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# Iridoid Glycosides from Underground Parts of *Ajuga remota*

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**Abstract:** Fractionation of EtOAc and aqueous MeOH extracts of *Ajuga remota* underground part has led to the isolation of four new iridoid glycosides identified as 2',3'-diacetylharpagide (**1**), 6'-*O*-rhamnosylharpagide (**2**), 6'-*O*-galloyl-7,8-dehydroharpagide (**3**) and 6-*O*-xylosylharpagoside-B (**4**). Together with these were known compounds 8-*O*-acetylharpagide(**5**), harpagide (**6**), cyasterone (**7**), 20-hydroecdysone (**8**), sengosterone (**9**), 3-*O*-β-glucopyranosylstigmasta-5, 25-diene (**10**), stigmasterol (**11**) and ergosterol-5, 8-endoperoxide (**12**). Their structures were determined using spectroscopic methods as well as comparison with data from known compounds. In the *in vitro* larvicidal tests using 2nd instar *Aedes aegypti* larvae, the EtOAc extract was found to be toxic with $LC_{50}$ of 5.30 ± 1.3 µg/mL, while the MeOH extract exhibited weak toxicity with an $LC_{50}$ value of 65.94 ± 0.4 µg/mL. Among the pure isolates tested, compound **12** associated with EtOAc extract was the active principal with $LC_{50}$ value of 4.40± 0.2.

**Keywords:** *Ajuga remota*; iridoid glycosides; phytoecdysteroids; sterol glycoside; larvicidal activity.

## 1. Introduction

*Ajuga remota* (Labiatae) is a medicinal plant used in Kenya as a remedy for malaria treatment [1, 2]. In previous pytochemical studies, secondary metabolites with biological activities including insect antifeedant [3, 4], antiplasmodial [5] and antibacterial [6] have been reported. In our two previous publications, flavonol and iridoid glycosides were reported from the plant aerial part [7, 8]. In the continuation with plant underground part phytochemical studies, we herein report the isolation and structure elucidation of four new iridoid glycosides (**1-4**) along with 8-*O*-acetylharpagide (**5**), harpagide (**6**) [9, 10]. Also isolated from this source were three known phytoecdysteroids, cyasterone (**7**), 20-hydroecdysone (**8**), sengosterone (**9**) [11-13] together with sterol glycoside, 3-*O*-β-glucopyranosylstigmasta-5, 25-diene (**10**) [14], stigmasterol (**11**) and ergosterol-5, 8-endoperoxide

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(**12**) [5]. This is the first report of compounds **7**, **8**, **9** and **10** which were isolated from this plant. Their structures were elucidated using spectroscopic methods as well as comparison with data of known compounds. The larvicidal activities of the plant extracts and isolated compounds are also presented.

## 2. Materials and Methods

2.1. *General experimental procedures*

Optical rotations were measured with JASCO DIP-370 digital Polarimeter. Melting points were determined using a Gallenkamp melting apparatus and are uncorrected. The UV and IR data were recorded on PYE UNICAM SP8-150 UV/Vis spectrophotometer and Perkins-Elmer FTIR 600 series, respectively. The ESI-MS data were taken in JOEL JMS-700 M station mass spectrometer. The NMR data were taken in $CDCl_3$+ one drop DMSO-$d_6$ and DMSO-$D_6$ on a Brucker Ultrashield-500 spectrometer operating at 500MHz and 125 MHz, respectively.

2.2. *Plant material*

*Ajuga remota* plants were collected at Doonholm estate in Nairobi in April 2005. Voucher specimen (Herbarium number: AR/04/2005) of whole plant was identified by Mr. Mwangangi after comparison with authentic sample at the herbarium department, National Museum of Kenya.

2.2 *Extraction of plant material*

Air-dried powdered underground part of *A. remota* (2 kg) was extracted sequentially with ethyl acetate (3 x 3L) and MeOH (3 x 3L) by cold percolation, in each case for one week. The extracts were conc. under reduced pressure to afford brown residues of 15.0 and 65g, respectively.

2.3. *Isolation of components in EtOAc extract*.

Approximately 11.0 g of the EtOAc extract was adsorbed onto silica gel in a minimum amount of EtOAc solvent and evaporated using a rotary evaporator. The dried material was subjected to flash chromatography (3.0 x 60 cm, $SiO_2$ 250g, pressure =1.5 bar) using petroleum ether - EtOAc gradient elution, EtOAc and elution concluded with MeOH, collecting 20 mL each. A total of 300 fraction were collected and their homogenity monitored by TLC: eluent; petroleum ether-EtOAc (9:1, 4:2 and 1:1) and $CH_2CI_2$-MeOH (95:5, 9:1). The eluants were combined to afford the following fraction pools.

