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STEROIDAL GLYCOSIDES OF *FURCRAEA SELLOEA* AND THEIR BIOLOGICAL PROPERTIES AGAINST DIFFERENT *SCHISTOSOMA MANSONI* STAGES

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تم اخضاع اوراق نبات فوركاريا سيلوي (عائلة الصبارات) لعمليات استخلاص تجزيئي مصحوبة باختبار سمية النواتج في كل مرحلة من مراحل التجزئة وذلك لفصل المواد المسئولة عن الفعالية البيولوجية للنبات. كما يلي: اولا تم اجراء اختبار سمية المستخلصات المختلفة للنبات ضد ديدان البلهارسيا المعوية خارج الجسم وذلك بعد زراعة الديدان في وسط ملائم. وظهر ان مستخلص الميثانول هو اقوي المستخلصات فاعلية ضد الديدان حيث سجل نسبة وفيات ١٠٠% عند تركيز ٥٠ ميكروجرام/مل بعد فترة ٢٤ ساعة. ولذلك فقد تمت تجزئة مستخلص الميثانول وبالحصول علي الصابونين الخام من هذا المستخلص وتجربته ضد الديدان اليافعة وصلت سميته الي ١٠٠% عند تركيز ٢٠ ميكروجرام/مل ودلت هذه النتيجة ان فاعلية هذا النبات تتركز في المحتوي الصابونيني كما انه يشكل المكون الرئيسي في هذا النبات. وبتوالي تجزئة الصابونين الخام عن طريق وسائل الفصل الكروماتوجرافي المختلفة مثل العمود الكروماتوجرافي باستخدام السيلكا جيل والسيفادكس وايضا كروماتوجرافي الطبقة الرقيقة والكروماتوجرافي الورقي امكن فصل ثلاثة مركبات صابونينية ستيرويدية. وعن طريق اجراء بعض التحاليل الطيفية و التجارب الكيميائية وتحليل بياناتها فقد تم التعرف علي التركيب الكيميائي الدقيق لكل من هذه المركبات المفصولة. ولتتبع المادة الفعالة فقد جري اختبار سمية كل من هذه المركبات ضد بعض الكائنات المختلفة التي تمثل عدة اطوار من البلهارسيا المعوية. ودلت النتائج ان المركب رقم (٣) هو الفعال ضد الديدان خارج الجسم حتي تركيز منخفض يساوي ٥ ميكروجرام/مل بينما كان المركبين ١و٢ عديمي الفاعلية. وايضا كان المركب (٣) فقط ذو سمية عالية حيث كانت الجرعة القاتلة ل ٩٠% من قواقع بيومفلاريا الكسندرينا = ٦ جزء في المليون. بينما المركبين الاخرين (١و٢) غير فعالين حتي ٥٠ جزء في المليون. كما تم اختبار فاعلية الصابونين الخام وكذلك المركبات المفصولة ضد كل من السركاريا والميراسيديا(الاطوار الحرة للبلهارسيا المعوية). كما لوحظ ان معدل فقس بيض ديدان البلهارسيا وتحوله الي ميراسيديا قد انخفض انخفاضا معنويا كبيرا عندما جري تعريض البيض لتركيز ٦ جزء في المليون من المركب (٣).

وبدراسة معدل عدوي قواقع بيومفلاريا الكسندرينا التي تمت معاملتها بثلاث جرعات تحت مميتة من بودرة النبات (٢٠ و٣٥ و٥٠ جزء في المليون) في فترتين مختلفتين: ٢٤ ساعة قبل التعريض للميراسيديا و٢٤ ساعة بعد التعريض للميراسيديا. وضح ان معدل عدوي القواقع المعاملة بالنبات قد انخفض انخفاضا معنويا شديدا عن معدل عدوي القواقع التي لم تتم معاملتها بالنبات.

وبذلك يتضح من هذه النتيجة ان عملية التجزئة المتتالية للنبات قد زادت من سمية النواتج المتحصل عليها ضد ديدان البلهارسيا والكائنات الاخري المختبرة بدرجة ملحوظة مما يعطي مؤشرا ان عملية التجزئة قد سارت في الطريق الصحيح. ولذلك جري تحديد الجرعات النصف مميتة (LD_{50}) من المستخلص الميثانولي ضد فئران التجارب البيضاء لتحديد سمية النبات وكانت تساوي ٤٦٥٦,٣ مجم/كجم وعند اعطاء الفئران جرعات ٢٥٠٠ مجم/كجم من وزن الفار من المستخلص الميثانولي بعد ٢ او ٤ او٧ أسابيع من العدوي انخفض العدد الكلي للديدان في الفئران المعالجة انخفاضا معنويا عن فئران المجموعة الضابطة. وتوصي هذه الدراسة باجراء مزيد من البحوث علي فاعلية هذا النبات والنباتات المحلية الاخري ربما يمكن التوصل الي عقار نباتي لمرض البلهارسيا.

