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PHYTOCHEMICAL INVESTIGATION AND MOLLUSCICIDAL ACTIVITY OF *OREOPANAX RETICULATUM* DONN

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باجراء تفاعل التصبن علي الجزء الذائب في الاثير البترولي لاوراق وسيقان نبات اوريوباناكس ريتيكيولاتم وباستخدام جهاز كروماتوجرافيا الغاز امكن فحص المحتوي الدهني لاوراق وسيقان النبات.. ففي الجزء الغير متصبن امكن التعرف علي خليط من الهيدروكربونات والاستيرولات ومن الجزء المتصبن أمكن التعرف علي ثمانية أحماض دهنية.

باجراء فصل كروماتوجرافي للمستخلص البيوتانولي لاوراق وسيقان النبات باستخدام كروماتوجرافيا العمود والطبقة الرقيقة امكن فصل ثمانية مركبات (**I-VIII**) وهي ستة مركبات صابونينية واثنين فلافونيدات جليكوزيدية و قد تم التعرف علي التركيب الكيميائي الدقيق لهذه المركبات باستخدام الطرق الطيفية والكيميائية المختلفة.

أظهرت اثنان من المركبات الصابونينية الثالث و السادس (**III** and **VI**) قدرة ابادية عالية ضد قواقع بيمفولاريا الكسندرينا العائل الوسيط لطفيل البلهارسيا المعوية في مصر (LC90= 7 and 6 ppm) على التوالى بعد زمن تعرض قدره ٢٤ ساعة.بينما كانت بقية المركبات غير فعالة حتي تركيز ٥٠ جزء في المليون. وتمت مناقشة هذه النتائج في ضوء العلاقة بين فعالية المركبات ضد القواقع وتركيبها الكيميائي.

*The unsaponifiable part of the petroleum ether fraction of the leaves and stems of Oreopanax reticulatum Donn (Family Araliaceae) was analysed by GLC to reveal the presence of a series of hydrocarbons, cholesterol, stigmasterol and β-sitosterol. Also, eight fatty acids were identified on the saponifiable part. The butanolic fraction was chromatographed over silica gel and sephadex-LH20 columns and preparative thin layer chromatography to afford two flavonoid glycosides **(I and IV)** as well as four bidesmosidic triterpenoidal saponins **(II, V, VII and VIII)**. Alkaline hydrolysis of two bidesmosidic saponins (**II and V)** gave two monodesmosidic **(III and VI)**. Their structures were elucidated using spectroscopic and chemical techniques.*

*Molluscicidal screening of the isolated compounds **(I-VIII)** against Biomphalaria alexandrina snails, the intermediate host of Schistosoma mansoni in Egypt revealed that the two monodesmosidic triterpenoidal saponins **(III and VI)** have strong activities (LC_{90}= 7 and 6 ppm) after 24 hours exposure whereas the two flavonoid glycosides and four bidesmosidic saponins were inactive up to 50 ppm.*

INTRODUCTION

Schistosomiasis is still one of the most important parasitic diseases of man in Egypt and some tropical and subtropical parts of the world.[1] The transmission of this disease requires vector snails as obligate intermediate hosts. Control of the vector snails is therefore, revelant to the control of this parasitic disease.[2,3] The use of plants with molluscicidal properties is a simple, inexpensive and appropriate technology for the snail control.[4,5] Since the discovery of the active saponins in the berries of *Phytolacca dodecandra*, naturally occurring molluscicides are receiving considerable attention.[5-7]

In a previous study, it was observed that the water suspension of the leaves and stems of *Oreopanax reticulatum* (Family Araliaceae) has molluscicidal activity against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt (LC_{90}= 160 ppm) after 24 exposure.[8] The available

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literatures revealed that nothing was reported on the chemistry of *Oreopanax reticulatum.* Meanwhile lupane and oleanane triterpenoidal saponins have been isolated from the other species; *Oreopanax guatemalensis*.[9,10] This promoted us to isolate and identify the chemical constituents of *O. reticulatum* and evaluate the potency of the isolated compounds as molluscicidal agents against *Biomphalaria alexandrina* snails.

EXPERIMENTAL

General

Melting points were uncorrected.IR spectra were measured on a Perkin-Elmer model FT-IR recording spectrophotometer. Nuclear magnetic resonance ^{1}H-NMR (270 MHz) and ^{13}C-NMR (100 MHz) spectra were done using TMS as internal standard, DMSO-d_6 as solvent and chemical shifts are given in (ppm) scale. UV spectrophotometer (Milton Roy 601). Mass spectra were measured on a Finnigan TSQ 700 GC/MC equipped with a Finnigan electrospray source (EI-MS and CI-MS).TLC was carried out on silica gel 60 GF254 and the spots were visualized by spraying with 40% H_2SO_4 followed by heating in oven at 120°. Column chromatography was carried out on silica gel 60 (sigma 28-200 mesh). Centrifugal chromatography device was used for performing preparative chromatographic separations. Sephadex LH-20 columns were performed using methanol as eluent. Paper Chromatography was performed on Whatmann paper No. 1 using descending technique and visualized with aniline phthalate.

GLC analysis of the unsaponifiable matter was carried out on a GCV Pye Unicam gas chromatograph equipped with a dual flame ionization detector and a dual channel reactor using a coiled glass column (2.8 m x 4 mm) packed with diatomite C (100-120 mesh) and coated with 1% OV-17. Oven temperature was programmed at 10°/min from 70-270° then run isothermally at 270° for 25 minutes. Nitrogen flow was 30 ml/min. Detector and injector temperatures were generally 300° and 280° respectively. Hydrogen and air flow rates were 33 and 330 ml/min respectively.

GLC analysis of the fatty acid methyl esters was performed adapting the previously mentioned conditions except the following. A coiled glass column (1.5 m x 4 mm) packed with 10% polyethylene glycol Adipate (PEGA) on diatomite C (100-120 mesh). Nitrogen was used as a carrier gas at a flow rate of (30 ml/min). the oven temperature was programmed at 80°/min from 70° to 190°, then kept isothermal at 190° for 30°.

Plant

The leaves and stems of *Oreopanax reticulatum* Donn (Family Araliaceae) were collected from El-Zohrea Botanical Garden, Giza, Egypt in March 2001. The plant was identified by Mrs. Traes Labib, general manager and head of specialist for plant identification in El-Orman Botanical Garden, Giza, Egypt. A voucher specimen was deposited in our laboratory. The leaves and stems of this plant were dried and powdered by electric mill and kept for extraction process.

Extraction and fractionation

The air-dried powdered leaves and stems of *O. reticulatum* (1.5 kg) were extracted with methanol. The methanolic extract was evaporated using rotatory evaporator under reduced pressure to dryness (198 g) and defatted with petroleum ether (60-80°). The petroleum ether extract (9 g) was saponified with methanolic potassium hydroxide (10%) for 6 hours. The unsaponifiable fraction (USM) and fatty acid methyl esters (FAME) were prepared[11,12] and analysed by GLC. Identification of the hydrocarbons and sterols was carried out by comparing their relative retention times with the same reference matters as well as the reported data in the literatures[12,13] The quantitative estimation of each compound was carried out based on a peak area measurement relative to other peaks in the chromatogram. Results of GLC analysis were listed in Tables 1 and 2.

