Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 143-146

ISSN 1110-5089

MOLLUSCICIDAL SAPONINS FROM *ATRIPLEX LEUCOCLADA*

Mortada M. El-Sayed

Laboratory of Medicinal Chemistry, Theodore Bilharz Research Institute, IMBABA, GIZA, EGYPT.

ABSTRACT

Silica gel column chromatography of the butanolic extract of the aerial parts of *Atriplex leucoclada* (Family chenopodiaceae) afforded five triterpenoidal saponins. Their structures have been established using spectroscopic methods and hydrolysis followed by identification of the aglycones and sugar moieties. The five saponins were found to possess molluscicidal activities with LC_{90} values of 6,10,18,12, and 30 ppm, respectively against Schistosomiasis transmitting snail *Biomphalaria alexandrina*.

INTRODUCTION

Although, various attempts are presently made in order to control Schistosomiasis by killing the transmitter snails of this endemic diseases, but there is still a need for continued screening for more selective and efficient molluscicides. Although, there are several commercial chemical (synthetic) molluscicides, the use of plants with molluscicidal properties appears to be simple, safe and inexpensive technology for the snail vector[1-5].

Genus *Atriplex* (Family Chenopodiaceae) comprises about 120 species mainly in desert, saline and waste placed[6]. About 15 species of *Atriplex* are known to occur in Egypt[7].

Previous work on plants of the genus *Atriplex* reported the isolation and structure determination of oleanolic acid and echinocystic acid as well as two triterpenoid saponins from the aerial parts of *Atriplex nummularia*[8,9]. Furthermore, other three triterpinoid saponins have been isolated from *Atriplex halimus* and their molluscicidal activities have been already studied[10]. On the other hand, the water suspension of *Atriplex leucoclada* dry powder exhibited molluscicidal activity with LC_{90} of 260 ppm against *Biomphalaria alexandrina* snails[11].

To our knowledge, no report dealing with saponin contents of *Atriplex leucoclada* could be traced in the literature. Thus, this study was carried out to investigate this plant in order to isolate and identify the constituents which might exhibit molluscicdal properties.

EXPERIMENTAL

Plant material:

The fresh aerial parts of *Atriplex leucolada* Boiss (Family chenopodiaceae) were collected in July 1993 from Borg El-Arab, Alexandria, Egypt. The plant was kindly identified by Prof. Dr. Nabil El- Hadidi professor of plant Taxonomy, Cairo University. The plant was shade dried and ground by electric mill.

Methods and apparatus :

All melting points were uncorrected. IR spectra were measured on a Perkin-Elmer model, IR-recording spectrophotometer. ^{1}HNMR were recorded on a Varian 270 MHz spectrometer using TMS as internal standard and chemical shifts are expressed in ppm. Mass spectra were recorded on MS Hewlet-Parckard 5988 with direct inlet techniques at 70 ev. Thin- layer chromatography was carried out on silica gel Merck CF_{254} and spots were visualized with 40 % H_2SO_4 in ethanol. Column chromatography was performed using glass columns (5 x 120 cm. and 3 x 100 cm) using silica gel as a stationary phase. Paper chromatography was performed on Whatman NO.1 sheets and aniline phathalate was used as a visualizing agent[12].

Solvent systems:

I- $CHCl_3$: MeOH : H_2O (65 : 35: 5)

II- $CHCl_3$: MeOH : H_2O (58 : 35: 7)

III- $CHCl_3$: MeOH : H_2O (65 : 40: 10)

IV- C_6H_6 : MeOH (80: 20)

V- n-BuOH : AcOH : H_2O (4 : 1: 5)

Snails:

Bomphalaria alexandrina, the intermediate host of *Schistosoma mansoni* in Egypt was used in this study. They were collected from irrigation canals previously untreated with any molluscicides in Abu-Rawash, ten kilometers from Giza Governorate (Egypt). The snails were kept in laboratory for three weeks in de-chlorinated tap water for acclimatization with laboratory conditions.