Furcraea selloea C. Koch dry powder (Family Agavaceae) was subjected to a bioassay-guided fractionation technique to isolate the active constituents responsible for the potency of this plant. The antischistosomal impact of different extracts of the leaves of F. selloea was

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screened against adult Schistosoma mansoni worms in vitro using a 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 hours. Owing to the high potency of the crude saponins obtained from the methanolic extract (100% mortality at 20 μg/ml),it was submitted to chromatographic separation using silica gel and Sephadex columns as well as preparative thin layer chromatography. Three steroidal saponins ***(I-III)*** *were isolated and their structures were elucidated using some spectroscopic and chemical methods as follows: 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**).*

Bioassay screening of the isolated saponins ***(I-III)*** *were carried out against certain Schistosoma mansoni stages. Compound* ***III*** *only proved to possess antischistosomal activity against S. mansoni worms at concentration as low as 5 μg/ml, while compounds I and II were inactive. Also, test against B. alexandrina snails revealed that only saponin* ***III*** *has high molluscicidal activity (LC_{90} = 6 ppm) whereas the other two saponins did not show any activity up to 50 ppm after 24 hours exposure. Different concentrations of the crude and the isolated saponins were evaluated against S. mansoni free larval stages (cercariae and miracidia). Hatchability of S. mansoni ova was markedly depressed when exposed to 6 ppm of compound III. The infection rate of B. alexandrina snails was significantly reduced when snails were exposed to three sublethal concentrations of the dry plant powder. Determination of the acute oral toxicity of F. selloea methanol extract against mice was carried out. When three groups of mice infected with S. mansoni were treated orally with a single dose of 2500 mg/kg of F. selloea methanol extract either at 2, 4 or 7 weeks post infection, the reduction rate in worm load was significantly lower when compared to infected untreated control.*

INTRODUCTION

Intestinal schistosomiasis is caused by the helminth *Schistosoma mansoni* and afflicts millions of people in many tropical and subtropical countries.[1] Control of this disease involves chemotherapy along with molluscicide the water bodies infested with the parasites intermediate host; snails of the genera *Biomphalaria*. Synthetic molluscicides and chemotherapy are relatively expensive and therefore not accessible in the developing countries.[2]

Praziquantel is still the drug of choice for schistosomiasis control, yet in the recent years, a number of reports indicated the apparent failure of the recommended doses of PZQ to yield the expected cure rates in human population of Kenya,[3] Brazil,[4] Senegal[5,6] and Egypt.[7]

Plants have provided a number of useful clinical agents that prove to have considerable potentials as sources of new drugs.[8] 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.[9,10]

Furcraea is a plant genus that contains specific compounds of numerous medicinal purposes. The dry powder of *F. selloea* plant (Agavaceae) was previously reported to possess strong molluscicidal potency against *Biomphalaria alexandrina* snails.[11] So it was thoughtful to continue work on this plant and to test the effect of its extracts on *S. mansoni* worms and to subject this plant to chromatographic technique to isolate and identify some of its constituents. This investigation also reported the impact of the isolated compounds on different *S. mansoni* stages such as snails, the larvae, *S. mansoni* ova and adult worms. The effect of the plant on the infectivity of *S. mansoni* to *B. alexandrina* and on worms *in vivo* was also evaluated.

EXPERIMENTAL

Plant Material

Furcraea selloea C. Koch plant (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. The plant leaves were shade dried and powdered by electric mill.

Organisms and animals

1- *Schistosoma mansoni* adult worms used in the *in vitro* test were obtained from Schistosoma Biological Supply Center (SBSC), Theodor Bilharz Research Institute through perfusion of mice experimentally infected with *S. mansoni* cercariae.
2- *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* were collected from irrigation canals in Giza governorate. Snails (10-12 mm shell diameter) were maintained in laboratory under optimum conditions.
3- *S. mansoni* cercariae and ova were obtained from infected *B. alexandrina* snails and liver of infected mice respectively.
4- Adult male CD-1 Swiss albino mice weighing 20±2 g at the beginning of the experiment were obtained from Theodor Bilharz Research Institute.

Extraction and preparation of crude saponins:

About 50 g of *F. selloea* dry powder was separately extracted using different solvents as reported previously.[11] Also, the crude saponins (50 g) were prepared from the dry plant powder (1.5 Kg) as mentioned in the same study.