Table 1: GLC analysis of the unsaponifiable fraction of lipid content of the leaves and stems of *Oreopanax reticulatum.*

Peak No.	RR_t	Relative %	Authentic hydrocarbons and sterols
1	0.205	0.237	n-Dodecane
2	0.216	0.289	n-tridecane
3	0.243	0.124	Unknown
4	0.252	0.201	Unknown
5	0.265	0.470	n-Tetradecane
6	0.281	1.616	n-Pentadecane
7	0.296	0.149	Unknown
8	0.312	0.256	Unknown
9	0.336	8.723	n-Hexadecane
10	0.365	0.243	n-Heptadecane
11	0.384	0.106	n-Octadecane
12	0.394	0.595	n-Eicosane
13	0.417	0.167	n-Heneicosane
14	0.434	0.306	n-Docosane
15	0.461	0.200	n-Tricosane
16	0.479	14.843	n-Tetracosane
17	0.490	2.374	n-Pentacosane
18	0.529	5.266	n-Hexacosane
19	0.565	7.071	n-Octacosane
20	0.638	0.139	Squalene
21	0.665	0.633	Unknown
22	0.789	4.284	Cholesterol
23	0.911	17.663	Stigmasterol
24	1	33.809	β-sitosterol

RR_t= Retention time relative to β-sitosterol R_t= 22.25

Table 2: GLC analysis of the fatty acid methyl esters of the leaves and stems of *Oreopanax reticulatum.*

Peak No.	RR_t	Relative %	Authentic fatty acid methyl esters
1	0.497	7.686	Lauric acid
2	0.593	17.078	Myristic acid
3	0.646	1.333	Unknown
4	0.706	2.133	Unknown
5	0.761	2.682	Isopalmitic acid
6	0.829	13.200	Palmitic acid
7	0.899	3.437	Stearic acid
8	1	41.862	Oleic acid
9	1.214	1.735	Linoleic acid
10	1.382	8.853	Arachidic acid

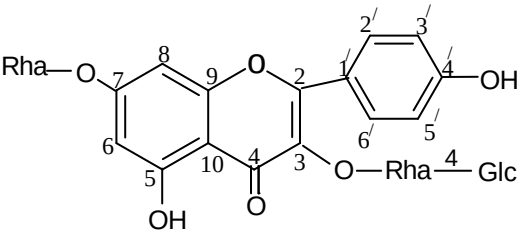
RR_t= Retention time relative to Oleic acid R_t= 20.00

Chromatographic separation of the butanolic fraction of *o. reticulatum*

The deffated methanolic extract was dissolved in water and successively extracted with $CHCl_3$, EtOAc and n-butanol.The butanolic fraction (45 g) was subjected to open column chromatography (120 x 5 cm) packed with silica gel 60 (70-230 mesh, Merck). Elution was started with petroleum ether followed by $CHCl_3$, gradient mixtures of $CHCl_3$: MeOH and ending with pure methanol. Five major fractions **A-E** were obtained by elution with different mixtures of $CHCl_3$: MeOH; 90:10; 85:15; 80:20; 70:30 and 60:40 respectively. Each of these fractions was separately purified on Sephadex LH-20 column using MeOH as eluent agent. The Sephadex column products of each fraction were collected and separately subjected to preparative TLC. Fraction **A** gave compound **I** (using solvent system $CHCl_3$:MeOH:H_2O, 30:10:1). Fraction **B** yielded compounds **II** and **IV** (using solvent system $CHCl_3$:MeOH:H_2O, 60:30:5). Fraction **C** yielded compound **V** (solvent system n-BuOH:MeOH:H_2O; 5:1:1). Fractions **D** and **E** gave compounds **VII** and **VIII** (solvent system n-BuOH:MeOH:H_2O; 5:3:2) respectively. Alkaline hydrolysis of compounds **II** and **V** gave compounds **III** and **VI** respectively**.**

Isolated compounds

Compound I, yellow powder, m.p 184-186°, R_f 0.47 ($CHCl_3$:MeOH:H_2O; 30:10:1). UV λ_{max} (MeOH) nm 266, 320, 344; ($AlCl_3$) 272, 344, 397; ($AlCl_3$/HCl) 272, 341, 395; (NaOAc) 266, 320, 346 and (NaOAc/H_3BO_3) 264, 324, 345. ^{1}H-NMR (DMSO-d_6) δ at ppm: 8.20 (5-OH), 7.79 (2H, d, J= 8.6 Hz, H-2$^/$ and H-6$^/$), 6.91 (2H, d, J= 8.6 Hz, H-3$^/$ and H-5$^/$), 6.72 (1H, d, J= 2.5 Hz, H-8), 6.42 (1H, d, J= 2.5 Hz, H-6), 5.53 (1H, d, J= 2.5 Hz, H-1$^{//}$ of Rha), 5.31 (1H, d, J= 2.5 Hz, H-1$^{///}$ Rha), 5.16 (1H, d, J= 7.5 Hz, H-1$^{//}$ of Gal), 1.14 (3H, d, J= 6.2 Hz, CH_3-Rha) and 0.82 (3H,d, J= 6.2 Hz, CH_3-Rha). CI-MS; m/z 741.8 $[M+H]^+$, 579.1 $[M\text{-}Glc]^+$ and 433.2 $[M\text{-}Glc\text{-}Rha]^+$, 287.2 $[M\text{-}Glc\text{-}2\ Rha]^+$. ^{13}C-NMR spectrum see Table (3).

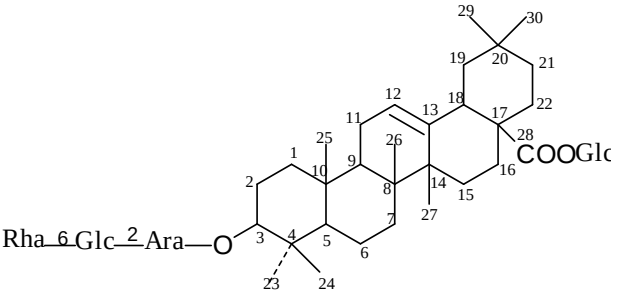


Compound I

Table 3: ^{13}C-NMR spectral data of compounds **I** and **IV** (in DMSO-d_6; TMS as internal standard).

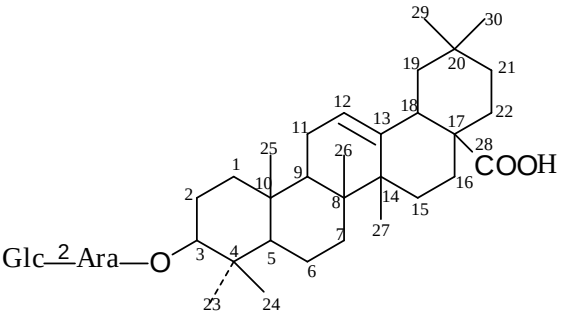
Aglycone part	Compound (**I**) δ C	Compound (**IV**) δ C
2	157.9	158.30
3	135.14	135.46
4	177.8	177.90
5	160.90	161.56
6	99.22	99.19
7	161.84	162.40
8	95.00	95.27
9	156.89	157.20
10	106.59	106.70
1/	120.37	120.50
2/	131.28	131.30
3/	116.36	116.27
4/	160.30	160.0
5/	116.36	116.27
6/	131.28	131.30
Sugar moieties		
	3-O-Rha	**3-O-Gal**
1//	98.62	100.20
2//	70.55	73.82
3//	72.80	75.92
4//	81.82	70.20
5//	70.10	76.07
6//	18.19	68.22
	7-O-Rha	**7-O-Rha**
1///	100.24	100.32
2///	69.97	70.40
3///	71.20	71.36
4///	71.68	71.76
5///	69.70	69.76
6///	18.50	18.58
	Glc (1→4 //) Rha	**Rha (1→6//) Gal**
1////	102.59	101.69
2////	72.40	70.63
3////	77.49	71.36
4////	70.55	71.64
5////	76.01	69.76
6////	61.83	18.08