(i). Fractions 9-50 (n-hexane-EtOAc, 95.5 and 9:1 eluates) contained mainly fatty acids and were discarded.

(ii). Eluate (n-hexane-EtOAc, 4:1, Fr 52-140, 5000mg) was rechromatographed over silica gel column using 10% of EtOAc in n-hexane to give stigmasterol (11, 120 mg, $R_f$=0.65) and ergosterol-5, 8-endoperoxide (12, 80 mg, $R_f$=5.80) [5].

(iii). The eluate (n-hexane ethyl acetate, 3:2 and 1:1, for 143-180, 7000mg) contained one major spot contaminated with minor impurities and were combined and further purified by flash chromatography over silica gel column (2.5 x 50cm, $SiO_2$ 200g) using n-hexane -EtOAc (3:2) to give 3-*O*-β-glucopyranosylstigmasta-5, 25-diene (10, $R_f$=0.21, 75 mg ) [14, 15].

(iv). Eluate (EtOAc, $CH_2CI_2$-MeOH, 95:5 and 9:1, Fr 182-300, 10.0g) was repeatedly fractionated over silica gel column (4.0 x 60cm, $SiO_2$, 300 gm), collecting 10 mL each to give cyasterone (7, $R_f$ =0.45, 55mg), 20-hydroecdysone (8, $R_f$ = 0.37, 35mg) and sengosterone (9, $R_f$ = 0.33, 33mg) [11, 15].

*2.4. Isolation of compounds from methanol extract*

TLC analysis of the MeOH extract using $CH_2CI_2$-MeOH (9:1) followed by (4:1) afforded six brick-reddish spots after spraying the plate with anisaldehyde-conc. $H_2SO_4$ mixture. The MeOH extract approx. 50g was adsorbed onto silica gel in a minimum amount of MeOH and removal of solvent under reduced pressure afforded a gummy material, which was left over night to dry. The resulting powder was loaded on top of column packed with silica gel (5.0 x 60cm, $SiO_2$, 500g, pressure = 1.5 bar) and using $CH_2CI_2$-MeOH gradient elution to give 400 fractions, each 20 mL. The collected fractions were combined depending on TLC profiles to constitute pools I-IV. Pool I (fractions 1-60, 6g) gave three spots stained by iodine vapor and were further resolved into individual compounds by flash chromatography using $CH_2CH_2$-MeOH (95.5) to give further 7 (30 mg), 8 (45 mg) and 9 (53 mg) [14]

Pool II (fractions 65-140, 8.0g) on TLC analysis using $CH_2CI_2$-MeOH (9:1) as mobile phase revealed two brick-reddish spots when sprayed with anisaldehyde-conc. $H_2SO_4$ mixture and were resolved into individual components over silica gel with $CH_2CI_2$-MeOH (9:1) to give 1 ($R_f$ =0.45, 35mg) , 5 ($R_f$ = 0.39, 60mg) and 6 ($R_f$=0.33, 45 mg).

Pool II (fraction 142-280, 10g) on TLC analysis was found to contain one major compound ($R_f$ =0.39) contaminated with two other minor compounds ($R_f$= 0.33 and 0.27). This pool was further purified by recrystallization in $CH_2CI_2$-MeOH-$H_2O$ (95:4:1) to give amorphous powder 350mg. The mother liquor upon rechromatography using $CH_2CI_2$-MeOH (9:1) followed by the same solvent system in the ratio (4:1) afforded further 5 (50mg), 2 ($R_f$ = 0.27, 65mg) and 3 ($R_f$ =0.33, 40mg).

Fractions 285-400 (constituted pool IV, 16 g) on TLC showed the presence of three spots already observed in pool III in addition to a new spot and were similarly resolved into compounds 5 (15 mg), 2 (35 mg), 3 ( 20 mg) and 4 ($R_f$=0.21, 45 mg).

