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STUDY OF THE SAPONIN CONTENT OF *ATRIPLEX STYLOSA* VIV. AND ITS MOLLUSCICIDAL EFFECT

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نبات القطف (الاتربلكس ستيلوزا) ينتمى إلى العائلة الرمرامية ، وقد تم فصل أربعة مركبات صابونينية من هذا النبات وتم التعرف على تركيبها الكيميائى عن طريق إجراء التحليلات الطيفية المختلفة مثل الأشعة تحت الحمراء ، الرنين النووى المغناطيسى البروتونى والكربوى وطيف الكتلة.
أظهرت ثلاثة مركبات من المركبات المفصولة فعالية عالية ضد قواقع بيومفلاريا الكسندرينا وليمنيا كايودى العوائل الوسيطة لطفيلى البلهارسيا المعوية والدودة الكبدية فى مصر بينما لم يظهر المركب الرابع أى قدرة أبادية ضد القوقعين.

From the aerial parts of Atriplex stylosa Viv, four triterpenoidal saponins have been isolated. Their structures were established as 3-O-α-L-rhamnopyranosyl-(l→3)-β-D-glucuronopyranosyl hederagenin; 3-O-β-D-glucopyranosyl-(l→3)-β-D- glucuronopyranosyl hederagenin;3-O-α-L-rhamnopyranosyl-(l→3)-β-D-glucuronopyranosylhederagenin28-O-β-D-glucopyranoside and 3-O-α-L-rhamnopyranosyl-(l→3)-[β-D-glucopyranosyl-(l→2)]-β-D-glucuronopyranosyl oleanolec acid by spectroscopic methods. (IR, ^{1}HNMR, ^{13}CNMR and FAB-MS) as well as on basis of acidic and alkaline hydrolysis. The three monodesmosidic saponins exhibited various molluscicidal activities against the two aquatic snails; Biomphalaria alexandrina and Lymnaea cailliaudi, the intermediate hosts of Schistosoma mansoni and Fasciola gigantica respectively in Egypt whereas the bidesmosidic saponin was inactive against the two snail species.

INTRODUCTION

Schistosomiasis and fascioliasis are among the most important health problems in many countries. Various attempts are presently made in order to control the two parasitic diseases by elimination of their intermediate hosts.[1-4] The use of plant with molluscicidal properties appears to be a simple and inexpensive technology for the control of the snail vector. Since the discovery of highly potent molluscicidal saponins in the berries of *phytolacca dodecandra* L' Herit, several reports on the plant molluscicides has been increased considerably.[5-8]

Atriplex L. species (Family Chenopodiacceae) occur throughout the semi-arid regions in the Western United States, the Middle East, Africa, Australia and Siberia.[9,10] The genus *Atriplex* is represented in Egyptian flora by 15 species.[11] Many plants belonging to this genus have been shown to cause various biological effects.[12-14]

This is a continuation of the course of our investigations on molluscicidal saponins from plants of the genus *Atriplex*[15,16] because of the high potency of the methanol extract of *Atriplex stylosa* Viv. against the two aquatic snails; *Biomphalaria alexandrina* and *Lymnaea cailliaudi*, the intermediate hosts of *Schistosoma mansoni* and *Fasciola gigantica* respectively in Egypt.[17] the present paper deals with the isolation and structure elucidation of certain saponins which are responsible for the molluscicidal activity. To our knowledge, these saponins are isolated for the first time from this plant.

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EXPERIMENTAL

General: Melting points are uncorrected. ^{1}HNMR and ^{13}CNMR were measured with TMS as an internal standard using a Bruker AM-300 spectrometer operating at 270 MHZ and 100 MHZ respectively with DMSO-d_6 as solvent. FAB-mass spectrum (negative ion mode) was measured with thioglycerol matrix bombardment with Xenon gas. Column chromatography was carried out on silica gel (sigma 28-200 nesh). TLC were performed using silica gel plates (Kiesel gel 60GF_{254}, Merck). Paper chromatography were performed on Whatmann No. 1 sheets and aniline phthalate was used as visualizing agent.