Bioassay procedures

***A- In vitro* schistosomicidal activity**

The *in vitro* tests of different concentrations of the prepared extracts, crude saponins 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 tested solution, while negative control worms were kept in the media only. All dishes were incubated at 37.2-37.5° for 48 hr during which the worms motility were microscopically examined after 2, 24 and 48 hr.[12,13]

B- Snails toxicity tests

Different concentrations of the three isolated compounds were prepared and snails were subjected to these molluscicidal concentrations in triplicate for 24 h with a similar recovery period at 25+2°.[14] Negative control snails were conducted at the same experimental conditions. The LC_{50}, confidence limits, LC_{90} and slope functions were calculated following Litchfield and Wilcoxon method.[15]

C- Hatchability test

Duplicate concentrations of the isolated compounds were conducted and supplied with *S. mansoni* ova. The hatchability of these ova was observed under stereomicroscope after different intervals comparing with ova in control test containing distilled water.[16]

D- Cercaricidal and miracidicidal test

Both the cercaricidal and miracidicidal impact of the isolated compounds was evaluated as previously recommended.[17,18] The tested solutions were prepared and supplied with freshly-emitted cercariae and miracidia freshly hatched from *S. mansoni* ova. Moralities of both motionless larvae were observed using stereomicroscope after different observation periods: 1/4, 1/2, 3/4, 1 and 2 hr. Thereafter all larvae were killed by Bouin solution and the cumulative mortality percentages were computed.

E- Snails Infectivity tests:

Biomphalaria alexandrina snails were cleaned thoroughly with dechlorinated tap water then exposed to 6-8 freshly hatched miracidia (1/2-hr age) per snail following the mass infection technique. Groups each of 30 snails were exposed for 24 hours to three sublethal concentrations (20, 35 and 50 ppm) of the dry plant powder in two ways; either one day pre-exposure to miracidia or one day post-exposure to miracidia. Snails were started to be individually examined for infection after 3 weeks by exposing each snail;in a test tube; to direct electric light for 2 hr. Snails shedding cercariae were counted as infected ones, while negative snails were re-examined twice weekly during the following 5 weeks till the end of the experiment (8 weeks). The infection rates were

evaluated as the No. of infected snails to those surviving at the first shedding of cercariae.[19]

F- Acute toxicity of *F. selloea* methanol extract

A pilot trial was conducted using a limited number of animals for determining the acute oral toxicity, 24 mice were divided into four groups each of six mice. These groups received increasing doses of *F. selloea* methanol extract administered orally by gastric intubations from 500 mg/kg to 4000 mg/kg. Animal groups were observed 24 hr after dosing and mortality data were then subjected to computer analysis (PCS Software, were used) for determination of LD_{50}.

G- Antischistosomal activity of *F. selloea* methanol extract

Twenty eight Swiss albino mice were infected with 80 cercariae /mouse of the Egyptian (CD) strain of *Schistosoma mansoni* using the body immersion technique. Guided by the LD_{50} estimation, the extract was given orally in a single dose of 2500 mg/kg. Infected animals were divided into 4 groups, three of them were treated orally with 2500 mg/kg either 2, 4 or 7 weeks post infection, while the last group was infected untreated group (Control). All animal groups were sacrificed 9 weeks post infection. Perfusion of the hepatic and portomesentric vessels were performed to study worm burden according to the procedure outlined by Duval and De Witt.[20]

H- Statistical methods

The LD_{50} (The dose at which died 50% of the mice) was evaluated using a computerized program "Pharm/PCS" Version 4.2 (Pharmacological calculation system) by a plot the number of dead mice versus total number of mice in each group against the dose of the drug administered.

The data obtained were summarized by the arithmetic mean and the standard deviation. Statistical analysis of results was carried out using Students t-test.[21] The degree of significance (probability p-value) was obtained from the corresponding tables.

Fractionation procedures

General

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 (270 MHz) and ^{13}C-NMR (100 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.

Chromatographic isolation of the crude saponins

Fractionation of the crude saponins of *F. selloea* was carried out using different chromatographic techniques. A glass column (120 X 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 together 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°.Two main groups of fractions were obtained from different eluent ($CHCl_3$: MeOH) mixtures.

1- Fractions collected by elution with $CHCl_3$: MeOH (90:10) gave residue (4.5 g) was 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 compound **I** (112 mg) and compound **II** (144 mg).

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

Acid hydrolysis

Each of the three compounds (15 mg) was refluxed with 4N HCl (40 ml) for 4 hours 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 saponin 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.

The Isolated Compounds

Compound 1 : Amorphous powder, m.p 256-285°, R_f 0.65 ($CHCl_3$: MeOH: H_2O; 7: 3: 0.5). IRν_{max} KBr 3401, 3939, 2886, 1641, 1454, 1377, 1069, 921, 898, 865 and 642 [Intensity 898 > 921; 25 R- spiroketal]. ^{1}HNMR (DMSO-d_6) 0.71 (3H, d, J=5.7 Hz, H-27), 0.78 [3H, s, H-18], 0.88 (3H, s, H-19), 1.12 (3H, d, J=6.8 Hz, H-21), 3.79 (1H, m, H-3), 3.66 (1H,m,H-6) 4.80 (1H, d, J=7.5 Hz, H-1 of Glc] and 4.88 (1H, d, J=7.7 Hz, H-1 of Glc]. CI/MS; m/z 757[M^++H], 595 [M^++H-Glc], 433[M^++H-2Glc], 415[M^++H-2Glc-H_2O] and 397[M^++H-2Glc-2H2O].

