Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(2): 259-264
Received: 04-01-2017
Accepted: 05-02-2017

El-Sayed M El-Ghaly
Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Correspondence
El-Sayed M El-Ghaly
Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Phytochemical and biological activities of *Asphodelus microcarpus* leaves

El-Sayed M El-Ghaly

Abstract
One monounsaturated fatty acid ester and three anthraquinones were isolated for the first time from the leaves of *Asphodelus microcarpus* and identified as oleic acid methyl ester (1), chrysophanol (2), 10-(chrysophanol-7-yl)-10-hydroxychrysophanol-9-anthrone (3) and asphodoside C (4). The compounds were identified on the basis of 1D and 2D NMR and MS analyses. The methanolic extract of *A. microcarpus* leaves demonstrated a higher antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* with an MIC value of 78µg/ml. The methanolic extract showed also a moderate antiviral activity against HAV-10 virus and a highest cytotoxic activity against lung carcinoma cell line (A-549) with IC_{50} value of 29.3µg/ml.

Keywords: *Asphodelus microcarpus,* anthraquinones, antimicrobial, antiviral and cytotoxicity.

Introduction
The genus *Asphodelus* comprises 187 genera and 2500 species. It is a circum-Mediterranean genus, which includes five sections and is represented by 16 species (Lifante and Aguinagalde, 1996) [20]. *Asphodelus microcarpus* Salzm. et Viv. (Xanthorrhoeaceae or Asphodelaceae) is a stout robust herb with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region (Täckholm and Drar, 1954, Täckholm, 1974) [27, 26]. Its bulbs and roots are used to treat microbial infections, psoriasis, jaundice and ectodermal parasites (Mohammed, 2014) [21]. Carbohydrates, sterols, triterpenes, lipids, anthraquinones, naphthalenes and arylcoumarins have been isolated from the roots of *A. microcarpus* (Rizk and Hammouda, 1970; Hammouda, *et al*., 1971; Yagi and Makino, 1978; El-Seedi, 2007; Jerkovic and Carlo, 2011; Ghoneim *et al*., 2013; 2014) [25, 13, 31, 7, 16, 9, 10] Surprisingly no intensive research work has been reported on the leaves of this species. In this report the isolation and identification of one monounsaturated fatty acid ester (1) and three anthraquinone constituents (2-4) from the leaves of *A. microcarpus* for the first time. The antimicrobial, antiviral and cytotoxic activities of the methanolic extract were also evaluated.

Results and discussion
Compound 1 was obtained as colorless oil. Its ESI-MS gave a molecular ion peak at *m/z* 297 $[M+H]^+$, corresponding to a molecular formula of $C_{19}H_{36}O_2$. Its IR spectrum showed characteristic absorption bands for aliphatic ester group (1735 cm^{-1}), olefinic double bond (1651 cm^{-1}) and long aliphatic chain (722 cm^{-1}). In the ^{1}H-NMR spectrum, two-proton triplet-like at d 5.32 was ascribed to vinylic H-9 and H-10 protons. A two-proton multiplet at d 2.28 was attributed to methylene H_2-2 protons adjacent to the ester group. A four- proton broad signal at d 1.98 was attributed to methylene H_2-8 and H_2-11 protons near by vinylic carbons. A four-proton multiplet at d 1.59 and a broad signal at d 1.24-1.29 (18 H) were associated with the remaining methylene protons, indicating the presence of a long aliphatic chain of fatty acid moiety (Knothe and Kenar, 2004) [17]. A three-proton triplet at d 0.92 (J=7.3 Hz) was due to C-18 primary methyl protons. The ^{13}C NMR spectrum of 1 exhibited signals for ester carbon at d 174.44 (C-1), vinylic carbons at d 130.28 and 130.29 (C-9 and C-10), methylene carbon between d 34.55-19.60 and methyl carbon at d 14.17 (C-18). A methyl ester group appeared at δ_H 3.65/δC 51.88 (OCH3) and δ_C 174.44 (C-1) which were correlated in the HMBC (OCH3/C-1) experiment. The above data suggested that 1 was possibly an oleic acid methyl ester and in close resemblance with published data (Oscar; *et al*., 2003 and Gerhard; *et al*., 2004) [23, 8].