*2',3'-diacetylharpagide* (**1**): Amorphous colorless powder (MeOH-$H_2O$, 9:1); mp 185-187 $^0$C; $[\alpha]_D^{25}$ -4.5$^0$ (MeOH, c 0.1); UV $\lambda_{max}$ nm: 214 (2.86) and 206 (3.10); IR (KBr) $\nu_{max}$ 3340, 2920, 2850, 1736 (ester C=O), 1640, 1472, 1031, 1010, 960 cm$^{-1}$; $^1$H and $^{13}$C NMR(500 MHz, $CDCl_3$+ one drop DMSO-$d_6$) δ (ppm): See Tables 1 and 2; HRESIMS *m/z* 471.24533 [M+Na]$^+$ (calcd. for $C_{19}H_{28}O_{12}Na$, 471. 23782).

*6-O-rhamnosylharpagide* (**2**): Amorphous colorless powder (MeOH-$H_2O$, 9:1); mp 232-235 $^0$C; $[\alpha]_D^{25}$ -9.3$^0$ (MeOH, c 0.5); UV $\lambda_{max}$ nm: 204 (2.70) and 216 (3.30); IR (KBr) $\nu_{max}$ 3410, 2920, 2852, 1734 (ester C=O), 1633, 1520, 1050, 1011, 920 cm$^{-1}$; $^1$H and $^{13}$C NMR(500 MHz, DMSO-$d_6$) δ (ppm): See Tables 1 and 2; HRESIMS *m/z* 533.48236 [M+Na]$^+$ (calcd. for $C_{21}H_{34}O_{14}$, 533.48335 ).

*6'-O-galloyl-7, 8-dehydroharpagide* (**3**): Obtained as colorless amorphous powder, mp 227-230 $^0$C; $[\alpha]_D^{25}$ -33$^0$ (MeOH, c 0.1); UV $\lambda_{max}$ nm: 202 (3.02); IR (KBr) $\nu_{max}$ 3350, 2922, 2850, 1734 (ester C=O), 1644, 1370, 1051, 1020, 850 cm$^{-1}$; $^1$H and $^{13}$C NMR (500 MHz, DMSO-$d_6$) δ (ppm): See Tables 1 and 2; HRESIMS *m/z* 521.43044 [M+Na]$^+$ (calcd. for $C_{22}H_{26}O_{15}Na$, 521.44064).

*6-O-xylosylharpagoside* (**4**): Obtained as colorless amorphous powder, mp 227-230 $^0$C; $[\alpha]_D^{25}$ -33$^0$ (MeOH, c 0.1); UV $\lambda_{max}$ nm: 206 (3.10), 216 (2,86) and 274 (2.58); IR (KBr) $\nu_{max}$ 3424, 2940, 2851, 1724 (C=O), 1635, 1240, 1061, 1021, 980, 672 cm$^{-1}$; $^1$H and $^{13}$C NMR (500 MHz, DMSO-d$_6$) δ (ppm): See Tables 1 and 2; HRESIMS *m/z* 649.60152 $[M+Na]^+$ (calcd. for $C_{29}H_{38}O_{15}Na$, 649.60161).

*8-O-acetylharpagide* (**5**): Amorphous powder from MeOH-$H_2O$, (9:1); mp 228-229 $^0$C; IR (KBr) $\nu_{max.}$ 3400, 1725, 1637, 1050, 1016, 980 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$+drop DMSO-d$_6$) δ (ppm): See Tables 1 and 2. ESIMS *m/z* 429.2 $[M+Na]^+$.

*Harpagide* (**6**): Amorphous powder from MeOH-$CH_2Cl_2$ (1:4); IR (KBr) $\nu_{max.}$ 3360, 1644, 1250, 1047, 1021, 980 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$+DMSO-d$_6$) δ (ppm): See Tables 1 and 2; ESIMS *m/z* 387.1 $[M+Na]^+$.

### 2.5. *Larvicidal activity assay*

The larvicidal activity assay against 2$^{nd}$ instar larvae of *Aedes aegypti* was done as described in reference [16]. The $LC_{50}$ values were calculated from the average of three observations for each concentration using Finney's probit analysis for quanta data [17].

### *2.6. Acid hydrolysis*

Compounds (1-4), each 10 mg) in a mixture of 8% HCl (1 mL) and MeOH (5 mL) were separately refluxed for 2 h at 100$^0$C, after which the reaction mixtures were cooled. The cooled residues were reduced *in vacuo* to dryness, dissolved in $H_2O$ (1 mL) and neutralized with NaOH. The neutralized products were subjected to silica TLC analysis (eluent: EtOAc-MeOH-$H_2O$-HOAc, 6:2:1:1) and PC (eluents: n-BuOH-HOAc-$H_2O$, 4:5:1 and $C_6H_6$-n-BuOH-$H_2O$-pyridine, 1:5:3:3). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 $^0$C. The sugars were identified after comparison of their $R_f$ values with those of authentic samples.