Plant material

Atriplex stylosa Viv. (Family chenopodiaceae) was collected in June 1996 from Borg El-Arab, Alexandria, Egypt. The plat was identified by prof. Dr N. El-Hadidi, Dept. of Botany, Faculty of Science, Cairo university as well as by specialists at El-Orman Botanical Garden. The plant was shade dried and powdered by electrical mill.

Extraction and isolation

The dried aerial parts of *A. stylosa* Viv. (1.5 Kg) was defatted with pet. ether (60-80°C). The defatted material was extracted with methanol. The methanolic extract (90 g) was dissolved in water and extracted with chloroform, ethyl acetate and n-butanol. The butanolic extract was evaporated to dryness to give a residue (30 g) which chromatographed on silica gel column using gradient elution with chloroform, chloroform-methanol mixture and finally pure methanol. The column products were monitored by TLC to afford three major fractions I-III. Fraction I was purified on preparative TLC using solvent system $CHCl_3$-MeOH-H_2O (58:35:7) to yield saponin 1. Fractions II and III were combined and rechromatographed on other silica gel column using gradient elution of $CHCl_3$-MeOH and pure methanol to give a mixture of saponins which was purified by preparative TLC solvent system; $CHCl_3$-MeOH-H_2O (58:35:7) to yield saponins 2-4. The final purification of each saponin was achieved on a Sephadex LH-20 column using methanol as eluent.

Saponin 1: mp 252-254 °C, R_f 0.34 ($CHCl_3$-MeOH-H_2O; 58:35:7). IRν(KBr) max cm^{-1} 3394 (OH), 2940, 1700 (COOH), 1640, 1461, 1388, 1158, 1041 (C-O-C) and 785. ^{1}HNMR δ 0.70-1.23 (each 3H, S, 6xMe), 1.58 (3H, D, ME-Rha), 4.45 (1H, d, GlcA, H-l), 5.10 (1H, d, Rha, H-1), and 5.20 (1H, s, H-12). FAB-MS; m/z 793 $(M-H)^-$, 647 $(M-H-Rha)^-$, 471 $(M-H-Rha-GlcA)^-$. ^{13}CNMR see Table 1.

Saponin 2: mp 234-235 °C, R_f 0.31 ($CHCl_3$-MeOH-H_2O; 58:35:7). IRν (KBr) max cm^{-1} 3398 (OH), 2941, 1694 (COOH), 1460, 1387, 1044 (C-O-C), 936, and 780. ^{1}HNMR δ 0.69-1.24 (each 3H, s, 6xMe), 4.44 (1H, D, GlcA, H-1), 4.89 (1H, d, Glc, H-1), 5.22 (1H, s, H-12). FAB-MS; m/z 809 $(M-H)^-$, 647 $(M-H-GlC)^-$, and 471 $(M-H-Glc-GlcA)^-$. ^{13}CNMR see Table 1.

Saponin 3: mp 216-218 °C,R_f 0.18 ($CHCl_3$-MeOH-H_2O; 58:35:7). IRν (KBr) max cm^{-1} 3374 (OH), 2944, 1731 (ester group), 1696 (COOH), 1459, 1387, 1154, and 1045 (C-O-C). ^{1}HNMR δ 0.69-1.23 (each 3H, s, 6xMe), 1.57 (3H, d, Me-Rha), 4.41 (1H, d, GlcA, H-1), 5.09 (1H, d, Rha, H-1), 5.23 (1H, d, H-12) and 5.32 (1H, ester Glc, H-1). FAB-MS; m/z 955 $(M-H)^-$, 793 $(M-H-Glc-Rha)^-$ and 471 $(M-H-Glc-Rha-GlcA)^-$. ^{13}CNMR see Table 1.

Saponin 4: mp 203-205 °C, R_f 0.23 ($CHCl_3$-MeOH-H_2O; 58:35:7). IRν (KBr) max cm^{-1} 3405 (OH), 2890, 1696 (COOH), 1458, 1638, 1358, 1158, 1042 (C-O-C) and 760. ^{1}HNMR δ 0.70-1.26 (each 3H, s, 7x Me), 1.58 (3H, d, Me-Rha), 4.44 (1H, d, GlcA, H-1), 4.94 (1H, d, Glc, H-1), 5.12 (1H, d, Rha, H-1) and 5.20 (1H, s, H-12). FAB-MS; 939 $(M-H)^-$, 793 $(M-H-Rha)^-$, 777 $(M-H-Glc)^-$, 631 $(M-H-Rha-Glc)^-$ and 455 $(M-H-Rha-Glc-GlcA)^-$. ^{13}(CNMR see Table 1.

