Extraction, Isolation and Biological activities of *Phanera nakhonphanomensis*

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Abstract

Five known compounds; (1) palmitic acid, (2) lupeol, (3) gallic acid, (4) *β*-sitosterol-3-*O*-tetra-*O*-acetyl-*β*-D-mannopyranose, and (5) methyl gallate, were isolated from the liana and leaves crude extracts of *Phanera nakhonphanomensis*. Their structures were elucidated by IR, NMR, and MS data. Gallic acid showed significant cytotoxic activities on against P-388, HT 29, A 549, and CL human cancer cell lines using SRB method with ED_{50} values of 2.57, 16.27, 11.42, and 12.23 μg/mL, respectively. The Anti-HIV-1RT activity of gallic acid, ethyl acetate extract, and methanol extract showed strong percentage inhibitory values of 91.50%, 92.08%, and 97.63%, respectively.

Keywords: *Phanera, liana, Palmitic acid, Lupeol, Gallic acid, β-Sitosterol-3-O-tetra-O-acetyl-β-D-mannopyranose, Methyl gallate*

1. Introduction

Phanera is a new liana species in the subfamily Caesalpinioideae (Leguminosae) (Chatan, 2013). This species has been distinguished from *Bauhinia* by molecular and morphological characters. The *rp12* intron is studied, it is found in chloroplast DNA of *Phanera* but not present from *Bauhinia*. The morphology characters of *Phanera* are investigated, such as habit, calyx, fertile stamens, distribution, and numbers of species, which are different from *Bauhinia* (Mackinder, & Clark, 2014). Many species in this genus have been transfers to *Phanera* such as *Phanera glabrifolia* (Bandyopadhyay, 2013), *Phanera glauca* (Kumar, Bandyopadhyay, & Sharma, 2014), *Phanera hekouensis* (Krishnaraj, 2014), *Phanera jampuiensis* (Bandyopadhyay, 2015; Darlong & Bhattacharyya, 2014), *Phanera larseniana* (Chantaranothai, Mattapha, & Wangwasit, 2017), *Phanera yunnansis* (Wunderlin, 2011), and *Phanera bracteata* (Udomputtimekakul et al., 2017). The pollen morphology of the genera *Bauhinia* and *Phanera* Caesalpinioideae (Leguminosae) are studied by light and scanning electron microscopy for support information (Udomputtimekakul et al., 2017). It is found that the sizes of the angulaperturate pollen grain are different from two species (Francisco, Danovan, & Luciano Paganucci, 2012). In 2014, *Phanera nakhonphanomensis* was distinguished from *Bauhinia* by Mackind A. B and Clark R. (Mackinder, & Clark, 2014). The phytochemical constituents and bioactivity of *P. nakhonphanomensis* are studied, and three major groups of compounds were found: Inositol, α-tocopherol, and phenol from the ethanolic leaves extract, which has a good antioxidant activity (Promprom, & Chatan, 2017).

In this research, the phytochemical investigation of the mixed liana and leaves *P. nakhonphanomensis* are studied. The structures elucidation are characterized by ^{1}H, ^{13}C-NMR spectroscopic data, mass spectroscopic data, and IR spectra.

2. Objectives

1. To investigate the chemical compositions from liana and leaves of *Phanera nakhonphanomensis.*
2. To test the anti-HIVs activity of the pure compounds from liana and leaves of *Phanera nakhonphanomensis.*

3. Materials and Methods

3.1 Plant material

P. nakhonphanomensis is a new species of tendrilled liana in the subfamily Caesalpinioideae (Leguminosae). The new species of plant was identified, and the voucher specimen (BKF no. 189123) has been deposited at the resident of Thailand famous only from Phulangkha National Park, Ban Pheang District, Nakhon Phanom Province, Thailand, and authenticated by Mr. Narong Nuntasaen. This species grows in evergreen forests that are dense at an altitude of 240-170 meters.

3.2 Extraction and Isolation

The air dried powdered of *P. nakhonphanomensis* (3.60 kg) was successively percolated with hexane for 8 times each (8 × 20 L) and then extracted with ethyl acetate for 8 times each (8 × 20 L) and methanol for 8 times each (8 × 20 L) at room temperature, respectively. The solvents of each extract were combined and evaporated to afford crude hexane extract, crude ethyl acetate extract and crude methanol extracts as 9.80 g, 220.61 g and 353.29 g, respectively.

