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Chemical constituents and their antibacterial and antifungal activity from the Egyptian herbal medicine *Chiliadenus montanus*

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ABSTRACT

Phytochemical investigations of the CH_2Cl_2/MeOH (1:1) extract of air-dried aerial parts of *Chiliadenus montanus* afforded eight metabolites, in addition to eight other previously reported compounds, of which two were isolated for the first time as free acids. Structures were established by spectroscopic methods, including HREIMS, ^1H, ^{13}C, DEPT, ^1H–^1H COSY, HMQC and HMBC NMR analysis. Antimicrobial activity against an array of common bacterial and fungal strains was measured via a colorimetric assay with minimal growth inhibition observed in the μg/mL range for one of the tested metabolites.

Introduction

The Sinai Peninsula is an epicenter of medicinal plants in the Arabian Desert with active plant constituents serving as a focal point for ecologists, taxonomists and phytochemists alike from around the globe (Abd El-Wahab et al., 2004; Bailey and Danin, 1981; Boulos, 1983; Bown, 1995; Batanouny et al., 1999; Hanafi and Abdel-Wahab, 2000). *Chiliadenus montanus* (Vahl.) Brullo [=*Jasonia montana*, *Varthemia montana* (Vahl.) Boiss.], an herb endogenous to the North Sinai region of Egypt, is a member of the Asteraceae (Boulos, 2002). Previous phytochemical investigations of it have established the presence of monoterpenes (Ahmed and Jakupovic, 1990), sesquiterpenes (Ahmed, 1991; Ahmed and Jakupovic, 1990; Ahmed et al., 1988; Ahmed et al., 2004; Mahmoud, 2006; Mohamed, 2007), diterpenes (Al-Howiriny et al., 2005), triterpenes, sterols (Eid et al., 1987) and flavonoids (Ahmed et al., 1989; El-Din et al., 1985; Soliman et al., 2009; Zaghloul, 1989). Locally known as Haneida (Täckholm, 1974), this threatened medicinal plant is used as a herbal tea for the treatment of renal troubles and select chemical components have been shown to exhibit anti-diabetic, antimicrobial, anti-obesity, antiatherogenic, and antioxidant activities (Al-Howiriny et al., 2005; Hussein, 2011).

Herein, elucidation of new compounds **1**–**8** isolated from the dry aerial parts of *C. montanus* is reported, in addition to previously reported compounds **9**–**16**, two of which (**11**–**12**) were isolated as free acids for the first time (Fig. 1). Chemical structures were established by comprehensive spectroscopic analysis and comparisons with previous literature reports when possible. Antimicrobial activity for all purified metabolites was assayed against an array of wide-spread bacterial and fungal species.

Results and discussion

The methylene chloride/methanol (1:1) extract of the air-dried aerial parts of *C. montanus* was subjected to normal and reversed phase chromatography to afford a series of metabolites as reported here, in addition to previously reported compounds **9**–**16** (Fig. 1).

Compound **1** was isolated as colorless needles, $[\alpha]_D^{25}$ = +8.3 (*c* 0.3, $CHCl_3$) and its IR spectrum showed absorption bands at 3250 (OH), 3020 (OH), 1725 (C=O), and 1650 (C=C) cm^{-1}. The low resolution mass spectrum had a molecular ion peak $[M]^+$ at *m*/*z* 410. Its HREIMS exhibited a molecular ion peak $[M]^+$ at *m*/*z* 410.1946 (calcd. for $C_{21}H_{30}O_8$: 410.1941). The molecular formula of $C_{21}H_{30}O_8$ was confirmed by ^1H and ^{13}C NMR spectroscopic analysis (Table 1). Acid

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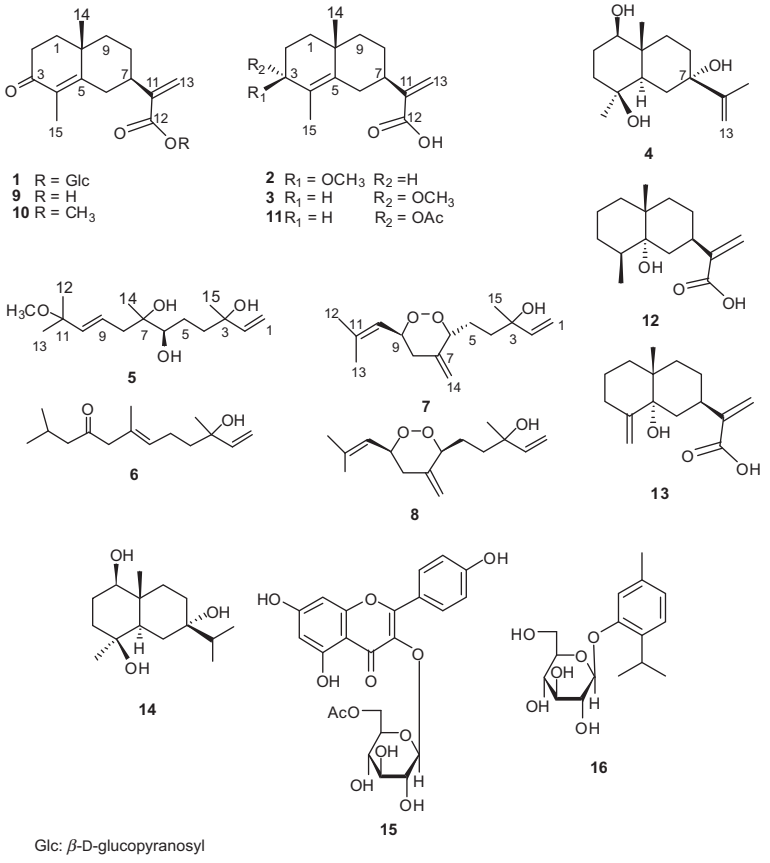

Fig. 1. Isolated metabolites from *Chiliadenus montanus*.

hydrolysis generated 3-oxo-γ-costic acid (**9**) and a sugar. Seven sugar signals were observed including: H-6′ protons that appeared at δ_H 3.81 (dd, J = 12.0, 2.0, H-6′) and 3.67 (dd, J = 12.0, 5.4, H-6′); an anomeric proton at δ_H 5.56 (d, J = 7.5 Hz); and other sugar signals at δ_H 3.33–3.43 (4H, m). Sugar proton resonances showed correlations in the HMQC spectrum with carbon signals at δ_C 94.7 (C-1′), 72.7 (C-2′), 76.8 (C-3′), 69.7 (C-4′), 77.6 (C-5′), and 60.9 (C-6′). The carbohydrate unit was confirmed to be D-glucose based on HPLC retention time comparison with a D-glucose authentic standard (retention time 7.1 min) and positive optical rotation. The β-anomeric configuration for glucose was determined from a relatively large $^3J_{H1,H2}$ coupling constant (J = 7.5 Hz) (Ye et al., 2002). Based on a correlation in the HMBC spectrum between the anomeric resonance at δ_H 5.56 and a carbonyl group at C-12 (δ_C 165.5) (Fig. 3), the sugar-terpene linkage was identified and **1** was named 3-oxo-γ-costic acid β-D-glucopyranoside ester, a new natural product.