Compound 2 was obtained as an orange-red powder. The UV spectrum of 2, showed absorption maxima at 224, 250, 280 and 410 nm, suggestive of an anthraquinone structure (Thomson, 1971) [28]. The IR spectrum showed absorption bands at 3440, 1678 and 1642 cm^{-1}, suggesting the presence of hydroxyl, free and chelated carbonyl functions, respectively

(Bloom, *et al*., 1959) [4]. ESI-MS gave an $[M+H]^+$ ion at *m/z* 255, consistent with the molecular formula of $C_{15}H_{10}O_4$. The ^1H NMR spectrum of 2 (Table 1) showed two highly deshielded singlets at δ 12.01 and 12.12 corresponding to the two chelated hydroxyl groups on C-1 and C-8, respectively (Hernandez-Medel *et al*., 1999) [14]. The ^1H NMR spectrum of 2 showed also a pair of meta-coupled protons at δ 7.09 (1H, dq, *J* = 1.9, 0.9 Hz, H-2) and 7.65 (1H, d, *J* = 2.2 Hz, H-4) with the C-3 attached methyl at *δ* 2.46 (3H, s). In addition to an ABX spin system was observed at δ 7.81 (1H, dd, *J* = 9.4, 1.4 Hz, H-5), 7.67 (1H, t, *J* = 9.1 Hz, H-6), and 7.28 (1H, d, *J* = 1.4 Hz, H-7), indicated the presence of chrysophanol moiety. The ^{13}C NMR and DEPT spectra of 2 (Table 2) displayed 15 carbon signals accounted for seven quaternary, five methine and one methyl carbons, as well as two carbonyl carbons. HMQC and HMBC correlations were confirmed the structure determination. Accordingly, compound 2 was identified as chrysophanol and its spectral data were in good agreement with the reported literatures (Bonsignore *et al*., 1998; Zhang, *et al*., 2012) [5, 32].

Compound 3 was obtained as orange powder. The UV spectrum of 3 indicated an anthraquinone structure. Its IR spectrum showed absorption bands for chelated carbonyls and for hydroxyl groups at 1635 and 3450 cm^{-1}, respectively. ESI-MS gave an $[M-H]^-$ ion at *m/z* 507, consistent with the molecular formula of $C_{30}H_{20}O_8$. The ^1H NMR spectrum (Table 1) showed the presence of four highly deshielded singlets at *δ* 12.23, 12.16, 11.99 and 11.45 due to the presence of four chelated hydroxyl groups; supported that the compound is a dimeric anthraquinone/anthrone derivative (Hernandez-Medel *et al*., 1999) [14]. The ^1H NMR spectrum showed two meta-coupled protons assigned to H-2 and H-4, respectively, at *δ* 6.75 ((1H, brs) and 6.60 ((1H, brs), with the C-3 attached methyl at *δ* 2.19 (3H, s). In addition to an ABX spin system corresponding to three aromatic protons resonated at δ 6.72 (1H, d, *J* = 7.4 Hz, H-5), 7.47 (1H, t, *J* = 8.0 Hz, H-6) and 6.90 (1H, d, *J* = 8.0 Hz, H-7) of the chrysophanol anthrone moiety as one-half of the molecule. The ^1H NMR spectral data of the other half of the molecule showed that it is of a chrysophanol moiety; two meta-coupled protons assigned to H-2` and H-4`, respectively, at *δ* 7.04 (1H, brs) and 7.36 (1H, brs), with the C-3` attached methyl at *δ* 2.30 (3H, s). Additionally, the ABX spin system in chrysophanol anthrone is replaced by an AX spin system in chrysophanol moiety at δ 8.63 (1H, d, *J* = 8.0 Hz, H-6') and δ 7.83 (1H, d, *J* = 7.9 Hz, H-5'). This indicated that the point of attachment in this half of the molecule is at C-7' (δ 141.91). The ^{13}C NMR and DEPT of 3 (Table 2) displayed 30 carbon signals, including two methyl groups at *δ* 21.96 (Me-C-3) and 21.77 (Me-C-3') as well as three carbonyl carbons at *δ* 192.84 (C-9), 191.93 (C-9') and 181.27 (C-10'). The analogy of these features with the spectral NMR data of the related metabolites previously reported, suggested the structure 3 as chrysophanol linked to a moiety of chrysophanol-10-anthrone (Adinolfi, *et al*., 1989; Lanzetta, *et al*., 1990) [1, 18]. The cross-peak observed in the HMBC spectrum between H-6' (δ 8.63) and C-10 (δ 69.72) indicates the site of attachment was between C-10 of chrysophanol-10-anthrone and C-7` of chrysophanol. Based on the above data compound 3 was identified as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone and the structure was confirmed by comparison of its physical properties and proton and carbon NMR data to literature (Lanzetta *et al*., 1990; Li *et al*., 2000) [18, 19].

