



## CHEMICAL CONSTITUENTS OF *HEDYOTIS PINIFOLIA* WALL. COLLECTED IN THUA THIEN HUE

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Received: 1 February 2018; Accepted for publication: 10 May 2018

**Abstract.** This study reports the chemical constituents from the whole plants *Hedyotis pinifolia* Wall. Ex G. Don (now accepted as *Oldenlandia pinifolia* (Wall. Ex G. Don) Kuntze) collected in Thua Thien Hue province. Thirteen compounds were isolated by chromatography method. Their structures were elucidated using MS and NMR analysis and compared with reported data. They contain three anthraquinones, a carotenoid, two triterpenes, four iridoid glucosides and three flavonoid glycosides. Three of them were found for the first time in this genus.

**Keywords:** *Hedyotis pinifolia* (*Oldenlandia pinifolia*), anthraquinone, carotenoid, triterpene, iridoid glucosides and flavonoid glycosides.

**Classification numbers:** 1.1.1, 1.1.6, 1.2.1

### 1. INTRODUCTION

The *Hedyotis* genus belongs to the Rubiaceae family, which has about 180 species and is native to tropical and subtropical Asia [1]. Numerous *Hedyotis* species are used in traditional medicine for the treatment of inflammation, cancer and other diseases [2-4] such as *Hedyotis biflora*, *H. corymbosa*, *H. diffusa*, *H. verticillata*, etc. For example, the leaves of *H. auricularia* were used for treatment of diarrhea, dysentery in India, while using as vegetable with reducing blood pressure effect in Sri Lanka [5]. Vietnamese folk medicine used *H. herbacea* as expectorant, hypothermia and tonic; *H. tenelliflora* as detoxify, analgesic agents [4]. From this genus, many compounds with the novel structures and unique biological activities of the alkaloid, anthraquinone, iridoid, triterpenoid and lignin classes have been reported [6-9].

*Hedyotis pinifolia* is a small herb growing in sandy areas from Hue to the south of Viet Nam [10]. Until now, according to our literature search, there has been only one report on the phytochemistry of this species [11]. In this paper, we isolated the chemical constituents from the *n*-hexane, ethyl acetate and *n*-butanol extracts of *H. pinifolia*. The structures of isolated compounds have been elucidated as three anthraquinones: 2-hydroxy-1-methoxy-anthraquinone (**1**); 1,6-dihydroxy-2-methylanthraquinone (**2**); digiferruginol (**3**); one carotenoid: lutein (**4**); two triterpenes: ursolic acid (**5**), oleanolic acid (**6**); four iridoid glycosides: asperuloside (**7**), deacetyl asperuloside (**8**), asperulosidic acid (**9**), scandoside methyl ester (**10**); three flavonoid glycosides: afzelin (**11**), rutin (**12**), isorhamnetin-3-*O*- $\beta$ -rutinoside (**13**). Among them, compounds **1**, **3** and **11** were isolated for the first time in this genus.

## 2. MATERIALS AND METHODS

### 2.1. Equipments and methods

ESI-MS: LC-MSD-Trap-SL. NMR: Bruker Avance 500 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ). The  $^1\text{H}$  chemical shifts were referenced to the internal TMS; the  $^{13}\text{C}$  chemical shifts to the solvent signals. The 2D experiments (HSQC and HMBC) were performed using standard Bruker pulse sequences at room temperature. Analytical TLC was performed on silica gel 60 F<sub>254</sub> plates (Merck). Spots were visualized using UV light and vanillin-H<sub>2</sub>SO<sub>4</sub> reagent. For preparative column chromatography silica gel 60, 60-200  $\mu\text{m}$  (Merck) and sephadex LH-20 were used.

### 2.2. Plant material

*Hedyotis pinifolia* was collected on October 2014 in Phu Vang, Thua Thien Hue province of Viet Nam and determined by Dr. Do Xuan Cam, Hue University. The voucher specimen (VHH.TTH 10.2014.1) is deposited at the Institute of Chemistry, Vietnam Academy of Sciences and Technology (VAST).

### 2.3. Extraction and isolation

The air-dried whole of *H. pinifolia* (2.1 kg) were ground and extracted three times with 95 % MeOH at room temperature. The organic solvent was evaporated under reduced pressure and the aq. solution was partitioned with *n*-hexane, ethyl acetate and *n*-butanol, successively.

The *n*-hexane extract (36.2 g) was given on silica gel column, eluting with gradient *n*-hexane: EtOAc (from 100 % *n*-hexane to 100 % EtOAc) to yield 10 fractions (H1-H10). Fraction H7 (150 mg) was chromatographed on silica gel column, *n*-hexane: EtOAc (15:1), followed by Sephadex LH-20, CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to furnish compound **1** (5 mg). Compound **2** (6 mg) and **4** (11 mg) were isolated when fraction H10 (410 mg) was purified over Sephadex LH-20, using CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) as eluent.

The ethyl acetate extract (34.2 g) was chromatographed over silica gel using gradient *n*-hexane:EtOAc to yield 7 fractions (E1-E7). Fraction E2 (4.42 g) was repurified over silica gel column, CH<sub>2</sub>Cl<sub>2</sub>: MeOH (10:1) to give 30 mg of **5**. Rechromatography of fraction E3 (1.62 g) was done on silica gel column, CH<sub>2</sub>Cl<sub>2</sub>: MeOH (10:1) furnished compound **6** (200 mg). Fraction E4 (1.15 g) was given on silica gel column, eluting with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (9.5:1), followed on Sephadex LH-20 to obtain compound **3** (10 mg).

