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RESEARCH ARTICLE

In vitro Evaluation of Crude Extracts and Isolated Compounds from *Goniothalamus rongklanus* and *Goniothalamus latestigma* for Bioactive Properties

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Abstract

The genus *Goniothalamus* belongs to the Annonaceae family. The pure compounds from *Goniothalamus rongklanus* and *Goniothalamus latestigma* were isolated by repeated column chromatography. The structures were recognized by NMR spectral methods. The chemical compounds of ethyl acetate extract (EtOAc) from the stems of *G. rongklanus* were identified as goniotriol (**1**) and stigmasterol glucoside (**2**). The EtOAc leaves extract of *G. latestigma* yielded a compound, which was identified as pinocembrin (**3**). The ethyl acetate (EtOAc) and methanol (MeOH) extract were tested for anti-HIV-1 RT and cytotoxic activities against P-388, KB, HT 29, MCF-7, A 549, ASK and CL cell lines. The EtOAc and MeOH extract of *G. rongklanus* showed evidence of anti-HIV-1 RT inhibition at 76.44 and 88.48 %, respectively. The EtOAc extract of *G. rongklanus* and *G. latestigma* showed cytotoxic activities on KB and HT 29 with an ED_{50} at <4 μg/mL. In addition, antibacterial study on extracts and isolated compounds was also performed. Antibacterial study was evaluated using nine strains (*Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli* 0157: H7, *Escherichia coli* (ETEC), *Escherichia coli* (EPEC), *Proteus mirabilis*, *Salmonella typhimuriam*, *Shigella flexneri* and *Vibrio cholera*) by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) method. Goniotriol was the most effective antibacterial with a MIC in the range <0.16 - 0.6 mg/mL and MBC in the range 0.3 - >5 mg/mL. The antibacterial activity of goniotriol has been reported for the first time.

Keywords: Antibacterial Activity, Anti-HIV-1 RT, Cytotoxicity, *Goniothalamus rongklanus, Goniothalamus lagestima*

1. Introduction

The genus *Goniothalamus* (Annonaceae) include about 185 species, of which 25 species are found in Thailand[1]. This genus is widely distributed in the tropical forests of Southeast Asia and are used in traditional medicine. Root barks of *G. cheliensis* and seeds of *G. amuyon* are used in the treatment of edema and arthritis[2,3]. The stem bark of *G. laoticus* has been applied as roborant[4]. *G. macrophyllus* has been used in the treatment of colds, fever, malaria, cholera, to stimulate blood flow in the body and after childbirth[5,6]. This genus has been previously reported for styryl lactones, flavonoids, naphthoquinones, azaanthraquinones, alkaloids, and acetogenins[7–11]. Compounds isolated from *Goniothalamus* genus has generally shown cytotoxicity, antimycobacterial, antimalarial, antiinflammatory, antitumor, antiviral, anti-larvicidal, anti-biofilm and

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antiplasmodial activity[7,9,12,13]. Styryl lactone compounds are the most isolated compounds from this genus for example, goniothalamin, goniotriol, cardiobutanolide, goniofufurone, altholactone, goniopypyrone, goniodiol, and goniothalamin oxide. Goniothalamin has shown cytotoxicity in oral cavity, breast, small cell lung, and vero cell lines[14].

G. rongklanus is a member of this genus, locally known as 'Pa-Nan-Rong-Kla' in Thai. This species is distributed in the Northern and North-Eastern Thailand. *G. latestigma* is locally known as 'Sa-Lao-Ton' and distributed around Peninsular Thailand. These species are small trees upto 8 m tall and slender, featured by flowers with whorl of three sepals and two whorls of three petals each, the inside petals are connivant and forming a distinctive dome over the stamens[1].

There are no reports related to isolation of compounds from *G. rongklanus* and *G. latestigma* and their bioactive evaluation. Hence, the study was planned to extract, isolate, characterize the compounds from *G. rongklanus* and *G. latestigma* and to evaluate them for Anti-HIV-1 RT (Reverse Transcriptase), cytotoxicity and antibacterial activity.

