEE).

Identification of phenolic compounds in C. cochinchinensis roots

Individual phenolic compounds were identified by Agilent LC30 system equipped with a quaternary pump, an autosampler, thermostated column compartment and a diode array detector. Separations were carried out on Agilent C18-H reverse-phase column (150 mm × 2.1 mm × 3.5 μm) at 30 °C. The injection volume was 5 μL. The solvent system, consisting of formic acid (0.1%) in Milli-Q water (A) and acetonitrile (B), was pumped into the HPLC system at 0.6 mL/min, and wavelength of UV detector was set at 280 nm. The gradient conditions were 0–5 min, 5% B; 5–25 min, 5–20% B; 25–30 min, 20–95% B; 30–35 min, 95–5% B.

For identification, an AB SCIEX Triple TOF 5600 + system (SCIEX, Foster City, CA, USA) was used, and the MS spectrum was obtained using electrospray ionization operating in negative modes in the range of m/z 50–1200. Other major parameters were set as follows: ion spray voltage

floating, − 4500 V; curtain gas, 35 psi; ion source gas1, 50 psi; ion source gas 2, 50 psi; source temperature, 550 °C; collision energy, 10 eV; declustering potential, − 80 V. Data were collected in centroid mode using MS^n to obtain both protonated molecular ions and/or adducts and product ions, and MassBank online database (http://www.massbank.jp) was used for analysis.

Determination of the antioxidant activity of phenolic extractsl scavenging activity

DPPH radical scavenging activity was measured using a modified colorimetric method reported by Fraga et al. [16]. A sample solution (2 mL, 0.02 mg/mL) was mixed with 2.0 mL of 95% ethanol solution of DPPH, and the absorbance of the mixture was measured at 517 nm after incubation for 30 min in the dark at the temperature. The scavenging activity of the DPPH radicals was expressed as mmol of Trolox equivalent (TE) per gram of dry weight of sample (mmol TE/g dw).

ABTS radical scavenging activity

The ABTS assay was conducted by the method reported by Fraga et al. [16]. The stock solution of ABTS radical cation (ABTS+) was generated by mixing ABTS solution (7 mM) and potassium persulfate (2.45 mM) in the dark at room temperature for 12–16 h. Prior to analysis, the solution was diluted with ultrapure water until the absorbance of 0.70 ± 0.02 at 734 nm. A sample solution (1 mL, 0.05 mg/mL) and 2 mL of ABTS+ solution were mixed and incubated at room temperature for exactly 10 min and the absorbance measured at 734 nm. The scavenging activity of the ABTS+ radicals was expressed as mmol of Trolox equivalent (TE) per gram of dry weight of sample (mmol TE/g dw).

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay of ethanol extracts was performed using the previous method [16], with some modifications. Briefly, the stock FRAP solution was prepared by mixing 300 mM acetate buffer (pH 3.6) and 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. A sample solution (400 µL, 0.1 mg/mL) was allowed to react with the FRAP solution (3 mL) at 37 °C under dark conditions. Absorbance was recorded at 593 nm after exactly 10 min of the reaction. The results of FRAP were expressed as mmol of Trolox equivalent (TE) per gram of dry weight of sample (mmol TE/g dw).

Cupric ion reducing activity (CUPRAC)

The cupric ion reducing activity was determined using the method reported by Tlili [17]. A sample solution (0.5 mL,

0.05 mg/mL) was added to a premixed reaction mixture containing 1 mL $CuCl_2$ solution (10 mM), 1 mL ethanolic neocuproine solution (7.5 mM) and 1 mL NH_4Ac buffer (1 M, pH 7.0). In addition, distilled water was used instead of $CuCl_2$ as a blank control. Absorbance against a blank reagent was measured at 450 nm after 30 min. The results of CUPRAC were expressed as mmol of Trolox equivalent (TE) per gram of dry weight of sample (mmol TE/g dw).

Phosphomolybdenum method

The total antioxidant capacity (TAC) was measured by phosphomolybdenum method based on the protocol reported by Mocan et al. [18]. To the sample solution (0.5 mL, 0.5 mg/mL), 3 ml of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added and the mixture was incubated at 95 °C for 90 min. Afterwards, the absorbance against a blank was read at 695 nm. The total antioxidant capacity was expressed as mmol of Trolox equivalent (TE) per gram of dry weight of sample (mmol TE/g dw).

Enzyme inhibitory activity

α-Glucosidase inhibition

The α-glucosidase inhibitory activity was analysed according to the previous method [19]. Briefly, the α-glucosidase solution was diluted to 700 U/mL with 10 mM phosphate buffer (pH 6.8). The inhibition activity was assayed by mixing 50 µL of sample solution (25 µg/mL) and 50 µL of α-glucosidase solution, and 50 µL of and incubated at 37 °C for 15 min. After that, 50 µL of 4-N-trophenyl-α-glucopyranoside (PNPG) was added, and incubate at 37 °C for 15 min. Finally, 100 µL of 0.2 M sodium carbonate solution was added to stop the reaction. The absorbance was measured at 405 nm using a microplate reader and compared with a control (buffer instead of the extracts). Acarbose was used as a positive control, and the α-glucosidase inhibitory activities were expressed as millimoles of acarbose equivalents per gram of dry weight of sample (mmol ACE/g dw).

Tyrosinase inhibition

Tyrosinase inhibitory activity was determined according to a method reported previously [20], with slight modifications. Briefly, 100 µL of phosphate buffer (pH 6.8), 100 µL of sample solution (5 µg/mL), and 50 µL of tyrosinase solution were added to a 96-well microplate and incubated at 37 °C for 15 min. Subsequently, 50 µL of L-DOPA was added and then incubated at 37 °C for 15 min. The absorbance was measured at 475 nm with a microplate reader and compared

with a control (without enzyme). Kojic acid was used as a positive control, and the tyrosinase inhibitory activities were expressed as millimoles of kojic acid equivalents per gram of dry weight of sample (mmol KAE/g dw).

Cell culture and cytotoxicity

HepG2 are immortalized cell line consisting of human liver carcinoma cells, derived from the liver tissue of a 15 year-old Caucasian male who had a well-differentiated hepatocellular carcinoma. In this study, HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin. Cells were cultured in cell culture CO_2 incubator at 37 °C under a humidified atmosphere containing 95% air and 5% CO_2.

The viability of HepG2 cells was assessed using the Cell Counting Kit-8 (CCK-8) [21]. Briefly, 100 μL of complete culture medium containing 10^4 HepG2 cells/well was plated in a 96-well plate. After 24 h, the complete medium was replaced by the samples and incubated for another 24 h. After reaction with 10% CCK-8 for 40 min, the optical density (OD) was read with a microplate reader at 450 nm.

$$\text{Cell viability (\%)} = \left[A_{sample} - A_{sample\ background}\right] / \left[A_{control} - A_{control\ background}\right] \times 100.$$

Statistical analysis

Results are presented as mean ± standard deviation ($n = 3$). The statistical differences between the different extracts were analyzed by using one-way analysis of variance (ANOVA), followed by Duncan's test with SPSS statistical 26 software. A p value < 0.05 was considered statistically significant.