The *n*-butanol extract (32.0 g) was purified over silica gel columns to obtain 12 fractions (B1-B12). The fraction B3 (2.9 g) was rechromatographed on Sephadex LH-20 column with solvent systems of CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to yield three subfractions (B3.1 – B3.3). Compound **7** (30 mg) was isolated by purification of subfraction B3.2 using Sephadex LH-20 column, MeOH. The purification of fraction B4 (1.35 g) on Sephadex LH-20 column, eluted with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to give compound **11** (10 mg). The purification of fraction B5 (2.42 g) was carried out on Sephadex LH-20 column with solvent systems of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:9) to give two subfractions (B5.1-B5.2). Repeated chromatography of the subfraction B5.2 on sephadex LH-20 column, MeOH yielded compound **10** (16 mg). The fraction B6 (2.95 g) was given over Sephadex LH-20 column eluted with MeOH to furnish compound **8** (11 mg). The fraction B9 (3.9 g) was chromatographed on Sephadex LH-20 column, eluted with solvent systems of CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to yield compound **12** (40 mg). The fraction 10 (1.5 g) was given on Sephadex LH-20 column, eluted with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to furnish 2 subfractions (B10.1 and B10.2). Compound **7** (10 mg) and **13** (10 mg) were obtained by repeated chromatography of subfraction B10.2. The fraction 12 (1.5 g) was chromatographed on Sephadex LH-20 column, eluted with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:9) to give two subfractions (B12.1-B12.2). Rechromatography of B12.2 on sephadex LH-20 column, MeOH furnished compound **9** (10 mg).

**2-hydroxy-1-methoxy-anthraquinone (1)**: Orange-red needles. (-)-ESI-MS *m/z*: 253 [M-H]<sup>-</sup>

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> (ppm) 7.36 (1H, *d*, 9.0 Hz, H-3), 8.14 (1H, *d*, 9.0 Hz, H-4), 8.27 (2H, *m*, H-5, H-8), 7.74 (2H, *m*, H-6, H-7), 4.04 (3H, *s*, OMe), 6.69 (1H, *s*, OH).

<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> (ppm) 146.6 (C-1), 155.6 (C-2), 120.3 (C-3), 125.8 (C-4), 127.1 (C-5), 133.9 (C-6, C-7), 126.9 (C-8), 182.7 (C-9), 182.1 (C-10), 133.0 (C-11), 134.5 (C-12), 125.7 (C-13), 127.6 (C-14), 62.3 (OMe).

**1,6-dihydroxy-2-methylanthraquinone (2)**: Orange powder. (-)-ESI-MS *m/z*: 253 [M-H]<sup>-</sup>

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ<sub>H</sub> (ppm) 7.61 (1H, *d*, 7.5 Hz, H-3), 7.55 (1H, *d*, 7.5 Hz, H-4), 7.44 (1H, *d*, 2.5 Hz, H-5), 7.21 (1H, *dd*, 2.5, 8.5 Hz, H-7), 8.08 (1H, *d*, 8.5 Hz, H-8), 13.08 (1H, *s*, 1-OH), 2.27 (3H, *s*, 2-CH<sub>3</sub>).

<sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): δ<sub>C</sub> (ppm) 159.9 (C-1), 114.6 (C-2), 136.8 (C-3), 118.6 (C-4), 112.5 (C-5), 163.9 (C-6), 121.4 (C-7), 129.8 (C-8), 187.6 (C-9), 181.7 (C-10), 131.1 (C-4a), 124.4 (C-8a), 134.2 (C-9a), 135.6 (C-10a), 15.7 (2-CH<sub>3</sub>).

**Digiferruginol (3)**: Orange-yellow needles. (-)-ESI-MS *m/z*: 253 [M-H]<sup>-</sup>

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ<sub>H</sub> (ppm) 7.77 (1H, *d*, 7.5 Hz, H-3), 7.92 (1H, *d*, 8.0 Hz, H-4), 8.20 (1H, *m*, H-5), 7.95 (2H, *m*, H-6, H-7), 8.25 (1H, *m*, H-8), 4.66 (2H, *s*, CH<sub>2</sub>OH), 5.46 (1H, *t*, 5.5 Hz, CH<sub>2</sub>OH), 12.77 (1H, *s*, OH).

<sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): δ<sub>C</sub> (ppm) 158.4 (C-1), 138.2 (C-2), 131.3 (C-3), 118.8 (C-4), 126.8 (C-5), 134.5 (C-6), 135.1 (C-7), 126.6 (C-8), 188.7 (C-9), 181.8 (C-10), 133.6 (C-5a), 133.2 (C-8a), 114.9 (C-9a), 132.8 (C-10a), 57.4 (CH<sub>2</sub>OH).

**Lutein (4)**: Orange-red powder. (+)-ESI-MS *m/z*: 569 [M+H]<sup>+</sup>

<sup>1</sup>H-, <sup>13</sup>C-NMR (500, 125 MHz, CDCl<sub>3</sub>): Table 1.

**Ursolic acid (5)**: White amorphous powder. (-)-ESI-MS *m/z*: 455 [M-H]<sup>-</sup>

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> (ppm) 2.98 (*m*, H-3), 5.11 (*m*, H-12), 2.09 (*d*, 11.3 Hz, H-18), 0.88 (*s*, Me-23), 0.66 (*s*, Me-24), 0.85 (*s*, Me-25), 0.73 (*s*, Me-26), 1.02 (*s*, Me-27), 0.79 (*d*, 6.4 Hz, Me-29), 0.89 (*d*, 8.7 Hz, Me-30).

$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  (ppm) 38.2 (C-1), 27.0 (C-2), 76.8 (C-3), 38.4 (C-4), 54.8 (C-5), 18.0 (C-6), 30.2 (C-7), 39.1 (C-8), 47.0 (C-9), 36.5 (C-10), 23.8 (C-11), 124.6 (C-12), 138.2 (C-13), 41.6 (C-14), 32.7 (C-15), 22.8 (C-16), 46.8 (C-17), 52.4 (C-18), 38.4 (C-19), 38.5 (C-20), 27.5 (C-21), 36.3 (C-22), 28.3 (C-23); 16.9 (C-24); 16.1 (C-25), 15.2 (C-26), 23.3 (C-27), 178.3 (C-28), 17.0 (C-29), 21.1 (C-30).

**Oleanolic acid (6):** White amorphous powder. (-)-ESI-MS  $m/z$ : 455 [M-H] $^-$

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  (ppm) 5.27 (1H, *t*, 3.5 Hz, H-12), 3.20 (1H, *dd*, 4.0, 11.0 Hz, H-3), 2.81 (1H, *dd*, 4.0, 13.5 Hz, H-18), 1.12, 0.97, 0.91, 0.90, 0.89, 0.76, 0.74 (each 3H, *s*, Me-23, 24, 25, 26, 27, 29, 30).

