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MOLLUSCICIDAL ACTIVITY OF SOME CONSTITUENTS ISOLATED FROM *CESTRUM PURPUREUM*

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Abstract

Purified compounds of *Cestrum purpureum* (family: Solanaceae), were used as plant molluscicides against the vector snails *Biomphalaria alexandrina.* Phytochemical screening tests aiming at identification of the different groups of the constituents of *Cestrum purpureum* were carried out and the results showed that the plant contains glycosides, sterols, saponins, tannins and flavonoids where alkaloids are absent. Three compounds were isolated from *Cestrum purpureum* by using silica gel column chromatography and thin layer chromatography and could be identified using spectroscopic analysis (IR, ^{1}HNMR, ^{13}CNMR and chemical ionization mass spectrum) and hydrolysis to be compound **(1)** was ursolic acid, compound **(2)** was 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-methylglucuronopyranosyl-21-cinnamoyloxy-hederagenin and compound **(3)** was 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-2,19-dihydroxyepihederagenin-28-O-1-[26-(3-acetyl-4-methoxycinnamoyl-oxyhexa-cosanoyl)] glycerol. Literature survey indicates that compounds **(2 and 3)** are a new compounds separated from *C. purpureum* for the first time. Compound **(2)** showed molluscicidal activity at a concentration of 25ppm.

Keywords: Solanaceae, hederagenin, *Cestrum purpureum* , snails, glycerol, chromatography

Introduction

Schistosomiasis, the most important trematode disease of man, is a world health problem. It is one of the most prevalent endemic diseases in tropical and subtropical regions, where it is spreading as cultivated areas increase [1]. This parasitic disease is affecting more than 390 million peoples over 73 countries [2] causing tremendous loss in economy and man power. Numerous schistosomiasis control projects in Egypt, Brazil. Ghana, Philippines, Venezuela and elsewhere, have shown that snail control by molluscicides either alone or in combination with other methods, can be a rapid and efficient means of reducing or eliminating transmission [3]. Since the 1930's more than 1000 plant species have been tested for molluscicidal activity. Only a few of them are used for snail control. Economic and ecologic considerations increasing favor the use of molluscicides that are selectively active, biodegradable, inexpensive and readily available in affected areas. The high cost of imported synthetic compounds along with increasing concern over the possible build up of snail resistance to these compounds and their toxicity in non target organisms have given new impetus to the study of plant molluscicides [4]. Phytochemically, the Solanaceae plants are characterized by the occurrence of numerous alkaloids. Steroidal glycoalkaloids (SGA) are nitrogenous secondary plant metabolites found in all parts of Solanum species [5]. It is generally thought that glycoalkaloids probably evolved as protective compounds in response to tissue invasion by fungi and/or to herbivory, but the information relating to antifungal and/or antifeedant / insecticidal properties is predominantly concerned with solanine and chacomine [6,7]. It was reported that monodesmosidic saponins showed strong molluscicidal activity, whereas aglycones as well as bidesmosidic saponins are inactive. These compounds occur in most plant tissues including the fruit, leaf, bud, flower and stem [8]. *Cestrum diurnum* is indigenous to South America and West Indies and it is cultivated as an ornamental plant in Pakistan as well as in other parts of the world. There are more than 300 species in the genus [9] and there are several applications of these species in folk medicine [10]. Tigogenin, and ursolic acid have been isolated from alcoholic and ethereal extracts of *C. diurnum* [11,12]. Solanidine was mainly found in Solanaceae [13] and also isolated from *Cestrum purpureum* [14]. The triterpenoids with one or more sugar side chain form mono-, bi-, or tridesmosidic glycoside, the sugar is often attached at C-3 through an ether linkage in monodesmosidic glycosides and the other is found to be attached at carbon C-28 through an ester linkage in bidesomosidic glycosides. Isolation of the tridesmosidic glycoside is rare, recently olean-12-en (quinoside A) with sugars at C-3, C-23, and C-28 of hederagenin was isolated [15]. In bidesmosidic glycoside, the removal of sugar chain from carboxylic group attached to C-17 by alkaline hydrolysis causes increasing in the activity of bidesmosidic glycoside, while the removal of sugar chain from C-3 of monodesmosidic glycoside give inactive aglycone [16]. The molluscicidal activities of glycosides vary with the sequence of sugar, the nature of sugar chain and the substitution patterns of the aglycone [17].

Methods

Experimental

IR spectra were recorded on PERKIN ELMER 1650 and on BRUKER vector 22 Germany apparatus, Melting points were detected by an electrothermal apparatus. ^{1}HNMR and ^{13}CNMR (δ (ppm), J (Hz)) spectra were recorded on a Varian GE-MHZ, a Varian (300) NMR, Oxford, and (75) ^{13}CNMR for Mercury spectrometer by using DMSO-d6, CD_3OD as a solvent and $SiMe_4$(TMS) as internal standard chemical shifts are expressed as ppm units. Mass spectra were operated on HP-5988 and on GC-MS-1000 EX with direct inlet equipments at 70 ev. Chromatographic glass plates (20 x 20 cm) and (10 x 20 cm) coated with silica gel (Merck FG_{254}), glass columns (120 x 7cm) packed with silica gel G_{60} Merck, paper chromatography Whatmann No. 1 sheets. The chromatograms and the chromatographic glass plates were visualized under UV light VilberLourmat (VL -6LC France) at 365and 254 nm.

Plant materials

Cestrum purpureum (Solanaceae), collected from El-Zohreia garden in Cairo during, July.

Extraction

The dry powder of the whole plant *Cestrum purpureum* (1kg) was extracted with 70% methanol at room temperature (4 times, 7 days). Methanol extract was evaporated under vacuum to give a dark brown residue (145 gm), which was successively washed with petroleum ether (60-80 °C), benzene, chloroform, ethyl acetate, acetone and the residue was dissolved in H_2O, then partitioned with n-butanol. The butanol extract was evaporated under reduced pressure to give (50 gm) mixture of saponin extract.

