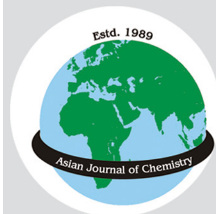

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Phytochemical Studies on the Toxic Compounds of *Gladiolus segetum*

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Gladiolus segetum (Iridaceae) is a toxic plant, lethal for livestock. The phytochemistry and biological activity of this plant have not been extensively investigated. The aim of present study is to elucidate the structure of the toxic compounds responsible for the lethal effect of this plant. We report here the isolation from the aerial parts, four anthraquinones: 3,8-dihydroxy-4,7-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**1**), 3,8-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester (**2**), 1-hydroxy-3,6,7-trimethoxy-8-methylanthraquinone (**3**), 3,8-dihydroxy-6-methoxy-1-methylanthraquinone-2-carboxylic acid (**4**) along with quercetin-3-O-β-D-glucopyranoside (**5**), kaempferol-3-O-β-D-glucopyranoside (**6**), betulinic acid (**7**), stigmasterol (**8**), ergosterol (**9**), cholesterol (**10**), ursolic acid (**11**) and the cytotoxicity effects toward MRC-5 cells of nine fractions obtained from a DCCC. The structures of new natural products anthraquinones **1** and **2** were elucidated by spectral (UV, IR, 1D and 2D NMR and HR-ESI-MS) methods.

Key Words: *Gladiolus segetum*, Iridaceae, Anthraquinones, Toxicity.

INTRODUCTION

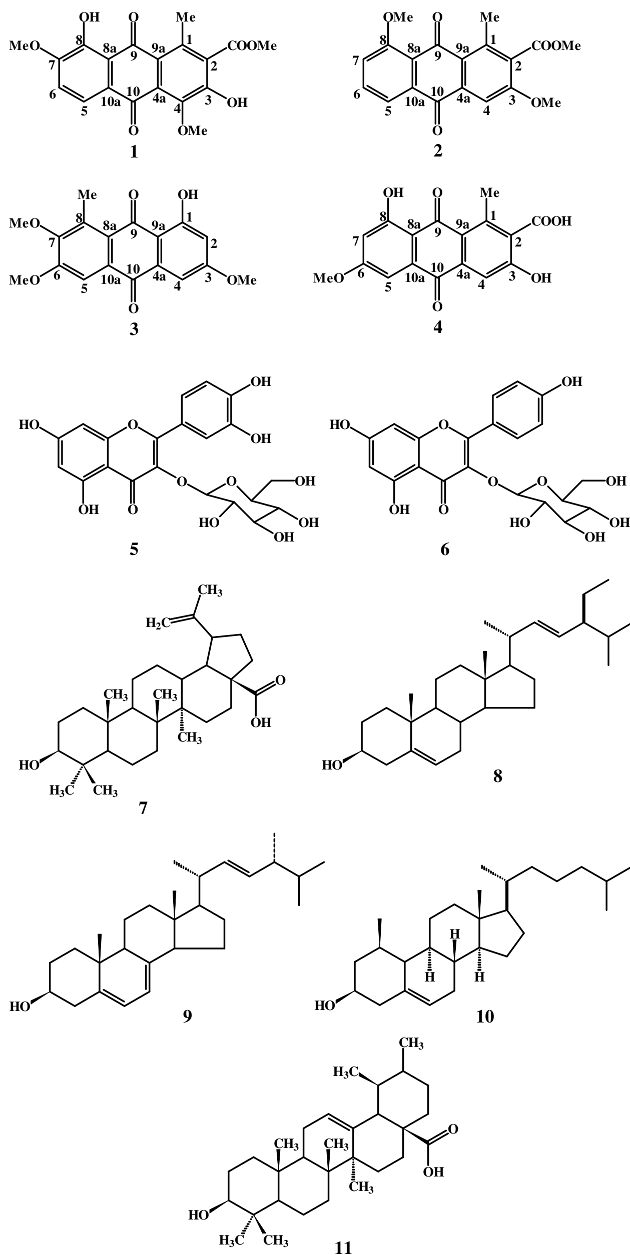
Gladiolus segetum (Iridaceae)[1,2] called "Sif er rorab"[1], is a beautiful ornamental plant. It is used in the Southern Algeria, in traditional medicine mixed with other known plants for their therapeutic effects as antiulceric. However, it is a toxic plant, lethal for livestock, growing in wheat fields and cultures. It is wide spread in high plains in Algeria. During four years of observation it is noticed that after ingestion of this plant, 75 % of the cattle presented signs of poisoning. Generally all the animals which consumed the gladiolus die after 72 h. The phytochemistry and biological activity of this plant have not been extensively investigated. The aim of present study is to elucidate the structure of the toxic compounds responsible for the lethal effect of this plant. Previous investigations led to isolation of saponins[3], anthraquinones[4]. In earlier work[5,6] we have reported the cytotoxicity effects toward MRC-5 cells on the $CHCl_3$ extract, EtOAc, MeOH extracts. The methanolic extract is the most toxic. Therefore we proceeded with the methanolic extract which has the highest toxicity. In the present paper we report the cytotoxicity effects toward MRC-5 cells of nine fractions obtained from a DCCC and isolation of two new anthraquinones: 3,8-dihydroxy-4,7-dimethoxy-1-methyl-anthraquinone-2-carboxylic acid methyl ester (**1**), 3,8-dimethoxy-1- methylanthraquinone -2-carboxylic acid methyl ester (**2**), in addition to 9 known compounds, 1-hydroxy-3,6,7-trimethoxy-8-methylanthraquinone[7] (**3**), 3,8-dihydroxy-6-methoxy-1-methylanthraquinone-2-carboxylic acid[4] (**4**) along with quercetin-3-O-β-D-glucopyranoside[8] (**5**), kaempferol-3-O-β-D-glucopyranoside[8] (**6**), betulinic acid (**7**), stigmasterol (**8**), ergosterol (**9**), cholesterol (**10**), ursolic acid[9] (**11**). The structures of the new anthraquinones were established by spectroscopic analyses (UV, IR, ^{1}H and ^{13}C NMR, HMBC and HR-ESI-MS) and those of the known isolates were determined by comparing their spectral data to those given in the literature and by TLC comparison with standard samples.