2. Materials and Methods

2.1 Plant Materials

The stems of *G. rongklanus* (BKF. 152884) were collected from Phuhin Rongkhla National Park, Amphoe Nakhonthai, Phitsanulok Province, Northern Thailand in 2017. The leaves of *G. latestigma* (BKF. 163333) were collected from Surat Thani provinces, Thailand in 2017. The plant materials were authenticated by Narong Nantasean, The Forest Herbarium, Ministry of Natural Resources and Environment, Bangkok.

2.2 General Procedures

^{1}H (500 MHz), ^{13}C (125 MHz) and 2D NMR spectra were noted on a BrÜker AV-500 spectrometer in deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD) solutions. The tetramethylsilane (TMS) was internal standard of chemical shifts (δ) in ppm. The mass spectra were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe) and EIMS were measured by a BrÜker Esquire apparatus. Infrared spectra (IR) were recorded at potassium bromide (KBr) solid with a Shimadzu 8900 FT-IR spectrophotometer and major bands (λ_{max}) were noted in wave number (cm^{-1}). The Column Chromatography (CC) were used silica gel 60 H from E. Merck. 70-230 mesh ASTM, cat. No. 7734 and Sephadex LH-20 (20–150 μm) for absorbents. Thin layer chromatography (TLC) technique was proceeded on silica gel 60 PF254 at aluminium sheets and isolated compounds were described below ultraviolet light. Melting points were defined by a Büchi 322 micro melting point apparatus and records in degree Celsius (˚C).

2.3 Extraction and Isolation

2.3.1 Isolation of Chemical Constituents from *G. rongklanus* Extracts

The air-dried milled stems of *G. rongklanus* (1.772 kg) was extracted with EtOAc (6 × 5 L) (ethyl acetate) and MeOH (6 × 5 L) (methanol) at room temperature for three days to yield EtOAc extract (47.98 g) and MeOH extract (103.86 g) after solvent removal by rotary evaporator.

The EtOAc extract was isolated to pure compounds by CC with gradient mixtures of Hexane: EtOAc (100:0–0:100) followed by mixtures EtOAc: MeOH (100:0–0:100) to give 8 fractions (A_1-A_8) which was characterized by TLC. Fraction A_7 (8.04 g) was further subjected to CC to furnish 5 subfraction (B_1-B_5). Subfraction B_3 (0.48 g) was crystallized by 95% ethanol to yield yellow needles of compound **1** (0.3 g).

Subfraction B_4 (1.70 g) was separated on CC by eluting with gradient mixtures of Hexane: EtOAc (100:0–0:100) followed by mixtures EtOAc: MeOH (100:0–0:100) to provide 4 fractions (C_1–C_4). Subfraction C_3 and C_4 showed similar TLC characteristic therefore it was combined and then further purified by flash column chromatography (FCC) with gradient mixtures of Hexane: EtOAc (100:0–0:100) followed by mixtures EtOAc: MeOH (100:0–0:100) to give 3 fractions (D_1-D_3) followed by crystallization of D_2 in MeOH : CH_2Cl_2 (1:1) to yield compound **2** (0.02 g) as white solid.

2.3.2 Isolation of Chemical Constituents from G. latestigma Extracts

The air-dried milled leaves of *G. latestigma* (1.035 kg) was extracted with EtOAc (6 × 5 L) and MeOH (6 × 5 L) at room temperature for three days to yield EtOAc extract (46.55 g) and MeOH extract (60.69 g) after solvent removal by rotary evaporator.

The EtOAc extract was separated by CC with gradient mixtures of Hexane: EtOAc (100:0–0:100) followed by mixtures EtOAc: MeOH (100:0–0:100) to yield 7 fractions (E_1–E_7) which was characterized by TLC. Fraction E_3 (9.09 g) was further subjected to column chromatography to give 5 subfraction (F_1-F_5). Subfraction F_2 (0.72 g) was subjected to CC by eluting with Hexane: EtOAc (100:0–0:100) and then with mixtures EtOAc : MeOH (100:0–0:100) to obtain 3 fractions (G_1-G_3). The G_2 (0.57 g) was recrystallized by 95% ethanol to yield compound **3** (0.44 g) as pale needles.