Compound II, white powder, m.p 226-228°, R_f 0.63 ($CHCl_3$:MeOH:H_2O; 60:30:5) IR ν (KBr)max cm^{-1} 3420.2, 2939.9, 1735.7, 1639.5, 1386.6, 1066.2 and 914.0. ^{1}H-NMR (DMSO-d_6) δ 0.75-1.11 (each 3H, s, 7 x Me), 1.59 (3H, d, J= 6.4 Hz, Me of Rha), 4.70 (1H, d, J= 7.7 Hz, H-1 of Glc), 4.78 (1H,d, J= 6.2 Hz, H-1 of Ara), 5.16 (1H,d, J= 7.8 Hz, H-1 of Glc), 5.22 (1H,d, J= 1.6 Hz, H-1 of Rha) and 5.25 (1H, br, s, H-12). CI-MS; m/z 1057 $[M+H]^+$, 908.9 $[M-Rha]^+$, 894.6 $[M-Glc]^+$, 879.2 $[M-Glc-O]^+$, 851 $[M-Glc-COO^-]^+$, 749.7 $[M-Glc-Rha]^+$, 705.5 $[M-Glc-Rha-COO^-]^+$, 587.4 $[M-2Glc-Rha]^+$, 543.5 $[M-2Glc-Rha-COO^-]^+$, 455$[M-2Glc-Rha-Ara]^+$, and 411.4 $[M-2Glc-Rha-Ara-COO^-]^+$. ^{13}C-NMR spectrum see Tables 4 and 5.



Compound II

Compound III, white powder, m.p 244-247°, R_f 0.74 ($CHCl_3$: MeOH: H_2O; 60: 30: 5). IR ν (KBr) max cm^{-1} 3434.5, 2931.9, 1691.4, 1462.2, 1387.9, 1078.1 and 999.1. ^{1}H-NMR (DMSO-d_6) δ 0.69-1.09 (each 3H, s, 7 x Me), 4.60 (1H, d, J= 7.9 Hz, H-1 of Glc), 4.80 (1H,d, J= 6.4 Hz, H-1 of Ara) and 5.18 (1H, br,s H-12). CI-MS; m/z 751.7 $[M+H]^+$, 704.7 $[M+H-COOH]^+$, 587.5 $[M+H-Glc]^+$, 542.4 $[M + H-Glc-COOH]^+$, 455.3 $[M-Glc-Ara]^+$, and 411.2 $[M-Glc-Ara-COO^-]^+$. ^{13}C-NMR spectrum see Tables 4 and 5.



Compound III

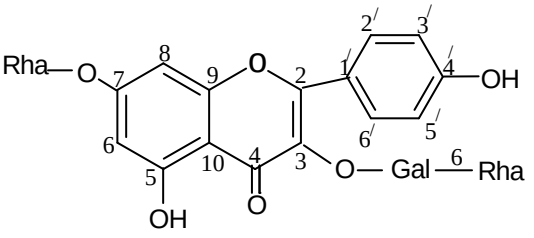
Table 4: ^{13}C-NMR spectral data of compounds **II, III, V, VI, VII, VIII** (in DMSO-d_6; TMS as internal standard).

Aglycone	**II**	**III**	**V**	**VI**	**VII**	**VIII**
1	38.85	38.70	39.02	38.70	39.47	39.48
2	26.36	26.1	26.18	26.30	28.17	26.17
3	88.43	87.2	88.93	88.50	88.53	88.91
4	39.73	38.80	39.45	39.20	39.74	39.76
5	55.72	55.79	55.84	55.60	56.30	55.85
6	18.43	18.39	18.50	18.70	18.43	18.45
7	33.00	33.12	33.04	33.20	37.20	33.44
8	39.73	39.80	39.72	39.50	40.29	39.48
9	47.81	47.50	47.84	48.70	46.29	47.85
10	37.03	36.90	36.96	39.20	38.40	36.97
11	23.67	23.70	23.29	24.60	24.09	23.26
12	122.04	121.90	122.41	123.2	131.40	122.44
13	144.14	144.70	144.11	144.30	133.40	144.11
14	42.4	42.3	42.01	42.60	56.32	42.02
15	28.38	28.35	28.30	28.50	26.51	28.32
16	24.10	24.5	24.08	26.70	26.17	24.10
17	46.71	46.80	46.66	46.30	49.09	46.69
18	42.04	42.16	41.50	42.50	54.60	41.15
19	46.31	46.31	46.28	46.90	39.02	46.32
20	30.97	30.97	30.30	31.2	36.02	30.97
21	34.00	34.00	33.09	34.50	30.96	34.00
22	33.01	33.01	33.44	33.70	36.02	33.44
23	27.97	27.97	27.91	28.20	18.43	27.99
24	17.14	17.14	17.42	16.80	28.27	17.41
25	15.89	15.89	15.88	15.50	15.87	15.89
26	17.14	17.14	17.79	17.40	18.43	17.10
27	26.19	26.19	26.18	26.10	177.90	26.17
28	175.97	179.85	176.49	179.80	175.96	176.06
29	33.43	33.43	33.04	33.4	17.10	33.40
30	23.67	23.67	23.66	23.6	20.80	23.65

Table 5: ^{13}C-NMR spectral data of sugar of compounds **II, III, V, VI, VII, VIII** (in DMSO-d_6; TMS as internal standard).

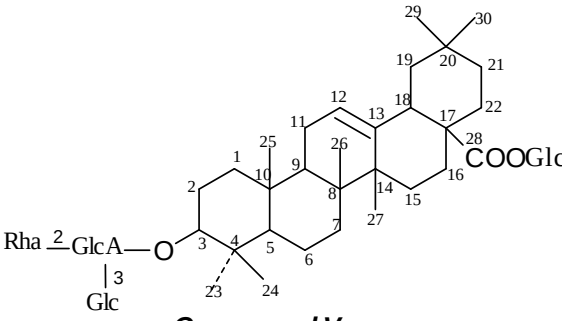
	II	III	V	VI	VII	VIII
	3-O-Ara	**3-O-Ara**	**3-O-GlcA**	**3-O-GlcA**	**3-O-Rha**	**3-O-GlcA**
1	106.38	106.20	104.30	104.20	101.30	103.34
2	77.61	78.30	78.41	77.50	77.00	78.60
3	76.21	76.34	82.15	82.20	83.59	82.91
4	69.36	69.44	72.77	71.90	73.7	72.73
5	65.36	65.20	74.23	74.70	69.34	74.50
6	--	--	173.71	172.90	18.43	173.39
	Glc (1→2) Ara	**Glc (1→2) Ara**	**Glc (1→3) GlcA**	**Glc (1→3) GlcA**	**Glc (1→3) Rha**	**Glc (1→2) GlcA**
1	103.32	104.23	104.71	105.30	104.98	104.29
2	75.97	76.30	74.23	74.40	72.97	74.50
3	77.40	77.30	77.40	76.90	74.49	76.75
4	71.75	72.40	70.33	70.50	70.68	71.50
5	77.30	76.90	77.48	77.20	76.21	76.40
6	69.36	61.89	61.47	61.80	61.73	61.31
	Rha (1→6) Glc		**Rha (1→2) GlcA**		**28-O-Glc**	**Xyl (1→3) GlcA**
1	101.30		101.40		94.76	104.72
2	72.70		70.33		77.52	76.8
3	72.96		72.77		78.52	73.4
4	73.41		73.13		71.38	69.2
5	70.09		70.33		75.96	65.41
6	17.45		17.41		60.73	
	28-O-Glc		**28-O-Glc**			**28-O-Glc**
1	94.76		94.87			94.80
2	73.41		74.23			74.03
3	77.40		77.40			77.60
4	70.09		70.66			70.30
5	77.40		77.48			77.40
6	61.39		61.63			61.47