Extraction and isolation of saponins:

The powdered aerial parts (3 Kg) of the plant were cold extracted with methanol (4 x 8L) . The methanolic extract (130g) was defatted with petroleum ether (60- 80°C) (4x 500 ml) and then suspended in water (700 ml) and partitioned with chloroform (4x 400 ml), ethyl acetate (4x 400 ml) and butanol (4x 400ml). The butanolic extract was concentrated to give (35g). The butanolic extract was chromatographed on silica gel column (800g; 5 x 120 cm), eluted with chloroform and polarity was increased with methanol. Fractions 250 ml each were collected and monitored by TLC. Two major fractions (A and B) were obtained. Trials for crystallization from different solvent mixtures failed to give pure compounds.

Isolation of saponins 1-3:

Fractions A (12g) was rechromatographed on a silica gel column (450 g: 3 x 100 cm) eluted with chloroform and polarity was increased with methanol . Similar (TLC) fractions were pooled to yield saponins 1-3.

Saponin 1:

Fractions 220-249 eluted with $CHCl_3$: MeOH (75: 25) upon concentrations and crystallization (Methanol) provided and amorphous powder (182 mg) with R_f 0. 42 (solvent system I) with mp 255-257°C; it gave strong froth on shaking with water and showed high molluscicidal activity (LC_{90} = 6 ppm) .

Saponin 2:

Fractions 288-312 eluted with $CHCl_3$-MeOH (50 50) on concentration gave amorphous white powder (155 mg) with R_f 0.27 (solvent system I), mp 268-271°C. This compound gave strong froth on shaking with water and have molluscicidal activity (LC_{90} = 10 ppm).

Saponin 3:

Fractions 370-391 eluted with $CHCl_3$: Me OH (20 : 80) after recrystallization yielded a white powder (125 mg) mp 215 - 215°C with R_f 0.16 (solvent system I). It exhibited molluscicidal activity (LC_{90} 18 ppm).

Isolation of saponins 4 and 5:

Fraction B (6g) was rechromatographed on silica gel column (300g; 3 x 100 cm); eluted with $CHCl_3$ and polarity was increased with methanol.

Fractions 100-134 eluted with $CHCl_3$- Me OH (40:60) upon evaporation and crystallization (Methanol) provided saponin 4 as an amorphous powder (105 mg) with R_f 0.32 (system II); mp 245-247°C, It gave froth on shaking with water and having molluscicidal activity (LC_{90} = 12 ppm) .

Fractions 280-335 eluted with $CHCl_3$-MeOH (10-90) were pooled. concentrated and residue was subjected to preparative chromatography on silica gel TLC using solvent (system III) to afford saponin 5 which on crystallization (methanol) gave amorphous powder (76 mg) with R_f 0.19 (system III) and mp 277 - 279°C. This saponin showed molluscicidal activity (LC_{90} = 30 ppm) and gave strong froth on shaking with water .

Acid hydrolysis of the isolated sponins :

Each saponin about 40 mg was dissolved in 7 % H_2SO_4 in aqueous ethanol (1:1) and was refluxed for 6 hours on water bath. The reaction mixture was concentrated under reduced pressure to remove the ethanol. It was diluted with water (500ml) and the sapongenin was extracted with chloroform (4x 300 ml). The chloroform extract was evaporated to dryness. Each sapogenin was identified from its spectral and physical data as well as by comparison with an authentic samples on TLC (system IV).

Saponins 1,2 and 3 afforded the same sapogenin which was identified as oleanolic acid by mp 303-305°c; MS: m/z (% rel.int.) 456 (M^+,0.87), 441(0.03), 438 (0.13), 423 (0.17), 395 (0.15), 300 (0.59), 248(100, 207 (20.75), 203(17.50), 189 (22.46), 175(15.98) and 133 (29.35); IR (KBr) : 3420,2900, 1685, 1460, 1350, 1264, 826, and 801 cm^{-1}. ^{1}HNMR δ 0.75 -1.15 (7 x CH_3), 5.12 (H-12) in addition to comparison with an authentic samples where as the sapogenins of saponins 4 and 5 were identified as hederagenin by mp 317-319°C; MS: m/z (%, rel int) 472 (M^+,0.7) 454 (0.4) 436 (0.97), 395 (3), 248 (100), 223(8), 203(93), 175(22) 133 (36) and 69 (42); ^{1}HNMR: δ 0.77-1.16 (6 x CH_3), 3.37 (23- CH_2OH) and 5.28 (H-12); IR (KBr) : 3400, 2900, 1685, 1445, 1376, 1250, 1025, and 975 cm^{-1} [12-15].

