Zagazig J. Pharm. Sci., June 1998
Vol. 7, No. 1, pp. 73-79

ISSN 1110-5089

# MOLLUSCICIDAL STEROIDAL SAPONINS FROM *AGAVE FEROX*

**Mortada M. El-Sayed**
Laboratory of Medicinal Chemistry, Theodor Bilharz Institute,
Imbaba, Giza - Egypt

## ABSTRACT

Four steroidal saponins were isolated from the methanol extract of the leaves of *Agave ferox*. On the basis of chemical and spectroscopic evidence, the structures of these saponins were established as yamogenin 3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranoside, yamogenin 3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, gentrogenin 3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and gentrogenin 3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. The four saponins were found to possess various molluscicidal activities against *Biomphalaria alexandrina* snails; the intermediate host of *Schistosoma mansoni* in Egypt.

## INTRODUCTION

Schistosomiasis is a parasitic disease caused by threadworms of the genus *Schistosoma* and is endemic throughout South America, Africa and the far East. Several ways to solve the problem of this disease is to destroy the carrier snails and thus remove a link in its life cycle. This may be achieved with the aid of synthetic molluscicides such as Bayluscide or alternatively with molluscicides from plant sources[1-3].

*Agave species* is a very common ornamental plant in Egypt and other tropical countries. Previous phytochemical studies of these species have demonstrated the presence of flavonoids and steroidal saponins [4-9]. It has been reported that, saponins of certain members of the Agavaceae are highly toxic to aquatic snails [10-11]. Also, in previous study, the aqueous suspension of the leaves of *Agave ferox* showed a considerable activity against *Biomphalaria alexandrina* and *Lymnaea cailliaudi* snails, the intermediate hosts of *Schistosoma mansonia* and *Fasciola gigantica* in Egypt, respectively [12]. Meanwhile, to our knowledge, no work has been reported on the saponins of the leaves of this plant. Therefore, our attention was drawn to isolate and structure elucidation of the molluscicidal saponins of *Agave ferox*.

## EXPERIMENTAL

### General :

Melting points were uncorrected ; IR spectra were measured on a Perkin - Elmer model FT-IR recording spectrophotometer. $^{1}H$-NMR and $^{13}C$-NMR spectra were recorded at 270 and 100 MHz respectively in DMSO-$d_6$ as solvent and TMS as internal standard. Mass spectra were measured on a finnigan TSQ 700 GC/MC equipped with a finnigan electrospray source (EI-MS and CI-MS). Column chromatography was carried out on silica gel 60 (Sigma 28 - 200 mesh) . TLC was performed on silica gel plates (Merck, Kieselgel 60 GF 254, 0.5 mm) . Spots were visualized by spraying with 40% $H_2SO_4$ followed by heating at 120°C . Paper chromatography were performed on Whatman paper No.1, using descending technique and visualised with aniline phthalate .

### Plant material :

The fresh leaves of *Agave ferox* Koch. (Family Agavaceae) were collected in June 1995 from the Botanical Orman Garden , Giza , Egypt . The plant was kindly authenticated by Eng. Badia H. Diwan (Agriculture engineer of Orman Garden) . The leaves were shade dried and powdered by electrical mill.

### Extraction and Isolation:

Fresh leaves of *Agave ferox* Koch(2.5 kg) were extracted with methanol (15 L) . The methanolic extract after removal of the solvent under reduced pressure (112 g) was defatted with petroleum ether (3 L) . The defatted residue (95 g) was partitioned between n-butanol and water. The butanolic extract (40 g) was subjected to column chromatography. Elution was started with $CHCl_3$ and the polarity was increased by adding a gradient mixtures of $CHCl_3$ : MeOH and finally pure methanol. Fractions with the same TLC profile were combined to yield four major fractions I-IV .

Fractions eluted with Chloroform - Methanol 90 : 10 and 80 : 20 respectively gave saponins 1 and 2. These saponins were purified on a sephadex LH -20 column with methanol as eluent followed by recrystallization with methanol. Fractions eluted with chloroform- methanol 70 : 30 and 60: 40 respectively were further purified on prep . TLC using solvent system $CHCl_3$ : MeOH: $H_2O$ (13: 6:1) to yield saponins 3 and 4.