$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  (ppm) 38.4 (C-1), 27.7 (C-2), 79.1 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 32.7 (C-7), 39.3 (C-8), 47.7 (C-9), 37.1 (C-10), 23.0 (C-11), 122.7 (C-12), 143.6 (C-13), 41.7 (C-14), 27.2 (C-15), 23.4 (C-16), 46.5 (C-17), 41.1 (C-18), 45.9 (C-19), 30.7 (C-20), 33.8 (C-21), 32.5 (C-22), 28.1 (C-23), 15.6 (C-24), 15.3 (C-25), 17.1 (C-26), 25.9 (C-27), 181.6 (C-28), 33.1 (C-29), 23.6 (C-30).

**Asperuloside (7):** white powder. (+) ESI-MS:  $m/z = 437$  [M+Na] $^+$ . NMR data: Table 2.

**Deacetyl asperuloside (8):** white powder. (-) ESI-MS:  $m/z = 371$  [M-H] $^-$ . NMR data: Table 2.

**Scandoside methyl ester (9):** white powder. (+) ESI-MS:  $m/z = 427$  [M+Na] $^+$ . NMR data: Table 2.

**Asperulosidic acid (10):** white powder. (+) ESI-MS:  $m/z = 455$  [M+Na] $^+$ . NMR data: Table 2.

**Afzelin (11):** yellow powder. (-) ESI-MS:  $m/z = 431$  [M-H] $^-$ .

$^1\text{H}$ -NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  (ppm) 6.22 (1H, *d*, 2.0 Hz, H-6), 6.40 (1H, *d*, 2.0 Hz, H-8), 7.79 (2H, *d*, 9.0 Hz, H-2', 6'), 6.96 (2H, *d*, 9.0 Hz, H-3', 5'), 5.40 (1H, *d*, 1.5 Hz, H-1''), 3.73 (1H, *dd*, 3.0, 9.0 Hz, H-2''), 3.36 (1H, *d*, 5.0 Hz, H-3''), 3.35 (1H, *d*, 5.0 Hz, H-4''), 4.24 (1H, *dd*, 2.0, 4.0 Hz, H-5''); 0.94 (3H, *d*, 6.0 Hz, H-6'').

$^{13}\text{C}$ -NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{C}}$  (ppm) 159.3 (C-2), 136.2 (C-3), 179.6 (C-4); 163.2 (C-5), 99.9 (C-6), 166.1 (C-7), 94.8 (C-8), 158.6 (C-9), 105.9 (C-10), 122.7 (C-1'), 131.9 (C-2', C-6'), 116.5 (C-3', C-5'), 161.6 (C-4'), 103.5 (C-1''), 72.0 (C-2''), 72.2 (C-3''), 73.2 (C-4''), 71.9 (C-5''), 17.6 (C-6'').

**Rutin (12):** yellow powder. (-) ESI-MS:  $m/z = 609$  [M-H] $^-$ .

$^1\text{H}$ -NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  (ppm) 6.22 (1H, *br s*, H-6), 6.42 (1H, *br s*, H-8), 7.71 (1H, *br s*, H-2'), 6.91 (1H, *d*, 8.0 Hz, H-5'), 7.64 (1H, *br d*, 8.0 Hz, H-6'), 5.07 (1H, *d*, 7.5 Hz, H-1''), 3.53 (1H, *t*, 9.0 Hz, H-2''), 3.81 (1H, *br d*, 9.5 Hz, H-6''), 4.54 (1H, *br s*, H-1'''), 3.69 (1H, *br s*, H-2'''), 3.58 (1H, *dd*, 3.5, 9.5 Hz, H-3'''), 3.31 (1H, *m*, H-4'''), 3.44 (1H, *m*, H-5'''), 1.14 (3H, *d*, 6.5 Hz, H-6''').

$^{13}\text{C}$ -NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{C}}$  (ppm) 158.4 (C-2), 135.6 (C-3), 179.3 (C-4), 162.7 (C-5), 100.0 (C-6), 166.0 (C-7), 95.0 (C-8), 159.4 (C-9), 105.6 (C-10), 123.1 (C-1'), 117.7 (C-2'), 145.6 (C-3'), 149.7 (C-4'), 116.1 (C-5'), 123.6 (C-6'), 104.8 (C-1''), 75.5 (C-2''), 78.0 (C-3''), 71.3 (C-4''), 77.1 (C-5''), 68.6  $\text{CH}_2$  (C-6''), 102.3 (C-1'''), 72.0 (C-2'''); 72.1 (C-3'''); 73.9 (C-4'''), 69.6 (C-5'''), 17.8 (C-6''').

