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CHEMICAL CONSTITUENTS FROM AERIAL PARTS OF *POLYALTHIA EVECTA* (PIERRE) FINET & GAGNEP. VAR. *ATTOPEUENSIS*

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ABSTRACT

The first phytochemical investigation from aerial parts of *Polyalthia evecta* (Pierre) Finet & Gagnep. var. *attopeuensis* led to the isolation of six compounds, including two triterpenoids; stigmasterol (**1**) and β-sitosterol (**2**), two aporphine alkaloids; oxostephanine (**3**) and dicentrinone (**4**), one diureide of glyoxylic acid; allantoin (**5**) and one styryl lactone; goniothalamin (**6**). Characterization of all compounds was carried out by extensive spectroscopic analysis and comparison with literature. All compounds were previously isolated from *Polyalthia*, excepting dicentrinone (**4**) and goniothalamin (**6**) that are being reported for the first time from this genus.

Key words: Annonaceae, *Polyalthia evecta* var. *attopeuensis*, Triterpenoids, Aporphine alkaloids, Diureide of glyoxylic acid.

INTRODUCTION

Polyalthia is a genus of flowering plants in family annonaceae, which consists about 120 species of shrubs and trees. This genus is extensively distributed in tropical and subtropical areas[1]. Previous chemical investigations of this genus showed that some

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aporphines[2-7], tetrahydroprotoberberines[4,8], sesquiterpenylindoles[9,10], isoquinolines[7,11], flavonoids[12], azafluorenes[13], terpenes[14], clerodane diterpenoids[15-18], furans[19], acetogenins[20], prenylated benzopyrans[21-22], polyacetylenes[23], styryl-lactones[24], chalcones[1], polyphenolics[25] and other chemical constituents reported from *Polyalthia* genus review[26]. Many constituents have cytotoxic[27,28], antifungal, antiviral[19,29], antimicrobial[19,30,31], antimalarial[6,7], anti-HIV[32], antibacterial[33], anticancer activity[19,25,34,35] from various *Polyalthia* species. In addition, it is widely used in traditional medicine in many tropical countries such as in India[26], Thailand[23] and Malaysia[1]. Due to the tremendous uses in medicinal applications of *Polyalthia*, this research proposal aims to investigate the chemical constituents of *P. evecta* var. *attopeuensis*, as no research work has been published on this variety till now.

EXPERIMENTAL

Materials and methods

Plant material

P. evecta var. *attopeuensis* is called "Khamhom" in Thai. The aerial parts of this plant were collected in Sakon Nakhon province and identified by Narong Nuntasaen. A voucher specimen (BKF No. 137701) has been deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Extraction and isolation

The air-dried powdered of *P. evecta* var. *attopeuensis* (1.5 kg) were successively percolated with hexane (3 L x 3 days x 4 times) and then extracted with EtOAc (3 L x 3 days x 4 times) and MeOH (3 L x 3 days x 4 times) at room temperature, respectively and followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to afford hexane, ethyl acetate and methanol extracts as 32.23, 29.83 and 69.95 g, respectively.

The hexane extract was separated by column chromatography (CC) over silica gel, eluted with various proportions of EtOAc:*n*-hexane (0:100 to 100:0), followed by the increasing amount of MeOH in EtOAc (0:100 to 100:0). Fractions were collected and combined on the basis of TLC behavior. The solvents were evaporated to dryness to afford eight fractions (F_1–F_3). Fraction F_2 (7.62 g) was eluted by EtOAc:*n*-hexane (5:95). Fractions were collected and combined, then solvent were removed under reduced pressure to afford subfraction A_1–A_3. Further, the subfraction A_3 (3.59 g) was separated by CC over silica gel

(*n*-hexane) and then recrystallized with 95% EtOH to give a mixture of compounds **1** and **2** (317.10 mg).

The EtOAc extract was separated by CC over silica gel. Gradient elution was conducted initially with *n*-hexane, gradually enriched with EtOAc (0:100 to 100:0), followed by increasing amount of MeOH in EtOAc (0:100 to 100:0). Fractions were collected and combined on the basis of TLC characteristic. The solvents were evaporated to dryness to afford five fractions (F_1'–F_5'). Fraction F_2' (6.63 g) on elution by EtOAc:*n*-hexane (30:70) afford subfractions I_1–I_6. Further, the subfraction I_4 was chromatographed over silica gel eluted with MeOH:CH_2Cl_2 (5:95 to 10:90) to afford subfraction M_1–M_5. Subfraction M_3 (16.90 mg) and M_4 (1.90 mg) were purified by Sephadex LH-20 column (MeOH) to yield compounds **6** (6.00 mg) and **3** (5.10 mg), respectively.