Compound **2** was isolated as a yellowish oil, ($[\alpha]_D^{25}$ = +73.0 (*c* 0.01, $CHCl_3$)) and its IR spectrum showed absorption bands at 3020 (OH), 1715 (C=O), and 1670 (C=C) cm^{-1}. HREIMS exhibited a molecular ion peak $[M]^+$ at *m/z* 264.1734 (calcd. for $C_{16}H_{24}O_3$: 264.1725). The ^{1}H- and ^{13}C-NMR spectrum (Table 1) confirmed the molecular formula $C_{16}H_{24}O_3$ and carbon signals were classified by DEPT NMR as follows: three methyl carbon resonances at δ_C 17.4, 23.0 and 56.9; six methylene carbon signals at δ_C 22.0, 28.0, 31.2, 34.2, 42.0 and 124.7; two methine carbon resonances δ_C 40.0 and 79.3; and five quaternary carbon signals at δ_C 35.0, 124.0, 140.2, 145.4, and 170.8. Based on NMR spectroscopic comparisons with other metabolites previously characterized from this species, **2** was very similar to 3-oxo-γ-costic acid (**9**) with the exo-methylene carboxylic acid group attached to C-7 in a β-configuration. However, in contrast to a C-3 carbonyl group present in **9**, a methoxy group functionality at δ_H 3.41 (s) was observed in **2**. The relative configuration of C-3 and C-7 was based on comparable NMR spectroscopic data for a published eudesmane sesquiterpene (3β,10α)-3-methoxyeudesma-4,11(13)-dien-12-oic acid (Zhang et al., 2013) equivalent to compound **2** at H-3 (δ_H 3.41 (s) versus δ_H 3.39 (s), respectively) and H-7 (δ_H 2.54, J = 13, 2.8 versus δ_H 2.44 J = 11.6, respectively). The relative beta methyl configuration at C-10 was assigned based on NOESY correlations between H-7_a and H-6_a and H-6_b and CH_3-14_b. These spatial correlations established a 3β-methoxy, 7α-H, and a 10β-methyl configuration. Therefore **2** is assigned to 3β-methoxy isocostic acid, a new natural product.

Compound **3** was isolated as a yellowish oil, ($[\alpha]_D^{25}$ = −13.6 (*c* 0.03, $CHCl_3$)) and its IR spectrum showed absorption bands at 3020 (OH), 1715 (C=O), and 1670 (C=C) cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **2** and **3** (Table 1) indicated the presence of epimers with a differing configuration at C-3. Relative to **2**, **3** has a downfield H-3 shift of δ_H 3.61 appearing as a triplet and H-15 appears up field at δ_H 1.67. Coupling constant (J = 6.8 Hz) and NOESY data for C-3 was consistent with methoxy placement in an α orientation (Jakupovic et al., 1988). Compound **3** was assigned to 3α-methoxy isocostic acid, a new natural product.

Compound **4** was isolated as a yellowish oil, $[\alpha]_D^{25}$ = −24.3 (*c* 0.03, $CHCl_3$) and its IR spectrum showed absorption bands at 3566 (OH), 3020 (OH), and 1670 (C=C) cm^{-1}. The low resolution mass spectrum had a molecular ion peak $[M]^+$ at *m/z* 254. Its HREIMS exhibited a quasi-molecular ion peak $[M]^+$ at *m/z* 254.1880 (calcd. for $C_{15}H_{26}O_3$: 254.1882). The ^{1}H- and ^{13}C-NMR spectra (Table 1) confirmed the molecular formula $C_{15}H_{26}O_3$ and the fifteen carbon signals were classified by DEPT as: three methyl carbon resonances at δ_C 11.7, 19.2, and 29.9; six methylene carbon signals at δ_C 26.8, 31.0, 31.2, 34.6, 39.8 and 109.1; two methine

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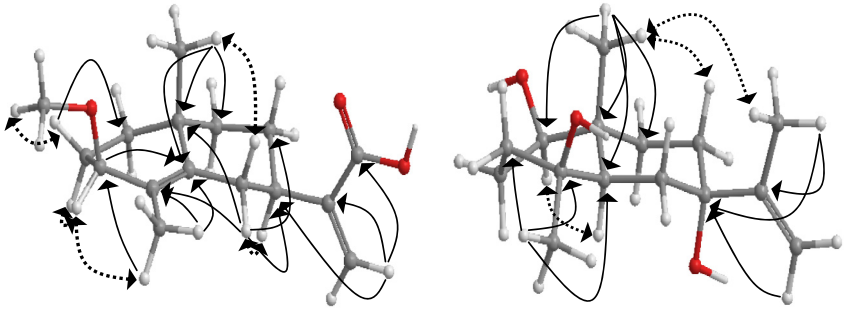
Table 1
^{1}H NMR and ^{13}C NMR spectroscopic data of **1–4** and **11–12** (600 MHz, δ-ppm).[a]