## 3. Results and Discussion

The powdered underground part of *Ajuga remota* was extracted sequentially with EtOAc and MeOH. Repeated column fractionation of ethyl acetate led to the isolation of cyasterone (**7**), 20-hydroecdysone (**8**), sengosterone (**9**) together with 3-*O*-β-glucopyranosylstigmasta-5, 25-diene (**10**). On the other hand, the MeOH extract upon fractionation over silica gel afforded new iridoid glycosides (**1-4**) together with the known 8-*O*-acetylharpagide (**5**) and harpagide (**6**). Their structures were established from spectroscopic analysis and also comparison with existing literature data.

Compound 1, obtained as an amorphous colourless powder, gave an ion peak at m/z (HR-ESIMS 471.2453, $[M+Na]^+$) for $C_{19}H_{28}O_{12}Na$, and showed a brick-reddish color upon spraying with anisaldehyde-conc. $H_2SO_4$ mixture suggesting an iridoid derivative [18]. It exhibited hydroxyl (3340cm$^{-1}$), ester (1736cm$^{-1}$) and olefinic bond (1640cm$^{-1}$) absorption bands in the IR spectrum. The $^1$H NMR spectral features of compound **1** (Table 1) were closely similar to those of harpagide (6) [10] except for the two acetoxy group signals evidenced by $^1$H NMR peaks at δ 1.98 and 1.96 with corresponding $^{13}$C NMR peaks centered at δ 171.15, 169.85, 21.60 and 21.0, respectively. Acid hydrolysis afforded glucose as the sugar residue confirmed by TLC and PC co-chromatography with authentic sample. The large coupling constant of the anomeric proton (*J*=7.7Hz) indicated that the sugar was present in the β-configuration. The presence of 9 carbon signals due to the aglycone moiety were also evident in the $^{13}$C

NMR spectrum (Table 2), thus further supporting the close structural relationship between compounds **1** and **6**.