Compound 4 was obtained as a yellowish amorphous powder. In the UV spectrum of 4, absorption maxima were observed at 210, 245, 285, 375 and 425 nm, suggestive of an anthraquinone structure as in 3 (Thomson, 1971) [28]. The IR spectrum showed absorption bands at 3455, 1680 and 1630 cm^{-1}, suggesting the presence of hydroxyl, free and chelated carbonyl functions, respectively (Bloom, *et al*., 1959) [4]. ESI-MS gave an $[M+H]^+$ ion at *m/z* 641, consistent with the molecular formula of $C_{35}H_{28}O_{12}$. The ^1H NMR spectrum showed two chelated phenolic proton signals at δ 11.63 and 11.96 attributed to OH-1`, OH-8`, respectively (Yagi, and Makino, 1978) [31]. The ^1H NMR spectrum showed one-half of the molecule; one signal at *δ* 7.31 ((1H, s, H-2), with the C-3 attached methyl *δ* 2.0 (3H, s), indicating C-4 is the site of attachment to the other half of the molecule. In addition, an ABX spin system was observed for three aromatic protons which resonated at δ 7.33 (1H, d, *J* = 7.9 Hz, H-5), 7.69 (1H, t, *J* = 7.9 Hz, H-6), and 7.38 (1H, d, *J* = 7.7 Hz, H-7) of the chrysophanol moiety. The ^1H NMR spectral data of the other half of the molecule showed that, it is of a chrysophanol oxanthrone moiety, and the ABX pattern in the chrysophanol is replaced by a pair of deshielded ortho-coupled protons with AX spin system at δ 7.26 (1H, d, *J* = 8.2 Hz, H-6') and δ 7.36 (1H, d, *J* = 7.7 Hz, H-5'). This indicated that C-7' (δ 128.65) is the point of attachment in this half. In the ^1H NMR spectrum the anomeric proton appeared at δ 3.10 (1H, d, H-1", *J* = 9.2 Hz) indicating the *β*- configuration of the anomeric proton at C-1" (Altona and Haasnoot, 1980) [2]. Oxidative hydrolysis of 4 confirmed the sugar component as arabinose by TLC analysis. The ^{13}C NMR and DEPT spectra of 4 (Table 2) displayed 35 carbon signals, 5 signals were assigned to the arabinose moiety and 30 signals were assigned to the aglycone including two methyl groups at *δ* 21.0 (Me-C-3) and 22.21 (Me-C-3') as well as three carbonyl carbons at *δ* 192.72 (C-9), 181.88 (C-10) and 191.76 (C-9'), supported that the compound is a dimeric derivative. The upfield shift of the anomeric carbon signal at *δ* 84.06 (C-1") in ^{13}C NMR spectrum indicated C-glycoside. The HMBC spectrum showed a cross-peak between H-3' and C-4, confirming the point of attachment of chrysophanol moiety to chrysophanol oxanthrone moiety was C-2 and C-4`, respectively. The linked site of arabinose to C-10` position was determined by the HMBC experiment where a correlation was observed between arabinose -H-1`` (δ 3.10) and C-10` (δ 75.77) of the aglycone. The relative configuration at C-10` was determined by comparison with literature. Therefore, compound 4 was established as asphodelin-10`-oxanthrone-(10`*S*)-D-arabinopyranoside (asphodoside C) and its spectral data were in good agreement with the reported literatures (Ghoneim, *et al*., 2014) [11].

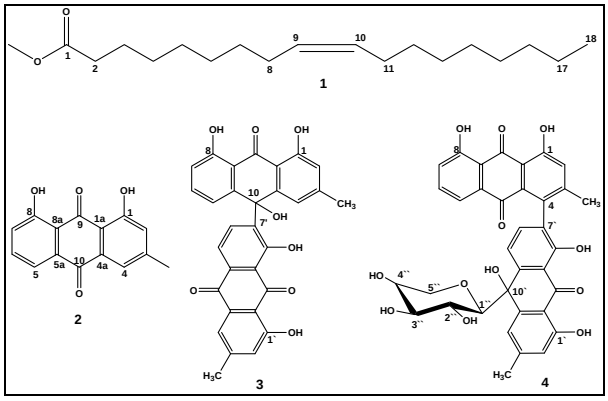

Fig 1: Compounds 1-4

Table 1: ^{1}H NMR data of compounds (2-4)

Position	^{1}H (*J* in Hz)			
	2[a]	3[b]	4[b]	4[c]
2	7.09;(dq, 1.9,0.90)	6.75, brs	7.31, s	7.35, s
4	7.65(d, 2.2)	6.60, brs	-	-
5	7.81;(dd, 9.4, 1.4)	6.72;(d, 7.4)	7.33;(d, 7.9)	7.25;(d, 7.8)
6	7.67;(t, 9.1)	7.47;(t, 8.0)	7.69;(t, 7.9)	7.60;(t, 7.8)
7	7. 28;(d, 1.4)	6.90;(d, 8.0)	7.38;(d, 7.7)	7.49;(d, 7.4)
3-CH_3	2.46, s,	2.19, s,	2.00, s	2.03, s
1-OH	12.01, s,	12.16, brs		
8-OH	12.12, s	12.23, brs		
2`	-	7.04, brs	6.75, s	6.72, s
4`	-	7.36, brs	7.31, s	7.17, s
5`	-	7.83;(d, 7.9)	7.36;(d, 7.7)	7.47;(d, 7.8)
6`	-	8.63;(d, 8.0)	7.26;(d, 8.20)	7.19;(d, 8.2)
3`-CH_3	-	2.30, s	2.37, s	2.41, s
1`-OH	-	11.45 brs	11.63, brs	-
8`-OH	-	11.99 brs	11.96, brs	-
1``	-	-	3.10;(d, 9.2)	3.21;(d, 9.2)
2``	-	-	3.18, m	3.31, m
3``	-	-	3.19, m	3.43; (t, 8.9)
4``	-	-	3.05, m	3.29,m
5``	-	-	3.50; (dd, 5.0, 10.8) 2.70; (t, 10.8)	3.69; (dd, 4.4, 10.7) 2.82; (t, 10.6)

