Isolation of New Cytotoxic Metabolites from *Cleome droserifolia* Growing in Egypt

Shahira M. Ezzat* and Amira Abdel Motaal

Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr-El-Ainy St., Cairo 11562, Egypt. E-mail: shahyelkomy@hotmail.com

* Author for correspondence and reprint requests

Z. Naturforsch. **67 c**, 266–274 (2012); received August 15, 2011/January 13, 2012

The sulforhodamine B (SRB) assay was used to assess the cytotoxicity of the aqueous (AqEx) and ethanolic (AlEx) extracts, respectively, of the aerial parts of *Cleome droserifolia* (Forssk.) Del. against two human cancer cell lines, breast (MCF7) and colon (HCT116) adenocarcinoma. AqEx exhibited higher cytotoxic activity, thus its four subfractions, namely *n*-hexane (HxFr), chloroform (ClFr), ethyl acetate (EtFr), and *n*-butanol (BuFr) fractions, were also tested. Purification of the more active ClFr and EtFr yielded nine compounds. Six terpenoids, guai-7(11),8-diene (**C_1**), 1-hydroxy-guai-3,10(14)-diene (**C_2**), 18-hydroxy-dollabela-8(17)-ene (**C_3**), (24*E*)-stigmasta-5,8-dien-3*β*-ol (**C_4**), teucladiol [1*α*,5*β*-guai-10(14)-ene-4*β*,6*β*-diol] (**C_5**), and buchariol (4,10-epoxy-6*α*-hydroxyguaiane) (**C_6**), were isolated from ClFr and three flavonol glycosides, isorhamnetin-3-*O*-*β*-D-glucoside (**F_1**), quercetin-3`-methoxy-3-*O*-(4``-acetylrhamnoside)-7-*O*-*α*-rhamnoside (**F_2**), and kaempferol-4`-methoxy-3,7-*O*-dirhamnoside (**F_3**), were isolated from EtFr. Compounds **C_3** and **F_2** are new in nature. The isolated compounds were identified using various spectroscopic methods (UV, IR, ^{1}H NMR,^{13}C NMR, HMQC, HMBC, and COSY). Compounds **C_1**, **C_3**, **F_2**, and **F_3** showed significant cytotoxic activities against the two tested cell lines comparable to those of the anticancer drug doxorubicin®. The new compound **C_3** was the most active as it had the lowest IC_{50} values, (1.9 ± 0.08) and (1.6 ± 0.09) *μ*g/ml corresponding to 6.5 and 5.4 *μ*M, against MCF7 and HCT116 cells, respectively.

Key words: Cytotoxic, *Cleome droserifolia*, Flavonols, Terpenes

Introduction

Plants possess activities which are beneficial in the treatment of different types of diseases. According to WHO, 80% of the world population's health problems can be treated by medicinal herbal drugs (Etkin, 1981; WHO, 2003). Although cancer belongs to the group of disorders difficult to treat and sometimes is incurable, many plants have a long history in the treatment of cancer. Active constituents from plants such as *Catharanthus roseus* and *Taxus brevifolia* have been used in the treatment of advanced stages of various malignancies (Medina *et al.*, 2006). Cytotoxicity screening models provide important preliminary data which help in selecting plant extracts with potential antineoplastic properties for future work (Cardellina *et al.*, 1999). As a part of a continuous screening program aiming to search for Egyptian medicinal plants with anticancer properties, the present investigation reports the cytotoxicity of an Egyptian plant, *Cleome droserifolia* (Forssk.) Del. The genus *Cleome* comprises annual and perennial herbs or low shrubs. It is represented in Egypt by nine species among which *Cleome droserifolia* (Forssk.) Delile, Descr. (syn. *Roridula droserifolia* Forssk.) is the most famous (Boulos, 1999). *Cleome droserifolia* is an aromatic shrub characterized by its orbicular leaves (Boulos, 1999). It is known in Egypt as Samwah, Afein or Reeh-El-Bard (Yang *et al.*, 1990). It grows in Egypt, Libya, Palestine, and Syria as it requires a stony and sandy soil (Boulos, 1999). This plant has a great fame as an antihyperglycemic agent (Abdel-Hady, 1998; Abdel-Kawy *et al.*, 2000; Motaal *et al.*, 2011; Nicola *et al.*, 1996; Yang *et al.*, 1990; Yaniv *et al.*, 1987) and lately has been found to contain antioxidant and hepatoprotective constituents (Abdel-Kader *et al.*, 2009; Nassar and Gamal-Eldeen, 2003). In the present study, both the ethanolic (AlEx) and aqueous (AqEx) extracts of the plant were subjected to bioscreening assays using the cultivated human breast (MCF7) and colon (HCT116) cancer cell lines with the aim of identifying their bioactive compounds.

