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Original Article

Cytotoxic activity of phenolic constituents from *Echinochloa crus-galli* against four human cancer cell lines

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ABSTRACT

Echinochloa crus-galli (L.) P. Beauv., Poaceae, grains are used as a feed for birds and millet for humans. The sulforhodamine B assay was used to assess its cytotoxicity against four human cancer cell lines. The ethanolic extract (70%) proved to be most active against HCT-116 and HELA cell lines (IC_{50} = 11.2 ± 0.11 and 12.0 ± 0.11 μg/ml, respectively). On the other hand, the chloroform and ethyl acetate fractions exhibited their highest activities against HCT-116 cell lines. The chloroform and ethyl acetate fractions were subjected to several chromatographic separations to render pure phenolic compounds (**1**–**8**). Compounds **1**–**8** were identified as: 5,7-dihydroxy-3′,4′,5′-trimethoxy flavone, 5,7,4′-trihydroxy-3′,5′-dimethoxy flavone (tricin), quercetin, flavone, apigenin-8-*C*-sophoroside, 2-methoxy-4-hydroxy cinnamic acid, *p*-coumaric acid and quercetin-3-*O*-glucoside. All the isolated phenolic compounds exhibited various significant activities against the four human carcinoma where the methoxylated flavones (**1** and **2**) were the most active, in a way comparable to the anticancer drug Doxorubicin®. Thus, these methoxylated flavonoids may be considered as lead compounds for the treatment of cancer, which supports previous claims of *E. crus-galli* traditional use. This is the first report of the occurrence of these phenolic compounds in *E. crus-galli*.

Introduction

Cancer is a leading cause of death in economically developed countries and the second leading cause of death in developed countries. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year. Breast cancer in females, and lung and prostate cancer in males are the most frequently diagnosed cancers and the leading causes of cancer death for each sex. About 30% of cancer deaths are due to the five leading behavioral and dietary risks; high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use (Jemal et al., 2011).

Plant derived compounds have played a major role in the development of several useful cytotoxic agents *viz.* vinblastine, vincristine, and paclitaxel (Taxol®). Other promising new agents are in clinical development stage, including flavopiridol and combretastatin, which clarifies the urge for screening native flora in search for new bioactive phytochemical compounds (Reddy et al., 2003; Cragg and Newman, 2005).

Echinochloa crus-galli (L.) P. Beauv., Poaceae, is a problematic summer weed found in rice fields and known as Barnyard grass. The grains are known as fodder for livestock and as millet for people in many Asian countries (Boulos and El Hadidi, 1984). It is known traditionally to reduce body weight, blood sugar, treat hypertension and help to detoxify liver and kidney. It is also used for carbuncles, hemorrhage, sores, spleen trouble, wounds and cancer ('t Mannetje and Jones, 1992). It was previously reported that the 70% hydroalcoholic extract of the grains of *E. crus-galli* showed significant antidiabetic activity in normal and alloxan induced diabetic rats (Devi et al., 2012), and that the methanol and aqueous extracts exhibited significant antioxidant activities (Ho et al., 2012; Mehta and Vadia, 2014). Several phenolic compounds; flavones, flavone glycosides, caffeoyl quinic acid derivatives were isolated from other *Echinochloa* species such as *E. utilis*, *E. frumentacea* and *E. colona* (Watanabe, 1999; Kim et al., 2008; Lazaro, 2009; Gomaa and Abd Elgawad, 2012; Hegab et al., 2013).

This study was carried out in order to prove the ethnopharmacological use of *E. crus-galli* grains as a remedy for cancer ('t Mannetje and Jones, 1992), and to specify the compounds responsible for its cytotoxic activity against four human cancer cell lines; MCF-7 (breast carcinoma), HCT-116 (colon carcinoma), HELA (cervical carcinoma) and HEPG-2 (liver carcinoma). The use of such

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relatively cheap millets in the Egyptian's daily diet could contribute to the prevention of cancer in the overgrowing number of cancer patients.