[a] *$CDCl_3$*, [b] DMSO-*d_6*, [c] *CD_3OD*

Table 2: ^{13}C and ^{13}C-DEPT NMR data of compounds (2-4)

Position	2[a]		3[b]		4[b]	
	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT
1	162.70	Cq	161.59	Cq	160.63	Cq
1a	113.72	Cq	112.39	Cq	114.02	Cq
2	124.35	CH	116.66	CH	125.06	CH
3	149.32	Cq	148.64	Cq	149.60	Cq
4	121.34	CH	120.66	CH	125.01	Cq
4a	133.26	Cq	148.05	Cq	131.06	Cq
5	119.91	CH	119.63	CH	134.83	CH
5a	133.63	Cq	148.14	Cq	134.26	Cq
6	136.93	CH	137.12	CH	137.36	CH
7	124.54	CH	116.95	CH	124.22	CH
8	162.40	Cq	161.31	Cq	161.60	Cq
8a	115.86	Cq	114.46	Cq	115.69	Cq
9	192.52	Cq	192.84	Cq	192.72	Cq
10	181.98	Cq	69.72	Cq	181.88	Cq
3-CH_3	22.26	CH_3	21.96	CH_3	21.00	CH_3
1`	162.70	Cq	161.65	Cq	161.90	Cq
1`a	113.72	Cq	113.83	Cq	114.90	Cq
2`	124.35	CH	124.13	CH	116.86	CH
3`	149.32	Cq	149.39	Cq	148.05	Cq
4`	121.34	CH	120.71	CH	118.06	CH
4`a	133.26	Cq	141.64	Cq	148.51	Cq
5`	119.91	CH	119.16	CH	118.46	CH
5`a	133.63	Cq	132.13	Cq	144.07	Cq
6`	136.93	CH	133.04	CH	119.01	CH
7`	124.54	CH	141.91	Cq	128.65	Cq
8`	162.40	Cq	157.89	Cq	158.28	Cq
8`a	115.86	Cq	115.70	Cq	115.73	Cq
9`	192.52	Cq	191.93	Cq	191.76	Cq
10`	181.98	Cq	181.27	Cq	75.77	Cq
3`-CH_3	22.26	CH_3	21.77	CH_3	22.21	CH_3
1``	-	-	-	-	84.06	CH
2``	-	-	-	-	77.81	CH
3``	-	-	-	-	71.49	CH
4``	-	-	-	-	69.27	CH
5``	-	-	-	-	70.16	CH_2

[a] *$CDCl_3$*, [b] DMSO-*d_6*

Biological activity

The antimicrobial activities of the methanolic extract of *A. microcarpus* leaves were evaluated. The results are presented as the diameter of inhibition zone (mm) and as MIC against tested bacteria and fungi (Table 3). The methanolic extract demonstrated variable antimicrobial activity against all organisms tested. The obtained MICs varied from 78 to 313µg/ml for the tested extract. The lowest MIC value (78µg/ml) was observed against *Staphylococcus aureus* and *Candida albicans*. The lowest level of activity was observed against *Salmonella* SP (313µg/ml).

Table 3: Results of antimicrobial activity of the methanolic extract of *A. microcarpus*

Tested microorganisms	Diameter of inhibition zone (mm)		MIC (µg/ml)
	Methanolic extract	Standard	
Fungi		Ketoconazol	
Aspergillus flavus (RCMB 002002)	10.26 ± 0.09	16.13 ± 0.81	125
Candida albicans (RCMB 05036)	13.25 ± 0.14	20.23 ± 1.37	78
Gram positive bacteria		Gentamycin	
Staphylococcus aureus (RCMB 010010)	10.31 ± 0.29	24.13 ± 1.21	78
Bacillus subtilis (RCMB 010067)	11.06 ± 0.14	25.97 ± 0.95	156
Gram negatvie bacteria		Gentamycin	
Salmonella SP. (RCMB 010043)	12.16 ± 0.23	16.97 ± 0.95	313
Escherichia coli (RCMB 010052)	13.11 ± 0.17	30.03 ± 1.05	125

Well diameter: 6.0 mm …. …. (100µl was tested), Sample concentration (5mg/ml),
NA: No activity, data are expressed in the form of mean ± Standard deviation.