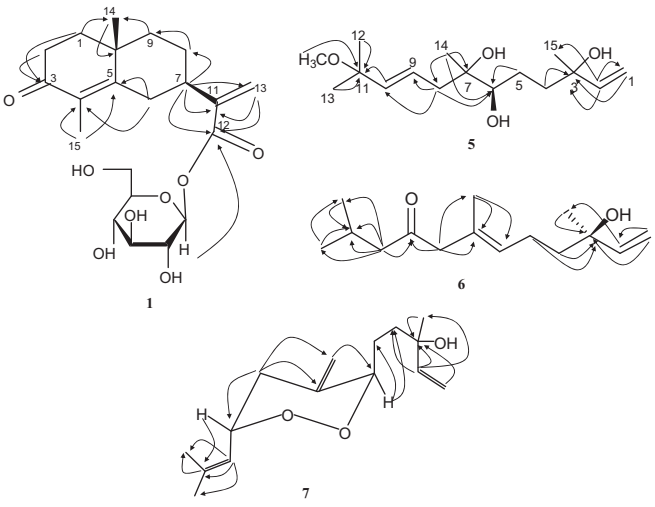
No	**1** (CD_3OD)		**2**		**3**		**4**		**11**	**12**
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_C	δ_C
1	1.78 *m*	37.1 *t*	1.56 *m* *	34.2 *t*	1.56 *m*	35.9 *t*	3.33 *dd* (11.5, 4.0)	79.4 *d*	35.0	38.2
			1.31 *m*		1.30 *m*					
2	2.32 *ddd* (16.5, 4.0, 4.0)	33.3 *t*	1.87 *m*	22.0 *t*	1.58 *m*	23.4 *t*	1.88 *m*	26.8 *t*	25.0	26.4
	2.54 *m*		1.58 *m**				1.62 *br dd* (7.0, 4.0)			
3	–	200.2 *s*	3.39 *br s like*	79.3 *d*	3.61 *t* (6.8)	80.0 *d*	1.69 *m*	39.8 *t*	74.1	34.8
							1.57 *m*			
4	–	128.5 *s*		124.0 *s*		124.8 *s*	–	71.5 *s*	141.1	41.3
5	–	163.1 *s*	–	140.2 *s*	–	139.4 *s*	1.57 *m*	44.7 *d*	124.0	75.8
6	2.89 *br d* (14.0)	33.2 *t*	2.62 *br dt* (13.0, 2.8)	31.2 *t*	2.62 *br d* (13.8)	31.7 *t*	1.84 *m**	31.0 *t*	31.8	28.1
	2.13 *t like* (14.0)		1.75 *br t* (13.0)		1.79 *m*		1.52 *br dt* (12.0, 1.3)			
7	2.58 *m*	40.2 *d*	2.44 *br tt* (13.0, 2.8)	40.0 *d*	2.39 *m*	40.3 *d*	–	74.3 *s*	40.3	34.5
8	1.78 *m*	26.9 *t*	1.68 *m*	28.0 *t*	1.63 *m*	27.9 *t*	1.84 *m* *	31.2 *t*	27.7	17.1
			1.52 *ddd* (13.0, 13.0, 3.5)		1.41 *ddd* (13.0, 13.0, 2.7)		1.44 *m*			
9	1.72 *m*	41.6 *t*	1.56 *m**	42.0 *t*	1.49 *m*	42.0 *t*	1.67 *m*	34.6 *t*	41.3	38.0
	1.50 *ddd* (13.5, 13.5, 4.0)		1.32 *m*		1.31 *m*		1.49 *br dd* (13.0, 4.0)			
10	–	35.7 *s*	–	35.0 *s*	–	35.0 *s*	–	38.9 *s*	36.0	36.8
11	–	144.0 *s*	–	145.4 *s*	–	144.6 *s*	–	151.4 *s*	144.4	145.9
12	–	165.5 *s*	–	170.8 *s*	–	170.2 *s*	1.83 *s*	19.2 *q*	171.4	171.8
13	6.37 *s*	124.5 *t*	6.27 *s*	124.7 *t*	6.31 *s*	126.4 *t*	5.06 *br s*	109.1 *t*	125.1	125.1
	5.83 *s*		5.58 *s*		5.68 *s*		4.81 *br d* (1.3)			
14	1.26 *s*	21.3 *q*	1.01 *s*	23.0 *q*	1.08 *s*	24.7 *q*	1.03 *s*	11.7 *q*	24.5	16.8
15	1.73 *br d* (1.3)*	9.7 *q*	1.71 *s*	17.4 *q*	1.67 *s*	15.6 *q*	1.13 *s*	29.9 *q*	15.2	38.2
1′	5.56 d (7.5)	94.7 *d*	–	–	–	–	–	–		
2′	3.33–3.43m*	72.7 *d*								
3′		76.8 *d*								
4′		69.7 *d*								
5′		77.6 *d*								
6′	3.81 dd (12.0,2.0)	60.9 *t*								
	3.67 dd (12.0, 5.4)									
$-OCH_3$			3.41 *s*	56.9 q	3.33 *s*	56.0 q				

[a] Recorded in $CDCl_3$.
* Overlapped.



2 **4**

Fig. 2. Observed HMBC (solid arrows) and NOESY correlations (dotted arrows) for **2** and **4**.



1 **5** **6** **7**

Fig. 3. HMBC correlations of **1**, **5**–**7**.

carbon resonances δ_C 44.7 and 79.4; and four quaternary carbon signals at δ_C 38.9, 71.5, 74.3, and 151.4. NMR comparisons with previous isolated compounds by Wang et al. (2007) indicated the presence of an eudesmane (sesquiterpene) framework. The ^{1}H NMR indicated the presence of exomethylene protons at δ_H 4.81 (H-13, d, J = 1.3 Hz) and 5.06 (H-13, s), an olefinic methyl at δ_H 1.83 (H-12, s), and a tertiary methyl at δ_H 1.03 (H-14, s). HMBC identified the position of the olefinic methyl protons at δ_H 1.83 (H_3-12, s) that correlated with signals at δ_C 151.4 (C-11), 109.1 (C-13), and 74.3 (C-7); tertiary methyl protons at δ_H 1.03 (H_3-14) that correlated with carbons at δ_C 79.4 (C-1), 44.7 (C-5), 34.6 (C-9), and 38.9 (C-10); and methyl protons at δ_H 1.13 (H_3-15, s) correlated with carbons at δ_C 71.5 (C-4), 39.8 (C-3), and 44.7 (C-5) (Fig. 2). HMBC was also used to infer the position of the three hydroxyl groups at C-1 (δ_C 79.4), C-4 (δ_C 71.5), and C-7 (δ_C 74.3). The relative stereochemistry of **4** was assigned on the basis of coupling constants and NOESY data (Fig. 2). The OH group at C-1 was placed in a beta position, according to coupling constants for H-1 [δ_H 3.12 (dd, J = 11.5, 4.0 Hz)] (Sung et al., 1992, 1988; Wang et al., 2007). The NOESY spectrum exhibited a correlation between δ_H 3.33 (H-1, dd, J = 11.5, 4.0 Hz) and δ_H 1.57 (H-5, m), indicating these protons were in an α configuration. The correlation between δ_H 1.03 (H_3-14, s) and δ_H 1.83 (H_3-12, s), together with δ_H 1.84 (H-8_b, m), indicated that the hydroxyl at C-7 is positioned on the opposite side relative to the hydroxyl groups at C-1 and C-4. Therefore **4** was assigned to eudesm-11,13-ene-1β,4β,7α-triol, a new natural product.