Material and Methods

Plant material

The aerial parts of *Cleome droserifolia* were collected in 2008 and 2009 from populations growing in the wild on El-Ketar mountain, Hurghada, Egypt. The plant was authenticated by Assist. Prof. Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

General

Silica gel H (Merck, Darmstadt, Germany) was used for vacuum liquid chromatography (VLC), silica gel 60 (70–230 mesh ASTM; Fluka, Steinheim, Germany) and Sephadex LH-20 (Pharmacia, Stockholm, Sweden) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on silica gel GF_{254} precoated plates (Fluka) using the following solvent systems: S_1, *n*-hexane/ethyl acetate (90:10 v/v); S_2, *n*-hexane/ethyl acetate (80:20 v/v); S_3, chloroform/methanol (95:5 v/v); S_4, chloroform/methanol (90:10 v/v); S_5, chloroform/methanol (80:20 v/v); and S_6, ethyl acetate/methanol/water (100:16.5:13.5 v/v/v). The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour and spraying with $AlCl_3$, as well as after spraying with anisaldehyde/sulfuric acid spray reagent. Melting points (uncorrected) were determined on a D. Electrothermal 9100 instrument (Labequip, 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) measurements. IR spectra were measured on an infrared spectrophotometer FT/IR-460, Plus (Jasco, Easton, MD, USA). The NMR spectra were recorded in $CDCl_3$ and DMSO-d_6, and chemical shifts are given in δ (ppm) relative to TMS as internal standard.

Extraction, fractionation, and isolation

The air-dried aerial parts of *Cleome droserifolia* (1 kg) were powdered, then extracted by boiling distilled water to yield 210 g of aqueous extract (AqEx). The residue was suspended in distilled water and partitioned successively using *n*-hexane, chloroform, ethyl acetate, and *n*-butanol saturated with water to yield the following subfractions: HxFr, ClFr, EtFr, and BuFr. Fractions were separately concentrated under reduced pressure to yield 5 g, 12.8 g, 11.8 g, and 48.9 g, respectively. A 95% ethanolic extract (AlEx) (250 g) was prepared from 1.5 kg of the dried aerial parts.

ClFr (12 g) was chromatographed over a VLC column (6 x 20 cm, silica gel H, 250 g). Gradient elution was carried out using *n*-hexane/methylene chloride mixtures and methylene chloride/ethyl acetate mixtures. Fractions of 200 ml each were collected and monitored by TLC to yield four main fractions (A_c–D_c). Fraction A_c (20–25% methylene chloride/*n*-hexane) was rechromatographed over a silica gel 60 column, using *n*-hexane as eluent, to give compound **C_1** (57 mg, oily compound, R_f = 0.37 in S_1). Fraction B_c (30% methylene chloride/*n*-hexane) was purified in the same way using *n*-hexane/ethyl acetate (9.9:0.1 v/v) as an eluent to give compound **C_2** (63 mg, oily compound, R_f = 0.32 in S_1) and compound **C_3** (71 mg, oily compound, R_f = 0.23 in S_1). Fraction C_c (35–40% methylene chloride/*n*-hexane) was similarly purified using *n*-hexane/ethyl acetate (9.5:0.5 v/v) as an eluent to give compound **C_4** (111 mg, white needle-shaped crystals, R_f = 0.38 in S_3, m.p. 169–170 °C). Finally, fraction D_c was purified using *n*-hexane/ethyl acetate (8.8:1.2 v/v) as an eluent to give compounds **C_5** (240 mg, oily compound, R_f = 0.44 in S_3) and **C_6** (113 mg, oily compound, R_f = 0.33 in S_3).

EtFr (10 g) was fractionated over a Sephadex LH-20 column (25 x 3 cm). Fractions were collected and purified using several Sephadex LH-20 columns to yield yellow microcrystalline compounds: **F_1** (22 mg, R_f = 0.62 in S_6, m.p. 242–243 °C), **F_2** (169 mg, R_f = 0.55 in S_6, m.p. 256–257 °C), and **F_3** (230 mg, R_f = 0.43 in S_6, 246–247 °C).

Assessment of cytotoxic activity

The cytotoxicities of AqEx and AlEx, the four subfractions of AqEx, and the compounds isolated from ClFr and EtFr were assessed using the sulforhodamine B (SRB) assay (Skehan *et al.*, 1990) against the two human cancer cell lines: colon (HCT116) and breast (MCF7) adenocarcinoma. The assessment was performed in the Na-

tional Cancer Institute in Egypt (NCI). The IC_{50} values (concentration which reduces survival to 50%) were calculated together with that of the reference drug doxorubicin®. Three separate experiments, each with three replicates, were performed for each sample.