2.4 Anti-HIV-1 RT (Reverse Transcriptase) Assay

The extracts of *G. rongklanus* and *G. latestigma* were tested for anti-HIV-1 RT assay and cytotoxicity at the Service Centre of Department of Microbiology, Mahidol University, Thailand. The anti-HIV activities were determined by testing RT inhibition[15,16]. The extracts were dissolved in 20 mg/mL of 100% dimethyl sulfoxide (DMSO) after removal of tannin by Polyvinylpyrrolidone (PVP). The final volume was 200 μg/mL in 10% DMSO and Nevirapine, 2 μg/ mL was used as positive control. The HIV-1 RT (Amersham Pharmacia Biotech Asia Pacific Ltd., Hong Kong) kit was used. The 96-well plate (100 U/μl, 4 μl/well) was filled with samples (2 μl/well) and then 2.5 μg/μL of poly A and 0.125 μg/mL of oligo dT16 primer was added to 4 μl/well and incubated at 37 ˚C for 20 min. The reaction was fixed by 0.2 M EDTA (2 μl/well) and incubated at 4 ˚C for 15 min. The signal of fluorescence was measurement at emission wavelength of 535 nm and excitation wavelength of 480 nm after Pico green dissolved in TE buffer (1: 2000) was added (volume 200 μl/well). The results were calculated as percentage of inhibition.

2.5 Cytotoxicity Assay

The extracts of *G. rongklanus* and *G. latestigma* were also studied for cytotoxicity, using the standard Sulforhodamine B (SRB) assay. Ellipticine was used as the positive control[15,17]. The concentration of the samples was 20 - 0.16 μg/mL in 0.5 % DMSO. The cancer cell lines used were Murine lymphocytic leukemia (P-388), Human oral cavity carcinoma (KB), Human colon adenocarcinoma (HT 29), Human breast adenocarcinoma (MCF-7), Human lung, adenocarcinoma (A 549), Rat glioma cell (ASK) and Chang liver (CL). MEM (minimum essential medium with Earles salt and L-glutamine) in 10% FBS were used for culturing of cell lines. The cell lines were maintained t 37 °C for 72 hours (48 hours for P-388) in 5% CO_2 and 100% relative humidity, followed by stabilizing with 20% trichloroacetic acid at 4 °C for 60 min and then stained for 30 min by 0.4 % SRB in 1% acetic acid at room temperature. The unbound dye was washed by 1% acetic acid, already dried stain was mixed with 10 mM Tris base with pH = 10. The absorbance was read at 510 nm on a microplate reader and 50% effective dose (ED_{50}) was calculated.

2.6 Bacterial Strains

In vitro antibacterial studies were carried out against nine strains (*Staphylococcus aureus* ATCC 25923 DMST 8840, *Enterobacter aerogenes* ATCC13048 DMST 8841, *Escherichia coli* 0157 : H7 DMST 12743, *Escherichia coli* (Enterotoxigenic, ETEC) DMST 30543, *Escherichia coli* (Enteropathogenic, EPEC) DMST 30546, *Proteus mirabilis* DMST 8212, *Salmonella typhimuriam* ATCC 13311 DMST 562, *Shigella flexneri* DMST 4423 and *Vibrio cholera* nonO1/nonO139 DMST 2873) which were obtained from Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand.

2.7 Minimum Inhibitory Concentration (MIC)[18]

The samples were dissolved in 10 % DMSO to obtain concentrations of 200 mg/mL (extracts), 5 mg/mL (isolated compounds) and 1 mg/mL (chloramphenicol). The extracts and isolated compounds were diluted further 2-fold dilution to a final concentration of 6.25 mg/mL and 0.16 mg/mL for extracts and isolated compounds respectively. The final concentration of *S. aureus, E. aerogenes, E. coli* 0157 : H7, *E. coli* (ETEC), *E. coli* (EPEC), *P. mirabilis*, *S typhimuriam*, *S. flexneri* and *V. cholera* in Mueller Hinton Broth (MHB) were $1x10^6$ cfu/mL, 50 μl/well (Mcfarland standard no. 0.5)

in a 96-well plate and were mixed into the samples (50 μl/well). The plates were incubated at 37 °C for 24 hours and the growth of organisms were investigated by the color change of resazurin (1 mg/mL, 20 μl/well). No colour change indicated the prevention of microbial growth.

