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ISOLATION AND IDENTIFICATION OF SOME COMPOUNDS FROM MOLLUSCICIDALY ACTIVE PLANT *YUCCA FILAMENTOSA "MARGINATA"*

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Abstract

In the aim to find a plant extract highly effective against *Biomphalaria alexandrina* snails the intermediate host of *Schistosoma mansoni*. We have tested the molluscicidal activity of the dry leaves water suspention together with the metabolites isolated from dry powdered leaves methanol extract of *Yucca filamentosa "marginata"* (fam. Agavaceae). Four compounds were isolated from the methanol extract by using silica gel column chromatography and thin layer chromatography and could be identified using spectroscopic analysis (IR, ^{1}HNMR, ^{13}CNMR and chemical ionization mass spectrum) to be compound **(1)** was tigogenin 3-O-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D glucopyranosyl-(1→4)-β-D-galactopyranoside, compound **(2)** was 25-hydroxyspirostan-3-O-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside, compound **(3)** was 3-O-α-L-rhamnopyranosyl-(1→2) β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-4-O acetyl-glucuronopyranosyl tigogenin and compound **(4)** was 3-β-linoleoyloxy,16-β-D-glucopyranosyl-22-acetoxy-2-hydroxycholestan-6-one. Compounds 1, 3 and 4 were separated for the first time from *Yucca filamentosa "marginata"*.The molluscicidal activities of these compounds against *B.alexandrina* snails were 30, 40 and 10 ppm for compounds **(1), (2)** and **(3)** respectively. These results showed that *Yucca filamentosa "marginata"* might be considered as a potential candidate for use in vector control of schistosomiasis.

Keywords: Agavaceae ; *Biomphalaria alexandrina*; plant extract; chromatography; compound; tigogenin.

Introduction

Schistosomiasis, the most important trematode disease of man, is a world health problem. In Egypt, the erection of irrigation projects and the opening of large areas to perennial irrigation increased the infection rate as the new areas added suitable habitats to the fresh water snails, the intermediate vectors of the parasite [1]. The use of molluscicides is a control measure that offers rapid means for extermination of the causative organism. Moreover, molluscicides have considerable economic importance as they destroy the vectors of other trematode infections, especially filariasis [2]. Baylucid has been used as a molluscicide since 1960's and remain the molluscicide of choice [3], but its preparation needs high price and its formulation causes high fish mortality at the application concentrations used to kill snail and their eggs [4]. So there has been a vigorous and systematic search for plant molluscicides which might provide cheap, locally produced, biodegradable and effective control agents for snail in developing countries where schistosomiasis is endemic [5,6]. Many fine ornamental foliage plants belong to this monocotyle-donous family, composed of about 20 genera and 670 species. Agave plants exhibited piscicidal and anticancer properties [7, 8].

The methanol extract of *Agave cantala* has been found to possess cytotoxicity against JTC-26 (originating from human cervical carcinoma) [9]. Aqueous extracts and genins obtained from *Agave Americana* showed anti-inflammatory properties by testing their effects on currageenin induced edema [10].The occurrence of steroidal saponins in several Agavaceae species, especially those belonging to such representative genera as Agave, Dracaena and Yucca is well documented. The plants with anti-inflammatory activity have a saponin – containing compound of the leaves of *Yucca schottii* (Liliaceae) inhibits carragenin-induced oedema in rats (145). Yuccoside C and protoyuccoside C were isolated from the methanol extract of *Y. filamentosa* root [11], It was reported that the extract of *Y. filamentosa* yields tigogenin, sarsapogenin, gitogenin, hecogenin and chlorogenin [12,13]. The saponins of diosgenin and tigogenin were also isolated from the methanol extract of *Y. filamentosa* root [14]. Spirostanol and furostanol glycosides are found in the plant [15,16]. In monodesmosidic glycoside the sugar side chain attached at C-3. Glycosides of the furstan type have ring F open and the sugar moiety attached at C-26 [17]. The molluscicidal activities of glycosides vary with the sequence of sugar, the nature of sugar chain and the substitution patterns of the aglycone [18]. The objective of the present work is a laboratory evaluation of the molluscicidal activity of the dry powder and different extracts of *Yucca filamentosa "marginata"* plant.

Materials and Methods

The leaves of the plant *Yucca filamentosa* "*marginata*" were collected from Orman garden in Giza city during October.

Preliminary phytochemical screening

The dry powder and different solvent extracts of *Y. filamentosa "marginata"* leaves were subjected separately to the following phytochemical tests and the results were recorded in table **(1)** for the dry powder and table **(2)** for the corresponding extracts.

1 - Test for carbohydrates and glycosides

One gm of the dry powder was extracted with 10 ml of 50 % aqueous ethanol. 5 ml of ethanol extract were mixed with 0.5 ml of ethanolic α-naphthol solution followed by one ml of H_2SO_4 poured carefully on the wall of the test tube. The appearance of a violet ring between the two layers indicates the presence of carbohydrates and/or glycosides [(19)].

