Phytochemical and Molluscicidal Investigations of *Fagonia arabica*

Eman A. El-Wakil

Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute (TBRI), Warrak El-Hader, Egypt. Fax: 2025408125. E-mail: e_elwakil@hotmail.com

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The aqueous methanolic extract of the aerial parts of *Fagonia arabica* L. (family Zygophyllaceae) was successively fractionated using certain organic solvents. From the ethyl acetate fraction, two flavonoid glycosides were isolated and identified as kaempferol-7-*O*-rhamnoside and acacetin-7-*O*-rhamnoside. Four triterpenoidal glycosides were isolated from the butanolic layer. Their structures were elucidated on the basis of the spectral and chemical data as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside oleanolic acid (**1**), 3-*O*-α-L-arabinopyranosyl quinovic acid 28-*O*-β-D-glucopyranoside (**2**), 3-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→3)-α-L-arabinosyl oleanolic acid (**3**) and 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabino-pyranosyl quinovic acid 28-*O*-β-D-glucopyranoside (**4**). The two monodesmosidic saponins **1** and **3** were found to possess strong molluscicidal activity against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt (LC_{90} = 13.33 and 16.44 μM), whereas the other two bidesmosidic saponins **2** and **4** as well as the two flavonoid glycosides were inactive up to 50 μM.

Key words: Fagonia arabica, Flavonoids, Triterpenoidal Glycosides, Molluscicides

Introduction

Schistosomiasis is among the most important health problems in many countries. Control of this disease can be achieved by eradication of its snail vectors (Ceplean *et al*., 1994; Ndamba *et al*., 1994). Molluscicidal activity has been observed in numerous plant families and attributed to several major classes of natural products including saponins, alkaloids, sesquiterpenes and other terpenes (Ceplean *et al*., 1994; Diallo *et al*., 2001). In a previous study, it has been found that the aqueous suspension of the aerial parts of *Fagonia arabica* has molluscicidal activity against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt (El-Wakil, 2001). This finding prompted us to investigate its chemical constituents. On the other hand, previous phytochemical studies on the genus *Fagonia* have led to the isolation of several types of compounds such as saponins, flavonoids, diterpenes and triterpenes which are regarded as characteristic constituents of plants of the genus *Fagonia* (Shaker *et al*., 1999; El-Negoumy *et al*., 1986; Abdel Khalik *et al*., 2000; Gedara *et al*., 2003; Miyase *et al*., 1996a). Therefore, this paper describes the isolation and characterization of some triterpenoidal and flavonoidal glycosides as well as the evaluation of the plant extracts and the isolated compounds as molluscicidal agents against *B. alexandrina* snail.

Experimental

General

Melting points (uncorrected) were measured using a digital melting point apparatus (Electrothermal IA 9200). ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD containing TMS as internal standard on a Bruker Avance 400 spectrometer equipped with a 5 mm normal configuration $^{13}C\{^{1}H\}$ probe with standard sequences operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Mass spectra were recorded using an Agilent G 1978A dual ESI and APCI spectrometer. Preparative HPLC was done on a Waters Model 590 pump with refractive index detector (RID 6A, Shimadzu) and separating using a reversed phase column, Lichroprep® RP-18 (5 μm, 250 × 21.2 mm). Different sizes of open glass chromatographic columns packed with silica gel 60 (70–230 mesh, Merck), polyamide 6S and Sephadex LH-20 (Sigma) were used. TLC for saponin compounds was performed over pre-coated silica plates (GF_{254}, Merck) and the spots were visualized by spraying with 40% sulphuric acid/methanol reagent followed by heating the plate at 110 °C for 15 min. Paper chromatography was carried out using Whatman No 1 and 3 paper for flavonoid compounds.

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Plant material

Fagonia arabica L. (family Zygophyllaceae) was collected from Suez-Cairo road during April 2004. The plant was identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt. The aerial parts of the plant were air-dried and ground to fine powder. A voucher specimen was kept at the laboratory of Medicinal Chemistry, TBRI, Egypt.

