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Molluscicidal Properties and Chemical Constituents of *Euphorbia peploides*

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ABSTRACT

The present study was carried out to evaluate the molluscicidal activities of the 70 % methanolic extract of *Euphorbia peploides* (Family Euphorbiaceae) and petroleum ether, chloroform, ethyl acetate and n-butanol fractions which were derived from the methanolic extract against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt . The results revealed that these extracts exhibited high molluscicidal activities as the recorded LC_{90} values were 30,32,35,26 and 52 ppm for these extracts respectively .The ethyl acetate fraction was the most active one (LC_{90} = 26 ppm) .Eleven compounds have been isolated and identified from $CHCl_3$,EtOAc and n-BuOH fractions using different chromatographic and spectroscopic techniques. These compounds were identified as β-amyrin ,cycloart-23-ene-3β,25-diol, gallic acid, stigmasterol-3-O-β-D-glucopyranoside , quercetin, P-hydroxybenzoic acid, methyl gallate ,luteolin, Kampferol-3-O-β-D-glucopyranoside ,quercetin - 3-O-β -D-glucopyranoside and kampferol -3-O-α - L-rhamnopyranosyl - (1→6)- β -D-glucopyranoside .It can finally be concluded that this plant is rich in triterpenoids and phenolic compounds . It can finally be concluded that this plant is rich in triterpenoids and phenolic compounds. It can finally be concluded that this plant is rich in triterpenoids and phenolic compounds. As this plant and its fractions showed strong molluscicidal activity, so it can be used as botanical molluscicides as part of integrated schistosomiasis control program.

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INTRODUCTION

Schistosomiasis, is a dreadful disease caused by parasitic trematode worms in humans as well as in animals. Various species of fresh water snails act as intermediate host of schistosomiasis. It is widespread in the world especially developing countries. Schistosomiasis is considered second most important disease after malaria as a target disease of the World Health Organization **(**WHO, 1993; Chitsulo *et al*., 2000**)**. Despite more than a century of control efforts and introduction of highly effective antischistosomal drug therapy, the disease just will not go away **(**WHO, 2010**)**. In Egypt, the disease is not only a prime health problem, but it is also an economic one, as it affects million of farmers at the early age diminishing their productivity and exerting a serious socioeconomic problem **(**El-Baz *et al*., 2003; Abdul-Samie *et al*., 2010). Thus, treatment of *Schistosoma* infection remains highly problematic. Mollusciciding is regarded as an important aggressive strategy in the control of the snail hosts of this disease (Giovanelli *et al*., 2001; Hamed, 2010).

Biomphalaria alexandrina is the intermediate host of *Schistosoma mansoni* in Egypt. It is prevalent in both Upper and Lower Egypt (WHO, 2002). Historically, plants and plant derived materials played an important role in the control of various pests including snail vectors of helminthetic diseases. Plant molluscicide appears to be a simple and inexpensive alternative to chemical molluscicides .Several plant species were screened and proved to have molluscicidal properties against different snail species (Abou Basha *et al*.,1994; El-Nahas, 2001; Ahmed and Rifaat, 2005; Sharaf *et al*., 2011; Hassan *et al*., 2012). *Euphorbia* (Euphorbiaceae) is the largest genus of flowering plants in Egyptian flora (Bakry and Hamdi, 2007) .In our previous studies, the dry powder of *Euphorbia peploides* showed molluscicidal properties again *Bulinus truncatus* (Shoeb *et al*., 1990). Therefore, the aim of this study was to evaluate the molluscicidal properties of 70 % methanolic extract of *E. peploides* against *B. alexandrina* snails as well as fractionation and chromatographic isolation of the methanolic extract of this plant.

MATERIALS AND METHODS

General experimental procedures:

Melting points were determined on an electrothermal apparatus and were uncorrected. The ^{1}H and ^{13}C-NMR spectra were recorded on a Brucker Avance -500 and JEOL GX-spectrometer (500 MHz for ^{1}H and 125

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MHz for ^{13}C). The chemical shifts were expressed in δ (ppm) with TMS as reference and coupling constant in (J) Hertz. UV spectra (max) were determined in methanol before and after addition of different reagents on a UV-601 UV-VIS recording spectrophotometer. Mass spectra were measured using Finningan SSQ 700 H, mass spectrometer. Silica gel (70-230 mesh, Merck) and Sephadex LH-20 (25-100 µm, Sigma) were used for column chromatography. Thin-layer chromatography and preparative TLC was performed on silica gel GF 254 pre-coated plates (Merck). Paper chromatography was carried out on Whatmann No.1 or No.3 paper sheets (Whatmann, England). Spots were visualized by absorption of UV radiation and spraying with ethanolic $AlCl_3$ (2 %), $FeCl_3$ and 10 % H_2SO_4 followed by heating for flavonoids phenolic compounds and triterpenoids.