The saponin mixture (50 gm) was chromatographed on silica gel column which was eluted successively using solvent systems with increasing polarity starting from petroleum ether, petroleum ether : chloroform ratios, chloroform, and chloroform : methanol increasing from 0-100. The elutes were collected in fractions each 250 ml, the separation monitored by TLC (table **3**). The chromatograms were examined by using UV light after spraying with 40% sulphuric acid in methanol followed by heating in oven at 120°C for 10 minutes.

Preliminary phytochemical screening

The dry powder and different solvent extracts of *C. purpureum* plant laves were subjected separately to the following phytochemical tests and the results were recorded in table **(1)** for the dry powder and table **(2)** for the corresponding extracts.

1. **Test for carbohydrates and glycosides:** One gm of the dry powder was extracted with 10 ml of 50 % aqueous ethanol. 5 ml of ethanol extract were mixed with 0.5 ml of ethanolic α-naphthol solution followed by one ml of H_2SO_4 poured carefully on the wall of the test tube. The appearance of a violet ring between the two layers indicates the presence of carbohydrates and /or glycosides [18].
2. **Test for sterols and/or triterpenes:** For each powdered sample, about 10 ml of ethanol extract were evaporated to dryness. The residue was dissolved in 20 ml of chloroform solution and filtered. The filtrate was subjected to the following tests.
   a. **Salkowski test:** To about 5 ml of the chloroform solution, an equal volume of sulphuric acid were added carefully. Formation of a red colour indicates the presence of sterols and /or triterpenes.
   b. **Liebermann-Burchard test:** Evaporate about 5 ml of the chloroform solution of each sample to a small volume, about 1ml of acetic anhydride was added followed by 2 ml of H_2SO_4 poured carefully on the wall of the test tube to form a lower layer. A reddish brown colour appeared at the junction between the two layers indicates the presence of unsaturated sterols and/or triterpenes [19].
3. **Test of saponins:** About 10 gm of the plant powder were shaken with distilled water and filtered. This filtrate was shaken strongly and allowed to stand for five minutes. The presence of voluminous froth indicates the presence of saponins [20].
3. **Test for tannins:** About 2 gm of the powdered plant were extracted by 20 ml of 50 % aqueous ethanol and filtered. Add few drops of ferric chloride solution; a green colour was obtained confirming the probability of existence of catechol tannins [21].
4. **Test for flavonoids:** 5 gm of the dry powdered sample were soaked over-night with 150 ml of 1% hydrochloric acid solution and filtered. The filtrate was subjected to flavonoid compounds tests as follows:
5. **About** 10 ml of the filtrate was rendered alkaline with NaOH solution. The appearance of a yellow colour indicates the presence of flavonoids.
6. **About** 5 ml of the filtrate was blended with 5 ml of HCl and few pieces of magnesium metal were added. The formation of a red colour proves the presence of flavonoids [22].
7. **Test for alkaloids and/or nitrogenous bases:**10 gm of the dry powdered plant were splitely extracted with 100 mlof dilute hydrochloric acid. The acidic extract was filtered, and rendered alkaline with ammonium hydroxide solution, followed by extraction with chloroform. The chloroform extract was evaporated till dryness and the extract was dissolved in 2 ml of HCl. The formation of very faint brown precipitate with Wagner's reagent and very slight precipitate with Mayer's reagent confirming the presence of nitrogen bases [23].

TLC systems:-

Benzene: toluene
1 : 1
Chloroform
Chloroform : methanol
9.5 : 0.5
Chloroform : methanol : H_2O
65 : 35 : 0.5
6.5 : 5 : 1
Butanol : acetic acid
4 : 1

Acid hydrolysis of compounds (2 and 3)

About 20 mg of the pure compound was hydrolyzed using 4N HCL (10 ml) and (5 ml) methanol on boiling water bath for about (3-4) hours. The mixture was concentrated under reduced pressure to remove the methanol solvent; then the mixture was partitioned between chloroform and water by using separating funnel. Evaporation for the chloroform extract under reduced pressure occurs and crystallized to give the

Molluscicidal activity tests

Snails

The intermediate host of *Schistosoma mansoni*, *Biomphalaria alexandrina* snails (shell diameter 6-8 mm), were collected from irrigation canals at Giza governorate which had not been subjected to molluscicides before. The snails were left to acclimatize in the laboratory for about three weeks within pH 7, in dechlorinated tap water before being used (temperature adjusted at 25±2 °C).

Prepare different concentration from the dry powder and methanol extract of the plant to produce an aqueous suspension on the basis of weight / volume (ppm) in dechlorinated tap water. The same technique was carried out with the compound isolated from the plant. Three replicate each of ten snails / liter for each experimental concentration, was used, as well as in control group. The exposure time was 24 hours followed by another 24 hours of recovery, and then the snail mortalities were recorded. The molluscicidal activities were expressed in terms of LC_{50} & LC_{90} values according to Litchfield and Wilcoxon's method, (1949) [24].

Results and Discussion

Compound (1): Compound **(1)**, amorphous solid (79.7 mg), m.p. 290-292 °C, R_f 0.52, [Chloroform : methanol (9.5 : 0.5)]. It responded positively to Liebermann-Burchard test suggesting that the compound was triterpene. IR ^{kBr} spectrum showed the presence of absorption bands at 3425.3 cm^{-1} (OH), 2928.2 cm^{-1} (CH and CH_2), 1382.5 cm^{-1} (gem. Dimethyl), 1692.8 cm^{-1} (C=O) [25]. The ^{1}HNMR (DMSO-d_6) spectrum showed signals at δ 5.112 (trisubstituted double bond), δ 1.265-2.073 (methylene absorption), five tertiary methyl groups at δ 0.736-1.220 ppm, two secondary methyl groups at δ 0.853 (C-29 (d)) and δ 1.026 ppm (C-30 (d)). The presence of a doublet at δ 1.026 ppm for (C-30) methyl groups in the ^{1}HNMR spectrum was evidence for an urs-12-ene triterpene [26]. On the basis of its spectral analysis the compound was suggested to be α-amyrin type (urs-12-ene-3β-ol) with C-28 carboxyl group .^{13}CNMR spectra of pentacyclictriterpene of the α-amyrin type and β-amyrin type have been documented [27-29].^{13}CNMR spectroscopy is the most precise tool for distinguishing between these two types of triterpenes which is not easy otherwise. The major difference between both series were found in the chemical shifts for the olefinic carbon atoms (C-12, C-13) and for the carbon atoms belong to ring E.