Extraction and isolation

The dry powder of the aerial parts of *F. arabica* L. (2.5 kg) was extracted with 80% methanol three times at room temperature. The methanolic extract was evaporated under reduced pressure to give a gummy residue (180 g). This residue was defatted with petroleum ether. The defatted matter (169 g) was dissolved in a small amount of water and successively extracted with chloroform, ethyl acetate and *n*-butanol affording 7, 10 and 49 g, respectively. The ethyl acetate extract was chromatographed on Whatman No 1 paper using the solvent system 15% acetic acid and *n*-BuOH/AcOH/H_2O, 4:1:5, v/v/v. The chromatograms were examined under both visible and UV light (254 and 366 nm) before and after exposure to the ammonia reagent. 10 g of the ethyl acetate extract were chromatographed over a polyamide open glass column using a gradient mixture of water and methanol. Two major fractions were collected guided by PC (solvent system *n*-BuOH/AcOH/H_2O, 4:1:5, v/v/v). Each fraction was passed through a Sephadex LH-20 column eluted with methanol. Two flavonoid compounds were purified using preparative paper chromatography (solvent system *n*-BuOH/AcOH/H_2O, 4:1:5, v/v/v and 15% acetic acid). The identification of the two flavonoids was confirmed by UV and ^{1}H NMR spectra guided by review of literature data of these kinds of compounds. 40 g of the *n*-butanolic fraction were chromatographed on a silica gel column using a gradient of methanol in $CHCl_3$ as eluent to give two major fractions, A and B. Each fraction was rechromatographed over a Sephadex LH-20 open column using pure methanol as eluent. The major fractions from the Sephadex columns were purified through preparative HPLC [reversed phase C18 column, isocratic elution (65 and 70% MeOH/H_2O) at flow rate 6 ml/min (with refractive index detection)]. Four saponin compounds were obtained, **1**–**4**.

Acid hydrolysis of saponin compounds

Each saponin (10 mg) was refluxed with 4 M HCl (30 ml) for 4 h. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was evaporated to dryness and the aglycone part was identified by TLC analysis comparing with authentic samples in each case using the solvent system C_6H_6/MeOH (8:2, v/v). The aqueous layers were neutralized with $NaHCO_3$, filtered, concentrated and compared with standard sugars on PC using the solvent system *n*-BuOH/AcOH/H_2O, 4:1:1, v/v/v).

3-O-β-D-Glucopyranosyl-(1→3)-α-L-arabinopyranoside oleanolic acid (saponin **1**): Amorphous powder; m.p. 214–216 °C. – R_f = 0.79 (*n*-BuOH/MeOH/H_2O, 4:1:1, v/v/v). – IR: ν_{max} = 3399 (OH), 1699 (COOH), 1664, 1388, 1075 and 815 cm^{-1}. – ^{1}H NMR (CD_3OD): δ = 0.79–1.23 (7 Me groups), 4.87 (1H, d, J = 7.2 Hz, Ara, H-1),

Table I. ^{13}C NMR spectral data of aglycone moieties of saponins **1**–**4** (CD_3OD as NMR solvent and TMS as internal standard).

C	1	2	3	4
1	38.60	39.01	38.55	39.09
2	26.40	26.88	26.20	26.08
3	88.02	89.74	88.50	88.02
4	39.71	40.61	38.90	39.09
5	55.69	55.43	55.75	55.30
6	18.20	18.60	18.31	18.01
7	32.90	36.30	32.88	37.03
8	38.95	40.22	39.02	39.09
9	47.80	46.86	47.94	47.02
10	36.95	36.46	36.78	36.86
11	23.60	23.21	23.40	23.01
12	122.50	128.96	122.30	128.98
13	143.90	132.38	143.60	132.67
14	42.01	55.85	41.79	56.30
15	27.89	25.63	27.70	25.13
16	23.40	26.08	23.30	26.08
17	46.30	48.01	46.40	48.05
18	41.82	54.04	41.90	54.25
19	46.01	38.34	45.98	37.16
20	30.89	36.61	30.85	38.75
21	34.02	30.13	34.06	29.95
22	32.69	35.80	33.40	36.01
23	28.50	27.92	28.01	27.86
24	16.06	16.54	16.90	16.04
25	15.20	16.24	15.30	16.55
26	17.03	20.18	17.15	18.86
27	26.12	177.57	26.50	177.91
28	179.60	176.36	179.50	176.05
29	33.20	13.36	33.40	17.91
30	23.40	21.07	23.80	21.05