**Saponin 1:**

An amorphous powder, m p 250 - 252°C , $R_f$ 0.62 ($CHCl_3$ MeOH $H_2O$; 13 : 6 : 1) . IR (KBr) $cm^{-1}$ 3412 (OH) , 2928 (CH), 1642, 1457, 1371, 1261,1071,980, 919 and 895 (intensity 919>895, 25S-spiroketal). $^1$H-NMR δ 0.70 (3H , s, Me-18), 0.83 (3H, d, Me-27), 0.94 (3H, s, Me-19), and 1.14 (3H, d, Me-21), 1.60 (3H , d, Rha-Me), 4.82 (1H, d, Ara, H-1), 4.96 (1H, d, Glc, H-1), 5.07 (1H,d, Rha , H-1) and 5.32 (1H,s, H-6) CI-MS, m/z 854 ($M^+$+H), 722 ($M^+$-Ara), 708 ($M^+$ Rha), 575 ($M^+$-Ara- Rha) and 413 ($M^+$-Ara -Rha - Glc)

**Saponin 2 :**

White powder , m.p 230-232°C , $R_f$ 0.61 ($CHCl_3$ : MeOH $H_2O$, 13 : 6: 1) . IR (KBr) $cm^{-1}$ 3404 (OH), 2930 (CH), 1656, 1462, 1347, 1262, 1072, 975 , 917, 900 and 875 ( intensity of 917 > 900 ; 25S- spiroketal). $^1$H-NMR δ 0.70 (3H,s, Me-18), 0.81 (3H, d, Me-27), 0.93 (3H, s, Me-19), 1.13 (3H, d, Me-21), 1.58 (3H, d, Rha-$CH_3$), 4.80 (1H, d, Glc, H-1), 4.94, (1H, d Glc, H-1) , 5.12 (1H, d, Rha, H-1) and 5.31 (1H, s, H-6). CI-MS. m/z 884 ($m^+$+H), 737 ($M^+$-Rha), 721 ($M^+$-Glc), 575 ($M^+$-Rha-Glc) and 413 ($M^+$-Rha - 2 x Glc).

**Saponin 3:**

White powder , m.p 245-246°C , $R_f$ 0.52 ($CHCl_3$ : MeOH $H_2O$; 13 : 6: 1) IR (KBr) $cm^{-1}$ : 3399 (OH), 2928 (CH), 1704 (C=O), 1656, 1441, 1347, 1065 and 985, 918, 900 and 870 (intensity 900 > 918, 25 R-spiroketal) . $^1$H -NMR δ 0.71 (3H, d, Me-27), 0.91( 3H, s, Me-19) , 1.01 (3H, s, Me-18) 1.13 (3H, d, Me-21), 4.75 (1H, d, Gal, H-1) , 4.81 (1H, d, Ara , H-1), 4.96 (1H, d, Glc, H-1), 5.09 (1H, d, Rha, H-1) and 5.30 (1H,s, H-6). CI-MS : m/z 1033 ($M^+$+H) , 899 ($M^+$-Ara), 885 ($M^+$-Rha), 753 ($M^+$-Ara -Rha) 591 ($M^+$- Ara - Rha-Glc) and 429 ($M^+$-Ara -Rha-Glc-Gal).

**Saponin 4 :**

An amorphous powder, m.p. 261 - 265°C with $R_f$ 0.44 ($CHCl_3$: MeOH : $H_2O$; 13 : 6: 1). IR KBr) $cm^{-1}$ 3406 (OH), 2927 (CH), 1706 (C=O), 1344, 1261, 1069,982, 920,898 and 863 (intesity 898 > 920 ; 25 R - spiroketal). $^1$H-NMR δ 0.69 (3H, d, Me-27), 0.90 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.12 (3H, d, Me-21), 1.60 (3H, d, Rha-Me), 4.77 (1H, d, Gal, H-1), 4.84 (1H, d, Glc, H-1), 4.98 (1H, d, Glc, H-1), 5.12 (1H, d, Rha, H-1) and 5.32 (1H, s, H-6). CI-MS , m/z 1061 ($M^+$+H) , 915 ($M^+$-Rha), 898 ($M^+$ - Glc) , 753 ($M^+$-Rha- Glc), 591($M^+$- Rha-2x Glc). and 429 (M-Rha-2 x Glc- Gal).