Materials and methods

Plant material

Grains of *Echinochloa crus-galli* (L.) P. Beauv., Poaceae, were collected between July and September, 2010 from plants grown in the Food Technology Research Institute, Faculty of Agriculture, Cairo University, Giza. They were identified by Prof. Dr. Osama El Kopacy, Professor of Botany, Faculty of Agriculture, Cairo University. A voucher specimen number 9010 was placed at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

General

Silica gel H (Merck, Darmstadt, Germany) was used for vacuum liquid chromatography (VLC), silica gel 60 (70-230 mesh ASTM; Fluka, Steinheim, Germany), sephadex LH-20 (Pharmacia, Stockholm, Sweden), polyamide and cellulose (Merck, Darmstadt, Germany) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on silica gel GF_{254} pre-coated plates (Fluka) using the following solvent systems: S_1, chloroform:methanol (9:1, v/v); S_2, petroleum ether:ethyl acetate:formic acid (45:16:3.6, v/v/v); S_3, chloroform:acetone:formic acid (65:15:1.5, v/v/v); S_4, chloroform:acetone:formic acid (75:16.5:8.5 v/v/v); S_5, ethyl acetate:methanol:water (100:16.5:13.5, v/v/v); S_6, chloroform:methanol (8:2, v/v). The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapor and spraying with $AlCl_3$, as well as after spraying with *p*-anisaldehyde/sulphuric acid spray reagent. Melting points (uncorrected) were determined on a D. Electrothermal 9100 instrument (Labe-quip, Markham, Ontario, Canada). UV spectra were recorded using a Shimadzu UV 240 (P/N 204-58000) spectrophotometer (Kyoto, Japan). A Varian Mercury-VX-300 NMR instrument (Palo Alto, CA, USA) was used for ^{1}H NMR (300 MHz) and ^{13}C NMR (75 or 125 MHz). The NMR spectra were recorded in DMSO-d_6 and chemical shifts were given in δ (ppm) relative to tetramethylsilane as an internal standard.

Extraction, fractionation and isolation

The air-dried powdered grains of *E. crus-galli* (1 kg) were percolated with ethanol 70% till exhaustion to yield 115 g of the alcoholic extract (AlEx). The residue was suspended in distilled water and partitioned successively using n-hexane, chloroform, ethyl acetate, and *n*-butanol saturated with water giving the fractions HxFr, ClFr, EtFr, and BuFr, respectively. The fractions were separately concentrated under reduced pressure to yield 58 g, 7.6 g, 5.8 g and 4.3 g, respectively. The ClFr (5 g) was chromatographed over a VLC column (5 cm × 20 cm, silica gel H, 250 g). Gradient elution was carried out using *n*-hexane/methylene chloride mixtures, methylene chloride/ethyl acetate mixtures, and ethyl acetate/methanol mixtures. Fractions of 200 ml each were collected and monitored by TLC to yield seventeen fractions ($A_c - Q_c$). Fraction G_c (60% methylene chloride in ethyl acetate) was re-chromatographed over silica gel 60 column using methylene chloride in ethyl acetate (9:1, v/v) as an eluent to give compound **1** (22 mg, yellow powder, R_f 0.72 in S_2, m.p. 251–253 °C) and compound **2** (17 mg, yellow powder, R_f 0.70 in S_2, m.p. 249–251 °C). Fraction I_c (90% ethyl acetate in methanol) was purified using several sephadex LH-20 column to yield compound **3** (23 mg, yellow powder, R_f 0.60 in S_2, m.p. 310–312 °C). Fraction E_c (20% methylene chloride in ethyl acetate) was re-chromatographed on a silica gel 60 column, using *n*-hexane/ethyl acetate (8:2 v/v) as an eluent to give compound **4** (15 mg, white powder, R_f 0.91 in S_2, m.p. 349–351 °C). The EtFr (5 g) was chromatographed over polyamide column (250 g, 5 cm × 120 cm). Gradient elution was carried out with water, followed by increasing amount of methanol starting with 5% up to 90% methanol. Fractions of 250 ml each were collected and monitored by TLC to yield five main fractions ($A_{Et} - E_{Et}$). Fraction B_{Et} (40% methanol in water) was re-chromatographed over cellulose column (50 g, 3.5 cm × 120 cm) using 10% methanol in water as an eluent to give compound **5** (15 mg, yellowish brown powder, R_f 0.15 in S_4, m.p. 216–218 °C). Fraction C_{Et} (60% methanol in water) was purified several times over cellulose columns (50 g, 3.5 × 10 cm) using 15% methanol in water to yield compound **8** (12 mg, yellow powder, R_f 0.42 in S_4, m.p. 239–240 °C). Fraction D_{Et} (70% methanol in water) was purified using several sephadex LH-20 columns to yield compound **6** (11 mg, yellowish white powder, R_f 0.71in S_4, m.p. 168–170 °C) and compound **7** (15 mg, yellowish white powder, R_f 0.73in S_4, m.p. 169–171 °C).