Compound II: Amorphous powder, m.p 270-272°, 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; 25S-spiroketal]. ^{1}HNMR (DMSO -d_6) 0.75[3H, s, H-18], 0.85 [3H, s, H-19], 1.12 (1H, d, J=6.8 Hz; H-21], 3.63-3.68 (2H, dd, J=0.2, 6.9; H-27], 4.11 [1H, d, J=11.50 Hz, H-2]; 4.64 (1H, m, H-16), 4.84 (1H,d, J=7.4 Hz, H-1of Glc) and 4.94 (1H, 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-2Glc-H_2O] and 394 [M^++H- 2Glc-2 H_2O].

Compound III :colorless needles, m.p 276-278°, 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}HNMR (DMSO-d_6) 0.76 (3H, s, H-19) 0.84 (3H, s, H-18), 1.09 (3H, d, J=6.9 Hz, H-27), 1.37 (3H, d, J=7.0 Hz, H-21), 4.84 (1H, d, J=7.5 Hz, H-1 of Glc), 4.97 (1H, d, J=6 Hz, H-1 of Glc) and 5.33 [1H, d, J=6.9 Hz, H-1of Xylose]. CI/MS, m/z 887 [M^++H], 725[M^++H-Glc], 593 [M^++H-Glc-Xyl], 431 [M^++H-2Glc-Xyl], 412, [M^++H-2Glc-Xyl-H_2O] and 394 [M^++H-2Glc-Xyl -2 H_2O].

RESULTS AND DISCUSSION

In the present study, the well-established bioactivity guided fractionation was followed. This technique involves systematic fractionation of the dry plant powder accompanied by bioassay tests of each systemic subfraction. The promising fractions were subjected to further fractionation, while the least active fractions were ignored or neglected. Fig (1) illustrates these fractionation steps. The same technique was previously used to isolate an active antischistosomic compound.[22]

Bioassay results

In this study the viability of *S. mansoni* adult worms was observed during *in vitro* incubation with various concentrations of the extracts and the isolated compounds. Data showed that the lethal effect was dependent on the chemical concentration and time of incubation.

Gradual concentrations of pet. ether, chloroform, acetone and methanol extracts (Table 1) showed that the first three extracts did not show any effect against worms at 100 µg/ml for 2 and 24 hr. After 48 hr, 100 and 50 µg/ml of pet. ether extract showed a weak schistosomicidal action as almost 20% of the parasites were dead. In negative control, worms were alive and viable till the end of the experimental period.

As to the methanol extract, 100 and 50 µg/ml exhibited 100% mortality in both male and female worms after 24 hr meanwhile at 100 µg/ml, worms began to slow down their motion and crawl after only 2 hr. When the concentration of methanol was lowered to 20 µg/ml, worm mortality was 10% and 20% for female and male after 24 hr; 20 and 40% after 48 hr. At 10 µg/ml worms recorded 10% and 20% mortality for female and male after 48 hr This clearly indicates that the antischistosomal action is restricted to the methanol extract, so further fractionation of methanol extract was carried out and all subfractions were subjected to series of bioassay tests till crude saponin was prepared (Fig. 1).