**Acid hydrolysis :**

Each saponin (15 mg) was refluxed with 2N HCl-MeOH (1:1, 25 ml) for 5 hours. The reaction mixture was diluted with water and extracted with chloroform. The chloroformic extract was evaporated to dryness and the aglycone part in each case was identified by TLC analysis with authentic samples using solvent system $C_6H_6$: EtOAc; 80 : 20 . The aqueous layer was neutralized with $NaHCO_3$, filtered and concentrated under reduced pressure. The residue was extracted with pyridine and filtered. The pyridine extract was concentrated and dissolved in 10% isopropanol and examined by PC using solvent system n-BuOH : AcOH : $H_2O$; 4 : 1: 5 against sugar samples.

**Saponin 1 and 2 :**

Yielded yamogenin , m.p 202 - 203°C [Lit. m.p 201°C] [13,14] with $R_f$ 0.36 ($C_6H_6$: EtOAc; 80 : 20 ) ; IR (KBr) $cm^{-1}$ 3400 (OH), 2927, 1650, 1400, 1380, 980, 919, 899, 804 (intensity 919> 899; 25 S-spiroketal). CI-MS m/z 414 ($M^+$+H) , 397 ($M^+$-$H_2O$), 355, 345, 300 and 139.

**Saponin 3 and 4 :**

Yielded gentrogenin , m.p 214-216°C (Lit. 217-219°C)[15]. CI-MS m/z 429 ($M^+$+H) , 410, 314, 296, 176, 139 and 126. IR (KBr) $cm^{-1}$ 3399 (OH), 2928, 1704 (C=O) , 980, 920, 900, 865 (intensity 900 > 920 25 R - spiroketal).

## Molluscicidal assay :

*Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt were collected from the irrigation canals in Abou Rawash, Giza Governorate. The snails were maintained in dechlorinated tap water in the laboratory conditions (temp 25 ± 2°C and pH 7-7.7) . Tests were performed in duplicate using ten snails for each test. Different dilutions of each saponin were prepared with dechlorinated tap water. The snails were exposed to the prepared dilutions for 24 hours follwed by 24 hours in dechlorinated tap water as recovery period. Procedures and statistical analysis of the data were carried out according to the WHO as well as Litchfield and Wilcoxon protocols [16-18].

## RESULTS AND DISCUSSION

The fresh leaves of *Agave ferox* were extracted with methanol. The methanolic extract was defatted with petroleum ether and the defatted part was partitoned between n-butanol and water. The butanol soluble - phase was fractionated through the combined use of repeated column chromatography on silica gel, sephadex and preparative TLC to furnish four saponins.

Zagazig J. Pharm. Sci., June 1998
Vol. 7, No. 1, pp. 73-79

**Table 1:** $^{13}C$-NMR spectral data for aglycone moieties of saponins 1-4 in DMSO-$d_6$

| Carbons | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 37.1 | 37.1 | 36.9 | 36.7 |
| 2 | 29.8 | 30.1 | 29.9 | 30.0 |
| 3 | 77.8 | 78.0 | 77.6 | 77.9 |
| 4 | 38.8 | 39.0 | 39.1 | 38.7 |
| 5 | 140.5 | 140.3 | 141.7 | 141.4 |
| 6 | 121.6 | 121.4 | 121.2 | 121.5 |
| 7 | 32.1 | 32.2 | 31.4 | 31.5 |
| 8 | 31.4 | 31.7 | 30.7 | 30.6 |
| 9 | 49.7 | 49.9 | 51.9 | 51.8 |
| 10 | 36.9 | 36.7 | 37.4 | 37.1 |
| 11 | 21.0 | 21.2 | 37.3 | 37.0 |
| 12 | 39.6 | 39.4 | - | - |
| 13 | 40.1 | 40.2 | 54.8 | 54.9 |
| 14 | 56.2 | 56.5 | 55.6 | 55.8 |
| 15 | 31.9 | 31.6 | 31.8 | 31.4 |
| 16 | 80.7 | 81.1 | 79.6 | 79.3 |
| 17 | 62.8 | 62.9 | 53.9 | 53.7 |
| 18 | 16.1 | 16.2 | 15.1 | 15.4 |
| 19 | 19.2 | 19.1 | 18.4 | 18.2 |
| 20 | 41.8 | 41.9 | 42.1 | 42.4 |
| 21 | 14.9 | 14.7 | 13.8 | 13.6 |
| 22 | 108.7 | 108.9 | 108.7 | 108.8 |
| 23 | 31.2 | 31.5 | 31.1 | 31.3 |
| 24 | 28.9 | 28.6 | 29.1 | 29.0 |
| 25 | 30.1 | 30.0 | 30.3 | 30.2 |
| 26 | 66.1 | 66.2 | 66.1 | 66.3 |
| 27 | 16.9 | 17.1 | 17.0 | 16.8 |