Plant materials:

The aerial parts of *E. peploides* (Family Euphorbiaceae) were collected from the fields of Giza Governorate, Egypt. The plant was kindly identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt. The fresh plant was washed with clean water and completely dried in shade place at room temperature and then powdered by electric mill. The dried powders were kept in dark places until subjected to the extraction process.

Snails:

Biomphalaria alexandrina snails (shell diameter 8-11 mm), the intermediate host of *Schistosoma mansoni* in Egypt were collected from irrigation canals in Abu-Rawash, Giza Governorate. The snails were maintained under laboratory conditions in aquaria filled with dechlorinated tap water (Temp. 25 ± 2 °C and pH 7.0-7.7) for three weeks before being used in experimental tests. Dried lettuce leaves were added daily as food.

Extraction and fractionation process:

The air-dried powder of *E.peploides* (3 Kg) was extracted with 70% methanol at room temperature and the solvent was removed under reduced pressure to give (150 g) of methanol extract .This extract was defatted with petroleum ether and this extract was evaporated to dryness to give 27 g . The defatted residue was dissolved in distilled water and the aqueous filtrate was successively extracted with chloroform followed with ethyl acetate and finally with n-butanol. The chloroform, ethyl acetate and n-butanol fractions were evaporated until dryness to give 18, 11 and 14 g respectively.

Bioassay for Molluscicidal activity:

Molluscicidal activity of the 70% methanolic extract of *E. peploides* and the fractions derived from it was evaluated against *Biomphalaria alexandrina* snails using the standard method according to the WHO (1965) and Lemma (1970) . Stock solution of each extract was prepared by dissolving requisite amount of each extract in minimum amount of absolute ethyl alcohol and was made up with dechlorinated water .Different dilutions were prepared from these stock solutions .Tests of these concentrations were performed in triplicate using ten snails for each test while the control was run using ten snails in only dechlorinated water. The snails were exposed to these concentrations for 24 hours exposure followed with 24 hours recovery period .The percent mortality of the snails were recorded using 10 % NaOH solution. Statistical analysis of the data was carried out according to Litchfield and Wilcoxon method **(**Litchfield and Wilcoxon, 1949**).**

Chromatographic separation of chloroform extract:

The chloroform extract (16 g) was subjected to silica gel column (5 x 120 Cm). Elution started with chloroform followed with $CHCl_3$-MeOH mixtures and finally with methanol. Fractions 250 ml each were collected and monitored by TLC to give four major fractions. Fractions eluted with $CHCl_3$: MeOH; 98: 5 gave two compounds 1 and 2 with few impurities. The two compounds 1 and 2 were separately purified on column Sephadex with MeOH as eluent. Fractions eluted with $CHCl_3$: MeOH; 90: 10 were rechromatographed on other silica gel column using gradient of $CHCl_3$-MeOH mixture to yield two subfractions A and B which were purified on preparative TLC and PPC to yield compounds 3 and 4. Fractions eluted with $CHCl_3$: MeOH; 75: 25 gave compound 5 with small amount of impurities.This compound was purified on Sephadex column with MeOH: H_2O (80 :20) as eluent . Fractions eluted with $CHCl_3$: MeOH; 15:85 gave a major subfraction C. The subfraction C was subjected to preparative paper chromatography (3 mM and 15 % Acetic acid as eluent) to give compound 6.

Compound 1 was obtained as colorless powder with melting point = 185-187 °C, R_f = 0.57 ($CHCl_3$: EtOAc). It responded positively to the Liebermann- Burchard test. IR υ max (KBr) 3400 (OH), 2945, 1660, 1618, 1450, 1380, 1067 cm^{-1} .EI-MS (m/z) 426.

Compound 2 was obtained as white powder ; m.p 180-182 °C, R_f = 0.54 ($CHCl_3$: EtOAc) .^{1}H-NMR δ 5.32 (2H,.m, H-23,H-24), 3.28 (1H, dd, J= 4.04 and 11.0 Hz, H-3), 1.28 (2 × 3H, s, H-26, H-27), 0.93 (2× 3H, s, H-18, H-30), 0.89 (3H, s, H-28), 0.81 (3H, d, J=6.20 Hz, H- 21), 0.81 (3H, s, H-29), 0.50, 0.30 (each 1H, d,

J=4.0 Hz, H-19a, b) ; ^{13}C-NMR δ: 31.45 (C-1), 30.02 (C-2), 78.25 (C- 3), 40.02 (C-4), 46.82 (C-5), 20.70 (C-6), 27.90 (C- 7), 47.67 (C-8), 19.40 (C-9), 25.82 (C-10), 25.04 (C-11), 32.18 (C-12), 45.10 (C-13), 48.03 (C-14), 35.05 (C-15), 26.02 (C-16), 51.60 (C-17), 17.41 (C-18), 29.30 (C-19), 36.05 (C-20), 17.50 (C-21), 38.50 (C-22), 124.70 (C-23), 138.50 (C-24), 70.40 (C-25), 29.48 (C-26), 19.18 (C-27), 60.65 (C- 28), 13.50 (C-29), 25.30 (C-30). EI-MS *m/z:* 442 [M]+, 427, 424, 381, 360, , 355, 315, 302, 267, 175.