Compound IV, yellow powder, m.p 202-205°, R_f 0.55 ($CHCl_3$:MeOH:H_2O; 60:30:5). UV λ_{max} (MeOH)nm 264, 325, 341; ($AlCl_3$) 272, 341, 395; ($AlCl_3$/HCl) 272, 341, 392; (NaOAc) 268, 322, 345 and (NaOAc/H_3BO_3) 268, 324, 343. ^{1}H-NMR (DMSO-d_6) δ 8.24 (5-OH), 7.79 (1H,d, J= 8.24 Hz, H-2$^{/}$-H-6$^{/}$), 6.94 (2H, d, J= 8.5 Hz, H-3$^{/}$, H-5$^{/}$), 6.78 (1H, d, J= 2.5 Hz, H-8), 6.42 (1H, d J= 2.5 Hz, H-6), 5.57 (1H,d, J= 2.5 Hz, H-1$^{////}$ of Rha), 5.53 (1H,d, J= 2.5 Hz, H-1$^{///}$ Rha), 4.94 (1H, d, J= 7.6 Hz, H-1$^{//}$ of Gal), 1.15 (3H, d, J= 6.1 Hz, CH_3-Rha) and 0.85 (3H, d, J= 6.1 Hz, CH_3-Rha) CI-MS; m/z 741.5 $[M]^+$, 595.3 $[M\text{-Rha}]^+$, 433.5 $[M\text{-Rha-Gal}]^+$ and 287 $[M\text{-2 Rha-Gal}]^+$. ^{13}C-NMR spectrum see Table 3.



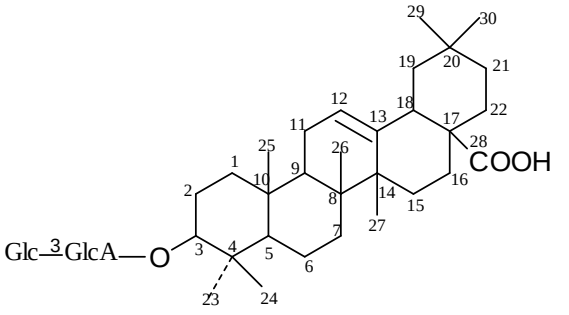
Compound IV

Compound V, white powder, m.p 255-257°, R_f 0.58 (n-BuOH: MeOH: H_2O; 5: 1: 1). IR ν (KBr)max cm^{-1} 3415.6 2943.2, 1732.7, 1617.4, 1417.8, 1077.9 and 950.9. ^{1}H-NMR (DMSO-d_6) δ 0.73-1.06 (CH_3, s, 7 x Me), 1.69 (3H, d, J= 6.2 Hz, CH_3 of Rha), 4.39 (1H, d, J= 7.6 Hz, H-1 of GlcA), 4.45 (1H, d, J= 7.6 Hz, H-1 of Glc), 5.14 (1H, d, J= 7.8 Hz, H-1 Glc), 5.21 (1H, d, J= 1.6 Hz, H-1of Rha) and 5.23 (1H, br,s,H-12). CI-MS; m/z 1101 $[M+H]^+$, 939.6 $[M+H\text{-}Glc]^+$, 893 $[M+H\text{-}Glc\text{-}COO^-]^+$, 793.7 $[M+H\text{-}Glc\text{-}Rha]^+$, 748.1 $[M\text{-}Glc\text{-}Rha\text{-}COO^-]^+$, 633.6 [M-2Glc-Rha], 585.5 $[M\text{-}2Glc\text{-}Rha\text{-}COO^-]^+$ and 455.4$[M\text{-}2Glc\text{-}Rha\text{-}GlcA]^+$. ^{13}C-NMR spectrum see Tables 4 and 5.



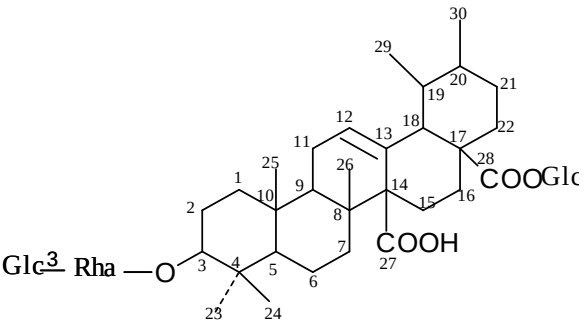
Compound V

Compound VI, white powder m.p 248-250°, R_f 0.76 (n-BuOH: MeOH: H_2O; 5: 1: 1). IR ν (KBr)max cm^{-1} 3419.5, 2930.7, 1695.5, 1461.6, 1167.4, 1078.2 and 978. ^{1}H-NMR (DMSO-d_6) δ 0.73-1.06 (each 3H, s, 7 x Me), 4.43 (1H, d, J= 7.7 Hz, H-1 of GlcA), 4.35 (1H, d, J= 7.8 Hz, H-1 of Glc) and 5.22 (1H, br, s, H-12). CI-MS; m/z 793.5 $[M + H]^+$, 749.1 $[M\text{-}COOH]^+$, 631.2 $[M+H\text{-}Glc]^+$, 587.2 $[M+H\text{-}Glc\text{-}COOH]^+$ and 455.4 $[M\text{-}Glc\text{-}GlcA]^+$. ^{13}C-NMR spectrum see Tables 4 and 5.