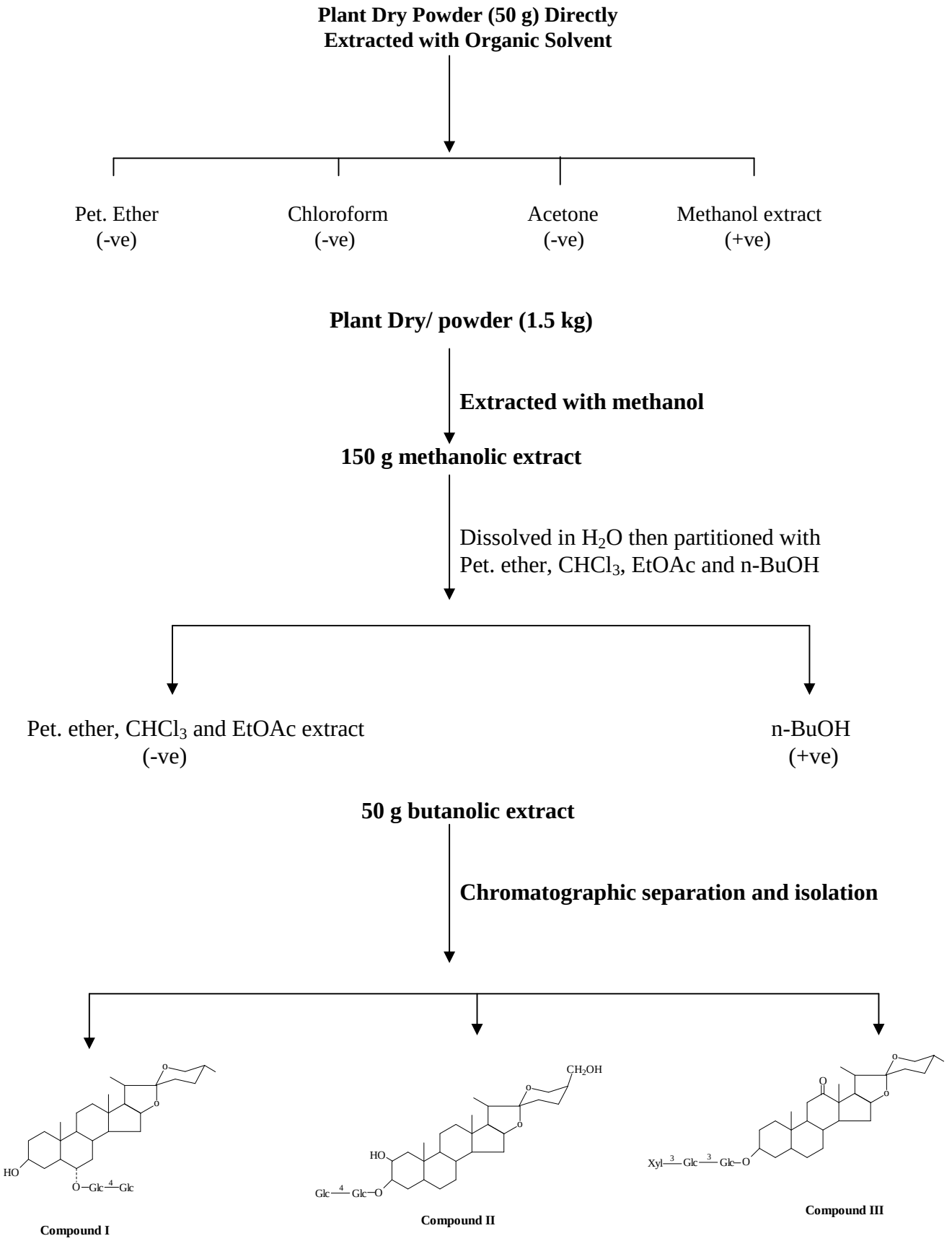

Fig. 1: Diagram illustrating the bioactivity- guided fractionation steps showing the bioassay results of each fraction against *S. mansoni* worms and other stages.

Table 1: Effect of different extracts and crude saponins of *Furcraea selloea* against adult S. *mansoni* worms using *in vitro* method.

Extract	Conc. (µg/ml)	% Mortality of worms after different observation intervals (hrs)					
		2		24		48	
		Female	Male	Female	Male	Female	Male
Pet. ether	100	0	0	0	0	20	30
	50	0	0	0	0	20	20
	20	0	0	0	0	0	0
Chloroform	100	0	0	0	0	0	0 very active
	50	0	0	0	0	0	0
Acetone	100	0	0	0	0	0	0 (weak)
	50	0	0	0	0	0	0
Methanol	100	0	0	100	100	100	100
	50	0	0	100	100	100	100
	20	0	0	10	20	20	40
	10	0	0	0	0	10	20
Crude saponins	100	100	100	100	100	100	100
	50	0	0	100	100	100	100
	20	0	0	100	100	100	100
	10	0	0	40	60	100	100
Control	------	0	0	0	0	0	0

The crude saponins showed a much stronger effect as 100% worms mortality was recorded after being exposed to 100 µg/ml for only 2 hr. Moreover, microscopical observation of worms showed that they started to die from the first hour. Reducing the concentration to 50 then 20 µg/ml, the mortality rate was still 100% after 24 hr. When worms were treated with 10 µg/ml for 24 hr, 40 and 60% mortalities were noticed for female and male worms respectively and 100% in both sexes after 48hr. It is evident from this data that female worms were slightly more resistant to the toxic action of the chemicals as they recorded lower mortality rates. This result agrees well with Sanderson *et al*.[13] on the potency of *Zingiber officinale* extract on both sexes of worms.

As the crude saponins proved to possess higher toxicity against *S. mansoni* worms than methanol extract and fractionation of the methanol extract lead to enhancement of the activity, therefore the potency of this plant could be attributed to the crude saponins which is the principal active component. This guided us to limit the chromatographic separation to the crude saponins and three compounds (**I-III**) were isolated by further purification.

