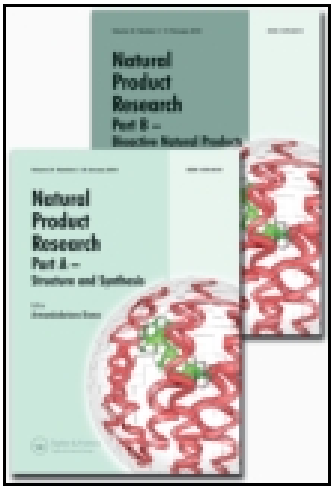

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Two new flavonol glycosides and biological activities of Diplotaxis harra (Forssk.) Boiss.

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Two new flavonol glycosides and biological activities of *Diplotaxis harra* (Forssk.) Boiss.

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Two new flavonol glycosides, isorhamnetin 3-*O*-β-glucopyranoside-4′-*O*-β-xylopyranoside (**1**) and kaempferol 3-*O*-β-glucopyranoside -4′-*O*-β-xylopyranoside (**2**), were isolated from the defatted aqueous methanol extract of the whole plant *Diplotaxis harra* along with 12 known flavonols (**3–14**). They were characterised by chemical and spectral methods. The 70% aqueous methanol, chloroform and defatted aqueous methanol plant extracts exhibited significant antioxidant effects (nitroblue tetrazolium reduction method). Their cytotoxic activity was carried out against 11 tumour cell lines (sulphorhodamine B assay). The three extracts expressed the greatest antiproliferative activity against colon 38, P388 and MKN-28 with GI_{50} (0.45, 0.4, 0.07 μg/mL) and against P388 [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide assay] with IC_{50} (0.26, 0.24, 0.25 μg/mL), respectively. The chloroform extract showed the highest activity as eukaryotic DNA topoisomerase II inhibitors of P388 with IC_{50} 0.24 μg/mL. Antiviral screening of the extracts and the pure compounds against foot-and-mouth disease virus types A and O revealed a prominent inhibition of its cytopathic effect.

Keywords: *Diplotaxis harra*; flavonols; antioxidant; cytotoxic; antiviral activity

1. Introduction

Diplotaxis harra (Forssk.) Boiss. is an annual desert plant belonging to the Brassicaceae family and widely distributed in Egypt (Boulos 1999). It is locally known as Harra, and is used in folk medicine as antibacterial, antifungal, anti-inflammatory and anticancer (Hartwell 1982). The non-methylated fatty acids and steroids from *D. harra* showed strong activity against bacteria than fungi (Hashem & Saleh 1999). Leaf flavonoids of 13 *Diplotaxis* species were reported to be glycosides of kaempferol, quercetin and isorhamnetin (Sanchez-Yelamo & Martinez-Laborde 1991). Quercetin, isorhamnetin 7-*O*-β-glucoside, apigenin 7-*O*-α-rhamnoside, 3-*O*-β-glucoside of kaempferol, quercetin and isorhamnetin, isorhamnetin 3-*O*-α-rhamnoside, isorhamnetin 3-*O*-rutinoside and isorhamnetin 3-*O*-β-glucoside 4′-*O*-α-rhamnoside were reported on aerial parts of *D. harra* (Atta et al. 2011; Mohammed et al. 2011). Despite the widespread use of *D. harra* as medicinal herb, we aimed to identify the active compounds from the whole plant and evaluated its antioxidant and cytotoxic activities as well as the antiviral activity against foot-and-mouth disease viruses (FMDV).

