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Isolation and identification of some steroidal glycosides of *Furcraea selloa*

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The antischistosomal impact of different extracts of the leaves of *Furcraea selloa* C. Koch (Family Agavaceae) were screened against adult *Schistosoma mansoni* worms *in vitro* using well established culture media. The methanol extract of the plant showed the highest activity as *S. mansoni* worms recorded 100% mortality at 50 μg/ml after 24 h ($EC_{50} = 29.78$ and 29.41 μg/ml for female and male worm respectively). Owing to the high potency of the crude butanolic extract (100% mortality at 20 μg/ml; $EC_{50} = 10.42$ and 8.94 μg/ml for female and male worm respectively) obtained from the methanolic extract, it was submitted to chromatographic separation and isolation using silica gel and Sephadex columns as well as preparative thin layer chromatography. Three steroidal glycosides (saponins) (**I–III**) were isolated and their structures were elucidated using some spectroscopic and chemical methods. The structure of the three compounds was formulated as 6-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside chlorogenin (**I**), 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside crestagenin (**II**) and 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside gloriogenin (**III**). Only compound **III** at 5 μg/ml led to 100% mortality of the *S. mansoni* ($EC_{50} = 2.25$ and 1.91 μg/ml for female and male worm respectively) whereas compounds **I** and **II** did not show any activity up to 50 μg/ml.

1. Introduction

Intestinal schistosomiasis is caused by the helminth *Schistosoma mansoni* and afflicts 200 million people in 76 tropical and subtropical countries (Eddlestan 1999). Control of this disease involves chemotherapy along with molluscicide the water bodies infested with the parasites intermediate host; snails of the genera *Biomphalaria* (Shuhua and Chollet 2000; Katz et al. 1991; Anna et al. 2000; Abdel-Gawad et al. 2004a).

Plants have provided a number of useful clinical agents that prove to have considerable potentials as sources of new drugs (Phillipson 1994). So the use of medicinal plants which grow abundantly in areas where schistosomiasis is endemic may become a useful complement either as molluscicides or chemotherapy for the control of this disease. However few studies have addressed the use of medicinal plants with antischistosomal activity as treatment for this disease (Liu and Weller 1996; Schulz et al. 1997).

Furcraea is a plant genus that contains specific compounds serving numerous medicinal purposes (Itabashi et al. 2000; Abdel-Gawad et al. 2002). The dry powder of *F. selloa* plant (Agavaceae) was previously reported to possess strong molluscicidal potency against *Biomphalaria alexandrina* snails (El-Sayed et al. 2002). So it seemed promising to continue work on this plant and to test the effect of its extracts on *S. mansoni* worms. In addition some of its constituents were isolated and identfied using chromatographic, spectroscopic and chemical techniques.

2. Investigations, results and discussion

In the present study, the well-established bioactivity guided fractionation was followed (Hostettmann et al. 1997; Marston et al. 1993). The bioactivity guided fractionation of some different organic solvent extracts of *F. selloa* leaves were evaluated for *in vitro* antischistosomal activity (Table 1, Fig.). Only the methanol extracts showed a high activity up to 50 μg/ml after 24 h incubation period ($EC_{50} = 29.78$ and 29.41 μg/ml for female and male worm respectively). Also, the butanolic crude fraction obtained from successive fractionation of the active methanol extract showed a high activity up to 20 μg/ml ($EC_{50} = 10.42$ and 8.94 μg/ml for female and male worm

Table 1: Effect of different extracts, crude butanolic extract and isolated compounds of *Furcraea selloa* against adult *S. mansoni* worms using *in vitro* method

Extracts and compounds	EC_{50} (μg/ml)		EC_{84} (μg/ml)	
	Female	Male	Female	Male
Pet. ether	−ve up to 100 μg/ml			
Chloroform	−ve up to 100 μg/ml			
Acetone	−ve up to 100 μg/ml			
Methanol	29.74	29.41	43.02	45.91
Crude butanolic extract	10.42	8.94	16.96	12.63
Compound **I**	−ve up to 100 μg/ml			
Compound **II**	−ve up to 100 μg/ml			
Compound **III**	2.25	1.91	4.10	3.76