Assessment of cytotoxic activity

The cytotoxicities of the AlEx, the four fractions (HxFr, ClFr, EtFr and BuFr) and the eight isolated compounds from ClFr, and EtFr were assessed using the sulforhodamine B assay (Skehan et al., 1990) against the four human cancer cell lines; colon (HCT-116), cervical (HELA), liver (HEPG-2) and breast (MCF-7) adenocarcinoma using Doxorubicin® as a reference standard. Active fractions and compounds were assessed against normal human fibroblast cell lines (HFB4). Dose-dependent activities were studied for all samples using concentrations from 5 to 50 μg/ml, and the IC_{50} values (concentration which reduced survival to 50%) were calculated. Three separate experiments, each with three replicates, were performed for each sample.

Results and discussion

The cytotoxic effects of the extracts of *E. crus-galli* grains at concentrations up to 50 μg/ml and 48 h of exposure showed that the AlEx exhibited IC_{50} values of 12.0 ± 0.11, 11.2 ± 0.11, 18.9 ± 0.12, and 14.2 ± 0.11 μg/ml against HELA, HCT-116, MCF-7, and HEPG-2 cells, respectively (Table 1). According to the US NCI plant screening program a crude extract is considered to possess an *in vitro* cytotoxic activity if its IC_{50} value is less than 20 μg/ml, following an incubation period of 48 and 72 h (Boik,

Table 1
Cytotoxic activities of alcoholic extract, fractions and the isolated compounds of *Echinochloa crus-galli* (IC_{50} values are given in μg/ml).

Tested sample	IC_{50} ± SD (μg/ml)			
	HELA	HCT-116	MCF-7	HEPG-2
AlEx	12.0 ± 0.11	11.2 ± 0.11	18.9 ± 0.12	14.2 ± 0.11
HxFr	29.5 ± 0.01	20.5 ± 0.03	17.1 ± 0.01	15.5 ± 0.04
ClFr	27.3 ± 0.21	17.1 ± 0.01	21.7 ± 0.01	23.9 ± 0.02
EtFr	21.3 ± 0.01	3.8 ± 0.01	16.8 ± 0.01	22.4 ± 0.04
BuFr	18.5 ± 0.01	4.2 ± 0.03	13.5 ± 0.01	14.3 ± 0.03
1	4.5 ± 0.03	18.5 ± 0.04	11.5 ± 0.01	4.5 ± 0.03
2	2.8 ± 0.01	3.6 ± 0.03	6.6 ± 0.03	2.4 ± 0.02
3	13.8 ± 0.01	20.4 ± 0.02	12.7 ± 0.02	11.3 ± 0.02
4	11.0 ± 0.03	27.0 ± 0.01	14.0 ± 0.02	9.3 ± 0.03
5	11.8 ± 0.03	14.5 ± 0.04	6.3 ± 0.01	7.4 ± 0.03
6	5.1 ± 0.01	2.7 ± 0.03	12.4 ± 0.03	17.3 ± 0.02
7	6.5 ± 0.01	4.5 ± 0.02	15.6 ± 0.02	13.5 ± 0.02
8	11.5 ± 0.03	13.5 ± 0.01	5.3 ± 0.02	11.3 ± 0.03
Doxorubicin	4.5 ± 0.03	4.5 ± 0.53	4.3 ± 0.03	4.2 ± 0.03