**Isorhamnetin-3-O- $\beta$ -rutinoside (13):** yellow powder. (-) ESI-MS:  $m/z = 623$  [M-H] $^-$ .

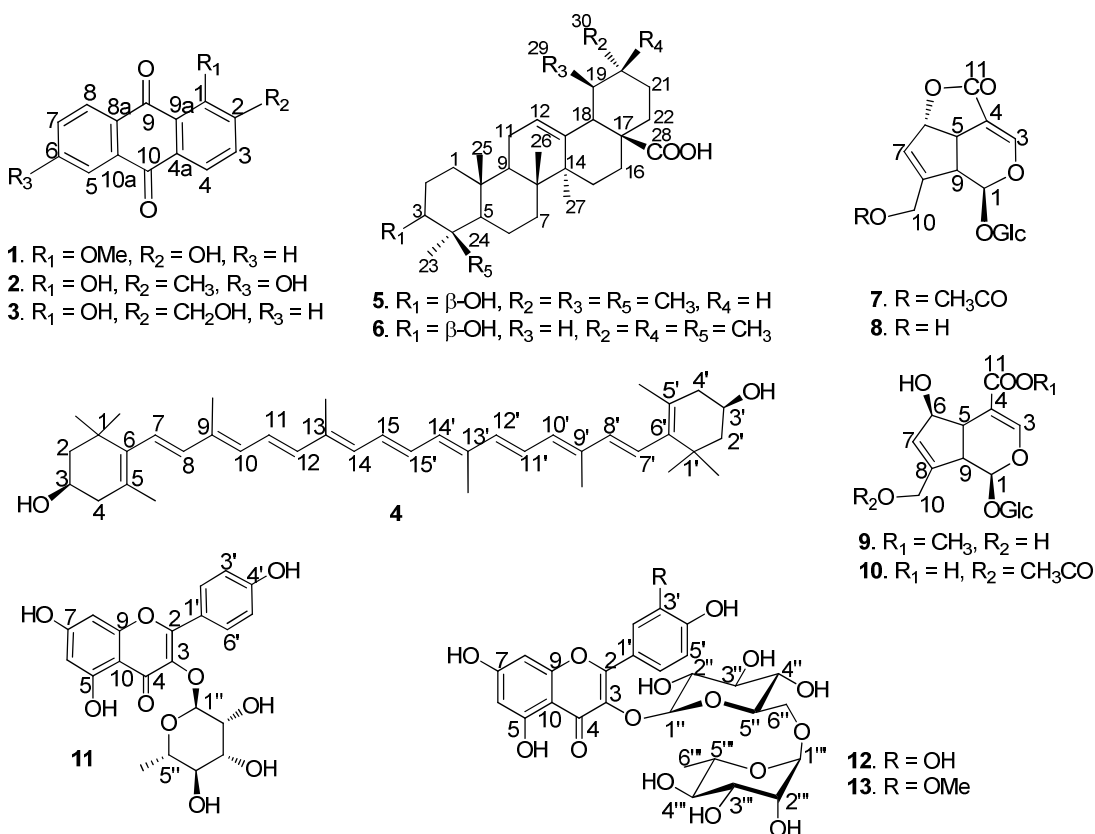
$^1\text{H}$ -NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  (ppm) 6.22 (1H, *d*, 1.5 Hz, H-6), 6.41 (1H, *d*, 1.5 Hz, H-8), 7.95 (1H, *d*, 2.0 Hz, H-2'), 6.93 (1H, *d*, 8.5 Hz, H-5'), 7.64 (1H, *dd*, 2.0, 8.5 Hz, H-6'), 5.24

(1H, *d*, 7.5 Hz, H-1''), 4.55 (1H, *d*, 1.0 Hz, H-1'''), 1.12 (3H, *d*, 7.5 Hz, H-6'''), 3.96 (3H, *s*, OMe).

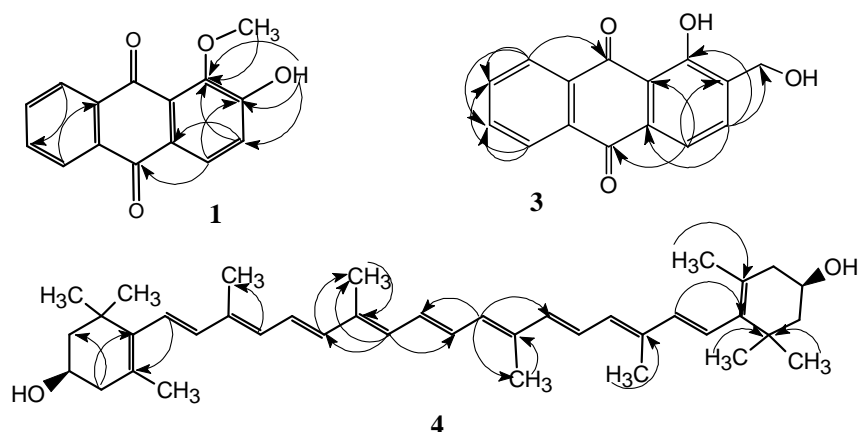
<sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD):  $\delta_c$  (ppm) 158.5 (C-2), 135.5 (C-3), 179.3 (C-4), 162.9 (C-5), 100.0 (C-6), 166.0 (C-7), 94.9 (C-8), 158.9 (C-9), 105.7 (C-10), 123.0 (C-1'), 114.6 (C-2'), 148.3 (C-3'), 150.8 (C-4'), 116.1 (C-5'); 124.0 (C-6'), 104.4 (C-1''), 75.9 (C-2''), 78.1 (C-3''), 71.6 (C-4''), 77.3 (C-5''), 68.5 (C-6''), 102.49 (C-1'''), 72.0 (C-2'''), 72.3 (C-3'''), 73.8 (C-4'''), 69.8 (C-5'''), 17.9 (C-6'''), 56.8 (-OMe).

### 3. RESULTS AND DISCUSSION

Thirteen compounds were isolated from *n*-hexane, ethyl acetate and *n*-butanol extracts of the whole plant of *H. pinifolia* by repeated column chromatography with the appropriate solvent systems. Their structures were identified as 2-hydroxy-1-methoxy-anthraquinone (**1**); 1,6-dihydroxy-2-methylantraquinone (**2**); digiferruginol (**3**); lutein (**4**); ursolic acid (**5**) and oleanolic acid (**6**) asperuloside (**7**), deacetyl asperuloside (**8**), asperulosidic acid (**9**), scandoside methyl ester (**10**), afzelin (**11**), rutin (**12**), isorhamnetin-3-*O*- $\beta$ -rutinoside (**13**) by the analysis of their 1D, 2D-NMR, ESI-MS spectra and compared with published data.