2.8 Minimum Bactericidal Concentration (MBC)

The MBC assay was determined for samples which did not showed any visible growth and was subsequently sub-cultured on to nutrient agar plate. These plates were incubated at 37 °C for 24 hours. MBC was recorded only for the lowest concentration of the bacteria that did not retrieve or a single colony.[18]

3. Results

3.1 Goniotriol (1)

Yellow needles (ethanol). $C_{13}H_{14}O_5$, m.p. = 174.4 – 175.7 ˚C, EI-MS (*m/z*) 250 $[M]^+$(42), 233 (100), 215 (75), 144 (18), 126 (38), 107 (45), 105 (22), 100 (24), 97 (81), 91 (37), 81 (27), 79 (83), 77 (91), 69 (21), 55 (34). IR (KBr) λ_{max} 3465 (O-H), 1718 (C = O), 2800-2900 (C-H), 1637, 1500 and 1450 (C=C) cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ 7.49 (2H, m, H-2', 6'), 7.37 (2H, m, H-3', 5'), 7.31 (1H, m, H-4'), 7.04 (1H, dd, *J* = 9.7, 5.7 Hz, H-4) , 6.11 (1H, d, *J* = 9.7 Hz, H-3), 4.77 (1H, d, *J* = 7.9 Hz, H-8), 4.62 (1H, m, H-6), 4.46 (1H, dd, *J* = 5.7, 3 Hz, H-5), 4.21 (1H, dd, *J* = 7.9, 3.8 Hz, H-7). ^{13}C NMR (CD_3OD, 125 MHz): δ 164.66 (C-2), 145.03 (C-4), 141.94 (C-1'), 127.77 (C-3', 5'), 127.43 (C-2', 6'), 127.36 (C-4'), 121.57 (C-3), 78.88 (C-6), 74.24 (C-7), 72.55 (C-8), 62.10 (C-5). HMBC correlations, H/C: 7.49 (H-2', 6')/C-1', 2', 3', 4', 5', 6', 8; 7.37 (H-3', 5')/ C-1', 2', 3', 4', 5', 6'; 7.31 (H-4')/C-2', 3', 5', 6'; 7.04 (H-4)/C-2, 3, 5, 6; 6.11 (H-3)/C-2, 4, 5; 4.77 (H-8)/C-6, 7, 1', 2'; 4.62 (H-6)/C-2, 4, 5, 7, 8; 4.46 (H-5)/C-3, 4, 6; 4.21 (H-7)/C-5, 6, 8, 1'. COSY correlations, H/H: 3/4, 4/5, 5/6, 6/7, 7/8, 2'/3', 3'/4', 4'/5', 5'/6' (Figure 1).

3.2 Stigmasterol Glucoside (2)

White solid (CH_2Cl_2: MeOH). $C_{35}H_{60}O_6$, m.p. = 290.6 – 291.1 ˚C, EI-MS (*m/z*) 574 $[M]^+$, 126 (100), 112 (28), 95 (12), 81 (22), 72 (88), 59 (75), 55 (93). IR (KBr) λ_{max} 3338 (O-H), 2868, 2933 (C-H), 1589 and 1400 (C=C) cm^{-1} [19]. The ^{13}C NMR (CD_3OD : $CDCl_3$, 125 MHz): δ 140.34 (C-5), 138.27 (C-22), 129.26 (C-23), 121.97 (C-6), 101.07 (C-1'), 79.16 (C-3), 76.42 (C-3'), 75.96 (C-5'), 73.49 (C-2'), 69.84 (C-4'), 61.23 (C-6'), 56.73 (C-14), 56.02 (C-17), 50.19 (C-24), 45.85 (C-9), 42.27 (C-13), 40.44 (C-20), 39.73 (C-4), 39.66 (C-20), 38.54 (C-12), 37.20 (C-1), 36.63 (C-10), 33.89 (C-7), 31.85 (C-25), 29.44 (C-2, 8), 28.13 (C-16), 24.17 (C-28), 23.00 (C-15), 20.99 (C-11), 19.53 (C-21), 19.08 (C-26), 18.78 (C-27), 18.59 (C-19), 11.71 (C-29), 11.66 (C-18). The ^{13}C NMR spectral data was compared with literature[19] (Figure 1).