EXPERIMENTAL

Melting points were determined on an Electrothermal apparatus. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. The IR and UV spectra were recorded on a Perkin-Elmer 281 spectrophotometer and a Shimadzu UV-3101 PC spectrophotometer, respectively. NMR data were recorded on a Bruker DMX spectrometer. Chemical shifts δ are reported in ppm. MS spectra (HR-ESI-MS) was carried out on a Q-TOF 98 micro instrument (Bruker Customer). DCCC: DC-300-G2, Tokyo Rikakikai Co., Tokyo, Japan) equipped with 72 long vertical glass-columns (60 cm) (2 mm i.d.) interconnected in series by capillary Teflon tubes.

Cell lines and cell culture: MRC-5 (human pulmonary embryonic fibroblasts) were obtained from BioMerieux institute

(Lyon, France). Cells were maintained in modified Eagle's medium (MEM, Invitrogen 41090) supplemented with 10 % decomplemented foetal bovine serum (FBS, Invitrogen 10270, lot 40Q5150K) without antibiotics at 37 ºC, 5 % CO_2, under a humid atmosphere. MRC-5 were plated at 104 cells/well in 96-well plates (Sarstedt 831835). Forty eight hours after plating, the growth medium was removed and replaced with the test solutions (100 µL). After 24 h exposure, cytotoxicity tests were performed.