Compound 3 was obtained as amorphous powder, 245-247^{o}C, R_f=0.48 (solvent system $CHCl_3$: EtOAc ; 85 :5 , TLC). IR υ $_{max}$ (KBr) 3396 ,3070, 2908, 2650,1705, 1618,1539, 1446, 1340, 1248, 1028, 860 cm^{-1}. ^{1}HNMR δ 6.93 (2H,s, H-2, H-6) , 3.79. ^{13}C-NMR δ 168.04 (C-7) ,144.05 (C-3,C-5), 139.06 (C-4),121.50 (C-1),108.75 (C-2,C-6) . Mass spectra of compound 3 gave base peak at m/z 170.

Compound 4 was obtained as amorphous powder. m.p 287 - 288^{o}C. IR υ max (KBr): 3400 (OH), 1640 (C=C) cm^{-1}.^{1}H-NMR: δ 0.67 (3H, s, Me-18) , 0.78 (3H, d, J = 6.2 Hz, Me-27), 0.80 (3H, t, J = 6.5 Hz, Me- 29) 0.80 (3H, d, J = 6.5 Hz, M-26),0. 93 (3H, s, Me-19), 0.89 (3H, d, J= 6.4 Hz, Me-21), 4.70 (1H, d, J = 7.5 Hz, H-1$^{/}$) .^{13}C-NMR δ 141.50 (C-5), 138.40 (C-22), 128.70 (C-23), 121.40 (C-6), 101.20 (C-1$^{/}$), 77.10 (C-3), ,76.50 (C-5$^{/}$),75.80(C-3$^{/}$), 73 .20 (C- 2$^{/}$), 71.69 (C-4$^{/}$), 61.20 (C-6$^{/}$), 56.30 (C-14), 55.30 (C-17), 51.40 (C-24),49.50 (C-9), 43.09 (C-4), 42.70 (C-13), 39.06 (C-20), 38.90 (C-12), 38.05(C-1), 36.05(C-10),32.03 (C-2), 31.80 (C-25), 31.50(C-24), 31.07 (C-7), 30.70 (C-8),28.63 (C-16), 24.60 (C-28), 24.05 (C-15),20.35 (C-21), 20.70 (C-27), 20.05 (C-11), 18.43 (C-19), 18.02 (C-26), 12.50 (C- 18), 12.20 (C-29).

Compound 5 was obtained as yellowish powder, m.p 312-314^{o}C,R_f = 0.48 ($CHCl_3$: MeOH ; 9 :1 ;TLC) .UV λ_{max}(MeOH) 262,295,368; (MeOH+NaOMe) 256,290,357 ;(MeOH+$Alcl_3$)274,298, 420; (MeOH+ $AlCl_3$+HCl) 270,302,419; (MeOH+NaOAc) 276,298,405; (Me+NaOAc +H_3BO_3) 257,302 ,402 . ^{1}H NMR: δ 12.30 1H,s, C5-OH) ,7.55 (1H, d, *J* = 2.5 Hz,H-2$^{/}$), 7.53 (1H,dd, *J* = 8.5, 2.1 Hz,H-6$^{/}$), 6.84 (1H, d, *J* = 8.5 Hz,H-5$^{/}$), 6.38 (1H, d, *J* =2.5 Hz,H-6), 6.18 (1H, d, *J* = 2.1 Hz,H-8). ^{13}C-NMR δ177.40(C-4), 164.50(C-7), 160.23(C-5), 157.30(C-9), 147.30(C-4$^{/}$), 146.01(C-2), 145.20(C-3$^{/}$), 136.20(C-3).122.76(C-1$^{/}$), 120.50(C-6$^{/}$), 116.45(C-5$^{/}$), 115.05 (C-2$^{/}$), 103.40(C-10), 98.30 (C-6), 94.30 (C-8).

Compound 6 was obtained as white powder, m.p = 212 – 214^{o}C, R_f = 0.62 (EtOAC –MeOH ; 9:1; TLC). IRυ max (KBr) 3450, 2800 1680 1600,1540,1440 ,845 cm^{-1} .^{1}H-NMR δ 8.23 (1H,brs, 4-OH), 7.75 (2H ,J=8.50 Hz, H-2,H-6), 6.80 (2H,d,J=8.65Hz, H-3,H-5).^{13}C-NMR δ 167.50(C-7),162.32(C-4),132.05(C-2,C-6),120.40(C-1),114.60(C-3,C-5). EI-MS (m/z) 138, 120, 90 and 75.