4.94 (1H, d, J = 7.7 Hz, Glc) and 5.25 (1H, t, J = 3.1 Hz, H-12). – ESI-MS (negative ion mode): m/z = 749 $[M-H]^-$, 587 $[M-H-Glc]^-$ and 455 $[M\text{-}H\text{-}Glc\text{-}Ara]^-$. – ^{13}C NMR: see Tables I, II.

3-O-α-L-Arabinopyranosyl quinovic acid 28-O-β-D-glucopyranoside (saponin **2**): White powder; m.p. 240–242 °C. – R_f = 0.70 (n-BuOH/MeOH/H_2O, 4:1:1, v/v/v). – IR: ν_{max} = 3400, 2928, 1693, 1460, 1076 and 840 cm^{-1}. – ^1H NMR (CD_3OD): δ = 0.74–1.22 (6 Me groups), 4.77 (1H, d, J = 7 Hz, Ara, H-1), 5.20 (1H, d, J = 7.9 Hz, ester Glc H-1) and 5.25 (1H, t, J = 3.1 Hz, H-12). – ESI-MS (negative ion mode): m/z = 779 $[M\text{-}H]^-$, 735 $[M\text{-}H\text{-}CO_2]^-$, 573 $[M\text{-}H\text{-}CO_2-Glc]^-$ and 441 $[M\text{-}H\text{-}CO_2\text{-}Glc\text{-}Ara]^-$. – ^{13}C NMR: see Tables I, II.

3-O-[β-D-Glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl oleanolic acid (saponin **3**): Amorphous powder; m.p. 260–262 °C. – R_f = 0.66 (n-BuOH/MeOH/H_2O, 4:1:1, v/v/v). – IR: ν_{max} = 3402, 2929, 1696, 1460, 1388, 1070 and 860 cm^{-1}. – ^1H NMR (CD_3OD): δ = 0.75–1.24 (7 Me groups), 4.78 (1H, d, J = 7 Hz, Ara, H-1), 4.88 (1H, d, J = 7.8 Hz, Glc, H-1), 4.92 (1H, d, J = 7.9 Hz, Glc, H-1) and 5.25 (1H, t, J = 3 Hz, H-12). – ESI-MS (negative ion mode): m/z = 911 $[M\text{-}H]^-$, 749 $[M\text{-}H\text{-}Glc]^-$, 586 $[M\text{-}H\text{-}2Glc]^-$ and 455 $[M\text{-}H\text{-}2\ Glc\text{-}Ara]^-$. – ^{13}C NMR: see Tables I, II.

3-O-β-D-Glucopyranosyl-(1→3)-α-L-arabinopyranosyl quinovic acid 28-O-β-D-glucopyranoside (saponin **4**): Amorphous powder; m.p. 267–269 °C. – R_f = 0.58 (n-BuOH/MeOH/H_2O, 4:1:1, v/v/v). – ^1H NMR (CD_3OD): δ = 0.78–1.24 (6 Me groups), 4.76 (1H, d, J = 7 Hz, Ara, H-1), 4.85 (1H, d, J = 7.7 Hz Glc, H-1), 5.30 (1H, d, J = 7.8 Hz, ester Glc, H-1) and 5.23 (1H, t, J = 3 Hz, H-12). – ESI-MS (negative ion mode): m/z = 941 $[M\text{-}H]^-$, 897 $[M\text{-}H\text{-}CO_2]^-$, 779 $[M\text{-}H\text{-}Glc]^-$, 735 $[M\text{-}H\text{-}Glc\text{-}CO_2]^-$, 573 $[M\text{-}H\text{-}2Glc\text{-}CO_2]^-$, 441 $[M\text{-}H\text{-}2Glc\text{-}Ara\text{-}CO_2]^-$ and 397 $[M\text{-}H\text{-}2Glc\text{-}Ara\text{-}2CO_2]^-$. – ^{13}C NMR: see Tables I, II.