A comparison in the Δ δ between olefinic carbon signals (C-12, C-13) for both series [27]. The difference between the two values has been rationalized by the presence of a 19 β (equatorial) methyl group which in close proximity of the double bond at (δ 125.280 and 138.883 ppm) for olefinic carbons (C-12, C-13) (table **4**) respectively, in the urs-12-ene series (α- amyrin type), thus these steric effects influence the chemical shifts of these carbons [30,31]. Analysis of ^{13}CNMR spectrum of compound **(1)** signals were assigned to ursene skeleton by considering the differences discernible for the corresponding oleanene carbons, especially of C-18, C-19 and C-20 signals as those of C-12, C-13 which were of most diagnostic value. ^{13}CNMR spectrum of the compound revealed the presence of signals at (δ 178.915 ppm) indicating the presence of (C-28) carboxyl group. The olefinic carbon signals at δ 125.280 and 138.883 ppm (C-12, C-13) in its ^{13}CNMR suggested that compound (**1**) possesses an urs-12-ene type carbon skeleton [32] .^{13}CNMR also revealed the presence of signal at (δ 77.538 ppm) suggesting the presence of C-3 hydroxyl group. The above data suggested that the compound was ursolic acid.

Compound (2): (98.4 mg), responded positively to the Liebermann-Burchard and Molish tests suggesting that it was triterpene glycoside, m.p. 112-115 °C, R_f 0.62, [$CHCl_3$: MeOH : H_2O (65 : 35 : 0.5)]. Its IR ^{kBr} exhibited absorption bands at 3399.6 (OH), 2927.2 (CH and CH_2), 1737.5 (CO ester), 1629.6 (C=C) and at 1075.1 cm^{-1} (anomeric protons) indicating the glycosidic nature of the compound. IR also showed absorption bands at 1660.2 and 1515.5 cm^{-1} (aromatic). The ^{1}HNMR (CD_3OD) spectra displayed signals for six methyl proton singlets at δ 0.888-1.318 ppm, a broad vinyl proton at δ 5.743 ppm [33] indicated that the aglycone possessed an olean-12-ene skeleton coupled with information from ^{13}CNMR spectrum which showed the presence of hydroxymethyl group at C-23 (δ 68.512 ppm) suggested by the presence of high field signal at δ 13.523 ppm (C-24) [34], also ^{13}CNMR showed the presence of six sp^3 hybrid carbons at δ 13.523, 16.667, 17.047, 26.350, 32.719 and 23.147 ppm and two sp^2 hybrid carbons at δ 121.981(C-12) and δ 143.343 ppm (C-13) [35,36], the small down field shift of C-13 was indicative of the presence of free carboxyl group for (C-28) at δ 179.927 ppm [37] (table **5**). ^{13}CNMR showed the presence of two methine carbons bearing oxygen were found at δ 80.490 (C-3) bearing glycoside and δ 78.091 ppm (C-21) to which oxygen was attached. An acyl group at δ 168.452 (C-1') confirmed by the down field signal at C-21 (78.091) ppm indicative of acylation [38]. The chemical shift of C-3 (δ 80.490) and C-28 (δ 179.927)

ppm indicated that compound (**2)** was a monodesmosidic triterpene glycoside. Comparison of the ^{1}HNMR and ^{13}CNMR data with literature [32,39] suggested the triterpene substructure as 21-hydroxyhederagenin, also ^{13}CNMR of the compound showed glycosylation shift around C-3, and acylation shift around C-21 indicating that the compound was 3-glycosyl and 21-O-acyl. The presence of hydroxymethyl group at δ 68.512 (C-23) suggested by the presence of high field signal at δ 13.523 ppm (C-24). Compound (**2**) displayed two anomeric protons in the ^{1}HNMR spectrum at δ 4.818 and δ 5.041, two anomeric carbons in the ^{13}CNMR spectrum at δ 108.435 and δ 104.635 ppm [37,40]. The ^{1}HNMR spectrum revealed signals at δ 7.668 (d) and at 7.316-7.553 ppm (m, aromatic) which characterized the presence of cinnamoyloxy group. The presence of these groups were confirmed by ^{13}CNMR spectrum which showed the presence of signals at δ 168.452 (C-1′), 117.373 (C-2′), 145.545 (C-3′), 135.779 (d, C-4′), 127.260 (d, C-5′ and C-9′ or C-6′ and C-8′) and at 130.560 ppm (C-7′) aided by comparison with cinnamic acid [41].

