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MOLLUSCICIDAL SAPONINS FROM BASSIA MURICATA (L.) MURR

Mortada M. El-Sayed

Laboratory of Medicinal Chemistry, Theodor Bilharz Institute,

Imbaba, Giza, Egypt.

ABSTRACT

Four triterpenoidal saponins have been isolated from the aqueous extract of Bassia muricata (L.) Murr (Chenopodiaceae). The structure of these saponins were elucidated by using some spectroscopic methods as well as acid hydrolysis followed by identification of both the aglycones and sugar moieties. The four saponins exhibited molluscicidal activities against Biomphalaria alexandrina snails, the intermediate host of Schistosoma mansoni in Egypt.

INTRODUCTION

The parasitic disease Schistosomiasis is widespread in many tropical and subtropical countries. The disease is linked with certain species of aquatic snails because they serve the parasite as intermediate hosts. Molluscicides or snail-killing activities of plants are of special importance for the control of Schistosomiasis as they seem to be less expensive than the synthetic compounds[1]. Therefore, the attention was drawn to use plant constituents which have molluscicidal activity. In this respect a number of saponins have demonstrated molluscicidal activity [2,5].

In the course of systematic screening studies of medicinal plants for molluscicidal activity, it has been noticed that water suspension of powdered whole plant of Bassia muricata (L.) Murr (Chenopodiaceae) showed an activity of 160 ppm within 24 hours against Biomphalaria alexandrina, the intermediate host of Schistosoma mansoni[6]. To the best

of our knowledge, survey of literature showed that there is no report of any chemical work that has been done on this species. Therefore, this work is an attempt to isolate and determine the principle molluscicidal compounds of Bassia muricata (L.) Murr.

EXPERIMENTAL

Melting points were uncorrected. ^{1}HNMR spectra were recorded on a Varian 200 MHz spectrometer using TMS as internal standard and chemical shifts are expressed in ppm. IR spectra were recorded on a Pye Unicam SP-1100 apparatus. Mass spectra were measured on HP-5988 with direct inlet techniques at 70 ev. Thin layer chromatography was carried out on silica gel Merck FG_{254} and spots were visualized with 40% H_2SO_4 in ethanol. Column chromatography was performed using a glass column 120 x 5 cm using silica gel as a stationary phase. Paper chromatography performed on Whatman No. 1 paper and aniline phthalate was used as a visualizing agent.The following systems were used:

A) $CHCl_3$: MeOH : H_2O (75 : 25 : 5).

B) $CHCl_3$: MeOH : H_2O (65 : 35 : 5).

C) n-BuOH : ACOH : H_2O (4 : 1 : 5).

Plant material:

The whole plant of Bassia muricata (L.) Murr (Chenopodiaceae) was collected from Cairo-Ismalia road. The plant was kindly identified by Prof. Dr. N. El-Hadidi, Prof. of Plant Taxonomy, Cairo University. A voucher specimen is deposited at Laboratory of Medicinal Chemistry, Theodor Bilharz Institute. The whole plant was shade-dried and powdered by electric mill.

Snails:

Biomphalaria alexandrina, the intermediate host of Schistosoma mansoni in Egypt, was used in this study. They were collected from irrigation canals not previously treated with any molluscicide in Abu-Rawash area, ten Killometers from Giza, Governorate (Egypt) and kept for 3 weeks in the laboratory in dechlorinated water for acclimatization with laboratory conditions.

Extraction and isolation of the saponins:

Dry powdered plant (3 Kg) was defatted with petroleum ether (60-80°C). The defatted material was exhaustively extracted with distilled water. The water extract was partitioned with n-butanol. The concentrated n-butanol layer (30 g) was chromatographed over silica gel column (750 g, 5 x 120 cm) and eluted with chloroform and gradient increase of methanol. Fractions were collected (250 ml each) and examined by TLC. Fractions containing similar spots were pooled and concentrated. Fractions (200-232) eluted with $CHCl_3$: MeOH (20 : 80) afforded compound **1** and fractions (267-300) eluted with $CHCl_3$: MeOH (10:90) gave compound **2** whereas fractions 330-368 eluted with (5:95) rechromatographed on preparative thin layer chromatography using solvent B afforded compounds **3** and **4**, respectively.

