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A new hexenoic acid glycoside with cytotoxic activity from the leaves of *Psychotria luzoniensis*

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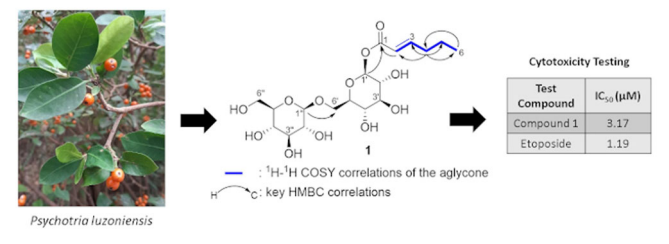
ABSTRACT

A new hexenoic acid glycoside (**1**) together with known compounds, flavonol glycosides (**2**–**4**), iridoid glycoside (**5**), megastigmane glycoside (**6**), and amino acid (**7**) were isolated from the leaves of *P. luzoniensis* by resin column chromatography and preparative HPLC. Their structures were determined based on spectroscopic analysis, including HRFABMS and NMR (^{1}H and ^{13}C, ^{1}H–^{1}H COSY, HMQC, and HMBC) data. All compounds tested for cytotoxicity were active (IC_{50} < 50 μM) with IC_{50} values ranging from 1.97 to 32.85 μM against human colon adenocarcinoma cell line, compared to etoposide (IC_{50} 1.19 μM).



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Psychotria luzoniensis; hexenoic acid glycoside; cytotoxicity

1. Introduction

Phytochemical and biological investigations from the genus *Psychotria* have resulted to the identification of many compounds (indole, monoterpene indole, quinolone, and isoquinoline alkaloids as well as flavonoids, coumarins, terpenoids, and cyclic peptides) with cytotoxic, analgesic, antiviral, antifungal and antibacterial activities (Calixto et al. 2016; Yang et al. 2016). The species-rich genus *Psychotria* comprises approximately 2000 species and considered to be the largest genera within the Rubiaceae family (Turner and Kumar 2018), of which 112 species can be found in the Philippines

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(Parungao et al. 2018). Plants from this genus have collective folkloric use in traditional medicine for the treatment of various illnesses such as bronchial and gastrointestinal disorders (Soares et al. 2017). *Psychotria luzoniensis* (Cham. & Schltdl.) Fern.-Vill, an endemic plant to the Philippines, is a terrestrial shrub growing to a height of 1.5 to 5 meters (Pelser et al. 2011). *P. luzoniensis,* locally known as tagpong-gubat, is used traditionally by Filipinos through leaf decoction for treating dysentery and ulcerated wounds (Carag and Buot 2017; Parungao et al. 2018). On the contrary, there is no published data on the phytochemical constituents and limited information as to the biological activities of *P. luzoniensis*.

The global burden of cancer is progressing unabated (Bray et al. 2018). Recently, the WHO reported that colorectal cancer remains as one of the leading causes of incidence and deaths worldwide affecting both sexes (WHO 2018). In the Philippines, among all types of cancer, colorectal cancer ranked 3rd as to incidence with 10,167 cases and 4th in terms of mortality with 6,004 deaths (Ferlay et al. 2018). In the search for new drug leads for colon cancer, the study presented the isolation and structural elucidation of bioactive compounds from the leaves of *P. luzoniensis* via direct phytochemical isolation approach. Herein, for the first time, we disclose the identification of one new hexenoic acid glycoside (**1**) and six known compounds, flavonol glycosides (**2**–**4**), one iridoid glycoside (**5**), one megastigmane glycoside (**6**), and amino acid (**7**). These isolated compounds except the amino acid were tested against HT29 cancer cells to assess their cytotoxic activities using the WST-8 assay.

2. Results and discussion

An EtOH extract of *P. luzoniensis* leaves was subjected to solvent partitioning and the H_2O sub-extract was initially purified to open column chromatography using a Diaion HP-20, a macroporous resin. Further purifications were applied using preparative HPLC with reversed phase columns. The chemical structure of a new compound (**1**) was determined based on the spectroscopic data, including ^{1}H and ^{13}C NMR and MS. Other isolated known compounds, quercetin 3-*O*-rutinoside (**2**) (Kazuma et al. 2003), quercetin-3-*O*-glucopyranoside (**3**) (Kazuma et al. 2003), kaempferol 3-*O*-β-D-apiosyl (1→2)-β-D-glucopyranoside (**4**) (Wu et al. 2007), asperuloside (**5**) (Li et al. 2006), (6*S*, 9*R*)-roseoside (**6**) (Yamano and Ito 2005), and tryptophan (**7**) were identified by comparing their spectroscopic data with those reported in the literature (Figure 1).