2 - Test for sterols and/or triterpenes

For each powdered sample, about 10 ml of ethanol extract were evaporated to dryness. The residue was dissolved in 20 ml of chloroform solution and filtered. The filtrate was subjected to the following tests.

i. **Salkowski test:** To about 5 ml of the chloroform solution, an equal volume of sulphuric acid were added carefully. Formation of a red colour indicates the presence of sterols and /or triterpenes.
ii. **Liebermann-Burchard test:** Evaporate about 5 ml of the chloroform solution of each sample to a small volume, about 1ml of acetic anhydride was added followed by 2 ml of H_2SO_4 poured carefully on the wall of the test tube to form a lower layer. A reddish brown colour appeared at the junction between the two layers indicates the presence of unsaturated sterols and/or triterpenes [20].

3 - Test of saponins: About 10 gm of each plant powder were shaken with distilled water and filtered. This filtrate was shaken strongly and allowed to stand for five minutes. The presence of

voluminous froth indicates the presence of saponins [21].

4 - Test for tannins: About 2 gm of each powdered plant were extracted by 20 ml of 50 % aqueous ethanol and filtered. Add few drops of ferric chloride solution; a green colour was obtained confirming the probability of existence of catechol tannins [22]

5 - Test for flavonoids: 5 gm of each dry powdered sample were soaked over-night with 150 ml of 1% hydrochloric acid solution and filtered. The filtrate was subjected to flavonoid compounds tests as follows:

i) About 10 ml of the filtrate was rendered alkaline with NaOH solution. The appearance of a yellow colour indicates the presence of flavonoids.
ii) About 5 ml of the filtrate was blended with 5 ml of HCl and few pieces of magnesium metal were added. The formation of a red colour proves the presence of flavonoids [23].

Test for alkaloids and/or nitrogenous bases

10 gm of each dry powdered plant were splitely extracted with 100 ml of dilute hydrochloric acid. The acidic extract was filtered, and rendered alkaline with ammonium hydroxide solution, followed by extraction with chloroform. The chloroform extract was evaporated till dryness and the extract was dissolved in 2 ml of HCl. The formation of very faint brown precipitate with Wagner's reagent and very slight precipitate with Mayer's reagent confirming the presence of nitrogen bases [24].

Apparatus

- Melting points were determined by an electrothermal apparatus.
- IR spectra were recorded on PERKIN ELMER 1650 and on BRUKER vector 22 Germany apparatus.
- ^{1}HNMR and ^{13}CNMR spectra were recorded on a Varian GE-MHZ, a Varian (300) NMR, Oxford, and Mercury spectrometer using DMSO-d_6, CD_3OD as a solvent and $SiMe_4$ as internal standard chemical shifts are expressed as ppm units.
- Mass spectra were measured on HP-5988 and on GC-MS-1000 EX with direct inlet equipments at 70 ev. The absence of peaks corresponding to (M+1) or (M+2) for some compounds may be due to deficiency in its resolution.

Chromatographic techniques

1. Thin-layer chromatography (TLC)

Chromatographic glass plates (20 x 20 cm) and (10 x 20 cm) were dried and cleaned, then coated with silica gel (Merck FG_{254}) of 0.2 mm thickness as an adsorbent layer and used for analytical separation and layers of 0.5-1mm thickness were applied for preparative separation. The two types of plates were activated in oven at 100-110°C before using and allow to cool. Spots were showed with 40% sulphuric acid in methanol, followed by heating at 120°C for 5 minutes.

2. Column chromatography (CC)

Glass columns (120 x 7cm) were used. They were packed with silica gel G_{60} Merck as stationary phase. A small amount of silica gel was mixed with the solution of the material to be fractionated and allowed to evaporate to dryness and the mixture was poured on the top of the column. The suitable solvent was applied to start the development process and continued in order of increasing polarity. The fractions were collected (250 ml each) and monitored by TLC.

3. Paper chromatography techniques

Whatmann No. 1 sheets were applied to paper chromatographic investigations. A starting line was drowning across each sheet along the shorter edge and 2 cm a way from the margin. The positions of spots were marked on this line using a pencil, and suitable volumes of the spotting solutions were spotted using glass capillaries along side with the respective authentic reference samples. After this study second techniques were used which are, the spots are dried and visualized in UV light before and after spraying with the suitable reagent.

Material and Methods

Plant material and Extraction, Chemical methods

The dry powder leaves of *Yucca filamentosa "marginata*" (2kg) was soaked in 70% methanol for one week, then evaporated to dryness to give a brown residue (250 gm) which was defatted using petroleum ether (60-80 °C) for several times to give residue (230 gm). The defatted methanol extract was concentrated in vacuo and dissolved in H_2O, then partitioned with n-butanol. The butanol extract (110 gm) was precipitated by acetone to give (80 gm) precipitate.