When worms were subjected to 100 µg/ml of saponins **I** and **II** no mortality was noticed (Table 2), so these compounds can be considered inactive. As to saponin **III**, 100 µg/ml of this plant caused complete death of the worms after 2 hr. Microscopical observation showed that worms started to die even after only 1/2 hr. By lowering the concentration to 50 and 20 µg/ml; and thereafter to 10 then 5 µg/ml; worms were totally dead at 24 hr. These results clearly proved that saponins **III** is the compound responsible for the antischistosomal property of the plant. This minimum lethal concentration (5 µg/ml) was markedly lower than concentrations reached by other authors of various botanical compounds against schistosomiasis worms *in vitro*; 200-1600 µg/ml.[12,23,24]

Results on the impact of the three isolated saponins on *B. alexandrina* snails (Table 3) showed that saponins **I** and **II** are totally inactive up to 50 ppm after 24 hr. While saponin **III** recorded higher molluscicidal effect as the LC_{50} and LC_{90} were 4.8 and 6 ppm respectively. This result confirmed the previous study on the molluscicidal action of the extracts and crude saponins of this plant.[11]

Table 2: Effect of the isolated steroidal saponins (**I-III**) against adult *S. mansoni* worms *in vitro*.

Compound	Conc. (µg/ml)	% Mortality of worms after different observation intervals (hrs)					
		2		24		48	
		Female	Male	Female	Male	Female	Male
Saponin **I**	100	0	0	0	0	0	0
Saponin **II**	100	0	0	0	0	0	0
Saponin **III**	100	100	100	100	100	100	100
	50	0	0 (weak motion)	100	100	100	100
	20	0	0	100	100	100	100
	10	0	0	100	100	100	100
	5	0	0	100	100	100	100
Control	-----	0	0	0	0	0	0

Table 3: Effect of the saponins isolated from *Furcraea selloea* on adult *Biomphalaria alexandrina* after 24 hours exposure.

Compound	LC_{50} (ppm) Confidence limits	LC_{90}(ppm)	Slope
Saponin **I**	- ve up to 50 ppm		
Saponin **II**	- ve up to 50 ppm		
Saponin **III**	4.8 (4.391-5.546)	6	1.63

The effect of 50 ppm of saponin **I** and **II** and 6 ppm of saponin **III** was examined against hatchability of *S. mansoni* ova after different intervals (Table 4). Data showed that saponins **I** and **II** significantly reduced the hatchability of *S. mansoni* ova ($P< 0.05$) till 3/4 hr. However after 1 hr, the reduction was insignificant from that of the control ($P> 0.05$). Observation of the hatched miracidia showed that their movements started to slow down by 3/4 hr. As to ova exposed to 6 ppm of saponin **III**, a very minor ratio of ova (5%) hatched till the end of the observation period. This value is very significantly lower from the control ($P < 0.001$) and the dark color of the dead embryo can easily be noticed inside the eggs. While 80% of the control eggs hatched by the end of the observation period and the hatched miracidia swim very rapidly.

Very minor literatures reviewed the effect of isolated botanical compounds on larvae of schistosomiasis.[25-26] In this study the effect of crude saponins and compound **III** on both larvae was remarkable (Tables 5 and 6) as 50 ppm of crude saponins killed 100% and 65% of miracidia and cercariae respectively. Meanwhile 6 ppm of saponin **III** caused 100 and 79% death of both larvae respectively and this ratio was significantly variable from control. These results agree well with Lyddiard *et al*[27] who reported on numerous biological potencies as antischistosomal, miracidicidal and molluscicidal bioactivity of isoflavonoids isolated from *Millettis thonningii*. From this data it can also be concluded that miracidia and cercariae were more susceptible to the tested saponins than the snails and this conclusion is in good accordance with other reported studies.[28]

The interesting finding about this plant is that it affects many stages of the parasite throughout the whole life cycle of schistosomiasis. So studying the effect of the dry plant powder on infection rate of snails was

Table 4: Effect of the isolated saponins on the hatchability of *S. mansoni* eggs.

Compound Conc. (ppm)	% Percent of *S. mansoni* eggs hatched after different intervals and motion of miracidia			
	1/4	1/2 h	3/4 h	1 h
Saponin **I** (50 ppm)	20*%	30*%	37*%, started to slow	60%, motion slowed
Saponin **II** (50 ppm)	25*%	50%	50*%, started to slow	70%, motion slowed
Saponin **III** (6 ppm)	5***%	5***%	5***%, Embryo dead and opaque	5***%, embryo dead and opaque
Control	40%	60%	80%, motion very fast	80%, motion very fast

Table 5: Effect of the crude saponins and the isolated compounds (**I-III**) against miracidia of *S. mansoni*.

Compound / Conc. (ppm)	% Mortality of miracidia after different intervals (hrs)				
	1/4	1/2	3/4	1	2
Crude saponins (50 ppm)	31***	48**	66***	69***	100***
Saponin **I** (50 ppm)	5	6	7	8	8
Saponin **II** (50 ppm)	6	7	8	9	10
Saponin **III** (6 ppm)	27***	43***	49***	56***	100***
Control	3	5	7	8	9

Table 6: Effect of the crude saponins and the isolated compounds (**I-III**) against cercariae of *S. mansoni*.