The crude hexane extract was separated by column chromatography (CC) over silica gel eluted with gradient mixture of ethyl acetate: hexane, to give five fractions (HF_1-HF_5). HF_3 was recrystallized by 95% hexane in ethyl acetate to yield palmitic acid (**1**). HF_4 was separated over silica gel column (100% hexane) and then purified by recrystallization with 95% ethanol to give lupeol (**2**).

The crude ethyl acetate extract was chromatographed on silica gel column eluted with ethyl acetate: hexane, to obtain ten fractions (EF_1-EF_{10}). EF_6 was fractionated by CC (60% hexane in ethyl acetate), then recrystallized by ethanol: acetone (2: 1) to give gallic acid (**3**). EF_8 was obtained as light brown solid. It is used in reaction acetylation with acetic anhydride in the ratio 2: 1. Then, the reaction was worked up and the crystals were recrystallized by ethanol: acetone (2: 1), *β*-Sitosterol-3-*O*-tetra-*O*-acetyl-*β*-*D*-mannopyranose (**4**).

The crude methanol extract was separated by CC with gradient systems of ethyl acetate: hexane, methanol: ethyl acetate and methanol to give eight fractions (MF_1-MF_8). MF_4 and MF_5 were recolumn, eluted with 80% hexane in ethyl acetate, then recrystallized by methanol to afford methyl gallate (**5**).

The experimental data for isolated compounds **1-5** were as follows: palmitic acid **(1) –** White solid; Chemical Formula: $C_{19}H_{36}O_2$, MP: 52.1-53.0°C. EI-MS (*m/z*) 55 (49), 87 (100), 129 (29), 143 (16), 157 (20), 171 (24), 185 (26), 199 (12), 213 (12), 256 (31). IR (KBr) ν_{max}: 2922, 2845, 1701, 1472, 1294, 935, 719 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.34 (2H, t, *J*=7.5 Hz, H-2), 1.63 (2H, p, *J* = 7.5 Hz, H-3), 1.25 (2H, m, H-4 to H-15), 0.88 (3H, t, *J* = 6.9 Hz, H-16).^{13}C NMR ($CDCl_3$, 125 MHz): 179.91 (C=O, C-1), 34.17 (CH_2, C-2), 32.09 (CH_2, C-14), 29.85 (CH_2, C-13), 29.84 (CH_2, C-12), 29.83 (CH_2, C-11), 29.81 (CH_2, C-10), 29.80 (CH_2, C-9), 29.75 (CH_2, C-8), 29.59 (CH_2, C-7), 29.51 (CH_2, C-6), 29.40 (CH_2, C-5), 29.23 (CH_2, C-4), 24.86 (CH_2, C-3), 22.84 (CH_2, C-15), 14.25 (CH_3, C-16).