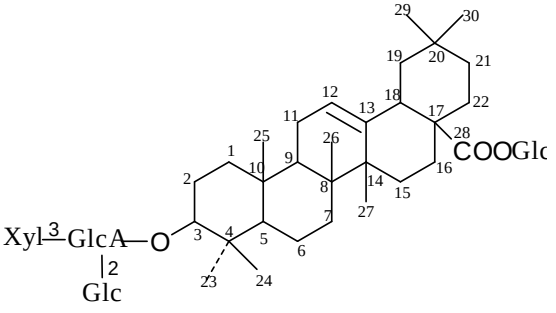
Compound VI

Compound VII, white powder m.p 223-225°, R_f 0.62 (n-BuOH: MeOH: H_2O; 5:3:2). IR ν (KBr) max cm^{-1} 3387.9, 2931.8, 1734.3, 1631, 1446, 1129.3, 1072.7 and 929.4. ^{1}H-NMR (DMSO-d_6) δ 0.69 -1.06 (each 3H, s, 6 x Me), 1.61 (3H, d, J= 6.5 Hz, CH_3 of Rha), 4.65 (1H, d, J= 7.6 Hz, H-1 of Glc), 4.70 (1H, d, J= 7.9 Hz, H-1 of Glc), 4.85 (1H, d, J= 1.65 Hz, H-1 of Rha) and 5.18 (1H, br,s, H-12). CI-MS; m/z 955.6 $[M+H]^+$, 911.7 $[M+H\text{-}COO^-]$, 793.9 $[M+H\text{-}Glc]^{+,}$ 749.7 $[M+H\text{-}Glc\text{-}COO^-]^{+,}$ 705.8$[M+H\text{-}Glc\text{-}2COO^-]^+$, 648.0 $[M+H\text{-}Glc\text{-}Rha]^+$, 604.1 $[M\text{-}Glc\text{-}Rha\text{-}COO^-]^+$ and 485.4 [M-2Glc- Rha]$^+$. ^{13}C-NMR spectrum see Tables 4 and 5.



Compound VII

Compound VIII, white powder, m.p 232-234°, R_f 0.51 (n-BuOH: MeOH: H_2O; 5:3:2). IR ν (KBr)max cm^{-1} 3422.2, 2927.1, 1732.8, 1602.2, 1386.0, 1078.1 and 940. ^{1}H-NMR (DMSO-d_6) δ 0.72-1.09 (each 3H, s, 7 x Me), 4.69 (1H, d, J= 7.6 Hz, H-1 GlcA), 4.80 (1H, d, J= 7.8 Hz, H-1 of Glc), 5.15 (1H, d, J= 7.9 Hz, H-1 of Glc), 5.20 (1H, d, J= 6.9 Hz, H-1 of Xyl) and 5.25 (1H, br, s, H-12). CI-MS; m/z 1087.2 $[M+H]^+$, 925.8 $[M\text{-}Glc]^+$, 880.4 $[M\text{-}Glc\text{-}COO^-]^+$, 749.6 $[M\text{-}Glc\text{-}Xyl\text{-}COO^-]^+$, 719.8 $[M\text{-}2Glc\text{-}COO^-]^+$, 618.7 [M-Glc-Xyl-GlcA], 587.5 $[M\text{-}2Glc\text{-}Xyl\text{-}COO^-]^+$, 455.4 [M-2Glc-Xyl-GlcA] and 411 $[M\text{-}2Glc\text{-}Xyl\text{-}GlcA\text{-}COO^-]$. ^{13}C-NMR spectrum see Tables 4 and 5.



Compound VIII

Acid hydrolysis of saponins

Each saponin (20 mg) was refluxed with 4 N HCl (30 ml) for 4 hours. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was evaporated to dryness and the aglycone part was identified by TLC analysis with authentic samples in each case using solvent system C_6H_6: MeOH, (8:2). The aqueous layers were neutralized with $NaHCO_3$, filtered, concentrated and compared with standard sugars on PC using solvent system (n-BuOH-AcOH-H_2O, 4:1:5). Spots were detected by spraying with a solution of aniline phthalate.

Alkaline hydrolysis of saponins

About 5 mg of bidesmosidic saponins **(II** and **V)** was refluxed with 5 ml 1M NaOH for 3 hours. The reaction mixture was neutralized and the prosapogenin was extracted with n-BuOH. The aqueous layer was subjected to paper chromatography to identify sugar part with authentic sugars.

Molluscicidal Assay

Biomphalaria alexandrina snails, the intermediate host of *Schistosoma mansoni* were collected from canals in Abu-Rawash, Giza Governorate. The snails were maintained in dechlorinated tap water in the laboratory conditions (Temp 25±2°, pH 7-7.7). Series of dilutions were prepared from each isolated compound to calculate the LC_{90} values. Ten snails were added in each concentration and the exposure time was 24 hours followed by 24 hours as recovery period. Three replicates were carried out for each case. Procedures and statistical analysis of data were carried out according to WHO and Litchfield and Wilcoxon procedures[14,15]

RESULTS AND DISCUSSION

The methanolic extract of the leaves and stems of *O. reticulaum* was defatted with pet.ether and the residue was dissolved in water and successively extracted with $CHCl_3$, EtOAc and n-BuOH.

The petroleum ether extract was saponified and each of USM and FAME was analysed by GLC. From Table (1), it was concluded that the major hydrocarbons of the unsaponifiable fraction of *O. reticulatum* is n-Tetracosane (14.843%) followed by n-Hexadecane (8.723%), n-Octacosane (7.071%), n-Hexacosane (5.266%) and n-Pentacosane (2.374%). The sterol part was represented by three components, cholesterol, stigmasterol and β-sitosterol where β-sitosterol was the highest percent (33.809%).

GLC analysis of the fatty acid methyl esters (Table 2) of *O. reticulatum* showed the presence of ten fatty acids, where eight of them were identified. Oleic acid recorded high percent (41.862%) whereas linoleic acid was the lowest one (1.735%).

The butanolic layer was chromatographed using silica gel and sephadex columns and preparative LC using different solvent systems. The structure of the following isolated compounds were elucidated through spectroscopic analysis as IR, ^{1}H-NMR, ^{13}H-NMR and CI-MS and identification of the product of acidic and alkaline hydrolysis of most of these compounds.

Compound I, gave positive tests for flavonoid glycosides. Its UV absorption bands with methanol and different reagents were similar to those for kampferol with substitution at 3-OH and 7-OH.[16-18] This was confirmed by the presence of the two signals in ^{1}H-NMR at upfield δ 7.79 (2H, d, H-2′, H-6′) and 6.91 (2-H, d, H-3′, H-5′) than original kampferol indicated the presence of substitution at 3-OH. Also, the presence of characteristic signals of H-6 and H-8 at downfield δ 6.72 (1H,d, H-8) and 6.42 (1H, d, H-6) than the usual pattern in kampferol (δ 6.5 for H-8 and 6.1 for H-6) indicated the presence of sugar substitution at C-7.[19,20] Appearance of two signals as doublets at δ 5.53 (H-1) and 5.31 (H-1) indicated the presence of two rhamnosyl units substituted at 3-OH and 7-OH respectively. This was supported by appearance of two methyl groups of two rhamnose units at δ 0.82 and 1.14.[19,20] The ^{13}C-NMR spectrum of this compound (Table 3) showed the characteristic signals of ketonic carbon at δ 177.8, acidic carbon C-7 at δ 161.84, C-5 at 160.90, C-4′ at δ 160.30, C-2 at δ 157.9 and C-9 at 156.89. Also, signal of C-3 appeared at δ 135.14 followed by non-oxygenated carbons C-2′ and C-6′ at δ 131.28 as well as C-3′ and C-5′ at δ 116.36. The most non-affected quaternary carbons C-1′ appeared

at δ 120.37 and C-10 at δ 106.59.[20-23] The signals of two carbons C-6 and C-8 appeared at δ 99.22 and 95.0 respectively. The anomeric carbons of the sugar units appeared at δ 98.62, 102.59 and 100.24. This was supported by the presence of three anomeric protons of sugar units in ^{1}H-NMR at δ 5.53, 5.31 and 5.16. Also, the two-methyl signals of two rhamnose units appeared at δ 18.19 and 18.50. The downfield shift of C-4$^{//}$ of rhamnose unit at δ 81.82$^{//}$ indicated that the terminal glucose unit is connected at this carbon,[21,22] CI-MS spectrum of compound **I**, gave m/z 741.8 [M^{+}], 579.1 [M^{+}-Glc], 433.2 [M^{+}-Glc-Rha], 287.2 [M^{+}-Glc-2Rha].[22,23] From the above data, the structure of compound **I** was elucidated as kampferol-3-O-[glucosyl (1→4$^{//}$) rhamnoside]–7-O-rhamnoside.