Results and discussion

Tentative identification of phenolics from the CEE, PEE, GD-PEE, ID-PEE

UPLC-TOF-MS/MS was used to identify the phytochemicals in the ethanol extracts from *C. cochinchinensis* roots and its digested samples. Fifteen compounds were tentatively identified, based on their retention times, related literature, molecular mass and by comparison of their MS/MS spectra with the online databases, including five phenolic compounds (Peaks 1, 2, 3, 9 and 12) and ten flavonoid compounds (Peaks 4, 5, 6, 7, 8, 10, 11, 13, 14 and 15). MS^2 spectra of tentatively identified compounds are shown in supplementary material. Total ion chromatograms (TIC) in negative mode of *C. cochinchinensis* samples are shown in

Fig. 1. Information of all compounds tentatively identified in this study is summarized in Table 1. Structures of the tentatively identified compounds of CEE, PEE, GD-PEE, and ID-PEE samples from *C. cochinchinensis* roots are shown in Fig. 2. The characteristics of these compounds are discussed as follows:

Compounds **1** and **2** exhibited the molecular ion $[M–H]^-$ at m/z 137.0301 and 153.0181, respectively, and their fragments generated highly significant peaks at m/z 93.0395 and 109.0302. The two compounds were preliminarily determined to be 4-hydroxybenzoic acid [22] and 3,5-dihydroxybenzoic acid [23], respectively. Compound **3** was proposed as 6,7-dihydroxycoumarin based on molecular ion $[M–H]^-$ at m/z 177.0231 with fragments at m/z 149.0273, 133.0318, 89.0415 and 77.0431 by matching the MS and MS/MS data with a report [24]. Compound **4**, with $[M–H]^-$ ion of m/z 449.1089, produced a secondary ion peak at m/z 287.0562 $[M–H–C_6H_{10}O_5]^-$ and 259.0629 $[M–H–C_6H_{10}O_5–CO]^-$, was tentatively identified as eriodictyol-7-O-glucoside, based on the literature [25]. Compounds **5** and **6**, showing a molecular ion at m/z 445.9921 and 609.1472, were identified as biochanin-A8-C-glucoside and luteolin-3',7-di-O-glucoside by matching the online databases. Specifically, compound **5** losing one glucose residue and produced fragment ion at m/z 283.0558, whereas compound **6** losing two glucose residues and produced fragment ions at m/z 447.0986 and 285.0434. Compound **7**, with an $[M–H]^-$ ion of m/z 317.0657, had typical fragments of m/z 177.0213 and m/z 151.0424. These were attributed to the neutral loss of $C_7H_7O_3$ and $C_8H_5O_4$, respectively, resulting in the identification of the compound as myricetin [26]. Compound **8**, with molecular ion $[M–H]^-$ at m/z 303.0540, produced fragments at m/z 285.0437, 217.0538, 177.0214 $[M–H–C_7H_2O_5]^-$, 149.0268 $[M–H–C_7H_2O_5–CO]^-$ and 125.0268, the fragment in accordance with the mass spectrometry fragmentation pattern of taxifolin [27]. Compound **9** was tentatively identified as 9,10-dihydro-2,3,5,7-phenanthrenetetrol by matching with online database with a $[M–H]^-$ at m/z 243.0667, fragmentation ions at m/z 225.0556, 199.0761 and 175.0762. Compound **10** exhibited molecular ion $[M–H]^-$ at m/z 287.0576 and fragments at m/z 259.0620 $[M–H–CO]^-$ and 125.0249 $[M–H–CO–C_8H_6O_2]^-$ and was tentatively identified as dihydrokaempferol by comparison with the data profile with a previous report [24]. Compound **11** was tentatively identified as kaempferol-3-O-glucoside by a related literature, followed by the fragmentation of m/z 447.0950, with a neutral loss of glucose and MS^2 peak at m/z 285.0410 [28]. Compound **12**, with $[M–H]^-$ ion at m/z 227.0750, was tentatively identified as resveratrol as its MS^2 exhibited a product ion at m/z 185.0623 $[M–H–C_2H_2O]^-$ and 143.0516 $[M–H–C_2H_2O–C_2H_2O]^-$, indicating the loss of C_2H_2O

Fig. 1 Total ion chromatogram (TIC) of (**A**) crude ethanol extracts (CEE), (**B**) purified ethanol extracts (PEE), (**C**) gastric digestion-purified ethanol extracts (GD-PEE) and (**D**) intestinal digestion-purified ethanol extracts (ID-PEE) samples in negative ion mode
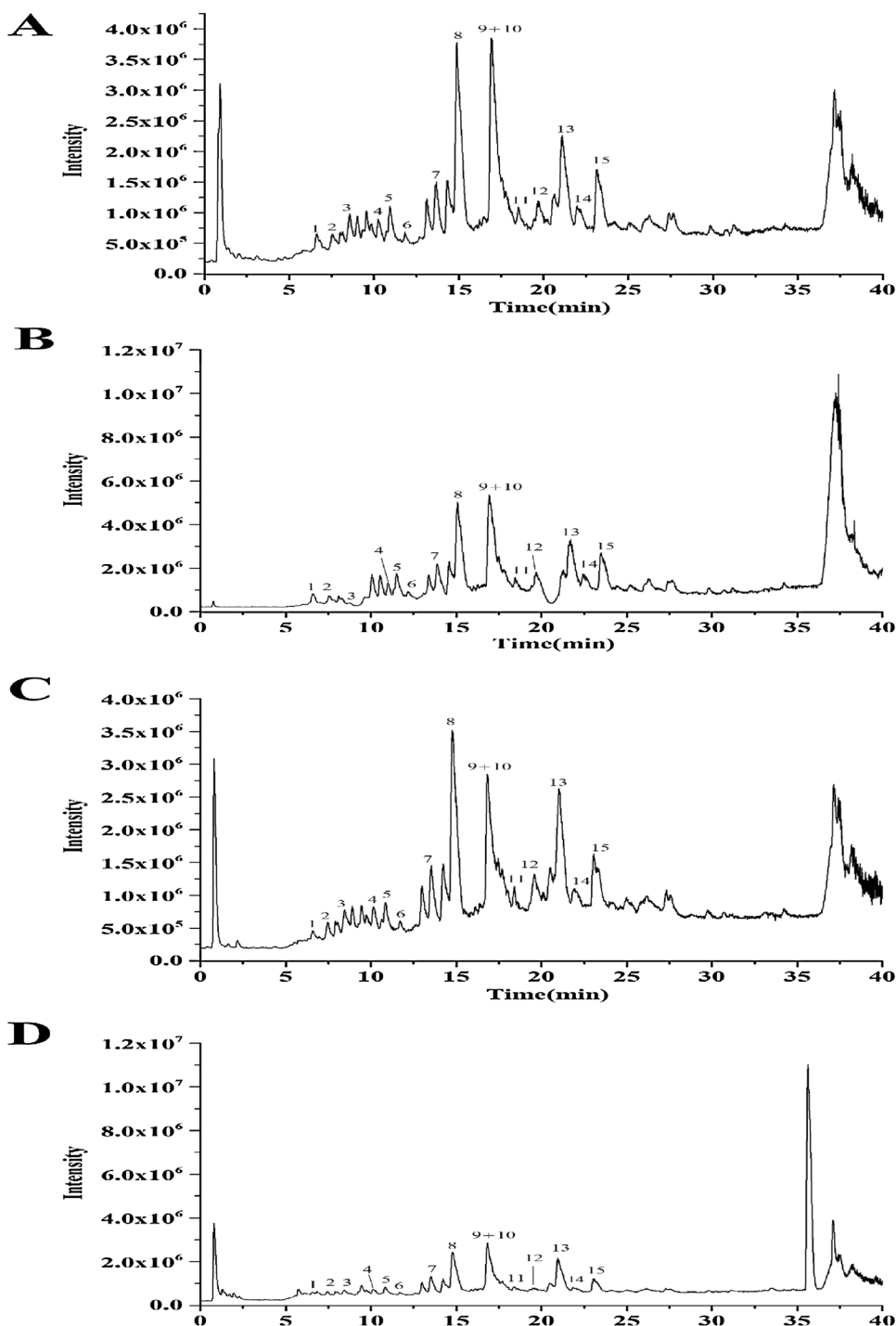
molecule and $2C_2H_2O$, respectively [29]. Compound **13** exhibited the $[M–H]^-$ ion at m/z 301.0368, which further fragmented into m/z 257.0425 $[M–H–CO_2]^-$, 229.0502 $[M–H–CO_2–CO]^-$, 151.0048 $[M–H–C_8H_6O_3]^-$ and 125.0242 $[M–H–C_9H_4O_4]^-$, and was identified as morin by matching the online database and literature [30]. Compound **14**, with $[M–H]^-$ at m/z 285.04 and released fragments at m/z 257.0512 $[M–H–CO]^-$, was thus tentatively

identified as 3,6,3',4'-tetrahydroxyflavone. Compound **15**, with $[M–H]^-$ at m/z 287.0596 and base peak at m/z 161.0252, 135.0456 and 125.0248, was tentatively identified as 3',4',5,7-tetrahydroxyisoflavanone.