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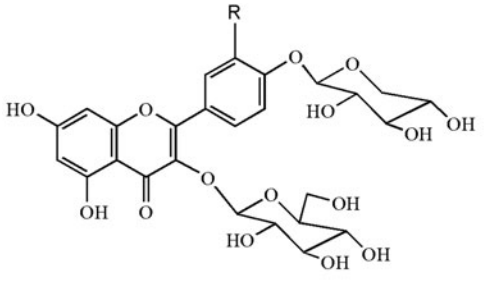
2. Results and discussion

2.1. *Identification of isolated compounds*

The phytochemical investigation of the defatted aqueous methanol extract (DAME) of the whole plant of *D. harra* afforded 14 flavonols (Figure 1). Compound **1** was isolated as a yellow amorphous powder. The positive-ion ESI-MS showed a molecular ion peak at *m/z* 611.38 $[M + H]^+$, corresponding to $C_{27}H_{30}O_{16}$. The UV spectral data using various shift reagents indicated a flavonol skeleton with free hydroxyl groups at C-5 and C-7, whereas those at C-3 and C-4′ are substituted (Mabry et al. 1970). Complete acid hydrolysis (2 N HCl, 1 h, 100°C) yielded glucose and xylose as the sugar moieties [co-paper chromatography (Co-PC)] and isorhamnetin (Co-PC, EI-MS and UV). The ^{1}H NMR spectrum of **1** showed the aromatic protons of ring A with two *meta* coupled protons for H-6 and H-8 at 6.23 and 6.47, respectively. The aromatic protons of the B-ring appeared as a doublet (δ7.97, $J = 2.0$ Hz) assigned to H-2′ and doublet of doublet at (δ7.54, $J = 2.0$, 8.5 Hz) assigned to H-6′ along with another doublet (δ7.24, $J = 8.5$ Hz) for H-5′ with a downfield shift indicating the substitution of the hydroxyl group at C-4′ (Park & Lee 1996). The spectrum also showed a singlet at 3.84, attributed to OCH_3 of isorhamnetin skeleton. In addition to two anomeric doublets at δ5.58 ($J = 7.5$ Hz) and 5.37 ($J = 4.6$ Hz) corresponding to β-glucopyranose and β-xylopyranose units at C-3 and C-4′, respectively (Markham & Geiger 1994). The ^{13}C NMR spectrum confirmed the presence of 16 carbon signals assigned for the isorhamnetin and 11 signals for the sugar units. The chemical shift of C-3 at 133.3 and C-4′ at 148.1 suggested their glycosylation (Agrawal 1989) which is confirmed by HMBC data, this showed the correlation from H-1″ (δ5.58) to C-3 (133.3) and from H-1‴ (δ5.37) to C-4′ (148.1). On the basis of the above data, **1** was identified as isorhamnetin 3-*O*-β-glucopyranoside-4′-*O*-β-xylopyranoside. Compound **2** was obtained as a yellow powder with a molecular formula $C_{26}H_{28}O_{15}$ as determined by the negative-ion ESI-MS at *m/z* 579.45 $[M-H]^-$. The UV spectral data using diagnostic shift reagents indicated a flavanol skeleton with free hydroxyl groups at C-5 and C-7, whereas those at C-3 and C-4′ are substituted (Mabry et al. 1970). Complete acid hydrolysis produced glucose, xylose and kaempferol (Co-PC). Its ^{1}H NMR spectrum showed two doublet protons at δ 8.13 and δ 7.19 (each $J = 9.0$ Hz), assigned to H-2′,6′ and H-3′,5′, respectively; this downfield chemical shift confirmed that C-4′ was substituted (Yoshitama et al. 1993). Two doublets at δ 6.47 and δ 6.23 (each $J = 2.0$ Hz) were assigned to H-8 and H-6, respectively. The spectrum also revealed two protons at δ 5.47 ($J = 7.2$ Hz) and δ 5.32 ($J = 4.6$ Hz), attributed to H-1″ and H-1‴ of the β-glucopyranose and β-xylopyranose units, respectively (Markham & Geiger 1994). In addition, the ^{13}C NMR spectrum confirmed the substitution of C-3 (134.1) and C-4′ (160.8). Therefore, **2** seems to be the kaempferol analogue of **1**; kaempferol 3-*O*-β-glucopyranoside-4′-*O*-β-xylopyranoside. Compounds (**3**–**14**) were elucidated by comparing their spectral data with those published previously (Agrawal 1989; Markham & Geiger 1994; Shang et al. 2006). They were identified as isorhamnetin 3-*O*-[2‴-*O*-acetyl-β-glucopyranosyl (1 → 6)-β-glucopyranoside] (**3**), isorhamnetin 3,4′-di-*O*-β-glucoside (**4**), isorhamnetin 3,7-di-*O*-β-glucoside (**5**), isorhamnetin 3-*O*-β-glucoside (**6**), isorhamnetin 3-*O*-α-rhamnoside (**7**), isorhamnetin 7-*O*-β-glucoside (**8**), quercetin 3-*O*-β-glucoside (**9**), quercetin 7-*O*-β-glucoside (**10**), quercetin 3-*O*-α-rhamnoside (**11**), kaempferol 3-*O*-β-glucoside (**12**), isorhamnetin (**13**) and quercetin (**14**).