Structures of compounds **1-11**

Lactate dehydrogenase (LDH): The detection procedures of lactate dehydrogenase release (Cyto-tox 96 kit non-radio-active cytotoxicity assay, Promega G1780) were in accordance with the manufacturer's instructions. Briefly, the 96-well plates were, centrifuged at 3000 g for 10 min to have "cell-free" supernatants. Then 50 µL of each sample were transferred to a 96-well plate (Greiner, 650161). Freshly prepared reaction mixture was added to each well and incubated up to 0.5 h in the dark at room temperature. Absorbance was measured at 540 nm using an ELISA plate reader (Multiskan EX, Thermo Electron Corporation, France). Eight wells per dose and time point were counted in only one experiment.

Plant material: The plant material was collected in Mai 2004 in the region of Batna (Algeria) and was identified by Dr. Bachir Oudjehih, Agro veterinary Department of the Batna University, where a voucher specimen (183DAUB2004) is deposited.

Extraction and isolation: We performed an extractive protocol on 800.22 g of a dry matter (aerial part) of *Gladiolus segetum*. The plant material was subjected to subsequent extraction by *n*-hexane (4 L), $CHCl_3$ (4 L), EtOAc (4 L) and MeOH (10 L). The MeOH extract, after filtering, was concentrated in vacuum at 35 ºC to yield 29.50 g of the crude extract. The extract was dissolved in a mixture of $CHCl_3$-MeOH-H_2O (43:37:20) and filtered through a 0.2 µm filter, then it is subjected to a DCCC. The ascending method was utilized in our DCCC separation with $CHCl_3$-MeOH-H_2O (43:37:20) (the heavy phase was the stationary phase; the light phase was the mobile phase). A first step consists to fill the whole system with stationary phase followed by the injection of the sample in a sample chamber. The mobile phase is then pumped through the sample chamber and inserted with the capillary tube into the bottom of the first glass column. Fractions of 10 mL were collected and analyzed by TLC (Silica gel 60 F_{254}, 0.25 mm), using $CHCl_3$-MeOH (9:1) as eluent and visualized by spraying with 10 % H_2SO_4 in ethanol. All the fractions containing similar compound were combined then concentrated. Nine fractions were obtained. Fractions F_1, F_3 and F_9 were subjected to a series of chromatographic techniques, such as silica gel column (50 mm × 800 mm) and prep. HPLC (a column of silica gel, 10 mm × 500 mm was used) to afford eleven compounds **1-11**.

F_1: Fractions 1-19 (200 mg, orange colored) containing anthraquinones was purified by repeated preparative HPLC using the binary gradient elution system consisted of EtOAc (solvent A) and MeOH (solvent B), to afford fractions A-E. Compounds **1** (10 mg), **2** (15 mg), **3** (17 mg) and **4** (15 mg) were obtained from fraction B (subfractions 15-30), fraction C (subfractions 35-40), fraction D (subfractions 45-50) and fraction E (subfractions 52-60), respectively. **F_3:** Fractions 30-44 containing flavonoids (95 mg) was submitted to column chromatography on silica gel eluted with a gradient of EtOAc-MeOH to give **5** (7 mg) and **6** (8 mg). **F_9:** Fractions 106-120 (75 mg) was rechromatographed on silica gel column using a gradient of $CHCl_3$-MeOH to yielded **7** (5 mg), **8** (4 mg), (**9**) (10 mg), **10** (5 mg) and (**11**) (9 mg). Compounds **3**, **4**, **5**, **6** and **11** were determined by comparing their UV, IR, MS and NMR data with those reported. Compounds **7**, **8**, **9** and **10** were identified by comparing them with authentic samples on TLC. Compounds **3**, **5**, **6**, **7**, **8**, **9**, **10** and **11** are reported for the first time from *Gladiolus segetum*.

