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Isolation of Bioactive Metabolites from Root Extracts of *Polyalthia Debilis*

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Abstract

Bioactive azafluorenone alkaloids from *Polyalthia debilis* were previously reported. Herein, bioactive root extracts of *P. debilis* (hexane and methanol) with antimalarial, antimicrobial and cytotoxic activities were further explored. Upon repeated chromatographic isolations of the extracts gave triterpenes; 3-acetylaleuritolic acid (**1**), suberosol (**4**) and stigmasterol (**2**) together with a mixture of stigmasterol and β-sitosterol (**3**) including many fractions of triterpenes mixture. Compounds **1** and **4** were isolated from the *P. debilis* for the first time. The sterol **3** exhibited antifungal action against *Saccharomyces cerevisiae* ATCC 2601 with a minimum inhibitory concentration of 64 μg/mL. Some of the isolated fractions were also shown to be antifungals. The results provide medicinal applications of the plant species.

Keywords: *Polyalthia debilis*, tetra and pentacyclic triterpenoids, antimicrobial, antimalarial, cytotoxic activities.

1. Introduction

The previous work of *Polyalthia debilis* (Annonaceae) reported the isolation of bioactive azafluorenone alkaloids for the first time together with a mixture of sterols from chloroform and ethyl acetate extracts (Prachayasittikul et al., 2009c). It is of interest to further investigate on the plant extracts obtained from different polarity which might give a diverse group of compounds with bioactivities. In this study we reveal the bioactive metabolites from nonpolar hexane and polar methanol extracts of the *P. debilis* as well as their antimicrobial, antimalarial and cytotoxic activities.

2. Materials and Methods

2.1. General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 NMR spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C). Infrared spectra (IR) were obtained on a Perkin Elmer System 2000 FTIR. Mass spectra were recorded on a Finnigan INCOS 50 and Bruker Daltonics (micro TOF). Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Analytical thin layer chromatography (TLC) was performed on silica gel 60 PF_{254} aluminium sheets (cat. No. 7747 E., Merck). Solvents were distilled prior to use. Reagents for cell culture and assays were of analytical grade as the following: RPMI-1640 (Rosewell Park Memorial Institute medium, Gibco and Hyclone laboratories, USA), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), L-glutamine, penicillin, streptomycin, sodium pyruvate and glucose (Sigma, USA), Ham's/F12 (Nutrient mixture F-12), DMEM (Dulbecco's Modified Eagle's Medium) and FBS (fetal bovine serum, Hyclone laboratories, USA), gentamicin sulfate (Government Pharmaceutical Organization, Thailand), MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, USA).