Lupeol **(2) –** White solid; Chemical Formula: $C_{30}H_{50}O$, MP: 203.1-205.2°C. EI-MS (*m/z*) 426.43 $[M+1]^+$ (18), 68 (11), 81 (12), 95 (24), 109 (22), 121 (18), 149 (20), 162 (17), 176 (16), 189 (33), 204 (32), 218 (45), 409 (100), 410 (33). IR (KBr) ν_{max}: 3325, 3067, 2945, 2872, 1638, 1462, 1379, 1042, 882 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.68 (1H, d, *J* = 2.5 Hz, 4.56 (1H, dq, *J* = 2.7, 1.4 Hz, H-29a), H-29b), 3.18 (1H, dd, *J* = 11.4, 4.9 Hz, H-3), 2.37 (1H, td, *J* = 11.0, 5.8 Hz, H-19), 1.94 (2H, m, H-21), 1.70 (3H, s, H-30), 1.66 (2H, m, H-12, H-2), 1.65 (2H, m, H-1), 1.60 (2H, m, H-12), 1.59 (2H, m, H-15), 1.51 (2H, m, H-6), 1.40 (2H, m, H-16), 1.38 (2H, m, H-7), 1.38 (1H, m, H-18), 1.25 (1H, m, H-9), 1.19 (2H, m, H-11), 1.20 (2H, m, H-22), 1.02 (3H, s, H-26), 0.96 (3H, s, H-23), 0.94 (3H, s, H-27), 0.82 (3H, s, H-25), 0.78 (3H, s, H-28), 0.76 (3H, s, H-24), 0.68 (1H, m, H-5).^{13}C NMR ($CDCl_3$, 125 MHz): 151.10 (C, C-20), 109.47 (CH_2, C-29), 79.18 (CH, C-3), 55.52 (CH, C-5), 50.66 (CH, C-9), 48.53 (CH, C-18), 48.17 (CH, C-19), 43.18 (C, C-17), 43.03 (C, C-14), 41.06 (C, C-8), 40.19 (CH_2, C-22), 39.04 (C, C-4), 38.92 (CH_2, C-1), 38.28 (CH, C-13), 37.38 (C, C-10), 35.78 (CH_2, C-16), 34.50 (CH_2, C-7), 30.06 (CH_2, C-21), 28.17 (CH_3, C-23), 27.65 (CH_2, C-2), 27.62 (CH_2, C-15), 25.37 (CH_2, C-12), 21.14 (CH_2, C-11), 19.49 (CH_3, C-30), 18.51 (CH_2, C-6), 18.18 (CH_3, C-28), 16.28 (CH_3, C-25), 16.17 (CH_3, C-26), 15.33 (CH_3, C-24), 14.75 (CH_3, C-27).

Gallic acid **(3) –** White solid; Chemical Formula: $C_7H_6O_5$, MP: 253.2-254.4 °C. EI-MS (*m/z*)

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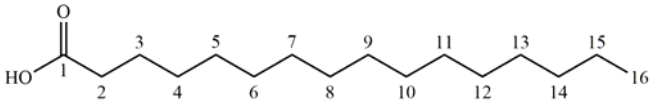
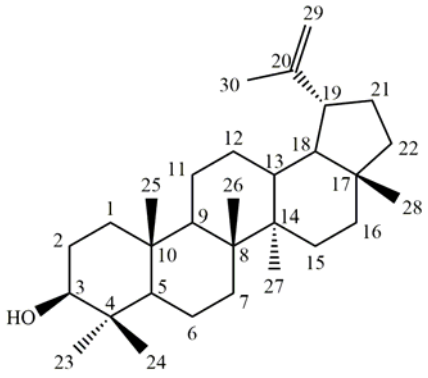
170.20 $[M+1]^+$ (100), 51 (16), 79 (11), 125.10 (10), 153 (85), 154 (32), 171 (23). IR (KBr) ν_{max}: 3387, 3285, 2664, 2577, 1701, 1624, 1618, 1541, 1452, 1346, 1256, 1036. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.01 (1H, s, H-2, H-6). ^{13}C NMR (CD_3OD, 125 MHz): 170.37 (C=O, C-7), 146.36 (C=C, C-3, C-5), 139.57 (C=C, C-4), 122.00 (C=C, C-1), 110.36 (CH, C-2, C-6).