The residual acidic solution (after extraction of the aglycones) was neutratalzed with barium carbonate and filtered. The filtrate was evaporated to dryness and the residue was extracted with pyridine and foltered the pyridine was evaporated and the residue was dissolved in 10% isopropanol and subjected to PC against sugars using solvent system V and aniline phathalate as visualizing agent [12-14].

The sugars obtained from the saponin hydrolysates were identified as glucose for saponin 1, glucuronic acid and rhamnose for 2 glucuronic acid , glucose and rhamnose for 3, glucose for 4 and glucuronic acid for 5.

Testing for molluscicidal activity:

Stock solutions (500ppm) from the methanol extract as well as the isolated saponins (in distilled water) were prepared (w/v) on different concentrations (ppm). The number of snails used in each experiment and control was ten . The exposure time was 24 hours followed by 24 hours period. standard procedures of WHO committee[16,17] were followed. Statistical analysis of the data was carried out according to Litchfield and Wilcoxon[18].

RESULTS AND DISCUSSION

The methanolic extract of the fresh aerial parts of *Atriplex leucoclada* exhibited molluscicidal activity at 110 ppm within 24 hours against Schiatosomiasis-transmitting snails *Biomphalaria alexandrina*. Thus, This extract was defatted with petroleum ether and then suspended on water and partitioned between chloroform, ethyl acetate and n-butanol.

Fractionation of the butanolic extract on silica gel column chromatography led to two major fractions. These fractions were further separated by another silica gel column and preparative thin-layer chromatography (PTLC)to afford five pure saponins 1-5. They positively responded to the triterpenoid saponins tests [19,20].

Saponin 1 showed IR at 3400 (br.OH), 1695(COOH), 1495 (methyl),1384(gem-dimethyl) and 1175-1040 for the glycosidic linkage[21-25]; the ^{1}HNMR spectrum of this saponin exhibited seven tertiary methyl groups of the aglycone at δ 0.76-1.25, a signal at 5.22 is ascribed to the vinylic proton at H-12 of the aglycone and signal of anomeric protons of the sugar at 4.89 (H-1,glucose)[21-24].

Acid hydrolysis of saponin 1 aglycone identified as oleanolic acid from its mass spectral fragmentation

Zagazig J. Pharm. Sci., December 1995,
Vol. 4, No. 2, pp. 143-146

ISSN 1110-5089

where the molecular ion peak m/z 456 consistant with molecular formula $C_{30}H_{48}O_3$ beside the characteristic fragments m/z 441, 441, 248, 207, 203, and 189. This structure was further confirmed by direct comparison (mp, CO, TLC, IR, ^{1}HNMR) with authentic sample. The sugar obtained from the saponin hydrolysate was identified as glucose by direct comparison with authentic sugar on PC(solvent system V). saponin 2 showed IR bands at 3437 (br,OH), 2925 (CH), 1690 (COOH), 1454 (Me), 1377 (gem- dimethyl) as well as 1181-1033 cm^{-1} which confirmed the glycosidic linkage[21-25]. The ^{1}HNMR spectra showed signals of seven tertiary methyl groups δ 0.77-1.28 and signal of the anomeric protons of the sugars at δ 4.46 (H-1, glucuronic acid) and 5.32 (H-1, rhamnose)[22-25].

Acid hydrolysis of saponin **2** gave an aglycone identical with oleanolinc acid as well as glucuronic acid and rhamnose as the sugar moieties. Moreover, saponin 2 showed high molluscicidal activity (LC_{90} = 10 ppm), therefore the sugar moiety are attached to the aglycone at C-3 (monodesmosidic saponin)[26-28]. From these data the structure of saponin **2** was established as oleanolic acid 3-0-glycoside.