2.2. Isolation

Plant (*P. debilis*) extracts obtained from 6 kg of dried root were separated by silica gel column and eluted by gradient solution with increasing polarity. Hexane extract (PDH, 36 g) was separated by silica gel (1000 g) and eluted by hexane : EtOAc and EtOAc : MeOH to give 8 fractions; H1 (3.90 g of yellow oil), H2 (2.34 g of yellow solid), H3 (4.80 g of browny gum), H4 (2.84 g of yellow-green oil) and dark brown waxes of H5 (9.22 g), H6 (1.91 g), H7 (2.10 g) and H8 (2.16 g). Fraction H2 (2.34 g from hexane:EtOAc, 8:2 elution) was separated on silica gel (80 g), eluting by hexane:CH_2Cl_2 to give 4 fractions of yellow oils; H2.1 (119.8 mg), H2.2 (118.0 mg from Hexane:CH_2Cl_2, 7:3 elution) H2.3 (338.4 mg) and H2.4 (1.02 g). The fraction H2.2 (118.0 mg) was further isolated by preparative TLC eluted by hexane:CH_2Cl_2, 7:3 to afford yellow oils of H2.2.1 (18.26 mg) and H2.2.3 (13.98 mg) and H2.2.2 (37.80 mg) as pale yellow solid. Recrystallization of H2.2.2 from methanol gave white solid (3.20 mg) of compound **1**; (3-*β*-*O*-acetyltaraxer-14-en-28-oic acid or 3-acetylaleuritolic acid) mp 299-302^{o}C (lit mp 299-300^{o}C, Prachayasittikul et al., 2009a); IR(KBr): υ_{max} 3434, 2935, 2868, 1734, 1686, 1466, 1364, 1242, 1026 cm^{-1}; ^{1}H NMR($CDCl_3$): δ 0.78(s, 3H H-25), 0.81(s, 3H, H-27), 0.86(s, 3H, H-24), 1.18(s, 3H, H-26), 2.05(s, 3H, CH_3COO), 4.46(dd, 1H, H-3, J = 5.04, 9.82 Hz), 5.52(dd, 1H, H-15, J = 3.15, 7.77H); ^{13}C NMR($CDCl_3$): δ 37.47(C-1), 23.46(C-2), 80.89(C-3), 37.68(C-4), 55.59 (C-5), 18.71(C-6), 40.78(C-7), 39.02(C-8), 49.06(C-9), 37.92(C-10), 17.31(C-11), 33.66(C-12), 37.34(C-13), 160.59(C-14), 116.76(C-15), 31.36(C-16), 51.38(C-17), 41.47(C-18), 35.33(C-19), 29.29(C-20), 33.32(C-21), 30.72(C-22), 27.96(C-23), 16.59(C-24), 15.62(C-25), 26.15(C-26), 22.42(C-27), 183.23(C-28), 31.89(C-29), 28.68(C-30), 21.30(CH_3), 171.00(CO). LRMS(EI): *m/z* (%) = 498(0.2)$[M]^{+}$, 438(4), 344(5), 329(8), 269(9), 248(36), 234(90), 190(112), 189(100).

Fraction H4 (2.80 mg) was placed on silica gel (100 g) eluting by hexane:CH_2Cl_2 and CH_2Cl_2:MeOH to yield 7 fractions of yellow and brown waxes; H4.1 (30.0 mg), H4.3 (130.1 mg),

H4.4 (68.1 mg), H4.5 (1.05 g), H4.6 (31.0 mg) and H4.7 (295.6 mg) and pale yellow solid of H4.2 (391.1 mg). Recrystallization of H4.2 (of hexane:CH_2Cl_2, 7:3 elution) from methanol gave solid (269.70 mg) of compound **2** (stigmasterol); mp 147-149^oC (lit mp 152-153^oC, Prachayasittikul et al., 2008); IR(KBr): υ_{max} 3435, 2937, 1654, 1459, 1388, 1054, 970 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.68-1.02 (m, 18H, 6xCH_3), 1.02-2.32 (m, 25H, 9xCH_2, 7xCH), 3.49-3.55 (m, 1H, CH-OH), 4.49-5.18 (m, 2H, CH=CH), 5.35(br s, 1H, CH=C); ^{13}C NMR($CDCl_3$) : δ 37.02(C-1), 31.62(C-2), 71.78(C-3), 42.26(C-4), 140.72(C-5), 121.69(C-6), 31.87(C-7), 31.87(C-8), 50.19(C-9), 36.48(C-10), 21.05(C-11), 39.74(C-12), 42.26(C-13), 56.84(C-14), 24.34(C-15), 28.90(C-16), 56.02(C-17), 12.02(C-18), 19.38(C-19), 40.48(C-20), 21.05(C-21), 138.30(C-22), 129.24(C-23), 51.21(C-24), 31.87(C-25), 21.20(C-26), 19.01(C-27), 25.39(C-28), 12.23(C-29). LRMS(EI): *m/z* (%) = 412(1.57)$[M]^+$, 273(29.89), 271(43.31), 255(65.40), 231(43.25), 213(69.37), 185(33.86), 159(100).