Column chromatography

The dried precipitate of n-butanol extract (80 gm) was subjected to column chromatography using

silica gel G_{60} as stationary phase. Silica gel column was constructed by packing a glass column (150 x 7 cm) with silica gel adopting the wet method using petroleum ether. The dried extract was adsorbed on 100 gm silica gel applied on the top of the prepared column. Elution started with petroleum ether (60-80°C) followed by chloroform, and chloroform: methanol ratio increasing from 0-100. The elutes were collected in fractions each 250 ml. The collected fractions were subjected to chromatographic identification using silica gel (Gf_{254}) plates (20x20 cm). The chromatographic plates were examined in ordinary and UV light after spraying with 40% sulphuric acid in methanol and heating in an oven at 100-120 °C for ten minutes to give compounds (1, 2, 3 and 4) table (3) .

Acid hydrolysis

20 mg of the compound was hydrolyzed using 4N HCL (10 ml) and methanol (5 ml) on boiling water bath for (3-4) hours. The reaction mixture was concentrated under reduced pressure to remove the methanol. The mixture was partitioned between chloroform and water using separating funnel, the chloroform extract was evaporated under reduced pressure and crystallized to give the aglycone. The aqueous layer was neutralized with $NaHCO_3$, filtered and concentrated then the sugar was extracted with pyridine. The sugar obtained was compared with authentic sugars on TLC silica gel plate, with system [Ethyl acetate:methanol : acetic acid:H_2O (13:4:3:3)]. The spots were detected by spraying with a solution of aniline phthalate in n-butanol (freshly prepared). The analysis showed that the sugars in compound **(1)** were galactose, glucose, rhamnose and xylose, in compound **(2)** were rhamnose and glucose, while in compound **(4)** was glucose.

Moreover, identification of the sugar was further confirmed by paper chromatography (whatmann) filter paper No. 1, by using solvent system [n-butanol:pyridine-d5:water (10:3:3)] with developing time 48 hours, the sugar spots were detected by spraying with freshly prepared aniline phthalate sugar reagent which showed the presence of four spots corresponding to rhamnose, xylose, glucose and glucuronic acid for compound (**3**).

TLC systems

Benzene

Benzene : methanol

9 : 1

8.5 : 1.5

Chloroform : methanol

9.8 : 0.2

9.5 : 0.5

9 : 1

7 : 3

6.5 : 3.5

7 : 4

Chloroform : methanol : H_2O

6.5 : 5 : 0.5

Snails

Biomphalaria alexandrina snails, the intermediate host of *Schistosoma mansoni* (shell diameter 6-8 mm) were collected from irrigation canals in Giza governorate which had not been treated with molluscicides. The snails were left to acclimatize in the laboratory, dechlorinated tap water with pH 7 (temperature 25±2°C) for three weeks before being used.

Molluscicidal activity tests

The dry powder of the plant was used as an aqueous suspension to prepare different concentrations on the basis of weight / volume (ppm) in dechlorinated tap water. The same technique was followed with the compounds isolated from the plant. For each experimental concentration, 3 replicate each of ten snails / liter was used, as well as in control group. The exposure period was 24 hours followed by another 24 hours of recovery, and then the snails mortalities were recorded. The molluscicidal activities of the two plants were expressed in terms of LC_{50} & LC_{90} values according to Litchfield and Wilcoxon's method, (1949) [25].

Results and Discussion

Compound (1): An amorphous solid (91.5 mg), m.p. 285 -287°C, R_f 0.63 [$CHCL_3$: MeOH (7:3)] gave Liebermann and Molish tests. IR ^{kBr} showed absorption bands at 3437.2 cm^{-1} (OH), 1066.6 cm^{-1} (anomeric protons) indicating the glycosidic nature of compound **(1)** and at 893.5 cm^{-1}, 920 cm^{-1} led to the prediction of existence of spirostan skeleton [26-28], which was moreover, evident by the presence of carbon resonance due to (C-22) at 109.0 [29]. ^{1}HNMR (DMSO-d_6) revealed the presence of two tertiary methyl groups at δ 0.86 (3H, s) and δ 1.05 (3H, s) ppm and two secondary methyl groups at δ 0.70 (3H, d) and at δ 1.14 (3H, d) ppm [30]. Furthermore signals for six anomeric protons at δ 4.20, 4.40, 4.50, 4.65, 4.70 and 4.75 ppm, and characteristic methyl doublets at 1.60 and 1.76 ppm of rhamnose were appeared.

Acid hydrolysis according to technique described gave galactose, rhamnose, glucose, and xylose, identified by comparison with authentic samples. Examination of ^{13}C NMR signals of the aglycone moiety showed that the structure of the aglycone to be tigogenin (25R spirostan- 3 β ol) [29] (Table 4) suggested by the appearance of chemical shifts at 109.0 (C-22) and 17.3 (C-27). Four methyl groups was observed at δ 16.5 (C-18), 12.3 (C-19), 14.9 (C-21), 17.3 (C-27), and δ 15.9 ppm for rhamnose methyl group. The ^{13}C NMR spectrum (DMSO-d_6) displayed six anomeric carbon atoms at δ 101.3, 101.8, 103.2, 103.4, 103.6 and 104.0 (Table 4). The chemical shift of C-3 in ^{13}C NMR spectrum at δ 77.7 revealed that compound **(1)** was glycosylated at position 3 of the aglycone [31]. Comparison of the ^{13}C NMR spectrum of the sugar moiety with that of reference methyl glycoside [32] from the literature led to the conclusion that monosaccharide chain was linked to C-3 of the aglycone. In support of the suggested structure for compound **(1)**, its CI-MS spectrum (DMSO) showed the molecular ion peak at 1342.9 $[M]^+$, its molecular formula was identified as $C_{62}H_{102}O_{31}$.