Compound **5** was isolated as a yellowish oily substance, $[\alpha]_D^{25}$ = +18 (c 0.03, $CHCl_3$) and its IR spectrum showed absorption bands at 3550 (OH), 2972 (OH) and 1684 cm^{-1} (C=C). Its low resolution mass spectrum had a quasi-molecular ion peak at m/z 218 [M−($2H_2O$+CH_3OH)]$^+$. Its HREIMS exhibited a quasi-molecular ion peak [M−($2H_2O$+CH_3OH)]$^+$ at m/z 218.1677 (calcd. for $C_{15}H_{22}O$: 218.1677). The molecular formula of $C_{16}H_{30}O_4$, was consistent with ^{1}H- and ^{13}C-NMR spectroscopic data (Table 2) and DEPT data established the presence of sixteen carbon signals assigned to: five methyls (one methoxy), four methylenes (one olefinic), four methines (three olefinic and one oxygen bearing), and three oxygen-bearing quaternary carbons respectively. A methoxy group at δ_H 3.14 (H_3-16, s) correlated with carbons at δ_C 74.9 (C-11), 25.8 (C-12) and 26.1 (C-13) as observed from the HMBC data (Fig. 3). The C-11 also correlated with an overlapping singlet at δ_H 1.25 (H_6-12/13), and correlated with an olefinic proton at δ_H 5.48 (H-10, d, J = 16.0 Hz), in the HMQC and HMBC spectra, respectively. The signal at δ_H 5.61 (H-9, ddd, J = 16.0, 8.2, 6.2 Hz) showed a correlation with an olefinic proton at δ_H 5.48 (H-10, d, J = 16.0 Hz) and methylene protons at δ_H 2.10 (H-8, dd, J = 13.7, 8.2 Hz) and 2.31 (H-8, dd, J = 13.7, 6.2 Hz); the appearance of a vinyl ABX system appeared at δ_H 5.85 (H-2, dd, J = 17.2, 10.5 Hz), 5.15 (H-1, dd, J = 17.2, 1.3 Hz), and 4.97 (H-1, dd, J = 10.5, 1.3 Hz). Additionally, the resonance at δ_H 3.79 (H-6, dd, J = 6.8, 6.8 Hz) correlated with a multiplet at δ_H 1.71 and 1.88 (H_2-4, m) and with a methyl singlet at δ_H 1.18 (H_3-14, s) in the COSY spectrum. The H-6 also correlated with δ_C 25.9 (C-5) in the HMQC spectrum and with δ_C 84.8 (C-6) and methylene carbons at δ_C 40.9 (C-8), in the HMBC spectrum. HMBC connectivities were also observed between: vinyl methylene protons at δ_H 5.15 (H-1a, J = 17.2, 1.3 Hz) and 4.97 (H-1b, dd, J = 10.5, 1.3 Hz,) and an oxygenated signal δ_C 73.3 (C-3); vinyl methine protons at δ_H 5.85 (H-2, dd, J = 17.2, 10.5 Hz) and δ_C 111.4 (C-1), 83.0 (C-3), and 26.8 (C-15); a methyl singlet at δ_H 1.18 (H_3-14, s) and δ_C 72.9 (C-7), 84.8 (C-6), and 40.9 (C-8). In addition, a correlation between a methoxy proton signal at δ_H 3.14 (H_3-16, s) and δ_C 74.9 (C-11) was observed.

The relative stereochemistry assignment was based on coupling constants and NOESY data. Correlations between δ_H 3.79 (H-6, dd, J = 6.8, 6.8 Hz) and δ_H 1.18 (H-14, s), δ_H 2.10 (H-8_a, dd, J = 13.7, 6.2 Hz) and δ_H (H-5_a, m), and δ_H 1.30 (H-15, s) and δ_H 1.88 (H-4_a and H-5_a, m, 2H) indicated that the secondary hydroxyl group is situated on the same side as the other hydroxyl groups at C-3 and C-7. These chemical data confirmed the structure of **5**, 3,6,7-trihydroxy-11-methoxy-3,7,11-trimethyldodeca-1,9-diene, assigned as a new natural product, Chiliadenol A.

Compound **6** was isolated as a yellowish oily substance, $[\alpha]_D^{25}$ = +3.67 (c 0.03, $CHCl_3$) and its IR spectrum showed absorption bands at 3550 (OH), 2972 (OH) and 1684 cm^{-1} (C=C). Its low resolution mass spectrum gave a molecular ion peak [M−H_2O]$^+$ at m/z

Table 2
^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **5**–**8** (600 MHz, δ-ppm).[a]

