Revista Brasileira de Farmacognosia 29 (2019) 483–487

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Short communication

Identification of compounds from chufa (*Eleocharis dulcis*) peels with inhibitory acrylamide formation activity

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ARTICLE INFO

Article history:
Received 30 August 2018
Accepted 21 November 2018
Available online 3 December 2018

Keywords:
Chinese water chestnut
Chufa peels
Acrylamide
Antioxidant
HPLC

ABSTRACT

Five compounds were isolated from the peels of chufa (*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch., Cyperaceae). The chemical structures were determined by various spectroscopic analysis methods, including 1D and 2D NMR, and by comparison with literature data. All compounds were isolated for the first time from the peels of chufa. Compounds orcinol glucoside, leonuriside A, 2-hydroxymethyl-6-(5-hydroxy-2-methyl-phenoxy-methyl)-tetra-hydro-pyran-3,4,5-triol, and 1,4-dihydroxy-3-methoxy-phenyl-4-O-β-D-glucopyranoside showed good acrylamide formation activity, and acrylamide inhibition rates were 30.24, 32.81, 30.53, and 28.18%, respectively.

Introduction

Cooking food has many advantages, including the destruction of microbes, elimination of heat-sensitive toxins, increase in the bioavailability of nutrients, and the development of desirable colors, flavors, and textures (Van et al., 2010). However, food processing can sometimes lead to the formation of toxic compounds. One such compound is acrylamide, a rodent carcinogen and human neurotoxin that is classified as a possible human carcinogen (Mottram and Friedman, 2008). Acrylamide is of particular concern worldwide (Marion and Reinhard, 2018). This compound was first detected in various heat-treated, carbohydrate-rich, and low-moisture containing food items in 2002 (Maurus and Koni, 2008; Zhang et al., 2008; Tateo et al., 2010; Bartkiene et al., 2015).

Previous studies have demonstrated the effectiveness of various antioxidants and antioxidant extracts in reducing acrylamide production (Bassama et al., 2010; Jin et al., 2013; Zhan et al., 2016). The Chinese water chestnut or chufa (*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch.), which belongs to the family Cyperaceae, is widespread in southern China, particularly in the Guangxi Province (Li et al., 2013), and is often used in Chinese folk medicine for the treatment of pharyngitis, laryngitis, enteritis, cough, hepatitis, and hypertension (Luo et al., 2014). It is one of the most popular hydrophytic vegetables in China due to its unique taste (Li et al., 2016). Chufa peels are often discarded; however, previous studies have demonstrated that they exhibit good antioxidant activities (Li et al., 2013; Luo et al., 2014), hence, they could be used to inhibit natural acrylamide formation during food processing.

Therefore, to clarify and inhibit acrylamide formation, it is necessary to identify the chemical compounds in chufa peels. In this study, HPLC, column chromatography and NMR analysis were used to purify and identify the chemical constituents of chufa peels. Compounds **1** and **4** showed good inhibitory activity with an inhibitory concentration of 1×10^{-3} mg/ml. Compounds **2** and **5** showed good inhibitory activity with an inhibitory concentration of 1×10^{-2} mg/ml.

Materials and methods

Chemicals and materials

1D and 2D NMR spectra were obtained on a Bruker Advance 400-MHz spectrometer. The HRESIMS data were carried out on a MAT 95XP mass spectrometer. Semi-preparative HPLC was performed using a reverse-phase column (Apollo C_{18} column, 5 μm, 10 mm × 250 mm, detection at UV 210 nm). For column chromatography (CC), Sephadex LH-20 (Amersham Pharmacia Biotech AB) and RP-C_{18} (LiChroprep 40-63 μm, Merck) were used. Centrifugal preparative TLC was performed using an apparatus from Qingdao

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https://doi.org/10.1016/j.bjp.2018.11.002

Table 1
Inhibition of acrylamide formation by extracts from chufa peels.[a]

Extract[b]	AA (μg/g)[c]						
	1.0×10^{-9}[d]	1.0×10^{-7}	1.0×10^{-5}	1.0×10^{-3}	1.0×10^{-2}	1.0×10^{-1}	1.0
PE	9.382	8.183	8.739	8.973	9.335	10.765	10.795
EE	9.449	9.342	9.221	8.391	9.362	9.583	9.737
NE	9.408	8.491	8.170	7.795	8.866	9.067	9.174

[a] All values are means of three independent experiments.
[b] PE, petroleum ether extract; EE, ethyl acetate extract; NE, *n*-BuOH extract.
[c] Content of acrylamide (per gram of potatoes).
[d] Concentration of extract (mg/ml).