The CI-MS spectrum revealed the presence of molecular ion at m/z 970 indicating the molecular weight of the compound. A fragment ion at m/z 808 $[M+H-163]^{+}$ indicating the loss of glucose unit, a moderate intense fragment ion at m/z 781.9 $[M+H-190]^{+}$ representing the loss of methyl glucuronate, a small fragment ion at m/z 618.2 $[M+H-353]^{+}$ corresponding to the loss of methyl glucuronate and glucose and at m/z 840.3 $[M+H-131]^{+}$ indicating the loss of cinnamoyl moiety. The above results suggested that glucose was attached to methyl glucuronate which was linked to C-3 of the aglycone through an ether linkage, whereas cinnamoyl moiety was linked to C-21 hydroxyl group. These results were supported by the presence of a signal at δ 80.490 (assigned to C-3) and at δ 78.091 ppm (assigned to C-21) of the aglycone. A low intense fragment ion at m/z 487.8 $[M+H-484]^{+}$ indicating the loss of methyl glucuronate, glucose, and cinnamic acid. Retro Diels Alder fragmentation of the radical ion involving the double bond at C-12 position give rise to a peak at m/z 224.9 (a) and 263.0 (b) scheme (1). Fragment ion (b) losses one molecule of water generating the fragment ion at m/z 245.1. Fragment ion (a) loss hydroxyl group producing fragment ion peak at m/z 207.0 scheme (1) [42]. On acid hydrolysis, compound (**2**) provided glucuronic acid and glucose, identified by comparison with authentic samples and melting point. The above results suggested that compound (**2**) was 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-methylglucuronopyranosyl-21-cinnamoyloxyhederagenin. Literature survey indicates that compound **(2)** is a new compound and separated from *C. purpureum* for the first time.

Compound (3): (80.5 mg), m.p. 118-122 °C, R_f 0.61 [Butanol: Acetic acid (4 : 1)]. It gave +ve Liebermann-Burchard test for triterpenes. IR ^{kBr} spectrum exhibited absorption bands at 3417.4 cm^{-1} (OH), 1076.7 cm^{-1} (anomeric protons), indicating the glycosidic nature of the compound, the IR spectrum also showed absorption bands at 2926.8 cm^{-1} (CH and CH_2), 1704.2 cm^{-1}(CO), and at 1514.3 cm^{-1} (aromatic). ^{1}HNMR spectrum (DMSO-d_6) revealed the presence of six methyl proton signals at (δ 0.772-1.349) (s, 3H), anomeric protons at δ 4.838 and 5.645 ppm and a broad vinyl proton signal at δ 5.532 (1H, br, H-12). The ^{13}CNMR spectrum (DMSO) revealed the presence of three oxygenated carbons (C-2, 69.569), (C-3, 80.877) and (C-19, 81.853), hydroxymethyl group (C-24, 61.512) [34] and carboxyl group (C-28, 177.639), the remaining 25 carbons of the aglycone was shown by ^{13}C NMR spectrum to have six quaternary carbon atoms at 39.691 (C-4), 39.981 (C-8), 36.425 (C-10), 42.422 (C-14), 34.106 (C-20) and 46.024 (C-17). Three methine carbons at 50.266 (C-9), 41.812 (C-18), 52.173 (C-5), eight methylene carbons at δ 39.416 (C-1) , 18.495 (C-6), 35.357 (C-7), 23.501 (C-11), 28.139 (C-15), 24.721 (C-16), 29.574 (C-21), 31.954 (C-22), six methyl groups at [δ 23.699 (C-23), 15.260 (C-25), 17. 717 (C-26), 25.148 (C-27), 31.695 (C-29), 22.737 ppm (C-30)] and trisubstituted double bond at 121.925 and 142.755 (C-12 and C-13) (table **4**). A comparison of these findings with results in the literature suggested that the aglycone had the characteristics of an olean-12-ene triterpene [32,39]. The presence of anomeric carbons at δ 100.913, δ 103.736 ppm and the chemical shift at δ 80.877 ppm (C-3) suggested that the triterpene was glycosylated at C-3. Also ^{1}H NMR spectrum exhibited signals at δ 6.413 (1H, d, H-2"), δ 7.677 (1H, d, H-3"), δ 7.109 (1H, d, H-2‴), δ 7.068 (1H, d, 5‴), δ 7.121 (1H, d, H-6‴), δ 3.758 (s) (OMe), δ 2.065 (s) (OAc) suggested the presence of 3-acetyl-4-methoxycinnamoyl moiety, and at δ 2.355 (t) (H-2'), δ 1.610 m (H-3'), δ 1.214 (br) (H4'-H-24'), δ 1.696 (H-25'), δ 4.198 (H-26'), suggesting the presence of hexacosanoyl moiety and at δ 3.675 dd (C-1a), 3.925 (C-2a), 3.553 (C-3a) suggesting the presence of glycerol moiety [43,44]. The ^{13}C NMR showed the presence of chemical shifts at δ 168.019 (C-1"), 115.669 (C-2"), 145.257 (C-3"), 127.953 (C-1‴), 109.077 (C-2‴), 148.660 (C-3‴), 147.530 (C-4‴), 116.294 (C-5‴), 122.704 (C-6‴), 56.461 (OCH_3),173.061(OAc) suggesting the presence