Saponin **3** was obtained as white powder from methanol. The IR spectrum of this saponin exhibited bands of the hydroxyl and carboxylic group at 3450 (br) and 1690 (COOH) beside the characteristic bands of the glycosidic linkage 1158-1073 cm^{-1}. The ^{1}HNMR spectrum revealed the presence of seven tertiary methyls δ 0.75-1.29, a vinylic group at δ 5.16 as well as the anomeric protons of the sugar signal at δ 4.55 (H-1, glucuronic acid), δ 4.86 (H-1, glucose) and δ 5.32 (H-1, rhamnose)[21-26].

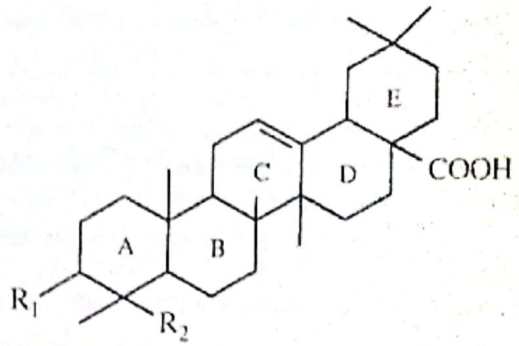
Acid hydrolysis of this saponin exhibited oleanolic acid beside glucuronic acid, glucose and rhamnose as sugar moieties. Moreover, it has shown potent molluscicidal activity (LC_{90} = 18 ppm). From these data saponin **3** was proposed the oleanolic acid 3-0-glycoside structure.

Saponin **4** exhibited molluscicidal activity (LC_{90} = 12 ppm). IR spectrum showed bands at 3450 (br,OH), 2935 (CH), 1700 (OH) and 1180-1020 cm^{-1} for the presence of hydroxyl and carboxylic groups as well as the glycosidic linkage. The ^{1}HMR spectrum showed signals of six methyl groups at δ 0.76-1.28 and the signal of the anomeric proton at δ 4.84 (H-1, glucose)[22-25].

Acid hydrolysis of this saponin gave hederagenin which was identified by mp 318-320°C; MS, m/z 472 and comparison with authentic sample on TLC (solvent system IV). The sugar moiety was identified as glucose by comparison with authentic sugar. From the above data, saponin 4 was proposed hederagenin 3-0-glycoside structure.

Saponin **5** crystallized from methanol with IR bands at 3400 (br,OH), 2927 (CH), 1700 (COOH), 1454 (methanol), 1384 (gem dimethyl), beside the characteristic bands of glycosidic linkage at 1125-1068 cm^{-1}. In the ^{1}HNMR spectrum, the signals at δ 0.79-1.28 (six tertiary methyls), 5.23(H-12) and δ 4.48 correponding to anomeric proton of glucuronic acid[21-23]. Acid hydrolysis of this saponin gave hederagenin and glucuronic acid by direct comparison with authentic samples. From these data, saponin **5** proposed as hederagenin 3-0-glycoside.

Finally, the saponin composition of *Atriplex leucocalada* appears to be complex and is formed of numerous glycosides of oleanolic acid and hederagenin with different sugar moieties. To the best of our knowledge this is the first report on the presence of these saponin in *A. leucoclada*. On the other hand, the high molluscicidal activity of the saponin contents of this plant recommended it as a good source for natural molluscicides.