Methanol extract (PDM, 110 g) was placed onto silica gel (150 g) eluting by $CHCl_3$: MeOH to give 12 fractions; M1 (122.6 mg of yellow oil) and dark brown gum of M2 (82.20 mg), M3 (3.03 g), M4 (832.20 mg), M5 (3.85 g), M6 (2.52 g), M7 (19.08 g), M8 (11.10 g), M9 (6.53 g), M10 (9.61 g), M11 (18.82 g) and M12 (21.94 g). Fraction M5 (3.85 g from $CHCl_3$:MeOH, 9:1) was reseparated on silica gel (135 g) and eluted by $CHCl_3$:acetone, acetone, acetone:MeOH to afford 15 fractions as brown gum of M5.1 (17.60 mg), M5.2 (14.80 mg), M5.4 (18.5 mg), M5.5 (2.59 mg), M5.7 (43.5 mg), M5.8 (154.8 mg), M5.9 (221.3 mg), M5.10 (3.7 mg), M5.11 (187.5 mg), M5.12 (79.4 mg), M5.13 (69.50 mg), M5.14 (24.10 mg) and M5.15 (39.5 mg) and as light brown solid of M5.3 (60.0 mg) and M5.6 (112 mg). Fraction M5.3 (from $CHCl_3$:acetone, 9:1) was recrystallized from methanol to provide white solid (7.2 mg) of compound **3** (a mixture of stigmasterol and *β*-sitosterol); mp 135-137^oC, IR(KBr): υ_{max} 3422, 2936, 2868, 1654, 1465, 1383, 1054, 959, 800 cm^{-1}; ^1H NMR($CDCl_3$): δ 0.69-1.02 (m, 18H, 6xCH_3), 1.06-1.73 (m, 22H, 11xCH_2): 1.88-2.39 (m, 7H, 7xCH), 3.50-3.75 (m, 1H, H-3; CH-OH), 4.49-4.18 (m, 2H, H-22, H-23), 5.35 (br s, 1H, H-6; C=C); ^{13}C NMR ($CDCl_3$): δ 37.20 (C-1), 31.61(C-2, C-7, C-8), 71.77(C-3), 42.25(C-4, C-13), 140.70(C-5), 121.68(C-6), 50.08(CI-9, CI-24), 51.19 (CII-9), 36.10(CI-10), 36.10(CII-10, CII-20), 21.17(C-11, C-26), 39.72(C-12), 56.72(C-14), 24.32(CI-15), 24.25(CII-15), 28.88(CI-16), 28.20(CII-16), 56.00(C-17), 12.00(CI-18), 12.00(CII-18, CII-29), 12.21(CI-29), 19.35(C-19, C-27), 40.25(CI-20), 21.17(CI-21), 18.73(CII-21), 138.28(CI-22), 33.89(CII-22), 129.22(CI-23), 26.01(CII-23), 45.78(CII-24), 31.85(CI-25), 129.09(CII-25), 25.36 (CI-28), 23.04(CII-28), 12.21(CI-29), CI is stigmasterol where CII is *β*-sitosterol. LRMS(EI): *m/z* (%) = 414(100)$[M]^+$, 412(79.45)$[M]^+$, 399(31.42), 396(48.12), 273(38.89), 271(38.24), 255(65.80), 231(62.18), 213(72.24). Solid of M5.6(eluted by hexane:acetone, 6:4) was rechromatographed on silica gel (12 g) and eluted by hexane:acetone, acetone:MeOH to give 7 fractions as dark gum; M5.6.1 (19.8 mg), M5.6.2 (5.4 mg), M5.6.3 (25.2 mg), M5.6.5 (18.0 mg), M5.6.6 (9.6 mg) and M5.6.7 (11.5 mg) and fraction M5.6.4 (21.0 mg) as pale yellow solid which was further purified by preparative TLC. Elution by hexane:acetone, 7:3 gave 2 bands of M5.6.4.1 (4.30 mg as white solid) and M5.6.4.2 (0.79 mg as yellow oil). The M5.6.4.1 was compound **4** (suberosol or 24-methylenelanosta-7,9(11)-dien-3*β*,15*α*-diol); mp 139-142^oC (lit mp 144-146^oC, Lue et al., 1998, lit mp 179-182^oC, Li et al., 1993); IR(KBr): υ_{max} 3614, 3448, 3038, 2932, 1640, 1466, 1376, 981, 909, 892; ^1H NMR($CDCl_3$): δ 0.61 (s, 3H, H-18), 0.88(s, 3H, H-29), 0.90(s, 3H, H-21, J=6.46 Hz), 0.94(s, 3H, H-28), 0.97(s, 3H, H-19), 1.00(s, 3H, H-30), 1.02(d, 3H, H-26, J = 6.84 Hz), 1.03(d, 3H, H-27, J = 6.84 Hz), 1.10(dd, 1H, H-5, J = 3.78, 11.79 Hz), 1.64-1.68(m, 1H, H-17), 2.06(dd, 1H, J = 6.94, 17.74 Hz, H-12), 2.30(d, 1H, J = 17.48 Hz, H-12), 3.25(dd, 1H, H-3, J = 4.50, 11.29 Hz), 4.28(dd, 1H, H-15, J = 5.70, 9.70 Hz), 4.65, 4.72(2s, 2x1H, H-31), 5.13(d, 1H, H-11, J = 6.23 Hz), 5.85(d, 1H, H-7, J = 6.59 Hz); ^{13}C NMR($CDCl_3$): δ 35.68(C-1), 27.72(C-2), 78.85(C-3), 38.62(C-4), 48.90(C-5), 22.89(C-6), 127.27 (C-7), 140.90(C-8), 146.05(C-9), 37.37(C-10), 116.01(C-11), 38.45(C-12), 44.80(C-13), 52.00(C-14), 74.70(C-15), 40.06(C-16), 48.81(C-17), 15.87(C-18), 22.81(C-19), 35.95(C-20), 18.41(C-21), 34.81(C-22), 31.16(C-23), 156.52(C-24), 33.75(C-25), 21.95(C-26), 21.81(C-27), 17.10(C-28),