of 3-acetyl-4-methoxycinnamoyl moiety. ^{13}C NMR spectrum also revealed the presence of chemical shifts at δ 29.192-29.741 (C-4'- C-23'), 25.286 (C-24'), 32.102 (C-25'), 61.878 (C-26') and at δ 174.999 (C-1') suggested the presence of hexacosanoyl moiety [45,46]. ^{13}C NMR spectrum exhibited signals at δ 63.099 (C-1a), 68.455 (C-2a), and at 63.099 (C-3a) suggesting the presence of glycerol. The above results suggesting esterification of 3-acetyl-4-methoxycinnamic acid with the hydroxyl end of hexacosanoic acid and of the latter to glycerol (C-1a). Esterification of 2,19-dihydroxyepihederagenin with (C-3a) of glycerol could be supported by the presence of ^{13}C NMR chemical shift at δ 177.639 corresponding to C-28 carboxyl group of the aglycone and high field signal at δ 63.099 (C-3a) of glycerol. The CI-MS (DMSO) spectrum revealed the presence of molecular ion peak at m/z 1498.5 $[M]^+$. A fragment ion peak at m/z 1336.8 [M+H-163] corresponding to the loss of glucose moiety, highly intense fragment ion at m/z 1191.5 $[M+H-308]^+$ representing the loss of glucose and rhamnose moieties and at m/z 1174 $[M+H-325]^+$ indicating the loss of glucose and rhamnose moieties together with glycosidic oxygen at C-3 of the aglycone. CI-MS spectrum also showed a fragment ions at m/z 796 [M+H-703] representing the loss of 1-(26-trans-3-acetyl-4-methoxycinnamoyloxyhexacosanoyl) glycerol linked to C-28 carboxyl group of the triterpene, this result can be supported by the presence of chemical shift at δ 177.639 ppm for C-28 in the ^{13}C NMR spectrum and the presence of fragment ion peak at m/z 703.6 in CI-MS spectrum. CI-MS spectrum also revealed the presence of fragment ions at m/z 263.1 (a) and at 239.1 (b) resulting from retro Diels-Alder cleavage of ring C suggested a structure of Δ^{12}-oleane triterpene having hydroxyl group and hydroxymethyl group in rings A/B, hydroxyl, carboxyl groups in rings D/E, and a double bond at C-12 position scheme (2) [42]. The presence of the double bond at C-12 was confirmed by ^{13}C NMR spectrum, by the chemical shifts [δ 121.925 (C-12) and δ 142.755 ppm (C-13)]. The above results suggested that the aglycone was 2, 19-dihydroxyepihederagenin was linked to rhamnose and glucose through an ether linkage, whereas 1-(26-3-acetyl-4-methoxycinnamoyloxyhexacosanoyl) glycerol through an ester linkage, thus compound **(3)** was 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamno-pyranosyl-2,19-dihydro-xyepihederagenin-28-O-1-[26-(3-acetyl-4-methoxycinnamoyl-oxyhexacosanoyl)] glycerol. Literature survey indicates that compound **(3)** is a new compound and separated from *C. purpureum* for the first time.

Molluscicidal activity bioassay

Molluscicidal activity of the dry leaves water suspension of *"Cestrum purpureum"* against *B. alexandrina* snails after 24 and 48 hours was LC_{50} =72 and 16 ppm respectively, also LC_{90} =120 and 52 ppm respectively; these observations indicate that the molluscicidal activity of the plant increased by increasing the exposure time from 24 to 48 hours (table 6). The molluscicidal activity of methanol extract LC_{50}, LC_{90} value were 39 ppm and 78 ppm respectively after exposure period 24 hours; so the chromatographic separation for the plant was carried out on the methanol extract. Compound (**2**) showed molluscicidal activity against *Biomphalaria alexandrina* snails (the intermediate host of *schistosoma-mansoni*) at a concentration of 25 ppm after 24 hours exposure.

Structure-activity relationship

The molluscicidal activity was confined to the compounds with glycoside linkage. There is a correlation between the molluscicidal activity and the presence of glucuronic acid in the sugar part of the molecule. The high molluscicidal activity of compound (**2**) was due to the presence of methyl glucuronate in the sugar chain attached to the aglycone [47].

Conclusion

The present study showed that three compounds were isolated from *Cestrum purpureum* plant and could be identified using different spectroscopic analysis and hydrolysis process, these compounds named ursolic acid **(1)**, 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-methylglucuronopyranosyl-21-cinnamoyloxy-hederagenin **(2)** and 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-2,19-dihydroxy-epihederagenin-28-O-1-[26-(3-acetyl-4-methox-ycinnamoyloxyhexacosanoyl)] glycerol **(3)**. Literature survey indicates that compounds **(2 and 3)** are a new compounds separated from *C. purpureum* for the first time. Compound **(2)** showed molluscicidal activity at a concentration of 25 ppm.

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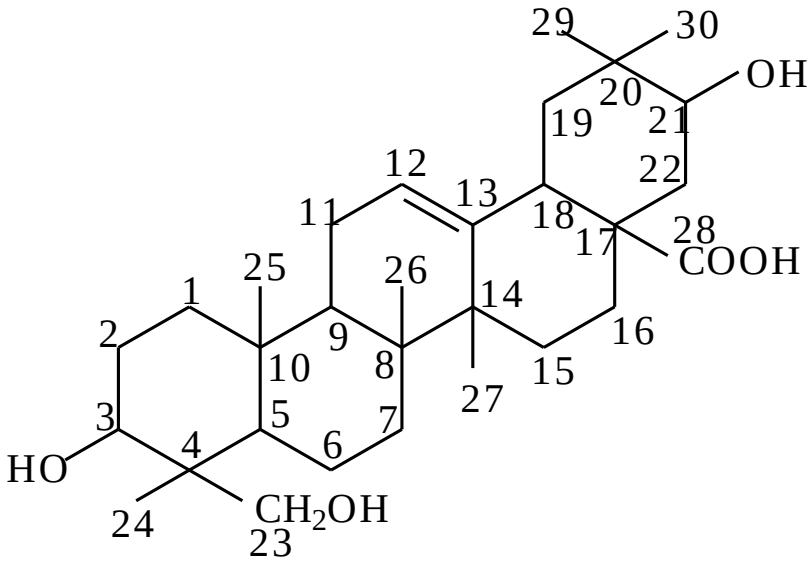
Scheme (1)



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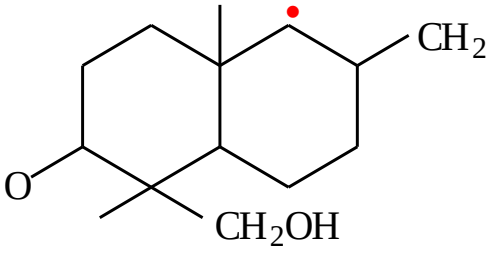
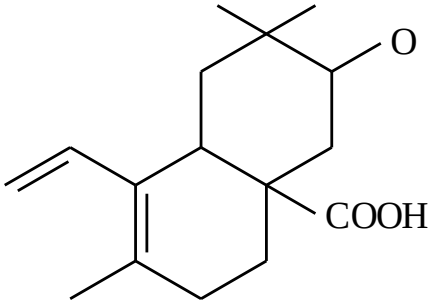