Isolation of compound 1:

Fractions (200-232) eluted with $CHCl_3$: MeOH (20 : 80) on concentration yielded amorphous compound mp 245-248°C, R_f 0.58 (system A). The compound gave strong froth on shaking with water and showed high molluscicidal activity (LC_{90} = 9 ppm).

Isolation of compound 2:

Fractions (267-300) eluted with $CHCl_3$: MeOH (10:90) on concentration gave amorphous white powder with R_f 0.36 (system B), mp 259-262°C. The compound gave strong froth on shaking with water and have high molluscicidal activity (LC_{90} = 14 ppm).

Isolation of compound 3 and 4:

Fractions (330-368) eluted with $CHCl_3$: MeOH (5:95) yielded two major spots on TLC (solvent B). This mixture was further purified by preparative silica gel TLC plates using (system B) which led to isolation of compounds **3** and **4.**

Compound 3: amorphous white powder with R_f 0.43 (system B), mp 242-244°C. The compound showed high molluscicidal activity (LC_{90} = 4 ppm) and gave strong froth on shaking with water.

Compound 4: amorphous white powder with R_f 0.28 (system B), mp 229-232°C, it gave strong froth on shaking with water and showed high molluscicidal activity (LC_{90} = 7 ppm).

Acid hydrolysis of the isolated saponins:

Each saponin was dissolved in 4N H_2SO_4 in 50% aqueous ethanol. The solution was refluxed for 6 hours. The aglycone was extracted with chloroform and identified by mp, IR, ^{1}HNMR and mass spectra. The saponin **1** afforded hederagenin with mp 318-319°C, IR (KBr): ν 3400, 2900, 2875, 1445, 1250, 1025 and 975 cm^{-1}; PMR : δ 0.74-1.05 (6 x CH_3), 3.35 (CH_2OH), 5.25 (H-12) ppm; MS : m/z (% rel. int.) 472 (M^+, 0.3) 454 (0.2), 395 (2), 248 (83), 223 (7), 206 (16), 203 (89), 189 (24), 175 (20), 133 (46), 105 (41) and 69 (47). Whereas saponins **2-4** gave oleanolic acid with mp 305-306°C; IR ν (KBr): 3400, 2900, 2875, 1670, 1370, 1025, 1000 and 975 cm^{-1}; PMP δ 0.75-1.2 (7 x CH_3), 5.12 (H-12); MS : m/z (% rel. int.) 456 (M^+, 0.87), 454 (0.33), 438

(0.15), 423 (0.17), 410 (0.07), 395 (0.11), 302 (0.16), 248 (100), 207 (20), 203 (97), 189 (22.46), 175 (15.98) and 133 (29.35).

The residual acidic solution (after extraction of the aglycone) was neutralized with barium carbonate and filtered. The filtrate was evaporated under vacuum to drynes and the residue was extracted with pyridine and filtered; The pyridine was evaporated and the residue was dissolved in 10% isopropanol and subjected to PC against authentic sugars using solvent C and aniline phthalate as visualizer to give glucose, arabinose and rhamose.

Testing for molluscicidal activity:

Stock solution (500 ppm) from the different extracts and the isolated saponins (in distilled water) were prepared (w/v) in 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 recovery time. Standard procedures were followed by WHO 1953 and WHO 1965[7,8] . Statistical analysis of the data were carried out according to Litchfield and Wilcoxon[9].

RESULTS AND DISCUSSION

Screening of some extracts, using organic solvents viz.; petroleum ether (60-80°C), benzene, acetone, chloroform and methanol of powdered whole plant of Bassia muricata showed no molluscicidal activity against Biomphalaria alexandria up to 300 ppm whereas the water suspension of the dry plant powder yield a highly potent solution killing the snails at a concentration 160 ppm within 24 hours. This is not surprising because, phytochemical tests of Bassia muricata showed triterpenoid saponins, also it has been reported that the bidesmosidic saponin (sugar in position 3 and 28) is inactive but during water extraction bidesmosodic saponins are

easily hydrolyzed to very active monodesmosidic saponins (sugar in position 3 only) [2]. A similar observation was made during the study of Phytolacca dodecandra (Phytolaccaceae), Polyscias dichroostachya (Araliaceae) and several other plants[1, 10-12].