Molluscicidal assay

Biomphalaria alexandrina snails, the intermediate host of *Schistosoma mansoni* in Egypt, were collected from the canals in Abu-Aawash, Giza Governorate. The snails were maintained in dechlorinated tap water under laboratory conditions

Table II. ^{13}C NMR spectral data of sugar moieties of saponins **1**–**4** (CD_3OD as NMR solvent and TMS as internal standard).

C	**1**	**2**	**3**	**4**
	3-O-Ara	3-O-Ara	3-O-Ara	3-O-Ara
1	105.01	104.61	103.65	104.01
2	71.80	73.19	75.80	71.80
3	82.60	74.50	83.20	82.89
4	68.10	69.01	68.97	68.50
5	65.45	66.07	65.80	65.80
	Glc1→3 Ara		Glc1→2 Ara	Glc1→3 Ara
1	104.50		104.20	103.01
2	75.40		75.60	75.41
3	78.63		78.30	78.30
4	71.50		71.98	70.61
5	78.30		78.01	78.01
6	62.10		62.30	62.06
			Glc1→3 Ara	
1			104.61	
2			75.03	
3			78.50	
4			71.70	
5			78.20	
6			62.40	
		28-O-Glc		28-O-Glc
1		95.06		95.03
2		74.01		73.60
3		78.70		78.50
4		71.30		71.02
5		79.02		78.18
6		62.04		62.08

[(25 ± 2) °C, pH 7–7.7]. Series of dilutions of extracts or isolates were prepared to calculate each of LC_{50} and LC_{90}. Ten snails were added at each concentration and the exposure time was 24 h followed by 24 h as recovery period. Three replicates were carried out for each case. Procedures and statistical analyses of data were carried out according to WHO (1965) and Litchfield and Wilcoxon procedures (1949).

Results and Discussion

The methanol extract of the aerial parts of *F. arabica* showed molluscicidal activity against *B. alexandrina* (LC_{90} = 85 ppm). Therefore, the aqueous defatted methanolic extract was successively fractionated by chloroform, ethyl acetate and *n*-butanol. The ethyl acetate extract was chromatographed and purified using a combination of polyamide 6S and Sephadex LH-20 column chromatography and preparative paper chromatography affording two flavonoid compounds. The two flavonoid compounds were identified using UV and ^{1}H NMR spectra guided by review of literature data of these compounds.

Flavonoid A: The substance was obtained as a yellow powder. Its UV spectrum showed all the absorption bands which are similar to kaempferol but the bathochromic shift in band II was not recorded after addition of sodium acetate. This indicated the presence of substitution at C-7 (Barakat *et al.*, 1991; Adel-Wahab *et al.*, 1990). ^{1}H NMR spectrum of this compound showed the characteristic signals of H-2′ and H-6′ at 8.14 (2H, d, J = 8.6 Hz), H-3′ and H-5′ at 6.86 (2H, d, J = 8.6 Hz), H-6 at 6.42 (1H, d, J = 2.5 Hz), H-8 at 6.90 (1H, d, J = 2.5 Hz), H1″ of rhamnose at 5.14 (1H, d, J = 2.5 Hz) and CH_3 of Rha at 1-H (3H, d, J = 6 Hz). The downfield of H-6 and H-8 at 6.42 and 6.90 like in case of kaempferol (at 6.18 and 6.40) indicated 7-OH substitution (Adel-Wahab *et al.*, 1990; Saleh *et al.*, 1990; Mabry *et al.*, 1970). Thus, this compound was assumed to be kaempferol-7-*O*-rhamnoside. This compound did not show any activity as molluscicidal agent up to 50 μM.

