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 ED₅₀ 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 O157: H7, Escherichia coli (ETEC), Escherichia coli (EPEC), Proteus mirabilis, Salmonella typhimurium, 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 latestigma

1. Introduction

The genus Goniothalamus (Annonaceae) include about 185 species, of which 25 species are found in Thailand¹. This genus is widely distributed in the tropical forests of Southeast Asia and are used in traditional medicine. Root barks of G. cheilensis and seeds of G. amuyon are used in the treatment of edema and arthritis²³. The stem bark of G. laoticus has been applied as roborant⁴. G. macrophyllus has been used in the treatment of colds, fever, malaria, cholera, to stimulate blood flow in the body and after childbirth⁵⁶. This genus has been previously reported for styryl lactones, flavonoids, naphthoquinones, azaanthraquinones, alkaloids, and acetogenins⁷–¹¹. 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. Styryl lactone compounds are the most isolated compounds from this genus for example, goniothalamin, goniotriol, cardiobutanolide, goniofurfurone, altholactone, goniopypyrone, goniodiol, and goniothalamin oxide. Goniothalamin has shown cytotoxicity in oral cavity, breast, small cell lung, and vero cell lines.

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.

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 Nakhtonthai, 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 Bruker AV-500 spectrometer in deuterated chloroform (CDCl$_3$) and deuterated methanol (CD$_3$OD) 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 Bruker 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$_2$Cl$_2$ (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 x 5 L) and MeOH (6 x 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 (E₁–E₇) which was characterized by TLC. Fraction E₃ (9.09 g) was further subjected to column chromatography to give 5 subfraction (F₁-F₅). Subfraction F₂ (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 (G₁–G₃). The G₂ (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. The extracts were dissolved in 20 mg/mL of 100% dimethyl sulfoxide (DMSO) after removal of tannin by Polyvinylpyrrolidone (PVP). The final concentration of 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 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. 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% CO₂ 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 (ED₅₀) 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 typhimurium 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)

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 typhimurium, S. flexneri and V. cholera in Mueller Hinton Broth (MHB) were 1x10⁶ 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_{24}O_3, 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), 1416, 1437, 1459 (C = C of aromatic) cm⁻¹. 1H NMR (CD_{3}OD, 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). 13C NMR (CD_{3}OD, 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: 7.45 (2H, m, 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_{2}Cl_{2}: MeOH). C_{35}H_{60}O_{8}, 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. The 13C NMR (CD_{3}OD : 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 13C NMR spectral data was compared with literature.19

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). 13C 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', 3', 4', 5', 6' (Figure 1).
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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, 2', 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 styrllactone) and (2) (a steroid glucoside) isolated from EtOAc stem extract of G. rongklanus were identified as goniotriol and stigmasterol glucoside respectively. Compound (3) (a flavanone) isolated from EtOAc leaves extract of G. latestigma was identified as pinocembrin based on Mass, NMR spectra and analogy with literature data.

| Organism          | Concentrations of MIC/MBC (mg/mL) |
|-------------------|-----------------------------------|
|                   | Goniotriol (1) | Stigmas terol glucoside (2) | Pinocembrin (3) | EtOAc extract G. rongkulanus | MeOH extract G. rongkulanus | EtOAc extract G. latestigma | MeOH extract G. latestigma | Chloramp henicol |
| 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 O157: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       |

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

| Crude extract                        | Anti-HIV-1 RTa (% inhibition) | P-388 | KB | HT 29 | MCF-7 | A 549 | ASK | CL |
|--------------------------------------|-------------------------------|-------|----|-------|-------|-------|-----|----|
| EtOAc extract of G. rongkulanus      | 76.44                         | 4.59  | <4 | <4    | 7.11  | 6.82  | 4.82 | 8.19|
| MeOH extract of G. rongkulanus       | 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|

*aAnt-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

*bCytotoxic assay: ED<sub>50</sub> 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. rongkulanus and G. latestigma
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_{2} as analyzed from the molecular ion peak at m/z 250 of EI-MS. The 1H 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), 4.63 (1H), 4.56 (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 13C NMR spectral data showed eleven carbons resonances, which were defined to a one carbonyl of ester at δ 164.94, 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_{42}O_{6} as analyzed from the molecular ion peak at m/z 574 of EI-MS. In comparison of 13C-NMR with literature 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 13C-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 13C-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_{19}H_{22}O_{4} as analyzed from the molecular ion peak at m/z 256 of EI-MS. The 1H 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 13C 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 against all tested micro-organisms. Extracts of G. rongklanus showed potent antibacterial activity against KB and HT 29 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 - >5mg/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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