HAIYANG (PR China). Analytical HPLC was conducted on an Agilent 1260 instrument (PDA detector, Agilent Zorbax SB-C_{18} column, 4.6 mm × 150 mm, 5 μm).

Materials

Fresh chufa peels (*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch., Cyperaceae) were collected from Guangxi Province in China in March 2016 and identified by Professor Baiming Pan, Hezhou University (Hezhou, Guangxi, China).

Acrylamide, >99% (CAS registry number 79-06-1), was supplied by Sigma-Aldrich (St. Louis, MO, USA) and deuterium labeled acrylamide-*d3* (99%) by LGC Standards (Teddington, Middlesex, UK).

Extraction and isolation of the compounds

Dry chufa peels (10 kg) were subjected to extraction three times with 70% aqueous acetone (32 l × 3), each for 24 h, at room temperature. The extract was evaporated *in vacuo* to give a crude extract. The residue was separated into petroleum ether (60–90%, 3 × 6 l), EtOAc (3 × 6 l), and *n*-BuOH (3 × 6 l), and H_2O layers using liquid–liquid partitioning. Each fraction was subjected to an acrylamide formation assay to determine their acrylamide reduction activities. The *n*-BuOH extract had the highest activity (Table 1). The *n*-BuOH extract (92.5 g) was divided into three fractions (A–C) using D101 macroporous resin chromatography (8 × 60 cm) eluting with a gradient of H_2O/EtOH (75:25, 45:55, 10:90, v/v). Fraction A (15 g) was subjected to RP-C_{18} column chromatography with H_2O/MeOH (90:10–0:100, v/v) to give four fractions A-(1-4). Subfraction A-1 was fractionated by TLC to give three fractions A-1-(1-3). Fraction A-1-1 (230 mg) was subjected to semi-preparative HPLC and eluted with H_2O/MeOH (95:5; flow rate: 2.5 ml/min) to yield compound **1** (17 mg, t_R = 25.0 min) and compound **2** (22 mg, t_R = 87.0 min). Fraction A-1-2 (890 mg) was purified using Sephadex LH-20 column chromatography (3 × 180 cm). The column was eluted with MeOH to yield compound **3** (4 mg). Fraction A-1-3 (910 mg) was subjected to semi-preparative HPLC and eluted with H_2O/MeOH (95:5; flow rate: 2 ml/min) to yield compound **4** (36 mg, t_R = 36.0 min) and compound **5** (5 mg, t_R = 87.0 min). The structures of all isolates were characterized based on their NMR data.

Acrylamide formation assays

Sample preparation

Potato tubers, purchased from a local market, were peeled, and cut into little cubes. The cubes were air-dried and ground into potato powder. Chips prepared from potato powder were shaped as circles with a thickness of 1 mm and a diameter of 4 cm, and baked using an electric oven (Ukeo HBD-5002, Zhuhai Jiabao de Science & Technology Co, Zhuhai, Guangdong Province, China) at 150 °C for 5 min. Each potato chip weighed 1.12 g.

Standard preparation

Stock solutions of acrylamide standard and the internal standard acrylamide-*d3*, 1 mg ml^{-1}, were prepared in H_2O/MeOH (95/5, v/v). Acrylamide was further separated by HPLC using an elution with H_2O/MeOH (95:5) and a flow rate of 0.8 ml/min. It eluted at 8.53 min.

Sample extraction

Potato chips were analyzed according to the LCI-internal validated method (Matissek et al., 2005; Carrieri et al., 2010). Potato chips were homogenized and 20 ml of water and 400 μl of internal standard acrylamide-*d3* (5×10^{-6} g ml^{-1}) were added to 2 g of the homogenized sample. Potato chips were extracted by ultrasonic treatment (15 min, 60 °C) and 20 ml of acetonitrile were added. Clean-up of the extracts was performed using 500 μl of Carrez I ($K_4Fe(CN)_6 \cdot 3H_2O$, 150 g l^{-1}) and Carrez II ($ZnSO_4 \cdot 7H_2O$, 300 g l^{-1}), and the samples were then centrifuged at 3155× g for 10 min at 4 °C. The supernatant was then passed through a syringe filter prior to injection into the HPLC system (0.22 μm, Millex® Syringe Filter Unite).