Chromatographic isolation of ethyl acetate extract:

The ethyl acetate fraction (10 g) was first fractionated on silica gel column with $CHCl_3$- MeOH gradient as eluent. Three major fractions were obtained. Two of them which eluted with $CHCl_3$ –MeOH 90: 10 and 70 :10 were collected and rechromatographed on other silica gel column and eluted with $CHCl_3$: MeOH gradient to give compounds 7 and 8 . The other fractions eluted with $CHCl_3$- MeOH; 60: 40 and 25: 75 were purified on Sephadex LH- 20 column using 80 % MeOH to give compound 9

Compound 7 was obtained as colorless powder**,** mp: 201 -203 °C, R_f = 0.62 ($CHCl_3$:MeOH ;9 :1), IR υ_{max} (KBr) 3455 (OH) ,2955,1740 ,1697 ,1618 ,1590,1070,830 cm^{-1} .^{1}H NMR: δ 7.05 (2H, s,H-2,H-6), 3.75 (3H, s,OCH_3). ^{13}C-NMRδ 167.56 (C-7), 144.60 (C-3, C5) 138.07 (C-4) 118.90 (C-1), 108.05 (C-2,C-6).50.40(C-8) . EI-MS (*m/z*) 184, 153,125, 107, 79, 53.

Compound 8 was obtained as yellow amorphous powder; mp 223-225 ^{o}C; R_f=0.04 (15% AcOH ,PC); UVλ $_{max}$(MeOH) 268,325,405; (MeOH+ NaOMe)270,318, 410; (MeOH+$AlCl_3$) 272, 295, 416;(MeOH+ $AlCl_3$+ HCl) 270, 304, 402;(MeOH+ NaOAc) 272,318, 400; (MeOH+ NaOAc+H_3BO_3) 269, 320,398 nm. ^{1}H-NMR δ 7.40 (1H, dd, J=8.5 Hz, J=2.0 Hz, H-6/),7.35 (1H,d, J=2.2 Hz ,H-2$^{/}$),6.80 (1H,J=7.9 Hz,H-5$^{/}$),6.40 (1H, J=2.0 Hz,H-8),6.20 (1H,J=2.1 Hz ,H-6).

Compound 9 was obtained as yellow powder, m.p 250-252 ^{o}C, R_f = 0.42(15%AcOH, PC).UV λ $_{max}$ (MeOH) 267,290,318 ; (MeOH+ NaOMe) 272,330,402; (MeOH+$AlCl_3$) 274,309,,398; (MeOH+ $AlCl_3$+ HCl) 270,305, 400; (MeOH + NaOAc) 270,297, 367; (MeOH + NaOAc + H_3BO_3) 267,289, ,348 nm .^{1}HNMR 12.42 (1H,s,5-oH) ,7.89 (2H,J=8.2 Hz,H-2$^{/}$,H-6$^{/}$) ,6.82 (2H,d, J= 8.5 Hz,H-3$^{/}$,H-5$^{/}$) ,6.40 1H,s,J=2.2 Hz,H-8),6.25 (1H,d,J=2.1 Hz,H-6),5.25 (1H,d, J=7.9 Hz,H-1$^{//}$). ^{13}CNMR δ 177.32 (C- 4),163.18 (C-7), 160.53 (C-5), 158.82 (C-4 $^{/}$), 156.24 (C-9), 156.05 (C-2), 133.12 (C- 3), 130.54 (C-2$^{/}$), C-6$^{/}$),120.90 (C-1$^{/}$), 115.05 (C-3$^{/}$, C-5$^{/}$), 103.75 (C-10),100.94 (C-1$^{//}$) 98.61 (C- 6), 93.900 (C-8), 77.49 (C-3$^{//}$, 76.32 (C-5$^{//}$), 74.05 (C-2$^{//}$), 71.69 (C-4$^{//}$),60.59 (C6$^{//}$).

Chromatographic isolation of the butanolic fraction:

The butanolic extract (9 g) was subjected to silica gel column. Elution started with chloroform followed with CHCl3-MeOH mixtures and finally with methanol. Fractions 250 ml each were collected and monitored by TLC. Fractions eluted with $CHCl_3$: MeOH ; 90:10 gave compound 10 with few impurities which was purified on preparative TLC with elution system CHCl3 : MeOH ; 9 : 1 .Fractions eluted with $CHCl_3$: MeOH ; 75 :25 gave three subfractions but only one of these subfractions can be purified using Sephadex column with MeOH as eluent and PPC using 15 % Acetic acid as solvent system to give compounds 11 .

