VIRAL PROTEIN R INHIBITORS FROM SOME MEDICINAL PLANTS COLLECTED IN MYANMAR

Prema[1], Hnin Htet Wai Nyunt[2], Yi Yi Win[3], Daw Hla Ngwe[4], Hiroyuki Morita[5]

Abstract

The aim of the study is to identity a viral protein R (Vpr) inhibitor from the bioactive selected medicinal plants collected in Myanmar. Their methanol and chloroform extracts were tested separately for their inhibitory effect on Vpr activities on the TREx-HeLa-Vpr cells. The chloroform extracts of P. javanica wood, G. sherwoodiana rhizomes, and *K. candida* roots and rhizomes showed the most potent anti-Vpr activities with cell proliferation percentages of 178%, 160%, and 160% at 10 µg/mL, respectively. Then, the thirty-nine compounds were isolated from these three bioactive samples, identified by UV, FT IR, NMR, HR-ESI-MS, and CD. Five new quassinoids (**1**-**5**), together with ten known compounds (**6**-**15**) were isolated from the active chloroform extract of the *P. javanica* wood. Isolated quassinoids **1**-**4**, **6**-**9**, and 10 displayed potent anti-Vpr activity, with the cell proliferation rate ranging from 148% to 181%, at the 5 µM concentration. Then, two new homodrimane type sesquiterpenoids (**16** and **17**), a new 16-norlabdane diterpenoid (**18**), two new naturally occurring compounds (**19** and **20**), together with eleven known compounds (**21**-**31**) were isolated from the chloroform extract of the *G. sherwoodiana* rhizomes. The isolated compounds **16**, **18**, **19**, **23**, **24**, **27**, and **29** showed moderate anti-Vpr activities at a concentration of 10 µM. In addition, three new compounds (**32**-**34**), together with five known compounds (**35**-**39**) were isolated from the chloroform extract of the *K. candida* roots and rhizomes. All of the isolated compounds showed moderate anti-Vpr activities on the TREx-HeLa-Vpr cells. Among the isolated compounds, **32** and **34**-**38** possessed higher anti-Vpr activity than **33** and **39**.

Keywords: Anti-Vpr activities, *P. javanica* wood, *G. sherwoodiana* rhizomes, *K. candida* roots and rhizomes, isolated compounds

Introduction

Viral protein R (Vpr) is a small basic protein (14 kDa) of 96 amino acids found in HIV-1, HIV-2, and the simian immunodeficiency virus (SIV), and plays an important role in the virus life cycle. Vpr seems to be essential for viral replication in non-dividing cells such as macrophages. It has several functions, including cell cycle arrest at G2/M phase, modulation of CD4 T cell apoptosis, nuclear import of the pre-integration complex (PIC), nuclear localization, cation selective channel activity, and transcriptional activation of HIV-1 LTR and other heterologous promoters (Morellet *et al*., 2003). Considering these functions, the Vpr inhibitor is one of the possible target molecules for anti-HIV drugs. Myanmar is the second largest country in Southeast Asia, and about half of the land area is covered with forest. Approximately 11,800 species belonging to 273 families of plants have been recorded in the Myanmar flora (Kress *et al.*, 2003). Several of these plant species are used in traditional cosmetics and/or folk medicine. However, most of the scientific evidence for the bioactivities of these medicinal plants and phytochemical constituents is still behind the scenes. In previous studies, we reported the chemical constituents such as isopimarane diterpenoids, picrasane quassinoids, terpenoids, iridoids, and bis-iridoid glycosides with potent anti-Vpr activities (Win *et al*., 2016). These results motivated me to continue the search for new Vpr inhibitors as a part of the anti-HIV drug discovery from Myanmar's natural resources. In this study, 11 species of plants, including *Picrasma javanica* wood (Simaroubaceae), *Stemona burkillii* tuber (Stemonaceae), *Zanthoxylum rhetsa* trunks

[1] Department of Chemistry, University of Myitkyina, Myanmar
[2] Department of Chemistry, Dagon University, Myanmar
[3] Department of Chemistry, Hpa-An University, Myanmar
[4] Department of Chemistry, University of Yangon, Myanmar
[5] Institute of Natural Medicine, University of Toyama, Japan

(Rutaceae), and eight rhizomes from the Zingiberaceae family, including *Amomum corynostachyum*, *Curcuma aeruginosa*, *C. comosa*, *C. petiolata*, *Globba sherwoodiana*, *Kaempferia candida*, *K. parvilora*, and *Zingiber zerumbet,* were collected in Myanmar, and their methanol and chloroform extracts with three different concentrations were tested separately for their inhibitory effect on Vpr activities on the TREx-HeLa-Vpr cells. Furthermore, the chloroform extracts of the *P. javanica* wood, *G. sherwoodiana* rhizomes, and *K. candida* roots and rhizomes showed the most potent anti-Vpr activities without any cytotoxicity on the Vpr-induced cells.