Compound II, its IR spectrum demonstrated the presence of hydroxyl group (3420.2 cm^{-1}), trisubstituted double bond (1639.5 cm^{-1}), ester group at (1735.7 cm^{-1}) and glycosidic linkage at 1066.0 cm^{-1}.[24,25] ^{1}H-NMR spectrum of compound **II** showed signals for seven methyl groups between a range δ 0.75-1.11, one trisubstituted olefinic proton at δ 5.25 and four anomeric protons at δ 4.70, 4.78, 5.16 and 5.22.[26,27] This was supported by presence of four anomeric carbons in ^{13}C-NMR spectrum (Table 5) at δ 106.38, 103.32, 101.30 and 94.76.[26,28] The mass spectrum of compound **II** revealed a diagnostically two important mass peaks at m/z 908.9 [M-Rha]$^{+}$ and m/z 894.6 [M-Glc]$^{+}$ indicating its molecular weight was 1056. Other fragments ions at m/z 851.0 [M-Glc-COO^{-}]$^{+}$, 749.7 [M-Glc-Rha]$^{+}$, 705.5 [M-Glc- Rha-COO^{-}]$^{+}$, 587.4 [M-2Glc-Rha]$^{+}$, 543.5 [M-2Glc-Rha-COO^{-}]$^{+}$, 455 [M-2Glc-Rha-Ara]$^{+}$ and 411.4 [M-2Glc-Rha-Ara-COO^{-}]$^{+}$ indicated the respective elimination of two terminal sugar units, one rhamnose and one glucose as well as two other saccharide units, one glucose and one arabinose and indicated that the arabinose unit is linked to the aglycone part.[26-29] The signals of the aglycone part in ^{13}C-NMR (Table 4) spectrum were identical with oleanolic acid as reported in the literature except for the signals of C-3 and C-28 which at downfield δ 88.43 and 175.97.[26,27] This was supported by the upfield shift of one anomeric carbon at δ 94.76 (Table 5) indicated esterification of the carboxyl group with sugar.[26-28] Therefore, compound **II** was consequently a bidesmoside triterpenoid saponin. Alkaline hydrolysis of compound **II** gave compound **III** (as prosapogenin) as well as D-glucose and L-rhamnose as sugar moieties which were identified by comparison with authentic sugars on PC using solvent system n-BuOH: AcOH: H_2O; (4:1:5). The signals of C-2 of arabinose and C-6 of the glucose units (Table 5) appeared at downfield δ 77.61 and 69.36 indicating that the two carbons are the position of linkages between the sugar units.[26,29] Therefore, the structure of **II** was elucidated as 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-oleanolic acid-28-O-β-D-glucopyranoside.

Compound III was obtained from alkaline hydrolysis of compound **II**. Its IR spectrum showed an absorption bands of hydroxyl groups at 3434.5 cm^{-1}, carboxylic group at 1691.4 cm^{-1} and the characteristic band of the glycosidic linkage at 1078.1 cm^{-1} (24, 25). The predominant fragments in CI-MS at m/z 751.7 [M+H]$^{+}$, 704.7 [M-COOH]$^{+}$, 587.5 [M-Glc]$^{+}$, 542.4 [M-Glc-COOH]$^{+}$, 455.3 [M-Glc-Ara]$^{+}$ and 411.2 [M-Glc-Ara-COOH]$^{+}$. This fragmentation pattern reflected the subsequent loss of glucosyl and arabinosyl units.[30,31] Also, the peak ascribable to the aglycone was observed m/z 455.3. Comparison of ^{1}H and ^{13}C-NMR data of compound **III** (Table 4 and 5) with those of compound **II** indicated that the aglycone part is the same (oleanolic acid) and the saccharide chain at C-3 lost of the rhamnosyl unit. Also, the saccharide chain at C-28 was absent where the signal C-28 was shifted at downfield δ 179.85 indicating that compound **III** is monodesmosidic triterpenoidal glycoside.[29,31] The ^{1}H and ^{13}C-NMR spectra showed the characteristic signals of the two anomeric protons at δ 4.60 and 4.80 and two anomeric carbons (Table 5) at δ 106.20 and 104.23 indicating that this compound has two sugar units.[26,29] The signal of C-2 of arabinose unit shifted at downfield δ 78.30 (Table 5) reflecting that this carbon is the position of the interglycosidic linkage between the two sugar units.[26,31] On the basis of these evidence, compound **III** was identified as 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabino-pyranosyl oleanolic acid.

Compound IV, its UV spectrum with methanol and different reagents exhibited all the characteristic absorption bands of kampferol with 3-OH and 7-OH substitutions.[17,18] This was confirmed by the appearing of two signals at upfield δ 7.79 (2H, d, H-2′,6′) and 6.94 (2H, d, H-3′,5′) in the ^{1}H-NMR spectrum than the original kampferol indicating the presence of substitution at C-3. Also, two signals at downfield δ 6.78 (1H,d, H-8) and 6.42 (1H, d, H-6) than kampferol suggesting the substitution at C-7.[32,33] In ^{13}C-NMR spectrum (Table 3), the two signals of C-3 and C-7 appearing at δ 135.46 and 162.40 than kampferol indicating the substitution of these carbon atoms.[19,33] CI-MS spectrum of compound **IV** exhibited a molecular ion at m/z 741.5 and other fragment peaks at m/z 595.3 [M^{+}-Rha], 433.5 [M^{+}-Rha-Gal] and 287 [M^{+}-2Rha-Gal] suggesting that compound **IV** has one galactosyl and two rhamnosyl units.[22,23] This was supported by presence of three signals of anomeric protons at δ 5.57, 5.53 and 4.94 as well as two methyl signals of the two rhamnosyl units at δ 1.15 and 0.85. Also, in ^{13}C-NMR spectrum (Table 3) the signals at the three anomeric carbon atoms were seen at δ 100.20, 101.69 and 100.32. The signal of C-6″ of the galactosyl unit was shifted at downfield δ 68.22 indicating the position of attachment of the terminal rhamnose of this carbon.[22,23] From the above data compound **IV** was consistent with the structure kampferol 3-O-[rhamnosyl (1→6″)galactoside]-7-O-rhamnoside.