Compound **1,** was isolated as a colorless amorphous powder, with a molecular formula $C_{18}H_{30}O_{12}$ based on HRFABMS *m/z* 461.1636 $[M+Na]^+$ (calcd for $C_{18}H_{30}O_{12}Na$, 461.1634). The ^{1}H NMR spectrum of **1** showed signals of a triplet methyl (δ 0.96, 3H, $J = 7.0$ Hz, H_3-6), two methylene (δ 1.52, 2H, m, H_2-5; 2.23, 2H, m, H_2-4), and two olefinic (δ 7.10, 1H, dt, $J = 16.0$, 7.0 Hz, H-3; 5.90, 1H, dt, $J = 16.0$, 1.0 Hz, H-2) proton resonances. Their corresponding carbons assigned as δ 152.5 (C-3), 121.7 (C-2), 35.4 (C-4), 22.4 (C-5), and 14.0 (C-6) and a carbonyl carbon at δ 166.7 (C-1) suggested the presence of a 2-hexenoyl moiety (Kon et al. 2009). Furthermore, the correlations from H-2 to H-3, H-3 to H_2-4, H_2-4 to H_2-5, and from H_2-5 to H_3-6 were confirmed by the ^{1}H-^{1}H COSY spectrum of **1**. The coupling constant between H-2 and H-3 ($J = 16.0$ Hz) suggested their *E* configuration (Kon et al. 2009). In the ^{13}C NMR spectrum of **1**, the other

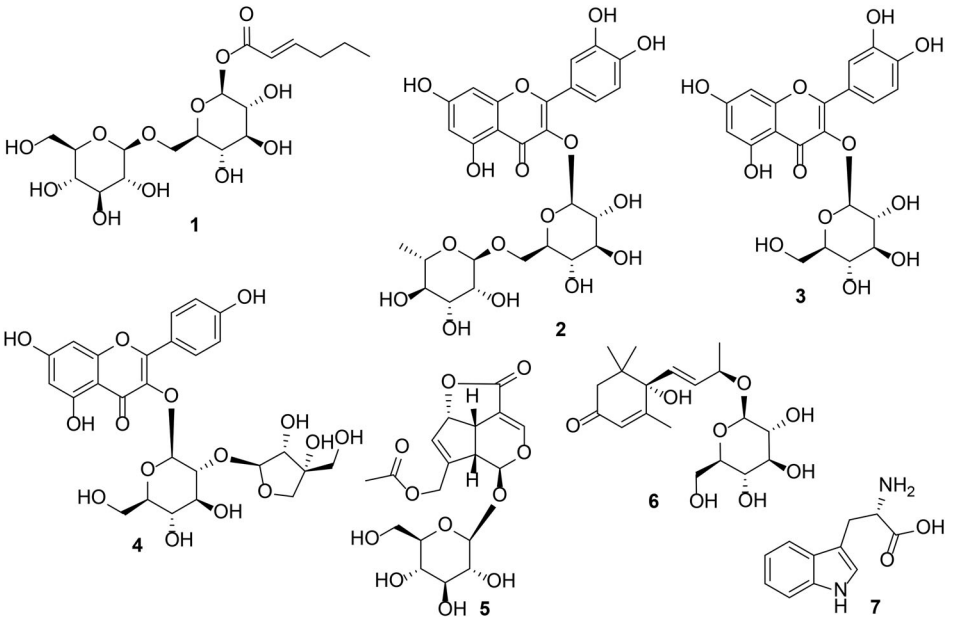

Figure 1. Structures of compounds **1–7.**

12 oxygenated carbon resonances (δ 95.7, C-1′; 73.9, C-2′; 77.9, C-3′; 71.0, C-4′; 77.8, C-5′; 69.5, C-6′;104.6, C-1′′; 75.1, C-2′′; 78.0, C-3′′; 71.5, C-4′′; 78.0, C-5′′; 62.7, C-6′′) and two anomeric proton resonances (δ 5.50, 1H, d, $J = 8.0$ Hz, H-1′; 4.32, 1H, d, $J = 7.5$ Hz, H-1′′) revealed the presence of two glucopyranosyl moieties (Yu et al. 2010). In the HMBC spectrum (Fig. S5), the H-1′′ resonance was long-range coupled with C-6′ and H-1′ coupled with C-1, which showed the (6-*O*-glucopyranosyl)-glucopyranosyl sugar moiety bonded to C-1 in the (*E*)-2-hexenoyl moiety. Sugar identification using HPLC indicated that the two glucosyl moieties were D-glucose (Tanaka et al. 2007), and the coupling constants $J = 7.0$ and 8.0 Hz showed their β-orientation. From these data, compound **1** was therefore established as 6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl (*E*)-hex-2-enoate (Figure 1).