Results and Discussion

The cytotoxic effects of the extracts of *Cleome droserifolia* (Forssk.) Del. aerial parts at concentrations up to 50 μg/ml and 48 h of exposure are presented in Table I and show that AqEx exhibited IC_{50} values of (10.2 ± 0.2) and (11.1 ± 0.02) μg/ml against MCF7 and HCT116 cells, respectively. 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, 2001). On the other hand, AlEx exhibited weaker cytotoxic activities against the two tested human cell lines [IC_{50} of (16.5 ± 0.11) and (19.9 ± 0.05) μg/ml against MCF7 and HCT116 cells, respectively]. Fractionation of the AqEx yielded three highly active subfractions, HxFr, ClFr, and EtFr (Table I). The two subfractions ClFr and EtFr, being more active, were subjected to further purification with the aim of identifying the corresponding cytotoxic compounds. ClFr yielded six terpenoids, $\mathbf{C_1}$–$\mathbf{C_6}$, and EtFr yielded three flavonol glycosides, $\mathbf{F_1}$–$\mathbf{F_3}$.

Table I. Cytotoxic effects of the extracts, fractions, and the isolated compounds.

Tested sample	IC_{50} ± SD [μg/ml][a]	
	MCF7	HCT116
AqEx	10.2 ± 0.2	11.1 ± 0.02
AlEx	16.5 ± 0.11	19.9 ± 0.05
HxFr	7.2 ± 0.17	6.8 ± 0.11
ClFr	4.4 ± 0.2	4.7 ± 0.09
EtFr	4.9 ± 0.04	5.5 ± 0.07
BuFr	20.45 ± 1.3	22.62 ± 2.1
Compound $\mathbf{C_1}$	4.0 ± 0.03	3.6 ± 0.03
Compound $\mathbf{C_2}$	22.6 ± 0.32	10.4 ± 0.24
Compound $\mathbf{C_3}$	1.9 ± 0.08	1.6 ± 0.09
Compound $\mathbf{C_4}$	24.5 ± 0.89	44.3 ± 1.09
Compound $\mathbf{C_5}$	28.1 ± 0.76	18.8 ± 0.54
Compound $\mathbf{C_6}$	48.5 ± 0.63	-
Compound $\mathbf{F_1}$	24.3 ± 0.98	-
Compound $\mathbf{F_2}$	3.35 ± 0.57	3.80 ± 1.01
Compound $\mathbf{F_3}$	4.35 ± 0.78	2.48 ± 0.42
Doxorubicin®	2.97 ± 0.05	3.73 ± 0.21

[a] Concentration which reduces the survival to 50%. HCT116, colon cancer cell line; MCF7, breast cancer cell line; AqEx, aqueous extract; AlEx, ethanolic extract; HxFr, *n*-hexane fraction; ClFr, chloroform fraction; EtFr, ethyl acetate fraction; BuFr, *n*-butanol fraction; SD, standard deviation for $n = 3$; -, no activity under the experimental conditions.

The ^{13}C NMR spectra of compounds $\mathbf{C_1}$ and $\mathbf{C_2}$ showed the fifteen signals for the guaiane-type skeleton (Table II) (Bohlmann and Jakupovic, 1979; Bruno *et al*., 1993; Oshima *et al*., 1983). The downfield shift of the two secondary methyl groups of the isopropyl unit in $\mathbf{C_1}$, which appeared as two singlets at δ_H 1.61 and 1.69 ppm in the H^1 NMR spectrum (Table III), indicated the presence of a double bond at C-7(11) which appeared in the ^{13}C NMR at δ_C 111.6 and 145.0 ppm for C-7 and C-11, respectively. The downfield shift of H-8 and H-9 (δ_H 5.00 ppm, 1H, d, J = 15.9 Hz and δ_H = 5.20 ppm, 1H, d, J = 15.9 Hz, respectively), besides the appearance of their carbon atoms at δ_C 122.6 and 124.1 ppm, indicated the presence of a double bond at C-8(9). Thus, compound $\mathbf{C_1}$ was identified as guai-7(11),8-diene (Fig. 1) which is reported here for the first time in *C. droserifolia*.