The structure of isolated compounds (1 -13)



### HMBC correlations of compounds 1, 3, 4

Compound **1** obtained as orange-red needles, gave pseudo molecular peak at  $m/z = 253$  [M-H]<sup>-</sup> in the negative ESI-MS. The <sup>1</sup>H NMR spectrum indicated the characteristic signals of the anthraquinone type, including the signals of the two aromatic rings: two *ortho*-coupled aromatic protons at  $\delta_{\text{H}}$  7.36 (*d*,  $J = 9.0$  Hz, H-3); 8.14 (*d*,  $J = 9.0$  Hz, H-4) of the first ring and typical aromatic protons of the A<sub>2</sub>B<sub>2</sub> substituted ring at  $\delta_{\text{H}}$  8.27 (2H, *m*, H-5, H-8); 7.74 (2H, *m*, H-6, H-7) of the second together with an aromatic methoxy group at  $\delta_{\text{H}}$  4.04. Beside these signals, the <sup>13</sup>C NMR spectrum gave the signals of the two carbonyl carbons at  $\delta_{\text{C}}$  182.7 and 182.1 and six aromatic quaternary carbons comprising one hydroxy-carbon at  $\delta_{\text{C}}$  155.6; a carbon connected to a methoxy group at  $\delta_{\text{C}}$  144.6 and four others. The position of the hydroxy group at C-2 was deduced from HMBC correlations among signals at  $\delta_{\text{H}}$  6.69 (2-OH),  $\delta_{\text{C}}$  120.3 (C-3); 155.6 (C-2) and 146.6 (C-1); among signals at  $\delta_{\text{H}}$  7.36 (H-3),  $\delta_{\text{C}}$  146.6 (C-1) and 127.6 (C-4a). The structure of **1** was thus determined to be 2-hydroxy-1-methoxy-anthraquinone or alizarin-1-methyl ether when compared to the literature [12]. This compound was isolated previously from *Hedyotis diffusa* and inhibited protein tyrosine kinases v-src and pp60src and the growth of Bcap37 cell line (IC<sub>50</sub> 65  $\mu\text{M}$ ). Furthermore, it could induce apoptosis on SPC-1-A cell (IC<sub>50</sub> 79  $\mu\text{M}$ ) with a close relationship to the mitochondrial apoptotic pathway [13].

Compound **2** was isolated as an orange powder, showed [M-H]<sup>-</sup> peak at  $m/z = 235$  in the negative ESI-MS. Its NMR spectra showed characteristic signals of an 9,10-anthraquinone very similar to those of **1**, revealing two carbonyl carbons at  $\delta_{\text{C}}$  187.6 and 181.7 as well as proton signals of two aromatic rings: two *ortho*-coupled aromatic protons and typical aromatic protons of the 1,3,4-substituted ring. These signals suggested that the substituted positions of **2** are the same in compound **1** but with other groups. The connection of hydroxy group at C-1 was confirmed based on a chelated hydroxy proton in <sup>1</sup>H NMR (measured in DMSO-*d*<sub>6</sub>) at  $\delta_{\text{H}}$  13.08, as well as the downshifted carbonyl carbon at  $\delta_{\text{C}}$  187.6. The position of the hydroxy group at C-6 was deduced from HMBC correlations among signals at  $\delta_{\text{H}}$  8.08 (H-8),  $\delta_{\text{C}}$  187.6 (C-9) and 163.9 (C-6); among signals at  $\delta_{\text{H}}$  7.44 (H-5),  $\delta_{\text{C}}$  181.7 (C-10) and 121.4 (C-7); and among signals at  $\delta_{\text{H}}$  7.21 (H-7),  $\delta_{\text{C}}$  112.5 (C-5) and 124.4 (C-8a). An aromatic methyl group [ $\delta_{\text{H}}$  2.27 (3H, *s*);  $\delta_{\text{C}}$  15.7] was determined to connect at C-2 by correlations among signals at  $\delta_{\text{H}}$  2.27 (CH<sub>3</sub>),  $\delta_{\text{C}}$  159.9 (C-1), 136.8 (C-3). The spectroscopic data of **2** was identical to those of 1,6-dihydroxy-2-methylanthraquinone in the literature [14]. This compound was first isolated from *Cinchona pubescens* in 1986 [14].

Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR (500 and 125 MHz) data of compound **4** and lutein (CDCl<sub>3</sub>).

No	$\delta_{\text{H}}$ (ppm, <i>J</i> , Hz)	$\delta_{\text{C}}$ (ppm)	$\delta_{\text{H}}$ (ppm, <i>J</i> , Hz)	$\delta_{\text{C}}$ (ppm)
	<b>Compound 4</b>		<b>Lutein [CDCl<sub>3</sub>]</b>	
1	-	37.1	-	37.1
2	1.48 ( <i>t</i> , 12.0)	48.5	1.48 ( <i>t</i> , 12.0)	48.4
3	400 ( <i>m</i> )	65.1	4.0 ( <i>m</i> )	65.1
4 ax	2.04 ( <i>dd</i> , 17.0; 10.0)	42.6	2.04 ( <i>dd</i> , 17.0; 10.0)	42.5
4 eq	2.33-2.42 ( <i>m</i> )		2.33-2.45 ( <i>m</i> )	
5	-	126.2	-	126.2
6	-	137.6	-	137.6
7	6.12 ( <i>s</i> )	125.6	6.12 ( <i>s</i> )	125.6
8	6.12 ( <i>s</i> )	138.5	6.12 ( <i>s</i> )	138.5
9	-	135.7	-	135.6
10	6.15 ( <i>m</i> )	131.3	6.15 ( <i>m</i> )	131.3
11	6.58-6.67 ( <i>m</i> )	124.8	6.55-6.71 ( <i>m</i> )	124.9
12	6.36 ( <i>d</i> , 15.0)	137.7	6.36 ( <i>d</i> , 15.0)	137.6
13	-	136.5	-	136.5
14	6.25 ( <i>br d</i> , 9.0)	132.6	6.26 ( <i>m</i> )	132.6
15	6.58-6.67 ( <i>m</i> )	130.1	6.55-6.71 ( <i>m</i> )	130.0
1-Me	1.07 ( <i>s</i> )	28.7	1.07 ( <i>s</i> )	28.7
1-gem Me	1.07 ( <i>s</i> )	30.3	1.07 ( <i>s</i> )	30.2
5 -Me	1.74 ( <i>s</i> )	21.6	1.74 ( <i>s</i> )	21.6
9 -Me	1.97 ( <i>s</i> )	12.8	1.97 ( <i>s</i> )	12.7
13 -Me	1.97 ( <i>s</i> )	12.8	1.97 ( <i>s</i> )	12.7
1'	-	34.0	-	34.0
2' ax	1.37 ( <i>dd</i> , 13.0; 7.0)	44.6	1.37 ( <i>dd</i> , 13.0; 7.0)	44.7
2' eq	1.84 ( <i>dd</i> , 13.0; 6.0)		1.84 ( <i>dd</i> , 13.0; 6.0)	
3'	4.25 ( <i>m</i> )	65.9	4.25 ( <i>m</i> )	65.9
4'	5.54 ( <i>br s</i> )	125.6	5.55 ( <i>s</i> )	125.6
5'	-	137.8	-	137.8
6'	2.33 – 2.42 <i>m</i>	55.0	2.33-2.45 ( <i>m</i> )	55.0
7'	5.43 ( <i>dd</i> , 10.0; 15.5)	128.7	5.43 ( <i>dd</i> , 15.5; 10.0)	128.6
8'	6.15 ( <i>m</i> )	138.0	6.15 ( <i>m</i> )	137.8
9'	-	135.1	-	135.0
10'	6.15 ( <i>m</i> )	130.8	6.15 ( <i>m</i> )	130.8
11'	6.58-6.67 ( <i>m</i> )	124.5	6.55-6.71 ( <i>m</i> )	124.5
12'	6.36 ( <i>d</i> , 15.0)	137.6	6.36 ( <i>d</i> , 15.0)	137.6
13'	-	136.4	-	136.5
14'	6.25 ( <i>br d</i> , 9.0)	132.6	6.26 ( <i>m</i> )	132.6
15'	6.58-6.67 ( <i>m</i> )	130.0	6.55-6.71 ( <i>m</i> )	130.0
1'-Me	0.85 ( <i>s</i> )	24.3	0.85 ( <i>s</i> )	24.3
1'-Me	1.00 ( <i>s</i> )	29.5	1.00 ( <i>s</i> )	29.5
5'-Me	1.63 ( <i>s</i> )	22.9	1.63 ( <i>s</i> )	22.8
9'-Me	1.91 ( <i>s</i> )	14.1	1.91 ( <i>s</i> )	13.2
13'-Me	1.97 ( <i>s</i> )	13.1	1.97 ( <i>s</i> )	12.7