As shown in Fig. 1, a total of 15 compounds were also found both in the TIC of GD-PEE and ID-PEE compared with PEE. The peak intensities of most compounds were decreased with sequential gastrointestinal

Table 1 Tentative identification of bioactive compounds in *C. cochinchinensis* root samples (CEE, PEE, GD-PEE, ID-PEE) by UPLC-TOF-MS/MS

| Peak	Error (ppm)	Molecular formula	[M-H]⁻ (m/z)	MS/MS (m/z)	Intensity (×10³)				Intensity change value versus PEE (%)		Tentative identification	Type of compound	Reference
					CEE	PEE	GD-PEE	ID-PEE	GD-PEE	ID-PEE			
1	0	$C_7H_6O_3$	137.0301	93.0395	124.8	167.4	58.4	49.8	34.9	29.7	4-Hydroxybenzoic acid	Phenolic	[22]
2	1.7	$C_7H_6O_4$	153.0181	109.0302	39.2	48.7	34.4	32.5	70.6	66.7	3,5-Dihydroxybenzoic acid	Phenolic	[23]
3	1.3	$C_9H_6O_4$	177.0231	149.0273, 133.0318, 89.0415, 77.0431	110.7	135.9	99.8	81.3	73.4	59.8	6,7-Dihydroxycoumarin	Phenolic	[24]
4	0	$C_{21}H_{22}O_{11}$	449.1089	287.0562, 259.0629	105.1	156.2	93.5	55.9	59.9	35.8	Eriodictyol-7-O-glucoside	Flavonoid	[25]
5	0.6	$C_{22}H_{22}O_{10}$	445.9921	325.0728, 297.0774, 283.0558	75.9	110.2	70.7	51.5	64.2	46.7	Biochanin A8-C-glucoside	Flavonoid	–
6	0.6	$C_{27}H_{30}O_{16}$	609.1472	447.0986, 285.0434	44.9	65.1	42.5	35.9	65.3	55.1	Luteolin-3',7-di-O-glucoside	Flavonoid	–
7	0.6	$C_{15}H_{10}O_8$	317.0657	177.0213, 151.0424	286.5	402.1	278.1	191.7	69.2	47.7	Myricetin	Flavonoid	[26]
8	− 0.5	$C_{15}H_{12}O_7$	303.0540	285.0437, 217.0538, 177.0214, 149.0268, 125.0268	937.8	1159	888.6	657	76.7	56.7	Taxifolin (3,3',4',5,7-pentahydroxyflavanone)	Flavonoid	[27]
9	0.1	$C_{14}H_{12}O_4$	243.0667	225.0556, 199.0761, 175.0762	809.7	928.3	641.7	676.1	69.1	72.8	9,10-Dihydro-2,3,5,7-Phenanthrenetetrol	Phenolic	–
10	0.7	$C_{15}H_{12}O_6$	287.0576	259.0620, 125.0249	246.8	343.7	229.0	174.6	66.6	50.8	Dihydrokaempferol	Flavonoid	[24]
11	0.9	$C_{21}H_{20}O_{11}$	447.0950	285.0410	111.5	161.4	105.0	71.3	65.1	44.2	Kaempferol-3-O-glucoside	Flavonoid	[28]
12	0.7	$C_{14}H_{12}O_3$	227.0750	185.0623, 143.0516	95.6	144.5	81.6	61.2	56.5	42.4	Resveratrol	Phenolic	[29]
13	0.4	$C_{15}H_{10}O_7$	301.0368	257.0425, 229.0502, 151.0048, 125.0242	553.5	715.5	496.1	573.9	69.3	80.2	Morin	Flavonoid	[30]
14	0	$C_{15}H_{10}O_6$	285.0421	257.0512	129.9	195.2	109.4	68.2	56.0	34.9	3,6,3',4'-Tetrahydroxyflavone	Flavonoid	–
15	0.6	$C_{15}H_{12}O_6$	287.0596	161.0252, 135.0456, 125.0248	342.4	463.0	295.8	220.5	63.9	47.6	3',4',5,7-Tetrahydroxyisoflavanone	Flavonoid	–
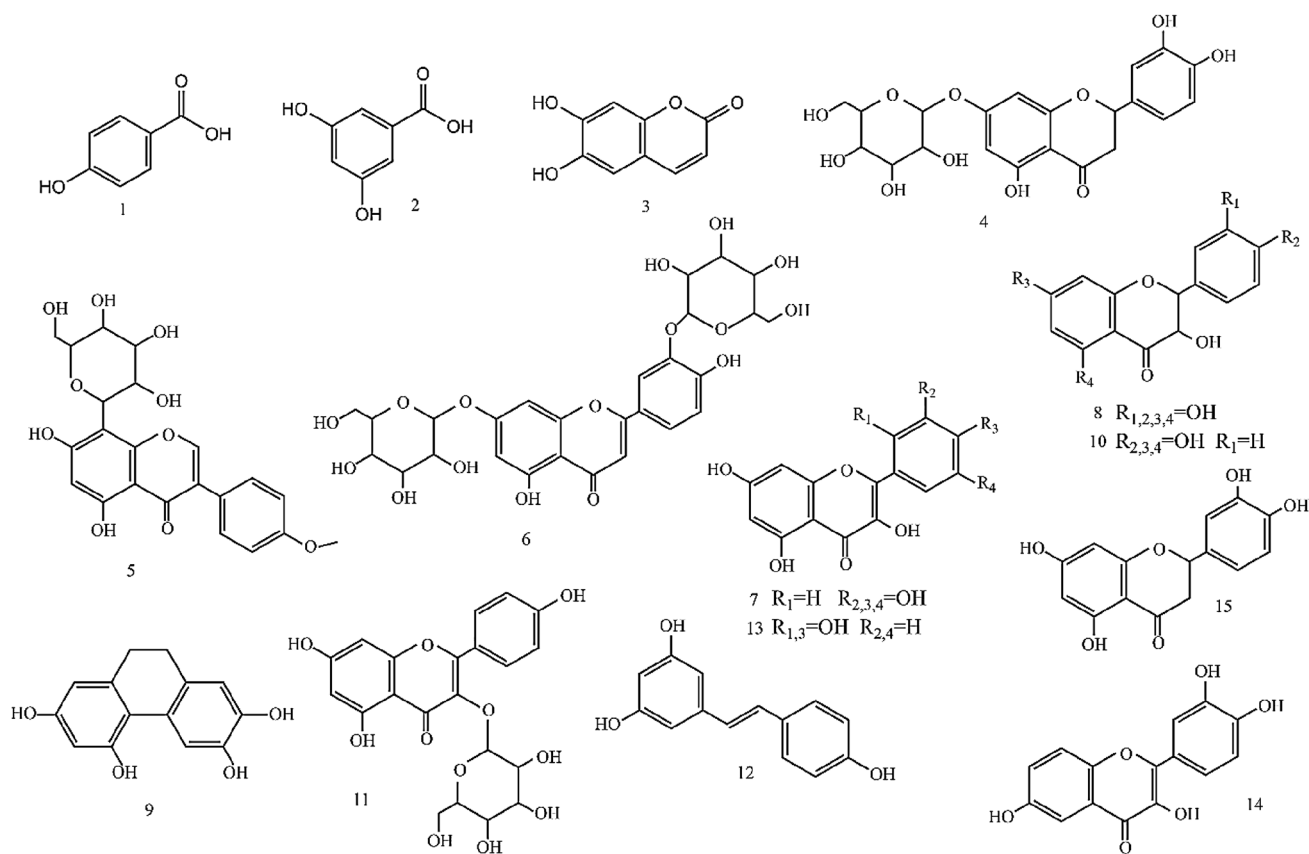
Fig. 2 Structures of the phenolic compounds identified in CEE, PEE, GD-PEE, ID-PEE samples from *C. cochinchinensis* roots. *CEE* crude ethanol extracts; *PEE* purified ethanol extracts; *GD-PEE* gastric digestion-purified ethanol extracts; *ID-PEE* intestinal digestion-purified ethanol extracts

digestion (Table 1). This may be due to the interaction between the compounds or the bonding of the compounds to the enzyme. Cao et al. [31] reported that simulated gastrointestinal digestion changed the structures of some flavonoids. Interestingly, the intensity of 9,10-dihydro-2,3,5,7-phenanthrenetetrol (compound 9) after intestinal digestion was 1.05 times than after gastric digestion, and the intensity of morin (compound 13) after intestinal digestion was 1.16 times than gastric digestion. These findings may be explained by the release of phenolic compounds from test matrix after intestinal digestion due to the break of the bonds of these compounds to sugar residues, proteins or fibers [32]. Quatrin et al. [33] reported the same phenomenon of phenolic compounds in jaboticaba (*Myrciaria trunciflora*) fruit peel after in vitro GID. A similar result was observed by Zhang et al. [34] in digested *Cinnamomum camphora* seed kernel.