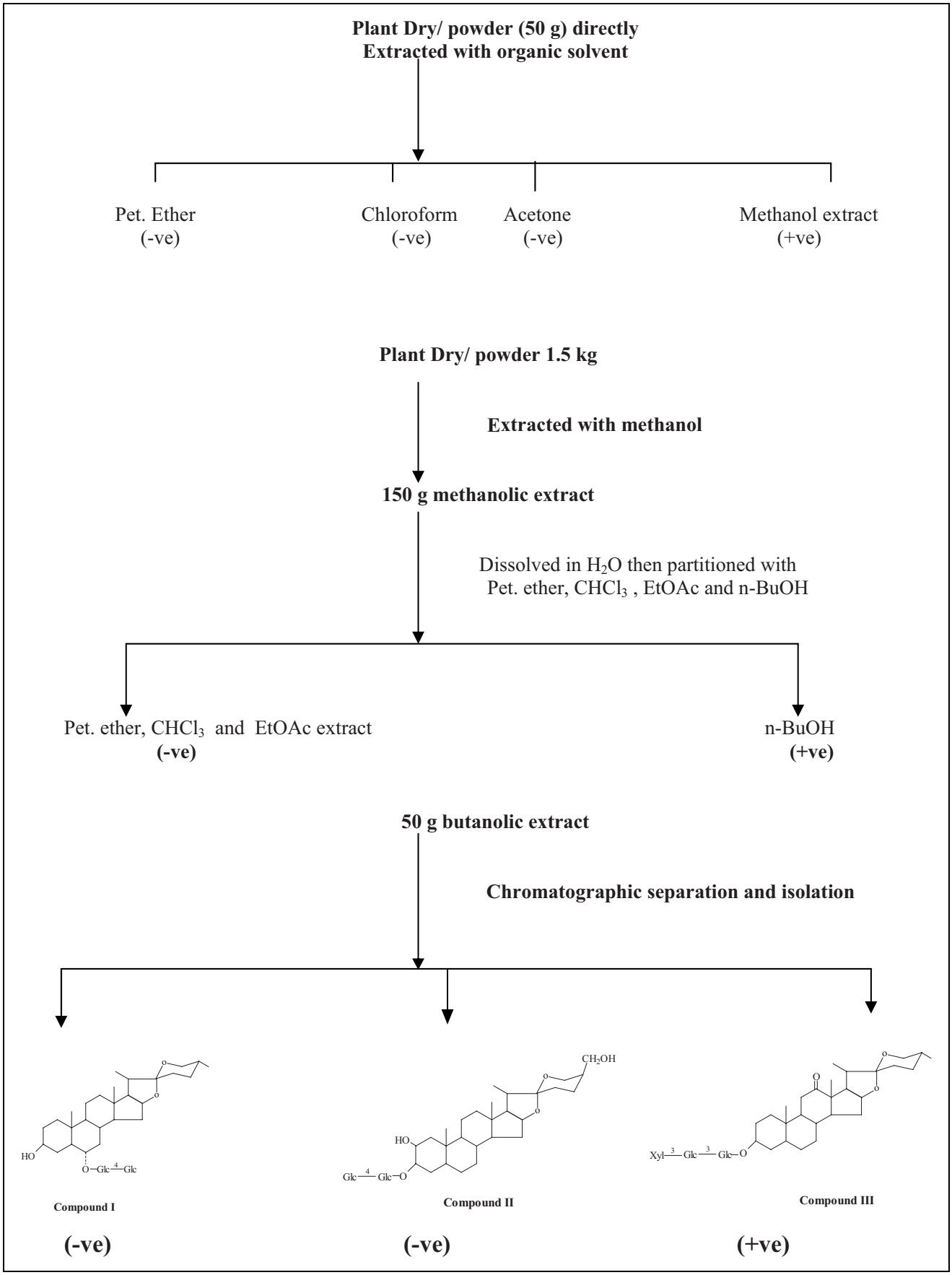

Fig.: Diagram illustrating the bioactivity-guided fractionation and isolation of *F. selloa* compounds against *S. mansoni* worms

respectively). Due to these results a chromatographic separation and isolation of the crude butanolic fraction was carried out leading to isolation of three compounds (**I**–**III**). The isolated compounds were identified guided by the obtained spectroscopic and chemical data as follows:

Compound **I** was obtained as an amorphous powder with the molecular formula $C_{39}H_{64}O_{14}$. This was deduced by appearance of the molecular ion peak [M+H] at m/z 757 in CI-MS spectrum and from the ^{13}C NMR spectrum (Tables 2 and 3) with 39 signals which were divided into 27 carbon signals due to their aglycone part and 12 carbon signals for the two sugar moieties. Compound **I** gave a negative reaction with Ehrlich reagent (Kieroda et al. 2001; Sati and Pant 1985; Mimaki et al. 1991). The glycoside nature of compound **I** was inferred from the strong absorption bands at 3401 and 1069 cm^{-1} in IR spectrum (Kieroda et al. 2001; Mimaki et al. 1991). The ^1H NMR spectrum showed signals for two tertiary methyl groups at δ 0.78 and 0.88 [each, s], two secondary methyl groups at δ 0.71 and 1.12 [each, d] and two anomeric protons at δ 4.80 and 4.88 [each, d], (Sati and Pant 1985; Mimaki et al. 1991). The presence of a disaccharide moiety was indicated by the fragment ion peaks at 757 [M^++H], 595 [M^++H−Glc] and 433 [M^++H−2 Glc] in CI-MS spectrum as well as two characteristic signals of two anomeric carbon signals of the two sugar moieties at δ 99.43 and 104.54 in the ^{13}C NMR spectrum (Mimaki et al. 1991; Yokosuka et al. 2000; Sharma and Sati 1982; Abdel-Gawad et al. 2004). Acid hydrolysis of compound **I** gave a steroidal sapogenin which was identified as chlorogenin by comparing of its IR and ^{13}C NMR spectra with the reported data of original chlorogenin signals (Mimaki et al. 1991; Yokosuka et al. 2000; Sharma and Sati 1982). The disacharide was concluded to be linked to the C-6 hydroxyl position of the aglycone because in the ^{13}C NMR spectrum of compound **I** the signal due to C-6 was shifted to a lower field by 10.76 ppm whereas the signals due to C-5 and C-7 moved to upper fields by 1.96 and 1.72 ppm as compared with those of chlorogenin signals (Kieroda et al. 2001; Mimaki et al. 1991; Yokosuka et al. 2000; Abdel-Gawad et al. 2004b). The structure of compound **I** is based upon a (25*R*)-spirostanol structure, this was suggested by presence of its characteristic bands in the IR spectrum at 921, 898 and 865 cm^{-1} where the intensity of band at 898 was greater than the band at 921 cm^{-1} (Kieroda et al. 2001; Mimaki et al. 1991; Yokosuka et al. 2000). The ^{13}C NMR signals of the disaccharide moiety of compound **I** revealed that C-4 of the inner glucose was shifted down field at δ 80.18 indicating that the terminal glucosyl unit was linked to the inner glucose unit through C4−OH of this glucose unit (Kieroda et al. 2001; Sati and Pant 1985; Mimaki et al. 1991). Therefore, the structure of compound **I** was formulated as 6-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside chlorogenin. This compound did not exhibit any activity against *S. mansoni* worm *in vitro* up to 50 μg/ml.

Compound **II** gave a negative reaction with Ehrlich reagent and showed broad absorption bands in its IR spectrum at 3400 and 1067 cm^{-1} indicating that this compound has spirostanol glycoside structure. Also the characteristic bands of (25*S*)-spirostane steroidal species appeared at 918, 897 and 865 where the intensity of the band at 918 is greater than the band at 897 (Kieroda et al. 2001; Sati and Pant 1985; Mimaki et al. 1991; Yokosuka et al. 2000). The appearing of the molecular ion peak in the CI-MS spectrum at m/z 755 [M^++H] exhibited the molecular weight is 754. Also, the two fragment ion peaks at 594 [M^++H-Glc] and 431 [M^++H−2 Glc] were corresponding to the loss of two glucose units (Sharma and Sati 1982; Abdel-Gawad et al. 2004b). This was supported by two anomeric carbon signals at δ 99.54 and 104.46 in its ^{13}C NMR spectrum (Mimaki et al. 1997; Yahara et al. 1994; Ding et al. 1989; Zou et al. 2001). The ^1H NMR spectrum of compound **II** showed the proton signals attributed to the C-18, C-19 methyl groups at δ 0.75 and 0.85 (each, s) as well as methyl group of C21 at δ 1.12 and C-27 methyl at range between at δ 3.63–3.68

Table 2: ^{13}C NMR spectral data of the aglycone parts of compounds I–III (in DMSO-d_6; TMS as internal standard)