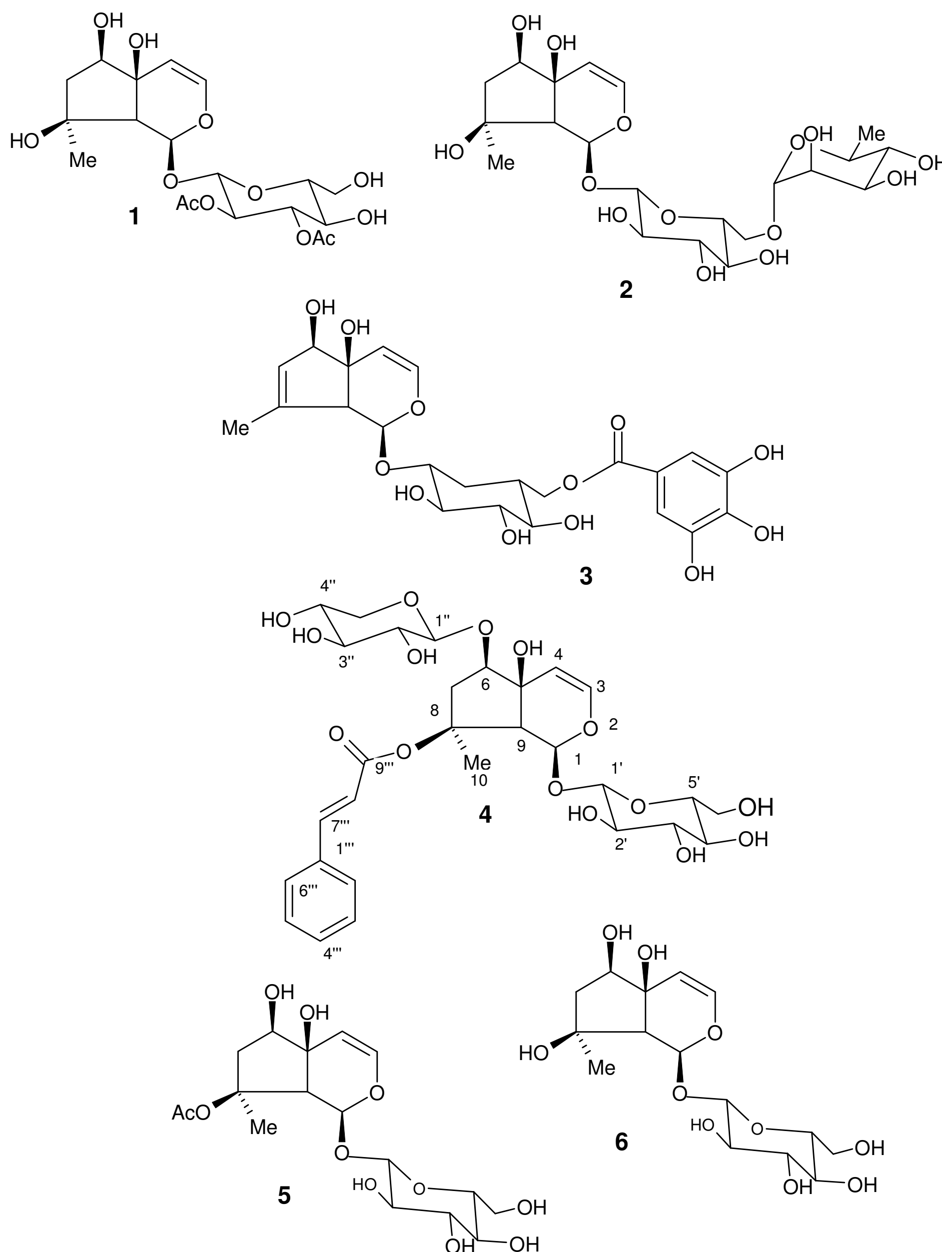


**Figure 1.** Isolated iridois from *Ajuga remota*

Unequivocal information on the aglycone and substitution mode in **1** was established from the $^{1}$H and $^{13}$C NMR, HSQC, HMBC and NOESY data. In fact, comparison of the $^{1}$H and $^{13}$C NMR data of compound **1** with those of 6 suggested that the two additional acetyl groups were substitutions in the sugar unit possibly at C-2' and C-3' positions.

This interpretation was supported by downfield shifts of $^{1}$H NMR peaks at H-2' (δ 4.57) and H-3' (δ 4.93), respectively and confirmed by HMBC long-range correlations ($^{3}J_{CH}$) between the carbon signal at $\delta_c$ 171.15 ($CH_3CO$) and proton at $\delta_H$ 4.57 (H-2') and between $\delta_c$ 169.85 ($CH_3CO$) and $\delta_H$ 4.93 (H-3'), respectively. The configurations at C-6, C-8 and C-9 of the aglycone were similarly confirmed from NOESY experiments due to cross-peaks between H-9 and H-1 and also in turn with $CH_3$-8. Similarly, a NOESY cross-peak between H-6 and H-7α confirmed the existence of 6-OH as β. Thus, on the basis of spectroscopic data and comparison with literature data, compound 1 was concluded to be 2', 3'-diacetylharpagide

Compound **2**, isolated as an amorphous powder exhibited the following physical data: mp 232-235$^{0}$C; $[\alpha]_D$ - 9.3$^{0}$ (MeOH, c 0.5). Its UV absorption maxima at 216 (3.10) and 204 (2.70) nm are typical of iridoid derivatives with OH groups both at C-5 and C-6 [19]. The NMR spectral data of **2** (Tables 1 and 2) were similar to those of compound **6** [10] with notable difference being the presence of a rhamnosyl moiety in the former compound, a fact confirmed by the anomeric proton at δ 4.46 (d, *J*=1.0 Hz) with corresponding $\delta_c$ 102.10 in the $^{13}$C NMR spectrum. Acid hydrolysis afforded glucose and rhamnose as the sugar residues similarly identified on the basis of TLC and PC co-chromatography with authentic samples. In the $^{1}$H NMR spectrum, an oxymethine proton at δ 3.62 (t, *J*=4.3 Hz, H-6) was observed to couple to two other protons, thus suggesting the saturation of C-6 to C-8 region of the aglycone moiety [10, 20].

The attachment of glucose to the aglycone was assigned to C-1 where it is in equatorial configuration, a fact further corroborated by HMBC correlation between H-1' ($\delta_H$ 4.66) and C-1 ($\delta_c$ 95.40), and confirmed by NOESY cross-peak between H-1 and H-9. Similarly, HSQC experiment was used to correlate the protons with corresponding carbons and this allowed the assignment of the interglycosidic linkage. In the $^{13}$C NMR, glycosylation shift was observed for C-6' at δ 67.60, thus suggesting the terminal rhamnose was linked to primary glucose through 1''→6' bond as in rutinosyl [21]. The foregoing evidence was confirmed by HMBC correlations between rhamnosyl-H-1' (δ 4.46) and glucosyl-C-6' (δ 67.60). Therefore, from the accrued spectroscopic data compound **2** was deduced as 6'-*O*-rhamnosylharpagide.