Structure and identification

Compound 1: Orange powder, $[\alpha]_D^{25}$ 284º (c 0.08 in MeOH), m.p. 240-242 ºC, UV (MeOH): λ_{max} (ε) = 220, 275, 315, 420 nm; IR (KBr, ν_{max}, cm^{-1}): 3337, 1720, 1665, 1620,

1591, 1503 and 1447. HR ESIMS m/z: negative mode 371.3137 [M-H]-(calcd. (%) for $C_{19}H_{15}O_8$, 371.3195). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC) δ = 13.17 (s, 8-OH), 8.05 (d, 1H, *J* = 8 Hz, H-5), 7.30 (d, 1H, *J* = 8 Hz, H-6), 2.67 (s, 3H, 1-Me), 3.96 (s, 3H, 2-OMe), 3.98 (s, 3H, 4-OMe), 3.94 (s, 3H, 7-OMe). ^{13}C NMR (Table-1).

Compound 2: Orange powder; $[\alpha]_D^{25}$ 274º (c 0.08 in MeOH), m.p. 234-236 ºC, UV (MeOH): λ_{max} (ε) = 224, 280, 289, 412 nm; IR (KBr, ν_{max}, cm^{-1}): 3386, 1730, 1665, 1622, 1589, 1504 and 1449. HR ESIMS m/z: negative mode 339.2969 [M-H]-(calcd. (%) for $C_{19}H_{15}O_6$, 339.2975). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC) δ: 7.84 (1H, dd, *J* = 8.0, 1.1 Hz, H-7), 7.73 (1H, dd, *J* = 7.8, 1.1 Hz, H-5), 7.66 (1H, dd, *J* = 8.0, 7.8 Hz, H-6), 7.62 (s 1H, H-4), 2.65 (s, 3H, 1-Me), 3.85 (s, 3H, 2-OMe), 3.98 (s, 3H, 3-OMe), 3.80 (s, 3H, 8-OMe). ^{13}C NMR (Table-1).

TABLE-1
^{1}H AND ^{13}H NMR SPECTRAL DATA OF COMPOUND **1** AND **2** IN $CDCl_3$ CHEMICAL SHIFTS ARE GIVEN IN ppm AND COUPLING CONSTANTS *J* IN Hz

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	132.3		137.5	
2	125.3		124.7	
3	155.1	162.4		
4	162.6		107.5	7.62 (s)
4a	118.0		132.0	
5	125.4	8.05 (d, 8.0)	12.0	7.73 (d, 8.0)
6	136.0	7.30 (d, 8.0)	135.8	7.66 (t, 8.0)
7	162.4		118.8	7.84 (d, 8.0)
8	159.9		165.5	
8a	116.9		110.2	
9	189.0		189.7	
9a	137.4		141.5	
10	188.9		182.4	
10a	132.3		131.2	
3-OH		12.89 (s)		
8-OH		13.60 (s)		
2-OMe		3.96 (s)		3.85 (s)
3-OMe			56.5	3.98 (s)
4-OMe	61.6	3.98 (s)		
7-OMe	61.2	3.94 (s)		
8-OMe			60.3	3.80 (s)
$2-CO_2Me$	166.9		167.5	
1-Me	19.7	2.67 (s)	20.1	2.65 (s)

Compound 3: Orange powder; m.p. 273-275 ºC, UV λ_{max} (MeOH): 220, 288, 300, 400 nm. IR (KBr, ν_{max}, cm^{-1}): 3380, 2950, 2850, 1622, 1620, 1570 and 1400. HR ESIMS, m/z: positive mode 328.3175 $[M]^+$ (calcd. (%) for $C_{18}H_{16}O_6$, 328.3028) ^{1}H NMR (300 MHZ, $CDCl_3$), δ: 13.18 (s, 1-OH), 6.80 (d, 1H, *J* =2.5 Hz, H-2), 7.00 (d, 1H, *J* = 2.5 Hz, H-4), 7.70 (s, 1H, H-5), 2.60 (s, 3H, 8-Me), 3.85 (s, 3H, 3-OMe), 3.81 (s, 3H, 6-OMe), 3.80 (s, 3H, 7-OMe). ^{13}C NMR (75 MHz, CD_3COCD_3), δ: 162.2 (C-1), 106.0 (C-2), 164.5 (C-3), 107.5 (C-4), 136.5 (C-4a), 109.0 (C-5), 155.5 (C-6), 154.5 (C-7), 120.5 (C-8), 129.0 (C-8a), 186.0 (C-9), 113.8 (C-9a) 183.3 (C-10), 133.2 (C-10a), 55.0 (3-OMe), 55.5 (6-OMe), 60.4 (7-OMe), 18.1 (Me).