Compound V, exhibited a molecular ion peak in CI-MS at m/z 1101.2 which in conjunction with the analysis of ^{13}C-NMR spectrum shown (Table 4, 5) to correspond to the molecular weight 1101. IR spectrum showed the characteristic signals for hydroxyl groups 3415.6 cm^{-1} and ester groups 1732.7 cm^{-1}.[24,25] The ^{1}H-NMR spectrum revealed the presence of seven tertiary methyl groups between δ 0.73 and 1.06, a double bond at δ 5.23 with typical ^{13}C-NMR resonances (Table 4) at δ 122.41 and 144.11 indicating an olean-12-ene triterpene.[25,29] In the CI-MS of compound **V**, the sequence of the sugar was established from diagnostic ions at m/z 939.6 $[M+H\text{-}Glc]^{+}$, 893 $[M+H\text{-}Glc\text{-}COO^{-}]^{+}$, 793.7 $[M+H\text{-}Glc\text{-}Rha]^{+}$, 748.1 $[M+H\text{-}Glc\text{-}Rha\text{-}COO^{-}]^{+}$, 633.6 $[M+H\text{-}2Glc\text{-}Rha]^{+}$, 585.5 $[M\text{-}2Glc\text{-}Rha\text{-}COO^{-}]^{+}$ and 455.4 $[M^{-}\text{-}2Glc\text{-}Rha\text{-}GlcA]^{+}$.[24,29] On acid hydrolysis, compound **V** liberated oleanolic acid as the genin which was identified with an authentic sample by Co-TLC, IR and NMR. Also, the sugar moieties were D-glucuronic acid, D-glucose and L-rhamnose while alkaline hydrolysis of compound **V** gave glucose and rhamnose.[25,30] In ^{13}C-NMR spectrum (Table 4), the glycosidation shifts were observed at C-3 and C-28 of the aglycone at δ 88.93 and 176.49 respectively[24,30] indicating that the two sugar chains were attached to these positions. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **V** showed signals for four anomeric protons at δ 4.39, 4.45, 5.14 and 5.21and four anomeric carbons (Table 5) at δ 104.30, 104.71, 101.40 and 94.87 respectively.[29,30] The interglycosidic linkages between the sugar moiety was established by ^{13}C-NMR spectrum (Table 5) where C-2 and C-3 of glucuronic acid were shifted at downfield δ 78.41 and 82.15 indicating the two carbons were positions of attachments of the terminal sugar units, glucose and rhamnose.[28-30] Thus the structure of compound **V** was assignated as 3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucuronopyranosyl oleanolic acid-28-O-β-D-glucopyranoside.

Compound VI was obtained from alkaline hydrolysis of compound **V**. Its ^{13}C-NMR spectrum (Table 4 and 5) showed 42 signals, of which 30 were assigned to a triterpenoid moiety and 12 to a saccharide moiety.[27,29] IR spectrum of this saponin reflected the characteristic band of carboxylic group at 1695.5 cm^{-1} as well as bands for hydroxyl group at 3419.5 cm^{-1} and glycosidic linkage at 1078.2 cm^{-1}.[24,29] This was supported by the resonances of C-3 and C-28 of the aglycone part of this saponin in ^{13}C-NMR spectrum (Table 4) at δ 88.50 and at δ 179.80 which reflected the fact that the sugar chain was linked to C-3 only.[28,29] The ^{1}H and ^{13}C-NMR spectra of this compound exhibited two anomeric protons located at δ 4.43 and 4.35 and two anomeric carbons (Table 5) at δ 104.20 and 105.30.[24,27] These data confirmed the sugar chain consists of two sugar units and this was supported by fragmentation pattern of this compound in CI-MS at m/z 793.5 [M+H]$^{+}$, 749.1 $[M\text{-}COO^{-}]^{+}$, 631.2 $[M+H\text{-}Glc]^{+}$, 587.2 $[M+H\text{-}Glc\text{-}COO^{-}]^{+}$ and 455.2 [M+H-Glc-

GlcA]. From the ^{13}C-NMR spectrum (Table 5) it was noticed that the C-3 of glucuronic acid was shifted at downfield δ 82.20 indicating that this carbon is the position of interglycosidic linkage between the sugar units.[24-27] From the above data the structure of compound **VI** was deduced as 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl oleanolic acid.

Compound VII, showed a molecular peak in its CI-MS spectrum at m/z 955.6 $[M+H]^+$. The predominant fragments at m/z 911.7 $[M+H-COO^-]^+$, 793.9 $[M + H-Glc]^+$ and 749.7 $[M+H-Glc-COO^-]^+$ suggesting the subsequent loss of a glucose unit with and without the ester glycosidic linkage. Other fragments at m/z 705.8 $[M+H-Glc- 2COO^-]^+$, 648.0 $[M+H-Glc-Rha]^+$ and 485.4 $[M+H-2Glc-Rha]^+$ reflected the lossing of two glucose units and one rhamnose unit.[34] This was confirmed by presence of three anomeric protons in ^{1}H-NMR spectrum at δ 4.65, 4.70 and 4.85 as well as appearing of three anomeric carbon atoms at δ 101.30, 104.98 and 94.76 in ^{13}C-NMR spectrum as shown in Table 5.[35,36] Acid hydrolysis of compound **VII** yielded glucose and rhamnose as sugar moiety. A comparison of ^{13}C-NMR spectrum of compound **VII** (Table 4) with the literature[34,36] revealed that this compound has quinovic acid as the aglycone part with two glycosidation sides at C-3 and C-28.[35] The first glycosidation side was confirmed by shifting of C-3 at downfield δ 88.53 in the ^{13}C-NMR spectrum (Table 4) compared with the same carbon of quinovic acid which appeared at δ 79.96.[35,36] Also, the other side was settled by shifting of C-28 at downfield δ 175.96 as well as by the upfield shift of the anomeric carbon of one glucose unit at δ 94.76 (Table 5). Its IR spectrum showed the characteristic bands of the hydroxyl groups at 3387.9 cm^{-1}, ester group at 1734.3 cm^{-1} and the glycosidic linkage at 1072.7 cm^{-1}.[34,35] The interglycosic linkages of the saccharide units was confirmed by the downfield shift of C-3 of the inner rhamnose unit in ^{13}C-NMR (Table 5) at δ 83.59.[32-34] Therefore compound **VII** was identified as 3-O-β-D-glucopyranosyl (1→3) α-L-rhamnopyranosyl-quinovic acid-28-β-D-glucopyranoside.

Compound VIII, exhibited an ester group absorption at 1732.8 cm^{-1} and hydroxyl group at 3422.2 cm^{-1} in its IR spectrum.[26,34] CI-MS investigation of the molecular ion afforded intense daughter ions at m/z 925.8 $[M-Glc]^+$, 880.4 $[M-Glc-COO^-]^+$, 749.6 $[M-Glc-Xyl-COO^-]^+$, 719.8 $[M-2Glc-COO^-]^+$, 618.7$[M-Glc-Xyl-GlcA]^+$, 587.5 $[M-2Glc-COO^--Xyl]^+$, 455.4 $[M^+-2Glc-Xyl-GlcA]^+$ and 411 $[M-2Glc-Xyl-GlcA-COO^-]^+$. These data suggested that compound **VIII** consisted of an aglycone with a molecular weight of 455 bound glycosidically through an acyl unit to a sugar chain consisting of one glucosyl unit, a second sugar chain consisting of one xylose, one glucose and one glucuronic acid bound O-glycosidically.[25-27] This was supported by alkaline hydrolysis which gave D-glucose only as sugar moiety while on acid hydrolysis of compound **VIII** yielded D-glucose, D-xylose and D-glucuronic acid as sugar moiety. ^{1}H and ^{13}C-NMR (Table 5) spectra showed four signals for anomeric protons (δ 4.69, 4.80, 5.15 and 5.20) and four anomeric carbons (δ 103.34, 104.29, 104.72 and 94.80).[32,35] This confirmed that compound **VIII** consisted of four sugar moieties. The ^{13}C-NMR spectrum of compound **VIII** showed 53 carbon signals, from which 30 were assigned, to the aglycone part, 23 to the saccharide moiety. The interglycosidic linkages were determined by observing a downfield shift of C-2 and C-3 of inner glucuronic acid in ^{13}C-NMR (Table 5) at δ 78.60 and 82.91, this proved that the terminal D-glucose and D-xylose were linked at these carbons. From these results compound **VIII** was established as 3-O-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-oleanolic acid-28-O-β-D- glucopyranoside.