Analysis of acrylamide

Acrylamide determination was carried out via water extraction, purification via solid phase extraction (Isolute Env+, 1 g) and quantitation via HPLC with acrylamide-*d3* as the internal standard, following a method similar to that proposed by Vural Gökmen (Vural and Hamide, 2007). The absorbance of the mixture was measured at 210 nm (t_R = 8.53 min). These tests were carried out in triplicate. The inhibition of acrylamide activity was expressed as the acrylamide inhibition rate (AIR) and was calculated as follows:

$$\left[\frac{AM_0 - AM}{AM_0}\right]$$

$$\text{AIR} \quad (\%) = \times 100\%,$$

where AM, acrylamide content in the experimental group; AM_0, acrylamide content in the blank control group.

The acrylamide inhibition rate of compounds 1-5, and using quercetin, catechin and epicatechin as positive controls, is shown in Fig. 1.

Results and discussion

The structures of all of the compounds were elucidated by NMR spectroscopy (^1H, ^{13}C, and HMBC) and comparison with previously reported values in the literature. They are orcinol glucoside (**1**), leonuriside A (**2**), L-tryptophan (**3**), 2-hydroxymethyl-6-(5-hydroxy-2-methyl-phenoxy-methyl)-tetra-hydro-pyran-3,4,5-triol (**4**), and 1,4-dihydroxy-3-methoxy-phenyl-4-*O*-β-D-glucopyranoside (**5**).

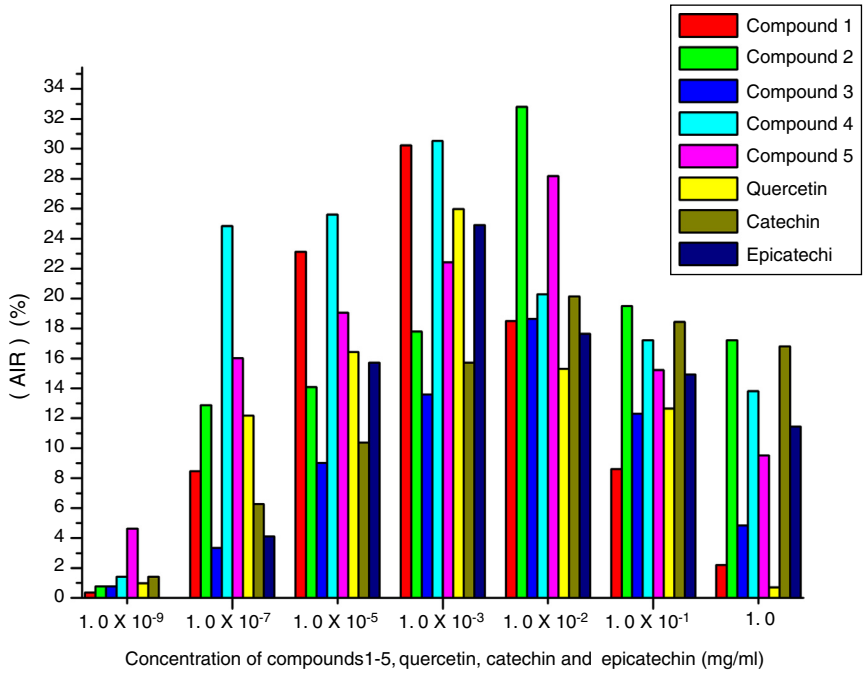
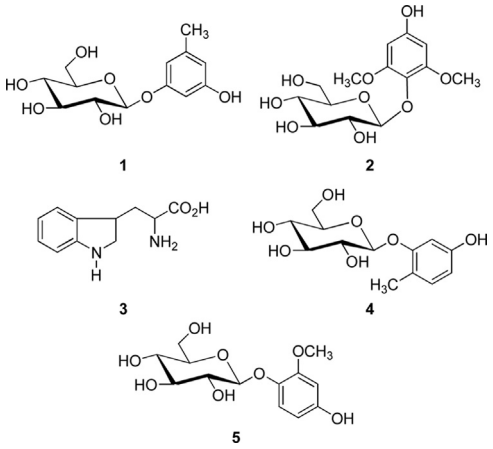


Fig. 1. The acrylamide inhibition rate of compounds **1**-**5**.



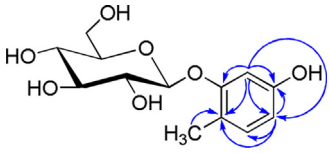


Fig. 2. Key HMBC correlations of compound **4** (H → C).