Compound 10 was obtained as yellow powder,m.p 232-234 ^{0}C, R_f = 0.25 (solvent system; $CHCl_3$: MeOH, 9 :1 ;TLC) and 0.42(n-BuOH : AcOH :H2O 4:1:5 ; PC). UV λ_{max}(MeOH) 257,285,350 ;(MeOH+ NaOMe) 270,385,400 ;(MeOH+AlCl3)275; 295,420 ; (MeOH+ $AlCl_3$+HCl) 268, 298,400; (MeOH+NaOAc) 270,312,380;(MeOH + NaOAC+H_3BO_3) 259, 289, 385 nm .^{1}H-NMR δ 12.52(1H,brs,5-OH),7.65(1H ,d, J=2.1 Hz,H-2$^/$).,7.52(1H,d,J=7.5 Hz, H-6$^/$), 6.70 (1H ,d, J=7.8 Hz,H-5$^/$) , 6.35 (1H ,d, J=2.0 ,H-8), 6.12 (1H, ,d, J= 2.5 Hz, H-6), 5.21 (1H, d, J=7.5,H-1$^{//}$) . ^{13}C-NMR δ 177.45 (C-4),164.25 (C-7),160.20 (C-5), 157.23(C-9),156.84 (C-2) ,149.80 (C-4$^/$),145.50 (C-3$^/$), 134.06 (C-3),122.32 (C-6$^/$), , 121.45 (C-1$^/$), 116.05 (C-5$^/$) ,115.48 (C-2$^/$) ,104.34 (C-10) ,101.05 (C-1$^{//}$) ,99.35 (C-6) ,94.25 (C-8),76.50 (C-5$^{//}$), 74.20 (C-3$^{//}$) ,72.50 (C-2$^{//}$), 72.50 (C-4$^{//}$),60.12 (C-6$^{//}$).

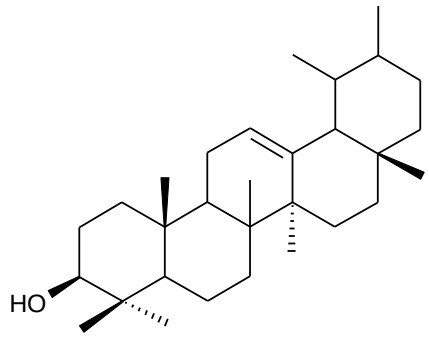
Compound 11 was obtained as yellow powder, m.p, 227-229 ^{o}C ,R_f: 0.44 (BAW, TLC), 0.50 (15%HOAc PC); . UV λ_{max}(MeOH 260,319, 360 ;(MeOH +NaOMe) 274, 340, 405 ; (MeOH +$AlCl_3$) 275, 318, 356, 397 ;(MeOH +$AlCl_3$+HCl) 272, 303, 352,399 ;(MeOH +NaOAc) 274, 312, 395; (MeOH+NaOAc/H_3BO_3) 265, 302,370. ^{1}H-NMR δ 12.42 (1H,s ,5-OH), 8.04 (2H, d, J= 8. 5 Hz, H-2$^/$,6$^/$), 6.89(2H, d, J= 8.7Hz, H-3$^/$,5$^/$), 6.27 (1H, d, J = 2.12 Hz, H-6), 6.22 (1H, d, J = 2.1Hz,H-8), 5.12 (1H, d, J = 7.5 Hz, Glc, H-1), 4.40 (1H, d, J = 1.55 Hz, Rha, H-1), 1.04 (3H, *d*, *J* = 6.20 Hz, Rha- Me). ^{13}C-NMR δ : □ 177.45 (C-4), 164.10 (C-7), 161.40 (C-5), 159.50 (C-4$^/$), 157.25 (C-9), 156.54 (C-2), 133.18 (C-3), 130.89(C-2$^/$, C-6$^/$), 121.40 (C-1$^/$), 115.43 (C-3$^/$, C-5$^/$), 104.30 (C-10), 99.14 (C-6), 94.30 (C-8);101.43 (C-1$^{//}$ of Glc), 76.50 (C-3 //), 76.21 (C-5 $^{//}$), 74.56 (C-2 $^{//}$), 71.48 (C-4 $^{//}$), 67.40 (C-6 $^{//}$); 100.50 (C-1$^{///}$of Rha), 72.80 (C-4 $^{///}$), 70.50 (C-3 $^{///}$), 70.20 (C-2 $^{///}$), 68.20 (C-5 $^{///}$), 17.86 (C-6 $^{///}$).