The screening antiviral activity of the methanolic extract of *A. microcarpus* leaves was determined using cytopathic effect inhibition assay. It showed moderate antiviral effect against HAV-10 and showed no effect against HSV-1.

The methanolic extract of *A. microcarpus* leaves was investigated for its cytotoxicity against four human tumor cell

lines: human lung carcinoma cells (A-549), human breast cancer cells (MCF-7), human colon cancer cells (HCT-116) and human prostate carcinoma cells (PC-3) using cell viability assay method. The methanolic extract exhibited cytotoxicity against the tested cell lines. The highest cytotoxic activity was observed against human lung carcinoma cells (A-549) with IC_{50} value of 29.3µg/ml (Table 4).

Table 4: Results of cytotoxicity evaluation of the methanolic extract of *A. microcarpus*

	Viability %							
	A-549		HCT-116		MCF-7		PC-3	
Sample conc. (µg/ml)	Extract	Standard	Extract	Standard	Extract	Standard	Extract	Standard
500	11.94	4.34 ±0.06	30.85	2.95 ±0.07	28.67	3.72 ±0.12	26.49	4.16 ±0.08
250	23.87	6.73 ±0.09	58.29	3.84 ±0.16	39.56	4.98 ±0.24	42.67	5.29 ±0.07
125	31.59	10.82 ±0.06	74.84	5.32 ±0.16	68.71	7.83 ±0.61	70.34	8.64 ±0.04
62.5	40.63	19.67 ±0.15	85.12	11.91 ±0.54	87.68	14.68 ±0.23	85.23	16.35 ±0.15
31.25	47.82	26.79 ±0.54	93.88	20.54 ±0.63	94.15	23.79 ±0.41	92.84	31.79 ±0.23
15.6	65.41	37.56 ±0.68	97.24	28.72 ±0.19	98.73	34.62 ±0.89	98.06	39.06 ±0.41
7.8	73.18	48.87 ±1.43	99.71	35.81 ±1.34	100	46.71 ±1.37	100	47.38 ±0.63
3.9	80.73	64.95 ±1.79	100	43.96 ±1.48	100	52.85 ±0.98	100	56.24 ±1.86
0	100	100	100	100	100	100	100	100
IC_{50} µg/ml	29.3	7.53	326	2.43	205	5.71	217	6.65

Cisplatin is included as a standard for cytotoxicity

Experimental

General experimental procedures: UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were obtained using a Bruker Tensor 27 instrument. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 (^{1}H) and 125 MHz (^{13}C), and a Varian Mercury 400 MHz spectrometer at 400 (^{1}H) and 100 MHz (^{13}C). ESI-MS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI). Column chromatographic (CC) separations were performed on silica gel 60 (Merck) and Sephadex LH-20 (Merck). TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min, and by spraying with ammonia solution.

Plant material

Asphodelus microcarpus leaves were collected from an area 70 km West of Marsa Matrouh, Egypt, during March 2011. The plant was kindly authenticated by Dr. Ibrahim El-Garf, Professor of Plant Taxonomy, Cairo University, Egypt. A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

The air-dried leaves (1.5 kg) was ground into fine powder and extracted exhaustively with methanol (3 x 8 L). The combined methanolic extracts was concentrated to dryness (280 g). The residue was suspended in H_2O (500 ml) and partitioned successively with petroleum ether (12 g), EtOAc (60 g) and *n*-BuOH (110 g). The EtOAc fraction was fractionated over Si gel column eluted successively with *n*-hexane and a gradient of *n*-hexane-EtOAc and finally with EtOAc to give eight fractions; A (7 g), B (3 g), C (4.3 g), D (5.1 g), E (4.2 g), F (2.3 g), G (2.9 g) and H (3.5 g). Fraction A was further chromatographed over Si gel column eluted with *n*-hexane-EtOAc (85:15-80:20) to give 4 sub- fractions; A1 (900 mg), A2 (1.2 g), A3 (2.2 g) and A4 (950 mg). Fraction A1 was rechromatographed on Si gel column and eluted with *n*-hexane-EtOAc (95:5-90:10) and final purification by gel filtration over Sephadex LH-20 eluted with MeOH to afford compound 1 (50 mg). Fractions A2 and A4 were separately subjected to Si gel column and eluted with *n*-hexane-EtOAc (90:10-80:20) and final purification by Sephadex LH-20 eluted with MeOH to afford compound 2 (100 mg) and compound 3 (60 mg), respectively. Fraction C was further chromatographed over Si gel column with chloroform-methanol: 95-5 to give three fractions; C1 (850 mg), C2 (730 mg) and C3 (910 mg). Fraction C3 was rechromatographed on Si gel column and eluted with $CHCl_3$-MeOH: 98-2 and final purification by Sephadex LH-20 eluted with MeOH to give compound 4 (30 mg).