Compound 4: Orange powder; m.p. 238-240 ºC, UV λ_{max} (MeOH): 226, 283, 320, 429 nm. IR (KBr, ν_{max}, cm^{-1}): 3385, 3325, 3300, 2850, 2600, 1722, 1700, 1623, 1570 and 1500. HR ESIMS, m/z: 328.0175 $[M]^+$ (calcd. (%) for $C_{17}H_{12}O_7$, 328.2606). ^{1}H NMR (300 MHZ, $CDCl_3$),δ: 13.18 (s, 8-OH), 7.15 (d, 1H, *J* = 2.5 Hz, H-5), 7.65 (s, Hz, H-4), 6.75 (d, *J* = 2.5 Hz, H-7), 2.65 (s, 3H, 1-Me), 3.88(s, 3H, 6-OMe). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 138.1 (C-1), 132.8 (C-2), 158.5 (C-3), 109.1 (C-4), 134.0 (C-4a), 110.0 (C-5), 163.5 (C-6), 112.5 (C-7), 162.2 (C-8), 114.5 (C-8a), 188.0 (C-9), 116.8 (C-9a) 182.0 (C-10), 130.2 (C-10a), 55.5 (6-OMe), 19.7 (Me). 168.0 (COOH).

Compound 5: Yellow amorphous powder, m.p.: 183-184 ºC, UV λ_{max} (MeOH): 360 and 264 nm. IR (KBr, ν_{max}, cm^{-1}): 3431, 1620 and 1604. HR ESIMS, m/z: 465.3608 $[M + H]^+$ consistent with a molecular formula $C_{21}H_{20}O_{12}$. ^{1}H NMR (300 MHZ, $CDCl_3$), δ_H: 6.20 (1H, d, *J* = 2.5 Hz, H-6), 6.40 (1H, d, *J* = 2.5 Hz, H-8), 7.67 (1H, d, *J* = 2.5 Hz, H-2'), 6.90 (1H, d, *J* = 8.5 Hz, H-5') and 7.62 (1H, dd, *J* = 8.5, 2.5 Hz, H-6'). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 154.60 (C-2), 132.65 (C-3), 175.70 (C-4), 159.50 (C-5), 97.00 (C-6), 162.70 (C-7), 91.70 (C-8), 154.47 (C-9), 102.00 (C-10), 119.60 (C-1'), 113.40 (C-2'), 143.00 (C-3'), 146.70 (C-4'), 114.42 (C-5'), 119.71 (C-6'), 99.30 (C-1''), 72.24 (C-2''), 74.67 (C-3''), 68.04 (C-4''), 75.59 (C-5''), 59.09 (C-6''). 4.89 (d, 1H, *J* = 7.7 Hz, H-1''), 3.43 (m, H-2''), 3.45 (m, H-3''), 3.46 (m, H-4''), 3.40 (m, H-5''), 3.89 (1H, dd, *J* = 2.2, 12 Hz, H-6''), 3.69 (1H, dd, *J* = 5.4, 12 Hz, H-6''),