Table 2
Cytotoxic activities of the isolated compounds of *Echinochloa crus-galli* (IC_{50} values are given in μM).

Tested compound	$IC_{50} \pm SD$ (μM)			
	HELA	HCT-116	MCF-7	HEPG-2
1	3.0 ± 0.04	53.7 ± 0.03	33.3 ± 0.02	3.0 ± 0.01
2	8.6 ± 0.02	10.8 ± 0.02	19.9 ± 0.04	7.2 ± 0.01
3	45.6 ± 0.03	67.4 ± 0.01	42.0 ± 0.02	37.3 ± 0.01
4	49.4 ± 0.02	121.4 ± 0.01	62.9 ± 0.02	41.8 ± 0.05
5	19.3 ± 0.03	23.7 ± 0.04	10.3 ± 0.01	12.1 ± 0.03
6	26.5 ± 0.03	13.9 ± 0.03	63.8 ± ±0.03	89.0 ± 0.01
7	39.5 ± 0.03	27.5 ± 0.02	95.0 ± 0.02	82.2 ± 0.02
8	24.7 ± 0.02	29.0 ± 0.03	11.4 ± 0.01	24.2 ± 0.03
Doxorubicin	8.2 ± 0.03	8.2 ± 0.05	9.3 ± 0.03	7.7 ± 0.03

2001). The ClFr exhibited its highest activity against the HCT-116 cell lines (17.1 ± 0.01 μg/ml), while the HxFr against HEPG-2 (15.5 ± 0.04 μg/ml). The EtFr and BuFr showed significantly higher cytotoxic activities against HCT-116 cell line with IC_{50} values of 3.8 ± 0.01 and 4.2 ± 0.03 μg/ml, respectively, comparable to the standard Doxorubicin (IC_{50} 4.5 ± 0.53 μg/ml) (Table 1). The two fractions, ClFr and EtFr, were subjected to further purification with the aim of identifying the corresponding cytotoxic compounds. Further studies are still running on the BuFr. The ClFr yielded four flavonoids (**1**–**4**) and the EtFr yielded four phenolic compounds (**5**–**8**).

The UV spectral data of compounds **1**, **2**, **4** and **5** indicated a flavone, while that of compounds **3** and **8** indicated the presence of a 3-substituted flavonol nucleus (Tables 3 and 4). The ^{1}H NMR (Tables 5 and 7) and ^{13}C NMR (Tables 6 and 8) spectral data of compounds **1**–**5** and **8** were in accordance with those reported for 5,7-dihydroxy-3′,4′,5′-trimethoxy flavone, 5,7,4′-trihydroxy-3′,5′-dimethoxy flavone (tricin), quercetin, flavone, apigenin-8-*C*-sophoroside and quercetin-3-*O*-glucoside, respectively (Mabry et al., 1970; Bhattacharyya et al., 1978; Agrawal, 1989; Nickavar et al., 2003; Moon et al., 2005; Gangwar and Saxena, 2010; Khanam et al., 2011; El-Sayed et al., 2013). While data of compounds **6** and **7** (Tables 4, 7 and 8) were similar to those reported for 2-methoxy-4-hydroxy cinnamic acid and *p*-coumaric acid, respectively (Kort et al., 1996; Sajjadi et al., 2012). All the compounds were isolated for the first time from genus *Echinochloa*, except for tricin (**2**) and *p*-coumaric acid (**7**) which were isolated for the first time from *E. crus-galli*. Tricin has been previously isolated from

Table 3
UV spectral data (λ_{max} in nm) for compounds **1**–**4**.