β-Sitosterol-3-*O*-tetra-*O*-acetyl-*β*-D-mannopyranose **(4) –** White powder; Chemical Formula: $C_{43}H_{68}O_{10}$, MP: 167.0-167.4ºC. EI-MS (*m/z*) 109 (43), 147 (45), 169 (39), 256 (32), 276 (21), 289 (17), 331 (16), 382 (21), 396 (100), 397 (61), 398 (14). IR (KBr) ν_{max}: 3462, 2961, 2868, 1753, 1376, 1223, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.34 (1H, d, J = 5.4 Hz, H-6), 5.19 (1H, t, J = 9.53 Hz, H-2'), 5.06 (1H, t, J = 9.7 Hz, H-4'), 4.94 (1H, dd, J = 9.7, 8.0 Hz, H-3'), 4.58 (1H, d, J = 8.1 Hz, H-1'), 4.09 (1H, dd, J = 12.2, 2.5 Hz, H-6'b), 4.24 (1H, dd, J = 12.2, 4.9 Hz, H-6'a), 3.66 (1H, ddd, J = 10.0, 4.9, 2.5 Hz H-5'), 3.47 (1H, td, J = 11.4, 5.6 Hz H-3), 2.24 (2H, m, H-1), 2.06 (3H, s, H-14'), 2.03 (3H, s, H-12'), 2.05 (2H, m, H-12), 2.03 (1H, m, H-8), 2.01 (3H, s, H-13'), 1.99 (3H, s, H-11'), 1.93 (2H, m, H-23), 1.84 (2H, m, H-2), 1.83 (2H, m, H-4), 1.69 (1H, m, H-25), 1.59 (2H, m, H-11), 1.50 (2H, m, H-15), 1.49 (2H, m, H-7), 1.37 (1H, m, H-20), 1.37 (2H, m, H-22), 1.26 (2H, m, H-28), 1.22 (1H, m, H-17), 1.18 (2H, m, H-16), 1.10 (1H, m, H-14), 0.97 (3H, s, H-19), 0.94 (1H, m, H-24), 0.92 (1H, m, H-9), 0.87 (3H, m, H-29), 0.84 (3H, d, m, H-26), 0.83 (3H, m, H-21), 0.81 (3H, m, H-27), 0.66 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): 170.74 (C=O, C-7'), 170.41 (C=O, C-10'), 169.48 (C=O, C-9'), 169.36 (C=O, C-8'), 140.50 (C, C-5), 122.26 (CH, C-6), 99.78 (CH, C-1'), 80.20 (CH, C-3), 73.09 (CH, C-2'), 71.86 (CH, C-5'), 71.70 (CH, C-3'), 68.77 (CH, C-4'), 62.28 (CH_2, C-6'), 56.92 (CH, C-17), 56.23 (CH, C-14), 50.35 (CH, C-9), 46.02 (CH, C-24), 42.48 (C, C-13), 39.91 (CH_2, C-12), 39.07 (CH_2, C-1), 37.35 (CH_2, C-2), 36.87 (C, C-10), 36.26 (CH, C-20), 34.12 (CH_2, C-22), 32.08 (CH_2, C-7), 32.03 (CH, C-8), 29.60 (CH_2, C-23), 29.36 (CH, C-25), 28.36 (CH_2, C-4), 26.29 (CH_2, C-16), 24.48 (CH_2, C-11), 23.25 (CH_2, C-28), 21.20 (CH_2, C-15), 20.85 ($\underline{CH_3}$-C=O, C-11'), 20.89 ($\underline{CH_3}$-C=O, C-12'), 20.73 ($\underline{CH_3}$-C=O, C-13'), 20.70 ($\underline{CH_3}$-C=O, C-14'), 19.93 (CH_3, C-26), 19.48 (CH_3, C-19), 19.19 (CH_3, C-27), 18.92 (CH_3, C-21), 12.12 (CH_3, C-29), 11.99 (CH_3, C-18).

Methyl gallate **(5) –** White solid; Chemical Formula: $C_8H_8O_5$, MP: 196.5-197.7 ºC. EI-MS (*m/z*) 184 $[M+1]^+$ (44), 21 (4), 79 (8), 125 (11), 153 (100), 185 (10). IR (KBr) ν_{max}: 3319, 1678, 1605, 1449, 1375, 1288, 1269, 1223, 1047. ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.01 (1H, s, H-2, H-6), 3.78 (3H, s, H-8). ^{13}C NMR (CD_3OD, 125 MHz): 169.06 (C=O, C-7), 146.50 (C=C, C-3, C-5), 139.77 (C=C, C-4), 121.51 (C=C, C-1), 110.11 (CH, C-2, C-6), 52.26 (−O$\underline{CH_3}$, C-8).