Compound **3** was obtained as yellow needle, showed [M-H]<sup>-</sup> peak at  $m/z = 235$  in the negative ESI-MS. Its NMR spectra predicted to be an 9,10-anthraquinone from similar signals to those of **1**. Beside the same signals as in **1**, the NMR spectra of **3** appeared additional characteristic signals of the hydroxy group at  $\delta_{\text{H}} 12.77$  and hydroxymethylen group [ $\delta_{\text{H}} 4.66$  (*d*,  $J = 5.0$  Hz, 2 -CH<sub>2</sub>OH);  $5.46$  (*t*,  $J = 5.5$  Hz, 2-CH<sub>2</sub>OH);  $\delta_{\text{C}} 57.4$ ]. The position of the hydroxyl group at C-1 and hydroxymethylen group at C-2 was deduced from HMBC correlations. Consequently, **3** was identified as digiferruginol by comparison with the published data [15]. Digiferruginol exhibited a significant cytotoxic activity against KB cancer cell line with an ED value of 0.09  $\mu\text{g/mL}$  [15].

Compound **4** was isolated as an amorphous red-orange powder, indicated [M+H]<sup>+</sup> peak at  $m/z = 569$  in the positive ESI-MS. The NMR spectrum of **4** appears characteristic signals of a carotenoid including conjugated olefins at  $\delta_{\text{H}} 5.43\text{-}6.67$ ;  $\delta_{\text{C}} 124.5\text{-}138.5$  together with 4 methylene groups. Besides, two hydroxy-methine groups [( $\delta_{\text{H}} 4.00$  (1H, *m*, H-3);  $\delta_{\text{C}} 65.1$  (C-3) and  $4.25$  (1H, *m*, H-3');  $65.9$  (C-3')] and 10 methyl groups were observed. The connection of two hydroxy groups at C-3 and C-3' was confirmed based on the downshifted carbons with  $\delta_{\text{C}} 65.1$  (C-3) and  $65.9$  (C-3'). The position of the allene moiety was determined to connect to alicyclic moiety at C-6 by HMBC correlations among signals at  $\delta_{\text{H}} 6.12$  (H-7) to  $\delta_{\text{C}} 126.2$  (C-5) and  $137.6$  (C-6). The conjugated alkadiene chain was deduced from HMBC correlations among protons H-14 with C-12 and C-15'; H-14' with C-12', C-15 and methyl group carbon connected to C-13'. The structure of **4** is concluded as lutein when compared with the spectral data in the literature [16]. Lutein plays significant roles in human health, particularly for eyes, linked to reduced risk of age-related macular degeneration and cataracts [17].

The structures of compounds **5** and **6** were elucidated as ursolic acid and oleanolic acid, respectively, by comparison with authentic sample in thin layer chromatography and <sup>1</sup>H, <sup>13</sup>C NMR spectral data [18, 19]. Oleanolic acid and ursolic acid are triterpenoid compounds that widely occur in nature in free acid form or as an aglycone of triterpenoid saponins. These compounds have shown pharmacological activities, such as hepatoprotective, anti-inflammatory, antioxidant, and anticancer effects [20].

Compound **7** showed the pseudo molecular peak at  $m/z = 437$  [M+Na]<sup>+</sup> in the positive ESI-MS spectrum. The molecular formula of C<sub>18</sub>H<sub>22</sub>O<sub>11</sub> was deduced from MS and NMR data. NMR spectra showed the signals of two double bonds -CH=C< at  $\delta_{\text{H}} = 7.32$  (1H, *d*, 2.0 Hz),  $\delta_{\text{C}} = 150.3$  CH, 106.1 C and  $\delta_{\text{H}} = 5.75$  (1H, *br s*),  $\delta_{\text{C}} = 128.9$  CH, 144.2 C; two oxygenated methine at  $\delta_{\text{H}} = 5.97$  (1H, *d*, 1.0 Hz),  $\delta_{\text{C}} = 93.3$  and  $\delta_{\text{H}} = 5.59$  (1H, *br d*, 6.5 Hz),  $\delta_{\text{C}} = 86.3$  CH. Besides these, two methine and an oxy-methylene have been observed in NMR spectra. The <sup>13</sup>C NMR spectrum indicated the signals of a lactone and an acetyl groups, revealing by signals at  $\delta_{\text{C}} = 172.3$  C and  $172.6$  C,  $20.6$  CH<sub>3</sub>, respectively. The presence of one  $\beta$ -D-glucopyranose unit in the molecule was confirmed by typical signals of anomer group at  $\delta_{\text{H}} = 4.71$  (1H, *d*, 8.0 Hz),  $\delta_{\text{C}} = 100.0$  and methylene group (Glc-6) at  $\delta_{\text{H}} = 3.94$  (*dd*, 12.0 & 2.0 Hz),  $3.70$  (*dd*, 12.0 & 6.0 Hz),  $\delta_{\text{C}} = 62.8$ . The correlations in the HMBC spectrum between H-1 ( $\delta_{\text{H}} 5.97$ ) with C-3, C-5, C-8, C-9 and Glc-1; H-3 ( $\delta_{\text{H}} 7.32$ ) with C-1, C-4, C-5 and C-11; H-7 ( $\delta_{\text{H}} 5.75$ ) with C-5, C-6, C-8, C-9 and C-10 determined the substituted positions in compound **7**. The above analysis and compared with the published data [21] led to conclude that **7** is asperuloside, a glucoside of iridoid C-9, which was already isolated from *Galium verum* [22].