Flavonoid B: The UV spectrum of the second flavonoid showed absorption maxima characteristic of a 5-hydroxy flavone and gave a bathochromic shift with $AlCl_3$ (Barakat *et al.*, 1991; Mabry *et al.*, 1970). The ^{1}H NMR spectrum showed the A-ring protons C-6 and C-8 separately as doublets (d, J = 2.5 Hz) at 6.21 and 6.40, respectively. The B-ring protons H-3′ and H-5′ and H-2′, H-6′ appeared at 6.84 and 7.7 (d, J = 8.5 Hz). H-3 appeared as singlet at 6.38, C4–OMe appeared as singlet at 3.82. H-1″ of the rhamnosyl appeared at 4.8 (1H, d, J = 2.5 Hz) and CH_3 of the rhamnosyl unit appeared at 1.12 (3H, d, J = 6 Hz) (Mabry *et al.*, 1970; Chatterjee *et al.*, 1981). By comparing this data with the literature, this compound was found to be acacetin-7-*O*-rhamnoside. This compound did not show any activity as molluscicidal agent up to 50 μM.

The butanolic fraction (40 g) was chromatographed and purified using a combination of silica gel and Sephadex LH-20 column chromatography and reversed phase (C18) preparative HPLC. Four triterpenoidal glycosides were isolated and their structures were elucidated using IR, ^{1}H NMR, ^{13}C NMR and ESI-MS as well as they were identified of the products of acid hydrolysis of each saponin.

Saponin **1** was obtained as amorphous powder. Its negative ESI mass spectrum exhibited $[M-H]^-$ at *m/z* 749. The fragment ions at *m/z* 587 $[M-H-Glc]^-$ and 455 $[M-H-Glc-Ara]^-$ suggested that the inner sugar unit is arabinose directly attached to the aglycone whereas the outer sugar unit is glucose (Ye *et al.*, 2000; Tain *et al.*, 1993; Zhong *et al.*, 2001). Acid hydrolysis of saponin **1** gave an aglycone that was identified as oleanolic acid by comparison with an authentic sample as well as L-arabinose and D-glucose as sugar moieties. The ^{1}H and ^{13}C NMR spectra of this compound exhibited the signals due to a triterpene aglycone at δ values that were in good agreement with literature data of oleanolic acid (Zhong *et al.*, 2001; Carvalho and Braz-Filho, 1999). Moreover, the ^{1}H NMR spectrum of saponin **1** showed the presence of two anomeric protons at δ 4.87 (d, J = 7.2 Hz) and 4.94 (d, J = 7.7 Hz) and also of two anomeric carbon signals at δ 105.01 and 104.50 (Tain *et al.*, 1993; Zhong *et al.*, 2001). The downfield shift of C-3 of the aglycone at δ 88.02 reflected that this carbon atom is attached of the sugar moiety. Two absorption bands of hydroxy and carboxylic groups appeared at 3399 and 1699 cm^{-1} (Amoros and Girre, 1987; Kirmizigül *et al.*, 1995; Fan and He, 1997). The observation of a downfield shift of the C-3 signal of the arabinose unit at δ 82.60 confirmed that the terminal glucose unit is linked to the C-3 position of the arabinose unit (Ye *et al.*, 2000; Tain *et al.*, 1993). On the basis of these results the structure of saponin **1** was determined to be 3-*O*-