Thus, the water extract of the defatted dry plant powder of Bassia muricata was partitioned with n-butanol. Butanol layer was chromatographed on silica gel column. Some column fractions were rechromatographed on preparative thin layer. Four compounds **1-4** were isolated and its structure have been elucidated as follows:-

Compound **1**: mp 245-248°C, it gave tests of saponins[13] and showed molluscicidal activity (LC_{90} = 9 ppm). The IR spectrum revealed a broad absorption at 3400 cm^{-1} (hydroxyl groups), bands at 1670 cm^{-1} (carboxyl group) and 1640 cm^{-1} (olefinic proton) besides the characteristic bands of glycosidic linkage at 1020-1175 cm^{-1} [14,16]. The ^{1}HNMR spectrum revealed six tertiary methyl signals at δ 0.8-1.2, CH_2OH at δ 3.6, signal at δ 5.25 is ascribed to the vinylic proton (H-12) and the anomeric proton signals of the sugar moiety at δ 4.76 (glucose, H-1), 4.38 (arabinose, H-1)[15,17-20].

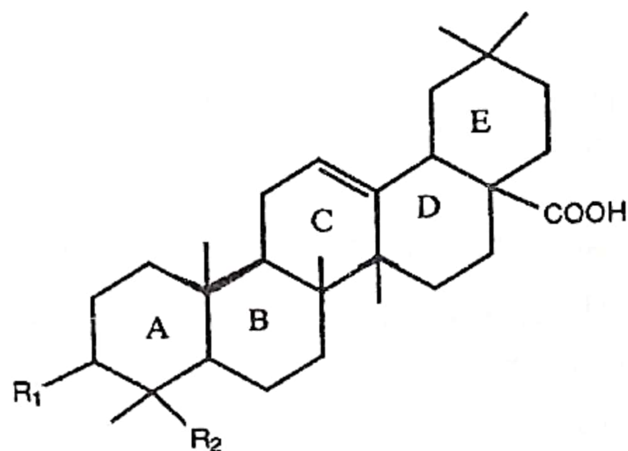
Acid hydrolysis of compound **1** yielded aglycone which was established by mp, IR ^{1}HNMR and mass spectra as well as by comparison with reported literature data as hederagenin[16,19]. It mass spectrum exhibited the typical oleanene skeleton with a 12-13 double bond. The base peak at m/z 248 corresponding to fragment **a** indicated the absence of hydroxyl groups in ring C,D, and E beside peak at m/z 203 due to the removal of the COOH group from fragment **a**; Fragment b2 at m/z 223 resulting from the susbtitution of the CH_3 group by CH_2OH at C-4[16,21,22]. On the other hand, the sugar components were identified as glucose and arabinose by comparison with authentic sugars on PC (solvent C).

From these observation it could be concluded that compound **1** was assigned 3-0-glycoside hederagenin.

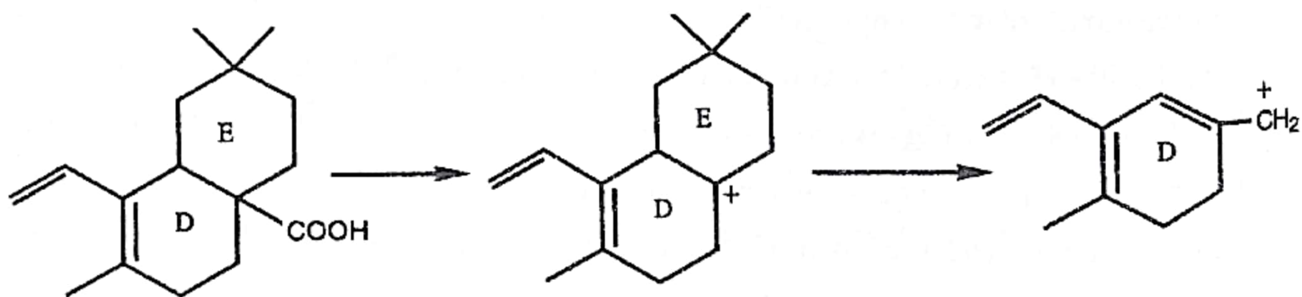
Compound **2:** mp 259-262°C, gave tests of saponin[13] and molluscicidal activity (LC_{90} = 14 ppm). The IR spectrum of the compound exhibited absorption broad band at 3450 cm^{-1} (hydroxyl groups) and band at 1685 cm^{-1} (carboxyl group) besides the glycosidic linkage at 1000-1150 cm^{-1} [14,15]. The ^{1}HNMR spectrum exhibited signals assignable to three anomeric proton at δ 4.76 (glucose, H-1), δ 4.37 (arabinose, H-1) and δ 5.35 (rhamnose, H-1). There were also seven tertiary methyl signals of aglycone at δ 0.77-1.23 and one olefinic proton at δ 5.25 (H-12)[15,17,18].