2.2. *Antioxidant assay*

The 70% aqueous methanol extract (AME), chloroform extract (CE) and DAME showed high-antioxidant activities (at a dose of 50 μg/mL) with percentage scavenging activity; 62.66, 31.15 and 51.66, respectively, relative to ascorbic acid (22.2%).



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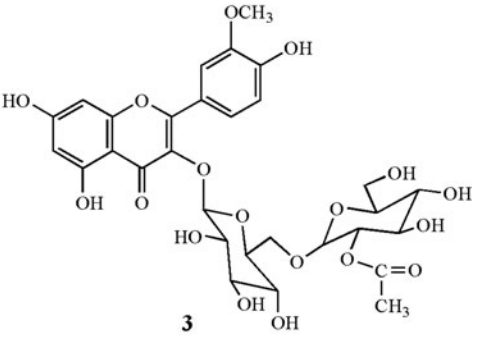

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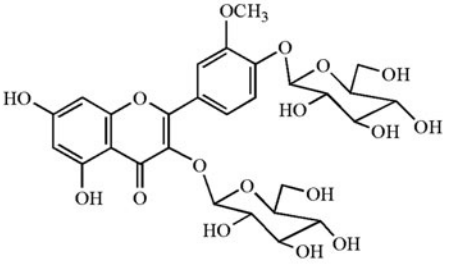

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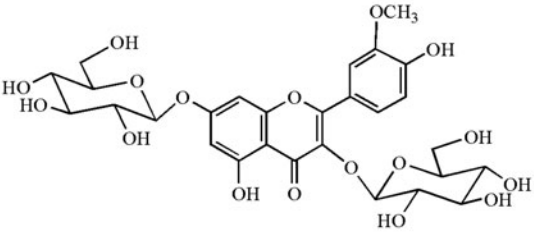

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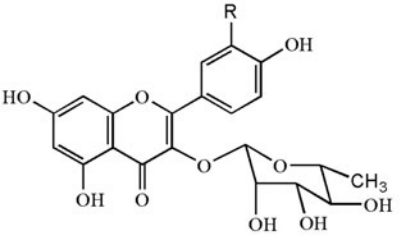

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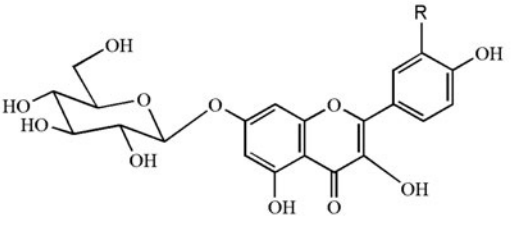

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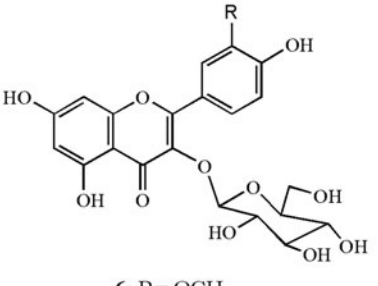
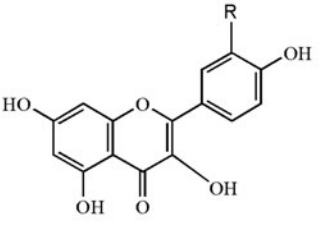


6: R= OCH_3
9: R= OH
12: R= H



13: R= OCH_3
14: R= OH

Figure 1. Chemical structure of compounds **1–14**.