Effect of gastrointestinal digestion on TPC, TFC and bioaccessibility

The crude ethanol extracts (CEE) from *C. cochinchinensis* roots were purified by AB-8 macroporous adsorption resin to obtain purified ethanol extracts (PEE). As shown in Fig. 3A, the TPC (596.18 ± 1.79 mg GAE/g dw) and TFC (379.64 ± 1.83 mg RE/g dw) in PEE were the highest. Compared with CEE, the contents of total phenolic and total flavonoid increased 52.92% and 36.73%, respectively. Zhu et al. [12] studied phenolic compounds from *Sanguisorba. officinalis* L. and found that TPC was increased from 31.50 ± 1.31 mg GAE/g dw to 338.92 ± 1.06 mg GAE/g dw after purification by macroporous resin adsorption. A similar result in *Potentilla discolor* Bge was reported [35]. By purification, the TPC of the ethanol extracts was significantly increased, which is helpful for us to study the effect of in vitro gastrointestinal digestion on phenolic compounds.

Simulated GID had a significant effect ($P < 0.05$) on TPC and TFC of PEE from *C. cochinchinensis* roots. As shown in Fig. 3A, the phenolic contents of GD-PEE and
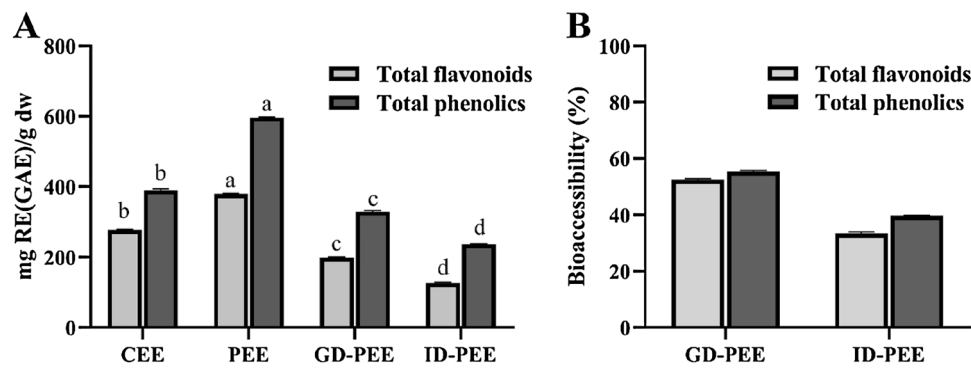
Fig. 3 Contents (**A**) of total phenolic and total flavonoid in CEE, PEE, GD-PEE, ID-PEE samples and bioaccessibility (**B**) of GD-PEE, ID-PEE from *C. cochinchinensis* roots. *CEE* crude ethanol extracts; *PEE* purified ethanol extracts; *GD-PEE* gastric digestion-purified eth- anol extracts; *ID-PEE* intestinal digestion-purified ethanol extracts. *GAE* gallic acid equivalent; *RE* rutin equivalent; *dw* dry weight. Data are mean ($n = 3$) ± standard deviation. Different letters represent sig- nificant differences ($p < 0.05$)

ID-PEE were significantly decreased by simulated in vitro digestion. The contents of total phenolic obtained in the *vitro* digestion were 329.85 mg GAE/g dw for gastric phase and 236.70 mg GAE/g dw for intestinal phase. Compared with the undigested PEE, the TPC was reduced by 44.67% after the gastric phase and by 60.29% at the end of the intestinal phase. The losses of phenolic compounds may be due to their interaction with other compounds and diges- tive enzymes or changes in molecular structure, which may influence their solubility [36]. In addition, the phenolic compounds in extracts had poor stability during GID. The decreased TPC may be caused by poor stability of phenolic compounds. Similar results have been reported by Quatrin et al. [33] and Bouayed et al. [37]. Moreover, the stability and bioaccessibility of phenolic compounds were found to mainly depend on pH value in the intestinal phase [38]. Some phenolic compounds may be degraded by non-enzymatic oxidation under near neutral conditions and in the presence of oxygen, which may be the reason for the further decrease of TPC in the intestinal stage. Similar results were previously found in the simulated GID. For example, Thomas-Valdés et al. [39] reported that the TPC in Chilean white strawberry were decreased by 18.0% after gastric phase and 55.7% after intestinal phase. Chilean red strawberry showed a high stability of TPC after the gastric phase (96.6%), followed by a significant reduction (43.0%) after the intestinal phase [40].

The total flavonoid contents were 199.09 mg RE/g dw and 126.66 mg RE/g dw for GD-PEE and ID-PEE, respectively. Compared with the PEE (undigested), the TFC was reduced by 47.55% after the gastric phase and by 66.63% at the end of the intestinal phase. As shown in Fig. 3, the loss of TFC was high than TPC. This phenomenon may be because fla- vonoids were less stable than phenolics in gastrointestinal digestion and may react with OH^- or be degraded under high pH conditions [11].

Gastrointestinal digestion affects bioaccessibility of phe- nolic and flavonoid compounds. At the gastric digestion stage, the bioaccessibility of total phenolic and flavonoids were 55.66% and 52.44%, respectively. After the intestinal digestion, the bioaccessibility of total phenolic and flavonoid contents were reduced to 39.70% and 33.36%, respectively (Fig. 3B). Different types of compounds were all affected by gastrointestinal digestion. As shown in Table 1, individual compounds that had less effect on bioavailability in gastric digestion were 3,5-dihydroxybenzoic acid, 6,7-dihydroxy- coumarin, myricetin, taxifolin, 9,10-dihydro-2,3,5,7-phen- anthreneterol and morin. However, individual compounds that had less effect on bioavailability in intestinal digestion were 3,5-dihydroxybenzoic acid, 9,10-dihydro-2,3,5,7-phen- anthreneterol and morin. The biotransformation of phenolic compounds during GID might affect their bioaccseeibility and be related to changes on their bioactivity [41], but the reasons still need further investigated.

Effect of gastrointestinal digestion on antioxidant activity

Five different antioxidant assays were used to investigate the antioxidant activity of CEE, PEE and its digested samples. As shown in Fig. 4, the antioxidant activity of PEE from *C. cochinchinensis* roots was significantly increased compared with CEE. Liao et al. [6] also reported that the extracts of *C. cochinchinensis* roots had higher DPPH radical scavenging activity and ferric ion reducing ability.