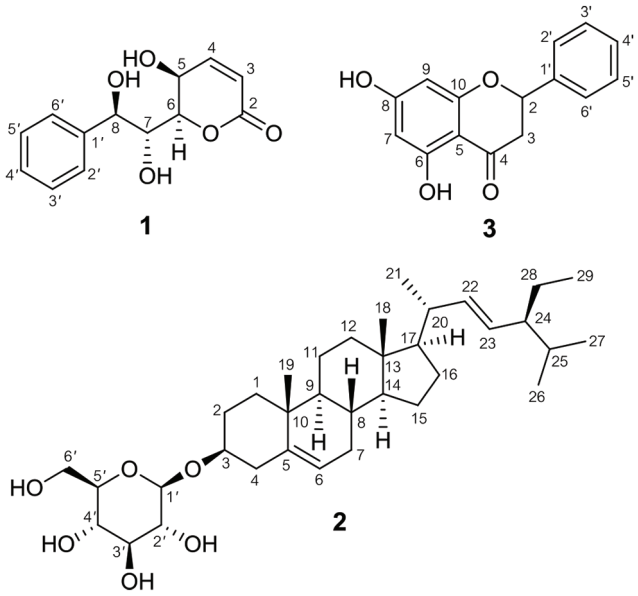

Figure 1. Chemical structures of compounds 1-3

3.3 Pinocembrin (3)

Pale needles (EtOAc: MeOH). $C_{15}H_{12}O_4$, m.p. = 195.9 -196.5 ˚C, EI-MS (*m/z*) 256 $[M]^+$ (86), 257 (100), 255 (58), 179 (36). IR (KBr) λ_{max} 3406 (O-H), 1631 (C = O), 1436, 1463 and 1487 (C = C of aromatic) cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 12.06 (1H, s, 5-OH), 7.45 (5H, m, H-2', 3', 4', 5', 6'), 6.04 (2H, d, *J* = 1.8 Hz, H-7, 9), 5.45 (1H, dd, *J* = 13.0, 3.1 Hz, H-2), 3.11 (1H, dd, *J* = 17.2, 13.0 Hz, H-3a), 2.85 (1H, dd, *J* = 17.2, 3.1 Hz, H-3b). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 195.76 (C-4), 164.81 (C-8), 164.34 (C-6), 163.16 (C-10), 138.31 (C-1'), 128.88 (C-3', 5'), 128.87 (C-4'), 126.12 (C-2', 6'), 103.17 (C-5), 96.78 (C-7), 95.52 (C-9), 79.20 (C-2), 43.31 (C-3). HMBC correlations, H/C: 7.45 (H-2', 3', 4', 5', 6')/C-2, 2', 3', 4',

5', 6'; 6.04 (H-7, 9)/C-4, 5, 6, 7, 8, 9; 5.45 (H-2)/C-4, 1', 2', 6'; 3.11 (H-3a)/C-2, 4, 1'; 2.85 (H-3b)/C-4, 5, 1'. COSY correlations, H/H: 2/3a, 2/3b, 3a/3b (Figure 1).

3.4 Biological Activities

The bioactivities screening of EtOAc and MeOH extract of *G. rongklanus* and *G. latestigma* comprised of anti-HIV-1 RT and cytotoxicity assay on P-388, KB, HT 29, MCF-7, A 549, ASK and CL cell lines. The results are shown in Table 1.

The extract and isolated compounds were studied for antibacterial activity against nine bacterial by micro well dilution assay. The tested concentrations of the extracts ranged between 6.25 – 200 mg/mL and 0.16 – 5 mg/mL for isolated compounds. The MIC and MBC of samples are shown in Table 2.