Compound 1: Colorless oil; UV λ_{max} (MeOH) nm: ; IR ν_{max} (KBr) cm^{-1}: 1735, 1651, 722. ESI-MS: An $[M+H]^+$ ion at *m/z* 297; ^{1}H NMR data (*$CDCl_3$*, 400 MHz) δ 5.32 (2H, t-like, 4.6 Hz, H-9, H-10), 2.28 (2H, m, H-2), 1.98 (4H, m, H-8, H-11), 1.59 (2H, m, H-3), 1.29-1.24 (20H, m, H_2-4 to H_2-7 and H_2-12 to H_2-17), 0.92 (3H, t, 7.3 Hz, H_3-18), 3.65 (3H, s, OCH_3); ^{13}C NMR data (*$CDCl_3$*, 100 MHz) δ 174.44 (C-1, Cq), 130.29 and 130.28 (C-9, C-10, CH), 51.88 (OCH_3), 34.55 (C-2, CH_2), 31.15-29.54 (C-4 to C-7 and C-12 to C-16, CH_2), 27.62 (C-8, C-11, CH_2), 25.46 (C-3, CH_2), 19.60 (C-17, CH_2), 14.17 (C-18, CH_3).

Compound 2: An orange-red powder; UV λ_{max} (MeOH) nm: 224, 250, 280, 410; IR ν_{max} (KBr) cm^{-1}: 3440, 1675, 1642. ESI-MS: An $[M+H]^+$ ion at *m/z* 255; ^{1}H and ^{13}C NMR data (*$CDCl_3$*, 500 and 125 MHz) see Tables 1 and 2.

Compound 3: An orange powder; UV λ_{max} (MeOH) nm: 261, 286, 395, 425; IR ν_{max} (KBr) cm^{-1}: 3450, 1678, 1635. ESI-MS: An $[M-H]^-$ ion at *m/z* 507; ^{1}H and ^{13}C NMR data (DMSO-*d_6*, 400 and 100 MHz) see Tables 1 and 2.

Compound 4: Yellowish amorphous powder; UV λ_{max} (MeOH) nm: 210, 245, 285, 375 425; IR ν_{max} (KBr) cm^{-1}: 3450, 1680, 1630; ESI-MS: An $[M+H]^+$ ion at *m/z* 641; ^{1}H and ^{13}C NMR data (DMSO-*d_6*, 400 and 100 MHz) see Tables 1 and 2.

Oxidative hydrolysis

Compound 4 (10 mg) was refluxed with 15 ml of 1N ferric chloride: methanol (1:1) at 100 ^{0}C, for four hours. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (20 ml) was extracted with EtOAc (3 x 20 ml). The EtOAc extracts were evaporated to afford asphodelin. The aqueous layer was neutralized with 2N KOH solution and concentrated under reduced pressure. The residue

showed a spot at the same R_f as arabinose on TLC (silica gel, $CHCl_3$-MeOH-H_2O-14:6:1).

Antimicrobial assays

The antimicrobial activity of the methanolic extract of *A. microcarpus* leaves was investigated in *vitro* against different bacteria and fungi. As fungal strains *Aspergillus flavus* (RCMB 002002) and *Candida albicans* (RCMB 05036) were used. The following bacterial strains were employed: Gram-positive bacteria; *Staphylococcus aureus* (RCMB 010010) and *Bacillus subtilis* (RCMB 010067), Gram-negative bacteria; *Salmonella* SP. (RCMB 010043) and *Escherichia coli* (RCMB 010052). The microbial species are environmental and clinically pathogenic microorganisms obtained from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. Antimicrobial tests were carried out by diffusion agar method (Bauer, *et al*., 1966) [3], using 100 µL of suspension containing 1 x10^8 colony forming units (CFU)/mL for tested bacteria and 1 x10^4 spore/ml fungi spread on nutrient agar and malt extract agar, respectively. After the media had cooled and solidified, wells (6 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested sample solution in 1 ml dimethyl sulfoxide (DMSO) with concentrations of 5mg/ml. Negative controls were prepared using DMSO employed for dissolving the tested sample, while gentamycin, gentamycin and Ketoconazol were used as positive controls for Gram positive bacteria, Gram negative bacteria and fungi respectively. The inoculated plates were then incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi and the diameter of any resulting zones of inhibition of growth was measured in millimeter (mm). Each experiment was performed in triplicates and the data was expressed as mean ± SD. The minimal inhibitory concentrations (MICs) were determined by using the two fold serial dilution technique (Rajbhandari and Schopke, 1999) [24]. The twofold serial dilutions of the tested compound solutions were prepared. The tubes were then inoculated with the test organisms, grown in their suitable broth for tested pathogenic bacteria (1 x 10^8 CFU/ml for bacteria and 1 x10^4 spore/ml for fungi); each 0.5 ml received 100 µL of the above inoculum and was incubated at 37°C for 24 h for bacteria and after 48 h of incubation at 28°C for fungi. MIC values were taken as the lowest compound concentration that prevents visible bacterial growth. Each experiment was made three times. The results are presented in Table 3.