**Saponin 1** was obtained as an amorphous powder. The glucosidic nature of saponin 1 was suggested by the strong absorption peaks at 3412 and 1071 $cm^{-1}$ in its IR spectrum (19,20). The $^{1}H$-NMR spectrum of saponin 1 showed singals for four typical steroid methyl groups, two of them appeared as singlets at δ 0.70 and 0.94 and the other two as doublets at δ 0.83 and 1.14 (21-23). The signal at δ 1.60 was due to the methyl group of 6-deoxyhexopyranose. Also, an olefinic proton and three anomeric proton signals were noted at δ 5.32, 4.82, 4.96 and 5.01 respectively (23,24). This was supported by presence of three anomeric carbon signals at δ 100.6, 102.4, 103.9 as well as the two signals corresponding to olefinic carbons at 140.5 and 121.6 ppm in the $^{13}C$-NMR spectrum (23,24).

The fundamental steroid structure of saponin 1 based upon (25 S) - spirostanol. This was suggested by presence of a quaternary carbon signal at δ 108.7 which was assignable to C-22 of the spirostanol skeleton in the $^{13}C$-NMR spectrum and by appearing the characteristic bands in IR spectrum at 980, 919, 895 $cm^{-1}$ with the absorption at 919 $cm^{-1}$ being of greater intensity than that at 895 $cm^{-1}$ (19-22). CI-MS of saponin 1 showed a molecular ion peak at m/z 854 ($M^{+}$+H). Fragments at m/z 722 ($M^{+}$-Ara), 708 ($M^{+}$-Rha) and 575 ($M^{+}$-Ara-Rha) indicated that the arabinosyl and rhamnosyl units are terminals and the glucose moiety is directly linked to the aglycone (yamogenin) at C-3 (22-24). This was supported by presence of the fragment ion at m/z 413 ($M^{+}$- Ara - Rha - Glc) and by $^{13}C$-NMR spectrum, C-3 of the aglycone part appeared at δ 77.8 whereas the chemical shift of this C-atom was at δ 71.7 in yamogenin (25).

In the $^{13}C$-NMR spectrum, the signals due to C-2 and C-3 of the inner glucose were shifted downfield at δ 79.5 and 86.7 indicating that the two carbon atom positions are the positions of the glycosidation linkages for the arabinosyl and rhamnosyl units (21-24). Saponin 1 showed high molluscicidal activity against *Biomphalaria alexendrina* snails ($LC_{90}$=9 ppm) (Table 3) indicating that this saponin was monodesmosidic type (26).

Acid hydrolysis of saponin 1 gave yamogenin as aglycone together with L-arabinose, L-rhamnose and D-glucose as sugar moieties. The aglycone part was identified by comparison of its mass fragmentation 414, 397, 355, 300 and 139 and its $^{13}C$-NMR spectrum with the reported data (13,25). Also, the sugar residue was identified by comparison with authentic sugars on PC using solvent system n- BuOH: AcOH: $H_2O$; (4 : 1 : 5). From these data, the structure of saponin 1 was elucidated as yamogenin 3-O-α - L- rhamnopyranosyl -(1→2) [α-L-arabinopyranosyl-(1→3)]- β -D- glucopyranoside.

**Saponin 2** showed a strong hydroxyl group absorption at 3404 as well as the characteristic bands of 25 (S) - spiroketal moiety at 975, 917, 900 and 875 with

the absorption at 917 $cm^{-1}$ being of greater intensity than that at 900 $cm^{-1}$ (19-22). This was supported by noting a quaternary carbon signal at δ 108.9 which was assignable to C-22 of the spirostanol skeleton of saponin 2 in the $^{13}C$-NMR spectrum (19-25). The $^{1}H$-NMR spectrum of this compound exhibited signal for an olefinic proton at δ 5.31, signals of three anomeric protons at δ 4.80, 4.94 and 5.12 and signals due to four typical steroid methyl, groups at δ 0.70, 0.81, 0.93 and 1.13. Also, the signal due to the methyl of 6-deoxyhexopyranose appeared at δ 1.58 ppm (22,23). This was confirmed by presence of three anomeric carbon signals at 101.4, 104.2 and 102.8 as well as two olefinic carbon signals at δ 140.3 and 121.4 (23,27,28).