Picrasma javanica Blume. is a bitter, medium-sized rainforest tree belonging to the Simaroubaceae family. It is locally known as 'Nann-paw-kyawt' in Myanmar. The plant is found spanning Southeast Asia, Papua New Guinea, India, Indonesia, the Solomon Islands, and Myanmar. *P. javanica* stem bark and leaves are widely used for the treatment of malaria in traditional medicine in Myanmar, Indonesia, Thailand, and northern India. The stem bark and leaves contain quassinoids, indole alkaloids, coumarins, sesquiterpenes, and triterpenes. *P. javanica* extracts and their constituents reportedly possess various biological properties; antimalaria, antioxidant, thrombolytic, cytotoxicity, antibacterial, antiproliferative, membrane stability, hypoglycemic, and anti-Vpr activities (Saiin and Sirithunyalug, 2017, and Win *et al*., 2016).

Globba sherwoodiana (Zingiberaceae) is a small perennial herb from 38 to 45 cm in height with compact rhizomes and tall leafy shoots. It was identified as a new species in 2012 based on its obvious morphology. In Myanmar, it is cultivated and locally known as 'Padein Gno'. *G. sherwooiana* is also cultivated in Thailand and India. The flowers of *G. sherwoodiana* are sold in markets and used for Buddhist offerings in Myanmar and Thailand. The previous phytochemical studies revealed that the genus *Globba* contains labdane diterpenoids, sesquiterpenoids, steroids, lipids, and phenolic compounds. Labdane diterpenoids were reported to possess antibacterial and antifungal activities (Shaaria *et al*., 2009).

The genus *Kaempferia* L. belongs to the Zingiberaceae family and comprises approximately 60 species distributed worldwide. Among them, *K. candida* is a 45-60 cm tall perennial herb that is distributed in Myanmar and Thailand. Its roots are small, tuberous, oblong, and pale brown in colour, and inflorescences are numerous, with white-yellow flowers emerging directly from rhizomes. It grows in the burnt bamboo forest along roadside and teak plantations in both shady and sunny habits. In Myanmar, it is locally known as "Pa-dat-sa", and the young inflorescences and tuberous roots are consumed as a fresh vegetable (Jenjittikul and Larsen, 2000). Previous studies on the plant species of the genus *Kaempferia* have reported the isolation of several sesquiterpenes, diterpenes, diarylheptanoids, essential oils, steroids, flavonoids, and phenolic compounds. These compounds have been reported to possess anticancer, anti-obesity, antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, anti-mutagenicity, and anti-Vpr activities (Win *et al*., 2016; and Chawengrum *et al*., 2018). To the best of our knowledge, the investigation of phytochemical constituents and biological activities of *G. sherwoodiana* and *K. candida* has not been reported yet.

Therefore, this study focused on the isolation and characterization of Vpr inhibitors from three Myanmar plants: *P. javanica* wood, *G. sherwoodiana* rhizomes, and *K. candida* roots and rhizomes.

Materials and Methods

Extraction and Preparation of Test Solution

Eleven plant samples were dried at room temperature and coarsely powdered. The dried powder was macerated with methanol and chloroform to obtain methanol and chloroform crude extracts. After the solvent was evaporated under reduced pressure, the crude extract was dissolved in DMSO and used for the anti-Vpr activities, according to the published procedure.