2.3. *Cytotoxic assay*

2.3.1. *Growth-inhibitory effect*

The results showed that AME, CE and DAME exhibited the most potent activities on MKN-28, colon 38 and P388, respectively, with GI_{50} (0.07, 0.45 and 0.4 μg/mL), TGI (0.65, 2.66 and 3.90 μg/mL) and LC_{50} (5.89, 6.18 and 29.56 μg/mL), respectively (Table 1). They were also tested against P388 using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay with IC_{50} (μg/mL); 0.26, 0.24 and 0.25, respectively (Table 2).

2.3.2. *Inhibition of topoisomerase II activity*

All extracts showed inhibition of topoisomerase II activity with lower concentration than that of etoposide. Among them, CE showed the highest activity where CE and etoposide show IC_{50} values of 0.24 and 0.30 μg/mL, respectively, which means that CE exhibited 1.182 times greater inhibitory effect than etoposide (Table 2).

2.4. *Antiviral assay*

The extracts and the pure flavonoids were found to be safe for baby hamster kidney cell culture (BHK-21). All of them showed antiviral effect against FMDV (types A and O) as indicated by the inhibition of the virus cytopathic effect (Table 3). The antiviral effect was varied from one sample to another depending on the active dilution. The samples which showed antiviral effect when they added to the infected cell culture before, after and simultaneously with the virus infection (1, 2, 8, 11 and DAME) were of choice to follow up such effect as virus inactivators. These samples began to show their action as inactivators by the first hour post starting of the process for either types of FMDV, although the required periods for complete inactivation were varied according to the used concentration. The study revealed that the highest concentration of the tested materials the faster inactivation of FMDV (types A and O). Such materials did not affect passively the antigenicity of FMDV as confirmed by the complement fixation test, which revealed the same results obtained before and after virus inactivation (titre 1/8) with different concentrations used.

Table 1. Cytotoxicity of AME, CE and DAME (SRB assay).

	AME			CE			DAME		
Cell line	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Colon 38	0.41	5.89	60.9	0.45[a]	2.66[a]	6.18[a]	6.44	50.78	124.9
WIDR	0.43	0.55	5.11	1.57	9.28	56.78	0.54	4.89	38.90
MKN-1	0.43	8.78	24.44	0.76	7.89	24.11	1.17	16.19	155.89
MKN-7	9.00	56.90	225.25	3.71	23.66	114.06	1.51	14.36	100.78
MKN-28	0.07[a]	0.65[a]	5.89[a]	1.52	7.44	58.66	2.60	19.56	112.98
MKN-74	0.07	0.71	6.90	1.63	5.66	24.30	0.68	5.88	53.89
HGC-27	0.09	0.87	8.66	1.42	3.14	14.08	0.98	5.66	40.89
GT3TKB	0.09	0.89	9.87	1.56	5.66	16.09	4.67	56.78	208.88
P388	1.88	12.77	34.99	0.51	4.88	25.00	0.40[a]	3.90[a]	29.56[a]
Lx-1	5.66	6.55	18.12	1.88	5.78	38.16	1.71	11.66	110.89
A549	0.065	0.89	7.88	1.42	12.45	67.45	5.78	43.67	307.78

Note: Concentration (μg/mL) required to inhibit cell growth by 50% (GI_{50}), concentration (μg/mL) required to produce total growth inhibition (TGI), concentration (μg/mL) required to cause net cell killing (LC_{50}).
[a] The most active extract with the least GI_{50} concentration.

Table 2. IC_{50} of AME, CE, DAME and etoposide (MTT assay) inhibited the topoisomerase II on DNA-relaxing activity of P388 topoisomerase II.