A significant loss of the antioxidant activity was observed throughout the in vitro GID (Fig. 4). The scavenging activ- ity of the samples towards the DPPH and ABTS+ radical decreased throughout the GID process (Fig. 4A, B). In DPPH assay, the values of PEE, GD-PEE and ID-PEE were 1.80 mmol TE/g dw, 1.27 mmol TE/g dw and 1.03 mmol TE/g dw. Compared with PEE, the values were 29.44% and
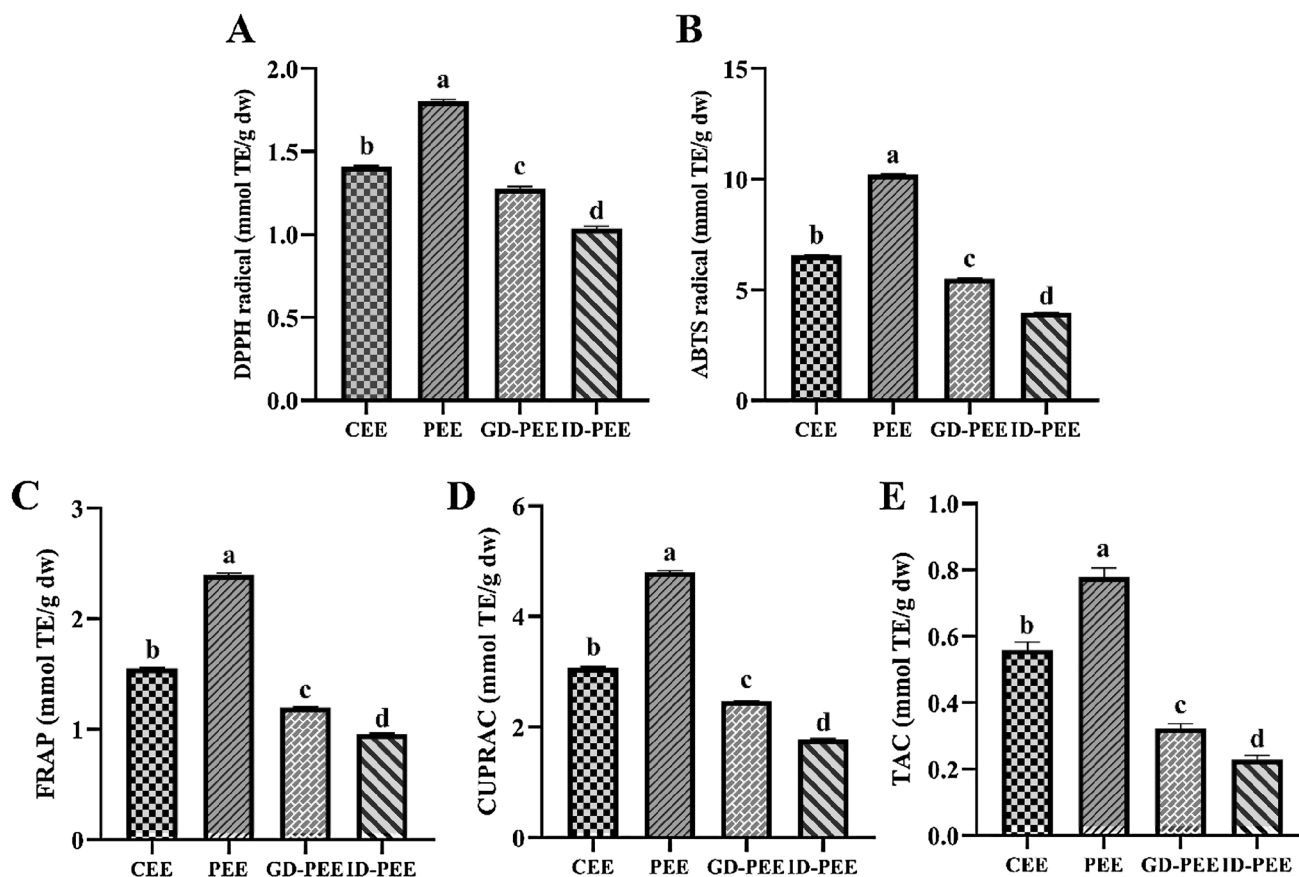
Fig. 4 Antioxidant activities of CEE, PEE, GD-PEE and ID-PEE expressed in mmol TE/g dw, including DPPH (**A**), ABTS (**B**), FRAP (**C**), CUPRAC (**D**) and total antioxidant activity (**E**) assays. *CEE* crude ethanol extracts; *PEE* purified ethanol extracts; *GD-PEE* gastric digestion-purified ethanol extracts; *ID-PEE* intestinal digestion-purified ethanol extracts; *TE* trolox equivalent; *dw* dry weight. Data are mean ($n = 3$) ± standard deviation. Different letters represent significant differences ($p < 0.05$)

42.77% lower after the gastric and intestinal phase, respectively. Similarly, the ABTS+ scavenging activity of PEE was the highest (10.19 mmol TE/g dw), followed by GD-PEE (5.49 mmol TE/g dw) and ID-PEE (3.95 mmol TE/g dw). ABTS activity was reduced 46.12% after the gastric digestion, with a further loss of 15.11% after the intestinal digestion. The results were similar to that of Lucas-González et al. [42] who found that DPPH scavenging activity of persimmon fruit co-products were decreased 13.1% and 30.8%, respectively, after gastric and intestinal digestion. Tagliazucchi et al. [43] also reported that ABTS radical scavenging activity of grape polyphenols decreased during in vitro digestion.

In the FRAP and CUPRAC assays, PEE also showed the highest reducing power activity (2.39 mmol TE/g dw and 4.81 mmol TE/g dw, respectively). After gastric digestion, the values of FRAP and CUPRAC were reduced by 50.21% and 48.75% compared with PEE, respectively. At the end of intestinal digestion, the values of FRAP and CUPRAC were reduced by 59.83% and 63.12% compared with PEE, respectively. Similar results were observed in Ribes magellanicum

[44] and different varieties of apple during in vitro GID [37]. On the contrary, Gullon et al. [45] found that FRAP of pomegranate peel flour was increased slightly after gastric digestion. This observation may be attributed to different types of phenolic compounds.

Total antioxidant capacity (TAC) is also an important indicator to assess the effect of in vitro digestion on antioxidant activity. A reduction of the TAC of the digested samples were observed throughout the digestion process. The TAC values at the gastric and intestinal digestion stage were reduced by 58.47% and 70.13%, respectively, with respect to the undigested PPE. Our results were in agreement with Kosti'c et al. [46] who found that TAC of phenolic from goat milk was decreased after in vitro digestion.

These results are significantly correlated with the changes in TPC during the GID process (Table 2, $p < 0.01$). Naeimi et al. [47] reported that myricetin, dihydrokaempferol and morin had a strong ability to scavenge DPPH free radicals, so the decrease in scavenging activity of DPPH free radicals may be closely related to the decrease in the contents of myricetin, morin and dihydrokaempferol, as shown in

Table 2 Coefficients of correlation between the TPC or TFC and antioxidant activity (DPPH, ABTS, FRAP, CUPRAC, TAC), enzyme inhibition activity (α-glucosidase, tyrosinase)

	DPPH	ABTS	FRAP	CUPRAC	TAC	α-Glucosidase	Tyrosinase
TPC	0.996**	1.000**	0.995**	0.999**	0.968**	0.982**	0.956**
TFC	0.989**	0.980**	0.977**	0.983**	0.990**	0.978**	0.927**

Data represent Pearson correlation coefficients. ** indicates $p < 0.01$

Table 1. In addition, the changes in ferric reducing antioxidant power may be closely related to myricetin and taxifolin contents [48]. Many previous studies have confirmed the relationship between TPC and antioxidant activity. Compounds with strong antioxidant activity, such as luteolin-3',7-di-o-glucoside (compound 6) and resveratrol (compound 12), also decreased significantly after GID process. The change in the pH value of the food matrix from the gastric to the intestine also had a certain effect on the phenolic compounds [49]. The hydroxyl groups on the aromatic ring were partially deprotonated, and the ionization balance changed [37], resulting in a reduction in antioxidant capacity.

Effect of gastrointestinal digestion on enzyme inhibitory activity

The PEE from *C. cochinchinensis* roots showed the highest α-glucosidase inhibitory activity (0.51 mmol ACE/g dw). However, the in vitro digestion significantly reduced the inhibitory activity after the gastric (39.21%) and intestinal phases (50.98%), respectively (Table 3). The loss of inhibitory activity was correlated with the changes in the TPC throughout the GID. Similar results were reported previously. For example, Thomas-Valdés et al. [40] found that the inhibitory activity of the phenolics from the native Chilean red strawberry towards α-glucosidase was decreased gradually by the gastric and intestinal digestion. Interestingly, Kasipandi et al. [50] reported that α-glucosidase inhibitory activity of

Opilia amentacea roxb fruit extracts increased slightly after gastric digestion and finally decreased significantly after the intestinal digestion. In addition, the type and structure of phenolic compounds in the samples may play an important role in enzyme inhibition. For example, resveratrol caused structural changes of the enzyme through non-competitive binding with the α-glucosidase region, thereby reducing enzyme activity [51]. Myricetin interacts with key amino acids of α-glucosidase through hydrogen bonds to inhibit α-glucosidase activity [52]. As shown in Table 1, the peak intensities of myricetin, resveratrol and most phenolic compounds were decreased gradually through in vitro digestion. This finding also explains why the inhibitory activity of α-glucosidase was reduced after GID.