(1)



(2)

Figure 1 The structures of compounds 1-5

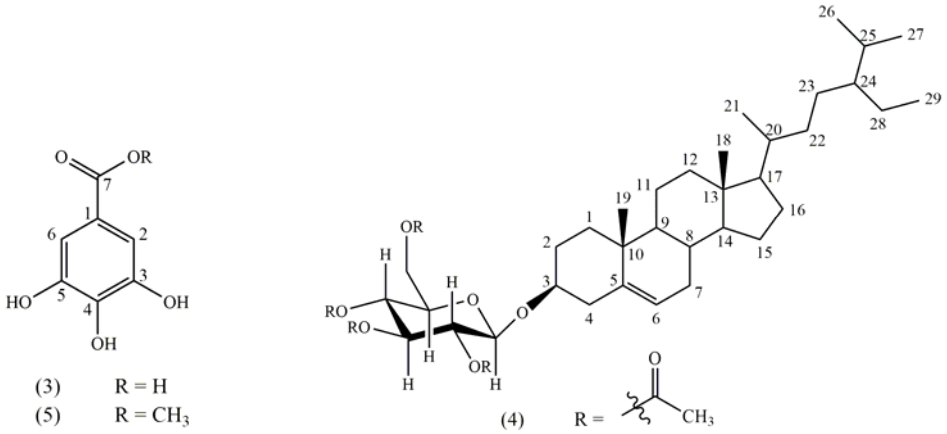


Figure 1 The structures of compounds 1-5 (continues)

3.3 Cytotoxicity

The cytotoxic activities of the tested crude extracts and compounds were carried out using the *in vitro* sulforhodamine B (SRB) method, and ellipticine was used as a positive control. Test samples were dissolved in DMSO as a stock concentration at 4 mg/mL. They were tested in triplicate with a final concentration of DMSO at 0.5%. The cancer cell lines were grown in a 96-well plate in the following media: P-388, in RPMI-1640 with 5% fetal bovine serum (FBS). The P-388, KB, HT 29, MCF-7, A 549, CL, and ASK cell lines were cultured in MEM (minimum essential medium with Earle's salt and L-glutamine) with 10% FBS. After drug exposure at 37°C for 72 h (48 h for P-388) with 5% CO_2 in air, and 100% relative humidity, cells were fixed with a final concentration of 10% trichloroacetic acid and stained with 0.4% sulforhodamine B in 1% acid. The bound and dried stain was solubilized with a 10 mM trizma base, after removal of the unbound dye by washing. The absorbance at wavelength 510 nm was read on a Fluostar optima BMG plate reader. The cytotoxic activity is expressed as a 50% effective dose (ED_{50}).

$$\text{Determine } ED_{50} \text{ value } \%\text{survival} = \frac{\text{OD (test sample)} - \text{OD (Day 0)}}{\text{OD (0.5\% DMSO control)} - \text{OD (Day 0)}} \times 100$$

Criteria of activity: extracts having an ED_{50} less than 20 μg/mL and pure compounds having an ED_{50} less than 4 μg/mL = Active; No Response = ED_{50} more than 20 μg/mL.

3.4 Anti-Human Immunodeficiency Virus (HIVs) effect

The Anti-human immunodeficiency virus (HIVs) activities of the isolate of crude extracts and compounds to a preliminary screening of inhibitory effect on HIV-1 RT at the cytotoxicity test Service Centre at the Department of Microbiology, Mahidol University, Thailand. Test samples were dissolved in 100% DMSO as a concentration at 20 mg/ml and continue further to remove tannins with Polyvinylpyrrolidone (PVP). The assay was carried out dilute in 5% DMSO, determining final concentration 200 μg/mL and nevirapine 2 μg/mL by negative control is 5% DMSO. The crude extracts, compounds, and nevirapine were used at positive controls, while DMSO was used at the negative control. The test samples presented 200 μg/mL. The results of test samples from the duplicate well plate were averaged and determined by the percentage inhibition (Tan, Pezzuto, Kinghorn, & Hughes, 1991; Chailungka, Junpirom, Pompimon, Nuntasaen, & Meepowpan, 2017).