Compound 6: Yellow amorphous powder, m.p. 180-181 ºC, UV λ_{max} (MeOH): 358 and 272 nm. IR (KBr, ν_{max}, cm^{-1}): 3160, 1630 and 1612 cm^{-1}. HR ESIMS, m/z: 449.3614 $[M + H]^+$ consistent with a m.f. $C_{21}H_{20}O_{11}$. ^{1}H NMR (300 MHZ, $CDCl_3$), δ_H: 6.20 (1H, d, *J* = 2.5 Hz, H-6), 6.38 (1H, d, *J* = 2.5 Hz, H-8), 6.80 (2H, d, *J* = 2.5, 8.5 Hz, H-5', H-3') and 7.95 (2H, d, *J* = 2.5, 8.5 Hz, H-6', H-2'). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 154.77 (C-2), 131.58 (C-3), 175.83 (C-4), 159.46 (C-5), 96.84 (C-6), 162.50 (C-7), 91.84 (C-8), 154.77 (C-9), 102.25 (C-10), 119.23 (C-1'), 129.15 (C-2'), 113.32 (C-3'), 158.1 (C-4'), 113.32 (C-5'), 129.15 (C-6'), 99.32 (C-1''), 72.30 (C-2''), 74.55 (C-3''), 68.04 (C-4''), 75.53 (C-5''), 58.98 (C-6''), 3.93 (d, 1H, *J* = 7.8 Hz, H-1''), 3.43 (m, H-2''), 3.45 (m, H-3''), 3.46 (m, H-4''), 3.40 (m, H-5''), 3.88 (1H, dd, *J* = 2.6, 12 Hz, H-6''), 3.70 (1H, dd, *J* = 4.6, 12 Hz, H-6'').

Compound 11: Ursolic acid, white amorphous powder, m.p. 259-261 ºC, HR ESIMS, m/z: 479.6640 $[M + Na]^+$ consistent with a m.f. $C_{30}H_{48}O_3$. ^{1}H NMR (300 MHz, $CDCl_3$), δ_H (ppm) 0.70 (3H, s, H-25), 0.75 (3H, d, *J* = 9 Hz, H-29), 0.80 (3H, d, *J* = 6 Hz, H-30), 0.88 (3H, s, H-24), 0.90 (6H, s, H-26, H-27), 1.05 (3H, s, H-23), 3.08 (1H, dd, *J* = 10.5, 4.8 Hz, H-3), 5.00 (1H, t, *J* = 3.6 Hz, H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ_H (ppm) 37.50 (C-1), 25.55 (C-2), 77.50 (C-3), 37.95 (C-4), 54.42 (C-5), 17.50 (C-6), 31.40 (C-7), 38.60 (C-8), 46.48 (C-9), 35.20 (C-10), 21.90 (C-11), 124.50 (C-12), 138.00 (C-13), 40.45 (C-14), 26.70 (C-15), 22.66 (C-16), 46.90 (C-17), 52.00 (C-18), 37.90 (C-19), 37.60 (C-20), 29.65 (C-21), 36.00 (C-22), 26.45 (C-23), 13.15 (C-24), 13.30 (C-25), 16.00 (C-26), 21.95 (C-27), 179.00 (C-28), 15.2 (C-29), 19.50 (C-30).

RESULTS AND DISCUSSION

The *in vitro* cytotoxic activities of the nine fractions from the DCCC of MeOH extract on MRC-5 cells, was determined using the lactate dehydrogenase assay. The cytotoxic effect is shown in Fig. 1.