Tyrosinase is a copper-containing polyphenol oxidase and is responsible for browning reactions through the phylogenetic scale. Over-activity of this enzyme was associated with human freckles, brown spots and neurodegenerative disorders [53]. As shown in Table 3, the PEE exhibited the highest inhibition activity, and the inhibitory activity was significantly decreased after the gastric digestion. It is worth noting that there were no significant difference in the inhibitory activity of tyrosinase after gastric digestion and intestinal digestion. This phenomenon may be due to the fact that the contents of phenolic compounds that inhibit tyrosinase were not significantly reduced after gastric digestion. The results suggested that phenolic compounds of *C. cochinchinensis* roots play an important role in the inhibitory activities for tyrosinase. It was also reported that the extracts of *C.*

Table 3 Enzyme inhibitory activities of *C. cochinchinensis* root extracts (CEE, PEE, GD-PEE, ID-PEE)

Assays	CEE	PEE	GD-PEE	ID-PEE
α-Glucosidase inhibition (mmol ACE/g dw)	0.38 ± 0.006^{b}	0.51 ± 0.014^{a}	0.31 ± 0.016^{c}	0.25 ± 0.021^{d}
Tyrosinase inhibition (mmol KAE/g dw)	64.10 ± 1.705^{b}	87.96 ± 2.294^{a}	54.30 ± 4.523^{c}	52.65 ± 2.467^{c}

CEE crude ethanol extracts; *PEE* purified ethanol extracts; *GD-PEE* gastric digestion-purified ethanol extracts; *ID-PEE* intestinal digestion-purified ethanol extracts; *ACE* acarbose equivalent; *KAE* Kojic acid equivalent; *dw* dry weight

Data are mean ($n = 3$) ± standard deviation

Different letters (a–d) in the same row indicate significant difference ($p < 0.05$)

Fig. 5 The viability of HepG2 cells after 24 h by crude ethanol extracts (CEE), purified ethanol extracts (PEE), gastric digestion-purified ethanol extracts (GD-PEE) and intestinal digestion-purified ethanol extracts (ID-PEE). CCK-8 assay was used to assess cell viability. Data are mean ($n = 6$) ± standard deviation. ** indicates $p < 0.05$
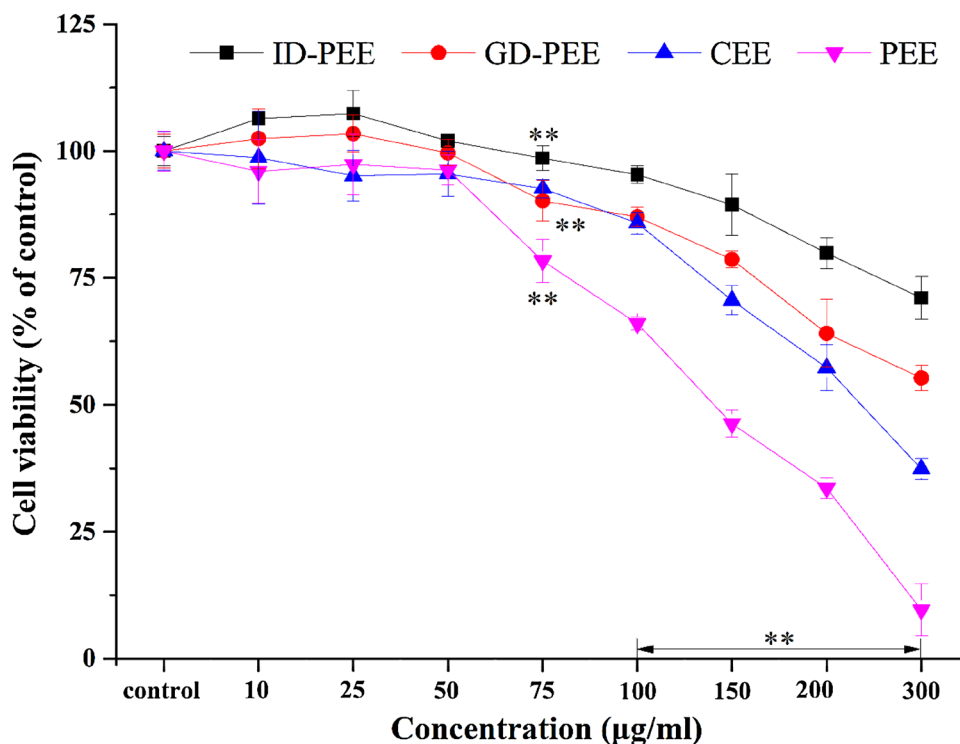
Cytotoxic effects on HepG2 cells

As shown in Fig. 5, compared with the control, there was no significant difference in the viability of HepG2 cells when the concentration of PEE, GD-PEE and ID-PEE was lower than 75 μg/mL. In addition, there was no significant difference in the cell viability when the concentration of CEE was lower than 100 μg/mL. However, compared with PEE, the cell viability significantly changed after simulated digestion, and the highest cell viability was observed in the ID-PEE group. This result differences may be due to the decrease in the contents of phenolic compounds from the *C. cochinchinensis* roots after simulated GID [54].

Correlation between the parameters

A Pearson's correlation analysis was used to determine the correlation coefficients between the TPC or TFC and the bioactivities of ethanol extracts from *C. cochinchinensis* during GID. As shown in Table 2, in the simulated GID process, there were significantly positive correlations between the TPC/TFC and the antioxidant or enzyme inhibitory activity, especially the ability to scavenge ABTS+ free radicals and cupric reducing antioxidant. These results further demonstrated that the TPC/TFC of ethanol extracts from

C. cochinchinensis roots during simulated GID play important roles in the bioactivity. These results were consistent with those of Lucas-Gonzalez et al. [55] and Arruda et al. [56] reporting that the phenolic and flavonoid compounds were the main contributors to the antioxidant activities from maqui berry and edible part of araticum fruit.

Conclusions

The study investigated the influence of simulated gastrointestinal digestion on phenolic bioaccessibility and bioactivity of *C. cochinchinensis* roots. Results showed that the total phenolic/flavonoid contents were generally decreased in the order of sequential digestions. Fifteen phenolic substances were tentatively identified by UPLC-TOF-MS/MS before and after simulated digestion, including ten flavonoids and five phenolics. These changes in the total phenolic/flavonoid contents were significantly correlated with the loss of antioxidant activity and enzyme inhibitory activity, as well as cytotoxicity on HepG2 cells. Overall, our results have helped understand the effects of gastrointestinal digestion on the bioactivity and bioaccessibility of ethanol extracts from *C. cochinchinensis* roots and provided a theoretical basis for potential applications in the food, cosmetics and pharmaceutical industries. However, these conclusions need to be confirmed by further studies in vivo and security evaluation.

Declarations

Conflict of interest All authors declare that they have no conflict of interests.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. Kubola J, Siriamornpun S (2008) Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. Food Chem 110(4):881–890. https://doi.org/10.1016/j.foodchem.2008.02.076

2. Dasgupta N, De B (2007) Antioxidant activity of some leafy vegetables of India: a comparative study. Food Chem 101(2):471–474. https://doi.org/10.1016/j.foodchem.2006.02.003

3. Kwon YI, Apostolidis E, Shetty K (2008) In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour Technol 99(8):2981–2988. https://doi.org/10.1016/j.biortech.2007.06.035

4. Wang LX, Zheng HR, Ren FC, Chen TG, Li XM, Jiang XJ, Wang F (2017) Polysubstituted Isoflavonoids from *Spatholobus suberectus*, *Flemingia macrophylla*, and *Cudrania cochinchinensis*. Nat Prod Bioprospect 7(2):201–206. https://doi.org/10.1007/s13659-017-0121-2

5. Zheng ZP, Zhu Q, Fan CL, Tan HY, Wang M (2011) Phenolic tyrosinase inhibitors from the stems of *Cudrania cochinchinensis*. Food Funct 2(5):259–264. https://doi.org/10.1039/c1fo10033e

6. Liao WC, Lin C-F, Lin C-C, He R-F, Chen C-C, Huang W-Y (2018) Biofunctionality studies of *Cudrania cochinchinensis* extracts. Am J Anal Chem 09(01):1–14. https://doi.org/10.4236/ajac.2018.91001

7. Zhou Q, Chen L, Yin HJ, Li B, Tian Y, Chen HW, Xiao YH, He XH, Zeng YL, Dong JX (2014) Two new flavonols from *Cudrania cochinchinensis*. J Asian Nat Prod Res 16(10):976–981. https://doi.org/10.1080/10286020.2014.923843

8. Hollebeeck S, Borlon F, Schneider YJ, Larondelle Y, Rogez H (2013) Development of a standardised human in vitro digestion protocol based on macronutrient digestion using response surface methodology. Food Chem 138(2–3):1936–1944. https://doi.org/10.1016/j.foodchem.2012.11.041

