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# Molluscicidal Activity of Steroidal Saponins Isolated from *Agave angustifolia*

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**Abstract:** In the search for new molluscicidal plants for controlling the snail vectors of schistosomiasis, laboratory evaluation was made to assess the molluscicidal activity of *Agave angustifolia* plant against *Biomphalaria alexandrina* snails. Results indicated that the plant have promising molluscicidal activity as the $LC_{90}$ of the dry powder was 120 ppm. The chloroform extract was the most active extracts among other extracts. Chromatographic isolation of the active constituents of *A. angustifolia* led to isolation of three steroidal saponins. Their structures were elucidated via, IR (KBr) $\nu_{max}$ $cm^{-1}$, $^{1}H$, $^{13}C$-NMR (500 MHz) spectral analyses and acid hydrolysis to be identified as; Stigmasterol-3-*O*-β-D-glucopyranoside 1, Tigogenin-3-*O*-β-D-glucopyranosyl-($^{1}J_{3}$)-β-D-xylopyranoside 2 and Rhodeasapogenin-1-*O*-benzoyl-3-*O*-[($^{1}J_{2}$)-α-L-rhamnopyranosyl-($^{1}J_{3}$)]-α-L-rhamnopyranosyl-β-D- galactopyranoside 3. Among them compound 2 proved to be the most active one as molluscicidal agent against *B. alexandrina* snails, with $LC_{90}$ value of 61.4 ppm after 24 h exposure period.

**Key words:** *Agave angustifolia* · Schistosomiasis · Chromatographic Isolation · Steroidal Saponins

## INTRODUCTION

Schistosomiasis is a parasitic disease from which more than 300 million people suffer throughout South America, Africa and the Far East. The disease is a public health problem and is endemic in Egypt and many other areas of the world [1-4]. In poor countries where schistosomiasis is common, chemical control of the snails that serve as intermediate hosts for Schistosoma and Fasciola appears feasible and cost effective. The use of molluscicides to control the snail vector is considered the method of choice to eliminate schistosomiasis [5].

The use of plant molluscicides is simple, inexpensive and appropriate technology for focal control of the snail vectors [6]. Also, they are highly effective, rapidly biodegradable, readily available and easily applicable with simple techniques.

*Agave angustifolia* is a Paniculata Agave with a broad distribution in Mexico and other warm countries. Reviewing the current literature showed that the chemical constituents of *A. angustifolia* was poorly investigated. However numerous chemical compounds were isolated from other Agave species. Species of the genus Agave constitute an important source of steroidal sapogenins, mainly hecogenin [7, 8]. Agavenosides H-j and cholestane steroid agavegenin D were isolated from *A. americana* leaves [9]. Steroidal sapogenins, hecogenin and diosgenin were isolated and identified from *Agave intermixta* [10,11]. Two steroidal saponin, named dongnoside B and A were obtained from the dried leaf-juices of *A. sislana* [12].

In present study, three compounds were isolated from *A. angustifolia* through different chromatographic techniques. Spectroscopic analysis was carried out on these compounds and they were identified. They were subjected to bioassay screening against *B. alexandrina* snails.

## MATERIAL AND METHODS

**Plant Materials:** *Agave angustifolia* (Family: Agavaceae) was collected from El-Orman Botanical garden in Giza Governorate. The Plant was kindly identified by Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. It was shade dried and finally powdered with an electrical grinder. Voucher specimen of the plant was kept at Department of Medicinal Chemistry, Theodor Bilharz Research Institute.

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**Preparation of Plant Extracts:** The powdered leaves of *A. angustifolia* (1.5 Kg) were extracted with 80% aqueous methanol at room temperature for several times. The methanolic extract was evaporated to dryness, using a rotatory evaporator at reduced pressure to give 110 g of methanolic extract. This extract was successively extracted with petroleum ether, chloroform and ethyl acetate. The remaining residue (70 g) was partitioned between n-butanol and water. The n-butanolic layer was evaporated to dryness under pressure to yield (40 g).