Table 1: ^{13}CNMR spectral data of saponins 1-4 (in DMSO-d_6; TMS as internal standard).

Carbon atom		1	2	3	4
Aglycone3	3	82.3	82.2	82.6	88.6
	12	122.2	122.3	122.1	122.4
	13	144.3	144.1	144.3	144.1
	23	65.1	65.2	65.4	28.1
	24	13.7	13.8	13.6	16.7
	28	179.8	179.7	176.2	179.9
3-0-Sugar moieties GlcA	1	105.8	105.9	105.8	106.1
	2	75.6	75.4	75.5	78.4
	3	82.1	82.3	82.1	82.1
	4	72.6	73.2	72.8	73.2
	5	76.8	76.2	77.1	76.9
	6	172.9	173.2	172.8	173.1
Rha	1	102.2		101.9	102.1
	2	71.7		71.3	71.5
	3	72.1		72.2	72.5
	4	73.9		73.8	73.7
	5	69.2		69.4	69.8
	6	18.3		18.4	18.3
Glc	1		104.2		104.5
	2		75.1		75.1
	3		77.9		78.1
	4		72.3		72.7
	5		78.2		78.4
	6		62.5		62.6
28-O-Sugar moieties Glc	1			94.9	
	2			73.8	
	3			78.2	
	4			70.9	
	5			78.4	
	6			62.2	

GlcA= ß-D-Glucuronopyranosyl ; Rha= α-L-rhamnopyranosyl ; Glc= ß-D-Glucopyranosyl

Alkaline hydrolysis

Saponin 3 (15 mg) was refluxed with 5% aqueous KOH (30 ml) on water bath for 2 hours. The reaction mixture was neutralized with dilute HCl and extracted with n-BuOH. The combined butanolic extract was washed with water and evaporated to dryness and analysed by TLC with $CHCl_3$-MeOH-H_2O (58:35:7). The aqueous layer was concentrated and neutralized with Na_2CO_3. After evaporation to dryness, the sugars were extracted with pyridine. The pyridine extract was evaporated and the residue was dissolved in 10% isopropanol and detected by PC with authentic sugars using solvent system n-BuOH-AcOH-H_2O (4:1:5) and aniline phthalate as visualizing agent.

Acid hydrolysis

Each saponin (20 g) was refluxed with 4N HCl (35 ml) for 6 hours on water bath. The

reaction mixture was diluted with water and the aglycone was extracted with chloroform. The chloroform extract was evaporated to dryness and identified by comparison with authentic sapogenins by TLC on silica gel with solvent system C_6H_6-MeOH (80:20). Saponins 1-3 gave hederagenin while saponin 4 yielded oleanolic acid. The aqueous layer has been treated as described above in alkaline hydrolysis and the sugars were identified by comparison with authentic samples.