9. Hur SJ, Lim BO, Decker EA, McClements DJ (2011) In vitro human digestion models for food applications. Food Chem 125(1):1–12. https://doi.org/10.1016/j.foodchem.2010.08.036

10. Correa-Betanzo J, Allen-Vercoe E, McDonald J, Schroeter K, Corredig M, Paliyath G (2014) Stability and biological activity of wild blueberry (*Vaccinium angustifolium*) polyphenols during simulated in vitro gastrointestinal digestion. Food Chem 165:522–531. https://doi.org/10.1016/j.foodchem.2014.05.135

11. Goulas V, Hadjisolomou A (2019) Dynamic changes in targeted phenolic compounds and antioxidant potency of carob fruit (*Ceratonia siliqua* L.) products during in vitro digestion. LWT-Food Sci Technol 101:269–275. https://doi.org/10.1016/j.lwt.2018.11.003

12. Zhu H-l, Chen G, Chen S-n, Wang Q-r, Wan L, Jian S-p (2019) Characterization of polyphenolic constituents from *Sanguisorba officinalis* L. and its antibacterial activity. Eur Food Res Technol 245(7):1487–1498. https://doi.org/10.1007/s00217-019-03276-2

13. Guimaraes Drummond ESF, Miralles B, Hernandez-Ledesma B, Amigo L, Iglesias AH, Reyes Reyes FG, Netto FM (2017) Influence of protein-phenolic complex on the antioxidant capacity of flaxseed (*Linum usitatissimum* L.) products. J Agric Food Chem 65(4):800–809. https://doi.org/10.1021/acs.jafc.6b04639

14. Tian J, Chen H, Chen S, Xing L, Wang Y, Wang J (2013) Comparative studies on the constituents, antioxidant and anticancer activities of extracts from different varieties of corn silk. Food Funct 4(10):1526–1534. https://doi.org/10.1039/c3fo60171d

15. Szczepaniak O, Cielecka-Piontek J, Kobus-Cisowska J (2020) Hypoglycaemic, antioxidative and phytochemical evaluation of Cornus mas varieties. Eur Food Res Technol 247(1):183–191. https://doi.org/10.1007/s00217-020-03616-7

16. Nascimento Fraga L, de Souza Oliveira AK, Pinheiro Aragao B, Alves de Souza D, Propheta Dos Santos EW, Alves Melo J, Mara de Oliveira ESA, Wisniewski Junior A, Bani Correa C, de Andrade Wartha ERS, Bacci L, Montezano de Carvalho IM (2021) Mass spectrometry characterization, antioxidant activity, and cytotoxicity of the peel and pulp extracts of Pitomba. Food Chem 340:127929. https://doi.org/10.1016/j.foodchem.2020.127929

17. Tlili N, Kirkan B, Sarikurkcu C (2019) LC–ESI–MS/MS characterization, antioxidant power and inhibitory effects on α-amylase and tyrosinase of bioactive compounds from hulls of *Amygdalus communis*: the influence of the extracting solvents. Ind Crop Prod 128:147–152. https://doi.org/10.1016/j.indcrop.2018.11.014

18. Mocan A, Moldovan C, Zengin G, Bender O, Locatelli M, Simirgiotis M, Atalay A, Vodnar DC, Rohn S, Crisan G (2018) UHPLC-QTOF-MS analysis of bioactive constituents from two Romanian Goji (*Lycium barbarum* L.) berries cultivars and their antioxidant, enzyme inhibitory, and real-time cytotoxicological evaluation. Food Chem Toxicol 115:414–424. https://doi.org/10.1016/j.fct.2018.01.054

19. Chen B, Long P, Sun Y, Meng Q, Liu X, Cui H, Lv Q, Zhang L (2017) The chemical profiling of loquat leaf extract by HPLC-DAD-ESI-MS and its effects on hyperlipidemia and hyperglycemia in rats induced by a high-fat and fructose diet. Food Funct 8(2):687–694. https://doi.org/10.1039/c6fo01578f

20. Yang D, Wang L, Zhai J, Han N, Liu Z, Li S, Yin J (2021) Characterization of antioxidant, alpha-glucosidase and tyrosinase inhibitors from the rhizomes of *Potentilla anserina* L. and their structure-activity relationship. Food Chem 336:127714. https://doi.org/10.1016/j.foodchem.2020.127714

21. Pietrzyk N, Zakłos-Szyda M, Koziołkiewicz M, Podsędek A (2021) *Viburnum opulus* L. fruit phenolic compounds protect against FFA-induced steatosis of HepG2 cells via AMPK pathway. J Funct Foods. https://doi.org/10.1016/j.jff.2021.104437

22. Assefa AD, Jeong YJ, Kim DJ, Jeon YA, Ok HC, Baek HJ, Sung JS (2018) Characterization, identification, and quantification of phenolic compounds using UPLC-Q-TOF-MS and evaluation of antioxidant activity of 73 *Perilla frutescens* accessions. Food Res Int 111:153–167. https://doi.org/10.1016/j.foodres.2018.05.017

23. Cheiran KP, Raimundo VP, Manfroi V, Anzanello MJ, Kahmann A, Rodrigues E, Frazzon J (2019) Simultaneous identification of low-molecular weight phenolic and nitrogen compounds in craft

beers by HPLC-ESI-MS/MS. Food Chem 286:113–122. https://doi.org/10.1016/j.foodchem.2019.01.198

24. Xiong Y, Zhang P, Warner RD, Shen S, Johnson S, Fang Z (2020) Comprehensive profiling of phenolic compounds by HPLC-DAD-ESI-QTOF-MS/MS to reveal their location and form of presence in different sorghum grain genotypes. Food Res Int 137:109671. https://doi.org/10.1016/j.foodres.2020.109671

25. Nie R, Zhang Y, Jin Q, Zhang S, Wu G, Chen L, Zhang H, Wang X (2021) Identification and characterisation of bioactive compounds from the seed kernels and hulls of *Paeonia lactiflora* Pall by UPLC-QTOF-MS. Food Res Int 139:109916. https://doi.org/10.1016/j.foodres.2020.109916

26. Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arraez-Roman D, Segura-Carretero A (2015) HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. Food Chem 166:179–191. https://doi.org/10.1016/j.foodchem.2014.06.011

27. Santos SAO, Villaverde JJ, Freire CSR, Domingues MRM, Neto CP, Silvestre AJD (2012) Phenolic composition and antioxidant activity of *Eucalyptus grandis*, *E. urograndis* (*E. grandis × E. urophylla*) and *E. maidenii* bark extracts. Ind Crop Prod 39:120–127. https://doi.org/10.1016/j.indcrop.2012.02.003

28. Peng H, Li W, Li H, Deng Z, Zhang B (2017) Extractable and non-extractable bound phenolic compositions and their antioxidant properties in seed coat and cotyledon of black soybean (*Glycinemax* (L.) merr). J Funct Foods 32:296–312. https://doi.org/10.1016/j.jff.2017.03.003

29. Zengin G, Diuzheva A, Jekő J, Cziáky Z, Bulut G, Dogan A, Haznedaroglu MZ, Rengasamy KRR, Lobine D, Bahadori MB, Mahomoodally MF (2018) HPLC–MS/MS-based metabolic profiling and pharmacological properties of extracts and infusion obtained from *Amelanchier parviflora* var. dentata. Ind Crop Prod 124:699–706. https://doi.org/10.1016/j.indcrop.2018.08.042

30. Díaz-de-Cerio E, Gómez-Caravaca AM, Verardo V, Fernández-Gutiérrez A, Segura-Carretero A (2016) Determination of guava (*Psidium guajava* L.) leaf phenolic compounds using HPLC-DAD-QTOF-MS. J Funct Foods 22:376–388. https://doi.org/10.1016/j.jff.2016.01.040

31. Cao Q, Teng J, Wei B, Huang L, Xia N (2021) Phenolic compounds, bioactivity, and bioaccessibility of ethanol extracts from passion fruit peel based on simulated gastrointestinal digestion. Food Chem 356:129682. https://doi.org/10.1016/j.foodchem.2021.129682