Compound **8** was obtained as white powder. The pseudo molecular ion peak at  $m/z = 371$  [M-H]<sup>-</sup> in the negative ESI-MS and NMR spectra led to conclude that the molecular formula of **8** is C<sub>16</sub>H<sub>20</sub>O<sub>10</sub>. Its NMR data were very similar to those of **7**, suggested it is an iridoid glucoside



too. The only difference between two compounds revealed by the replacement of acetyl signal group in **7** by hydroxy-methylene signals in compound **8** ( $\delta_{\text{H}} = 4.21$ , 2H, br *s*,  $\delta_{\text{C}} = 60.1$ ). The loss of 42 mass units ( $\text{CH}_3\text{CO}$ -) of compound **2** compared with **7** confirmed the above mentioned suggestion. Therefore, the structure of **8** was determined as deacetyl asperuloside by comparison with the data in literature [21].

Compound **9** was isolated as white powder. The positive ESI-MS gave pseudo molecular ion peak at  $m/z$  427  $[\text{M}+\text{Na}]^+$ . Its molecular formula was determined as  $\text{C}_{17}\text{H}_{24}\text{O}_{11}$  from MS and NMR data. NMR spectra of **9** showed similar signals to those of **8**. The upshifted of C-6 ( $\delta_{\text{C}} 82.6$ ) and C-11 ( $\delta_{\text{C}} 170.3$ ) together with the addition of methoxy group at  $\delta_{\text{H}} = 3.77$ ,  $\delta_{\text{C}} = 52.0$  suggested that lactone group in **8** was opened to form hydroxyl and methyl ester groups in **9**. Thus, the structure of **9** was elucidated as scandoside methyl ester when compared with published data in [21].

NMR spectra of **10** suggested that is an iridoid glucoside, very similar to those of **9** with iridoid and  $\beta$ -D-glucopyranose signals. But, the methyl ester and hydroxy signals in **9** were replaced by carboxylic at  $\delta_{\text{C}} 170.0$  (C-11) and acetyl at 172.5, 2.03/20.8. The position of substituted groups were confirmed by HMBC correlations. These analysis combined the published data in [23] led to conclude that **10** is asperulosidic acid. This compound exhibited cytotoxic activity on HL-60 and HCT15 cancer cell lines [24].

Compound **11** was obtained as yellow powder. The molecular formula was determined as  $\text{C}_{21}\text{H}_{20}\text{O}_{10}$  from pseudo molecular ion peak at  $m/z = 431$   $[\text{M}-\text{H}]^-$  in the negative ESI-MS and NMR spectra. The  $^1\text{H}$ -NMR spectrum exhibited two meta-coupled protons at  $\delta_{\text{H}} 6.22$  (1H, *d*,  $J = 2.0$  Hz, H-6), 6.40 (1H, *d*,  $J = 2.0$  Hz, H-8), characteristic proton signals of para-substituted aromatic ring at  $\delta_{\text{H}} 7.79$  (2H, *d*,  $J = 9.0$  Hz, H-2', 6'); 6.96 (2H, *d*,  $J = 9.0$  Hz, H-3', 5'). Besides these, the presence of an  $\alpha$ -L-rhamnopyranose have been observed, revealing by a proton anomer at  $\delta_{\text{H}} 5.40$  (1H, *d*,  $J = 1.5$  Hz) and a methyl group at  $\delta_{\text{H}} 0.94$  (3H, *d*,  $J = 6.0$  Hz). The  $^{13}\text{C}$ -NMR spectrum of **11** indicated 21 carbons, among them 15 belong to flavonoid skeleton and 6 carbons of sugar unit. The above analysis combined the comparison with the published data [25] led to conclude that **11** is 3-*O*- $\alpha$ -L-rhamnopyranoside kaempferol, named afzelin. It was isolated for the first time from *Afzelia doussie* [26]. Afzelin inhibited the growth of prostate cancer cells *in vitro* [27].

Compound **12** was isolated as yellow powder and showed the pseudo molecular ion peak at  $m/z = 609$   $[\text{M}-\text{H}]^-$  in the negative ESI-MS, corresponds to  $\text{C}_{27}\text{H}_{30}\text{O}_{16}$ . Its NMR spectra indicated that is a flavonoid glycoside, revealing by the signals of two meta-protons in A ring at  $\delta_{\text{H}} = 6.22$  br *s* (H-6), 6.42 br *s* (H-8), three protons of ABX spin system in ring B at  $\delta_{\text{H}} = 7.71$  br *s* (H-2'), 6.91 *d*, 8.0 Hz (H-5'), 7.64 br *d*, 8.0 Hz (H-6'), ketone group in ring C at  $\delta_{\text{C}} = 179.3$  (C-4). Furthermore, the characteristic signals of  $\beta$ -D-glucopyranose unit at  $\delta_{\text{H}} = 5.07$  *d*, 7.5 Hz (H-1''),  $\delta_{\text{C}} = 104.8$  (C-1'') and  $\alpha$ -L-rhamnopyranose at  $\delta_{\text{H}} = 4.54$ , br *s* (H-1'''), 1.14 *d*, 6.5 (H-6''');  $\delta_{\text{C}} = 102.3$  (C-1'''), 17.8 (C-6''') have been observed. The NMR data of **12** are identical with those of rutin in the literature [28]. Rutin exists widely in the nature, for example in barley, fruit types of *Citrus* genus and medicinal plant as *Sophora japonica* L. Rutin has been proven having many biological effects as inflammatory, antioxidant, etc. [29].