Carbon Number	I	II	III
1	38.76	45.12	37.40
2	30.99	70.24	29.35
3	70.24	84.33	77.60
4	33.40	34.65	34.45
5	49.84	44.50	44.30
6	78.76	27.29	28.50
7	41.18	33.35	31.30
8	34.60	36.20	33.90
9	53.08	53.11	55.80
10	36.20	36.92	35.85
11	20.53	20.55	38.73
12	40.15	40.39	212.40
13	40.43	40.60	55.60
14	55.56	55.53	56.40
15	32.10	31.50	31.70
16	81.40	81.24	79.90
17	63.89	63.20	54.41
18	16.71	16.31	17.12
19	13.19	14.30	11.57
20	42.81	42.20	42.90
21	16.21	14.70	13.43
22	108.41	110.45	108.46
23	31.51	30.73	30.60
24	28.87	23.80	28.50
25	29.88	39.28	29.40
26	66.06	64.96	65.40
27	17.13	65.89	17.20

Table 3: ^{13}C NMR spectral data of the sugar moieties of compounds I–III (in DMSO-d_6; TMS as internal standard)

Carbon Number	I	II	III
	6-O-Glc	3-O-Glc	3-O-Glc
1	99.43	99.54	101.60
2	73.63	73.63	75.60
3	75.43	75.43	87.50
4	80.18	80.47	70.45
5	76.42	76.52	76.25
6	61.22	61.22	61.52
	Glc (1→4) Glc	Glc (1→4) Glc	Glc (1→3) Glc
1	104.54	104.46	102.50
2	76.52	73.93	74.90
3	78.76	78.76	86.60
4	70.30	70.24	69.55
5	76.77	76.77	77.45
6	61.41	61.40	62.30
			Xyl (1→3) Glc
1			103.91
2			75.60
3			78.81
4			71.94
5			67.45

(Ding et al. 1989; Zou et al. 2001) Also, in the ^{1}H NMR spectrum the two anomeric proton signals appeared at δ 4.84 and 4.94 (each, d) representative of the β-configuration of the two sugar units (Yahara et al. 1994; Zou et al. 2001). On comparison between ^{1}H and ^{13}C NMR spectra of compound **II** with those of compounds isolated previously from the leaves of *Digitalis conariensis* and from the seeds of *Allium tuberosum,* it was observed that the aglycone signals of compound **II** were in good agreement with crestagenin (2α,3β,5α,25*S*)-spirostan-2,3,27-triol signals (Zou et al. 2001; Dolgado et al. 1969; Gonzalez et al. 1983). The linkage of the sugar chain was concluded to be at the C-3 hydroxyl position of the aglycone because, in the ^{13}C NMR spectrum of compound **II**, the signal due to C-3 shifted lower field by δ 7.9 whereas the signals due to C-2 and C-4 moved to upper fields by 2.86 and 2.75 ppm as compared with three carbon signals of original crestagenin (Kieroda et al. 2001; Zou et al. 2001). The interglycosidic linkage between the two sugar units of the dissaccharide of this compound was deduced from the down field shift of C-4 of the inner glucose at δ 80.47 in the ^{13}C NMR spectrum (Yahara et al. 1994; Ding et al. 1989; Zou et al. 2001). From the above data, the structure of compound **II** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside crestagenin. This compound did not exhibit any activity against *S. mansoni* worm *in vitro* up to 50 μg/ml.

Compound **III** was crystallized in the form of fine needles from methanol and gave a negative reaction with Ehrlich reagent (Mimaki et al. 1991; Yokosuka et al. 2000). The appearing of the ion peak at m/z 887 [M^++H] in the CI-MS spectrum indicated that the molecular weight of this compound was 886. Also the fragment ion peaks at m/z 412 and 394 were suggestive of a saturated monohydroxyl spirostane nucleus (Abdel-Gawad et al. 2004b; Zou et al. 2001). The IR spectrum exhibited strong absorption bands at 920, 898 and 812 cm^{-1} characteristic for the spirostane steroidal saponins. Weaker intensity of the band 920 cm^{-1} than 898 cm^{-1} showed that the saponin belongs to 25*R* series of spirostanes (Zou et al. 2001; Dolgado et al. 1969; Gonzalez et al. 1983). Also the absorption band at 1705 cm^{-1} in the IR spectrum of compound **III** and a ^{12}C-resonance at δ 212.40 in the ^{13}C NMR confirmed the presence of carbonyl carbon and its position at C-12 (Gonzalez et al. 1983; Debella et al. 1999). Also the ^{13}C NMR spectrum showed signals of 44 carbons, 27 of which arose from the aglycone moiety whereas 17 carbon signals for three sugar units (Abdel-Gawad et al. 2004b; Zou et al. 2001; Dolgado et al. 1969; Gonzalez et al. 1983; Debella et al. 1999; Nakanu et al. 1991). The carbon signals of the aglycone part were in good agreement with those reported in the literature of gloriogenin [(25*R*)-3-β-hydroxy-5-β-spirostan-12-one] (Mimaki et al. 1991; Yokosuka et al. 2000). Fragment ions at m/z 725 [M^++H−Glc], 593 [M^++H−Glc−Xyl] and 431 [M^++H−2 Glc−Xyl] reflected the loss of three sugar units, two of them are glucose units and one is xylose unit (Debella et al. 1999; Xu et al. 2000). The point of attachment of the trisaccharide part with the aglycone part and the interglycosidic linkages between the sugar units were established by ^{13}C NMR spectrum where C-3 of the aglycone part was shifted at downfield at δ 77.60 indicating that the trisccharide was linked with the aglycone part through OH at this carbon. Also, each of C-3 of the two inner glucose units were shifted at down field δ 87.50 and 86.60 reflecting that the two carbons are positions of sugar linkage and the xylose unit is outer sugar unit (Debella et al. 1999; Nakanu et al. 1991; Xu et al. 2000). From the above data, the structure of compound **III** was identified as 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside gloriogenin. Compound **III** at 5 μg/ml led to 100% mortality *S. mansoni* worm *in vitro* (EC_{50} = 2.25 and 1.91 μg/ml for female and male worm respectively after 24 h incubation period).