32. Qin Y, Wang L, Liu Y, Zhang Q, Li Y, Wu Z (2018) Release of phenolics compounds from *Rubus idaeus* L. dried fruits and seeds during simulated in vitro digestion and their bio-activities. J Funct Foods 46:57–65. https://doi.org/10.1016/j.jff.2018.04.046

33. Quatrin A, Rampelotto C, Pauletto R, Maurer LH, Nichelle SM, Klein B, Rodrigues RF, Maróstica Junior MR, Fonseca BdS, de Menezes CR, Mello RdO, Rodrigues E, Bochi VC, Emanuelli T (2020) Bioaccessibility and catabolism of phenolic compounds from jaboticaba (*Myrciaria trunciflora*) fruit peel during in vitro gastrointestinal digestion and colonic fermentation. J Funct Foods. https://doi.org/10.1016/j.jff.2019.103714

34. Zhang G, Yan X, Wu S, Ma M, Yu P, Gong D, Deng S, Zeng Z (2020) Ethanol extracts from *Cinnamomum camphora* seed kernel: potential bioactivities as affected by alkaline hydrolysis and simulated gastrointestinal digestion. Food Res Int 137:109363. https://doi.org/10.1016/j.foodres.2020.109363

35. Cheng D, Wang P, Huang J, Yang B, Ma M, Yu P, Zeng Z, Gong D, Deng S (2020) Antioxidant, antidiabetic and identification of phenolic constituents from Potentilla discolor Bge. Eur Food Res Technol 246(10):2007–2016. https://doi.org/10.1007/s00217-020-03551-7

36. Bohn T (2014) Dietary factors affecting polyphenol bioavailability. Nutr Rev 72(7):429–452. https://doi.org/10.1111/nure.12114

37. Bouayed J, Hoffmann L, Bohn T (2011) Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: bioaccessibility and potential uptake. Food Chem 128(1):14–21. https://doi.org/10.1016/j.foodchem.2011.02.052

38. Alminger M, Aura AM, Bohn T, Dufour C, El SN, Gomes A, Karakaya S, Martinez-Cuesta MC, McDougall GJ, Requena T, Santos CN (2014) In vitro models for studying secondary plant metabolite digestion and bioaccessibility. Compr Rev Food Sci Food Saf 13(4):413–436. https://doi.org/10.1111/1541-4337.12081

39. Thomas-Valdes S, Theoduloz C, Jimenez-Aspee F, Burgos-Edwards A, Schmeda-Hirschmann G (2018) Changes in polyphenol composition and bioactivity of the native Chilean white strawberry (*Fragaria chiloensis* spp. chiloensis f. chiloensis) after in vitro gastrointestinal digestion. Food Res Int 105:10–18. https://doi.org/10.1016/j.foodres.2017.10.074

40. Thomas-Valdes S, Theoduloz C, Jimenez-Aspee F, Schmeda-Hirschmann G (2019) Effect of simulated gastrointestinal digestion on polyphenols and bioactivity of the native Chilean red strawberry (*Fragaria chiloensis* ssp. chiloensis f. patagonica). Food Res Int 123:106–114. https://doi.org/10.1016/j.foodres.2019.04.039

41. Del Rio D, Costa LG, Lean ME, Crozier A (2010) Polyphenols and health: what compounds are involved? Nutr Metab Cardiovasc Dis 20(1):1–6. https://doi.org/10.1016/j.numecd.2009.05.015

42. Lucas-Gonzalez R, Viuda-Martos M, Perez Alvarez JA, Fernandez-Lopez J (2018) Changes in bioaccessibility, polyphenol profile and antioxidant potential of flours obtained from persimmon fruit (*Diospyros kaki*) co-products during in vitro gastrointestinal digestion. Food Chem 256:252–258. https://doi.org/10.1016/j.foodchem.2018.02.128

43. Tagliazucchi D, Verzelloni E, Bertolini D, Conte A (2010) In vitro bio-accessibility and antioxidant activity of grape polyphenols. Food Chem 120(2):599–606. https://doi.org/10.1016/j.foodchem.2009.10.030

44. Burgos-Edwards A, Jimenez-Aspee F, Thomas-Valdes S, Schmeda-Hirschmann G, Theoduloz C (2017) Qualitative and quantitative changes in polyphenol composition and bioactivity of *Ribes magellanicum* and *R. punctatum* after in vitro gastrointestinal digestion. Food Chem 237:1073–1082. https://doi.org/10.1016/j.foodchem.2017.06.060

45. Gullon B, Pintado ME, Fernández-López J, Pérez-Álvarez JA, Viuda-Martos M (2015) In vitro gastrointestinal digestion of pomegranate peel (*Punica granatum*) flour obtained from co-products: changes in the antioxidant potential and bioactive compounds stability. J Funct Foods 19:617–628. https://doi.org/10.1016/j.jff.2015.09.056

46. Kostic AZ, Milincic DD, Stanisavljevic NS, Gasic UM, Levic S, Kojic MO, Lj Tesic Z, Nedovic V, Barac MB, Pesic MB (2021) Polyphenol bioaccessibility and antioxidant properties of in vitro digested spray-dried thermally-treated skimmed goat milk enriched with pollen. Food Chem 351:129310. https://doi.org/10.1016/j.foodchem.2021.129310

47. Naeimi AF, Alizadeh M (2017) Antioxidant properties of the flavonoid fisetin: an updated review of in vivo and in vitro studies. Trends Food Sci Technol 70:34–44. https://doi.org/10.1016/j.tifs.2017.10.003

48. Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L (2005) Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochim Biophys Acta 1721(1–3):174–184. https://doi.org/10.1016/j.bbagen.2004.11.001

49. Kim I, Moon JK, Hur SJ, Lee J (2020) Structural changes in mulberry (Morus Microphylla. Buckl) and chokeberry (*Aronia melanocarpa*) anthocyanins during simulated in vitro human digestion. Food Chem 318:126449. https://doi.org/10.1016/j.foodchem.2020.126449

50. Kasipandi M, Manikandan A, Sreeja PS, Suman T, Saikumar S, Dhivya S, Parimelazhagan T (2019) Effects of in vitro simulated gastrointestinal digestion on the antioxidant, α-glucosidase and α-amylase inhibitory activities of water-soluble polysaccharides from Opilia amentacea roxb fruit. LWT-Food Sci Technol 111:774–781. https://doi.org/10.1016/j.lwt.2019.05.079

51. He H, Lu YH (2013) Comparison of inhibitory activities and mechanisms of five mulberry plant bioactive components against alpha-glucosidase. J Agric Food Chem 61(34):8110–8119. https://doi.org/10.1021/jf4019323

52. Tang H, Huang L, Sun C, Zhao D (2020) Exploring the structure-activity relationship and interaction mechanism of flavonoids and alpha-glucosidase based on experimental analysis and molecular docking studies. Food Funct 11(4):3332–3350. https://doi.org/10.1039/c9fo02806d

53. Zengin G, Uysal S, Ceylan R, Aktumsek A (2015) Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: a phytochemical study. Ind Crop Prod 70:1–6. https://doi.org/10.1016/j.indcrop.2015.03.012

54. Boaventura BCB, Amboni RDdMC, da Silva EL, Prudencio ES, Di Pietro PF, Malta LG, Polinati RM, Liu RH (2015) Effect of in vitro digestion of yerba mate (*Ilex paraguariensis* A. St. Hil.) extract on the cellular antioxidant activity, antiproliferative activity and cytotoxicity toward HepG2 cells. Food Res Int 77:257–263. https://doi.org/10.1016/j.foodres.2015.05.004

55. Lucas-Gonzalez R, Navarro-Coves S, Pérez-Álvarez JA, Fernández-López J, Muñoz LA, Viuda-Martos M (2016) Assessment of polyphenolic profile stability and changes in the antioxidant potential of maqui berry (*Aristotelia chilensis* (Molina) Stuntz) during in vitro gastrointestinal digestion. Ind Crop Prod 94:774–782. https://doi.org/10.1016/j.indcrop.2016.09.057

56. Arruda HS, Pereira GA, de Morais DR, Eberlin MN, Pastore GM (2018) Determination of free, esterified, glycosylated and insoluble-bound phenolics composition in the edible part of araticum fruit (*Annona crassiflora* Mart.) and its by-products by HPLC-ESI-MS/MS. Food Chem 245:738–749. https://doi.org/10.1016/j.foodchem.2017.11.120