Bioassay screening

Screening of the isolated compounds **I-VIII** as molluscicidal agent against *B. alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt exhibited that only monodesmosidic saponins **III** and **VI** have high activities (LC_{90}= 7 and 6 ppm) for the two compounds respectively within 24 hours exposure whereas all other compounds (bidesmosidic saponins and flavonoids glycosides) did not show any activity up to 50 ppm. From these results it appeared that the leaves and stems of *O. reticulatum* have a lot of

inactive bidesmosidic triterpenoidal saponins such as saponins **II, V, VII** and **VIII.** This bidesmosidic saponin content probably hydrolyze to active monodesmosidic saponins when the water suspension of the dry plant powder was molluscicidally tested.[8] This result is in full agreement with the previous Hosttetmann *et al.*, studies**.**[3,4] They reported that bidesmosidic triterpenoidal saponins (have two sugar chains at C-3 and C-28 of the aglycone part) are molluscicidally inactive against different snail species while the monodesmosidic triterpenoidal saponins (have one sugar chain at C-3 of the aglycone part) showed strong potencies against snails. This finding also supports the previous conclusion that explained the absence of any molluscicidal effect of all extracts of this plant.[8] In this study, it is also evident that the two flavonoid compounds **I** and **IV** gave negative results in the bioassay test against the snails. This is in good accordance with previous reports on most flavonoid compounds as they are molluscicidally inactive.[7]

REFERENCES

1- E. K. Markel, M. Voge and D. T. John, Control of Schistosomiasis in: Medical parasitology 6ed. Saunder W. B. Co. (ed) p. 169 (1986).
2- A. Marston, M. Maillard and K. Hostettmann, J. Ethnopharmacol., 38, 215-223 (1993).
3- K. Hostettmann and J. L. Wolfender, Pestic. Sci., 51, 471-482 (1997).
4- S. Y. Liu, F. Sporer, M. Wink, J. Jourdane, R. Henning, Y. L. Li and A. Ruppel, Tropical Medicine and International Health 2, 179-188 (1997).
5- K. Hostettmann, H. Kizu and T. Tomimori, Planta Medica 44, 34-35 (1982).
6- M. M. Abdel-Gawad, M.M. El-Sayed and E. S. Abdel-Hameed, Fitoterapia 70, 371-381 (1999).
7- H. Kloos and F. S. McCullough, Plant molluscicide (Mott, K. E., ed.), pp. 45-108, John Wiley, Chichester (1987).
8- H. H. El-Nahas, M. M. Abdel-Gawad, M. M. El-Sayed and E. S. Abdel-Hameed, Egypt. J. Biomed. Sci., 9, 28-40 (2002).
9- F. R. Malek, T. Miyase, S. M. Abdel-Khalik, M. H. Hetta and I. I Mahmoud, Phytochemistry 60, 185-195 (2002).
10- H. A. Shoeb, I. F. Zeid, L. A. Refahy and A. M. Kalifa, Az. J. Pharm. Sci., 13, 33-40 (1994).
11- R. A. Johnsonand and J. B. Devenpart. Biochemistry and Methodology of Lipids. A division of John Wiley and Sons-Inc New York (1971).
12- A. S. El-Kashoury, Zag. J. Pharm., 3, 116-119 (1994).
13- T. I. Khalifa, H. A. Ammer, H. A. Azizi and M. Hosny, Az. J. Nat. Prod., 3, 23-49 (1989).
14- WHO. Molluscicide screening and evaluation. Bull. Wld. Hlth. Org. 33, 567-581 (1965).
15- J. T. Litchfield and F. E. Wilcoxon, J. Pharm. Exp. Ther., 96, 99-113 (1949).
16- S. M. Abdel-Wahab, M. A. Selim and N. M. El-Fiki, Bull. Fac. Pharm. Cairo Univ., 28, 67-70 (1990).
17- A. H. Abou-Zeid, N. E. Awad and M. M. El-Missiry, Egypt J. Pharm. Sci., 35, 445-452 (1994).
18- T. T. Mabry, K. R. Markham and M. B. Thomas, The Systematic Identification of Flavonoids. Springer Verlag, New York (1970).
19- O. Barbera, J. F. Sanz, N. Sanchez, J. Parareda and J. A. Marco, Phytochemistry 33, 2360-2365 (1986).
20- F. De Simon, A. Dini, C. Pizza, P. Satwnimo and O. Schettino, Phytochemistry 29, 3690-3692 (1990).
21- K. R. Markham, B. Ternai, R. Stanely, H. Geiger and T. J. Mabry, Tetrahedron 34, 1389-1397 (1978).
22- A. Sakushima and S. Nishibe, Phytochemistry 27, 915-919 (1988).
23- L. Rastrelli, P. Santurnino, O. Schettino and A. Dini., J. Agric. Food Chem., 43, 2020-2024 (1995).
24- B. Acebes, M. Bernabe, A. D. Diaz-Lanza and C. Bartolome, J. Nat. Prod., 61, 1557-1559 (1998).
25- Z. Jia, K. Koike and T. Nikaido, J. Nat. Prod., 61, 1368-1373 (1998).
26- V. D. Huan, S. Yamamura, K. Ohtani, R. Kasai, K. Yamasaki, N. T. Nham, and H. M. Chau, Phytochemistry 47, 451-457 (1998).

27- G. Carvalho, M. Carvalho and R. Braz-Filho, Phytochemistry 52, 1617-1619 (1999).
28- A. Marouf, S. Desbene, T. C. Khanh, H. Wagner, M. Correia, B. Chauffert and M. A. Lacaille-Dubois, Pharmaceutical Biology 39, 263-267 (2001).
29- S. M. Abdel-Khalik, T. Miyase, H. A. El-Ashaal and F. R. Melek, Phytochemistry 54, 853-859 (2000).
30- E. Stahl, Chem. Ztg., 82, 323-327 (1958).
31- J. Tian, F. Wu, M. Qiu and R. L. Nie, Phytochemistry 32, 1539-1542 (1993).
32- K. Xiao, Y. H. Yi, Z. Wang, H. Tang, Y. Li and H. W. Lin, J. Nat. Prod., 62, 1030-1032 (1999).
33- S. Yahara, M. Kohjyouma and H. Kohoda, Phytochemistry 53, 469-471 (2000).
34- R. Aquino, N. De Tommasi, F. De Simone and C. Pizza, Phytochemistry 54, 1035-1040 (1997).
35- G. Fan and Z. He, Phytochemistry 44, 1139-1143 (1997).
36- H. A. Hassanean, M. A. El-Hammouly, S. A. El-Moghazy and D. W. Bishay, Phytochemistry 33, 667-670 (1993).