The IR spectrum of compound $\mathbf{C_2}$ showed the presence of a hydroxy group at 3414 cm^{-1} (Table IV). The ^1H NMR spectrum of compound $\mathbf{C_2}$ displayed two secondary methyl groups due to the isopropyl unit at δ_H 0.80 (d, J = 6.7 Hz) and 0.89 ppm (d, J = 6.7 Hz) and a tertiary methyl group at δ_H 1.73 ppm assigned to Me-15. The downfield shift of Me-15 indicated the presence of a double bond at C-3 which appeared at δ_H 5.50 ppm (1H, m, H-3) and δ_C 121.8 and 133.6 ppm assigned for C-3 and C-4, respectively. The ^1H and ^{13}C NMR spectra of compound $\mathbf{C_2}$ showed signals for an exocyclic methylene group (δ_H 5.12 ppm, 1H, br.s, H-4B; δ_H 4.78 ppm, 1H, br.s, H-4A; δ_C 154.1 and 105.8 ppm for C-10 and C-14, respectively). A hydroxy group at the C-1 position (δ_C 72.9 ppm) was also noticed. From these data, compound $\mathbf{C_2}$ was identified as 1-hydroxy-guai-3,10(14)-diene (Fig. 1); it is reported for the first time in *C. droserifolia*.

The IR spectrum of compound $\mathbf{C_3}$ showed the presence of a hydroxy moiety (3454 cm^{-1}). The data acquired for compound $\mathbf{C_3}$ were found to agree with those reported for the dolabellane diterpenes indicating that it possesses the same core (Aboushoer *et al.*, 2010). The ^1H NMR and

Table II. ^{13}C NMR chemical shifts (δ in ppm) for compounds $\mathbf{C_1}$–$\mathbf{C_6}$ ($CDCl_3$, 75 MHz) and $\mathbf{F_1}$–$\mathbf{F_3}$ (DMSO, 125 MHz).

C	$\mathbf{C_1}$	$\mathbf{C_2}$	$\mathbf{C_3}$	$\mathbf{C_4}$	$\mathbf{C_5}$	$\mathbf{C_6}$	C	$\mathbf{F_1}$	$\mathbf{F_2}$	$\mathbf{F_3}$
1	42.0	72.9	49.4	37.2	42.8	53.3	2	156.4	156.1	156.0
2	27.8	27.0	28.9	31.6	27.2	23.8	3	132.8	134.3	134.5
3	40.3	121.8	27.9	71.8	40.4	37.5	4	177.1	177.7	177.8
4	25.6	133.6	27.5	42.3	80.8	74.4	5	161.1	161.8	160.1
5	46.6	49.5	29.6	140.7	59.5	68.2	6	98.9	98.5	98.4
6	21.3	21.7	22.6	121.6	72.7	75.9	7	165.1	160.4	161.6
7	111.6	48.4	22.7	31.9	48.4	38.5	8	93.7	94.6	94.5
8	122.6	23.6	151.1	138.1	24.8	20.2	9	156.9	157.8	157.6
9	124.1	33.1	26.0	129.1	35.5	48.2	10	103.6	105.8	105.7
10	23.7	154.1	33.8	36.5	152.4	74.4	1`	121.4	120.4	120.2
11	145.0	29.3	41.1	21.1	29.1	32.7	2`	113.4	113.0	130.5
12	22.6	22.6	50.0	39.8	21.5	21.1	3`	146.8	147.4	115.3
13	22.7	22.7	29.3	42.3	21.6	21.1	4`	149.3	149.8	160.8
14	22.6	105.8	39.3	56.7	108.1	21.9	5`	115.0	115.5	115.3
15	26.1	26.9	16.3	24.3	23.2	25.8	6`	121.9	120.7	130.5
16			15.7	29.2			1``	100.8	101.4	101.8
17			107.2	56.0			2``	74.3	71.7	70.2
18			80.7	11.9			3``	76.4	70.1	70.5
19			22.4	19.3			4``	69.8	69.8	71.5
20			26.9	40.5			5``	77.3	68.0	70.0
21				21.2			6``	60.5	17.4	17.8
22				33.9			1```		99.5	99.3
23				28.2			2```		70.3	70.2
24				51.2			3```		69.9	70.3
25				31.9			4```		73.2	71.1
26				19.0			5```		67.5	69.7
27				21.0			6```		17.1	17.3
28				26.1			OCH_3-3`	55.6	55.7	
29				12.2			OCH_3-4`			55.8
							$O\underline{C}OCH_3$		169.9	
							$OCO\underline{C}H_3$		20.8	