RESULTS AND DISCUSSION

Plants belonging to family Euphorbiaceace exhibited molluscicidal properties against the different snail species **(**Mendes *et al*., 1997; Bakry, 2009; Hassan *et al*., 2012**)**. In the present study, the 70% methanolic extract of *E.peploides* showed high molluscicidal properties (LC_{90} = 30 ppm) against *B.alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt as shown in table (1). Therefore, the methanolic extract was submitted to fractionation by petroleum ether, chloroform, ethyl acetate and n-butanol followed by chromatographic separation of $CHCl_3$, EtOAc and n-BuOH fractions . The results of the molluscicidal activity in table 1 revealed that these fractions have activity (LC_{90}=32, 35, 26 and 52 ppm)respectively.The ethyl acetate fraction was the most active fraction (LC_{90}=26 ppm) while n-BuOH fraction showed lowest activity (LC_{90} = 52 ppm) . The literature indicated that the ideal concentrations of plant extracts for molluscicidal activity must be below 100 ppm **(**WHO, 1993). Thus, the present results indicated that the 70% methanolic extract of *E.peploides* and the different fractions derived from it could be recommended as strong molluscicidal agent for controlling *B.alexandrina* snails the intermediate host of *Schistosoma mansoni* in Egypt.

The $CHCl_3$, EtOAc and n-BuOH fractions derived from the 70% methanol were submitted to chromatographic isolation and the isolated compounds were elucidated by using certain spectroscopic analysis and physical properties.

IR spectrum of compound 1 exhibited the presence of a hydroxyl function and the olefinic moiety at 3400 and 1660 and 1618 cm^{-1} and its MS spectrum showed a parent ion peak at m/z 426. It was identified as β-amyrin by co-chromatography with a reference substance and by mixed mp **(**Saha *et al*., 2011; Abdel-Monem *et al*., 2008**).** By comparison of UV spectra of compounds 5 and 8 in MeOH before and after addition of the different shift reagents and their NMR spectrum with the reported data in the literature (Bylka and Matlawska,2001; Gudei, 2003 ; Guvenalp and Demirezer,2005) as well as by their mp and mixed mp with authentic sample , compounds 5 and 8 were identified as quercetin and luteolin .Also compound 3 was identified as gallic acid by direct comparison of its UV and NMR spectrum with the previous reported data **(**El-Dahshan, 2011; Rao *et al*.,2012).



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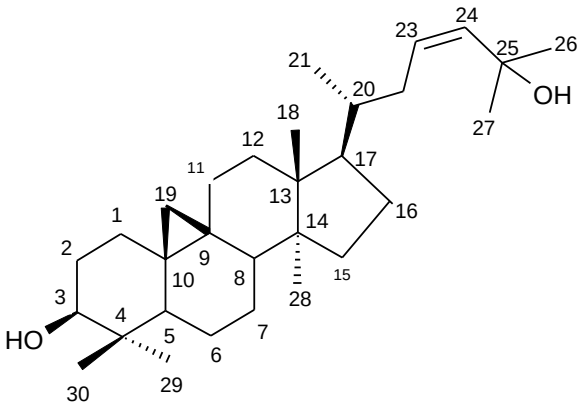

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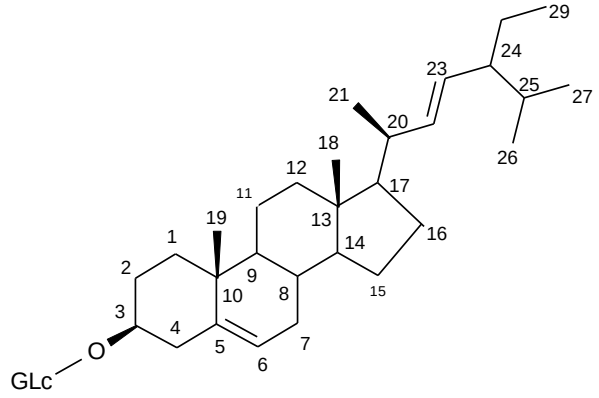

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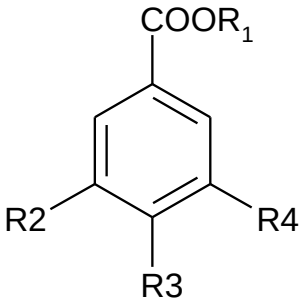
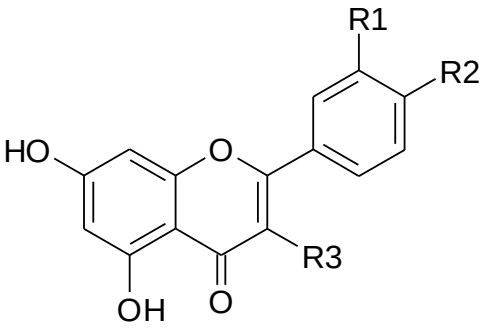


Compound	R_1	R_2	R_3	R_4
3	H	OH	OH	OH
6	H	H	OH	H
7	CH_3	OH	OH	OH



Compound	R_1	R_2	R_3
5	OH	OH	OH
8	OH	OH	H
9	OH	H	O-GLC
10	OH	OH	O-GLC
11	OH	H	O-GLC$_{(1\rightarrow6)}$Rha

Fig. 1: The structure of the isolated compounds.