Compound **3** was obtained as an amorphous powder (MeOH-$H_2O$, 9:1), $[\alpha]_D$ -33$^{0}$ (MeOH, c 0.1) and assigned a molecular formula $C_{22}H_{26}O_{13}$ deduced from the sodiated $[M+Na]^+$ ion at *m/z* 521.43044 in the HRESI-MS as well as from its NMR spectroscopic data. The $^{1}$H NMR data (Table 1) were characteristic of an irridoid derivative containing a sugar unit (evidenced by an anomeric proton, δ 4.70) and galloyl chromophore (δ 6.90) [22]. Acid hydrolysis yielded glucose as the sugar residue identified on the basis of TLC and PC co-chromatography with authentic sample. The $^{1}$H NMR data of the aglycone showed two adjacent olefinic protons at δ 6.24 (dd, *J*=6.6, 2.0 Hz) and 4.88 (dd, *J*=6.6, 2.0 Hz) ascribable to H-3 and H-4, respectively [8, 19]. Additional signals due to olefinic proton (δ 5.75, H-7), two oxymethine protons (δ 5.98, H-1 and 4.60, H-6) and vinyl methyl (δ 1.35, Me-10) signified the presence of >C(OH)-CH(OH)-CH=C($CH_3$)-CH system in the C-5 to C-9 region of the aglycone [23, 24]. This allowed the glucose unit to be assigned at position C-1 of the aglycone where it is in equatorial configuration, a fact corroborated by the HMBC correlation between H-1 ($\delta_H$ 4.70, d, *J*=7.6 Hz) and C-1 ($\delta_C$ 93.70), and confirmed by NOESY cross-peaks between H-1 (δ 5.98, d, *J*=1.0 Hz) and H-9 (δ 2.86, br s). In the $^{13}$C NMR, a downfield shift observed for glucose-C-6' (δ 66.40) suggested that glucose moiety was linked to the galloyl unit through 6'→7'' bond. The foregoing evidence was confirmed by the HMBC correlations between the glucose-H-6' (δ 4.01) and the galloyl-C-1'' (δ 168.50). Thus based on the above spectroscopic accrued data, compound **3** was concluded to be 6'-*O*-galloyl-7,8-dehydroharpagide.