Compound **1** (17 mg): $C_{13}H_{18}O_7$, colorless acicular. ^{1}H NMR (400 MHz, CD_3OD): 6.42 (1H, *s*, H-2), 6.36 (1H, *s*, H-4), 6.29 (1H, *s*, H-6), 4.59 (1H, *s*, H-1′), 3.36-3.45 (4H, *m*, glc. H), 3.70 (1H, *dd*, *J* = 12.0, 5.0 Hz, Ha-6′), 3.89 (1H, *dd*, *J* = 12.1, 1.8 Hz, Hb-6′), 2.22 (3H, *s*, –CH_3); ^{13}C NMR (101 MHz, CD_3OD): 160.1 (C-1), 102.2 (C-2), 159.2 (C-3), 109.8 (C-4), 141.3 (C-5), 111.2 (C-6), 102.1 (C-1′), 74.9 (C-2′), 78.0 (C-3′), 71.4 (C-4′), 77.9 (C-5′), 62.5 (C-6′), 21.6. Compound **1** was identified as orcinol glucoside by comparison with the spectral data in the literature (Li et al., 2003). Orcinol glucoside has been reported to be an antioxidant (Wu et al., 2008) and antidepressant (Ge et al., 2014).

Compound **2** (22 mg): $C_{14}H_{20}O_9$, white amorphous powder. ^{1}H NMR (400 MHz, DMSO-d_6): 6.06 (2H, *s*, H-3/5), 3.68 (6H, *s*, –OCH_3), 4.64 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.59 (1H, *d*, *J* = 11.3 Hz, H-6′), 3.40-3.45 (4H, *m*, glc. H), 9.24 (1H, *s*); ^{13}C NMR (101 MHz, DMSO-d_6): 127.5 (C-1), 153.2 (C-2/6), 93.8 (C-3/5), 153.9 (C-4), 103.5 (C-1′), 74.2 (C-2′), 77.1 (C-3′), 70.0 (C-4′), 76.5 (C-5′), 61.1 (C-6′), 56.1 (C-OCH_3). Compound **2** was identified as leonuriside A by comparison with spectral data in the literature (Wu et al., 2008). The phenolic glucoside leonuriside A has been evaluated for antioxidant activity (Sugaya et al., 1998).

Compound **3** (4 mg): $C_{11}H_{12}N_2O_2$, yellow solid. ^{1}H NMR (400 MH_Z, DMSO-d_6), 10.93 (1H, s, NH), 7.20 (1H, *s*, H-2), 7.56 (1H, *d*, *J* = 7.8 Hz, H-4), 6.97 (1H, *t*, *J* = 7.3 Hz, H-5), 7.06 (1H, *t*, *J* = 7.3 Hz, H-6), 7.34 (1H, *d*, *J* = 8.0 Hz, H-7); ^{13}C NMR (101 MHz, DMSO-d_6), 124.1 (C-2), 109.6 (C-3), 118.3 (C-4), 118.4 (C-5), 120.9 (C-6), 111.4 (C-7), 136.4 (C-8), 127.3 (C-9), 27.1 (C-10), 54.8 (C-11), 170.3 (C-COOH). This compound was identified as L-tryptophan by comparison with spectral data in the literature (Li et al., 2004). L-Tryptophan has been reported to have antifungal properties (Diego et al., 2016).

Compound **4** (36 mg): $C_{13}H_{18}O_7$, yellow oil, HRSEI-MS *m*/*z* 309.0941 $[M+Na]^+$ (calcd for $C_{13}H_{18}O_7Na$, 309.0941). ^{1}H NMR (400 MHz, CD_3OD): 6.28 (1H, *s*, H-3), 6.24 (1H, *d*, H-5), 6.24 (1H, H-6), 2.16 (3H, *s*, –CH_3), 3.31-3.33 (4H, *m*, glc. H); ^{13}C NMR (101 MHz, CD_3OD): 168.1 (C-1), 148.8 (C-2), 110.4 (C-3), 167.6 (C-4), 119.2 (C-5), 117.4 (C-6), 110.1 (C-1′), 82.8 (C-2′), 86.1 (C-3′), 79.2 (C-4′), 86.5 (C-5′), 70.2 (C-6′), 30.9 (C-CH_3). The ^{1}H and ^{13}C NMR spectroscopic data for this compound include peaks for one 1,2,4-trisubstituted aromatic ring system. Its HMBC spectrum shows the following correlations: H-3/C-2, H-3/C-4, H-3/C-5, H-5/C-3, H-5/C-4, H-5/C-6, H-6/C-1, H-6/C-2, H-6/C-5 (Fig. 2). We identified this compound as 2-hydroxymethyl-6-(5-hydroxy-2-methyl-phenoxy-methyl)-tetra-hydro-pyran-3,4,5-triol by comparison with spectral data in the literature (Takashi et al., 2007).