Acid hydrolysis of compound **2** afforded oleanolic acid as aglycone which was established through mp, ^{1}HNMR and mass spectra as well as by comparison with reported with reported literature data[23,24]. Its mass spectrum exhibited the typical oleanene skeleton[25]. The strong peak at m/z 248 (fragment **a**) were observed as well as peak at m/z 207 (fragment **b**). The sugar components were identified by comparison with authentic samples upon PC (solvent C) as glucose, arabinose and rhamnose from the above data compound **2** was assigned the structure oleanolic acid-3-0-glycoside.

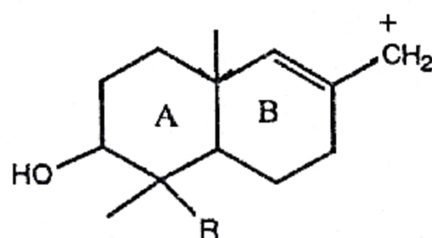
Compound **3:** mp 242-244°C, it showed molluscicidal activity (LC_{90} = 4 ppm) and gave tests of saponins[13]. ^{1}HNMR showed signals of seven tertiary methyl groups of the aglycone at δ 0.76-1.29 as well as the anomeric protons of the sugars at δ 4.62 (glucose, H-1) and 4.39 (arabinose, H-1). The IR spectrum of compound **3** showed absorption bands at 3400 (OH), 1690 (COOH) and the characteristic glycosidic linkage at 1020-1150 cm^{-1}. Acid hydrolysis of compound **3** exhibited oleanolic acid as aglycone and the suagr moiety was glucose and arabinose. From these informations, compounds **3** was proposed the structure oleanolic acid-3-0-glycoside.



Compound	R_1	R_2
1	sugar moiety	CH_2OH
2-4	sugar moiety	CH_3



a) m/z 248 c) m/z 203 d) m/z 133



m/z 207 (R = CH_3)

m/z 223 (R = CH_2OH)

Compound **4:** mp 229-232°C, it showed molluscicidal activity (LC_{90} = 7 ppm) and gave tests of saponins[13]. The IR spectrum exhibited bands at 3400 cm^{-1} (broad, OH), 1695 cm^{-1} (COOH) and 1100-1180) cm^{-1}), respectively. ^{1}HNMR exhibited the anomeric protons of glucose at δ 4.69 besides signals of seven tertiary methyl group at δ 0.8-1.19 of the aglycone and δ 5.30 of olefinic proton (H-12). Acid hydrolysis of compoun **4** afforded oleanolic acid as aglycone besides glucose as sugar residue. The above data indicated that compound **4** was suggested to have the structure 3-0-glycoside oleanolic acid.

In all the isolated saponins **1-4** the sugars are attached to the aglycone at position 3 only (monodesmosidic saponins), this was evident by its high molluscicidal activity with LC_{90} at 9, 14, 1 and 7 ppm within 24 hours respectively. To our knowledge, this is first report of presence of these saponin glycosides in Bassia muricata.

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صابونينات البازيا موريكاتا كمبيدات لقواقع البلهارسيا

مرتضى محمد السيد

معمل الكيمياء العلاجية معهد تيودوربلهارس - أمبابة - الجيزة

أمكن فصل أربع مركبات صابونية من المستلخص المائي لنبات البازيا موريكاتا كما أمكن التعرف علي تركيبها الكيميائي بواسطة الدراسات الطيفية بالإضافة الي تحديد كل من الاجليكونات والسكريات المصاحبة لها . أيضا أظهرت الصابونينات المفصولة فعالية عالية ضد قواقع بيموفلاريا الكسندرينا ، العائل الوسيط للبهارسيا المعوية .