**Table 1**. [1] H NMR of compounds **1-6**.

| **position aglycone** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| 1 | 5.64 d (1.4) | 5.76 d (1.0) | 5.98 d (1.2) | 5.75 d (1.1) | 5.97 d (0.9) | 5.92 d (1.0) |
| 3 | 6.22 d (6.2) | 6.12 d (6.4) | 6.24 d (6.6) | 6.31 d (6.0) | 6.42 d (6.3) | 6.40 d (6.5) |
| 4 | 4.80 dd (6.6, 1.2) | 4.74 dd (6.4, 1.6) | 4.88 dd (6.6, 2.0) | 4.88 dd (6.0, 1.8) | 5.02 dd (6.5, 1.5) | 4.94 dd (6.5, 1.4) |
| 5 | | | | | | |
| 6 | 3.50 d (4.4) | 3.62 t (4.3) | 4.60 d (6.1) | 3.85 dd (11.6, 3.5) | 3.84 d (4.4) | 3.60 d (4.4) |
| 7β | 2.00 dd (15.4, 4.6) | 1.88 dd (14.6, 4.7) | 5.75 br s | 1.92 dd (16.0, 5.0) | 2.00 dd (15.6, 5.3) | 2.20 dd (16.0, 1.5) |
| 7α | 1.95 dd (15.4, 3.4) | 1.78 dd (14.6, 3.2) | | 1.81 dd (16.0, 3.3) | 1.96 dd (15.6, 3.7) | 2.01 dd (16.0, 4.5) |
| 8-OAc | | | | | | 2.01 s |
| 9 | 2.61 s | 2.78 s | 2.86 br s | 2.58 s | 2.81 br s | 2.72 s |
| 10 | 1.30 s | 1.35 s | 1.35 s | 1.62 s | 1.54 s | 1.40 s |
| 1' | 4.76 d (7.7) | 4.66 d (7.3) | 4.70 d (7.6) | 4.40 d (7.7) | 4.71 d (7.1) | 4.56 d (7.4) |
| 2' | 4.57 m | 3.33 m | 3.28 m | 3.30 m | 3.34 m | 3.27 m |
| 3' | 4.93 m | 3.54 m | 3.47 m | 3.48 m | 3.52 m | 3.52 m |
| 4' | 3.30 m | 3.37 m | 3.35 m | 3.33 m | 3.38 m | 3.34 m |
| 5' | 3.40 m | 3.44 m | 3.42 m | 3.44 m | 3.48 m | 3.41 m |
| 6'β | 3.75 dd (12.0, 5.4) | 3.77 dd (12.4, 5.5) | 4.01 dd (12.2, 5.3) | 3.80 dd (12.2, 5.3) | 3.76 dd (12.4, 2.4) | 3.80 dd (12.4, 6.0) |
| 6'α | 3.54 dd (12.0, 3.0) | 3.69 dd (12.4, 3.1) | 3.89 dd (12.2, 2.0) | 3.68 dd (12.2, 2.0) | 3.66 dd (12.4, 4.8) | 3.59 dd (12.4, 2.5) |
| 1'' | | 4.46 d (1.0) | | 4.52 d (7.4) | | |
| 2'' | | 3.41 m | | 3.26 m | | |
| 3'' | | 3.39 m | | 3.36 m | | |
| 4'' | | 3.01 m | | 3.52 m | | |
| 5'' | | 3.50 m | | 3.66 m | | |
| 6'' | | 1.10 d (6.3) | | | | |
| 2''' | | | 6.90 s | 7.70 dd (7.2, 2.1) | | |
| 3''' | | | | 7.50 dd (6.5, 6.5) | | |
| 4''' | | | | 7.52 dd (6.5, 6.5) | | |
| 5''' | | | | 7.52 dd (6.5, 6.5) | | |
| 6''' | | | 6.90 s | 7.70 dd (7.2, 2.1) | | |
| 7''' | | | | 7.82 d (16.0) | | |
| 8''' | | | | 6.60 d (16.0) | | |
| 2'-OAc | 1.98 s | | | | | |
| 3'-OAc | 1.96 s | | | | | |

**Table 2**. $^{13}$C NMR of compounds **1-6**.

| position aglycone | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| 1 | 95.70 | 95.40 | 93.70 | 94.03 | 95.21 | 94.86 |
| 3 | 145.42 | 144.31 | 144.20 | 143.40 | 145.01 | 143.01 |
| 4 | 106.89 | 106.22 | 107.02 | 104.70 | 106.70 | 105.23 |
| 5 | 75.30 | 73.70 | 73.51 | 74.60 | 75.22 | 73.70 |
| 6 | 80.12 | 78.80 | 78.04 | 81.50 | 79.32 | 77.41 |
| 7 | 47.00 | 48.00 | 130.00 | 43.00 | 47.10 | 46.92 |
| 8 | 91.13 | 88.40 | 141.50 | 87.30 | 87.60 | 91.02 |
| 9 | 54.60 | 52.60 | 50.01 | 53.70 | 54.38 | 55.22 |
| 10 | 23.60 | 25.10 | 13.60 | 22.90 | 23.30 | 23.40 |
| 8-OAc | | | | | 169.30 | |
| | | | | | 23.60 | |
| 1' | 102.01 | 101.20 | 100.80 | 99.90 | 101.40 | 102.02 |
| 2' | 74.10 | 75.11 | 74.40 | 73.70 | 75.12 | 74.52 |
| 3' | 80.00 | 77.70 | 78.00 | 76.90 | 77.61 | 77.80 |
| 4' | 78.30 | 71.50 | 71.65 | 70.70 | 71..20 | 72.03 |
| 5' | 76.22 | 77.80 | 78.35 | 77.40 | 78.70 | 78.40 |
| 6' | 62.40 | 67.60 | 66.40 | 61.50 | 62.90 | 63.01 |
| 1" | | 102.10 | | 102.40 | | |
| 2" | | 70.20 | | 72.20 | | |
| 3" | | 68.80 | | 71.65 | | |
| 4" | | 72.70 | | 67.50 | | |
| 5" | | 70.65 | | 63.80 | | |
| 6" | | 17.61 | | | | |
| 1''' | | | 122.22 | 137.31 | | |
| 2''' | | | 114.10 | 130.10 | | |
| 3''' | | | 147.80 | 131.43 | | |
| 4''' | | | 138.61 | 132.64 | | |
| 5''' | | | 147.80 | 131.43 | | |
| 6''' | | | 114.10 | 130.10 | | |
| 7''' | | | 168.50 | 147.01 | | |
| 8''' | | | | 121.23 | | |
| 9''' | | | | 169.60 | | |
| 2'-OAc | 171.15 | | | | | |
| | 21.60 | | | | | |
| 3'-OAc | 169.85 | | | | | |
| | 21.00 | | | | | |