No	**5**		**6**		**7**		**8**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	4.97 *dd* (10.5, 1.3) 5.15 *dd* (17.2, 1.3)	111.4 *t*	5.07 *dd* (11.0, 1.3) 5.22 *dd* (17.0, 1.3)	111.9 *t*	5.04 *dd* (10.5, 1.3) 5.22 *dd* (17.2, 1.3)	111.8 *t*	5.03 *dd* (10.5, 1.5) 5.21 *dd* (17.0, 1.5)	111.8 *t*
2	5.85 *dd* (17.2, 10.5)	143.7 *d*	5.90 *dd* (17.0, 11.0)	144.9 *d*	5.84 *dd* (17.2, 10.5)	145.4 *d*	5.87 *dd* (17.0, 10.5)	145.2 *d*
3	–	83.0 *s*	–	73.3 *s*	–	72.7 *s*	–	72.7 *s*
4	1.88 *m*[*] 1.71 *m*	37.4 *t*	2.05 *m*	23.1 *t*	1.75 *br dd (14.0, 7.0)* 1.69 *m*[*]	39.0 *t*	1.68 *m*	37.5 *t*
5	1.88 *m*[*] 1.82 *m*	25.9 *t*	1.56 *m*	41.7 *t*	1.62 *m*	30.1 *t*	1.81 *m* 1.62 *m*	29.1 *t*
6	3.79 *dd* (6.8, 6.8)	84.8 *d*	5.24 *br t* (7.5)	129.6 *d*	4.40 *br t* (5.5)	80.5 *d*	4.28 *br t* (2.0)	80.7 *d*
7		72.9 *s*	–	129.1 *s*	–	151.8 *s*	–	151.7 *s*
8	2.31 *dd* (13.7, 6.2) 2.10 *dd* (13.7, 8.2)	40.9 *t*	3.00 *s*	54.3 *t*	2.64 *ddd* (15.0, 6.2, 1.4) 2.31 *m*	39.8 *t*	2.60 *dd like* (15.1, 5.0) 2.26 *m*	40.3 *t*
9	5.61 *ddd* (16.0, 8.2, 6.2)	125.2 *d*	–	209.4 *d*	4.65 *ddd* (8.2, 8.2, 6.2)	73.7 *d*	4.52 *ddd* (10.5, 8.2, 5.0)	75.2 *d*
10	5.48 *d* (16.0)	139.0 *d*	2.26 *d* (7.0)	50.9 *d*	5.19 *m*	124.9 *d*	5.23 *m*	124.7 *d*
11	–	74.9 *s*	2.12 *m*	24.5 *s*	–	136.7 *s*	–	137.3 *s*
12	1.25 *s*[*]	25.8 *q*	0.89 *d* (6.9) [*]	22.6 *q*	1.70 *br s*	25.9 *q*	1.72 *d* (1.4)	25.9 *q*
13	1.25 *s*[*]	26.1 *q*	0.89 *d* (6.9) [*]	22.6 *q*	1.68 *br s*[*]	18.3 *q*	1.68 *br s*	18.4 *q*
14	1.18 *s*	24.3 *q*	1.59 *s*	16.6 *q*	4.97 *dd* (4.8, 2.0) 4.83 *dd* (4.8, 2.0)	105.0 *q*	4.95 *dd* (4.8, 2.0) 4.80 *dd* (4.8, 2.0)	104.4 *t*
15	1.30 *s*	26.8 *q*	1.27 *s*	28.1 *q*	1.25 *s*	28.6 *q*	1.26 *s*	28.3 *q*
–OCH_3	3.14 *s*	50.4 *q*	–	–	–	–	–	–

[a] Recorded in $CDCl_3$.
[*] Overlapped.

220. Its HREIMS exhibited a quasi-molecular ion peak $[M-H_2O]^+$ at *m*/*z* 220.1833 (calcd. for $C_{15}H_{24}O$: 220.1827). The molecular formula of $C_{15}H_{26}O_2$ was supported by ^{1}H- and ^{13}C-NMR spectroscopic analysis (Table 2). DEPT NMR analysis showed the presence of fifteen carbon signals assigned to four methyls, five methylenes (one olefinic), three methines (two olefinic), and three quaternary carbons (one olefinic, one oxygen-bearing, and one free keto group), respectively. An isopropyl group appeared as one methyl doublet signal integrating for six protons at δ_H 0.89 (H-12/13, d, *J* = 6.9 Hz) that was correlated with a methane at δ_H 2.12 (H-11, m). The methyl protons also correlated with δ_C 24.5 (C-11) in the HMBC spectrum. Two singlet signals established the presence of two methyl groups which appeared at δ_H 1.59 (H-14, s) with δ_C 16.6 (C-14) and 1.27 (H-15, s) with δ_C 28.1 (C-15) that showed correlations with a quaternary olefinic carbon at δ_C 129.1 (C-7) and a quaternary oxygenated carbon at δ_C 73.3 (C-3), respectively. Additionally, a vinyl ABX system appeared as three double doublets at δ_H 5.90 (H-2, dd, *J* = 17.0, 11.0 Hz), 5.22 (H-1, dd, *J* = 17.0, 1.3 Hz), 5.07 (H-1, dd *J* = 11.0, 1.3 Hz); a doublet signal at δ_H 2.26 (H_2-10, d, *J* = 7.0 Hz); isolated methylene protons appearing as a singlet at δ_H 3.00 (H_2-8, s) and correlating with a keto group and a quaternary olefinic carbon at δ_C 209.4 (C-9) and 129.1 (C-7), respectively; and two methylenes appearing as multiplets at δ_H 1.56 (H_2-5) and 2.05 (H_2-4). Examination of the connectivities in the HMBC spectrum (Fig. 3) indicated: a vinyl methine at δ_H 5.90 (H-2, dd, *J* = 17.0, 11.0 Hz) correlated with δ_C 111.9 (C-1), 73.3 (C-3), and 28.1 (C-15); an oxygenated quaternary carbon at δ_C 73.3 (C-3) correlated with δ_H 1.27 (H_3-15, s), 2.05 (H_2-4, m), and 1.56 (H_2-5, m); an olefinic methyl singlet at δ_H 1.59 (H_3-14, s) correlated with δ_C 129.1 (C-7), 129.6 (C-6), and 54.3 (C-8); and δ_H 3.00 (H_2-8, s) correlated with δ_C 16.6 (C-14), 129.1 (C-7), and 209.4 (C-9). Additionally a correlation was observed between δ_H 2.26 (H_2-10, d, *J* = 7.0 Hz) and δ_C 209.4 (C-9), 24.5 (C-11), and 22.6 (C-12/13). The above chemical data confirmed the structure of **6** as a new natural product, 3-hydroxy-3,7,11-trimethyl-1,6-dodecadien-9-one, Chiliadenol B.