28.10(C-29), 15.76(C-30), 106.09(C-31); LRMS(EI): *m/z*(%) = 454(100)$[M]^+$, 421(28), 327(95), 286(43), 273(23), 239(25), 187(26), 185(29), 171(29), 159(27).

2.5. Antimicrobial Assay

Antimicrobial activity of the tested compounds was carried out using agar dilution method (Prachayasittikul et al., 2009b). Briefly, the tested compounds dissolved in DMSO were individually mixed with 1 mL Müller Hinton (MH) broth while the negative control was the MH broth without the tested compounds. The solution was then transferred to the MH agar solution to yield the final concentrations of 32-900 μg/mL. Nineteen strains of microorganisms, cultured in MH broth at 37°C for 24 h, were diluted with 0.9% normal saline solution to adjust the cell density of 3×10^9 cell/mL. The organisms were inoculated onto each plate and further incubated at 37°C for 18-48 h. Compounds which displayed high efficacy to inhibit bacterial cell growth were examined.

2.6. Antimalarial Assay

Antimalarial activity of the tested compounds was evaluated against chloroquine resistant *Plasmodium falciparum* (T9.94) using the literature method (Satayavivad et al., 2004). Human erythrocytes (type O) infected with chloroquine resistant *P. falciparum* (T9.94) were maintained in continuous culture, according to the method described previously (Trager and Jensen, 1976). RPMI-1640 culture medium supplemented with 25 mM of HEPES, 40 mg/L gentamicin sulfate and 10 mL of human serum was used in continuous culture. Before performing the experiment, *P. falciparum* culture was synchronized by using sorbitol induced hemolysis according to the literature method (Lambros and Vanderberg, 1979) to obtain only ring stage-infected red blood cells and then incubated for 48 h prior to the drug testing to avoid effect of sorbitol.