Molluscicidal assay

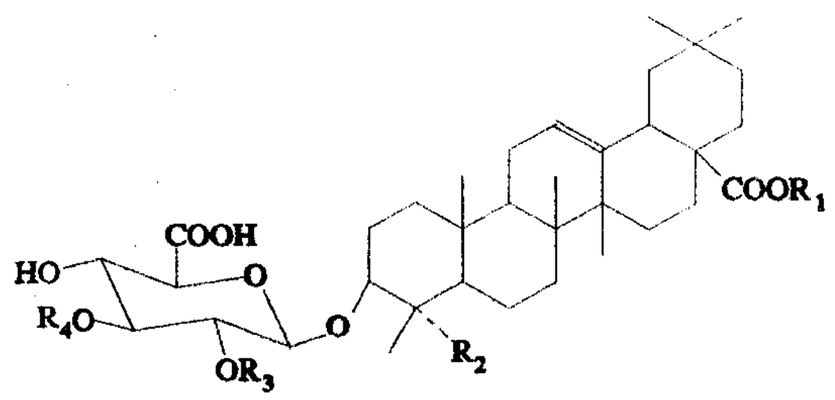
Biomphalaria alexandrina and *Lymnaea cailliaudi*, the intermediate hosts of *Schistosoma mansoni* and *Fasciola gigentica* respectively in Egypt were collected from the irrigation canals in Abou-Rawash, Giza Governorate. The snails have been maintained in dechlorinated tap water in the laboratory conditions (Temperatures 25°C±2 and pH 7-7.7). Tests were performed in duplicate using ten snails for each test. Compounds were initially dissolved is small amount of absolute ethanol then desired dilutions were prepared with dechlorinated tap water. The snails were exposed for 24 hours followed by 24 hours in dechlorinated water as recovery period. Procedures and statistical analysis of the data were carried out according to the WHO and Litchifield and Wilcoxon protocol.[18-20]

RESULTS AND DISCUSSION

The defatted methanolic extract of the dried parts of *Atriplex stylosa* Viv. was suspended in water and partitioned with chloroform, ethyl acetate and n-butanol. The butanol-soluble fraction was subjected to silica gel column using gradient elution $CHCl_3$, $CHCl_3$-MeOH mixture and pure MeOH and the column products were monitoried by TLC. The saponin fractions were further separated and purified by prep. TLC and Sephadex-LH-2O TO afford four pure triterpenoid saponins. The structures of the isolate saponins were established by IR, ^{1}HNMR, ^{13}CNMR and FAB-MS spectra as well as on the basis of acidic and basic hydrolysis.

Saponin 1 was obtained as an amorphous powder. The IR spectrum showed absorptions at 3394 (OH), 1700 (COOH), and 1041 glycosidic linkage.[21-23] Acid hydrolysis of saponin 1 yielded an aglycone identified as hederagenin by comparison with authentic sample. The sugars obtained from the saponin hydrolysates were identified as D-glucuronic acid and L-rhamnose. ^{1}HNMR spectrum of saponin 1 revealed the presence of six tertiary methyl groups between the range δ 0.70-1.23, one trisubstituted olefinic proton (δ 5.20) and two anomeric protons of the suger moiety at δ 4.45 and 5.10 (22-25). The FAB-MS spectrum (thioglycerol matrix, negative ion mode) of saponin 1 showed a quasi-molecular ion $(M-H)^-$ at m/z 793 indicating the molecular weight as 794. Other significant peaks at m/z 647 $(M-H-Rha)^-$ and m/z 471 $(M-H-Rha-GlcA)^-$ indicated the elimination of one rhamnose and one glucuronic acid and showed that the rhamnosyl moiety is the terminal sugar (26-28). In the ^{13}CNMR spectrum of compound 1 (Table 1), two signals corresponding to two olefinic carbon atoms appeared at δ 122.2 and 144.3. Also, the signals of the two anomeric carbon atoms of the sugar moiety appeared at δ 105.8 and 102.2. The above data indicated that compound 1 has to be monodesmosidic triterpenoid saponin in which two sugar units must be bounded by a glycosidic linkage to the aglycone at C-3 (27-30). This was confirmed by shifting of C-3 signal at downfield 82.3 in the ^{13}CNMR spectrun whereas in hederagenin, this C-atom was observed at δ 73.8 ppm (23,31) and appearing of the characteristic band of the carboxylic at δ 179.8 in ^{13}CNMR spectrum and 1700 cm^{-1} in IR spectrum. Also, saponin 1 showed high molluscicidal activity against *Biomphalaria alexandria* and *Lymnaea cailliaude* snails (LC_{90}= 8 and 6 ppm respectively). This demonstrated that saponin 1 is monodesmosidic type.[8,32] The nature of the interglycosidic linkage was established by the data of ^{13}CNMR spectroscopy; C-3 of glucuronic acid was shifted at downfield δ 82.1 whereas the other C-atoms remained almost unaffected.[28-30] This indicated that the terminal rhamnosyl is linked to the glucuronic acid at C-3. Consequently the

structure of saponin 1 is 3-O-α-L-rhamnopyranosyl-(1→3)-ß-D-glucuronopyranosyl hederagenin.