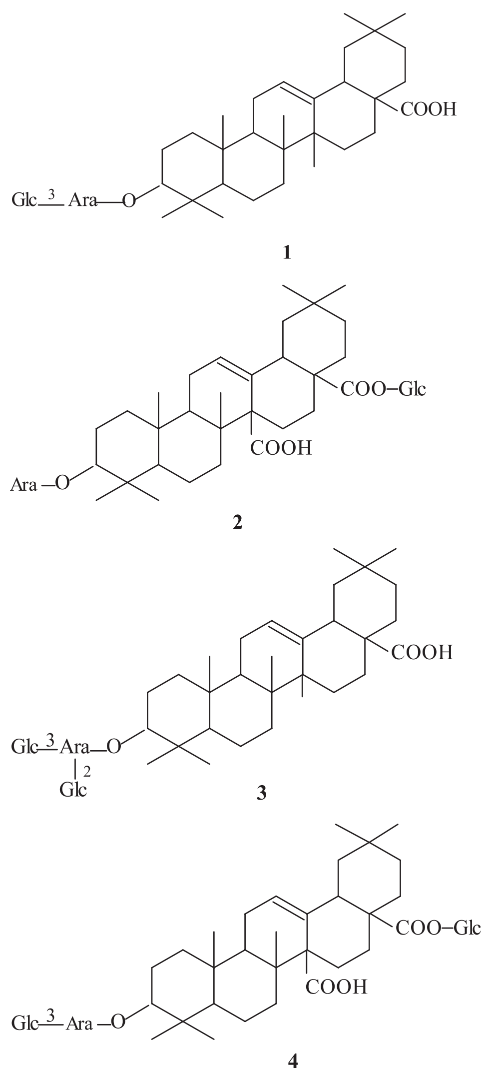

Fig. 1. Triterpenoidal glycosides isolated from aerial parts of *F. arabica*.

β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside oleanolic acid (Fig 1).

Saponin **2** had a relative molecular mass of 780 as determined by ^{13}C NMR and negative ESI-MS. The fragment ions at *m/z* 735 $[M-H-CO_2]^-$ and 573 $[M\text{-}H\text{-}162\text{-}44]^-$ exhibited the loss of one hexose unit as well as CO_2. Another fragment ion at *m/z* 441 $[M\text{-}H\text{-}162\text{-}132\text{-}CO_2]^-$ reflected that a pentose unit was directly attached to the aglycone part (Elgamal *et al.*, 1995; Fan and He, 1997). By comparison of ^{1}H and ^{13}C NMR data of saponin with the literature it appeared that the aglycone part is quinovic acid (Elgamal *et al.*, 1995; Hassanan *et al.*, 1993). This was confirmed by the identification of the product of acid hydrolysis of saponin **2** where the aglycone part was quinovic acid and the sugar moieties were L-arabinose and D-glucose. Two anomeric proton signals appeared in the ^{1}H NMR spectrum at δ 4.77 (d, J = 7 Hz) and 5.20 (d, J = 7.9 Hz). In the same time two anomeric carbon signals were recorded in the ^{13}C NMR spectrum at δ 104.61 and 95.06. The upfield signal at δ 95.06 indicated that one bound is a glycosyl ester. This was confirmed by the presence of a C-28 signal at δ 176.36 (Fan and He, 1997; Elgamal *et al.*, 1995; Hassanan *et al.*, 1993; Attia, 1999). The downfield shift of C-3 of the aglycone part at δ 89.74 exhibited that the C-3 is the position of the other glycoside (Amoros and Girre, 1987; Kirmizigül *et al.*, 1995). Thus, the structure of saponin **2** was 3-*O*-α-L-arabinopyranosyl quinovic acid 28-*O*-β-D-glucopyranoside (Fig. 1).

Saponin **3** was obtained as white powder. It had a relative molecular mass of 912 as determined by negative ESI-MS and ^{13}C NMR spectrum. The ^{13}C NMR showed 47 signals of which 30 were assigned to a triterpenoid moiety and 17 to a saccharide moiety. Acid hydrolysis of saponin **3** afforded L-arabinose and D-glucose as the component sugar moieties as well as oleanolic acid as aglycone part. Both of the sugar moieties and the aglycone were compared with authentic samples on PC and TLC respectively. Moreover, the ^{1}H and ^{13}C NMR spectra of saponin **3** exhibited signals due to a triterpene aglycone which were in good agreement with literature data of oleanolic acid (Tain *et al.*, 1993; Carvalho and Braz-Filho, 1999). The ^{1}H NMR spectrum of saponin **3** showed three anomeric proton signals at δ 4.78 (d, J = 7 Hz), 4.88 (d, J = 7.8 Hz) and 4.92 (d, J = 7.9 Hz). This was confirmed by the presence of three anomeric carbon signals in the ^{13}C NMR spectrum at δ 103.65, 104.20 and 104.61 (Zhong *et al.*, 2001; Ye *et al.*, 2000). Prominent fragment ions occurred at *m/z* 749 [M-H-162], 586 [M-H-2×162] and 455 [M-H-2×162-132] corresponding to the subsequent loss of two glucose units and one arabinose unit (Zhong *et al.*, 2001). This fragmentation pattern exhibited that the arabinose unit is directly attached to the aglycone part. The downfield shift of C-3 of the aglycone part at 88.50 reflected that at this carbon atom the sugar moiety is attached to the aglycone part (Tain *et al.*, 1993; Ye *et al.*, 2000). The downfield shifts of C-2 and C-3 of the arabinose unit at δ 75.80 and 83.20 confirmed that