Compound **5** (5 mg): $C_{13}H_{17}O_7$, colorless amorphous powder. ^{1}H NMR (400 MHz, DMSO-d_6): 7.09 (1H, *s*, H-2), 6.66 (1H, *s*, H-5), 6.44 (1H, *s*, H-6), 4.93 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.90 (1H, *s*, H-6′), 3.72 (3H, *s*, –OCH_3), 3.60-3.68 (4H, *m*, glc. H), ^{13}C NMR (101 MHz, DMSO-d_6): 147.8 (C-1), 107.9 (C-2), 151.0 (C-3), 152.4 (C-4), 115.2 (C-5), 107.9 (C-6), 102.5 (C-1′), 74.7 (C-2′), 78.2 (C-3′), 70.4 (C-4′), 79.7 (C-5′), 67.3 (C-6′), 55.5 (C-OCH_3). We identified compound **5**

Table 2
Inhibition of acrylamide formation by compounds **1**-**5**.[a]

Compounds	AA (μg/g)[b]							
	1.0×10^{-9}[d]	1.0×10^{-7}	1.0×10^{-5}	1.0×10^{-3}	1.0×10^{-2}	1.0×10^{-1}	1.0	0
1	9.375	8.612	7.232	6.563	7.667	8.598	9.200	9.408
2	9.335	8.196	8.083	7.734	6.321	7.574	7.788	9.408
3	9.335	9.094	8.558	8.129	7.654	8.250	8.953	9.408
4	9.275	7.071	6.998	6.536	7.500	7.788	8.109	9.408
5	8.973	7.902	7.614	7.299	6.757	7.975	8.511	9.408
Quercetin[c]	9.315	8.263	7.862	6.964	7.969	7.969	9.342	9.408
Catechin[c]	9.408	9.020	7.929	7.065	7.748	8.002	8.330	9.408
Epicatechin[c]	9.275	8.819	8.431	7.929	7.513	7.674	7.828	9.408

[a] All values are means of three independent experiments.
[b] Content of acrylamide (per gram of potatoes).
[c] Quercetin, catechin and epicatechin are used as positive control.
[d] Concentration of compounds **1**-**5**, quercetin, catechin and epicatechin (mg/ml).

as 1,4-dihydroxy-3-methoxy-phenyl-4-*O*-β-D-glucopyranoside by comparison with spectral data in the literature (Wu et al., 2008; Li et al., 2017).

Compounds **1**, **2**, **4**, and **5** exhibited good inhibition of acrylamide formation activity, inhibiting acrylamide formation better than quercetin, catechin, and epicatechin.

In the present study, we isolated and identified five compounds, orcinol glucoside, leonuriside A, L-tryptophan, 2-hydroxymethyl-6-(5-hydroxy-2-methyl-phenoxy-methyl)-tetra-hydro-pyran-3,4,5-triol, and 1,4-dihydroxy-3-methoxy-phenyl-4-*O*-β-D-glucopyranoside from chufa peels for the first time. Compounds **1**, **2**, **4**, and **5** exhibited good inhibition of acrylamide formation activity (Table 2). Compounds **1** and **4** exhibited inhibitory concentrations of 1×10^{-3} mg/ml, and acrylamide inhibition rates of 30.24%, 30.53%. Compounds **2** and **5** exhibited inhibitory concentration of 1×10^{-2} mg/ml, and acrylamide inhibition rate are 32.81% and 28.18%. Interestingly, the compounds with a glucose group exhibited greater activity than the one that lacked. Compounds **1**, **2**, **4**, and **5** inhibited acrylamide formation more strongly than **3**. Therefore, our study provides a good starting point for a theoretical basis for a better understanding of how to effectively inhibit acrylamide formation. Chufa peels are a valuable natural resource rich in beneficial bioactive compounds, and should be further developed as a functional food.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Author's contribution

HN and SQH contributed equally to this paper on structural determination, writing this paper, and running the laboratory work; JXG conducted the acrylamide formation activity; YHL performed the NMR investigations; XCL, YML, SJW, and JYY carried out the extraction, isolation, and purification.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This project was supported by the National Key R&D Progrom of China (2018YFD0901003), the National Natural Science Foundation of China (21365011), the Youth Teachers' Ability Promotion Project of Guangxi (2018KY0561), the Open Research Fund Program of the State Key Laboratory for the Food Science and Technology (1541001), the Doctor Scientific Research Foundation of Hezhou University (HZUBS201608), the Professor Scientific Research Foundation of Hezhou University (HZUJS201613), the Food Science and Engineering First-rate discipline in Guangxi (cultivation, GXYLXKP1802), the Students' Platform for Innovation and Entrepreneurship Training Program (201811838140), and the Open Research Fund Program of the Guangxi Talent Highland of Preservation (2017XJSS02).

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