Shift reagent	**1**	**2**	**3**	**4**
MeOH	270,309sh,332	244,269,309sh,350	256,269sh,301sh,370	250,294,307sh,370
NaOMe	287,300sh,364	263,275sh,330,416	249sh,321,394	250,295,309sh
$AlCl_3$	252sh,278,300,347,381sh	258sh,277,303,366sh,393	272,304sh,331,455	251,292,306sh
$AlCl_3$/HCl	280,295sh,340,282sh	259sh,277,302,360,386	265,301sh,359,428	250,293,309sh
NaOAC	273,299sh,359	264,276sh,321,414	257sh,274,329,390	248,292,307
NaOAC/H_3BO_3	272,313sh,330	270,304sh,350,422sh	261,303sh,385	255sh,294,307sh

Table 4
UV spectral data (λ_{max} in nm) for compounds **5**–**8**.

Shift reagent	**5**	**6**	**7**	**8**
MeOH	270,304sh,336	332,277sh	327,275sh	257,268sh,291sh,361
NaOMe	279,327,394sh	–	–	272,328,411
$AlCl_3$	276,305,350,384	–	–	276,305sh,332sh,438
$AlCl_3$/HCl	278,303,343,380	–	–	268,295sh,357sh,405
NaOAC	280,300,379	–	–	274,326,381
NaOAC/H_3BO_3	271,329sh,344	–	–	261,296sh,376

Table 5
^{1}H NMR chemical shifts (δ in ppm) for compounds **1**–**4** (DMSO, 300 MHz, *J* in Hz).

H	**1**	**2**	**3**	**4**
3	6.53	6.56	–	6.89
–	1H,S	1H,S	–	1H,S
5	–	–	–	7.43–7.53
–	–	–	–	4H, m
6	6.19	6.21	6.18	7.43–7.53
–	1H, d, *J* = 1.8	1H, d, *J* = 1.8	1H, d, *J* = 1.8	4H, m
7	–	–	–	7.43–7.53
–	–	–	–	4H, m
8	6.90	6.93	6.41	7.43–7.53
–	1H, d, *J* = 1.8	1H, d, *J* = 1.8	1H, d, *J* = 1.8	4H, m
2′	7.28	7.30	7.52	8.06
–	2H, d, *J* = 2.1	2H, d, *J* = 2.1	1H, d, *J* = 9.3	2H, m
3′	–	–	–	7.81
–	–	–	–	2H, m
4′	–	–	–	–
5′	–	–	6.89	7.81
–	–	–	1H, d, *J* = 9.3	2H, m
6′	7.28	7.30	7.66	8.06
–	2H, d, *J* = 2.1	2H, d, *J* = 2.1	1H, dd, *J* = 1.8 and 9.3	2H, m
OCH_3-3′, 5′		3.9		
–	3.7	6H, s		
OCH_3-3′, 4′, 5′	9H, m			

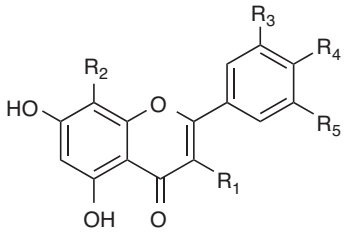
Table 6
^{13}C NMR chemical shifts (δ in ppm) for compounds **1**–**4** (DMSO, 75 MHz, *J* in Hz).