Compound / Conc. (ppm)	% Mortality of cercariae after different intervals (hrs)				
	1/4	1/2	3/4	1	2
Crude saponins (50 ppm)	17***	25**	33**	47***	65***
Saponin **I** (50 ppm)	5	7	7	11	12
Saponin **II** (50 ppm)	4	5	8	10	13
Saponin **III** (6 ppm)	16***	28**	41***	58***	79***
Control	4	6	7	9	11

*P< 0. 05, ** p< 0.01, ***p< 0.001

recommended. Snails exposed to 20, 35 and 50 ppm of the dry plant powder 1-day pre-exposure to miracidia showed significant reduction in the ratio of infected snails. Meanwhile the infection rates of snails exposed to the same concentrations I day post-exposure to miracidia was more significantly depressed. This suppression in infection rates of snails treated with sublethal concentrations of the plant was inagreements with studies carried out on other molluscicidal plants.[29,30] Moreover, El-Ansary *et al*.[31] reported that this depression in infection rate could be attributed to the inhibition in some enzymes with the subsequent disturbance of the glycolytic pathways responsible for the success of *B. alexandrina* sporocyst interaction (Table 7).

As to the acute toxicity of the methanol extract of the plant, the value of LD_{16} was 627.4 mg/kg, LD50 was 4656.3 mg/kg and LD_{84} was 34555.8 mg/kg (Table 8). These high values proved the relative safety of this extract towards mammals, so the *in vivo* antischistosomal impact of it was studied.

Mice infected with *S. mansoni* were treated with *F. selloea* methanol extract either 2,4 or 7 weeks post infection which represents early immature, late immature and mature infection. The three groups received a single dose of 2500 mg/kg orally and showed a highly significant decrease in worm load at $p< 0.01$ and $p < 0.001$. The percents of reduction were 31.8, 27.8 and 31.4 for the three groups respectively (Table 9). It can also be noticed the high significant decrease in the total No. of coupled worms compared with the control. This means that the extract enhanced the separation of coupled worms which may lead to subsequent depression in No. of egg count. These results confirmed those obtained on the impact of the methanol extract on *S. mansoni* worms *in vitro* and is considered a great privilege of this plant over other plants. The bioactivity of Ginger ethyl acetate extract was determined *in vitro* at concentration of 200 g/ml, while oral and subcutaneous delivery of this extract produced no significant reduction in worm numbers.[13]

Table 7: Effect of sublethal concentrations of *Furcraea selloea dry* powder on infectivity of *B.alexandrina* snails with *S. mansoni* miracidia.

Plant treatment	Plant con. (ppm)	No. of exposed snails	Snails surviving first shedding		Infected snails	
			No.	%	No.	%
Control	-	30	28	93.3	23	82.1
1 day pre-exposure to miracidia	20	30	26	86.7	19	73.1*
	35	30	23	76.7*	17	73.9*
	50	30	21	70.0*	15	71.4*
1 day post-exposure to miracidia	20	30	25	83.3	13	52.0**
	35	30	24	80.0	10	41.7***
	50	30	20	66.7**	6	30.0***

*P< 0. 05, ** p< 0.01, ***p < 0.001

Table 8: Acute toxicity of *F. selloea* methanol extract on albino mice.

Dose	No of animals	No of mortality	% Mortality	Calculated LD
500 mg/kg	6	1	16.66	LD_{16}=627.4 mg/kg LD_{50} =4656.3 mg/kg LD_{84}=34555.8 mg/kg
1000 mg/kg	6	1	16.66	
2000 mg/kg	6	2	33.33	
4000 mg/kg	6	3	50.00	

Table 9: Effect of F. selloea extract on worm load in mice infected with *S mansoni* and sacrificed 9 weeks post infection.

Animal group (No of animals)	Dose	Worm count			Total No. of Worms X±SE	% Reduction
		No. of Males X±SE	No. of Females X±SE	No. of Couples X±SE		
Untreated control (10)	-	7.4±0.4	5.1±1.1	6.0±0.5	24.5±0.92	
2 weeks post infection (6)	2500 mg/kg	6.3±1.5	3.5±0.6	3.5±0.6 **	16.7±1.2 ***	31.8%
4 weeks post infection (6)	2500 mg/kg	6.2±1.3	2.8±1.0	4.3±1.1	17.7±1.4 **	27.8%
7 weeks post infection (6)	2500 mg/kg	3.5±1.0	8.2±1.7	2.5±0.7 **	16.8±1.3 ***	31.4%

*Significant differences versus untreated control **p < 0.01, ***p < 0.001

Finally it can be concluded that these preliminary results on the action of this plant extract in reducing worm load is promising and needs a follow up comprehensive studies on this plant and other species for the hope of reaching a botanical, safe and potent antischistosomal drug.