The results obtained from this study encourage to continue this work with another study on the butanolic extract and the isolated compound. Complete evaluation against all different stages of *S. mansoni* cycle including an *in vivo* study on infected mice is in progress.

3. Experimental

3.1. Equipment

Melting points were determined by a micro melting point apparatus and were uncorrected. IR spectra were measured on a Perkin-Elmer model FT-IR recording spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were done using TMS as internal standard, DMSO-d_6 as solvent and chemical shifts were given in (ppm) scale. Mass spectra were measured on a Finnigan TSQ 700 GC/MS equipped with a Finnigan electrospray source (EI-MS and CI-MS). For detection of sugar, paper chromatography was performed on Whatmann paper No. 1 using descending technique and visualized with aniline phthalate.

3.2. Plant material

Furcraea selloa leaves (Family Agavaceae) was collected from El-Orman Botanical Garden, Giza, Egypt. The plant was identified by Mrs. Traes Labib, general manager and head of specialists of Plant Taxonomy in this Garden. A specimen has been deposited at the laboratory of medicinal chemistry, TBRI. The plant leaves were shade dried and powdered by electric mill.

3.3. Extraction and isolation

1.5 kg of the dried powdered leaves was extracted and fractionated as shown in Fig. 1. Isolation of the crude butanolic fraction of *F. selloa* was carried out using different chromatographic techniques. A glass column (120 × 5 cm) packed with silica gel 60 (70–230 mesh, Merck) as stationary phase was first used. The column was successively eluted with pet. ether followed with $CHCl_3$, ($CHCl_3$: MeOH) mixtures and finally pure methanol. Similar obtained fractions were collected using glass plates coated with silica gel $GF_{245,}$ Merck (TLC). The spots on TLC were visualized by spraying with 40% H_2SO_4 followed by heating in oven at 120 °C. Two main groups of fractions were obtained from different eluent ($CHCl_3$: MeOH) mixtures.

1-Fractions A (4.5 g) collected by elution with $CHCl_3$: MeOH (90 : 10) were washed with acetone. The residual part (500 mg) was subjected to preparative TLC (solvent system $CHCl_3$: MeOH: H_2O; 30 : 10 : 1) to give compounds **I** (112 mg) and **II** (144 mg).

2-Fractions B (3.9 g) collected by elution with $CHCl_3$: MeOH (80 : 30) were purified on Sephadex LH-20 column using methanol as eluent to give compound **III** (971 mg).

Compound **I**: Amorphous powder, m.p. 256–285 °C, R_f 0.65 ($CHCl_3$: MeOH : H_2O; 7 : 3 : 0.5). IR ν_{max} KBr 3401, 3939, 2886, 1641, 1454, 1377, 1069, 921, 898, 865 and 642 [Intensity 898 > 921; 25*R*-spiroketal]. ^{1}H NMR (DMSO-d_6) 0.71 (3 H, d, J = 5.7 Hz, H-27), 0.78 [3 H, s, H-18], 0.88 (3 H, s, H-19), 1.12 (3 H, d, J = 6.8 Hz, H-21), 3.79 (1 H, m, H-3), 3.66 (1 H, m, H-6) 4.80 (1 H, d, J = 7.5 Hz, H-1 of Glc] and 4.88 (1 H, d, J = 7.7 Hz, H-1 of Glc]. CI/MS; m/z 757[M^++H], 595 [M^++H−Glc], 433[M^++H−2 Glc], 415[M^++H−2 Glc−H_2O] and 397[M^++H−2 Glc−2 H_2O]. ^{13}C NMR see Tables 2 and 3.