Tested extracts	IC_{50} (μg/mL)	Topoisomerase II inhibition relative potency
AME	0.26	1.11
CE	0.24	1.182
DAME	0.25	1.125
Etoposide	0.30	1

Notes: Supercoiled pBR-322 DNA was incubated with topoisomerase II purified from P388 cells in the presence or absence of an inhibitor. Concentration (μg/mL) that causes 50% inhibition per unit enzyme (IC_{50}).

Table 3. The safe samples dilution on BHK-21 cells, the preliminary antiviral activity against FMDV strains (O and A) and their antiviral effect according to the time of their addition to the infected cell culture.

			The antiviral effect according to the time of their addition to the infected cell culture		
Sample	Safe sample dilution (non-toxic)	Preliminary antiviral activity against FMDV[a]	Before VI	After VI	Simultaneously with VI
1	10^{-3}	+	+	+	+
2	10^{-3}	+	+	+	+
3	10^{-4}	+	−	−	+
4	10^{-4}	+	−	−	−
5	10^{-1}	+	−	−	−
6	10^{-2}	+	−	−	+
8	10^{-2}	+	+	+	+
11	10^{-2}	+	+	+	+
13	10^{-3}	+	−	−	−
14	10^{-2}	+	−	−	−
AME	10^{-2}	+	+	−	+
CE	10^{-3}	+	−	−	+
DAME	10^{-5}	+	+	+	+

[a] Antiviral effect = no cytopathic effect (CPE), positive antiviral effect determined by inhibition of the cytopathic effect of either type of FMDV. VI, virus infection.

3. Experimental

3.1. *General*

NMR experiments were recorded on Jeol EX-500 spectrometer. EI-MS was measured on Finnigan-Mat SSQ 7000 spectrometer and ESI-MS was measured on LCQ Advantage Thermo Finnigan spectrometer. UV spectra were recorded on Shimadzu model-2401 CP spectrophotometer. Column chromatography (CC) was carried out on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia, Uppsala, Sweden). Paper chromatography (PC, descending) Whatman No. 1 and 3 mm papers, using solvent systems (1) H_2O, (2) 15% HOAc (H_2O:HOAc, 85:15), (3) BAW (*n*-BuOH:HOAc:H_2O, 4:1:5, upper layer), (4) BBPW (C_6H_6:*n*-BuOH:pyridine:H_2O, 1:5:3:3, upper layer).

3.2. *Plant material*

D. harra was freshly collected and identified by Dr Mona M. Marzouk in 20 March 2007 from Cairo–Suez desert road at 35 km. A voucher specimen (no. 808) has been deposited in the herbarium of National Research Centre, Cairo, Egypt.

3.3. *Extraction and isolation*

The air-dried powdered whole plant (1.2 kg) was extracted with 70% MeOH (3 × 4 L). The solvent was evaporated under reduced pressure at 50°C. The dried aqueous methanol extract; AME (228 g) was defatted with $CHCl_3$ (3 × 1 L) afforded CE (32 g). The DAME (180 g) was then subjected to polyamide CC (5.5 cm × 125 cm) eluted with H_2O and followed by H_2–MeOH mixtures of decreasing polarity. A total of 42 fractions were collected, each 400 mL; these were controlled by PC to give four main fractions (A–D). Fraction A was chromatographed on Sephadex column using H_2O:MeOH (1:1) for elution yielding three sub-fractions, each was subjected to preparative paper chromatography (PPC) using BAW and followed by Sephadex column using methanol to yield compounds **1** (23 mg) **2** (18 mg) and **3** (38 mg). CC of fraction B on polyamide column (2.5 cm × 45 cm), gradient elution with H_2–MeOH, gave two major sub-fractions: B1 and B2. Repeated CC on Sephadex of B1 using MeOH:C_6H_6:H_2O (60:38:2), followed by PPC using BAW, afforded compounds **4** (12 mg) and **5** (25 mg). Compounds **6** (31 mg) and **7** (10 mg) were obtained from B2 by repeated CC on Sephadex LH-20 using saturated BuOH. Fraction C was applied on PC using BAW which yielded compounds **8** (26 mg), **9** (10 mg) and **11** (18 mg). Fraction D yielded compounds **10** (22 mg), **12** (8 mg), **13** (16 mg) and **14** (36 mg) by separation on PC using 15% HOAc and followed by BAW two times.