Fractionation and purification results

The isolated compounds were identified guided by the obtained spectroscopic and chemical data as follows:

Saponin I, was obtained as an amorphous solid 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 10 and 11) with 39 signals which were divided into 27 carbon signals due to aglycone part and 12 carbon signals for the two sugar moieties. Saponin **I** gave a negative reaction with Ehrlich reagent.[32-34] The glycoside nature of saponin **I** was obtained from the strong absorption bands at 3401 and 1069 cm^{-1} in IR spectrum.[32,33]

The ^{1}HNMR 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].[33,34] The presence of disaccharide moiety was indicated by appearing 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

Table 10: ^{13}C-NMR spectral data of the aglycone parts of saponins **I-III** (In DMSO-d_6; TMS as internal standard).

Carbon No.	Saponin **I**	Saponin **II**	Saponin **III**
1	38.67	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 11: ^{13}C-NMR spectral data of the sugar moieties of saponins **I-III** (In DMSO-d_6; TMS as internal standard).

Carbon No.	Saponin **I**	Saponin **II**	Saponin **III**
	-60-Glc	-30-Glc	-30-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
			Xy(1⟶3) Glc
1			103.91
2			75.60
3			78.81
4			71.94
5			67.45

characteristic signals of two anomeric carbon signals of the two sugar moieties at δ 99.43 and 104.54 in ^{13}C-NMR spectrum.[34-37]

Acid hydrolysis of saponin **I** gave 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.[34-36] The dissacharide was concluded to be linked to the C-6 hydroxyl position of the aglycone because in the ^{13}C-NMR spectrum of saponin **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.[32,34,35,37]

The structure of saponin **I** based upon a (25R)-spirostanol derivatives, 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}.[32,34,35] The ^{13}C-NMR signals of the disaccharide moiety of saponin **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.[32-34] Therefore, the structure of saponin **I** was formulated as 6-O-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside chlorogenin.

Saponin 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 saponin has spirostanol glycoside structure. Also the characteristic bands of 25 S spirostane steroidal species were appeared at 918, 897 and 865 where the intensity of the band at 918 is greater than the band at 897.[32-35] The appearing of the molecular ion peak in CI-MS spectrum at m/z 755 [M$^+$+H] exhibited the molecular weigh is 754. Also, the two fragment ion peaks at 594 [M$^+$+H-Glc] and 431 [M$^+$+H-2Glc] were corresponding to the loss of two glucose units.[36,37] This was supported by appearing of

two anomeric carbon signals at δ 99.54 and 104.46 in its ^{13}C-NMR spectrum.[38-40] The ^{1}HNMR spectrum of saponin **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.[40,41] Also, in ^{1}HNMR 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.[39,41]

On comparison between^{1} H and^{13} C-NMR spectra of saponin **II** with those of saponin isolated previously from the leaves of *Digitalis conaripnsis* and from the seeds of *Allium tuberosum*, it was observed that the aglycone signals of saponin **II** were in good agreement with crestagenin (2α, 3β, 5α, 25 S)- spirostan-2, 3, 27-triol signals.[41-43] 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 saponin **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.[32,41]

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.[39-41] From the above data, the structure of saponin **II** was elucidated as 3-O-β-D-glucopyranosyl-(1→4)-β-D-gluco-pyranoside crestagenin.

Saponin III, was crystallized in the form of fine needles from methanol and gave a negative reaction with Ehrlish reagent.[34,35] The appearing of the ion peak at m/z 887 [M^{+}+H] in the CI-MS spectrum indicating that the molecular weight of this saponin was 886. Also the fragment ion peaks at m/z 412 and 394 were suggestive of a saturated monohydroxyl spirostane nucleus.[37,41] The IR spectrum exhibited strong absorption bands at δ 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 **III** belongs to 25R series of spirostanes.[41-43] Also the absorption band at 1705 Cm^{-1} in the IR spectrum of saponin **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.[43-44] Also ^{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.[37-44] The carbon signals of aglycone part were in good agreement with those reported in the literature of gloriogenin [(25 R) - 3 - β - hydroxy- 5 β spirostan-12-one].[1,3,14] Fragment ions at m/z 725 [M^{+}+H-Glc], 593 [M^{+}+H-Glc-Xyl] and 431 [M^{+}+H-2Glc- Xyl] reflected the loss of three sugar units, two of them are glucose units and one is xylose unit.[44,46]

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, 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.[44-46] From the above data, the structure of saponin **III** was identified as 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyrano-side gloriogenin.

We can finally conclude that in this study, the activity guided isolation of saponins of *F. selloea* lead to separation of a steroidal saponin compound (**III**) that accounts in part, for the observed antischistosomal and other biological potentials of this plant.

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