$C_{14}H_{24}O_2$

m/z = 224

-OH

m/z = 207

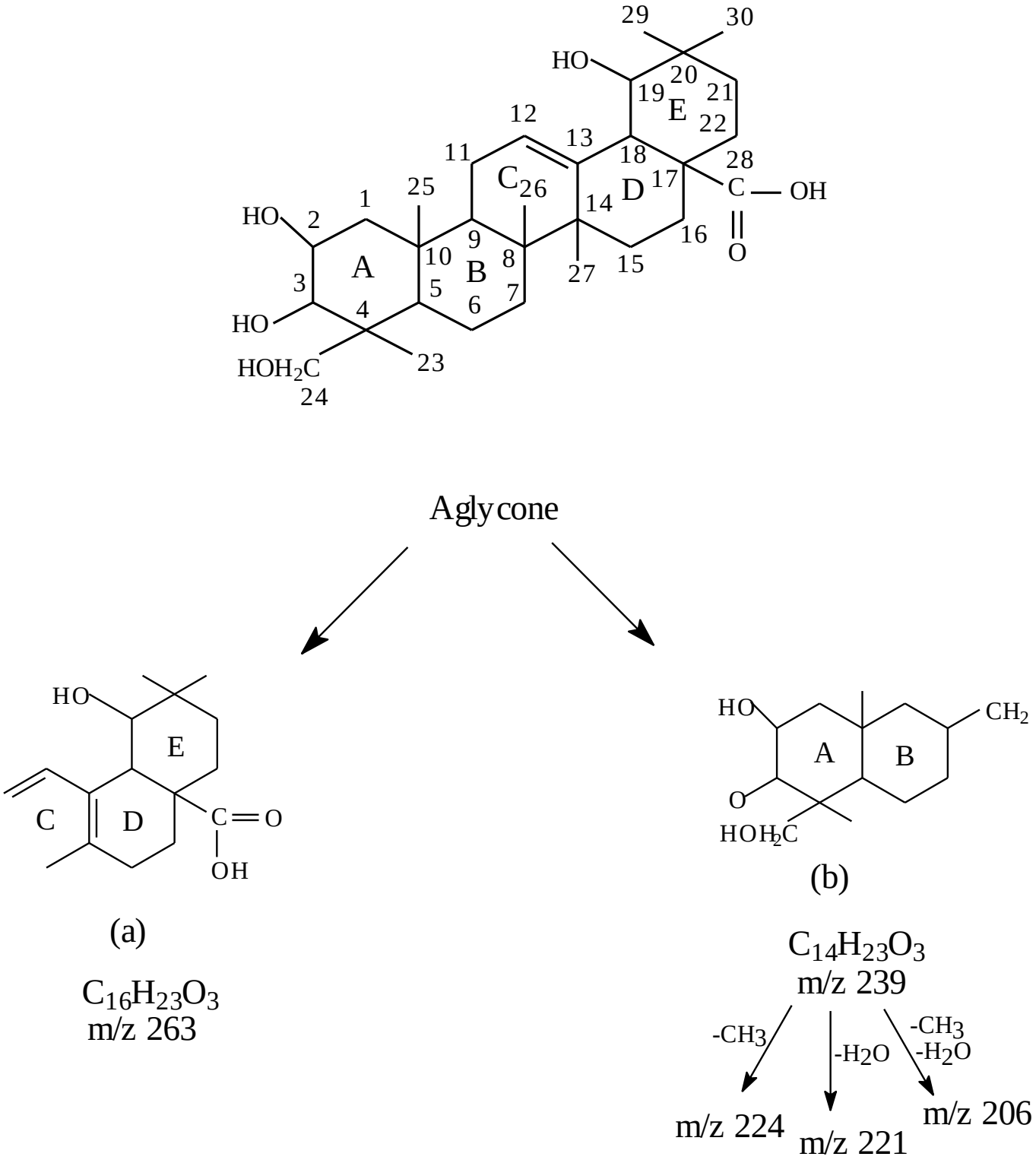
$C_{16}H_{23}O_3$

m/z = 263

$-H_2O$

m/z = 245

Scheme (2)


Aglycone
(a)
$C_{16}H_{23}O_3$
m/z 263
(b)
$C_{14}H_{23}O_3$
m/z 239
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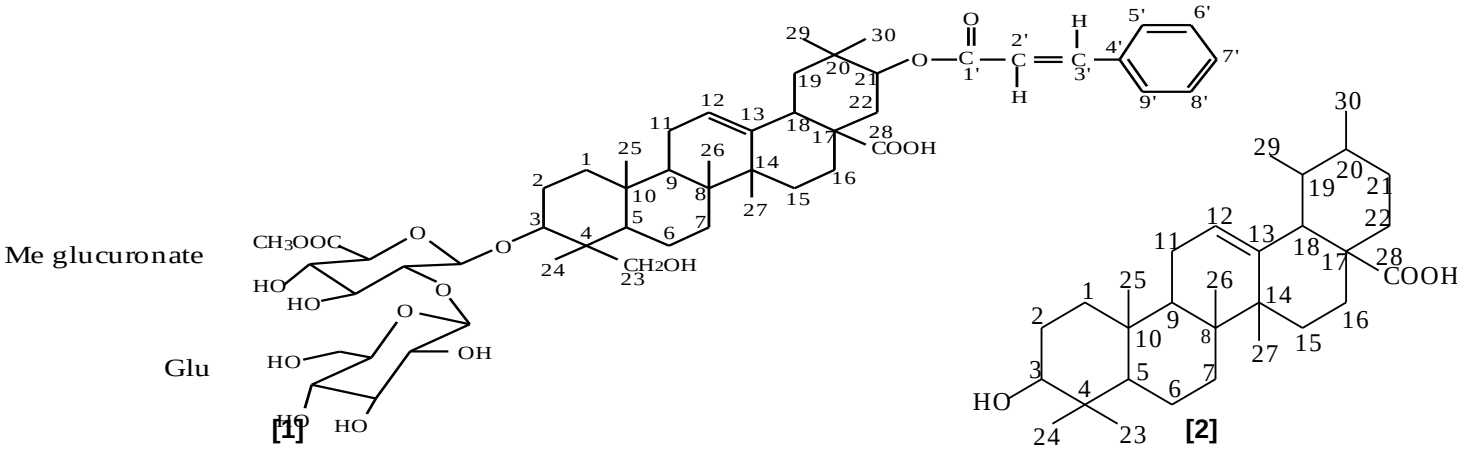
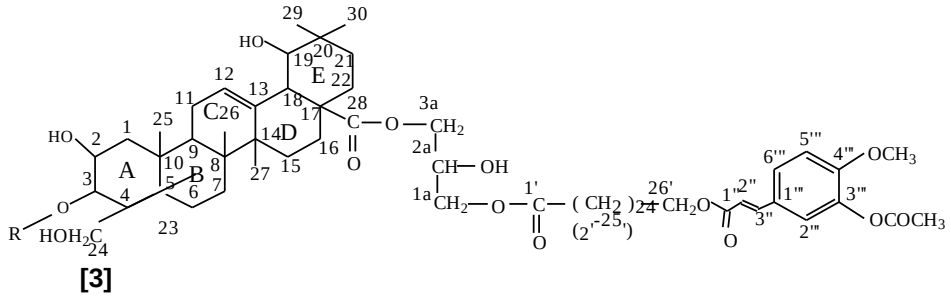
-H_2O
-CH_3
-H_2O
m/z 224
m/z 221
m/z 206