CI-MS of saponin **2** exhibited a molecular ion peak at m/z 884 ($M^{+}$+H). Fragment ions at m/z 737 ($M^{+}$-Rha) and 721 ($M^{+}$-Glc) demonstrated that the rhamnosyl and the glucosyl units are terminals. Other fragments at m/z 575 ($M^{+}$- Rha- Glc) and 413 ($M^{+}$-Rha -2 x Glc) indicated that the inner glucose is directly attached to the aglycone moiety (23,25).

In the $^{13}C$-NMR spectrum, the signal of the aglycone part was identical to yamogenin except for C-3 which shifted downfield at δ 78.0 whereas the signal of this carbon in yamogenin appeared at δ 71.7 (25). This illustrated that the inner glucose must be attached to the hydroxyl at C-3 of yamogenin. Furthermore, the points of attachments of the sugar residue were determined by $^{13}C$-NMR spectrum; the signals of C-2 and C- 4 of the inner glucose were shifted downfield at δ 80.1 and 78.5 whereas its other carbon signals were almost unaffected. Therefore, C-2 and C-4 positions are the positions of the glycosidation linkages for both glucose and rhamnose units (23-28).

Molluscicidal test revealed that saponin **2** have a considerable activity ($LC_{90}$ = 13 ppm). From the above data, the structure of saponin 2 was formulated as yamogenin 3 - O -α - L- rhamnopyranosyl- (1→2) -[(β-D- glucopyranosyl-(1→4) ]-β- D- glucopyranoside.

**Saponin 3** was obtained as a white powder. Its IR spectrum showed a strong absorptions for hydroxyl groups at 3399 $cm^{-1}$, a carbonyl group on six - membered ring at 1704 $cm^{-1}$ as well as the characteristic bands of 25 R-spirostanol at 985, 918, 900 and 870 with intensity of 900 > 918 (19,29).

**Table (2):** $^{13}C$-NMR chemical shifts of sugar moieties of saponins **1-4** in DMSO-$d_6$.

| Carbons | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| | 1 | 100.6 | 101.4 | | |
| | 2 | 79.5 | 80.1 | | |
| Glc | 3 | 86.7 | 76.7 | | |
| | 4 | 70.1 | 78.5 | | |
| | 5 | 77.1 | 77.4 | | |
| | 6 | 62.3 | 62.8 | | |
| | 1 | | | 102.1 | 102.4 |
| | 2 | | | 73.2 | 73.4 |
| Gal | 3 | | | 74.8 | 75.0 |
| | 4 | | | 80.0 | 80.2 |
| | 5 | | | 75.1 | 75.2 |
| | 6 | | | 60.3 | 60.7 |
| | 1 | | 104.2 | 104.0 | 104.3 |
| | 2 | | 74.9 | 79.8 | 79.9 |
| Glc | 3 | | 77.6 | 87.3 | 87.1 |
| (1→4) | 4 | | 70.2 | 69.6 | 70.0 |
| | 5 | | 77.5 | 77.2 | 77.3 |
| | 6 | | 62.3 | 62.1 | 62.3 |
| | 1 | | | | 103.8 |
| | 2 | | | | 74.4 |
| Glc | 3 | | | | 77.3 |
| (1→3) | 4 | | | | 70.3 |
| | 5 | | | | 77.6 |
| | 6 | | | | 62.6 |
| | 1 | 102.4 | 102.8 | 102.5 | 102.2 |
| | 2 | 72.1 | 72.3 | 72.3 | 72.2 |
| Rha | 3 | 72.5 | 72.7 | 72.7 | 72.8 |
| (1→2) | 4 | 74.1 | 74.3 | 74.3 | 74.2 |
| | 5 | 69.2 | 69.4 | 69.4 | 69.5 |
| | 6 | 18.4 | 18.2 | 18.2 | 18.5 |
| | 1 | 103.9 | | 104.5 | |
| | 2 | 71.7 | | 72.1 | |
| Ara | 3 | 73.8 | | 74.0 | |
| (1→3) | 4 | 69.1 | | 69.3 | |
| | 5 | 67.2 | | 67.4 | |