4. Results and Discussion

4.1 Structure Elucidation

The palmitic acid (**1**) was separated from the crude hexane extract of liana and leaves of *P. nakhonphanomensis*. It was obtained as a white solid with a melting point of 52.1-53.0°C, which suggested the molecular formula $C_{19}H_{36}O_2$. The IR spectrum presented C-H stretching at 2922 cm^{-1}, 2845 cm^{-1}, 719 cm^{-1} and C=O stretching at 1701 cm^{-1}. The ^{13}C-NMR spectrum of palmitic acid showed 16 signals corresponding to one carbonyl carbon at □ 179.91 (C-1), fourteen methylene carbon at□□ 34.17 (C-2), □24.86 (C-3), 29.23 (C-4), 29.40 (C-5), 29.51 (C-6), 29.59 (C-7), 29.75 (C-8), 29.80 (C-9), 29.81 (C-10), 29.83 (C-11), 29.84 (C-12), 29.85 (C-13), 32.09 (C-14), 22.84 (C-15) and one methyl carbon at □ 14.25 (C-16). The HMBC correlations from H- 2 to C-1, C-3, and C-4, from H-3 to C-1, C-2, C-4, and C-5, from H-14 to C-15 and C-16, from H-15 to C-14 and C-16, from H-16 to C-14 and C-15. This evidence assigns the structure of palmitic acid. It was confirmed by G. Vlahov and coworker (Bulama, Dangoggo, Halilu, Tsafe, & Hassan, 2014).

The lupeol (**2**) was separated from the crude hexane extract of liana and leaves of *P. nakhonphanomensis*. It was obtained as a white solid with a melting point of 203.1-205.2°C. The EIMS presence the molecular ions peak in the mass spectrum of lupeol at *m/z* 426 $[M+1]^+$, which suggested the molecular formula $C_{30}H_{50}O$. The IR spectrum suggested O-H stretching at 3325 cm^{-1}, C-H stretching at 3067 cm^{-1}, 2945 cm^{-1}, 2872 cm^{-1}, 1462 cm^{-1}, 1379 cm^{-1} and C-O bending at 1042 cm^{-1}. The ^{13}C-NMR spectrum of lupeol showed 30 signals corresponding to double bond at □ 151.10 (C-20) and methylene carbon (CH_2) at □ 109.47 (C-29), methine carbon (CH) of alcohol at □ 79.18 (C-3) and methyl carbon (CH_3) at □ 19.49 (C-30). The HMBC correlation from H-29 to C-19, C-20, and C-30, from H-3 to C-4, C-23, and C-24, from H-30 to C-19, C-20, and C-29, from H-9 to C-7, C-8, C-10, C-11, C-25, and C-26, from H-18 to C-12, C-13, C-14, C-16, C-17, C-20, C-21, and C-22, from H-19 to C-13, C-18, C-20, C-21, C-29, and C-30. The double bond was supported located at the C-20 position by the HMBC correlation proton. This evidence assigns the structure of lupeol. It was confirmed by A. T. M Silva and coworker (Silva et al., 2018).

The gallic acid (**3**) was separated from the ethyl acetate extract of liana and leaves from *P. nakhonphanomensis*. It was obtained as a white solid with a melting point of 253.2-254.4°C. The EI-MS presents the molecular ions peak in the mass spectrum of gallic acid at *m/z* 170 $[M+1]^+$, which suggested the molecular formula $C_7H_6O_5$. The IR spectrum appeared O-H stretching at 3387 cm^{-1}, 3285 cm^{-1}, C=O stretching at 1701 cm^{-1}, C=C stretching 1624 cm^{-1}, 1541 cm^{-1}, and 1452 cm^{-1} indicated of aromatic ring. The ^{13}C-NMR spectrum of gallic acid showed 7 signals corresponding to one carbonyl on the aromatic ring at □ 170.37 (C-7), aromatic ring (C=C) appeared at □ 146.36 (C-3 and C-5), 139.57 (C-4), and 122.00 (C-1) of quaternary carbon (C) and methine carbon (CH) at □ 110.36 (C-2 and C-6). The HMBC correlations from H-2 and H-6 to C-1, C-3, C-4, C-7 and C-1, C-4, C-5, C-7, respectively. This evidence assigns the structure of gallic acid. It was confirmed by H. A. Aglan and coworker (Aglan, Ahmed, El-Toumy, & Mahmoud, 2017).