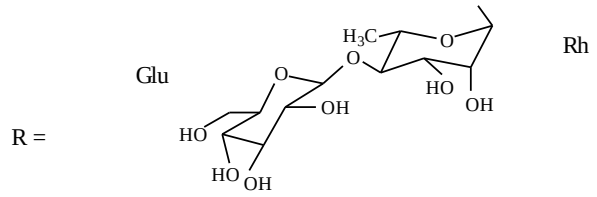


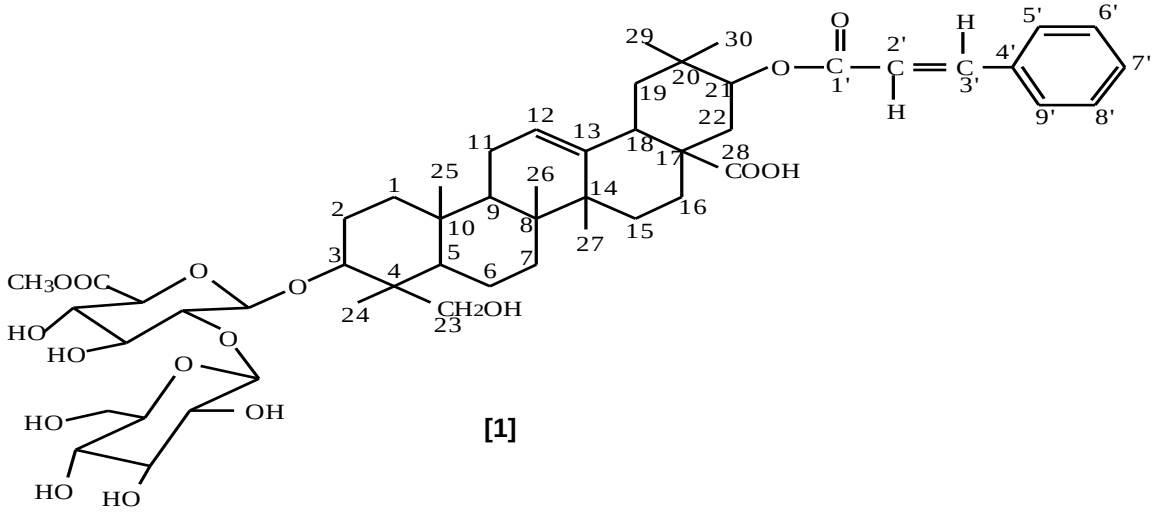
Figure 1. Structure of compounds [1, 2 and 3]

Table 1. Phytochemical screening of *C. purpureum* dry powder.

Test	*C. purpureum*
Carbohydrates and/or glycosides	+
Sterols and/or triterpenes	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids and/or nitrogenous bases	-

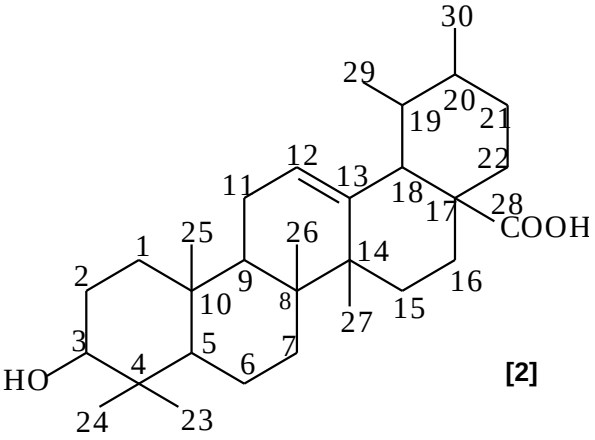
+ Signified the presence of the constituents
- Signified the absence of the constituents