Saponin	R_1	R_2	R_3	R_4
1	H	CH_2OH	H	Rha
2	H	CH_2OH	H	Glc
3	Glc	CH_2OH	H	Rha
4	H	CH_3	Glc	Rha

Rha = α-L-rhamnopyranosyl;
Glc = ß-D-glucopyranosyl
GlcA= ß-D-glucuronopyranosyl

Saponin 2 had an IR spectrum which indicated the presence of hydroxyl groups (3398 cm^{-1}), carboxylic group (1694 cm^{-1}) and glycosidic linkage at 1044 cm^{-1}.[21-23] Saponin 2 showed the presence of six methyl group signals at δ 0.69-1.24 in the ^{1}HNMR spectrum., an olefinic proton at δ 5.22 and two anomeric protons at δ 4.44 and 4.89 indicating the presence of two sugar moieties.[22-26] The ^{13}CNMR spectrum of saponin 2 showed the presence of an olefinic bond at 122.3 (C-12) and 144.1 (C-13) in addition to all the other carbon signals of the aglycone moiety which was identical to those reported for hederagenin.[21-24] The ^{13}CNMR spectrum of saponin 2 also showed the presence of two anomeric carbon signals at δ 105.9 and 104.2. also, the presence of a 3-O-glycosidic linkage in saponin 2 was easily seen by attendant downfield shift of C-3 at δ 82.2 whereas in hederagenin this C-atom signal was observed at δ 73.8.[23,24] This was confirmed by presence of free COOH group at δ 179.7 and from the highly molluscicidal potency of this saponin against the two aquatic snails; *B. alexandrina* and *L. cailliaudi* (LC_{90}= 12 and 11 ppm respectively). FAB-MS of saponin 2 showed a quasi-molecular ion at m/z 809 $(M-H)^-$ indicating a molecular weight as 810. Fragments at m/z 647 $(M-H-Glc)^-$ and 471 $(M-H-Glc-GlcA)^-$ indicated an elimination of one glucosyl and one glucuronic acid.[27-31] Acid hydrolysis of saponin 2 gave D-glucuronic acid and D-glucose as sugar moiety and hederagenin as aglycone. The interglycosidic linkage was determined by appearance of the downfield signal of C-3 of the inner glucuronic acid at δ 82.3; in the ^{13}CNMR spectrum whereas the other carbon atoms of this sugar moiety remain almost unaffected.[27-30] From the above data, the structure of saponin 2 was determined as 3-O-ß-D-glucopyranosyl-(1→3)-ß-D-glucuronopyranosyl hederaganin.

Saponin 3 was crystallized from aqueous ethanol to afford colourless needles. The IR spectrum of this saponin showed absorption bands indicating hydroxyl groups (3374 cm^{-1}), ester group (1731 cm^{-1}), carboxylic group (1696 cm^{-1}), and glycosidic linkage at 1045 cm^{-1}.[21-24] Acid hydrolysis of saponin 3 yielded hederaganin as aglycone and D-glucuronic acid, D-glucose and L-rhamnose. The individual monosaccharides were identified by Co-chromatography with authentic samples. Basic hydrolysis of saponin 3 yielded prosapogenin which was identical to saponin 1 (CO-TLC using solvent system $CHCl_3$-MeOH-H_2O (58:35:7) and the sugar moiety was D-glucose. The negative ion FAB-MS spectrum of saponin 3 exhibited a quasi-molecular ion at m/z 955 $(M-H)^-$, 647 (M-H-Mlc-Rha)$^-$ and 471 (M-H-Glc-Rha-GlcA)$^-$ corresponding to the loss of one glucosyl, one rhamnosyl and one glucuronic acid.[27,33,34] On comparison of the ^{13}CNMR spectrum of saponin 3 with saponin 1, D-glucose unit proved to be attached to the 28-carbonyl group of saponin 3 as well as a similar sugar chain attached to C-3 position of hederagenin. This was confirmed by presence of an anomeric carbon atom signal at upfield δ 94.9 in ^{13}CNMR, signal at δ 5.32 in the ^{1}HNMR and presence of an ester group at 1731 cm^{-1} in the IR spectrum.[22-27] Because of its bisdesmosidic nature of saponin 3, this compound was inactive against the two snail species up to 50 ppm but its corresponding prosapogenin (product of alkaline hydrolysis of