Also a fragment ion peak of low intensity at m/z 1211.9$[M+H-132]^+$ indicating the loss of xylose moiety, a fragment ion of a moderate intensity at m/z 1049.3$[M+H-294]^+$ representing the loss of xylose and glucose indicating that xylose was the terminal sugar. The fragment ion peak at m/z 903.7$[M+H-440]^+$ corresponding to the loss of xylose, glucose and rhamnose moieties. The fragment ion peak at m/z 741.4$[M+H-602]^+$ representing the loss of xylose, two glucose and rhamnose units, and that at m/z 417.3$[M+H-926]^+$ indicating the loss of one xylose, three glucose, one galactose and one rhamnose moieties. The molecular weight of the aglycone could be calculated from CI-MS spectrum by the presence of an intense fragment ion peak at m/z 417.3 which suggested that the aglycone was tigogenin. A highly intense fragment ion at m/z 399.3 $[M_{aglycone} - H_2O]$ representing the loss of one molecule of water. The above data suggested that compound **(1)** could be identified as tigogenin hexaglycoside. The last data suggested that this compound was tigogenin 3-O-β-D-xylo-pyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-α-L-rhamno-pyranosyl-(1→2)-β-D-gluc-opyranosyl-(1→3) –β -D-glucopyranosyl- (1→4)- β-D-galactopyranoside (fig. 1). Literature survey indicates that compound **(1)** is a new compound and separated from *Y. filamentosa "marginata"* for the first time.

Compound (2): White crystals (100.3 mg), m.p. 257-260°C, R_f 0.52 [$CHCL_3$: MeOH (6.5:3.5)]. It gave positive Liebermann-Burchard, and Molish tests for steroids. IR ^{kBr} spectrum showed absorption bands at 3411.5 cm^{-1} (OH) and 1072.9 (anomeric protons) indicating the glycosidic nature of the compound and characteristic absorption at 875.3 and 937.5 cm^{-1} (875>937) indicating the presence of (25R) spiroketal side chain [26-28].The ^{1}HNMR spectrum (CD_3OD) of the compound showed signals for four methyl groups at δ 0.784 (s, Me-18), δ 0.855 (s, Me-19), δ 0.959 (d, Me-21) and δ 1.318 ppm (s, Me-27) [33] and anomeric proton at δ 5.013. The signal at 1.318 in ^{1}HNMR spectrum assigned to (Me-27) was consistent with a methyl group being attached to a furanose ring of spirostan skeleton in α-orientation [33].This result can be supported by the IR spectrum which showed absorption below 1000 cm^{-1} indicated that the free hydroxyl group was located at ring F [34-35]. Acid hydrolysis afforded D-glucose and L-rhamnose which was identified by comparison TLC with authentic samples. ^{13}C NMR spectrum (CD_3OD) revealed the presence of signals at δ 108.8 (C), 81.0 (CH), 67.9 (C), 62.0 (CH) (Table 4) assigned to C-22, C-16, C-25, and C-17 positions respectively in the spirostan skeleton [29] ^{13}C NMR spectrum suggested that the aglycone was a spirostanol derivative having two hydroxyl groups, one at C-3 (from biogenetic consideration) and the other at C-25. ^{13}C NMR spectrum also revealed the presence of two anomeric carbons at δ 103.5 and 101.5 ppm, it also showed that one glucose and one rhamnose moieties were linked to C-3 of the aglycone through an ether linkage, of the two sugars, one was thought to be terminal (D-glucose), while the other (L-rhamnose) was interior linked at C-3 of the aglycone. This was suggested by the C-3 chemical shift of rhamnose (δ 77.0 ppm) which was almost 4 ppm further down field than C-3 of the normal rhamnose [36].

The CI-MS spectrum (DMSO) showed the presence of molecular ion peak at m/z 739 $[M]^+$. A high intense fragment ion at m/z 578.0 $[M+H-162]^+$ indicating the loss of glucose moiety and at m/z 431.1$[M+H-309]^+$ corresponding to the loss of glucose and rhamnose moieties. The last data suggested that rhamnose and glucose were linked to C-3 of the aglycone (25R), (3 β, 25) dihydroxyspirostan through an ether linkage and that glucose was linked to C-3 of rhamnose. CI-MS spectrum also showed characteristic signals at m/z 431.1 $[M_{aglycone}]^+$ and at m/z 416.6 $[M_{aglycone} - 15]^+$ representing the loss of methyl group and at m/z 399.4 $[M_{aglycone} +H-33]^+$ corresponding to the loss of methyl group and one molecule of water. A fragment ion at m/z 318.0 $[M_{aglycone} +H-114]^+$

indicating the loss of F ring of the aglycone and at m/z 384 [$M_{aglycone}$ +H-48]$^+$ representing to the loss of two methyl groups and one molecule of water. The above data suggested that compound (**2**) was 25-hydroxyspirostan-3-O-β-D-glucopyranosyl-(1→3)-α-L rhamnopyrano-side (fig. 1).