^{13}C NMR spectra of this compound showed the presence of four methyl groups and an exocyclic methylene group at C-8 (17) (δ_H 4.72 ppm, 1H, br.s; δ_H 4.43 ppm, 1H, br.s; δ_C 151.1 and 107.2 ppm, for C-8 and C-17, respectively). The two methyl groups at δ_H 1.21 and 1.26 ppm appeared as singlets assigned to Me-19 and Me-20 and are attached to C-18 forming an isopropyl group, which could be deduced from its HMBC spectra. The significant downfield shift of C-18 (δ_C 80.7 ppm) indicated its attachment to a hydroxy group. The third methyl group appeared as a singlet at δ_H 0.71 ppm, δ_C 16.3 ppm assigned to Me-15 which is attached to C-1 appearing at δ_C 49.4 ppm. The last methyl group appeared as a doublet at δ_H 0.89 ppm, δ_C 15.7 ppm, assigned to Me-16 attached to C-4 which appeared at δ_C 27.5 ppm. Correlations in HMQC and HMBC spectra confirmed these assignments. Thus, compound $\mathbf{C_3}$ was identified as 18-hydroxy-dollabela-8(17)-ene (Fig. 1), which is a novel natural product.

The ^1H NMR spectrum of compound $\mathbf{C_4}$ demonstrated the presence of a C-24 ethyl sterol nucleus. This was confirmed by the presence of 6 methyl groups at δ_H 0.69–1.26 ppm. A signal at δ_H 5.40 ppm (1H, br.s) corresponded to the olefinic proton at H-6, and a multiplet at δ_H 3.50 ppm (1H, m) was assigned to H-3 (Good and Akisha, 1997). The ^{13}C NMR spectrum showed the presence of a C-24 ethyl sterol nucleus and four olefinic carbon atoms could be assigned to two bouble bonds. The first was at δ_C 121.6 and 140.7 ppm corresponding to the double bond at C-5(6), and the second was assigned to a double bond at C-8(9). So, compound $\mathbf{C_4}$ was identified as (24*E*)-stigmasta-5,8-dien-3β-ol (Fig. 1). This is the first report of the compound in *C. droserifolia*.

Table III. ^{1}H NMR chemical shifts (δ in ppm) for compounds **C$_1$**–**C$_6$** ($CDCl_3$, 300 MHz, J in Hz).

H	**C$_1$**	**C$_2$**	**C$_3$**	**C$_4$**	**C$_5$**	**C$_6$**
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	5.50 1H, m	-	3.50 1H, m	-	-
4	-	5.12 1H, br.s (4B) 4.78 1H, br.s (4A)	-	-	-	-
5	2.34 1H, dd, J = 7.2, 16.5	2.34 1H, m	-	-	1.88 1H, dd, J = 9.6, 11.6	-
6	-	-	-	5.40 1H, br.s	4.09 1H, dd, J = 3.9, 6.9	4.00 1H, d, J = 1.5
7	-	-	-	-	-	2.14 1H, dd, J = 9.1, 3.1
8	5.00 1H, d, J = 15.9	-	-	-	-	-
9	5.20 1H, d, J = 15.9	-	-	-	2.56 1H, dd, J = 9.1, 3.1 (9B) 2.14 1H, dd, J = 9.1, 3.1 (9A)	-
10	-	-	-	-	-	-
11	-	-	-	-	-	
12	1.69 3H, s	0.89 3H, d, J = 6.7	-	-	1.00 3H, d, J = 6.7	0.93 3H, d, J = 6.7
13	1.61 3H, s	0.80 3H, d, J = 6.7	-	-	0.95 3H, d, J = 6.7	0.92 3H, d, J = 6.7
14	1.23 3H, d, J = 6.7	-	-	-	4.76 1H, br.s (14B) 4.73 1H, br.s (14A)	1.18 3H, s
15	0.91 3H, d, J = 6.7	1.73 3H, s	0.71 3H, s	-	-	1.42 3H, s
16	-	-	0.89 3H, d, J = 6.7	-	-	-
17	-	-	4.72 1H, br.s (17B) 4.43 1H, br.s (17A)	-	-	-
18	-	-	-	1.07 3H, s	-	-
19	-	-	1.21 3H, s	1.26 3H, s	-	-
20	-	-	1.26 3H, s	-	-	-
21	-	-		0.92 3H, d, J = 6.4	-	-
22	-	-		-	-	-
23	-	-		-	-	-
24	-	-		-	-	-
25	-	-		-	-	-
26	-	-		0.69 3H, d, J = 5.7		-
27	-	-		0.79 3H, d, J = 5.7	-	-
28	-	-		-		-
29	-	-		0.81 3H, t		