3.3.1. *Isorhamnetin 3-O-β-glucopyranoside-4′-O-β-xylopyranoside (**1**)*

A bright yellow powder, R_f 0.34 (BAW). Positive ESI-MS; *m/z* 611.38 $[M + H]^+$. UV spectral data, λ_{max} (nm): (MeOH) 255 sh, 262, 356; (+NaOMe) 270, 330 sh, 419 (dec.); (+$AlCl_3$) 263, 300 sh, 359 sh, 402; (+$AlCl_3$/HCl) 264, 301 sh, 360 sh, 401; (+NaOAc) 272, 320, 406; (NaOAc/H_3BO_3) 254, 266 sh, 357. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, *J*/Hz: 7.97 (1H, d, $J = 2.0$, H-2′); 7.54 (1H, dd, $J = 2.0$, 8.5, H-6′); 7.24 (1H, d, $J = 8.5$, H-5′); 6.47 (1H, d, $J = 2.0$, H-8); 6.23 (1H, d, $J = 2.0$, H-6); 5.58 (1H, d, $J = 7.5$, H-1″); 5.37 (1H, d, $J = 4.6$, H-1‴), 3.84 (3H, s, OCH_3); 3.69 (1H, dd, $J = 5.3$, 11.5, H-5‴); 3.61 (1H, dd, $J = 5.2$, 11.6, H-6″); 3.41–3.48 (2H, m, H-3″, H-4″, overlapped); 3.38 (1H, m, H-5″); 3.29 (1H, m, H-2″); 3.19–3.25 (2H, m, H-3‴, H-4‴, overlapped); 3.08 (1H, m, H-2‴).^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.4 (C-4), 164.2 (C-7), 161.1 (C-5), 156.3 (C-2), 155.6 (C-9), 148.1 (C-3′), 148.1 (C-4′), 133.3 (C-3), 123.5 (C-6′), 121.2 (C-1′), 114.4 (C-5′), 113.4 (C-2′), 104.1 (C-10), 100.7 (C-1″), 99.4 (C-1‴), 98.7 (C-6), 93.9 (C-8), 76.9 (C-5″), 76.8 (C-3″), 76.3 (C-3‴), 74.2 (C-2″), 73.1 (C-2‴), 69.7 (C-4″), 69.4 (C-4‴), 66.4 (C-5‴), 60.5 (C-6″), 55.6 (OCH_3).

3.3.2. *Kaempferol 3-O-β-glucopyranoside -4′-O-β-xylopyranoside (**2**)*

A pale yellow powder, R_f 0.32 (BAW). Negative ESI-MS; *m/z* 579.45 $[M-H]^-$. UV spectral data, λ_{max}(nm): (MeOH) 262, 352; (+NaOMe) 270, 328sh, 409 (dec.); (+$AlCl_3$) 263, 300, 354, 400; (+$AlCl_3$/HCl) 264, 301, 356, 399; (+NaOAc) 272, 388; (NaOAc/H_3BO_3) 266, 353. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, *J*/Hz: 8.13 (2H, d, $J = 9.0$, H-2′, 6′); 7.19 (2H, d, $J = 9.0$, H-3′, 5′); 6.47 (1H, d, $J = 2.0$, H-8); 6.23 (1H, d, $J = 2.0$, H-6); 5.47 (1H, d, $J = 7.2$, H-1″); 5.32 (1H, d, $J = 4.6$, H-1‴); 3.68 (1H, dd, $J = 5.4$, 11.4, H-5‴); 3.62 (1H, dd, $J = 5.4$, 11.5, H-6″); 3.54 (1H, m, H-4″); 3.36-3.44 (2H, m, H-3″, H-5″, overlapped); 3.31 (1H, m, H-4‴); 3.16 (1H, m, H-3‴); 3.04 (1H, m, H-2″), 2.98 (1H, m, H-2‴). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 178.1 (C-4), 162.5 (C-7), 161.4 (C-5), 160.8 (C-4′), 158.6 (C-2), 156.5 (C-9), 134.1 (C-3), 131.2 (C-2′, C-6′), 121.3 (C-1′), 116.2 (C-3′, C-5′), 106.2 (C-10), 100.9 (C-1″), 99.9 (C-1‴), 98.9 (C-6), 93.9 (C-8), 77.6 (C-5″), 76.6 (C-3″), 76.5 (C-3‴), 74.5 (C-2″), 73.8 (C-2‴), 70.1 (C-4‴), 69.9 (C-4″), 65.8 (C-5‴), 60.6 (C-6″).