the two sugar units are linked in these positions. In view of the above evidence, the structure of saponin **3** was determined as 3-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinosyl oleanolic acid (Fig 1).

Saponin **4**: The ESI mass spectrum exhibited the $[M-H]^-$ ion at *m/z* 941. The fragment ions at *m/z* 779 $[M-H-Glc]^-$, 735 $[M-H-Glc-CO_2]^-$ and 573 $[M-H-2Glc-CO_2]^-$ showed the sequential loss of two glucose units plus CO_2 and the fragment at *m/z* 441 $[M-H-2Glc-Ara-CO_2]^-$ the loss of an arabinose unit (Attia, 1999; Aquino *et al.*, 1989). Acid hydrolysis of saponin **4** gave an aglycone which was identified as quinovic acid by comparison with an authentic sample and by comparison of its ^1H, ^{13}C NMR spectra with those in the literature (Aquino *et al.*, 1989; Atimad *et al.,* 1993). The sugar moieties were identified as L-arabinose and D-glucose. The ^{13}C NMR spectrum of saponin **4** showed 47 signals of which 30 were assigned to a triterpenoid moiety and 17 to a saccharide part. The ^1H NMR spectrum exhibited three anomeric proton signals at δ 4.76 (1H, d, J = 7 Hz), 4.85 (1H, d, J = 7.7 Hz) and 5.30 (1H, d, J = 7.8 Hz) (Aquino *et al.*, 1989; Miyase *et al.*, 1996b). The downfield signal at δ 5.30 in the ^1H NMR spectrum was consistent with the ^{13}C NMR upfield signal of the anomeric carbon atom at δ 95.03, this locating one sugar unit at C-28 (Hassanan *et al.*, 1993; Aquino *et al.*, 1989). This glycosyl ester linkage was also confirmed by the presence of a C-28 signal upfield at 176.05. Also, in the ^{13}C NMR spectrum the glycosylation of C-3 appeared by the presence of the downfield shift of C-3 of the aglycone at 88.02 (El-gamal *et al.*, 1995; Hassanan *et al.*, 1993; Atimad *et al.*, 1993). The presence of a downfield signal due to C-3 of arabinose at δ 82.89 reflected the glycosylation of the terminal glucose with an inner arabinose unit at this position (Shaker *et al.*, 1999; Miyase *et al.*, 1996b). Thus, the structure of saponin **4** was proposed as 3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl quinovic acid 28-*O*-*β*-D-glucopyranoside (Fig. 1).

Molluscicidal screening of the aerial parts of *F. arabica* L. against *B. alexandrina* snails revealed that the aqueous methanolic extract has strong activity (LC_{90} = 85 ppm). The *n*-butanolic fraction obtained from successive fractionation only showed activity of LC_{90} = 22 ppm, whereas all other extracts did not show any activity up to 50 ppm. The bioassay test of the four isolated compounds from the *n*-butanolic fraction showed that only the two monodesmosidic saponins **1** and **3** showed high potency (LC_{90} = 13.33 and 16.44 μM) whereas the bidesmosidic saponins **2** and **4** did not show any activity up to 50 μM. This result is in full agreement with previously reported works on saponins as molluscicidal agents (Hostettmann and Marston, 1995; Lemmich *et al.,* 1995; Ribeiro *et al.*, 1995).

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