**General Experimental Procedures:** $^{1}$H-NMR (500 MHZ, DMSO-$d_6$) and $^{13}$C-NMR (125 MHZ, DMSO-$d_6$) spectra were recorded on JEOL GX-500, Centre for Analysis and Synthesis, Lund University, Getingevgen Lund, Sweden. The chemical shifts were expressed in (ppm) with reference TMS and coupling constant (*J*) in Hertz. Infrared spectra were determined in Fourier Transform Infrared Spectrometer (FT/IR)-6100 JASCO, National Research Center (NRC) Giza-Egypt. Silica gel 60 GF254 (Fluka) was used for analytical TLC and silica gel (70-230 mesh, Merck) was used for column chromatography. Spots were visualized by absorption of UV radiation and spraying 40% $H_2SO_4$ followed by thermal activation.

**Material and Chemicals:** All solvents and reagents used were of analytical grade. All solvents and acids [petroleum ether, chloroform, methylene chloride, ethyl acetate, n-butanol, acetone, methanol, acetic acid and sulphuric acid], were purchased from (Sigma-Aldrich Co.).

**Chromatographic Isolation of Butanolic Extract:** The butanol soluble part from the methanolic extract of *A. angustifolia* (35 g) was subjected to open column chromatography (120 X 5 cm) packed with silica gel 60 (70-230 mesh, Merck) as stationary phase. Elution was started with pure chloroform followed by chloroform-methanol mixtures and ending with pure methanol. Each fraction was concentrated and examined on TLC and similar fractions were collected together. Elution with $CHCl_3$:$CH_3OH$ (95: 5) yielded compound 1, while elution with $CHCl_3$: $CH_3OH$ (90: 10) gave compound 2 and $CHCl_3$:$CH_3OH$ (70: 30) yielded compound 3.

**Acid Hydrolysis:** Each isolated compound (5 mg) was refluxed with 10% HCl (3.5 ml) in aqueous methanol at 100°C for 2 hrs., then the reaction mixture was concentrated under reduced pressure and diluted with water and extracted with chloroform [13,14]. The chloroform extract was evaporated to dryness and each aglycone was identified by comparison with authentic sample on TLC, using a solvent system; $C_6H_6$:$CH_3OH$ (80:20). The aqueous layers were neutralized with $NaHCO_3$, filtered and concentrated under reduced pressure. The residue obtained in each case was compared with standard sugars on PC using solvent system (n- BuOH: pyridine: $H_2O$; 10: 3: 3). Spots were detected by spraying with a solution of aniline phthalate.

**Molluscicidal Activity Tests:** The dry powder of the plant was first evaluated against *B. alexandrina* snails (8-10 mm) as aqueous suspensions. A serial of gradual concentrations was prepared from the dry powder and each compound on basis of weight /volume, using dechlorinated tap water and expressed in terms of ppm (part per million). Three replicates were prepared for each experimental concentration and for control group (each of 10 snails / L). Both exposure and recovery periods were 24 hrs each unless otherwise stated. Standard WHO procedures were followed [15] and determination of $LC_{50}$, $LC_{90}$ and slope function was carried out following Litchfield and Wilcoxon method [16]. At the end of the exposure period, snails were removed from the experimental concentrations, washed with dechlorinated tap water and transferred to another container with fresh dechlorinated water for 24 hrs (recovery period). At the end of this period, death of snails was defined by immersing snails in 15-20% sodium hydroxide solution in a Petri dish. If bubbles or blood comes out of the shell, it is recorded alive and if not, it is considered dead.

## RESULTS

**Compound 1**

***Stigmasterol-3-O-β-D-glucopyranoside:*** Yellowish powder $^{1}$H-NMR: 0.6-1.03, 5.08, 5.24, 5.33. EI-MS: 412 [M-Hexose sugar], $^{13}$C-NMR: Table (1).