3.4. *Antioxidant assay*

Superoxide radicals were generated by xanthine/xanthine oxidase and measured by the nitroblue tetrazolium reduction method (Chung et al. 2004).

3.5. *Cytotoxicity activity*

3.5.1. *Cell lines used for anti-tumour activity test*

Murine colon, colon 38; murine leukaemia, P388 and Lung human, LX-1 cancer cell lines were obtained from the Cancer Chemotherapy Centre (Tokyo, Japan). Human gastric cancer cell lines, MKN-1, MKN-7, MKN-28 and MKN-74, were obtained from Immuno Biology Laboratory (Gunma, Japan) and HGC-27 and GT3TKB were from Riken Cell Bank (Ibaraki, Japan). Lung human, A549 and colon, WIDR cancer cell lines were obtained from Dainippon Pharmaceutical, Co. Ltd (Osaka, Japan).

3.5.2. *Growth-inhibitory effect*

The cytotoxicity of AME, CE and DAME against 11 cancer cell lines was evaluated using sulphorhodamine B (SRB) assay (Skehan et al. 1990). Three dose response parameters for the extracts, GI_{50}, TGI and LC_{50}, were calculated (Table 1) (Nakamura et al. 2002). Their cytotoxicity on P388 cell line was also determined by MTT assay for assessment of cell viability (Table 2) (Hsiang et al. 1989).

3.5.3. *Inhibition of DNA topoisomerase II activity*

The inhibitory effects on DNA topoisomerase II activity were evaluated by relaxation assay of supercoiled DNA (Saijo et al. 1990) in comparison with etoposide as reference drug (Table 2).

3.6. *Antiviral activity*

3.6.1. *Viral material*

Serotypes O (1/3/93) and A (A/Egypt/2006) of FMDV and BHK-21 clones, using Eagle's medium with 10% new born bovine serum, were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

3.6.2. *Detection of material cytotoxicity*

Three-day BHK-21 cell cultures in micro-titre plates were used to detect the toxicity of the tested samples. Ten-fold dilutions were prepared from each sample in phosphate buffer saline (PBS). Then 25 μL of each concentration was inoculated in each of the 5 wells of 96-well tissue culture plates.

3.6.3. *Investigation of* in vitro *antiviral effect*

BHK-21 cell culture plates were infected with FMDV strains using 25 μL/well of 10^8 $TCID_{50}$ of the used virus for 1 h. Then the plates were washed twice with PBS. Each one of the prepared concentrations was added to each of five tissue culture wells with 150 μL of maintenance medium. The cytopathic effect on BHK-21 cells of each sample was observed under light microscope (Lefevre & Diallo 1990).

3.6.4. *Complement fixation test*

Complement fixation test (CFT) was carried out according to Health protection Agency (2009) using the complement obtained from normal healthy Guinea pigs and the sheep RBCs from normal healthy sheep for preparation of the haemolytic system. It was used as an indicator in CFT consisting of four minimum haemolytic doses of Hemolysin and equal volume of 2% sheep RBCs in PBS diluents and synthesised at 37°C for 15 min.