In Vitro Anti-Vpr Activity Against TREx-HeLa-Vpr Cells

Cell culture and Transfection: TREXTM-HeLa cells were grown in α-minimal essential medium (α-MEM, Wako) supplemented with 10% fetal bovine serum (FBS, Nichirei Bioscience), 1% antibiotic antimycotic solution (Sigma–Aldrich), and 5 μg/mL of blasticidin at 37 °C under a 5 % CO_2 and 95 % air atmosphere. When the cells were 40–50 % confluent, the inducible expression vector (Vpr-c-myc-His6), pc DNA4/TO plasmid encoding full-length Vpr from HIV-1NL4-3 (Genbank) and c-myc-His6-Tag provided by Professor Ikuro Abe at the University of Tokyo, were co-transfected with pcDNA6/TR into TREx-HeLa cells using the calcium chloride transfection method. The medium was supplemented with 200 μg/mL of zeocin, and the culture was continued to select a single stable cell line expressing both the Tet repressor and Vpr genes. Screening the assay system: The anti-Vpr activities of the extracts and the isolated compounds with the three different concentrations were tested on the Vpr induced TREx-HeLa cells, as described previously. Briefly, TREx-HeLa-Vpr cells (12×10^3 cells/well) were seeded in 48-well plates and cultured in Minimum Essential Medium (α-MEM) at 37 °C with a 5% CO_2 and 95% air atmosphere for 24 h. After 24 h of culture, tetracycline solution (50 μL of 10 μg/mL) was added to induce Vpr expression, and the cells were incubated further for 24 h. After incubation, three different concentrations of the extracts and compounds (50 μL) were added to the wells and incubated for 48 h. Afterwards, a 10% MTT solution (50 μL) was added to each well and further incubated for 3 h. The supernatant was then removed, and DMSO (200 μL) was added to each well and incubated for 10–15 min. The absorbance was measured with a microplate reader at 570 nm. The cell proliferation was calculated, and the data was expressed as mean values and standard deviation by the following equation: cell proliferation (%) = 100 × [Abs (treated cell) - Abs (blank)/Abs (control cell)-Abs (blank)]. The cytotoxicities of the extracts and compounds were also evaluated using the same MTT method, without tetracycline induction. Damnacanthal (Dam) was used as a positive control.

General Experimental Procedures

Specific optical rotations were determined by the JASCO P2100 polarimeter (Japan) at standard room temperature (22 °C). Ultravoilet (UV) spectra were recorded on a NanoDrop TM 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) in methanol. Circular dichroism (CD) spectra were recorded on a JASCO-J-805 spectropolarimeter (Japan) in methanol. Fourier-transform infrared (FT IR) spectra were recorded on a JASCO FT/IR-460 Plus spectrometer (Japan). Melting points were measured with a YANACO melting point apparatus (Japan) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) on a JEOL ECA500II spectrometer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in $CDCl_3$. High-resolution mass spectrometry (HR-ESI-MS) data was taken on a SHIMADZU LCMS-IT-TOF (Shimadzu, Kyoto, Japan) spectrometer. Normal phase silica gel (silica gel 60 N, spherical, neutral, 40–50 μm; Kanto Chemical, Tokyo, Japan) and reversed phase silica gel (Cosmosil 75C18-OPN; Nacalai Tesque, Kyoto, Japan) were used for open column chromatography (C.C.). Thin layer chromatography (TLC) was performed using silica gel GF254 precoated (Merck) plates. Reverse-phase HPLC column chromatography

with COSMOSIL 5C18-AR-II (10 × 250 mm) columns was used for separation on an Agilent Technologies 1260 quat pump with a JEOL detector. The absorbance for the biological assay was measured at 570 nm by SH-1200 Microplate Reader (Corona, Hitachinaka, Japan).

Plant Materials for Isolation

The *P. javanica* wood was collected from Kayin State (September 2016), fresh *G. sherwoodiana* rhizomes (10.0 kg) were collected from Pyin Oo Lwin Township, Mandalay Region (October 2018), and fresh (5.0 kg) *K. candida* roots and rhizomes were collected from Pathein Township, Ayeyawady Division (June 2018), Myanmar. The samples were identified by Dr. Khin Cho Cho Oo and Dr. New Ni Tun, botanists at the Department of Botany, University of Yangon. The samples were washed, cut into small pieces, air dried at room temperature, and made into powder by using a grinding machine, and stored in airtight container.