Compound **7** was isolated as an oily material, $[\alpha]_D^{25}$ = +22.6 (*c* 0.03, $CHCl_3$) and its IR spectrum showed absorption bands at 3450 (OH), 1675 (C=C), and 1576 (C=C). Its low resolution mass spectrum had a molecular ion peak $[M-H_2O]^+$ at *m*/*z* 234. Whereas the HREIMS exhibited a quasi-molecular ion peak $[M-H_2O]^+$ at *m*/*z* 234.1627 (calcd. for $C_{15}H_{22}O_2$: 234.1620). By contrast, the FAB-MS had a molecular ion peak $[M+Na^+]$ at *m*/*z* 275. Its molecular formula of $C_{15}H_{22}O_2$ was supported by ^{1}H- and ^{13}C-NMR spectroscopic data (Table 2). The DEPT spectrum exhibited 15 carbon signals including: three methyls, five methylenes, four methines, and three quaternary carbon resonances. A vinyl ABX system similar to **5** and **6** was observed as three double doublets at δ_H 5.84 (H-2, dd, *J* = 17.2, 10.5 Hz), 5.22 (H-1, dd, *J* = 17.2, 1.3 Hz), 5.04 (H-1, dd, *J* = 10.5, 1.3 Hz) that indicated the presence of an allyl unit. A quaternary oxygenated carbon signal, indicated from DEPT analysis, appeared at δ_C 72.7 (C-3) and which showed a correlation with a vinyl group at δ_C 111.8 (C-1) and 145.4 (C-2), as well as a methyl carbon at δ_C 28.6 (C-15) in the HMBC spectrum thereby establishing a methyl group on the oxygenated quaternary carbon (C-3) (Fig. 3). A broad triplet signal at δ_H 4.40 (H-6, t, *J* = 5.5 Hz) had a correlation with an oxygenated δ_C 80.5 (C-6) and a methylene proton at δ_H 1.62 (H_2-5) in HMQC and ^{1}H–^{1}H COSY spectra, respectively. Two doublet of doublets at δ_H 4.83 (H-14, dd, *J* = 4.8, 2.0 Hz) and 4.97 (H-14, dd, *J* = 4.8, 2.0 Hz) were characteristic for exomethylene protons. These exomethylene protons correlated with methylene protons at δ_H 2.31 (H-8, m) and 2.64 (H-8, ddd, *J* = 15.0, 6.2, 1.4 Hz) in ^{1}H–^{1}H COSY spectrum, as well as with δ_C 105.0 (C-14) and δ_C 151.8 (C-7) in HMQC and HMBC spectra, respectively. A doublet doublet of doublets at δ_H 4.65 (H-9, *J* = 8.2, 8.2, 6.2 Hz) COSY correlated with a methylene proton δ_H 2.31 (H-8, m) and 2.64 (H-8, ddd, *J* = 15.0, 6.2, 1.4 Hz), as well as an olefinic proton at δ_H 5.19 (H-10, m). In the HMQC spectrum, the H-9 signal correlated with an oxygenated δ_C 73.7 (C-9). Two broad singlet signals for olefinic methyl groups at δ_H 1.68 (H_3-13, s), and 1.70 (H_3-12, s) correlated in the HMBC spectrum with low-field olefinic signals at δ_C 136.7 (C-11, quaternary carbon) and δ_C 124.9 (C-10, methine carbon). The position of the 2-methyl-2-propene unit was confirmed by HMBC analysis, in which the H-10 correlated with δ_C 136.7 (C-11), 73.7 (C-9), 25.9 (C-12), and 18.3 (C-13). These NMR spectroscopic data, combined with

Table 3
Antimicrobial Minimum Inhibitory Concentration (MIC) for **1**–**16**.

Compounds	Minimum inhibitory concentration (μg mL^{-1})				
	Gram positive bacteria		Gram negative bacteria		Yeast
	Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Alcaligenes faecalis*	*Candida albicans*
1	–	–	–	–	12.500
2	–	–	–	–	12.500
3	–	–	–	–	12.500
4	25.000	–	–	–	12.500
5	–	–	–	–	6.250
6	–	–	–	–	12.500
7	–	–	–	–	12.500
8	–	–	–	–	3.125
9	25.000	–	–	–	12.500
10	–	–	–	–	6.250
11	–	–	–	–	25.000
12	–	–	–	–	12.500
13	–	–	–	–	6.250
14	–	–	–	–	6.250
15	25.000	25.000	25.000	12.500	3.125
16	–	–	–	–	6.250
Chloramphenicol	<3.125	<3.125	<3.125	<3.125	–

(–) No inhibition observed at any tested concentration.

HREIMS data, established **7** to be a dioxane derivative. Its relative stereochemistry was assigned on the basis of coupling constants, with the coupling constants of H-9 (δ_H 4.65, ddd, $J_{9,10}$ = 8.2 Hz, $J_{9,8eq}$ = 8.2 Hz, $J_{9,8ax}$ = 6.2 Hz) determining the relative stereochemistry of H-9 to be axial. Additionally, the coupling constants of H-6 (δ_H 4.40 Hz, br t, J=5.5 Hz) determined the relative stereochemistry of H-6 to be in an axial position (Ibrahim et al., 2008; Kobayashi et al., 1993; Yosief et al.,1998). This is in agreement with NOESY data which showed correlations between H-6$_{ax}$ and H-5$_{ax}$ and between H-9$_{ax}$ and H-8$_{ax}$. The above chemical data confirmed the structure of **7**, 3-hydroxy-3,11-dimethyl-6β,9α-epidioxy-dodeca-1,7(14),10-triene, as a new natural product Chiliadenol C.

Compound **8** was isolated as an oily material, $[\alpha]_D^{25} = -8.6$ (*c* 0.03, $CHCl_3$) and its IR spectrum showed absorption bands at 3450 (OH), 1675 (C=C), and 1576 (C=C). Its low resolution mass spectrum had a molecular ion peak $[M-H_2O]^+$ at *m*/*z* 234, with the HREIMS exhibits a quasi-molecular ion peak $[M-H_2O]^+$ at *m*/*z* 234.1627 (calcd. for $C_{15}H_{22}O_2$: 234.1620). Its molecular formula of $C_{15}H_{24}O_3$ was supported by analysis of ^{1}H- and ^{13}C-NMR spectroscopic data (Table 2). Its DEPT spectrum exhibited 15 carbon signals with three methyl resonances at δ_C 18.4, 25.9, and 28.3; five methylene signals at δ_C 29.1, 37.5, 40.3, 104.4 and 111.8; four methine resonances δ_C 75.2, 80.7, 124.7 and 145.2; and three quaternary signals at δ_C 72.7, 137.3 and 151.7. The ^{1}H and ^{13}C NMR spectroscopic data of **8** was quite similar to **7** (Table 2), except for a downfield chemical shift of 1.5 ppm (δ_C 37.5, C-4 and δ_C 75.2, C-9). This carbon chemical shift was accompanied by an upfield chemical shift for H-6 to appear as broad triplet at δ_H 4.28 (J = 2.0). From these data, it appears that **8** is an epimer of **7** at position C-6. The relative stereochemistry of **8** was assigned on the basis of study of the coupling constants, where the coupling constants of H-9 (δ_H 4.52, ddd, $J_{9,10}$ = 5.0, $J_{9,8eq}$ = 8.2, $J_{9,8ax}$ = 10.5 Hz) determined the relative stereochemistry of H-9 to be axial. Additionally, the coupling constants of H-6 (δ_H 4.28, brt, $J_{6,5ax}$ = $J_{6,5eq}$ = 2.0 Hz) determined the relative stereochemistry of H-6 to also be axial (Ibrahim et al., 2008; Kobayashi et al., 1993; Yosief et al., 1998). This suggested that **8** is the 6- axial epimer of **7**, which has a clear effect of the carbon signal of C-9 resonate at δ_C 75.2 in compound **8** instead of δ_C 73.7 in **7** related to the position of hydroxyl group at C-3 (δ_C 72.7) (Ibrahim et al., 2008; Weyerstahl et al., 1999). This is in agreement with NOESY data that showed correlations between H-6$_{ax}$ and H-5$_{ax}$ and between H-9$_{eq}$ and H-8 eq. The above chemical data confirmed the structure of 8, 3-hydroxy-3,11-dimethyl-6α,9α-epidioxy-dodeca-1,10,7(14)-ene, as a new compound Chiliadenol D. In addition to new compounds (**1**–**8**), eight known compounds were isolated: 3-oxo-γ-costic acid (**9**) (Garcez et al., 2010), 3-oxo-γ-costic acid methyl ester (**10**) (Ceccherelli et al., 1989; Bohlmann and Borthakur, 1982), 3α-acetyl-γ-costic acid (**11**), 5α-hydroxy-4α,15-dihydrocostic acid (**12**), 5α-hydroxycostic acid (**13**) (Ahmed and Jakupovic, 1990), eudesmane-1β,4β,7α-triol (**14**) (Sung et al., 1992), kaempferol-3-O-(6″-*O*-acetyl)-β-D-glucopyranoside (**15**) (Merfort, 1988), and thymol β-D-glucopyranoside (**16**) (Shimoda et al., 2006). Since metabolites **11**–**12** were previously isolated as methyl esters, the ^{13}C NMR spectroscopic data for the isolated acids are given (Table 2).