The *β*-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose (**4**) was obtained from the ethyl acetate extract of liana and leaves of *P. nakhonphanomensis*. It was separated as a white powder with a melting point of 167.0-167.4°C, which indicated the molecular formula $C_{43}H_{68}O_{10}$. The IR spectrum showed C-H stretching at 2961 cm^{-1}, 2868 cm^{-1}, C=O stretching at 1753 cm^{-1}, O-H bending at 1223 $cm^{-1}$and 1040 cm^{-1}. The ^{13}C-NMR spectrum of *β*-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose showed 43 signals corresponding to methine carbon at □ 122.26 (CH, C-6), quaternary carbon at □ 140.50 (C, C-5) and methine carbon of ether at □ 80.20 (CH, C-3) and tetra-*O*-acetyl-D-mannopyranose was determined to four carbonyl carbon at □ 170.74 (C=O, C-7′), 170.41 (C=O, C-10′), 169.48 (C=O, C-9′), and 169.36 (C=O, C-8′), five methine carbon at □ 99.78 (CH, C-1′), 73.09 (CH, C-2′), 71.86 (CH, C-5′), 71.70 (CH, C-3′), and 68.77 (CH, C-4′), methylene carbon at 62.28 (CH_2, C-6′), four methyl carbon at □ 20.85 ($\underline{CH_3}$-C=O, C-11′), 20.87 ($\underline{CH_3}$-C=O, C-12′), 20.73 ($\underline{CH_3}$-C=O, C-13′), and 20.70 ($\underline{CH_3}$-C=O, C-14′). The HMBC experiment was also confirmed double bond (C=C) of sitosterene by correlation from H-6 to C-7, C-8, and C-10. The five methine carbon (CH) were supported located of tetra-*O*-acetyl-D-mannopyranose by correlation from H-1′to C-1, from H-2′to C-3′, C-4′, and C-10′, from H-3′to C-1′, C-2′, and C-4′, from H-

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4′ to C-2′, C-3′, C-5′, and C-8′, from H-5′ to C-4′, methylene carbon from H-6′ to C-4′, C-5′, and C-7′, methyl carbon from H-11′ to C-2′ and C-10′, from H-12′ to C-3′ and C-9′, from H-13′ to C-4′ and C-8′ and from H-14′ to C-7′ position by the HMBC correlation proton. This evidence assigns the structure of *β*-sitosterol-3-*O*-Tetra-*O*-acetyl-*β*-D-mannopyranose. It was confirmed by Peshin and Kar (2017).

The methyl gallate (**5**) was separated from the methanol extract of liana and leaves of *P. nakhonphanomensis*. It was obtained as a white solid with a melting point of 196.5-197.7°C. The EI-MS presence the molecular ions peak in the mass spectrum of methyl gallate at *m/z* 184 $[M+1]^+$, which suggested the molecular formula $C_8H_8O_5$. The IR spectrum presented O-H stretching at 3319 cm^{-1} and C=C stretching at 1678 cm^{-1}, 1605 $cm^{-1,}$ and 1375 cm^{-1} indicated of the aromatic ring. The ^{13}C-NMR spectrum of methyl gallate showed 8 signals corresponding to one carbonyl carbon (C=O) on the aromatic ring at □ 169.06 (C-7), signals to double bond in the aromatic ring (C=C) appeared at □ 146.50 (C-3 and C-5), 139.77 (C-4), 121.51 (C-1) of quaternary carbon (C) and methine carbon (CH) at □ 110.11 (C-2 and C-6) and methyl carbon (CH_3) at □ 52.86 (C-8). The HMBC experiment was also confirmed methine carbon at H-2 and H-6 by correlation with C-1, C-3, C-4, C-7, and C-1, C-4, C-5, C-7, respectively, which this signal presented HMBC correlation to carbon signal at H-8 with C-7 of methyl carbon. This evidence assigns the structure of methyl gallate. It was confirmed by M. D. Ahmed and coworker (Ahmed, Taher, Maimusa, Rezali, & Mahmud, 2017).