4. Discussion

Compound (**1**) (a styryllactone) and (**2**) (a steroid glucoside) isolated from EtOAc stem extract of *G. rongklanus* were identified as goniotriol[20] and stigmasterol glucoside[19] respectively. Compound (**3**) (a flavanone) isolated from EtOAc leaves extract of *G. latestigma* was identified as pinocembrin[21] based on Mass, NMR spectra and analogy with literature data.

Table 1. Anti-HIV-1 RT and cytotoxicity study of crude extracts of *G. rongklanus* and *G. latestigma*

Crude extract	Anti-HIV-1 RT[a] (% inhibition)	Cytotoxicity ED_{50} (µg/mL)[b]						
		P-388	KB	HT 29	MCF-7	A 549	ASK	CL
EtOAc extract of *G. rongklanus*	76.44	4.59	<4	<4	7.11	6.82	4.82	8.19
MeOH extract of *G. rongklanus*	88.48	-	-	-	-	-	-	-
EtOAc extract of *G. latestigma*	45.75	3.99	<4	<4	6.83	7.23	8.54	8.16
MeOH extract of *G. latestigma*	18.03	-	-	-	-	-	-	-
Ellipticine (positive control)	-	0.47	0.55	0.63	0.51	0.57	0.68	0.53

*[a]Anti-HIV-1RT activity express as % inhibition at 200 µg/mL (radioactive) or 667 µg/mL (non-radioactive): very active = >70% inhibition, moderately active = 50% to 69% inhibition, weakly active = 30% to 50% inhibition and inactive = <30% inhibition
*[b]Cytotoxic assay: ED_{50} less than 20 µg/mL were considered active for extracts. 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, ASK: Rat glioma cell, and CL: Chang Liver

Table 2. Determination of MIC and MBC for crude extracts and isolated compounds from *G. rongklanus* and *G. latestigma*

Organism	Concentrations of MIC/MBC (mg/mL)							
	Goniotriol (1)	Stigmasterol glucoside (2)	Pinocembrin (3)	EtOAc extract G. rongklanus	MeOH extract G. rongklanus.	EtOAc extract G. latestigma	MeOH extract G. latestigma	Chloramphenicol
S. aureus	0.3/0.3	-	5/5	<6.25/<6.25	12.5/50	<6.26/<6.25	50/200	<0.03/<0.03
E. aerogenes	0.6/2.5	-	-	12.5/>200	100/>200	200/>200	200/>200	<0.03/<0.03
E. coli 0157:H7	0.3/>5	-	5/>5	<6.25/200	50/>200	100/>200	100/>200	<0.03/<0.03
E. coli (ETEC)	0.3/2.5	-	-	12.5/25	100/>200	200/>200	100/>200	<0.03/<0.03
E. coli (EPEC)	0.3/1.25	-	-	12.5/200	25/>200	200/>200	100/>200	<0.03/<0.03
P. mirabilis	0.3/>5	-	-	<6.25/200	200/>200	100/>200	200/>200	<0.03/<0.03
S. typhimuriam	<0.16/>5	-	-	<6.25/200	25/>200	50/>200	200/>200	<0.03/<0.03
S. flexneri	0.3/>5	-	5/>5	<6.25/25	12.5/>200	50/>200	100/>200	<0.03/<0.03
V. cholera	0.3/>5	-	5/>5	-	100/>200	50/>200	200/>200	<0.03/<0.03

Compound **1** was isolated as pale-yellow needles with a melting point of 174.4 – 175.7 ˚C. The molecular formula was $C_{13}H_{14}O_5$ as analyzed from the molecular ion peak at *m/z* 250 of EI-MS. The ^{1}H NMR spectral data of compound **1** showed resonances at δ 7.49 (2H), 7.37 (2H), 7.31 (1H), 7.04 (1H) , 6.11 (1H), 4.77 (1H), 4.62 (1H), 4.46 (1H) and 4.21 (1H) which was of signals for five protons on aromatic, two protons of conjugated ester carbonyl, and four oxygenated methine proton. The ^{13}C NMR spectral data showed eleven carbons resonances, which were defined to a one carbonyl of ester at δ 164.66, one quaternary aromatic carbon atoms at δ 141.94, three aromatic carbon atoms at δ 127.77, 127.43 and 127.36, two methine carbon atoms at δ 121.57 and 145.03, one oxymethine carbon atoms at δ 78.88 and three secondary alcohol carbon atoms at δ 74.24, 72.55 and 62.10, respectively. The NMR spectral data from compound **1** were conforming goniotriol structure in literature.