C	1	2	3	4
2	164	164.19	146.72	162.47
3	103.70	103.50	135.62	106.87
4	181.77	181.67	175.72	176.99
5	161.41	161.29	160.59	125.39
6	98.88	98.81	98.15	124.70
7	163.64	163.53	163.89	134.15
8	94.21	94.12	93.30	118.40
9	157.35	157.23	156.06	155.59
10	103.70	103.50	102.90	123.26
1′	120	120.34	121.89	131.68
2′	104.39	104.33	114.99	126.24
3′	148.21	148.11	144.89	129.01
4′	139.89	136.79	147.62	131.68
5′	148.21	148.11	115.55	129.01
6′	104.39	104.33	119.92	120.59
OCH_3-3′	56.38	56.29	–	–
OCH_3-4′	48.64	–	–	–
OCH_3-5′	56.64	56.29	–	–

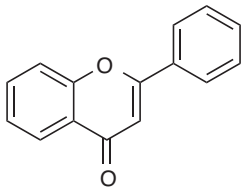
three other species of *Echinochloa* namely *E. utilis*, *E. frumentacea* and *E. colona* and proved to possess strong antioxidant and phytotoxic activities (Watanabe, 1999; Kim et al., 2008; Gomaa and Abd Elgawad, 2012; Hegab et al., 2013).

According to the US NCI plant screening program, pure compounds are considered to possess an *in vitro* cytotoxic activity if their IC_{50} values in cancer cells are less than 4 μg/ml (Boik, 2001). The methoxylated flavone tricin (**2**) was the most active as it possessed the lowest IC_{50} values (7.2 ± 0.01, 8.6 ± 0.02, 10.8 ± 0.02 and 19.9 ± 0.04 μM) against the four cancer cell lines; HEPG-2, HELA, HCT-116 and MCF-7, respectively, compared to 7.7 ± 0.03, 8.2 ± 0.03, 8.2 ± 0.05 and 9.3 ± 0.03 μM for Doxorubicin® (Tables 1 and 2). While compound **1** showed significantly high activities against HELA and HEPG-2 cell lines (IC_{50} = 4.5 ± 0.03 and 4.5 ± 0.03 μg/ml corresponding to 3.0 ± 0.04 and 3.0 ± 0.04 μM, respectively). It was previously reported that tricin isolated from the leaves of *Sasa senanensis* showed high cytotoxic activity against oral squamous carcinoma cell lines, whereas luteolin glycosides showed no cytotoxicity up to 0.8 mg/ml (Matsuta et al., 2011). Methoxylated flavones (8-hydroxy-7,3′,4′,5′-tetramethoxyflavone and 8,4′-dihydroxy-7,3′,5′-trimethoxyflavone) formerly isolated from the stem bark of *Muntingia calabura* exhibited effective cytotoxicities (ED_{50} values 3.56 and 3.71 μg/ml, respectively) against the P-388 cell line *in vitro* (Chen et al., 2004). Also methoxylated flavonols isolated from *Cleome droserifolia* herb showed highly significant cytotoxic activities against HCT-116 and MCF-7 cell lines (Ezzat and Abdel Motaal, 2012).

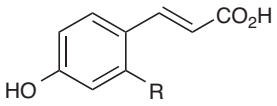
Compounds **6** and **7** exhibited highest cytotoxic activities against HCT-116 (IC_{50} 2.70 ± 0.034 and 4.53 ± 0.021 μg/ml;



1 R_1=R_2=H; R_3=R_4=R_5=OCH_3
2 R_1=R_2=H; R_3=R_5=OCH_3; R_4=OH
3 R_1=OH; R_2=H; R_3=R_4=OH; R_5=H
5 R_1=H; R_2=*C*-sophoroside; R_3=R_5=H; R_4=OH
8 R_1=*O*-β-D-glucopyranoside; R_2=H; R_3=R_4=OH; R_5=H



4



6 R=OCH_3
7 R=H

Table 7
^{1}H NMR chemical shifts (δ in ppm) for compounds **5**–**8** (DMSO, 300 MHz, *J* in Hz).