Among all the isolates, metabolite **15** showed antimicrobial activity inhibiting growth of *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Alcaligenes faecalis* and *Candida albicans* with MIC values 25, 25, 25, 12.5 and 3.125 μg/mL, respectively (Table 3) using chloramphenicol (less than 6 μg/mL) as a positive control. As with other flavonol structures **15** may contain an appropriate structural configuration to complex with bacterial cell walls and inhibit microbial growth (Cowan, 1999).

3. Conclusion

Among the Sinai herbal medicinal plants, *Jasonia montana*, locally known as Haneida, has a strong aromatic odor and is used in traditional medicine for diarrhea, stomachaches, and chest diseases (Täckholm, 1974). Haneida owes its therapeutically activity to an array of metabolites. In this phytochemical investigation of a closely related species, solvent extraction of the air-dried aerial parts of *C. montana* resulted in isolation and characterization of new compounds **1**–**8** as well as previously reported compounds **9**–**16**. Antimicrobial activity against an array of common bacterial and fungal strains was measured via a colorimetric assay with minimal growth inhibition observed in the μg/mL range for one of the tested metabolites (**15**).

Experimental

General procedures

Instrumentation included a Horiba SEPA-300 digital polarimeter (l = 5 cm) for specific rotation; a Shimadzu FTIR-8100 spec-

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trometer for IR analysis; JEOL JMS-GCMATE mass spectrometer for EI-MS and HR-EI-MS; a JEOL JNM-ECA 600 spectrometer with tetramethylsilane as an internal standard for ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra; a Shimadzu RID-10A refractive index detector for HPLC analysis; and a COSMOSIL-Pack type (C_{18}-MS-II) (250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns for analytical and preparative separation, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography (cc), silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reverse-phase silica gel cc, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with silica gel 60_{F254} (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with (1:9) H_2SO_4-MeOH followed by heating.

Plant material

Air-dried aerial parts of Chiliadenus montanus (Vahl.) Brullo. were collected in 2010, from Wadi Gebal, North Sinai, Egypt. Plant material was identified by Dr. El-Bialy E. Hatab, Egyptian Environmental Affairs Agency, Nature Conservation Sector, Siwa Protected Area, Siwa, Egypt. A voucher specimen SK-1001 has been deposited in the Herbarium of St. Katherine Protectorate, Egypt.

Extraction and isolation

Aerial parts (2.0 kg) of *C. montana* were powdered and extracted with CH_2Cl_2:MeOH (1:1) (10 L, 3 days x 2) at room temperature. The combined extract was concentrated *in vacuo* to obtain a residue (175 g), which was fractionated on a silica gel column (6 × 120 cm) eluting with *n*-hexane (3 L) followed by a gradient of *n*-hexane-$CHCl_3$ up to 100% $CHCl_3$ and then a $CHCl_3$–MeOH studies at up to 15% MeOH (3 L each of the solvent mixture). The *n*-hexane:$CHCl_3$ (3:1) fraction was applied to a Sephadex LH-20 column (3 × 90 cm) eluted with *n*-hexane:CH_2Cl_2:MeOH (7:4:0.25), to give **10** (12 mg). The *n*-hexane:$CHCl_3$ (1:1) fraction was subjected to silica gel cc (4 × 120 cm) eluted with *n*-hexane:EtOAc (4:1). Fractions were obtained and combined into three parts A, B and C on the basis of their TLC profiles. Sub-fraction A, was re-purified using silica gel cc (6 × 90 cm) eluted with *n*-hexane:EtOAc (6:1) to afford **9** (5.5 g). Sub-fraction B was re-purified by reversed phase HPLC, using MeOH/H_2O (75:25 v/v) at a flow rate 3.5 mL/min to afford **2** (7 mg), **3** (3 mg), **7** (5 mg), and **11** (20 mg); sub-fraction C was re-purified by reversed phase HPLC using MeOH/H_2O (70:30 v/v) to afford **5** (3 mg), **6** (8 mg), **7** (6 mg), and **8** (4 mg) and **1** (15 mg). The $CHCl_3$:MeOH (95:5) fraction was applied to a silica gel column (6 × 120 cm) eluted with *n*-hexane:$CHCl_3$:MeOH (7:4:1), followed by reversed phase HPLC using MeOH:H_2O (1:1) to afford **4** (10 mg), **12** (12 mg), **13** (10 mg), **7** (20 mg), and **14** (15 mg). The $CHCl_3$:MeOH (75:15) fraction was subjected to silica gel cc (6 × 120 cm) eluted with *n*-hexane:$CHCl_3$:MeOH (7:4:2), followed by reversed phase HPLC using MeOH:H_2O (1:1) at a flow rate 5.0 mL/min to afford **15** (30 mg), and **16** (20 mg).