Saponin	R_1	R_2
1	Glucose	CH_3
2	Glucuronic acid + rhamonse	CH_3
3	Glucuronic acid + glucose + rhamnose	CH_3
4	Glucose	CH_2OH
5	Glucuronic acid	CH_2OH

REFERENCES

1. Kloos, H. and Mc Cullough, F.S.; **Planta Medica,** 46, 195 (1982).
2. Lemma, A., **Ethioplan Med. J,** 3, 84 (1965).
3. Hostettmann, K., **Helv. Chim. Acta,** 63, 606-609 (1980).
4. Marston, A. and Hostettmann, K., **Phytochemistry,** 24, 639-652 (1985).
5. Hostettmann, K., Kizu, K. and Tomimori, T., **Planta Medica,** 44, 34-35 (1982).
6. Bailey, L.H., The **Standard Cyclopedia of Hortlcutture,** Vol. 1 A.E., 29, 427 (1944); McMillan Co., New York, Toronto.
7. Tackholm, V.; **Student's Flora of Egypt,** 2 nd Ed, Cairo Uiversity Press, pp 108-115 (1974).
8. Omar, A.A., Sarg, T.M., Mansour, E.S., and Abdel-Salam, N.A., **Fitoterapia,** 55, 59 (1984).
9. Broegger Christensen S., Omer, A.A., **J. Nat. Prod.**, 48 (1) 161 (1985).

10. El-Sayed, M.M., Ph.D., Faculty of Science, Al Azhar University, Egypt (1989).
11. Shoeb, H., El-Emam, M.A., Saad, A.M., and Mohamed, M.A., **J. Egypt. Soc. Parasit.**, 17 (2) 539-546 (1989)
12. Tiwari, K.P., and Singh, R.B., **Phytochemistry**, 17, 1991-1994 (1978).
13. Masood, M., Pandey, A. and Tiwari, K.P., **Phytochemistry**, 18, 1539-1542 (1979).
14. Tiwari, K.P. and Masood, M., **Phytochemistry**, 19, 1244-1247 (1980).
15. Srivastava, S.K., and Jain, D.C., **Phytochemistry**, 28, 644-647, (1989).
16. WHO (1953): Export Committee on Bilharziasis Control Methods Who 65, 33-38.
17. WHO (1965): Snail Control In The Preventation of Bilharziasis WHO Monograph Ser. 50, 124-138.
18. Litchfield, J.T., and Wilcoxon, F., **Exp. Therap**, 96: 99-113 (1949).
19. Wall, W., Krider, M.M., Krewson, C.F., Willama, J.J., Corell, D.S. and Gentry, H.S., **J. Amer. Pharm. Assoc**, 43, 1-7 (1954).
20. Sannie, C.H., **Analyt. Biochem. Med.**, 9, 175 (1984).
21. Dubois, M.A., Benze, S. and Wagner, H.; **Planta Medica**, 56, 445-455 (1990).
22. Sati, O.P., Bahuguni, S. and Uniyal, S., **J. Nat. Prod.**, 53, 466-469 (1990)
23. Higuchi, R., Tokimitsu, Y., Fujioka, T., Komori, T., and Oakenful, D.G., **Phytochemistry**, 26, 229-235 (1987).
24. Amoros, M., Girre, R.L., **Phytochemistry**, 26, 287-791 (1987).
25. Kizu, H., and Tomimori, T., **Chem. Pharm. Bull.**, 28 (9) 2827-2830 (1980).
26. Joshie, B., Moore, K.M. and Pellietier, S.W.; **J. Nat. Prod.**, 55, 1468-1476 (1992).
27. Dorsaz, A.C., and Hostettmann, K., **Helv. Chim. Acta.**, 69, 2038-2047 (1986).
28. Domon, B., and Hostettmann, K., **Helv. Chim. Acta.**, 67, 1310-1315 (1984).

صابونيات الاتربلكس ليوكوكلادا وتقييمهم كمبيدات لقواقع البلهارسيا

مرتضى محمد السيد

معهد تيودور بلهارس للأبحاث - امبابة - جيزة - مصر

من مستخلص البيوتانول لنبات الاتربلكس ليوكوكلادا أمكن فصل خمسة صابونينات وأمكن التعرف على تركيبها الكيميائى بواسطة الدراسات الطيفية وتحديد كل من الاجليكونات والسكريات المصاحبة لها. كما أظهرت الصابونينات المفصولة فعالية عالية ضد قواقع البيومفلاريا الكسندرينا العائل الوسيط لطفيل البلهارسيا المعوية فى مصر مما يرشح استخدام النبات كبديل لمبيدات القواقع التخليقية.