Compound (3): Compound **(3)** (87.4 mg) **,** m.p. 268-271°C, R_f 0.49 [$CHCL_3$: MeOH (7 : 4)] showed positive Libermann-Burchard test for steroids. IR ^{kBr} spectrum showed absorption bands at 3422.7 cm^{-1} (OH), 1074.6 cm^{-1} (anomeric protons) indicating the glycosidic linkage of the compound and a characteristic absorption bands of (25 R)-spiroketal moiety at 850.4, 892.1, 920.9 cm^{-1}(892.1> 920.9) [26-28,37].

H^1NMR spectrum revealed the presence of four methyl signals at δ 0.746 (3H, s, 18 Me), δ 0.838 (3H, s, 19 Me), δ 0.781 (3H, d, 27 Me), δ 1.092 (3H, d, 21 Me), a multiplet signal at δ 4.965 (1H, m, H-16), δ 4.899 (1H, ddd, 3H) and at δ 2.170 ppm (OAc) [38]. The H^1NMR also revealed the presence of signals at δ 5.575 , 5.206 , 4.994 and 4.678 ppm (anomeric protons) [33,37].

The ^{13}C NMR spectrum showed chemical shift values of the signals due to the aglycone moiety were superimposable on those of tigogenin; the site of linkage of the sugar units was at C-3 of the aglycone. The ^{13}C NMR spectrum also showed a total of 27 carbons for the aglycone in addition to sugar units. These 27 carbons were readily devidable into four methyl groups, 11 methylene carbons, 9 methine carbons, three quaternary carbon atoms were in good agreement with those of tigogenin [29]. The ^{13}C NMR spectrum of the compound showed chemical shift values of the signals due to the aglycone moiety at δ 16.5 (C-18), 12.7 (C-19), 14.7 (C-21), 18.4 (C-27) and a characterestic chemical shift for (C-22) at δ 108.9 ppm were superimposable on those of tigogenin. ^{13}C NMR spectrum also showed the presence of anomeric carbons at 103.9, 103.5, 103.1, 101.7 ppm (Table 4) [39,40].

The ^{13}C NMR assignment completed the location of linkage sites between the sugar residues were indicated by the characteristic downfield shifts (3 to 10 ppm) induced by the formation of the glycosidic bonds. This approach also led to the conclusion that there was terminal rhamnose moiety, as this sugar showed the typical chemical shift for unsubstituted α-rhamnose moiety (table **4**). This chemical shift was established by analysis of CI-MS fragmentation patterns (DMSO).

The characteristic glycosidic fragmentations of the oligosaccharide moiety aided greatly in confirming the sugar sequence, in addition to the [M]$^+$ molecular ion (m/z 1074.0), high intense fragment ion at m/z 929.1 [M+H-146]$^+$ indicating the loss of rhamnose moiety, a small intense fragment ion at m/z 797.0 [M+H-278]$^+$ corresponding to the loss of rhamnose and xylose moieties, and fragment ion at m/z 635.3 [M+H-440]$^+$ representing the loss of rhamnose, xylose and glucose moieties and a fragment ion at m/z 416 [M+H-659]$^+$ indicating the loss of rhamnose, xylose, glucose and 4-acetylglucuronic acid moieties. The above data indicates that compound **(3)** was 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl- (1→3)-β-D-4-O-acetylglucuronopyranosyl tigogenin (Fig.1). Literature survey indicates that compound **(3)** is a new compound and separated from *Y. filamentosa " marginata"* for the first time.

Compound (4): Compound **(4),** amorphous solid (79.1 mg), m.p. 240°C, R_f 0.59 [$CHCL_3$: MeOH: H_2O (6.5:5:0.5)]. It gave positive Liebermann – Burchard test for steroids. IR ^{kBr} spectrum showed absorption bands at 3420.4 cm^{-1} (OH) and 1073.4 cm^{-1} (anomeric protons) indicating the glycosidic nature of the compound. IR spectrum also exhibited absorption bands at 1376.6 cm^{-1} (gem. Dimethyl), 1707.1 cm^{-1} (C=0), 1259.4 cm^{-1} (OAC). H^1 NMR spectrum (DMSO-d_6) suggested to be steroidal derivative by the presence of two tertiary methyl groups in compound **(4)** at δ 0.530 (s, Me-18), δ 0.752 (s, Me -19) and three secondary methyl groups at 0.925 (d, Me – 26, Me-27) and at δ 1.123 ppm (d, Me-21). Appearance of the Me-18 signal in the rather high field position (δ 0.530) ppm indicated that compound **(4)** was a steroid [41]. The signals at δ 3.555, 4.177 and 4.330 ppm were assigned to H-2, H-3 and H-16 respectively, which were attributable to protons attached to hydroxylated carbons. H^1NMR spectrum also showed signal at δ 4.847 ppm assignable to H-22, this signal suggested that acetoxyl group was situated at 22-position of the steroid (free OH at 22- position appeared at δ 3.80 ppm) [42]. Moreover, the characteristic signal at δ 2.992 ppm (dd, H-5) suggested that compound **(4)** was a cholestan derivative having a carbonyl group at 6-position [41,43] and one anomeric proton at δ 5.209 ppm. The H^1NMR spectrum also showed signals at δ 5.616 (m), 2.251 (2H) and at δ 0.886 ppm (3H, t) were attributed to the olefinic protons of the double bonds, methylene protons of (C-2"), methylene protons of (C-8"), (C-14") and terminal (C-18") methyl proton of linoleoyl moiety attached to C-3 hydroxyl of the steroid derivative [44].