*3-Oxo-γ-costic acid β-D-glucopyranoside ester (**1**)*

Colorless needles; $[\alpha]_D^{25}$ = +8.3 (*c* 0.03, $CHCl_3$); for ^{1}H (CD_3OD, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 1; EIMS *m/z* 410 $[M]^+$, 290, 275, 248, 233, 220, 202; HREIMS *m/z* 410.1946 (calcd. for $C_{21}H_{30}O_8$: 410.1941); IR (ν_{max} cm^{-1}) = 3250, 3020, 1725, 1650 cm^{-1}.

*3β-Methoxy isocostic acid (**2**)*

Yellowish oily material; $[\alpha]_D^{25}$ = +73.0 (*c* 0.01, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 1; EIMS *m/z* 264 $[M]^+$, 249, 232 217, 199, 187, 171; HREIMS *m/z* 264.1734 (calcd. for $C_{16}H_{24}O_3$: 264.1725); IR (ν_{max} cm^{-1}) = 3020, 1715, 1670 cm^{-1}.

*3α-Methoxy isocostic acid (**3**)*

Yellowish oily material; $[\alpha]_D^{25}$ = −13.6 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 1; EIMS *m/z* 264 $[M]^+$, 249, 232 217, 199, 187, 171; HREIMS *m/z* 264.1734 (calcd. for $C_{16}H_{24}O_3$: 264.1725); IR (ν_{max} cm^{-1}) = 3020, 1715, 1670 cm^{-1}.

*Eudesmane-1β,4β,7α-triol-11,13-en (**4**)*

Yellowish oily material; $[\alpha]_D^{25}$ = −24.3 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 1; EIMS *m/z* 254 $[M]^+$, 236, 218, 195, 185, 179, 165; HREIMS *m/z* 254.1880 (calcd. for $C_{15}H_{26}O_3$: 254.1882); IR (ν_{max} cm^{-1}) = 3566, 3020, 1670 cm^{-1}.

*Chiliadenol A (**5**)*

Yellowish oily material; $[\alpha]_D^{25}$ = +18.0 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 2; EIMS *m/z* 250 $[M-2(H_2O)]^+$, 232 $[M-3(H_2O)]^+$, 218 $[M-[2(H_2O)+CH_3OH]]^+$, 203, 178, 155; HREIMS *m/z* 250.1578 (calcd. for $C_{16}H_{22}O_2$: 250.1933), 218.1677 (calcd. for $C_{15}H_{18}O$: 218.1671); IR (ν_{max} cm^{-1}) = 3550, 2972, 1684, 1338, 1219, and 771 cm^{-1}.

*Chiliadenol B (**6**)*

Yellowish oily material; $[\alpha]_D^{25}$ = +3.67 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 2; EIMS *m/z* 220 $[M-H_2O]^+$, 202, 187, 151, 120; HREIMS *m/z* 220.1833 (calcd. for $C_{15}H_{24}O$: 220.1827); IR (ν_{max} cm^{-1}) = 3550, 3020, 1684 cm^{-1}.

*Chiliadenol C (**7**)*

Yellowish oily material; $[\alpha]_D^{25}$ = +22.6 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 2; EIMS *m/z* 234 $[M-H_2O]^+$, 219, 201 179, 150; HREIMS *m/z* 234.1627 (calcd. for $C_{15}H_{22}O_2$: 234.1620); IR (ν_{max} cm^{-1}) = 3450, 1675, 1576 cm^{-1}.

*Chiliadenol D (**8**)*

Yellowish oily material; $[\alpha]_D^{25}$ = −8.6 (*c* 0.03, $CHCl_3$); for ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectroscopic data, see Table 2; EIMS *m/z* 234 $[M-H_2O]^+$, 219, 201 179, 150; HREIMS *m/z* 234.1627 (calcd. for $C_{15}H_{22}O_2$: 234.1620); IR (ν_{max} cm^{-1}) = 3450, 1675, 1576 cm^{-1}.

*Acid hydrolysis of **1***

A solution of **1** (1.0 mg) in 5% aq. H_2SO_4–1,4-dioxane (1:1, v/v, 1.0 mL) was heated under conditions where reflux began, this being maintained for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH− form) and the resin was removed by filtration. After removal of the solvent from the filtrate, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CNH_2O (85:15, v/v); flow rate 0.8 mL/min]. Identification of D-glucose was carried out by comparison of its retention time

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and optical rotation with that of authentic sample (Wako Pure Chemical Industries, Japan; t_R: 7.1 min (D-glucose, positive optical rotation).

Antimicrobial assay

Assays were performed according to Eloff (1998) with some modifications. Microorganisms used in the present study were collected from the culture collection of Chemistry of Microbial and Natural Products Department at the National Research Centre (NRC), Dokki, Cairo, Egypt. Bacterial strains used were the gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and the gram negative bacteria, *Klebsiella pneumoniae*, *Alcaligenes faecalis* and *Escherichia coli*. Fungal yeasts used were *Saccharomyces cerevisiae* and *Candida albicans*. All microorganisms were cultured on nutrient agar medium (purchased from Lab M limited, Lancashire BL9 6AS, United Kingdom) at 37 °C for 24 h prior to use, while the fungi was cultured on potato dextrose agar (purchased from Becton, Dickinson and company Sparks, MD 21152, USA) at 37 °C for 24 h then suspended in potato dextrose broth prior to use. After incubation, the microbial colonies were scrapped off the agar and transferred to nutrient and potato dextrose broth solutions for bacteria and yeast respectively, to prepare 0.5 McFarland of microbial cultures with turbidity standard 10^7 CFU/mL (colony-forming units). Tested pure compounds were prepared by dissolving each (1 mg) in DMSO (1 mL), and then diluting with deionized H_2O to prepare stock solutions of 10% DMSO. All compounds were serially diluted 50% with sterile media from 25 to 3.12 μg mL^{-1} by addition of each compound 100 μL to the first well of 96-well micro plates. Subsequently, inoculated broths (100 μL) were added to each well. The inoculated 96-well plates were incubated at 37 °C for 24 h. The chloramphenicol antibiotic (⩾98%, Sigma–Aldrich) was used as a positive control reference in the assay. Extract-free solution was used as a negative control, and 10% DMSO solution was used as blank control.

As an indicator of microbial growth, *p*-iodonitrotetrazolium violet salt (40 μL) (INT, Sigma) (0.2 mg mL^{-1}) dissolved in H_2O was added to the wells. Plates were incubated at 37 °C for 1 h until the purple color of formazan appeared. MIC values were recorded as the lowest concentration of the extract that completely inhibited microbial growth, *i.e.* a clear well.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.03.027.

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