Compounds **1–6** showed active (<50 μM) cytotoxicity against HT29 cancer cells with IC_{50} values ranging from 1.97 to 32.85 μM (Table S1) with reference to etoposide (IC_{50} 1.19 μM). The cytotoxicity of the new hexenoic acid glycoside (**1**) can be linked to the presence of (6-*O*-glucopyranosyl)-glucopyranosyl sugar moiety in its structure. Although the individual effects of 2-hexenoyl group and two glucopyranosyl moieties of the new compound against colorectal cancer cells are unknown, previous studies of some compounds containing two glucopyranosyl moieties reported to possess cytotoxicity against HL-60 and JB6 cell lines (Liu et al. 2001; Akihisa et al. 2012) while a recent study indicated no cytotoxic effect of a compound with 2-hexenoyl group (Di et al. 2017). The structurally related cytotoxic effects of the isolated flavonoids (**2–4**) can be attributed to the presence of a double bond between C-2 and C-3 in ring C, and the hydroxy moieties at the C-3′ and C-4′ positions on the B-ring (Chang et al. 2008; Jaramillo-Carmona et al. 2014). Besides, previous studies reported that rutin found to display selective cytotoxicity against CHME, Neuro-2a, U-118, LN-229, and SK-N-SH cancer cells with IC_{50} values between 15-20 μM (Yan et al. 2019) and isoquercitrin

showed cytotoxicity on T24 cancer cells with IC_{50} values between 20-80 μM (Wu et al. 2017). Similarly, the cytotoxic activities were previously reported for asperuloside with IC_{50} values of 16.5 μM on HL-60 and 26.9 μM on HCT-15 (Wang et al. 2018) and (6*S*, 9*R*)-roseoside indicated an IC_{50} value of 21.3 μM and 44.2 μM on HepG2 and MCF7, respectively (Fan et al. 2017).

3. Experimental part

Detailed general experimental procedures, plant material, extraction and isolation, sugar identification and cytotoxicity testing are available as Supplementary Material.

3.1. 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl (E)-hex-2-enoate (1)

Colorless amorphous powder, $[\alpha]^{25}_{D}$ − 13.3 (*c* 0.12, MeOH); HRFABMS *m/z* $[M + Na]^+$ 461.1636 (Calcd for $C_{18}H_{30}O_{12}Na$, 461.1634); ^{1}H NMR (400 MHz, CD_3OD) δ 7.10 (1H, dt, *J* = 16.0, 7.0 Hz, H-3), 5.90 (1H, dt. *J* = 16.0, 1.0 Hz, H-2), 5.50 (1H, d, *J* = 8.0 Hz, H-1′), 4.32 (1H, d, *J* = 7.5 H, H-1″), 4.15 (1H, dd, *J* = 11.0, 2.0 Hz, H-6′), 3.84 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″), 3.76 (1H, dd, *J* = 11.0, 5.0 Hz, H-6′), 3.66 (1H, dd, *J* = 12.0, 5.5 Hz, H-6″), 3.55 (1H, m, H-5′), 3.43 (overlapping, H-4′ and H-3″), 3.25-3.40 (overlapping, H-2′, H-3′, H-4″, and H-5″), 3.20 (1H, dd, *J* = 9.0, 7.5 Hz, H-2″), 2.23 (2H, m, H_2-4), 1.52 (2H, m, H_2-5), 0.96 (3H, t, *J* = 7.0 Hz, H_3-6); ^{13}C NMR (100 MHz, CD_3OD) δ 166.7 (C-1), 152.9 (C-3), 121.7 (C-2), 104.6 (C-1″), 95.7 (C-1′), 78.0 (C-3″), 78.0 (C-5″), 77.9 (C-3′), 77.8 (C-5′), 75.1 (C-2″), 73.9 (C-2′), 71.5 (C-4″), 71.0 (C-4′), 69.5 (C-6′), 62.7 (C-6″), 35.4 (C-4), 22.4 (C-5), 14.0 (C-6).

4. Conclusion

A new hexenoic acid glycoside, together with other known compounds, quercetin 3-*O*-rutinoside, quercetin-3-*O*-glucopyranoside, kaempferol 3-*O*-*β*-D-apiosyl (1→2)-*β*-D-glucopyranoside, asperuloside, (6*S*, 9*R*)-roseoside, and tryptophan were isolated from the leaves of *P. luzoniensis*. The new compound showed active cytotoxicity against HT29 cell line with IC_{50} of 3.17 μM, hence a potential anti-cancer lead compound.

Disclosure statement

No potential conflict of interest was reported by the authors.

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