The experiments were started with synchronized suspension of 0.5% to 1% infected red blood cell during ring stage. Parasites were suspended with culture medium supplemented with 15% human serum to obtain 10% cell suspension. The parasite suspension was put into 96-well microculture plate; 50 μL in each well and then added 50 μL of various tested drug concentrations. These parasite suspensions were incubated for 48 h in the atmosphere of 5% CO_2 at 37°C. The percents parasitemia of control and tested compounds were analyzed by microscopic technique using methanol-fixed Giemsa stained of thin smear blood preparation. The efficacy of the drugs were evaluated by determining the drug concentration that reduced the growth of parasite by 50% (IC_{50}).

2.7. Cytotoxic Assay

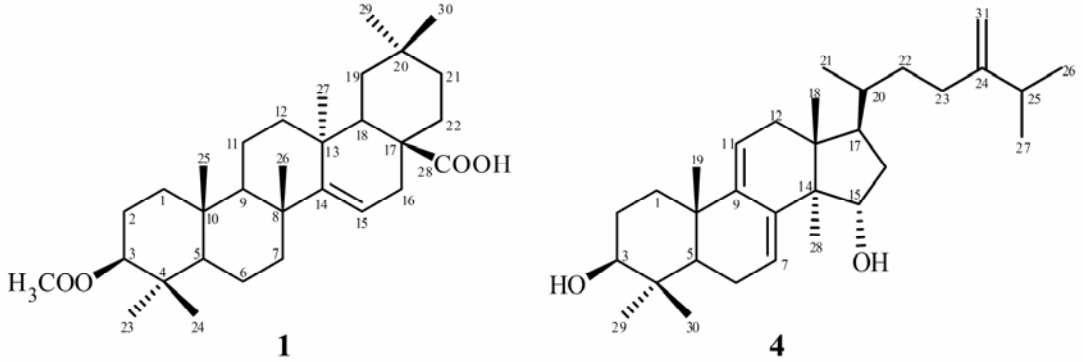
Cells were grown in Ham's/F12 medium containing 2 mM L-glutamine supplemented with 100 U/mL penicillin, streptomycin and 10% FBS. Except HepG2 cell was grown in DMEM. Cytotoxic assay was performed using the modified method as previously described (Tengchaisri et al., 1998). In brief, cell lines suspended in RPMI-1640 containing 10% FBS were seeded at 1×10^4 cells (100 μL) per well in 96-well plate and incubated in humidified atmosphere, 95% air, 5% CO_2 at 37°C. After 24h, additional medium (100 μL) containing the tested compound and vehicle was added to a final concentration of 50 μg/mL, 0.2% DMSO, and further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which absorbance was measured at 550 nm. Whereas A549 and HepG2 cells were stained by MTT. IC_{50} values were determined as the drug and sample concentrations at 50% inhibition of the cell growth.

3. Results and Disscussion

3.1. Isolation

Roots hexane (PDH) and methanol (PDM) extracts of *P. debilis* were screened for antimicrobial, antimalarial and cytotoxic activities. Extensive isolation and purification of the two extracts were performed by repeated silica gel column and preparative TLC using gradient elution with increasing polarity. The nonpolar extract (PDH) gave two isolates; 3-*O*- acetylaleuritolic acid (**1**, 3.20 mg) from fraction H2.2.2 and stigmasterol (**2**, 269.70 mg from fraction H 4.2). The polar extract (PDM) afforded a mixture of stigmasterol and *β*–sitosterol (**3**, 7.20 mg from fraction M5.3) and 24-methylenelanosta–7,9(11)–dien-3*β*,15*α*-diol (**4**, 4.30 mg from fraction M5.6.4.1). Structures of these triterpenoids (**1**-**4**, Figure 1) were identified by comparison of their spectral data; IR, ^{1}H and ^{13}C NMR with the literature reported. 2D-NMR; COSY, HMQC, HMBC, DEPT 90 and DEPT 135 were also studied. The triterpenes **1** and **4** were isolated from the *P. debilis* for the first time. Previously, **1** was isolated from other families like Euphorbiaceae e.g. *Phytolacca americana* (Woo and Wagner, 1977), *Croton urucurana* Baillon (Peres et al., 1997), *Maprounea africana* (Pengsuparp et al., 1994) and *Alerurites montana* (Misra and Khastgir, 1970) and recently from Compositae; *Spilanthes acmella* Murr. (Prachayasittikul et al., 2009a). The 24-methylenetetracyclic triterpene **4** was reported to be isolated from *P. lancilimba* (Lue et al., 1998) and *P. suberosa* (Li et al., 1993). The sterol **3** was previously isolated from chloroform and ethyl acetate extracts of the *P. debilis* (Prachayasittikul et al., 2009c).