saponin 3) showed almost the same potency of saponin 1. Therefore, saponin 3 was identified as 3-O-α-L-rhamnopyranosyl-(1→3)ß-d-glucuronopyranosyl hederagenin 28-O-glucopyranoside. This saponin has been previosly isolated from the aerial parts of *Dumasia truncata* and was also obtained from leaves and stems of *Opilia celtidifolia*.[27,33]

Saponin 4 was obtained as white powder. The negative-ion FAB- mass spectral data m/z 939 $(M-H)^-$ in combination with the ^{13}CNMR spectral data (Table 1) indicating that the molecular weight of this saponin is 940. Its IR spectrum revealed the presence of a hydroxyl group (3405 cm^{-1}), free carboxylic group (1696 cm^{-1}), double bond (1638 cm^{-1}) and glycosidic likage at 1042 cm^{-1}. The ^{1}HNMR spectrum showed signals of seven methyl groups in the range at δ 0.70-1.26, olefinic proton at δ 5.20, three anomeric protons of sugar moiety at δ 5.12, 4.94 and 4.44 as well as the characteristic signal of methyl group of rhamnosyl moiety at δ 1.58.[29,35,36] Acid hydrolysis of saponin 4 yielded oleanolic acid as aglycone which was identified by comparison with authentic sample. The sugars obtained from the saponin hydrolysates were also identified as D-glucuronic acid, D-glucose and L-rhamnose. Signals for the oleanolic acid moiety in the ^{13}CNMR spectrum was identified to those already reported[37,38] except for C-3 which appeared at δ 88.6 ppm. This indicated that the sugar chain is substituted at this position, thus saponin 4 is monodesmosidic type.[8,28] On the other hand, the appearing of fragment ions at m/z 793 $(M-H-Rha)^-$ and 777 $(M-H-Glc)^-$ FAB-MS indicated that one rhamnosyl and one glucosyl were eliminated from the quasi-molecular ion of saponin 4 and the two sugar units are terminals. Other fragments at m/z 631 $(M-H-Rha-Glc)^-$ and 455 $(M-H-Rha-Glc-GlcA)^-$ showed that the glucuronic acid is directly attached to the aglycone at C-3 and the two terminal sugar are linked to the glucuronic acid.[28,34,38] From the ^{13}CNMR spectral data, the substitution of the glucuronic acid were found to be at positions C-2 and C-3 and this was confirmed by shiftion of these carbon signals at downfiels δ 78.4 and 82.1 respectively.[27,28,33] Therefore, the sugar moiety contains three monosaccharide units and this was confirmed by presence of the characteristic signals of the three anomeric carbon atoms at δ 106.1, 102.1 and 104.5 in the ^{13}CNMR spectrum (35-38). The results of biological tests whowed that saponin 4 has a considerable molluscicidal activity against the two aquatic snails; *B. alexandrina* and *L. cailliaudi* (LC_{90}= 29 and 27 ppm respectively). On the basis of the above evidence, the structure of saponin 4 was elucidated to be 3-O-α-L-rhamnopyranosyl-(1→3)-[ß-D-glucopyranosyl-(1→2)]-ß-D-glucuronopyranosly oleanolic acid.