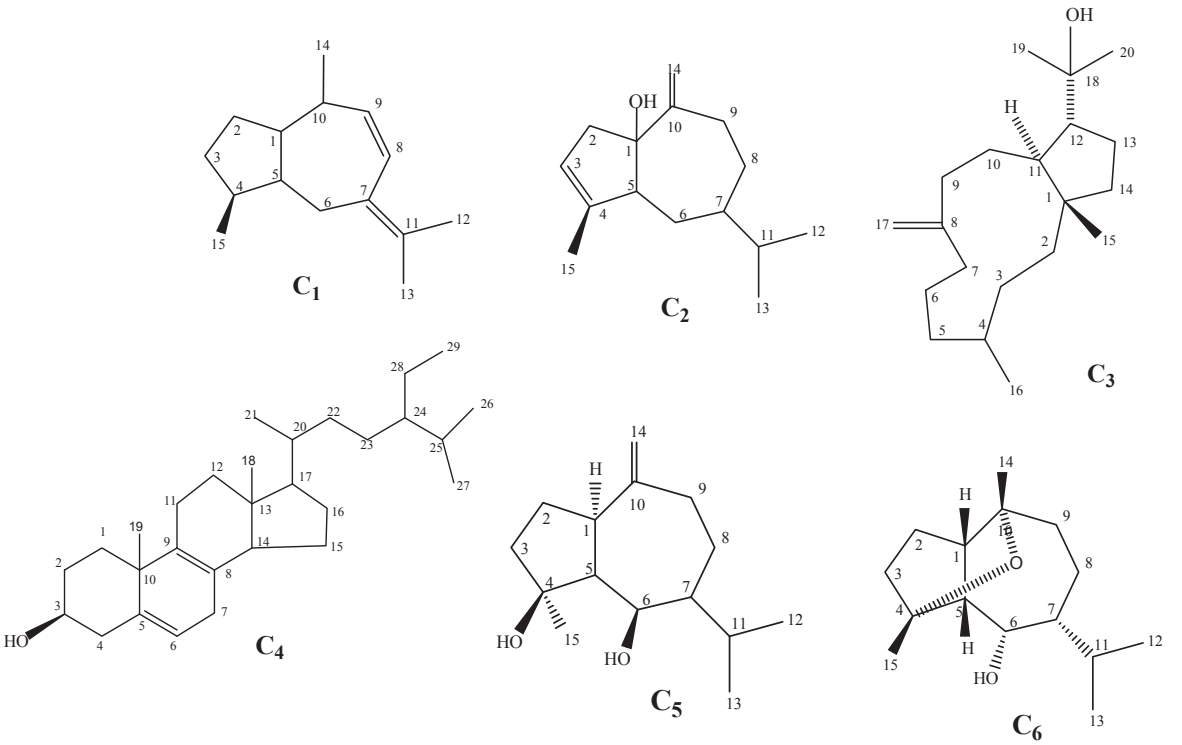

Fig. 1. Chemical structures of compounds isolated from the chloroform fraction (ClFr).

The ^{1}H and ^{13}C NMR spectral data of compound **C_5** were similar to those reported for teucladiol (Fig. 1) (Bruno *et al*., 1993). This compound was previously isolated from *C. droserifolia* (Abdel-Kader *et al*., 2009). Spectral data of **C_6** were in accordance with reports on buchariol (4,10-epoxy-6*α*-hydroxyguaiane) (Fig. 1) (Ahmad *et al*., 1999), previously isolated from an Egyptian collection of *C. droserifolia* (El-Askary, 2005).

The data of compound **F_1** were in accordance with those reported for isorhamnetin-3-*O*-*β*-D-glucoside (Fig. 2) (Halim *et al*., 1995). This compound has been isolated for the first time from *C. droserifolia.*

Table IV. IR spectral data [ν_{max} ($CHCl_3$) in cm^{-1}] for compounds **C_1**–**C_6**.

Compound	ν_{max} ($CHCl_3$) [cm^{-1}]
C_1	3054, 1660 (C-H, C=H)
C_2	3414 (OH), 3057, 1656 (C-H, C=H)
C_3	3454 (OH), 3089, 1666 (C-H, C=H)
C_4	3434 (OH), 3079, 1667 (C-H, C=H)
C_5	3412 (OH), 3070, 1669 (C-H, C=H)
C_6	3410 (OH), 2859 (C-H)