**Compound 2**

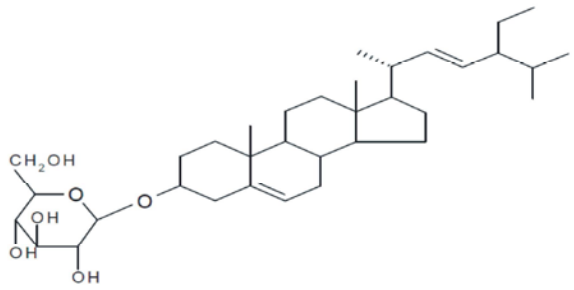
***Tigogenin-3-O-β-D-glucopyranosyl-($^{1}J_3$)-β-D-xylopyranoside:*** $^{1}$H-NMR: δ 0.75, 0.77, 0.85, 1.15, 4.84, 4.98. EI-MS: m/z 416 [M-(Glc+ xylose)], 399, 327, 273, 302, 287, 139 (100 %), 115, IR (KBr) $\nu_{max}$ cm$^{-1}$: 3397 cm$^{-1}$, 900, 897, 864 cm$^{-1}$, $^{13}$C-NMR: Table (1).

**Compound 3**

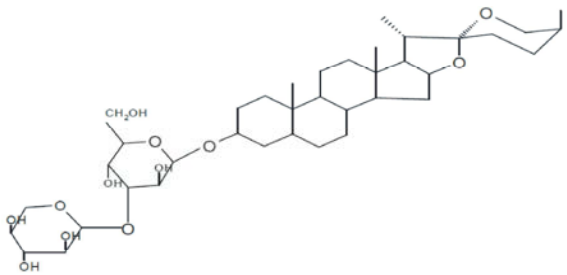
***Rhodeasapogenin-1-O-benzoyl-3-O-[($^{1}J_2$)-α-L-rhamnopyranosyl-($^{1}J_3$)]- α-L-rhamnopyranosyl-β-D-galactopyranoside:*** $^{1}$H-NMR: δ 8.45, 7.46, 0.71, 0.77, 0.90, 1.23, 4.76, 5.010, 5.60 cm$^{-1}$. EI-MS: 430, 397, 327, 289, 253. IR (KBr) $\nu_{max}$ cm$^{-1}$: 3420 cm-1, 982,919,895, 868, 899 cm$^{-1}$, 1720, 1640, 633 cm$^{-1}$, $^{13}$C-NMR: Table (1).

Table 1: $^{13}$C-NMR spectral data of compounds 1-3 (in DMSO-$d_6$, TMS as internal standard, in ppm, J in Hz)

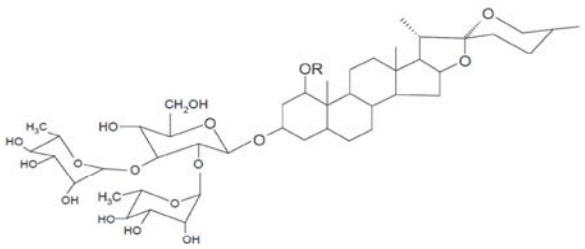
| | | | | Sugars | Compound 1 | Compound 2 | Compound 3 |
|---|---|---|---|---|---|---|---|
| Carbon No. | Compound 1 | Compound 2 | Compound 3 | Carbon No. | Glc | Glc | Gal |
| 1 | 38.6 | 37.50 | 79.35 | 1' | 100.77 | 99.30 | 101.50 |
| 2 | 31.41 | 30.66 | 31.30 | 2' | 73.45 | 75.40 | 76.52 |
| 3 | 76.76 | 78.69 | 77.50 | 3' | 76.76 | 78.77 | 84.99 |
| 4 | 45.13 | 34.56 | 35.28 | 4' | 70.03 | 73.55 | 69.40 |
| 5 | 140.37 | 44.80 | 28.90 | 5' | 76.90 | 76.68 | 76.38 |
| 6 | 121.17 | 28.77 | 27.70 | 6' | 62.50 | 61.34 | 61.32 |
| 7 | 31.41 | 33.25 | 26.50 | | | Xyl | Rha |
| 8 | 31.36 | 34.56 | 37.40 | 1" | | 104.67 | 101.90 |
| 9 | 49.59 | 53.03 | 44.03 | 2" | | 75.45 | 72.40 |
| 10 | 36.20 | 36.13 | 40.50 | 3" | | 78.69 | 73.51 |
| 11 | 20.91 | 20.46 | 29.78 | 4" | | 70.15 | 74.10 |
| 12 | 39.36 | 39.80 | 35.28 | 5" | | 65.81 | 70.50 |
| 13 | 41.84 | 40.20 | 41.50 | 6" | | ----- | 18.15 |
| Rha | | | | | | | |
| 14 | 56.70 | 55.45 | 55.59 | 1"' | | | 102.41 |
| 15 | 23.85 | 30.66 | 32.40 | 2"' | | | 72.70 |
| 16 | 28.70 | 80.44 | 80.95 | 3"' | | | 72.90 |
| 17 | 55.60 | 64.88 | 63.50 | 4"' | | | 73.80 |
| 18 | 12.50 | 16.40 | 16.16 | 5"' | | | 70.8 |
| 19 | 19.70 | 12.60 | 14.60 | 6"' | | | 17.80 |
| Benzoyl | | | | | | | |
| 20 | 39.19 | 40.70 | 41.60 | 1"" | | | 131.70 |
| 21 | 20.58 | 14.80 | 15.50 | 2"" | | | 101.50 |
| 22 | 138.20 | 109.03 | 108.34 | 3"" | | | 76.52 |
| 23 | 129.40 | 31.30 | 31.90 | 4"" | | | 84.99 |
| 24 | 49.59 | 28.77 | 29.50 | 5"" | | | 69.40 |
| 25 | 32.50 | 30.66 | 30.50 | 6"" | | | 76.38 |
| 26 | 18.83 | 65.81 | 65.88 | 7"" | | | 61.32 |
| 27 | 20.91 | 17.17 | 17.50 | | | | |
| 28 | 24.84 | ---- | ---- | | | | |
| 29 | 12.10 | ---- | ----- | | | | |