REFERENCES

1- P. H. Joubert and S. J. Pretorius, Annals. Trop. Med. Parasit., 85: 447 (1991).
2- P. Andrew J. Thyssen and D. lORKE, Pharmacology and Therapeutics, 19: 245 (1983).
3- P. F. D. Arcy and D. W. Harron, Pharm. Int., 4: 16 (1983).
4- G. Webb and K. E. Mott, Planr Molluscicides Ed John Wiley and Sans Chichester, p 1 (1987).
5- O. A. Ekabo and N. R. Farnsworth, J. Nat. Prod, 59: 431 (1996).
6- C. O. Adewunimi, B. O. Oguntimein and P. Furu, Planta Medica, 56: 374 (1990).
7- A. Lemma, Ethiop. Med. J., 3: 187 (1965).
8- A. Marston and K. Hostettmann, Phytochemistry, 24: 639 (1985).
9- L. H. Bailey, Mannual of cultivated plants, The Macmillan Company, New York, 352 (1949).
10- J. C. Willis, A Dictionary of the flowering plants and Ferns, The University press Cambridge 8th ed 238 (1943).
11- V. Tackholum, Student's flora of Egypt 2th ed, Cairo. University, 108 (1974).
12- A. A. Omar, T. M. Sarg, E. S. Mansour and N. A. Abdel-Salam, Fitoterapia, 55 (1984).

13- S. Broegger Christensen and A. A. Omar, J. Nat. Prod. 48 (1), 161 (1985).
14 H. A. Soeb, M. A. El-Emam, A. M. Saad and M. A. Mohamed, J. Egypt. Soc. Parasit. 17 (2), 539 (1989).
15- M. M. El-Sayed, Ph. D., Faculty of Science, Al-Azhar University (1989).
16- M. M. El-Sayed, Zag. J. Pharm. Sci., 4: 143 (1995).
17- M. M. El-Sayed and H. A. El-Nahas, Bull. Pharm. Sci., Assiut. Uni., 20: 105 (1997).
18- WHO: Expert Committee on Bilharziasis Control Methods, WHO, 65: 33 (1953).
19- WHO: Snail control, WHO Monograph Ser, 50: 124 (1965).
20- J. T. Litchfield and F. Wilcoxon, Exp. Therap., 96: 99 (1949).
21- J. Tian., E. Feng, M. Wu, Qiu and R. L. Nie, Phytochemistry, 32: 1539 (1993).
22- S. Kirmizigul, H. Anil and M. E. Rose, Phytochemistry, 39: 1171 (1995).
23- X. Li, C. R. Yang, Y. Y. Liu, R. Kasai, K. Ohthani, K. Yamasaki, K. Miyahara and K. Shingu, Phytochemistry, 39: 1175 (1995).
24- C. Lavaud, G. Massiot, M. Becchi, G. Misra and S. K. Nigam, Phytochemistry, 41: 887 (1996).
25- J. Kinjo, K. Suyama and T. Nohara, Phytochemistry, 40: 1765 (1995).
26- O. P. Sati, S. Bahuguna and J. Uniyal, J. Nat. Product., 53: 466 (1990).
27- C. F. Olliver, C. Lavaud, A. Babadzamian, R. Faure, L. Debrauwer, G. Balansard and G. Boudon, Phytochemistry, 33: 657 (1993).
28- C. Borel and K. Hostettmann, Helv. Chim. Acta., 70: 570 (1987).
29- Y. Shao, O. Poobrasert, C. K. Chin and G. A. Cordell, Phytochemistry, 43: 195 (1996).
30- Z. L. Liu, N. Owen, D. Grant, R. Cates and Z. Jia, J. Nat. Products. 58: 1632 (1995).
31- S. K. Srivastana, and D. C. Jain, Phytochemistry, 28: 644 (1989).
32- K. Hostettmann, K. Kizu and T. Tomimori, Planta Medica, 44: 34 (1982).
33- S. S. Kang, J. S. Kim and E. B. Lee, Int. J. Pharmacognosy, 34: 199 (1996).
34- C. Borel, M. P. Gupta and K. Hostettmann, Phytochemistry, 26: 2685 (1987).
35- T. Miyase, K. Shiokawa, D. M. Zhang and A. Ueno, Phytochemistry, 5: 1411 (1996).
36- M. A. Dubois, S. Benze and H. Wagner, Planta Medica, 451 (1990).
37- S. Sakai, M. Katsumata, Y. Satoh, M. Nagasao, M. Kmiyakoshi, Y. Inda and J. Shozi, Phytochemistry, 35: 1319 (1994).
38- C. Lavaud, S. Beauviere, G. Massiot, L. Le Men-Olivier and G. Bourdy, Phytochemistry, 43: 189 (1996).