^{13}C NMR spectrum (DMSO-d_6) revealed the presence of chemical shifts at δ 129.3, δ 132.21and δ 132.4 ppm attributed to the olefinic carbons of linoleoyl moiety [44]. Two carbonyl carbon signals were present in the ^{13}C NMR spectra of compound **(4)** as they occurred at chemical shifts appropriate for C-6 unsaturated ketone at δ 210.6 ppm [42] and at δ 169.8 ppm for C-22 acetoxyl group. The upfield shifts in ^{13}C NMR of C-3, C-22 and C-16 at δ 74.3, 77.2, and 77.5 ppm indicated that linoleoyl moiety, acetoxyl group and glycosyl moiety were linked to C-3, C-22, and C-16 of the aglycone respectively [43]. Also the ^{13}C NMR spectrum showed the presence of one anomeric carbon atom at δ 103.5 ppm. The CI-MS spectrum showed the presence of molecular ion at m/z 916 corresponding to $C_{53}H_{88}O_{12}$. A fragment ion at m/z 857.9 $[M_{glycoside} + H - 60]^+$ indicating the loss acetic acid, a fragment ion at m/z 754.9 $[M+H-163]^+$ indicating the loss of glucosyl moiety. A frgment ion at m/z 617.6 $[M_{glycoside} -298]^+$ representing the loss of linoleic acid and one molecule of water.

The CI-MS spectrum also revealed the presence of fragment ions at m/z 431.4 $[M_{aglycone} +H-H_2O]^+$ indicating that the molecular weight of the aglycone was 450.0 $[M]^+$, m/z 399.2 $[M_{aglycone} – (CH_3+2H_2O)]^+$ and m/z 357.2[$M_{aglycone}$ - $(2H_2O+ CH_3+ C<^{CH_3}_{CH_3})$].The CI-MS spectrum also showed the presence of intense fragment ion at m/z 493.0 $[(M_{aglycone} + COCH_3)]^+$ and small fragment ion at m/z 756 $[M_{aglycone} + (COCH_3+$ linoleoyl moiety$)]^+$. The CI-MS also revealed the presence of fragment ions due to the fragmentation of the aglycone at m/z 60 ($C_2H_4O_2$), m/z 88.0 ($C_4H_8O_2$), m/z 101.0($C_5H_9O_2$), m/z 129.0 ($C_6H_9O_3$) and at m/z 156.0 ($C_8H_{12}O_3$) indicating the presence of keto group in rings A and B. The last data suggested that the aglycone was 2α, 3β, 16, 22- tetrahydroxy-6-cholestanone linked to linoleoyl moiety at C-3, acetoxyl group at C-22 and glycosyl moeity at C-16. Acid hydrolysis afforded glucose from the acid hydrolysate identified by comparison TLC with authentic samples. The above data suggested that compound **(4)** was 3-β-linoleoyloxy, 16-β-D-glucopyranosyl-22-acetoxy-2-hydroxycholestan-6-one (fig. 1). Literature survey indicates that compound **(4)** is a new compound and separated from *Y. filamentosa* " *marginata*" for the first time.

Molluscicidal activity bioassay

Molluscicidal activity of the dry leaves water suspension of *Yucca filamentosa "marginata"* against B.alexandrina snails after 24 and 48 hours was LC50 =125 and 88 ppm respectively, also LC90 =130 and 118 ppm respectively ;these observations indicate that the molluscicidal activity of the plant increased by increasing the exposure time from 24 to 48 hours (Table 5). The molluscicidal activity of Compounds **(1, 2 and 3)** were LC_{50} =30, 40 and 10 ppm respectively against *Biomphalaria alexandrina* snails, after 24 hours exposure time (the intermediate host of *schistosoma-mansoni*).

Structure-activity relationship

The molluscicidal activity was confined to the glycosidal compounds. There is a correlation between the molluscicidal activity and the presence of glucuronic acid in the sugar part of the molecule [45]. High molluscicidal activity can also be observed in compound **(3)** was due to the presence of 4-acetylglucuronic acid in the sugar chain linked to the aglycone part of compound.