Glc = β-D-glucopyranosyl  Gal = β-D-galactopyranosyl
Rha=α-L-rhamnopyranosyl  Ara=α-L-arabinopyranosyl

Zagazig J. Pharm. Sci., June 1998
Vol 7, No 1, pp. 73-79

In $^{1}$H-NMR spectrum of this compound, four anomeric proton signals were observed at δ 4.75, 4.81, 4.96 and 5.09 confirming the presence of four sugar units. Also, the signal due to olefinic proton appeared at δ 5.30 (15,30). This was supported by presence of four anomeric carbon signals at δ 102.1, 104.0 and 102.3 and 104.5 in $^{13}$C-NMR spectrum (15,30,31). Furthermore, two olefinic carbon signals were noted at δ 141.7 and 121.2 in the $^{13}$C-NMR spectrum as well as the characteristic signals of C-20, C-22 and C-27 at δ 42.1, 108.7 and 17.0 . This confirmed that the structure of the aglycone of this saponin is based on the 25 R-spirostanol skeleton (15,31).

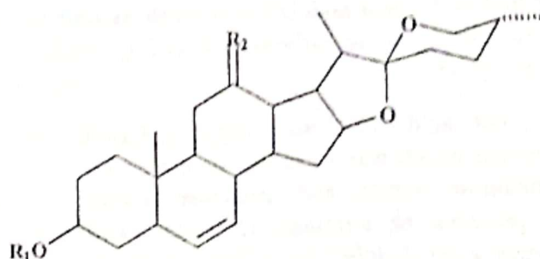
CI-MS of saponin **3** exhibited a molecular ion peak at m/z 1033 ($M^{+}$+H). Other fragment ions at m/z 899 ($M^{+}$-Ara), 885 ($M^{+}$-Rha) and 753 ($M^{+}$- Ara- Rha) indicated the loss of one arabinosyl and one rhamnosyl units and showing that two sugar units are terminals. The MS fragments at m/z 591 ($M^{+}$- Ara - Rha - Glc) and 429 ($M^{+}$- Ara- Rha- Glc-Gal) demonstrated that the galactose unit is directly attached to the aglycone and the sugar residue is a branched tetrasaccharide (29,30).

Acid hydrolysis of saponin **3**, it gave D-galactose, D-glucose, L-rhamnose and L-arabinose as well as gentrogenin. The aglycone part was confirmed by comparison of its mass fragmentation and $^{13}$C-NMR with those reported in the literature (15,30). The point of attachment of sugar residue to the aglycone was determined by $^{13}$C-NMR ; C-3 of the aglycone was shifted downfield at δ 77.6 while the signal of this carbon atom was at δ 71.4 in gentrogenin(15). This showed that the glycosidation linkage must be attached to the hydroxyl group at C-3 of gentrogenin. Also, in the $^{13}$C-NMR spectrum ; the signals of C-2 and C-3 of the inner glucose were shifted downfield at δ 79.8 and 87.3 ppm illustrated that the two carbon positions are positions of attachments of the terminals arabinosyl and rhamnosyl units to the inner glucose . Also, the signal of C- 4 of the galactose was shifted downfield at δ 80.0 suggesting that C-4 position was the glycosylated position to the inner glucose (30-31).

The results in Table 3 revealed that saponin 3 is active against *B. alexandrina* ($LC_{90}$ = 21 ppm). Accordingly, the structure of saponin 3 was elucidated as gentrogenin 3-O-α-L- rhamnopyranosyl -(1→2)-[α-L- arabinopyranosyl-(1→3) ]- β- D - glucopyranosyl-(1→4) - β-D- galactopyranoside.

**Table ( 3) :** Molluscicidal activities of saponins 1-4 against *Biomphalaria alexandrina* snails after 24 hour exposure period.

| Saponin | $LC_{50}$ | $LC_{90}$ | Slope function |
|---|---|---|---|
| 1 | 6<br>(4.689-8.739) | 9 | 1.21 |
| 2 | 10<br>(7.476-12.123) | 13 | 1.29 |
| 3 | 17<br>(13.219-20.101) | 21 | 1.31 |
| 4 | 28<br>(25.106-31.31) | 33 | 1.30 |




| Saponin | $R_1$ | $R_2$ |
|---|---|---|
| 1 | – Glc (1→3) Ara; (1→2) Rha | $H_2$ |
| 2 | – Glc (1→2) Rha; (1→4) Glc | $H_2$ |
| 3 | – Gal (1→4) Glc (1→2) Rha; (1→3) Ara | O |
| 4 | – Gal (1→4) Glc (1→2) Rha; (1→3) Glc | O |