Antiviral activity using CPE inhibition assay

The antiviral activities of the methanolic extract of *A. microcarpus* leaves against Human Herpes Simplex virus type 1 (HSV-1) and Hepatitis A virus (HAV-10) by using cytopathic endpoint (CPE) inhibition assay (Hu and Hsiung, 1989; Dargan, 1998; Vijayan, *et al*., 2004) [15, 6, 29] at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Briefly, 100µL of serial 10-fold dilutions of the sample were incubated with 100µL of Vero E6 cells, giving a final cell count of 20,000 cells per well in a 96-well plate. The incubation period was 24h at 37 °C in a humidified incubator with 5% CO_2. Ten micro liters of virus at a concentration of 10,000 PFU/well was then added to each of the test wells. The plates were incubated at 37 °C in a humidified incubator with 5% CO2 for 3 days and observed daily for CPE. The end point was the sample dilution that inhibited 100% of the CPE in quadruplicate wells. To determine cytotoxicity, 100µL of serial 10-fold dilutions of the sample was incubated with 100µL of Vero E6 cells, giving a final cell count of 20,000 cells per well in a 96-well plate, without viral challenge. The plates were then incubated at 37 °C in a humidified incubator with 5% CO2 for 3 days and examined for toxicity effects by using an inverted microscope. MTT, a tetrazolium salt (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) has been applied to measure cytopathic effect produced by viral infection.

Cytotoxicity evaluation using viability assay

The cytotoxicity of the methanolic extract of *A. microcarpus* leaves was tested against four human tumor cell lines; human lung carcinoma cells (A-549), human colon carcinoma cells (HCT-116), human breast cancer cells (MCF-7) and human prostate carcinoma cells (PC-3). The cells were obtained from VACSERA Tissue Culture Unit. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37 ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times a week. The cytotoxic activity was determined by using cell viability assay method (Mosmann, 1983; Gomha, *et al*., 2015) [22, 12]. The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested sample were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells at 37 °C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested sample. All experiments were carried out in triplicate. The cell cytotoxic effect of tested sample was calculated. The optical density was measured with the microplate reader (Sun Rise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified sample**.** The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA). The results are presented in Table 4.

Statistical analysis
All data were expressed as mean ± SE. Student's t test was applied for detecting the significance of difference between each sample; P\0.05 was taken as the level of significance (Woodson, 1987) [30].

Acknowledgement
Thanks are due to Dr. Mohammed Ghoneim, Lecturer of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, for NMR and MS analyses. National center for natural products research (NCNPR), School of Pharmacy, University of Mississippi, USA.