The methanol extract was fractionated to CC on silica gel eluted with gradient EtOAc:*n*-hexane (80:20 to 100:0) and gradually enriched with MeOH:EtOAc (0:100 to 100:0) to afford four fractions (F_1''–F_4''). Fraction F_3'' (12.60 g) was recrystallized with 95% EtOH to yield compound **5** (19.80 mg). The residue of fraction F_3'' was subjected to silica gel CC eluted with gradient MeOH:CH_2Cl_2 (0:100 to 100:0) to obtain subfractions L_1–L_4. Subfractions L_2 (69.20 mg) was separated by Sephadex LH-20 column (MeOH) to afford subfractions P_1–P_2. Then the subfractions P_2 (37.30 mg) was separated by Sephadex LH-20 column (MeOH) to afford subfractions Q_1–Q_3. Subfraction Q_2 (12.60 mg) was rechromatographed over Sephadex LH-20 column (MeOH) to obtain subfractions R_1–R_3. Finally R_3 (12.00 mg) was recrystallized with MeOH: CH_2Cl_2 (80:20) to yield compound **4** (15.60 mg). Subfractions L_3 (115.00 mg) was purified by preparative TLC on silica gel plate, eluted with MeOH:CH_2Cl_2 (2:98) to give compound **3** (9.30 mg).

RESULTS AND DISCUSSION

Phytochemical study

P. evecta var. *attopeuensis* were extracted, isolated, purified and identified to obtain two triterpenoids **(1-2)**, two aporphine alkaloids **(3-4)**, one diureide of glyoxylic acid **(5)** and one styryl lactone **(6)**. The structures of compounds were elucidated by spectroscopic techniques (^{1}H, ^{13}C, 2D-NMR and MS) and comparison with the previously literature data[36-40] (Fig. 1).

In this investigation, all the compounds except for compound **4** and **6** were isolated from *Polyalthia* genus. The steroid mixtures of compound **1** and **2** were revealed recently from *P. rumphii*[41]. Compound **3** was previously reported from *P. cauliflora* var. *beccarii*, *P.*

suaveolens, *P. stenopetala*, *P. suberosa*, *P. insignis*[2,8,26], *P. rumphii*[42]. Compound **5** was displayed from *P. longifolia* var. *pendula*[18,30], *P. sclerophylla*[41].

Structure elucidation and identification

Mixture of stigmasterol (1) and β-sitosterol (2)

White plates. **(1)** $C_{29}H_{48}O$ (*m/z* 412), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.36 (1H, m, H-6), 5.15 (1H, dd, *J* = 15.1, 8.6 Hz, H-23), 5.02 (1H, dd, *J* = 15.1, 8.7 Hz, H-22), 3.53 (1H, m, H-3), 1.02 (3H, d, *J* = 6.5 Hz, H-21), 1.01 (3H, s, H-19), 0.86 (3H, d, *J* = 6.8 Hz, H-26), 0.81 (3H, d, *J* = 7.6 Hz, H-29), 0.80 (3H, d, *J* = 6.2 Hz, H-27), 0.70 (3H, s, H-18). **(2)** $C_{29}H_{50}O$ (*m/z* 414), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.36 (1H, m, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 6.4 Hz, H-21), 0.85 (3H, d, *J* = 7.6 Hz, H-29), 0.84 (3H, d, *J* = 6.5 Hz, H-26), 0.81 (3H, d, *J* = 6.2 Hz, H-27), 0.68 (3H, s, H-18) (Lit.[36]).

Oxostephanine (3)

Yellow powder. $C_{18}H_{11}NO_4$ (*m/z* 305), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (1H, d, *J* = 5.2 Hz, H-5), 8.21 (1H, d, *J* = 8.1 Hz, H-11), 7.62 (1H, d, *J* = 5.2 Hz, H-4), 7.56 (1H, t, *J* = 8.3 Hz, H-10), 7.08 (1H, s, H-3), 7.02 (1H, d, *J* = 8.4 Hz, H-9), 6.27 (2H, s, H-12), 3.98 (3H, s, 8-OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.74 (C-7), 161.91 (C-8), 151.71 (C-2), 147.58 (C-1), 146.72 (C-3a), 144.84 (C-5), 135.33 (C-6a), 135.23 (C-7a), 134.59 (C-10), 123.29 (C-4), 122.13 (C-1b), 120.88 (C-1a), 119.74 (C-11), 112.35 (C-9), 108.76 (C-11a), 103.11 (C-3), 102.19 (C-12), 56.44 (8-OCH_3) (Lit.[37]).