Compound **2** was obtained as a white solid with m.p. 290.6 – 291.1 ˚C. The molecular formula was $C_{35}H_{60}O_6$ as analyzed from the molecular ion peak at *m/z* 574 of EI-MS. In comparison of ^{13}C-NMR with literature[19] suggested that the compound **2** is a stigmasterol glucoside as it showed resonances of glucoside at δ 101.07(C-1'), 73.49 (C-2'), 76.42 (C-3'), 69.84 (C-4'), 75.96 (C-5'), and 61.23 (C-6') together with endo cyclic double bond in stigmasterol at δ 140.34 (C-5) and 121.97 (C-6). The ^{13}C-NMR spectrum presented thirty-five carbons resonances. The DEPT NMR spectrum showed six methyl, ten methylene, sixteen methane, and three quaternary carbons. In comparison with the ^{13}C-NMR of literature data compound **2** was confirmed as stigmasterol glucoside.

Compound **3** was obtained as pale needles with m.p. 195.9 -196.5 ˚C. The molecular formula was $C_{15}H_{12}O_4$ as analyzed from the molecular ion peak at *m/z* 256 of EI-MS. The ^{1}H NMR spectral data of compound **3** showed resonances at δ 12.06 (OH), 7.45 (5H), 6.04 (2H), 5.45 (1H), 3.11 (1H) and 2.85 (1H), which was of signals for one hydroxyl proton, seven protons on aromatic, one oxymethine proton and two proton on position 3. The ^{13}C NMR spectral data of compound **3** showed thirteen carbons resonances, which were defined to a ketone carbonyl at δ 195.76, three oxygenated carbon atoms at δ 164.81, 164.34 and 163.16, five aromatic carbon atoms at δ 128.88, 128.87, 126.12, 96.78 and 95.52, two quaternary aromatic carbon atoms at δ 138.31 and 103.17, one methylene at δ 43.31 and one oxymethine carbon atoms at δ 79.20. The NMR spectral data of compound **3** were in consistence with pinocembrin.

The EtOAc and MeOH extract of *G. rongklanus* and *G. latestigma* were tested for anti-HIV-1 RT and cytotoxicity on P-388, KB, HT29, MCF-7, A 549, ASK and CL cell lines. The EtOAc and MeOH extract from *G. rongklanus* showed very active % inhibition of 76.44 and 88.48, respectively. The EtOAc extract of *G. rongklanus* displayed highly potent cytotoxicity against KB, HT 29 and inhibited P-388, MCF-7, A 549, ASK and CL cell lines with an ED_{50} values of <4, <4, 4.59, 7.11, 6.82, 4.82 and 8.19 μg/mL, respectively. In addition, the EtOAc extract from *G. latestigma* showed highly potent cytotoxicity against KB, HT 29 and inhibited P-388, MCF-7, A 549, ASK and CL cell lines with an ED_{50} values of <4, <4, 3.99, 6.83, 7.23, 8.54 and 8.16 μg/mL, respectively. (Table 1)

Inhibitory effects of the compound **1** was bactericidal with MIC values falling in the range <0.16 - 0.6 mg/mL and MBC values falling in the range 0.3 - >5 mg/mL. The crude extracts of both the plants showed the inhibitory effect in the range of values MIC/MBC at <6.25 - >200 mg/mL, of which the EtOAc extract of *G. rongklanus* was had the best inhibition effect compared to other extracts. (Table 2).

5. Conclusion

Crude extracts of *G. rongklanus* and *G. latestigma* yielded three compounds which were identified as goniotriol (**1**), stigmasterol glucoside (**2**) and pinocembrin (**3**). Extracts of *G. rongklanus* showed effective activity in anti-HIV-1 RT assay. Extracts of *G. rongklanus* and *G. latestigma* were cytotoxic against KB and HT 29 cell lines. Goniotriol showed potent antibacterial activity against all tested micro-organisms.

6. Acknowledgement

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7. References

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