H	5	8	H	6	7
3	6.74	–	2	–	7.56
–	1H, S	–	–	–	2H, d, *J* = 9.3 Hz
6	6.22	6.20	3	7.27	6.82
–	1H, S	1H, d, *J* = 1.8 Hz	–	1H, S	2H, d, *J* = 9.3 Hz
8	–	6.40	5	6.81	6.82
–	–	1H, d, *J* = 1.8 Hz	–	1H, d, *J* = 8.3 Hz	2H, d, *J* = 9.3 Hz
2	8.02	7.60	6	7.09	7.56
–′	2H, d, *J* = 8.4 Hz	1H, d, *J* = 9.3 Hz	–	1H, d, *J* = 8.3 Hz	2H, d, *J* = 9.3 Hz
3	6.91	–	7	7.53	7.51
–′	2H, d, *J* = 8.4 Hz	–	–	1H, d, *J* = 15.8 Hz	1H, d, *J* = 15.8 Hz
5	6.91	6.90	8	6.34	6.33
–′	2H, d, *J* = 8.4 Hz	1H, d, *J* = 9.3 Hz	–	1H, d, *J* = 15.8 Hz	1H, d, *J* = 15.8 Hz
6	8.02	7.70			
–	2H, d, *J* = 8.4 Hz	1H, dd, *J* = 1.8 and 9.3 Hz			
–′	–	5.45			
1″	5.1	1H, d, *J* = 7.5 Hz			
–	1H, d, *J* = 20.9 Hz	–			
1‴	5.3	–			
–	1H, d, *J* = 3 Hz	–			

Table 8
^{13}C NMR chemical shifts (δ in ppm) for compounds **5**–**8** (DMSO, 75 MHz, *J* in Hz).

C	**5**	**8**	C	**6**	**7**
2	163.60	156.28	1	144.59	144.17
3	103.70	133.31	2	147.99	125.25
4	181.90	177.40	3	115.73	129.99
5	160.40	161.20	4	149.15	159.54
6	98.10	98.60	5	122.86	129.99
7	162.40	164.04	6	125.89	125.25
8	103.80	93.44	7	115.73	115.78
9	156.10	156.15	8	111.26	115.29
10	103.70	103.96	9	168.9	167.97
1′	121.60	121.55	OCH_3	55.80	–
2′	128.80	115.16			
3′	115.80	144.75			
4′	161.2	148.40			
5′	115.80	116.17			
6′	128.80	121.14			
1″	71.40	100.87			
2″	81.80	74.06			
3″	78.65	77.50			
4″	59.91	69.91			
5″	81.72	76.48			
6″	58.15	60.95			
1‴	106.03				
2‴	71.78				
3‴	78.10				
4‴	66.65				
5‴	74.02				
6‴	60.02				

13.9 ± 0.03 and 27.5 ± 0.02 μM), and HELA (IC_{50} 5.15 ± 0.013 and 6.50 ± 0.013 μg/ml; 26.5 ± 0.03 and 39.5 ± 0.03 μM) cell lines, respectively (Tables 1 and 2). In a previous report, 4-hydroxy-3-methoxycinnamic acid inhibited proliferation and induced apoptosis in human breast cancer cells (Hamdan et al., 2013).

Thus the activity of the crude ethanolic extract of *E. crus-galli* could be directly correlated to its phenolic content, and methoxylation is the key for enhancing the cytotoxic activity when considering structure–activity relationship of the isolated compounds. In general methoxylated compounds (**1**, **2** and **6**) exhibited significantly higher cytotoxic activities compared to the other phenolic compounds and the anticancer drug Doxorubicin® (Tables 1 and 2). All the tested active fractions and compounds showed no cytotoxicity against the normal melanocytes HFB4.

Conclusion

Here we reported the cytotoxic activity of the ethanolic extract of the edible grains of *E. crus-galli* against four human cancer cell lines, which supports previous claims of *E. crus-galli* traditional use. Eight phenolic constituents were isolated for the first time from *E. crus-galli*, and six of them were reported for the first time in the genus. Two methoxylated flavones and one methoxylated cinnamic acid exhibited highly significant cytotoxic activities compared to Doxorubicin®.

Author contributions

AA, HE and AE participated in study concept and design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content. AA drafted the manuscript. SG carried out the extraction, fractionation and isolation of pure compounds, and participated in drafting the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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