Figure 1: Chemical structures and numbering of triterpenoids 1 and 4.



3.2. Antimicrobial activity

Two plant extracts (PDH and PDM), isolated fractions H2-H5, H7, H8 and M1-M12 including sterols **2** and **3** were tested for antimicrobial action. The results (Table 1) showed that some of the tested compounds exerted activity against gram positive bacteria and fungus. The PDH extract exhibited growth inhibition against *Streptococcus pyogenes* with minimum inhibitory concentration (MIC) of 64 μg/mL as well as *Bacillus subtilis* ATCC 6633 and *Corynebacterium diphtheriae* NCTC 10356 with MIC of 256 μg/mL. The fractions H3 and H7 containing a mixture of triterpenes displayed the activity against *Saccharomyces cerevisiae* ATCC 2601 with MIC of 256 and ≤ 900 μg/mL, respectively. The *S. cerevisiae* ATCC 2601 was also inhibited by the mixture of stigmasterol and *β*-sitosterol (**3**) with MIC of 64 μg/mL. Obviously, the sterol **3** and PDH extract showed the highest antimicrobial activity with MIC of 64 μg/mL against *S. cerevisiae* ATCC 2601 and *S. pyogenes*, respectively. Stigmasterol (**2**) was tested at 64 μg/mL, but found to be inactive. The same result (Prachayasittikul et al., 2009a) was noted for the acetylaleuritolic acid (**1**). No antimicrobial activity was observed for the PDM extract and fractions; H2, H4, H5, H8, M1-M12 when tested at 256 μg/mL. Unfortunately, the suberosol (**4**) was not tested due to its unavailable quantity. Triterpenoid **1** was reported, previously, to show

antimicrobial activity (Peres et al., 1997) and to inhibit vitality of *Onchocerca gutterosa* (Nyasse et al., 2006).

Table 1: Antimicrobial activity[a] of *Polyalthia debilis*.

Compound[b]	Microorganism	MIC[c] (μg/mL)
PDH	*B. subtilis* ATCC 6633,	256
	C. diphtheriae NCTC 10356	
	S. pyogenes	64
H3	*S. cerevisiae* ATCC 2601	256
H7	*S. cerevisiae* ATCC 2601	≤ 900
3	*S. cerevisiae* ATCC 2601	64

Note: Nineteen strains of microorganisms used for antimicrobial activity testing; **gram positive bacteria:** *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 33186, *Micrococcus lutens* ATCC 10240, *Corynebacterium diphtheriae* NCTC 10356, *Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes*; **gram negative bacteria:** *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Serratia marcescens* ATCC 8100, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas stutzeri* ATCC 17587, *Shewanella putrefaciens* ATCC 8071, *Achromobacter xylosoxidans* ATCC 2706 and **diploid fungus (yeast):** *Saccharomyces cerevisiae* ATCC 2601, *Candida albicans* ATCC 90028.

a: Ampicillin at 50 μg/mL was used as a control of the testing system; it showed 100% inhibition against *E. coli* ATCC 25922, *S. typhimurium* ATCC 13311, *P. stutzeri* ATCC 17587, *S. putrefaciens* ATCC 8071, *A. xylosoxidans* ATCC 2706, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 33186, *M. lutens* ATCC 10240, *B. subtilis* ATCC 6633, *C. diphtheriae* NCTC 10356, *S. pyogenes* and *S. cerevisiae* ATCC 2601.

b: Stigmasterol (**2**) was tested at 64 μg/mL, but found to be inactive; extract (PDM) and fractions H2, H4, H5, H8, M1-M12 were inactive to all the tested organisms at 256 μg/mL.

c: MIC is a minimum inhibitory concentration.