Extraction and Isolation Procedure of Three Selected Samples

The dried powdered *P. javanica* wood sample (1.0 kg) was macerated in methanol (5.0 L × 5) for 2 h with sonication. 97.6 g of methanol extract was obtained after filtration and removal of the solvent by a rotary evaporator, and it was triturated in 50 mL of water and successively partitioned with chloroform to obtain (43.3 g) extract. The chloroform extract (42.4 g) was subjected to normal phase silica gel open C.C., using solvent systems composed of *n*-hexane: $CHCl_3$ and $CHCl_3$: MeOH, to give seven main fractions (F1-F7) after TLC profiling. The fractions (F1-F5) were further subjected to a series of chromatographic separation: **1** (6.0 mg), **2** (20.0 mg), **3** (2.0 mg, R_f = 0.50), **4** (7.9 mg, R_f = 0.34), **5** (0.4 mg, tR 52.0 min), **6** (1.1 g), **7** (45.0 mg), **8** (7.0 mg, tR 42.00 min), **9** (15.0 mg, tR 47.00 min), **10** (17.0 mg, tR 60.5 min), **11** (80.0 mg, tR 74.0 min), **12** (123.0 mg), **13** (2.0 mL/min, tR = 21.65 min, 10.0 mg), **14** (19.0 mg), and **15** (2.0 mL/min, tR = 11.23 min, 5.0 mg) totaling fifteen compounds, were obtained. The dried powder rhizomes of *G. sherwoodiana* (3.2 kg) were extracted with chloroform by sonication (7.0 L, 90 min, × 5) at room temperature to give a residue (80.4 g). The chloroform extract (80.0 g) was subjected to silica gel C.C. eluted with *n*-hexane: EtOAc including (9.5:0.5 to only EtOAc) and EtOAc: MeOH (9:1 and 7:3) to give ten main fractions (F1 to F10) after TLC profiling. From fractions F2, F3, F5, F6, F7, and F8, **16** (25.0 mg), **17** (10.0 mg), **18** (11.0 mg), **19** (5.0 mg), **20** (20.0 mg), **21** (1.5 mg), **22** (2.0 mg), **23** (30.0 mg), **24** (25.7 mg), **25** (0.9 mg), **26** (2.0 mg), **27** (35.0 mg), **28** (5.0 mg), **29** (10.0 mg), **30** (1.0 mg), and **31** (7.2 mg), totaling sixteen compounds, were obtained. by silica gel C.C, eluted with an *n*-hexane: CH_2Cl_2: EtOAc (40:40:1 and 20:20:1). The dried powdered roots and rhizomes of *K. candida* (1.4 kg) were extracted with methanol (7.0 L, 90 min × 5) at room temperature by sonication, and it was evaporated under reduced pressure to obtain the extract (158.0 g). The residue was suspended in water and partitioned with chloroform (500 mL×8) to yield a chloroform soluble fraction (37.1 g). The chloroform extract (36.0 g) was chromatographed on a silica gel C.C. with gradient elution using *n*-hexane: EtOAc (9:1 to EtOAc only) to yield five fractions (F1-F5), which were combined based on TLC analysis. The fractions (F2-F5) were chromatographed by normal phase silica gel C.C. eluted with *n*-hexane: EtOAc and EtOAc: $CHCl_3$ (7:3 and 1:1) as solvent systems, to give eight compounds, **32** (2.5 mg), **33** (5.0 mg), **34** (2.5 mg), **35** (59.3 mg), **36** (10.0 mg), **37** (11.3 mg), **38** (11.0 mg), and **39** (50.0 mg).

DFT/TDDFT Calculations

A conformation based on the X-ray crystallographic structure of compound-**10** was optimized, using RI-DFT at the M06-L/TZVP level of theory. Excited states calculations (oscillator and rotatory strengths) were performed on the optimised ground state geometry at the

TDDFT M06-L/augcc-pVTZ level of theory. All DFT and TDDFT calculations were performed using Turbomole 7.1.

Results and Discussion

Anti-Vpr Activity of Various Crude Extracts of Eleven Medicinal Plants

In this study, methanol and chloroform extracts (Figures 1 and 2) of 11 species of plants were collected in Myanmar and tested separately for their inhibitory effect on Vpr activities on the TREx-HeLa-Vpr cells. The screening results revealed that the chloroform extracts possessed stronger anti-Vpr activities than the methanol extracts. Furthermore, the chloroform extracts of the *P. javanica* wood, *G. sherwoodiana* rhizomes, and *K. candida* roots and rhizomes showed the most potent anti-Vpr activities without any cytotoxicity on the Vpr-induced cells, with cell proliferation percentages of 178 %, 160 %, and 160 % at 10 μg/mL, respectively.