References
1. Adinolfi M, Corsaro MM, Lanzetta R, Parrilli M, Scopa A. A bianthrone C-glycoside from *Asphodelus ramosus* tubers Phytochemistry. 1989; 28(1):284-288.
2. Altona C, Haasnoot CAG Prediction of anti and gauche vicinal proton-proton coupling constants in carbohydrates: a simple additivity rule for pyranose rings Org. Magn. Reson. 1980; 13(6):417-429.
3. Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standardized single method American J. Cl. Patho. 1966; 45(4):493-496.
4. Bloom H, Briggs LH, Cleverly B. Physical properties of anthraquinone and its derivatives. Part I. Infrared spectra J. Chem. Soc. 1959, 178-185.
5. Bonsignore L, Cottiglia F, Loy G, Begala M, Sanna L Scordo F, Serpi M. " Preliminary study on the chemical constituents and microbiological activity of extracts of *Asphodelus microcarpus* (Salzm. et Viv.) Boll. Chim. Farm. 1998; 137:186-190.
6. Dargan DJ. Investigation of the anti-HSV activity of candidate antiviral agents. In: Methods in Molecular Medicine. Herpes Simplex Virus Protocols (Edited by: Brown, S.M. and MacLean, A.R.) Humana Press Inc., Totowa, NJ. 1998; 10(1):387-405.
7. El-Seedi HR. Antimicrobial arylcoumarins from *Asphodelus microcarpus* J. Nat. Prod. 2007; 70:118-120.
8. Gerhard KN, James AK. Determination of the fatty acid profile by 1H-NMRspectroscopy Eur. J. Lipid Sci. Technol. 2004; 106:88-96.
9. Ghoneim MM, El-Hela AA, Mohammad AI, Kottob S, El-Ghaly S, Cutler SJ, Ross SA. Biologically active secondary metabolites from *Asphodelus microcarpus* " Nat. Prod. Commun. 2013; 8:1117-1119.
10. Ghoneim MM, Elokely KM, Atef AA, Mohammad AI, Jacob M, Cutler SJ *et al*. "Isolation and characterization of new secondary metabolites from *Asphodelus microcarpus*" Med. Chem. Res. 2014; 23:3510-3515.
11. Ghoneim MM, Elokely KM, El-Hela AA, Mohammad AI, Jacob M, Radwan MM *et al*. Asphodosides A-E, anti-MRSA metabolites from *Asphodelus microcarpus*" Phytochemistry. 2014; 105:79-84.
12. Gomha SM, Riyadh SM, Mahmmoud EA, Elaasser MM. Synthesis and anticancer activities of thiazoles, 1,3-thiazines, and thiazolidine using chitosan-grafted-polyvinylpyridine as basic catalyst Heterocycles. 2015; 91(6):1227-1243.
13. Hammouda FM, Rizk AM, Abdel-Gawad MM. "Alkaloids of *Asphodelus microcarpus*" Current Science. 1971; 40(23):631-632.
14. Hernandez-Medel MDR, Ramirez-Corzas CO, Rivera-Dominguez MN, Ramirez-Mendez J, Santillan R, Rojas-Lima S. Diastereomeric C-glycosyloxanthrones from *Picramnia antidesma* Phytochemistry. 1999; 50:1379-1383.
15. Hu JM, Hsiung GD. Evaluation of new antiviral agents I In vitro prospectives Antiviral Res. 1989; 11(5):217-232.
16. Jerkovic I, Carlo IG. Volatile Compounds of Asphodelus microcarpus Salzm. et Viv. Honey Obtained by HS-SPME and USE Analyzed by GC/MS Chemistry & biodiver. 2011; 8:586-598.
17. Knothe G, Kenar AJ. Determination of the fatty acid profile by ^{1}H-NMR Spectroscopy Eur. J. Lipid Sci. Technol. 2004; 106:88-96.
18. Lanzetta R, Parrilli M, Adinolfi M, Aquila T, Corsaro MM. Bianthrone-C-glycosides 2. Three new compounds from *Asphodelus ramosus* tubers Tetrahedron. 1990; 46(4):1287-1294.
19. Li C, Shi JG, Zhang YP, Zhang CZ. Constituents of *Eremurus chinensis* J. Nat. Prod. 2000; 63:653-656.
20. Lifante ZD, Aguinagalde I. The use of random amplified polymorphic DNA (RAPD) markers for the study of taxonomical relationships among species of *Asphodelus* sect. Verinea (Asphodelaceae) Am. J. Bot. 1996; 83:949-953.
21. Mohammed MM. Isolation and Structure Elucidation of Bioactive Compounds with Cytotoxic Activity From Some Egyption Plants Belonging to Family Liliaceae Ph D. Thesis (Pharmacognosy), Faculty of Pharmacy, Al-azhar University, Cairo, Egypt. 2014.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays J Imm. Meth. 1983; 65(1):55-63.
23. Oscar L, Maritza D, Jesús M. 1H-NMR Spectroscopy Study of Oleic Acid and MethylOleate Ozonation in different reaction conditions. Revis. CENIC Cienc. Quím. 2003; 34:3-8**.**
24. Rajbhandari M, Schopke T. Antimicrobial activity of some Nepalese medicinal plants Pharmazie. 1999; 54:232-233.
25. Rizk AM, Hammouda FM. Phytochemical studies of *Asphodelus microcarpus* (Lipids and carbohydrates)" Planta Med. 1970; 18:168-172.
26. Täckholm V. Students Flora of Egypt, 2nd ed.; Cairo University Press, Cairo, 1974, 629-630.
27. Täckholm V, Drar M. Flora of Egypt, Bulletin of the Faculty of Science, No. 30, Cairo University. 1954, 3.
28. Thomson RH. Naturally Occurring Quinones. London, New York, Chapman and Hall, 1971; 3:39.
29. Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris" Indian J. Med. Res. 2004; 120(1):24-29.
30. Woodson RR. Statistical methods for the analysis of biochemical data. Series in probability and mathematical statistics Wiley, New York, 1987, 315-316.
31. Yagi A, Makino K. Nishioka I. Studies on the constituents of *Aloe saponaria* H_{AW}. IV. The structures of bianthraquinoid pigments Chem. Pharm. Bull. 1978; 26:1111-1116.
32. Zhang H, Guo Z, Wu N, Xu W, Han L, Li N, Han Y. Two Novel Naphthalene Glucosides and an Anthraquinone Isolated from *Rumex dentatus* and Their Antiproliferation Activities in Four Cell Lines". Molecules. 2012; 17:843-850.