Dicentrinone (4)

Orange needles. $C_{19}H_{13}NO_5$ (*m/z* 335), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.87 (1H, d, *J* = 5.2 Hz, H-5), 7.96 (1H, s, H-11), 7.95 (1H, s, H-8), 7.73 (1H, d, *J* = 5.2 Hz, H-4), 7.11 (1H, s, H-3), 6.36 (2H, s, H-12), 4.08 (3H, s, 10-OCH_3), 3.99 (3H, s, 9-OCH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 181.23 (C-7), 153.87 (C-10), 151.55 (C-1), 149.53 (C-9), 147.02 (C-2), 145.48 (C-6a), 144.79 (C-5), 135.55 (C-3a), 127.75 (C-7a), 125.93 (C-11a), 123.96 (C-4), 122.64 (C-1b), 109.61 (C-11), 108.85 (C-8), 108.34 (C-1a), 102.76 (C-3), 102.37 (C-12), 56.27 (10-OCH_3), 56.14 (9-OCH_3) (Lit.[38]).

Allantoin (5)

White crystals. $C_4H_6N_4O_3$ (*m/z* 158), ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.54 (1H, s, NH-1), 8.05 (1H, s, NH-3), 6.88 (1H, d, *J* = 8.2 Hz, NH-6), 5.78 (2H, s, NH-8), 5.23 (1H, d, *J* = 8.2 Hz, H-4). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.63 (C-5), 157.39 (C-7), 156.80 (C-2), 62.44 (C-4) (Lit.[39]).

Goniothalamin (6)

White crystals. $C_{13}H_{12}O_2$ (*m/z* 200), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40 (1H, d, *J* = 7.4 Hz, H-11, 13), 7.34 (1H, t, *J* = 7.5 Hz, H-10, 14), 7.29 (1H, d, *J* = 7.2 Hz, H-12), 6.93 (1H, dt, *J* = 9.8, 4.3 Hz, H-4), 6.74 (1H, d, *J* = 16.0 Hz, H-8), 6.28 (1H, dd, *J* = 16.0, 6.4 Hz, H-7), 6.10 (1H, dt, *J* = 9.8, 1.7 Hz, H-3), 5.11 (1H, dd, *J* = 14.2, 6.6 Hz, H-6), 2.55 (2H, m, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.79 (C-2), 144.44 (C-4), 135.81 (C-9), 133.17 (C-8), 128.69 (C-11, 13), 128.36 (C-12), 126.71 (C-10, 14), 125.70 (C-7), 121.77 (C-3), 77.91 (C-6), 29.92 (C-5) (Lit.[40]).

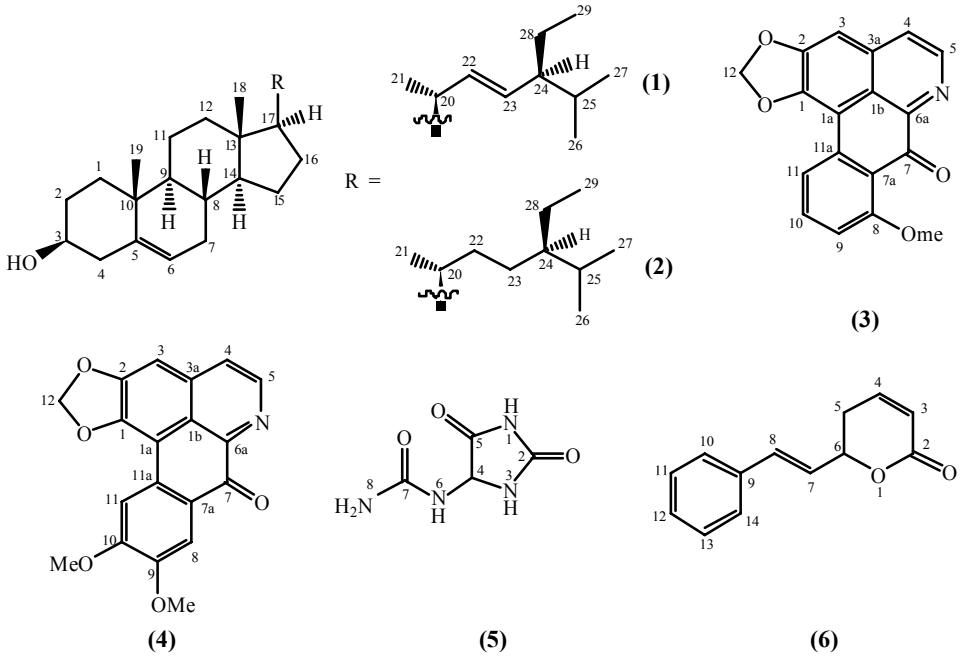

Fig. 1: Chemical structure of the isolated compounds from *P. evecta* var. *attopeuensis*

To the best of our knowledge, this is the first report of oxostephanine **(3)**, dicentrinone **(4)**, allantoin **(5)** and goniothalamin **(6)** from *P. evecta*. In addition, this is the first isolation of dicentrinone **(4)** and goniothalamin **(6)** from *Polyalthia* genus. Consequently, these compounds perhaps serve as potential chemotaxonomic markers for *P. evecta* var. *attopeuensis* and may be used to discriminate among varieties of *P. evecta*.

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