Compound **II**: Amorphous powder, m.p. 270–272 °C, R_f 0.53 [$CHCl_3$: MeOH : H_2O; 7 : 3 : 0.5]. IR ν_{max} KBr 3400, 2929, 2826, 1649, 1453, 1165, 1067, 918, 897, 865 and 582 [Intensity 0f 918 > 897; 25*S*-spiroketal]. ^{1}H NMR (DMSO-d_6) 0.75 [3 H, s, H-18], 0.85 [3 H, s, H-19], 1.12 (1 H, d, J = 6.8 Hz; H-21], 3.63–3.68 (2 H, dd, J = 0.2, 6.9; H-27], 4.11 [1 H, d, J = 11.50 Hz, H-2]; 4.64 (1 H, m, H-16), 4.84 (1 H,d, J = 7.4 Hz, H-1 of Glc) and 4.94 (1 H, d, J = 7.6 Hz, H-1 of Glc]. CI-MS, m/z 755 [M^++H], 594 [M^++H−Glc], 431 [M^++H−2 Glc], 413, [M^++H−2 Glc−H_2O] and 394 [M^++H−2 Glc−2 H_2O]. ^{13}C NMR see Tables 2 and 3.

Compound **III**: Colorless needles, m.p. 276–278 °C, R_f 0.54 [BuOH : MeOH : H_2O; 4 : 1 : 1]. IR ν_{max} KBr 3407, 2930, 2827, 1705, 1639, 1454, 1072, 920, 898, 812 and 591 [Intensity of 898 > 920; 25*R*-spiroketal]. ^{1}H NMR (DMSO-d_6) 0.76 (3 H, s, H-19) 0.84 (3 H, s, H-18), 1.09 (3 H, d, J = 6.9 Hz, H-27), 1.37 (3 H, d, J = 7.0 Hz, H-21), 4.84 (1 H, d, J = 7.5 Hz, H-1 of Glc), 4.97 (1 H, d, J = 6 Hz, H-1 of Glc) and 5.33 [1 H, d, J = 6.9 Hz, H-1 of Xylose]. CI/MS, m/z 887 [M^++H], 725

$[M^+ + H - Glc]$, 593 $[M^+ + H - Glc - Xyl]$, 431 $[M^+ + H - 2\,Glc - Xyl]$, 412, $[M^+ + H - 2\,Glc - Xyl - H_2O]$ and 394 $[M^+ + H - 2\,Glc - Xyl - 2\,H_2O]$. ^{13}C NMR see Tables 2 and 3.

3.4. Acid hydrolysis

Each of the three compounds (15 mg) was refluxed with 4 N HCl (40 ml) for 4 h on water bath then diluted with water and extracted with chloroform. The chloroform extract was evaporated to dryness and the aglycone parts were detected by TLC against authentic samples. Sugar units were obtained from the aqueous layer of each compound by extraction with anhydrous pyridine. The pyridine layer was evaporated to dryness and dissolved in 10% isopropanol and detected on PC against authentic sugars using system *n*-BuOH : AcOH : H_2O; 4 : 1 : 5 and aniline phthalate as visualizing agent.

3.5. Bioassay procedures

Schistosoma mansoni adult worms used in the *in vitro* test were obtained from the Schistosoma Biological Supply Center (SBSC), Theodor Bilharz Research Institute through perfusion of mice experimentally infected with *S. mansoni* cercariae. The *in vitro* tests of different concentrations of the prepared extracts, crude compounds and the pure isolated compounds were carried out using adult *S. mansoni* worms in culture medium. The medium consisted of RPMI-1640 supplemented with fetal calf serum and sterilizing antibiotics then buffered to pH 7.4–7.5. Concentrations were run in duplicate and 10 adult male and 10 adult female worms were added to each test solution, while negative control contained the media only. All dishes were incubated at 37.2–37.5 °C for 48 h during which the worms motility was microscopically examined after 2, 24 and 48 h (Jiwajinda et al. 2002; Sanderson et al. 2002). The EC_{50} was evaluated using a computerized program "Pharm/PCS" version 4.2 (Pharmacological calculation system).

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