Compound 1: Stigmasterol-3-*O*-β-D-glucopyranoside




Compound 2: Tigogenin-3-*O*-β-D- glucopyranosyl ($^1J_3$)-β-D-xylopyranoside



Compound 3: Rhodeasapogenin-1-*O*-benzoyl -3-*O*-[($^1J_2$)-α-L-rhamnopyranosyl – ($^1J_3$)]α-L-rhamnopyranosyl-β-D-galactopyranoside.

## DISCUSSION

**Compound 1:** Compound 1 was obtained as yellowish powder, it responded positively to Liebermann-Burchard and Molish tests [17]. $^1$H-NMR data exhibited the characteristic signals of methyl groups between 0.65-1.03 and two olefinic protons δ 5.24, δ 5.08 [H-22 and H-23]. The characteristic signal of Δ5-sterol appeared at δ 5.33 [18-20]. It also showed anomeric proton at δ 4.40 ppm.

$^{13}$C-NMR spectrum displayed 35 carbon signals in which 29 identified for stigmasterol where it showed six methyl signals at 12.50, 19.70, 20.58, 18.83, 20.91 and 12.10 [C-18, C-19, C-21, C-26, C-27, C-29]. The olefinic carbon gives signals at 140.37, 121.17, 138.20 and 129.40 [C-5, C-6, C-22, C-23]. The remaining carbon signals represented glucose moiety, this indicated by the presence of signal at 100.77 of anomeric carbon [19-21]. From the previous data compound 1 was established as stigmasterol-3-*O*-β-D-glucopyranoside. Compound 1 did not show any molluscicidal activity against *B. alexandrina* snails up to 100 ppm.