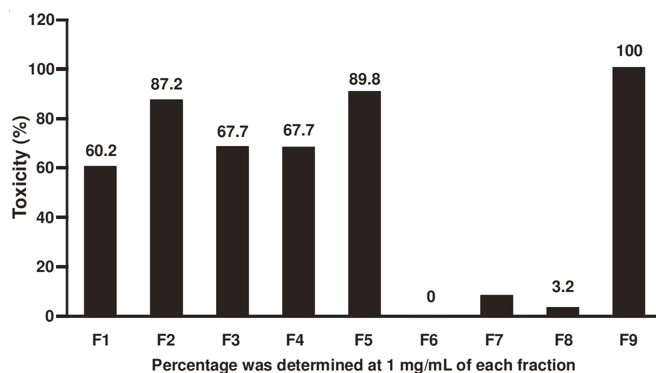


Fig. 1. *In vitro* cytotoxic activities of the **9** fractions

From the first fraction we isolated four anthraquinones. Compound **1** was obtained as orange powder. Its molecular formula was determined by HR ESIMS at m/z 371.3137 $[M-H]^-$ (calcd. 371.3195 for $C_{19}H_{15}O_8$). m.p. 240-242 ºC. The IR spectrum indicated the presence of hydroxy (3337 cm^{-1}), an ester carbonyl (1720 cm^{-1}), unchelated (1665 cm^{-1}) quinone carbony1groups and 1620 (chelated quinone CO) cm^{-1} and aromatic rings (1591, 1503 and 1447 cm^{-1}). Along with the IR data, UV-vis absorption maxima at 220, 275, 315 and 420 nm, two carbonyl carbons resonating at δ: 188.9 and 189.0 in the ^{13}C NMR spectrum.

The ^1H NMR spectrum, showed a singlet at δ 13.17 (1H, s, 8-OH) confirming a chelated hydroxy group, 12.89 (1H, s, 3-OH), two aromatic proton resonances at δ 8.05 (1H, d, J = 8 Hz, H-5) and 7.30 (1H, d, J = 8 Hz, H-6). These assignments were in agreement with the HMBC correlations observed between H-5 and C-6 and C-10a; H-6 and C-7 and C-5. The HMBC correlation between the chelated hydroxy group signal at δ: 13.17 and C-8a (δ 116.9) was used to place the hydroxy group on C-8. Signals attributable to an α methyl and 3 methoxy groups were observed at δ: 2.67, 3.94(C7), 3.96 (C2) and 3.98 (C4). The signal at δ 2.67 was assigned to a methyl group on C-1. This assignment was further confirmed from the HMBC correlations between the methyl signal and C-9a (δ 137.4), C-1 (δ 132.3) and C-2 (δ 125.3). The proton H-6 and a methoxy group (δ 3.94) correlated with C-7 (δ 162.4) in the HMBC experiment, thus the methoxy group could only be located on C-7. HMBC correlations between signals at δ 3.98 and C-4, C-4a and C-3 were used to place the second methoxyl on C-4. The third methoxy group resonating at δ 3.96 was located on C-2 by its HMBC correlation with C-2, C-1 and C-3. On the basis of the above evidences the new compound **1** is established to be 3,8-dihydroxy-4,7-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester which is reported for the first time (Fig. 2).

Compound **2** was obtained as an orange powder. The HR-EIMS of compound **2** showed a molecular ion peak at m/z: 339.2969 $[M-H]^-$ (calcd. 339.2975 for $C_{19}H_{15}O_6$) corresponding

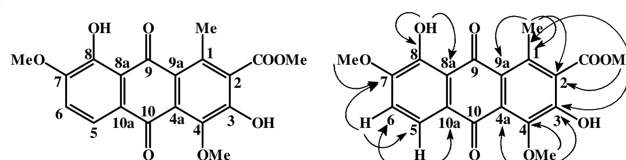


Fig. 2. Structures of the isolated compound **1** and the key HMBC (H→C) correlations

to the m.f. $C_{19}H_{16}O_6$. m.p. 234-236 ºC. The IR spectrum of **2** exhibited the presence of the following functionalities: hydroxy (3386 cm^{-1}), unchelated carbonyl (1672 cm^{-1}) and an ester carbonyl (1730 cm^{-1}). Along with the IR data, UV-vis absorption maxima at 224, 280, 289 and 412 nm, two carbonyl carbons resonating at δ: 189.7 and 182.4 in the ^{13}C NMR spectrum. The ^1H NMR spectrum reveals signals for a four aromatic protons at δ: 7.84, 7.73, 7.66 and 7.62, that could be assigned to H-7, H-5, H-6 and H-4, respectively.