**Saponin 4** was obtained as an amorphous powder. The glycosidic nature of this compound was inferred from the strong absorption bands at 3406 and 1069 $cm^{-1}$ in the IR spectrum. Also, the band due to a carbonyl on six-membered ring appeared at 1706 $cm^{-1}$. Its structure based upon a (25R) - spirostanol nature. This concluded from the IR spectrum (bands at 982, 920, 898 and 863, intensity 898 > 920), CI-MS (peaks at 139 and 115), and $^{13}$C-NMR (signal at δ 108.8 for a quaternary carbon atom C-22) (15,19).

The $^{1}$H-NMR spectrum of saponin 4 displayed signals of typical steroid methyls : two of them appeared as singlets at δ 0.90 and 1.02 for Me-19 and Me-18 whereas the other two as doublets at δ 0.69 and 1.12 for Me-27 and Me-21 respectively (27-30). Also, the signal due to methyl group of 6-deoxyhexopyranose appeared at δ 1.60, a signal of olefinic proton at δ 5.32 and signals due to four anomeric protons at δ 4.77, 4.84, 4.98 and 5.12 (30-32). This was supported by $^{13}$C-NMR spectrum ; the signals due to two olefinic carbon atoms appeared at δ 141.4 and 121.5 as well as four signals due to four anomeric carbons at δ 102.4, 104.3, 103.8 and 102.2 (15,31).

On acid hydrolysis, saponin 4 gave steroidal sapogenin which was identified as gentrogenin as well as rhamnose, glucose and galactose as sugar residue. The presence of terminals rhamnosyl and glucosyl moieties were deduced from appearance of the three fragments ions at m/z 915 ($M^+$-Rha), 898 ($M^+$-Glc) and 753 ($M^+$-Rha - Glc) in CI-MS spectrum. Also, fragments at m/z 591 ($M^+$-Rha - 2x Glc) and 429 ($M^+$-Rha-2 x Glc - Gal) demonstrated that the inner glucose in directly attached to galactose unit which is linked to the aglycone part (gentrogenin) (15,31,32).

The tetrasaccharide was deduced to be linked to the C-3 hydroxyl position of the aglycone part because, the signal due to C-3 in $^{13}$C-NMR was shifted downfield at δ 77.9 whereas the signal of this carbon atom in gentrogenin was at δ 71.4 (15). Also, it was observed that the signal of C-4 of the galactose was shifted downfield at δ 80.2 suggesting that C-4 position of galactose was the glycosylated position of the inner glucose.

Furthermore, the signals of C-2 and C-3 of the inner glucose were shifted downfield at δ 79.9 and 87.1. This demonstrated that the two terminal sugars; rhamnose and glucose are linked at C-2 and C-3 positions of the inner glucose (30-32). Results in table 3 showed that saponin 4 have molluscicidal activity against *B. alexandrina* snails ($LC_{90}$ = 33ppm). From the data presented above, full structure of saponin 4 was established as gentrogenin 3-O-α-L-rhamnopyranosyl - (1→2)-[β-D- glucopyranosyl (1→3)] -β-D- glucopyranosyl - (1→4)-β-D -galactopyranoside.

## REFERENCES


1-Hostettmann, K. and Wolfender, J. L.; **Pest. Sci.** 51, 471-482 (1997).

2-Kloos, H. and McCullough, F. S. ; **Planta Medica** 46, 195 - 209 (1982).

3-Lemma, A. ; **Ethiopian Med. J.** 3, 84 (1965).

4-Sati, O. P. and Pant, G.; **J. Nat. Products** 48, 395-399, (1985).

5-Jain, D. C.; **Phytochemistry** 26, 1789-1790, (1987).

6-El-Sayed, M. M.; **Bull. Pharm. Sci. Assiut Univ. Egypt** 20, 147-153 (1997).

7- Sharma, C. S. and Sati, O. P. ; **Phytochemistry** 21, 1820-1821 ( 1982).