4. Conclusion

In the present study, to the best of our knowledge, compounds **1** and **2** had not been isolated from plants before. In addition six flavonols; **3**–**5**, **10**, **11** and **13** were isolated for the first time from *D. harra.* Biological investigation of extracts demonstrated promising antioxidant and anti-cancer properties. They are targets for DNA topoisomerase, and their cytotoxicity is dependent on tumour cell type. These activities could be presumably ascribed in part to the flavonoids as well as other constituents of the plant. The pure compounds (**1**, **2**, **8**, **11**) and DAME are of choice as virus inactivators in the antiviral assay on FMDV, and their activity is concentration dependent. Therefore, this study provides the biochemical rationale for further chemical and biological analysis.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S10.

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References

Agrawal PK. 1989. Carbon-13 NMR of flavonoids. New York: Elsevier.

Atta EM, Hashem AI, Ahmed AM, Elqosy SM, Jaspars M, El-Sharkaw ER. 2011. Phytochemical studies on *Diplotaxis harra* growing in Sinai. Eur J Chem. 2(4):535–538.

Boulos L. 1999. Flora of Egypt. Cairo: Al Hadara.

Chung JE, Kurisawa M, Kim YJ, Uyama H, Kobayashi S. 2004. Amplification of antioxidant activity of catechin by polycondensation with acetaldehyde. Biomacromolecule. 5:113–118.

Hartwell JL. 1982. Plants used against cancer: a survey. Lawrence. MA: Quarterman.

Hashem FA, Saleh MM. 1999. Antimicrobial components of some Cruciferae plants (*Diplotaxis harra* and *Erucaria microcarpa*. Phytother Res. 13(4):329–332.

Health protection Agency. 2009. Complement fixation tests, standards unit, 3:1-23. London: Department for Evaluations, Standards and Training, Centre for Infections.

Hsiang YH, Libou MG, Liu LF. 1989. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res. 49:5077–5082.

Lefevre PC, Diallo A. 1990. Pest des petit ruminants. Rev Sci Tech Off Int Epiz. 9:951–965.

Mabry TJ, Markham KR, Thomas MB. 1970. The systematic identification of flavonoids. Berlin: Springer.

Markham KR, Geiger H. 1994. ^{1}H-NMR of flavonoids and their glycosides in DMSO-d_6. In: Harborne JB, editor. The flavonoids: advances in research since 1986. London: Chapman & Hall.

Mohammed MMD, El-Sharkawy ER, Matloub AA. 2011. Cytotoxic flavonoids from *Diplotaxis harra* growing in Sinai. J Med Plant Res. 5(20):5099–5103.

Nakamura K, Sugumi H, Yamaguchi A, Uenaka T, Kotake Y, Okada T, Kamata J, Niijima J, Nagasu T, Koyanagi N. 2002. Antitumour activity of ER-37328, a novel carbazole topoisomerase II inhibitor. Mol Cancer Ther. 1:169–175.

Park YK, Lee CY. 1996. Identification of isorhamnetin 4′-glucoside in onions. J Agric Food Chem. 44:34–36.

Saijo M, Enomoto T, Hanaoka F, Ui M. 1990. Purification and characterization of type II DNA topoisomerase from mouse FM3A cells: phosphorylation of topoisomerase II and modification of its activity. Biochemistry. 29:583–590.

Sanchez-Yelamo MD, Martinez-Laborde JB. 1991. Chemosystematic approach to (Cruciferae: Brassicaceae) and related species. Biochem Syst Ecol. 19:477–482.

Shang XY, Wang YH, Li C, Zhang CZ, Yang YC, Shi JG. 2006. Acetylated flavonol diglucosides from *Meconopsis quintuplinervia*. Phytochemistry. 67(5):511–515.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 82:1107–1112.

Yoshitama K, Kawasoe T, Ishikura N. 1993. Isolation of a new flavonol glycoside and its effects on the blue color and seed coat of *Ophiopogon jaburan*. J Plant Res. 106:223–227.