The ^{1}HNMR spectrum of compound 2 exhibited characteristic signals of six tertiary methyl groups at δ 1.28 (2 × 3H, s, H-26, H-27), 0.93 (2× 3H, s, H-18, H-30), 0.89 (3H, s, H- 28) and 0.81 (3H, s, H-29), one secondary methyl group at δ 0.81 (3H, d, *J*=6.4 Hz, H- 21), a pair of doublets at up-field δ 0.50 and 0.30 (2H, d, *J*=4.50 Hz, H- 19a,b) and two olefinic protons at 5.32 (2H,H-23 and H-24) (Ferreira et al.,2001). The position of double bond between C-23 and C-24 was confirmed by presence of the two carbon signals at δ 124.70 and 138.50 and the signal of C-22 at δ 38.50 (C-22) in the ^{13}C-NMR spectrum (Ghanadian *et al*.,2013) .Also, the positions of the two hydroxyl groups at C-3 and C-25 were reflected by presence of the signals of the two carbons at δ 78.25 and 70.40 respectively (Abdel-Monem *et al*.,2008; Ghanadian *et al*.,2013). The molecular ion peak of this compound appeared at m/z 442 and its fragmentation pattern exhibited two characteristic ions of tetracyclic triterpens at *m/z* 355 and 302, as well as three ions at m/z 381 (M $-H_2O-C_3H_7$), 355 [M-H_2O-C_5H_9] and 315 (M-side chain) (Ferreira et al.,2001; Abdel-Monem et al.,2008; Ghanadian et al.,2013). By comparing the above data and with the reported data in the literature (Ferreira *et al*.,2001; Abdel-Monem *et al*.,2008 ; Ghanadian *et al*.,2013) compound 2 was identified as cycloart -23-ene-3 β, 25-diol.

Compound 4 was isolated as amorphous powder. The NMR of it was similar to stigmasterol except for additional signals of the sugar residue especially the anomeric proton at δ 4.70 (1H, d, J= 7.5 Hz,H-1$^{/}$) in ^{1}HNMR and signal of anomeric carbon at δ 101.20 in ^{13}C-NMR (Falodun *et al*.,2008; Ridhay *et al*.,2012**) .**The down field of C-3 of the aglycone at δ 77.10 as compared of stigamsterol (at δ 71.90) reflected that C-3 is position of attachment of glucose **(**Falodun *et al*.,2008) . The ^{13}C-NMR displayed 35 carbon signals, 29 carbons for the stigmasterol moiety while the remaining 6 for the sugar molecule (Falodun *et al*., 2008; Ridhay *et al*.,2012)**.**Thus, the structure of compound 4 was determined as stigmasterol 3-O- β - D-glucopyranoside**.**

Compound 6 gave positive with 5% $NaHCO_3$ solution indicating the presence of carboxylic group. This was confirmed by presence of absorption band at 1680 cm^{-1} the IR spectrum .The ^{13}C- NMR displayed 7 carbon signals resonating at δ 167.50,162.32,132.05,120.40 and 114.60 for (C-7),(C-4),(C-2,C-6),(C-1) and (C-3 ,C-5) respectively **(**Pandey *et al*.,2011; Ali *et al*.,2014**)** . EI-MS mass spectrum of the compound showed the molecular ion and base peak at m/z 138 and the other fragmentation peaks at 120, 90 and 75 **(**Pandey *et al*., 2011; Ali *et al*., 2014**)**. By comparison with authentic sample (Co-TLC, melting point, mixed melting point), compound 6 was elucidated as P-hydroxybenzoic acid.

IR spectrum of compound 7 exhibited the characteristic bands of the double bond at 1618, the hydroxyl group at 3455 and C-H stretching at 2955 (El-Dahshan, 2011; Rao *et al*.,2012) . The ^{1}HNMR spectrum showed two aromatic protons at δ 7.05 (2H,H-2,H-6) and one methyl at δ 3.75 (3H, s,OCH_3) .This was confirmed by presence of the signal of C-2 and C-6 at δ 108.05 and the ester carbonyl at δ 167.56 in ^{13}C-NMR spectrum (Ekaprasada *et al*.,2009; El-Dahshan, 2011) .The molecular ion of compound 7 appeared at m/z 184 .The fragment ions was observed at m/z 153 , m/z 125 due to loss of OCH_3 followed by CO (Ekaprasada *et al*., 2009; Rao *et al*.,2012) .From the above data, compound 7 was elucidated as methyl gallate.