**Compound 2:** Compound 2 was obtained as amorphous powder. It was positive to the Liebermann-Burchard reaction and Molish reagent [17] Its IR spectrum (KBr) $\nu_{max}$ cm$^{-1}$ exhibited a strong absorption band of hydroxyl group at 3397 cm$^{-1}$ and the characteristic absorption bands of spirostane F-ring at 900, 897 and 864 cm$^{-1}$ [22,23]. This was confirmed by presence of the characteristic signals of spiroketal structure in the $^{13}$C-NMR at 109.03 (C-22), 31.30 (C-23), 28.77 (C-24), 30.66 (C-25) and 65.81(C-26) [23-25]. The $^1$H-NMR spectrum of compound 2 exhibited the presence of four methyl group signals of the aglycone moiety at δ 0.75, 0.77, 0.85 and 1.15 as well as two anomeric proton signals of the sugar residue at δ 4.84 and 4.98 ppm [25, 26]. $^{13}$C-NMR spectrum showed presence of 27 carbon signals of the aglycone (Tigogenin), whereas the other carbon signals of the two sugar moieties (11 carbon signals) were assigned to glucose and xylose units. Also, the C-3 of the aglycone part was shifted at 78.69 as compared to the tigogenin C-3 appeared at 70.6 [24].This suggested that the sugar moiety was attached at C-3 of the aglycone part [25, 26]. C-3 of the inner glucose was shifted downfield at 78.77, indicating that the terminal xylose is linked at this carbon. $^{13}$C-NMR spectrum showed two anomeric carbon signals appeared at 99.30 and 104.67 ppm [23, 24]. On acid hydrolysis, compound 2 gave tigogenin as the aglycone moiety which was identified by comparison with authentic sample on TLC. The sugar moieties were detected as glucose and xylose by comparison with authentic sugars on TLC and PC. EI-MS of compound 2 exhibited a peak at m/z 416 [M-(Glc+ xylose)] as well as the characteristic fragmentation of the aglycone part (tigogenin) at m/z 416, 399, 327, 273, 302, 287, 139 (100 %) and 115 [23-25]. This compound showed a moderate molluscicidal activity against *B. alexandrina* snails, with $LC_{90}$ value of 61.4 ppm after 24 hrs exposure period. On the basis of the above data, compound 2 was elucidated to be tigogenin-3-*O*-β-D- glucopyranosyl ($^1J_3$)-β-D-xylopyranoside.

**Compound 3:** Compound 3 was obtained as amorphous powder. It was positive to the Liebermann-Burchard reaction and Molish reagent [17]. Its IR spectrum (KBr) $\nu_{max}$ cm$^{-1}$ showed characteristic absorptions due to hydroxyl groups at 3420 cm$^{-1}$ and spiroketal groups at 982,919,895 and 868 cm$^{-1}$, the absorption of 919 cm$^{-1}$ was of greater intensity than 899 cm$^{-1}$ which indicate (25 s) spiroketal, The existence of a benzoyl group in the molecule was indicated by the characteristic absorptions at 1720, 1640 and 633 cm$^{-1}$ [27]. This was confirmed by $^1$H-NMR data, where a signal of benzoyl group appeared at 8.45 (2H) and 7.46 (3H) together with signals at 131.70, 130.50, 128.30, 132.90 and 166.50 cm$^{-1}$ for benzoyl group as well as the quaternary carbon signal of C-22 of the aglycone moiety at 108.34 [27]. The $^1$H-NMR showed the signal of methyl groups of aglycone moiety at δ 0.71, 0.77, 0.90 and 1.23 cm$^{-1}$ and three anomeric proton signals at δ 4.76, 5010 and 5.60 cm -1, three anomeric carbon signals were shown in its $^{13}$C-NMR at δ101.5, 101.9 and 102.41 cm$^{-1}$ [28]. The interglycosidic linkages among the sugar chain and the aglycone part was confirmed by the shift of C-3 of the aglycone at δ 77.50 as compared to the aglycone part (C-3 appeared at δ 73.1) [28,29]. Also the benzoyl group was linked with the aglycone at C-1. This was confirmed by the downfield of this carbon (C-1) of aglycone of compound 3 at δ 79.35 as compared with the aglycone only [29]. The linkages between the sugar

moieties were confirmed by downfield of C-2 and C-3 of the inner galactose moiety at δ 76.50 and 84.99). This indicated that these carbons are the positions of attachments of the outer two rhamnose units [30, 31].

CI-MS spectrum of this compound showed peaks at 430, 397, 327, 289 and 253. Acid hydrolysis of this compound gave D-galactose and L-rhamnose beside the aglycone part. The $^{1}$H and $^{13}$C-NMR signals for the aglycone part were closely similar to those of rhodeasapogenin except that the carbon signals of C-1 and C-3 were shifted at downfield due to the attachment of benzoyl and the sugar chain [30, 31]. Compound 3 did not show any molluscicidal activity against *B. alexandrina* snails up to 100 ppm. On basis of the above data, compound 3 was determined as: Rhodeasapogenin-1-*O*-benzoyl-3-*O*-[($^{1}$J$_{2}$)-α-L- rhamnopyranosyl-($^{1}$J$_{3}$)]-α-L-rhamnopyranosyl-β-D-galactopyranoside.

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