4.2 Biological Activities

4.2.1 Cytotoxicity

The isolated compounds and crude extracts from liana and leaves of *P. nakhonphanomensis* were estimated for cytotoxicity against P-388, KB, HT 29, MCF-7, A 549, CL, ASK cancer cell lines using SRB method were shown in Table 1. The results of gallic acid were exhibited as follows strong growth inhibitory activity against four cancer cell lines with ED_{50} values of 2.57, 16.27, 11.42, and 12.23 *μ*g/mL for P-388, KB, HT 29, MCF-7, A 549, CL, and ASK, respectively. Also, the crude extracts were not responded to cytotoxicity. The ellipticine was antibiotic drug used as positive control presented the activity with ED_{50} values in the range at 0.47, 0.55, 0.63, 0.51, 0.57, 0.53, and 0.68 *μ*g/mL which were P-388, KB, HT 29, MCF-7, A 549, CL, and ASK, respectively.

Table 1 Cytotoxicity of crude extracts and compound from liana and leaves of *P. nakhonphanomensis*

Crude extracts/pure compound	Cytotoxicity ED_{50} (*μ*g/mL)*						
	Cancer cell lines					Cancer cell lines	
	P-388	KB	HT 29	MCF-7	A 549	CL	ASK
Ellipticine (positive control)	0.47	0.55	0.63	0.51	0.57	0.53	0.68
Gallic acid	2.57	NR	16.27	NR	11.42	12.23	NR
Hexane	NR	NR	NR	NR	NR	NR	NR
Ethyl acetate	NR	NR	NR	NR	NR	NR	NR
Methanol	NR	NR	NR	NR	NR	NR	NR

*Cytotoxic assay: ED_{50} less than 20 *μ*g/mL were considered active for extracts and less than 4 *μ*g/mL for pure compounds. P-388: Murine lymphocytic leukemia, KB: human oral cavity carcinoma, HT 29: human colon adenocarcinoma, MCF-7: human breast adenocarcinoma, A 549: human lung, adenocarcinoma, CL: Chang Liver (HeLa derivative), ASK: Rat glioma cell, NR: no response (ED_{50} more than 20 *μ*g/mL).

4.2.2 Anti-human immunodeficiency virus (Anti-HIV-1RT activities)

The isolated compounds and crude extracts from liana and leaves of *P. nakhonphanomensis* were evaluated for Human Immunodeficiency Virus (HIVs) activity (Table 2). The results were exhibited of gallic acid, crude ethyl acetate, and crude methanol extract showed strong inhibitory activity values of 91.50%, 92.08%, and 97.63%, respectively. On the other hand, *β*-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose and the crude hexane extract have been no inhibition.

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Table 2 Anti-HIV-1RT activities of crude extracts and compounds from liana and leaves of *P. nakhonphanomensis*

Crude extracts/ pure compounds	Anti-HIV-1RT activities*	
	% inhibition	activity
Azidothymidine (positive control)	100	VA
Gallic acid	91.50	VA
β-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose	-9.01	I
Hexane	1.62	I
Ethyl acetate	92.08	VA
Methanol	97.63	VA

*Syncytium assay: Anti-HIV-1RT activity express as % inhibition at 200 µg/mL (radioactive) or 667 µg/mL (non-radioactive): VA = very active (>70% inhibition), M = moderately active (50% to 69% inhibition), W = weakly active (30% to 50% inhibition), I = inactive (<30% inhibition).

5. Conclusions

The results presented are a derivative from *P. nakhonphanomensis* that were carried out of the isolated and characterization palmitic acid (1) and lupeol (2) from hexane extract, gallic acid (3) and *β*-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose (4) from ethyl acetate extract, methyl gallate (5) from methanol extract. The cytotoxicity results of gallic acid (3) were exhibited as follows strong growth inhibitory activity against four cancer cell lines with ED_{50} values of P-388. Besides, the No response to cytotoxicity against hexane extract, ethyl acetate, and methanol. The Anti-HIV-1RT activity results were exhibited of gallic acid (3), ethyl acetate, and methanol extract showed strong inhibitory activity. In contrast, *β*-sitosterol-3-*O*-tetra-*O*-Acetyl-*β*-D-mannopyranose (4) and hexane extract have been no inhibition. This genus is discovered chemical diversity in the structure of compounds. Also, these two compounds represented a vital role in medicine. *P. nakhonphanomensis* has not been previously reported in the study of phytochemical for this plant.

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