Compound 9 was assumed to be a kampferol- monoglucoside from its UV spectra in methanol before and after additions of the different reagents as well as from its NMR spectrum . The anomeric proton signal of the glucose was appeared at δ 5.25 (1H, d, J=7.9 Hz, H-1$^{//}$). This was confirmed by presence of the carbon signal at δ 100.94 (C-1") . The chemical shifts of other glycosidic carbon signals at δ 77.49 (C-3"), 76.32 (C-5"), 74.05 (C-2"), 71.69 (C-4") and 60.59 (C-6") suggested the presence of a β-glucopyranosyl group. The down field of C-3 of the aglycone moiety at δ 133.12 as compare with the same carbon in kampferol indicated that this carbon is position of attachment of the sugar moiety .This was confirmed with the presence of the anomeric proton at δ 5.25 in ^{1}HNMR and the anomeric carbon at δ 100.94 in ^{13}CNMR spectrum (Hua *et al*., 2001; Anwal *et al*., 2008). From the above data compound 9 is kampferol 3–O-β-D-glucopyranoside .

The ^{1} HNMR spectrum of compound 10 showed three aromatic proton signals at δ 7.65 (1H ,d, J=2.1 Hz,H-2$^{/}$) ,7.52(1H ,d, J=7.5 Hz, H-6$^{/}$),6.70 (1H ,d, J=7.8 Hz,H-5$^{/}$) in the form of an ABD spin-system suggesting a flavonol with 3`,4`- disubstituted B-ring and showed a pair of meta coupling proton signals at δ 6.35 (1H ,d, J=2.0 ,H-8), 6.12 (1H, ,d, J= 2.5 Hz, H-6) for the A –ring (Bylka and Matlawska, 2001; Gudei,2003). It also showed anomeric signal for glucose moiety at δ □ 5.21 (1H,d, J=7.5, H-1``) (Gudei,2003). The ^{13}CNMR spectra supported this hypothesis and showed 21 signals including carbonyl signal at δ □ 177.45 (C-4). It revealed chemical shifts at δ □ 134.06 (C-3), 160.20 (C-5), 164.25 (C-7), 145.50 (C-3`), 149.80 (C-4`) that suggested the 3, 5, 7, 3`, 4`- oxygenated flavone nucleus. It showed significant glucose signals at δ□ 101.05 (C-1$^{//}$), 72.50 (C-2$^{//}$), 74.20 (C-3$^{//}$),72.50 (C-4$^{//}$),76.50 (C-5$^{//}$) and 60.12 (C-6$^{//}$) (Hua *et al*.,2001; Gudei,2003). Thus, compound 10 was identified as quercetin-3-O- β -D-glucopyranoside.

Compound 11 , its UV spectra in methanol before and after addition of different reagents and NMR spectrum were identical with compound 9 except for appearing of additional rhamnose unit .This was suggested by appearing of two anomeric protons for glucose and rhamnose units at δ 5.12 (1H, d, J = 7.5 Hz, Glc, H-1) , 4.40 (1H, d, J = 1.55 Hz, Rha, H-1), and the methyl of rhamnose unit at δ 1.04 (3H, *d*, *J* = 6.20 Hz, Rha- Me) in ^{1}HNMR spectrum (Hou *et al*., 2005; Anwal *et al*.,2008). In ^{13}CNMR spectra , two anomeric carbon signals appeared at δ 101.43 (C-1$^{//}$ of Glc) and 100.50 (C-1$^{///}$of Rha) .The point of the attachment of the two sugar

units was confirmed by the down field of the signal C-6 of glucose at δ 67.40 (C-6$^{//}$) (Hua *et al*.,2001; Hou *et al*., 2005; Anwal *et al*.,2008).Therefore, compound 11 was elucidated as kaempferol 3-*O*- α -L-rhamnopyranosyl-(1→6)- β -D-glucopyranoside.

Table 1: Molluscicidal activity of 70% methanolic extract of *E.peploides* and some fractions derived from it against *B.alexandrina* snails.

Extract	LC_{50}(ppm)	LC_{90}(ppm)	Slope function
70% MeOH	19 (14.56 - 24.52)	30 (26.82 - 35.35)	1.35
Pet.ether fraction	21 (15.62 – 26.94)	32 (27.26 – 38.45)	2.05
$CHCl_3$ "	23 (18.65 - 29.55)	35 (29.23 - 40.72)	1.64
EtOAc "	15 (10.54 - 20-21)	26 (20.74 – 32.32)	1.52
n-BuOH "	33 (26.81 - 39.51)	52 (46.66 - 57.22)	2.06

Conclusion:

It can finally be concluded that this plant is rich in triterpenoids and phenolic compounds. As this plant and its fractions showed strong molluscicidal activity, so it can be used as botanical molluscicides as part of integrated schistosomiasis control program.

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