Compound **13**, an yellow powder, showed the pseudo molecular ion peak at  $m/z = 623$   $[\text{M}-\text{H}]^-$  in the negative ESI-MS. Its molecular formula was deduced as  $\text{C}_{28}\text{H}_{32}\text{O}_{16}$  ( $M = 624$ ) from MS and NMR data. The NMR data of **13** were very similar to those of **12** with only one difference. The signal of hydroxy in ring B of **12** was replaced by methoxy signals ( $\delta_{\text{H}} = 3.96$  *s*,

3H and  $\delta_C = 56.8$ ) in compound **13** together with the downshifted of C-3' ( $\Delta\delta_C = 2.67$ ) when compared to those of compound **12**. Finally, the structure of **13** was established as isorhamnetin-3-*O*- $\beta$ -rutinoside by comparison with the published spectroscopic data in [30]. The other name of this compound is narcissin.

Table 2.  $^1\text{H}$  and  $^{13}\text{C}$ -NMR (500 and 125 MHz) of compounds **7** – **10** ( $\text{CD}_3\text{OD}$ ).

	<b>7</b> $\delta_{\text{H}}$ (J, Hz)	<b>8</b> $\delta_{\text{H}}$ (J, Hz)	<b>9</b> $\delta_{\text{H}}$ (J, Hz)	<b>10</b> $\delta_{\text{H}}$ (J, Hz)	<b>7</b> $\delta_{\text{C}}$	<b>8</b> $\delta_{\text{C}}$	<b>9</b> $\delta_{\text{C}}$	<b>10</b> $\delta_{\text{C}}$
<b>1</b>	5.97 <i>d</i> , 1.0	5.97 <i>d</i> , 1.5	5.83 <i>br s</i>	5.07 <i>d</i> , 9.0	93.3 CH	93.3	98.3	101.2
<b>3</b>	7.32 <i>d</i> , 2.0	7.31 <i>d</i> , 2.0	7.53 <i>br s</i>	7.63 <i>s</i>	150.3CH	150.2	153.9	154.6
<b>4</b>	-	-	-	-	106.1 C	106.5	110.8	109.0
<b>5</b>	3.70 <i>m</i>	3.70 <i>m</i>	3.04 <i>m</i>	3.05 <i>m</i>	37.4 CH	37.5	45.6	42.7
<b>6</b>	5.59 <i>br d</i> , 6.5	5.58 <i>dd</i> , 1.5 & 6.5	4.57 <i>br s</i>	4.85 <i>m</i>	86.3 CH	86.6	82.6	75.5
<b>7</b>	5.75 <i>br s</i>	5.66 <i>br s</i>	5.21 <i>t</i>	6.04 <i>br s</i>	128.9 CH	125.7	130.1	131.9
<b>8</b>	-	-	-	-	144.2 C	149.8	147.5	145.9
<b>9</b>	3.32 <i>m</i>	3.32 <i>m</i>	3.23 <i>br t</i> 8.0	2.65 <i>t</i> , 8.0	45.2 CH	45.0	47.1	46.4
<b>10</b>	4.69 <i>dd</i> , 14.0 & 1.0 4.80 <i>dd</i> , 14.0 & 1.0	4.21 <i>br s</i>	4.21 <i>br d</i> , 15.0 4.36 <i>br d</i> , 15.0	4.97 <i>br d</i> , 14.5 4.83 <i>br d</i> , 14.5	61.9 CH <sub>2</sub>	60.1	61.0	63.8
<b>11</b>	-	-	-	-	172.3 C	172.8	170.3	173.0
<b>CH<sub>3</sub>CO</b>	-	-	-	-	172.6 C	-	-	172.5
<b>CH<sub>3</sub>CO</b>	2.10 <i>s</i>	-	-	2.03 <i>s</i>	20.6 CH <sub>3</sub>	-	-	20.8
<b>OCH<sub>3</sub></b>	-	-	3.77 <i>s</i>	-			52.0	-
<b>Glc-1</b>	4.71 <i>d</i> , 8.0	4.70 <i>d</i> , 8.0	4.69 <i>d</i> , 8.0	4.75 <i>d</i> , 8.0	100.0 CH	99.9	100.3	100.6
<b>Glc-2</b>	3.22 <i>dd</i> , 9.0 & 8.0	3.21 <i>dd</i> , 9.0 & 8.0		3.41 <i>br t</i> , 8.5	74.6 CH	74.7	74.8	74.9
<b>Glc-3</b>	3.31 – 3.40 <i>m</i>	3.30 – 3.60 <i>m</i>	3.20 – 3.34 <i>m</i>	3.25 – 3.33 <i>m</i>	78.3 CH	78.4	78.4	78.5
<b>Glc-4</b>					71.6 CH	71.6	71.5	71.6
<b>Glc-5</b>					77.8 CH	77.9	77.9	77.9
<b>Glc-6</b>	3.94 <i>dd</i> , 12.0 & 2.0 3.70 <i>dd</i> , 12.0 & 6.0	3.94 <i>dd</i> , 12.0 & 2.0 3.84 <i>dd</i> , 12.0 & 6.0	3.88 <i>br d</i> , 11.5 3.66 <i>br d</i> , 11.5	3.87 <i>br d</i> , 10.0 3.64 <i>dd</i> , 5.0, 12.0	62.8 CH <sub>2</sub>	62.8	62.7	63.0

#### 4. CONCLUSION

In summary, this report deals with the isolation and structural elucidation of thirteen compounds from the *n*-hexane, ethyl acetate and *n*-butanol extracts of *Hedyotis pinifolia*. Three of them (compounds **1**, **3** and **11**) were obtained for the first time from this genus.

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