Conclusion

The results in the present work evaluate the molluscicidal activity of the dry leves water suspension and the compounds isolated from *Yucca filamentosa "marginata"*, it would appear that the compounds may prove to be useful in the molluscicidal applications. Four compounds separated from the methanol extract of the plant and defined as compound **(1)** was tigogenin 3-O-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl- (1→4)-β-D-galactopyranoside, compound **(2)** was 25-hydroxyspirostan-3-O-β-D-glucopyranosyl-(1→3)-α-L-rha-mnopyranoside, compound **(3)** was 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-4-O acetyl-glucuronopyranosyl tigogenin and compound **(4)** was 3-β-linoleoyloxy,16-β-D-glucopyranosyl-22-acetoxy-2-hydroxycholestan-6-one. Compounds 1, 3 and 4 were separated for the first time from *Yucca filamentosa "marginata"*.The molluscicidal activities of these compounds against *B.alexandrina* snails were 30, 40 and 10 ppm for compounds **(1), (2)** and **(3)** respectively. These results showed that *Yucca filamentosa "marginata"* might be considered as a potential candidate for use in vector control of schistosomiasis.

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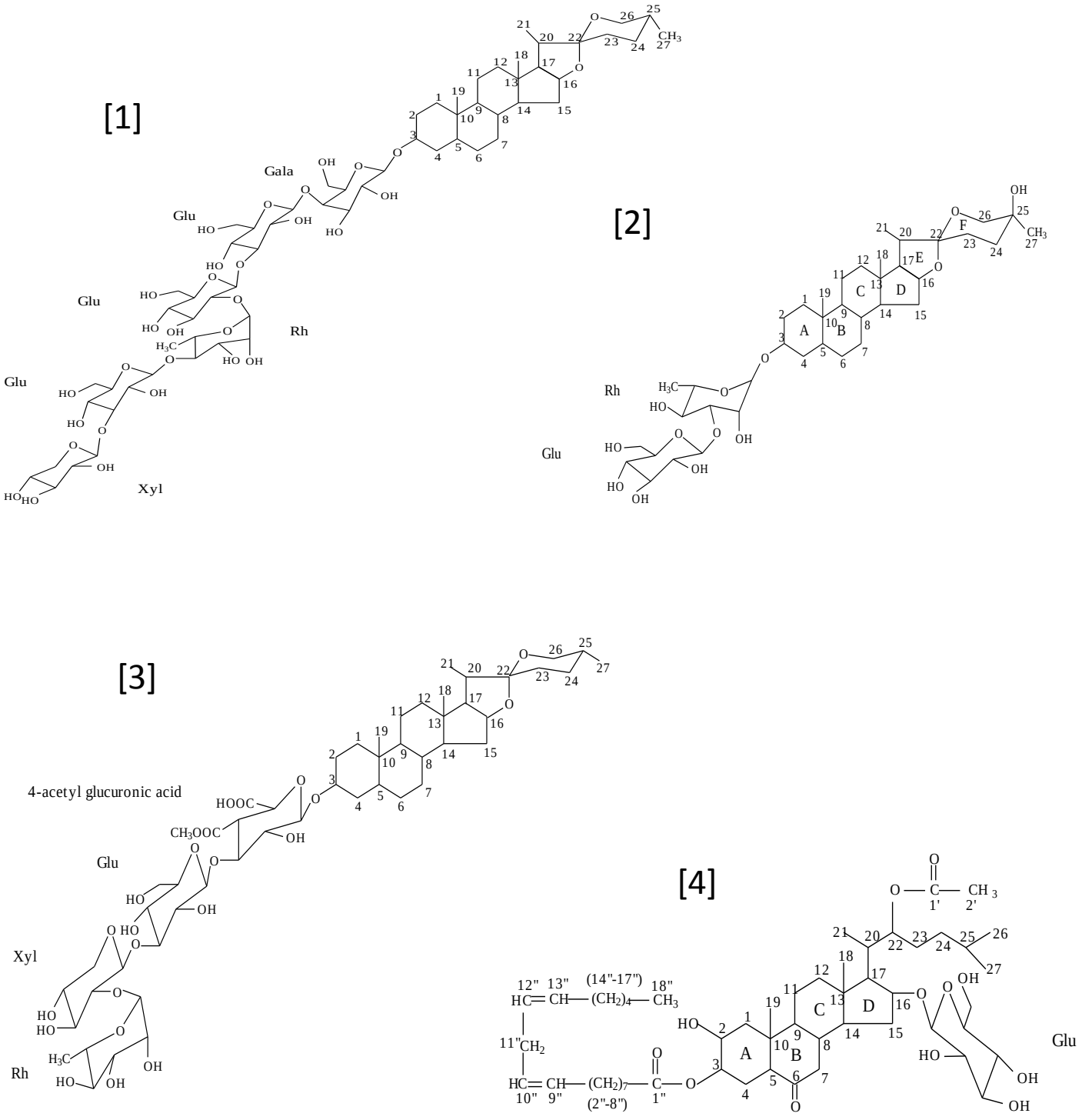

Figure 1. Chemical skeletons of the compounds isolated from *Yucca filamentosa "marginata"* leaves.