Compound **4**, obtained as an amorphous colorless powder exhibited a molecular ion peak at *m/z* corresponding to $C_{29}H_{38}O_{15}$ formula. Its IR spectrum indicated the presence of a hydroxyl group (3424 $cm^{-1}$), ester carbonyl (1724 $cm^{-1}$), double bond (1635 $cm^{-1}$) and an ether linkage (1021 $cm^{-1}$). The UV spectrum showed absorption bands at 206, 216, and 274 nm typical of iridoid derivative with enol ether system and a cinnamoyl chromophore [25]. The $^{13}$C NMR spectrum (Table 2) showed 29 carbon signals of which 9 were assigned to the aglycone part, eleven to the sugar units and 9 to the cinnamoyl moiety. The combined interpretation of the $^{1}$H and $^{13}$C NMR (Tables 1 and 2) aided by the HSQC allowed association of most protons with corresponding carbon signals, and by the HMBC spectrum, which was vital in connecting the various spin systems, the sugar units were confirmed to be attached glycosidically at C-1 and C-6. Similarly, in the $^{13}$C NMR, the C-8 shifted downfield at δ

85.75, suggesting that the C-8 hydroxyl group was involved in the ether linkage with the cinnamoyl moiety [22], a fact further corroborated by downfield shift of Me-10 (δ 1.62) in the $^1$H NMR spectrum. In fact, the foregoing evidence is compatible with harpagide-B, where Me-10 shifted downfield at δ 1.55 on cinnamoylation of the C-8 hydroxyl group [26]. Acid hydrolysis yielded glucose and xylose as sugar residues identified by TLC and PC co-chromatography after comparison with authentic samples and confirmed by the $^1$H NMR spectrum two anomeric proton signals at δ 4.40 (d, *J*=7.7 Hz) and 4.52 (d, *J*=7.4 Hz), with corresponding $^{13}$C NMR peaks at δ 99.90 and 103.40, respectively.

The attachment of the glucose unit at C-1 and xylose at C-6 were confirmed by long-range couplings between H-1 (δ 5.75) and glucose-C-1 ($\delta_c$ 99.90), and between H-6 (δ 3.85) and xylose-C-1 (δ 103.40), respectively in the HMBC spectrum. Similarly, the relative configuration at C-1 and C-6 was evident from NOESY cross-peaks between H-1 and H-9/Me-10, and between H-6 with H-7α, thus indicating the β-configuration of the C-6, C-1 and C-8 (cinnamoyl) substituents. Thus, the structure of compound **4** was established as 6-*O*-xylosylharpagoside-B.

### *3.1. Larvicidal activity on 2$^{nd}$ instar Aedes aegypti*

In this study, crude extracts and pure compounds of *A. remota* were screened for larvicidal activities against 2$^{nd}$ instar *Aedes aegypti* larvae. Deaths of the larvae were realized after 24h of exposure to EtOAc and MeOH extracts. The EtOAc showed relatively potent activity of 5.30 ± 1.3 µg/mL at $LC_{50}$ while the MeOH extract showed an activity of 65.94±0.4 µg/mL at 24 h. Of all pure isolates tested, only three exhibited activities with compound 12 showing the highest toxicity of $LC_{50}$ value 4.40 ±0.2 µg/mL. This was followed by compound 4 which showed weak activity of $LC_{50}$ values of 84.01± 0.4 while 2 afforded $LC_{50}$ value of 89.08 µg/mL (Table 3). The slightly higher toxicity for compound 2 than 4 could be due to presence of cinnamoyl moiety.

**Table 3**. Larvicidal activity of *A. remota* extracts and pure compounds against 2$^{nd}$ instar *Aedes aegypti.*

| **Sample tested** | **$LC_{50}$ (µg/mL) 24h** |
|---|---|
| EtOAc extract | 5.30 ± 1.3 |
| Methanol extract | 65.94 ± 0.4 |
| Compound **2** | 89.08 ± 0.1 |
| Compound **4** | 84.01 ± 0.4 |
| Compound **12** | 4.40 ± 0.2 |
| DMSO | > 100 |
| $H_2O$ | > 100 |

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