These assignments were in agreement with the HMBC correlations observed between H-5 and C-10a and C-6; H-6 and C-7 and C-5; H-7 and C-8, C-6 and H-4 and C4a and C3 (Fig. 3). The signal at δ 2.37 was assigned to a methyl group on C-1. This assignment was further confirmed from the HMBC correlations between the methyl signal and C-2 (δ 124.7), C-1 (δ 137.5) and C-9a (δ 141.5). The proton H-4 and a methoxy group (δ 3.98) correlated with C-3 (δ 162.4) in the HMBC experiment, thus the methoxy group could only be located on C-3. The methoxy group resonating at δ 3.80 showed HMBC cross peaks with C-8 and C-7 thus could only be located on C-8. The third methoxy group resonating at δ 3.85 was located on C-2 by its HMBC correlations with C-1, C-2 and C-3. From the foregoing discussion compound **2** was established to be 3,8-dimethoxy-1-methylanthraquinone-2-carboxylic acid methyl ester. Compound **2** is a new natural product. However it has been reported as a synthetic intermediate[10].

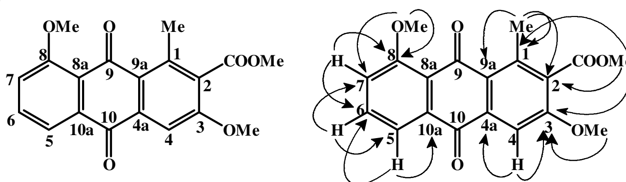


Fig. 3. Structures of the isolated compound **2** and the key HMBC (H→C) correlations

Compounds **3** and **4** were identified as 3,8-dihydroxy-6-methoxy-1-methylanthraquinone-2-carboxylic acid[4] (**3**) and 1-hydroxy-3,6,7-trimethoxy-8-methylanthraquinone[7] (**4**). The first fraction showing 60.2 % of toxicity at 1 mg mL^{-1}. Many anthraquinones exhibit various biological activities as pulmatin chrysophanein and physcionin[11]. These anthraquinone glycosides were found to exhibit cytotoxic activity against several types of carcinoma cells. Cytotoxic effect of hydroxy and 3-alkylaminopropoxy-9,10-anthraquinone derivatives against Hep G2, Hep 3B and HT-29 cell lines *in vitro*[12]. Glucose-6-phosphate translocase as inhibitors, antifungal and antibacterial[13], antibiotics[14]. From the third fraction we isolated quercetin-3-O-β-D-glucopyranoside (**5**) and kaempferol-3-O-β-D-glucopyranoside[8] (**6**). The third fraction is considered to have a signi-

ficant cytotoxic activity showing 67.7 % at 1 mg mL^{-1}. Many flavonoids, isolated from medicinal herbs used in traditional medicines, are endowed with desired biological activities such as antimicrobial, antihepatotoxic, antiinflammatory, cardioprotective, antiosteoporotic, antiulceric, anticancer, reducing total body fat[15]. These findings have contributed to the dramatic increase in the consumption and use of dietary supplements containing high concentrations of plant flavonoids. However, more research on the toxicological properties of flavonoids is warranted given their increasing levels of consumption[16]. From the ninth fraction we isolated betulinic acid (BA) (**7**), stigmasterol (**8**), ergosterol (**9**), cholesterol (**10**) and ursolic acid (UA)[9] (**11**). The ninth fraction is considered to have the most cytotoxic activity at 1 (mg mL^{-1}). Many reports have tempted to demonstrate the potent bioactivity of terpenes and more particularly UA and BA, cytotoxic assays demonstrated that two compounds induced a significant cytotoxicity[17,18].

Conclusion

In this study, an investigation on the *in vitro* cytotoxic activities, of *Gladiolus segetum* was carried out to verify the toxicity and to elucidate the structure of the toxic compounds and claimed traditional uses of the plant. Results have revealed that the methanolic extract of the aerial parts of the plant was the most active. Fractionation of this extract through DCCC led to the isolation of nine fractions (Fig. 1). The ninth fraction shows the most toxicity. Possibly several other constituents in less polar and more polar extracts from the plant may have a cytotoxic activity. In order to reveal the complete activity profile of this plant, the active fractions and their constituents of these extracts should also be investigated thoroughly.

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