8-Shoeb, H. A. ; Tammam, G. H.; El-Amin, S. M. and Abdel- Motagally, M. Az. **J. Nat. Product** 8, 236-246 (1992.)

9-Uniyal, G. C. ; Agrawal, P. K., Thakur, R. S. and Sati, O. P.; **Phytochemistry** 29, 937-940 (1990).

10-Pant, G. , Sati, O. P., Miyahara, K. and Kawasaki, T.; **Phytochemistry** 25, 1491-1494 (1986).

11-Kishor, N. and Sati, O. P.; **J. Nat. Products** 53, 1557-1559 (1990.)

12-El-Sayed, M. M. and El-Nahas, A. H. ; **Bull. Pharm. Sci. Assiut University** 20,105-112 (1997).

13-Sati, O. P. and Pant, G.; **Phytochemistry** 24, 123-126 (1985).

14-Sati, O. P. and Pant, G.; **J. Nat Products** 48, 390-394 (1985).

15-Xing-Cong, L.; Yang, C. R.; Ichikawa, M.; Matsuura, H., Kasai, R. and Yamasaki, K.; **Phytochemistry** 31, 3559-3563 (1992).

16-WHO ; Export Committee on Bilharziasis Control methods WHO 65, 33, (1953).

17-WHO : WHO Monograph Ser 50, 124-138, (1965).

18-Litchfield, J. T. and Wilcoxon, F. **J. Pharm. Exp. Therap.** 96, 99-113 (1949).

19-Wall, M. E., Eddy, C. R., McChennan, M. L. and Klumpp, M. E. **Analyt. Chem.** 24, 1337-1341 (1952).

20-Takeda, K., Minato, H., Shimaoka, A. and Matsui, Y.; **J. Chem. Society** 4815 (1963).

21-Mimaki, Y., Ishibashi, N., Ori, K. and Sashida, Y.; **Phytochemistry** 31, 1753-1758 (1992).

22-Mimaki, Y.; Naksmura, O.; Sashida, Y., Nikaido, T. and Ohmoto, T.; **Phytochemistry** 38 , 1279-1286, (1995)

23-Ahmed, V. U., Baqai, F.T. ; Fatima, I. and Ahmed, R.; **Phytochemistry** 30 , 3057-3061 (1991).

24-Sashida, Y., Kubo, S., Mimaki, Y., Nikaido, T. and Ohmoto, T.; **Phytochemistry** 31, 2439-2443, (1992).

25-Liu, H. W. and Nakanishi , K. ; **Tetrahedron** 38, 513-519 (1982)

26-Marston, M. and Hostettmann, K.; **Phytochemistry** 24 , 639-652 (1985).

27-Gupta, R. K.; Jain, D. C. and Thakur, R.; **Phytochemistry** 24 , 2399-2401 (1985).

28-Aquino, R.; Behar, I.; Simone, F.; D'Agostino, M. and Pizza, C.; **J. Nat. Products** 49, 1096 - 1101 (1986).

29-Yong, J. and Jia, Z. J.; **Phytochemistry** 33, 1193-1995 (1993).

30-Xing-Cong, L.; Yong, C. R. ; Matsuura, H.; Kasai, R. and Yamasaki, K.; **Phytochemistry** 33 , 465-470 (1993).

31--Xing-Cong, L.; Wang, D. Z. and Yang, C. R. ; **Phytochemistry** 29 , 3893-3898 (1990).

32-Wang, Y. ; Ohtanik, L.; Kaai, R. and Yamasaki , K. , **Phytochemistry** 45, 811-817 (1997).

---

**Received : Nov. 16, 1997**
**Accepted : Dec. 24, 1997**

# صابونينات الاجاف فيروكس وتقييمهم كمبيدات لقواقع البلهارسيا

مرتضى محمد السيد

قسم الكيمياء العلاجية - معهد تيودور بلهارس للأبحاث

أمبابة - الجيزة - مصر

أمكن فصل أربع مركبات صابونينية من المستخلص الميثانولى لأوراق نبات الاجاف فيروكس . وقد تم تحديد التركيب الكيميائى لتلك الصابونينات بواسطة الطرق الكيميائية والتحليلات الطيفية المختلفة.

أظهرت الصابونينات المفصولة قدرة عالية عل ابادة قواقع بيمفولاريا الكسندرينا ( العائل الوسيط لطفيل البلهارسيا المعوية ) الموجودة فى مصر.