The UV spectral data of compound **F_2** indicated the presence of a 3-substituted flavonol nucleus with a free OH group at C-4`, no *ortho*-dihydroxy group in ring B, and an occupied OH group at C-7 (Table V). The ^{1}H NMR (Table VI) and ^{13}C NMR spectra (Table II) showed the characteristic signals for a quercetin nucleus and a methoxy group at δ_H 3.87 ppm and δ_C 55.7 ppm which exhibited a correlation to C-3` in the HMBC spectra. Its position was also confirmed by the upfield shift of C-2` (δ_C 113.0 ppm). The spectra showed two anomeric protons (δ_H 5.55 and 5.31 ppm; δ_C 101.4 and 99.5 ppm) which appeared as singlets and two doublets (δ_H 1.13 and 0.82 ppm; δ_C 17.4 and 17.1 ppm) which were assigned to the two methyl groups of rhamnose moieties attached to 3-OH and 7-OH, respectively. This was confirmed by the COSY and HMBC spectra. An acetyl group appeared at δ_H 2.00 ppm ($OCOC\underline{H}_3$), δ_C 20.8 ($OCO\underline{C}H_3$)

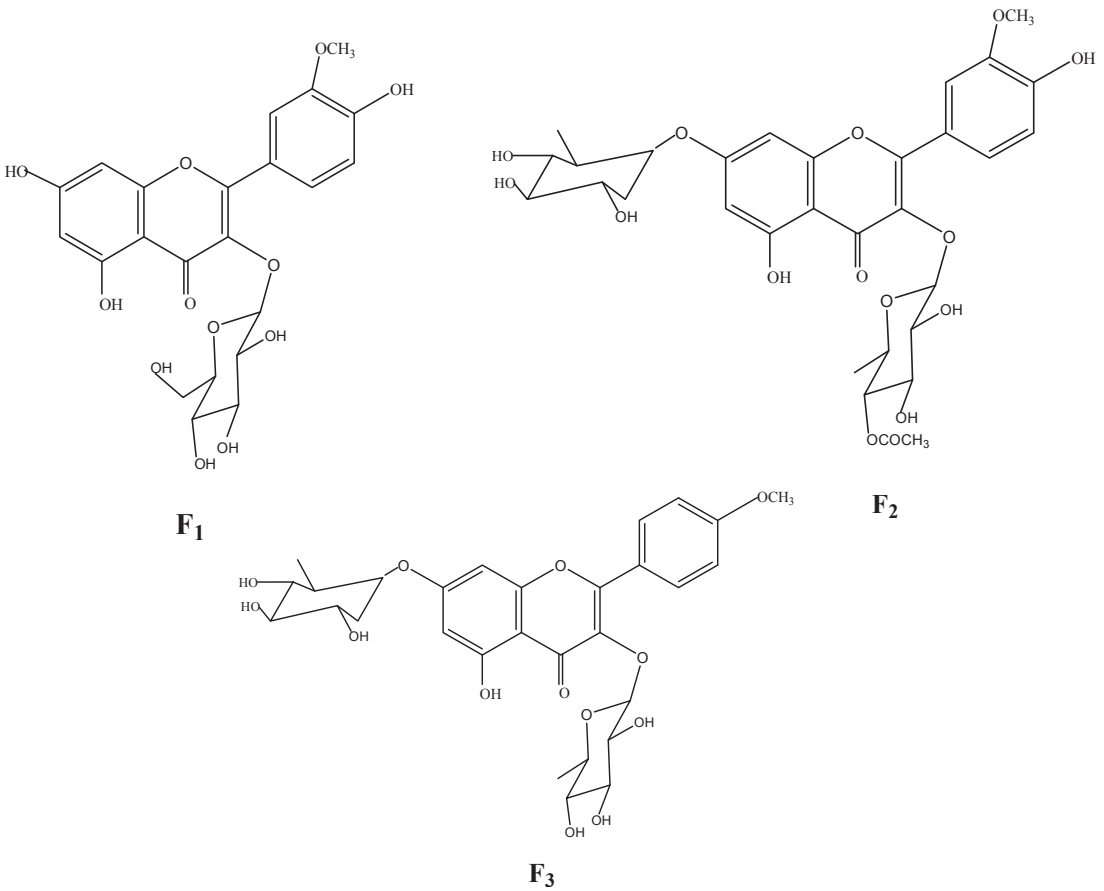

Fig. 2. Chemical structures of compounds isolated from the ethyl acetate fraction (EtFr).

Table V. UV spectral data (λ_{max} in nm) for compounds **F**$_1$–**F**$_3$.