3.3. Antimalarial activity

The PDH and PDM extracts were tested against chloroquine resistant *P. falciparum* (T9.94). It was found that (Table 2) the nonpolar PDH extract showed good antimalarial activity with an IC_{50} of 10 - < 100 μg/mL , whereas fair activity was noted for the polar PDM extract (IC_{50} 100 - 1000 μg/mL). Moderate polar; chloroform and ethyl acetate extracts were also shown to be fair antimalarials (Prachayasittikul et al., 2009c).

Table 2: Antimalarial activity[a] of *Polyalthia debilis* extracts.

Extract	Activity	IC_{50} (μg/mL)[b]
PDH	good	10 - <100
PDM	Fair	100 – 1000

Note: a: Chloroquine hydrochloride was used as a reference drug.

b: IC_{50} : for the extract; 10 - <100 μg/mL denotes good activity; 100 – 1000 μg/mL for fair activity.

3.4. Cytotoxic activity

Cytotoxic tests were performed on the two extracts using three cancer cell lines; HepG2, A549 and HCC-S102. Only the nonpolar PDH extract showed cytotoxic activity against all the tested cell lines with the IC_{50} values ranging from 21.5-27.5 μg/mL. Similar activity was also noted for rather polar chloroform extract of the plant species (Prachayasittikul et al., 2009c). Previously, the triterpene **1** isolated from other plant species displayed strong cytotoxic activity against human lung carcinoma

A549 cells and strong inhibition of DNA topoisomerase II (Wada and Tanaka, 2006). In addition, the lanostane type triterpene **4** was reported to show anti-HIV replication activity in H9 lymphocyte cells with an EC_{50} of 3 μg/mL (Lue et al., 1998).

Table 3: Cytotoxic activity of *Polyalthia debilis* extracts.

Extract	IC_{50} (μg/mL)[a,b,c]		
	Hep G2	**A549**	**HCC-S102**
PDH	24.5±2.1	21.5±0.7	27.5±0.7
PDM	>50	>50	>50
Etoposide	0.20	0.34	0.32

Note: a: Cancer cell lines are HepG2 (human hepatocellular liver carcinoma cell line); A549 (human lung carcinoma cell line); HCC-S102 (hepatocellular carcinoma cell line).
b: When IC_{50} > 50 μg/mL denotes inactive cytotoxic activity.
c: The assays were performed in triplicate using etoposide as a reference drug.

4. Summary and Future perspectives

The hexane and methanol extracts of *P. debilis* were tested for their bioactivities and found that the nonpolar extract exhibited good antimalarial as well as antimicrobial and cytotoxic activities. The polar extract was also shown to be fair antimalarials. The two bioactive extracts were isolated and characterized by spectroscopic methods to give acetylaleuritolic acid **1**, suberosol **4**, stigmasterol **2** and a mixture of sterol **3** together with many fractions containing a mixture of triterpenes. In particular, the triterpenes **1** and **4** were not reported to be isolated from the *P. debilis*. Moreover, the compound **1** was found for the first time in the Annonaceae family. The sterol **3** showed antifungal action against *S. cerevisiae* ATCC 2601 with MIC of 64 μg/mL. The fractions H3 and H7 also exhibited the antifungal activity. The triterpene **1** when isolated from plants of other families exerted antimicrobial (Peres et al., 1997), antifilarial (Nyasse et al., 2006) and strong cytotoxic activities (Wada and Tanaka, 2006) including as a strong DNA topoisomerase II inhibitor (Wada and Tanaka, 2006). Similarly, the suberosol **4** was reported to show anti-HIV activity in H9 lymphocyte cells (Lue et al., 1998). The results provide potential medicinal applications of the plant species and suggest further bioactivity testings.

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