Me glucuronate

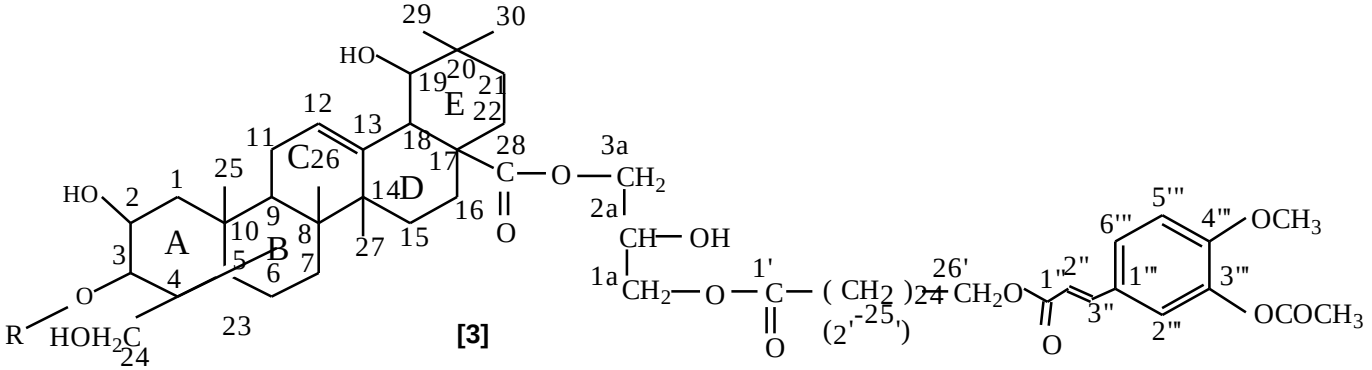


Glu

[1]



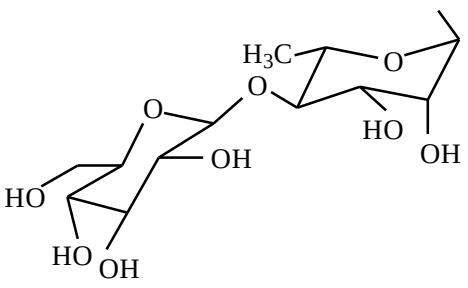
[2]



[3]

R =

Glu



Rh

Table 2. Phytochemical screening for different extracts of *C. purpureum*

Test	Methanol	Chloroform	Petroleum ether	Ethyl acetate	Ether	Benzene	Acetone
Carbohydrates and/or glycosides	+	+	+	+	+	+	+
Sterols and/or triterpenes	+	+	+	-	+	+	+
Saponins	+	+	+	-	+	+	+
Tannins	+	+	+	-	+	+	-
Flavonoids	+	+	+	+	-	+	-
Alkaloids and/or nitrogeneous bases	-	-	-	-	-	-	-

+ Signified the presence of the constituents
- Signified the absence of the constituents

Table 3. Column chromatographic fractions of saponin mixture of *Cestrum purpureum*

Elute	Fraction no.	No. of spots	Isolated compounds	TLC system
petroleum ether	1–20	3	---------	1
Petroleum ether : chloroform 1 : 1	21–30	--------	----------	1
Chloroform	31–39	2	Mixture	2
Chloroform: methanol 95 : 5	40–110	1	Compound 1	3
93 : 7	111–193	1	Compound 2	4
85 : 15	194–289	2	Mixture	5
70 : 30	290–320	1	Compound 3	6

Table 4. ^{13}C NMR for spectral data of compounds **(1** and **3)** [in DMSO $-d_6$ TMS as internal standard]

No. of carbon	Compound 1 δc	Compound 3 δc
1	39.211	39.416
2	27.706	69.569
3	77.538	80.877
4	38.956	39.691
5	55.497	52.173
6	18.711	18.495
7	37.243	35.357
8	39.715	39.981
9	47.730	50.266
10	37.022	36.425
11	23.567	23.501
12	125.280	121.925
13	138.883	142.755
14	41.107	42.422
15	28.255	28.139
16	24.528	24.721
17	47.539	46.024
18	53.097	41.812
19	39.147	81.853
20	38.956	34.106
21	30.899	29.574
22	33.590	31.954
23	28.255	23.699
24	15.934	61.512
25	16.777	15.260
26	17.639	17.717
27	23.983	25.148
28	178.915	177.639
29	17.639	31.695
30	21.770	22.737
Glycerol moiety		
1a		63.099
2a		68.455
3a		63.099
Hexacosanoyl moiety		
1'		174.999
2'		30.489
3'		25.896
4'-23'		29.192-29.741
24'		25.286
25'		32.102
26'		61.878
3-acetyl-4-methoxycinnamoyl moiety		
1"		168.019
2"		115.669
3"		145.257
1'"		127.953
2'"		109.077
3'"		148.660
4'"		147.530
5'"		116.294
6'"		122.704
OMe		56.461
OAc		173.061
Rhamnose		
1		100.913
2		72.759
3		71.797
4		88.919
5		68.731
6		18.833
Glucose		
1		103.736
2		74.193
3		75.192
4		71.400
5		77.550
6		62.602

Table 5. ^{13}C NMR for spectral data of compound **(2)** [in CD_3OD TMS as internal standard]

Carbon no.	Compound 2 δc
1	39.941
2	28.679
3	80.490
4	40.265
5	56.855
6	18.200
7	30.762
8	39.725
9	48.470
10	38.253
11	24.261
12	121.981
13	143.343
14	42.477
15	33.139
16	20.913
17	49.889
18	39.804
19	47.952
20	31.903
21	78.091
22	37.816
23	68.512
24	13.523
25	16.667
26	17.047
27	26.350
28	179.927
29	32.719
30	23.147
Cinnamoyl moiety	
1'	168.452
2'	117.373
3'	145.545
4'	135.779
5'	127.260
6'	127.260
7'	130.560
8'	127.260
9'	127.260
For C-3	
Methyl glucuronate [37]	
1"	108.435
2"	82.203
3"	77.966
4"	68.677
5"	77.843
6"	173.221
OMe	52.319
Glucose [40]	
1'"	104.635
2'"	74.147
3'"	78.251
4'"	70.127
5'"	77.473
6'"	60.903

Table 6. Effect of dry leaves water suspension of *"Cestrum Purpureum"* against *Biomphalaria alexandrina* snails at 25 °C

Exposure time	LC_{50} (ppm)	LC_{90} (ppm)	Slope
24 hours	72 (60.5-85.9)	120	1.51
48 hours	16 (9.4-27.2)	52	1.70