Shift reagent	**F**$_1$	**F**$_2$	**F**$_3$
MeOH	255, 368sh, 303sh, 357	256, 369sh, 358	244sh, 266, 312sh, 351
NaOMe	272, 327, 415	244, 270, 396	246, 269, 301sh, 350sh, 389
$AlCl_3$	269, 299sh, 365sh, 407	276, 300sh, 343sh, 414	255sh, 274, 354, 401
$AlCl_3$/HCl	267, 298sh, 356, 403	270, 305sh, 366sh, 412	274, 298sh, 348, 398
NaOAC	274, 316, 387	260, 294sh, 370, 416sh	265, 318sh, 384
NaOAC/H_3BO_3	257, 268sh, 307sh, 361	262, 298sh, 377	265, 319sh, 352

and 169.93 ppm (O<u>C</u>OCH$_3$) which was correlated to C-4`` ($\delta_H$ 4.72 ppm; $\delta_C$ 69.8 ppm) of the rhamnose moiety in the HMBC and COSY spectra. From the above data compound **F**$_2$ was identified as quercetin-3`-methoxy-3-*O*-(4``-acetylrhamnoside)-7-*O*-*α*-rhamnoside (Fig. 2) which is a novel compound.

The data of compound **F**$_3$ were compatible with those reported for kaempferol-4`-methoxy-3,7-*O*-dirhamnoside (Fig. 2), previously isolated from an

Table VI. ^{1}H NMR chemical shifts (δ in ppm) for compounds $\mathbf{F_1}$–$\mathbf{F_3}$ (DMSO, 300 MHz, J in Hz).

H	$\mathbf{F_1}$	$\mathbf{F_2}$	$\mathbf{F_3}$
6	6.17 1H, d, J = 1.2	6.45 1H, d, J = 1.2	6.45 1H, d, J = 1.2
7	-	-	-
8	6.40 1H, d, J = 1.2	6.78 1H, d, J = 1.2	6.77 1H, d, J = 1.2
9	-	-	-
10	-	-	-
1`	-	-	-
2`	7.93 1H, br.s	7.45 1H, br.s	7.78 2H, d, J = 8.4
3`	-	-	6.92 2H, d, J = 8.4
4`	-	-	-
5`	6.89 1H, d, J = 8.4	6.94 1H, d, J = 8.4	6.92 2H, d, J = 8.4
6`	7.48 1H, dd, J = 2.1, 8.4	7.44 1H, dd, J = 2.1, 8.4	7.78 2H, d, J = 8.4
1``	5.54 1H, d, J = 6.9	5.55 1H, s	5.55 1H, s
2``	-	-	-
3``	-	-	-
4``	-	4.72 1H, t	-
5``	-	-	-
6``	-	1.13 3H, d, J = 6	1.13 3H, d, J = 6
1```	-	5.31 1H, s	5.31 1H, s
2```	-	-	-
3```	-	-	-
4```	-	-	-
5```	-	-	-
6```	-	0.82 3H, d, J = 6	0.82 3H, d, J = 6
OCH3–3`	3.83 3H, s	3.87 3H, s	-
OCH3–4`	-	-	3.86 3H, s
OCOCH3	-	2.00 3H, s	-

Egyptian collection of *C. droserifolia* (Nassar and Gamal-Eldeen, 2003).

These isolated compounds had been proven before to possess significant hypoglycemic activities in C2C12 skeletal muscle cells and 3T3-L1 adipocytes (Motaal *et al*., 2011).

Here they were assessed for their cytotoxicity against two human carcinoma cell lines. 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). Thus, the two new compounds $\mathbf{C_3}$ and $\mathbf{F_2}$ showed the highest cytotoxicity [(1.9 ± 0.08), (1.6 ± 0.09) and (3.35 ± 0.57), (3.80 ± 1.01) μg/ml corresponding to 6.5, 5.4 and 5.0, 5.7 μM] against MCF7 and HCT116 cells, respectively. Their effects were comparable to those of the standard drug doxorubicin® [(2.97 ± 0.05) and (3.73 ± 0.21) μg/ml which corresponds to 5.0

and 6.4 μM, respectively]. On the other hand, compounds $\mathbf{C_1}$ and $\mathbf{F_3}$ showed certain selectivity against HCT116 cells [(3.6 ± 0.03) and (2.48 ± 0.42) μg/ml corresponding to 17.6 and 3.9 μM, respectively] compared to doxorubicin® (Table I).

This work introduces some of the compounds isolated from *Cleome droserifolia* ($\mathbf{C_1}$, $\mathbf{C_3}$, $\mathbf{F_2}$, and $\mathbf{F_3}$) as valuable lead compounds possessing *in vitro* cytotoxic activities against human MCF7 and HCT116 cell lines similar to those of the positive control doxorubicin®. Further *in vivo* and clinical studies are required to define safe and effective curative doses.

Acknowledgement

The authors are deeply thankful to Prof. Dr. Ahmed K. Hegazy, Professor of Botany, Faculty of Science, Cairo University, Egypt, and to the project "Development and Extraction of New Materials and its Applications" funded by Cairo University, Egypt, for providing the plant material.

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