Table 1. Phytochemical screening of *Y. filamentosa "marginata"* dry powder.

Test	*Y. filamentosa "marginata"*
Carbohydrates and/or glycosides	+
Sterols and/or Triterpenes	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids and/or nitrogenous bases	-

+ Signified the presence of the constituents
- Signified the absence of the constituents

Table 2. Phytochemical screening for different extracts of *Y. filamentosa "marginata"* leaves

Test	Methanol	Chloroform	Petroleum Ether	Ethyl acetate	Ether	Benzene	Acetone
Carbohydrates and/or glycosides	+	+	+	+	+	+	+
Sterols and/or triterpenes	+	-	-	+	-	+	+
Saponins	+	-	+	-	+	+	+
Tannins	+	+	+	+	+	+	-
Flavonoids	+	-	-	-	-	-	+
Alkaloids and/or nitrogeneous bases	-	-	-	-	-	-	-

Table 3. Column chromatographic fractions of crude mixture of *Yucca filamentosa " marginata*"

Elute	Fraction no.	No.of spots	Isolated compounds	TLC system
Petroleum ether	1-------30	2	----------	1
Chloroform	31-----50	4	mixture	2
Chloroform: methanol	51------62	3	mixture	3
99:1				
95:5	63------78	2	mixture	4
90:10	79-----100	5	mixture	5
80:20	101----140	3	mixture	6
75:25	141-----170	1	Compound 1	7
65:35	171----300	1	Compound 2	8
60:40	301-----380	1	Compound 3	9
55:45	381-----503	1	Compound 4	10

Table 4. ^{13}C NMR for spectral data of compounds **(1, 2, 3 and 4)**.

Carbon	Comp. 1 δc	Comp. 2 δc	Comp. 3 δc	Comp. 4 δc
1	37.2	35.6	37.2	38.8
2	29.7	28.7	29.7	68.1
3	77.7	72.0	77.4	74.3
4	39.9	34.2	34.7	32.1
5	44.8	44.8	44.7	54.8
6	29.0	28.9	28.9	210.6
7	32.5	32.0	32.4	42.5
8	35.3	35.6	34.0	41.1
9	54.5	54.6	54.2	37.0
10	35.9	35.5	36.0	40.5
11	20.6	21.0	21.3	22.7
12	40.2	39.0	40.2	39.4
13	40.8	40.5	40.8	44.7
14	56.4	56.4	58.7	56.5
15	31.8	31.4	32.1	24.0
16	80.9	81.0	80.0	77.5
17	62.3	62.0	62.0	54.6
18	16.5	16.3	16.5	11.4
19	12.3	11.5	12.7	23.0
20	42.0	41.7	41.0	40.8
21	14.9	13.2	14.7	14.5
22	109.0	108.8	108.9	77.2
23	31.6	23.8	31.6	29.6
24	28.9	34.1	29.7	36.1
25	30.3	67.9	30.6	30.5
26	67.5	69.5	66.6	23.1
27	17.3	29.5	18.4	22.8

Carbon	Comp. 1 δc	Comp. 2 δc	Comp. 3 δc	Comp. 4 δc
For C-3				
Galactose (45)				
1	103.4			
2	73.7			
3	75.5			
4	79.2			
5	76.4			
6	60.5			
Glucose				
1	101.3			
2	76.5			
3	87.2			
4	78.1			
5	77.5			
6	61.3			
Glucose (36)				
1	103.2	103.5	103.1	103.5
2	80.4	74.7	76.7	75.7
3	78.8	76.2	86.7	76.6
4	72.5	70.4	70.8	71.3
5	77.5	77.2	78.2	78.1
6	61.5	62.6	61.8	61.8
Rhamnose (36)				
1	101.8	101.5	101.7	
2	73.1	72.0	70.4	
3	69.5	77.0	69.8	
4	80.4	74.4	73.0	

Carbon	Comp. 1 δc	Comp. 2 δc	Comp. 3 δc	Comp. 4 δc
For C-3				
5	68.5	66.6	66.6	
6	15.9	15.4	18.2	
Glucose (36)				
1	104.0			
2	77.0			
3	86.5			
4	78.1			
5	75.5			
6	62.2			
Xylose (39)				
1	103.6		103.5	
2	75.1		79.7	
3	78.2		76.3	
4	70.9		71.3	
5	67.5		65.0	
Glu A (39)				
1			103.9	
2			75.6	
3			87.5	
4			72.6	
5			74.5	
6			176.3	
$\underline{C}H_3CO$			53.6	
$CH_3\underline{C}O$			174.1	
Acetyl 1'				169.8
2'				21.3

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Table 5. Effect of dry leaves water suspension of *Yucca filamentosa* "*marginata*" against *Biomphalaria alexandrina* snails at 25° C.

Exposure time	LC_{50} (ppm)	LC_{90} (ppm)	Slope
24 hours	125 (120.1-130.0)	130	1.08
48 hours	88 (78.5-98.5)	118	1.22