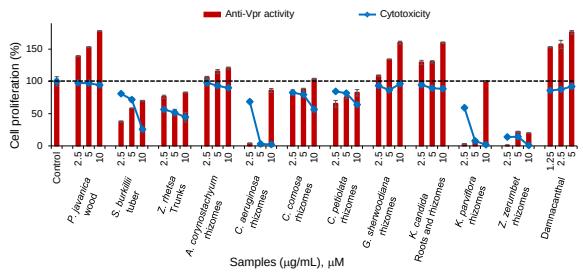

Figure1. Inhibitory effects of the chloroform extract of tested samples and positive control, damnacanthal, on Vpr expression. Data are represented as mean ± SD (n = 3)

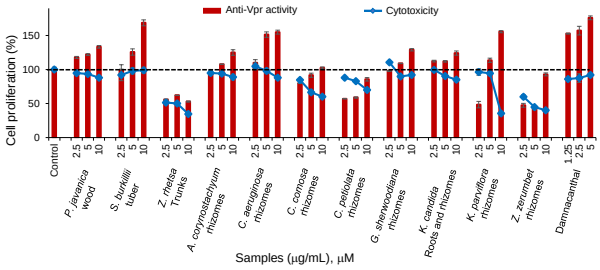

Figure 2. Inhibitory effects of the methanol extract of tested samples and positive control, damnacanthal, on Vpr expression. Data are represented as mean ± SD (n = 3)

Structure Elucidation of Isolated Compounds (1-15) from the Wood of *P. javanica*

Five new quassinoids (**1-5**) together with ten known compounds (**6-15**) (Figure 3) were isolated from the active chloroform extract of the wood of *P. javanica*.

(16*R*)-Methoxyjavanicin B (1): white amorphous powder; $[\alpha]_D^{22}$ −14.9° (*c* 0.1, MeOH); CD (*c* 0.01, MeOH): 245 (Δε −5.75), 268 (Δε 19.11) nm; UV (MeOH) λ_{max}: 218, 259 nm; IR (KBr) ν_{max}: 2945, 2839, 1690, 1638, 1454, 1370, 1213, 1130, 1042 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 5.46 (dd, *J* = 5.7, 2.3 Hz, H-3), 2.12 (dt, *J* = 18.9, 5.7 Hz, H-4*α*), 2.21 (m, H-4*β*), 2.44 (m, H-5), 1.57 (dt, *J* = 14.3, 2.9 Hz, H-6*α*), 1.86 (m, H-6*β*), 3.31 (t, *J* = 2.9 Hz, H-7), 3.17 (s, H-9), 1.96 (dd, *J* = 12.6, 4.6 Hz, H-14), 1.67 (dt, *J* = 12.6, 2.9 Hz, H-15*α*), 2.03 (m, H-15*β*), 4.35 (dd, *J* = 9.7, 2.9 Hz, H-16), 1.48 (s, H_3-18), 1.05 (s, H_3-19), 1.82 (s, H_3-20), 3.55 (s, 2-OCH_3), 3.64 (s, 12-OCH_3), 3.46 (s, 16-OCH_3); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 197.9 (C-1), 149.4 (C-2), 109.6 (C-3), 27.4 (C-4), 36.5 (C-5), 29.4 (C-6), 77.6 (C-7), 39.1 (C-8), 46.7 (C-9), 46.2 (C-10), 193.2 (C-11), 148.4 (C-12), 137.9 (C-13), 49.8 (C-14), 32.5 (C-15), 102.7 (C-16), 11.6 (C-18), 21.9 (C-19), 15.3 (C-20), 55.1 (2-OCH_3), 59.2 (12-OCH_3), 56.2 (16-OCH_3); HR-ESI-MS: *m/z* 391.2100 $[M+H]^+$ (calcd. for $C_{22}H_{31}O_6$, 391.2115) (Prema *et al*., 2019)

(16*S*)-Methoxyjavanicin B (2): colorless solid; m.p. 228–230 °C; $[\alpha]_D^{22}$ +14.3° (*c* 0.1, MeOH); CD (*c* 0.01, MeOH): 244 (Δε −5.82), 268 (Δε 19.11) nm; UV (MeOH) λ_{max}: 219, 260 nm; IR (KBr) ν_{max}: 2941, 2840, 1690, 1639, 1453, 1369, 1213, 1042 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 5.46 (dd, *J* = 5.7, 2.3 Hz, H-3), 2.10 (dt, *J* = 18.3, 5.7 Hz, H-4*α*), 2.23 (m, H-4*β*), 2.35 (m, H-5), 1.44

(dt, *J* = 14.4, 2.9 Hz, H-6*α*), 1.90* (m, H-6*β*), 3.60 (t, *J* = 2.9 Hz, H-7), 3.17 (s, H-9), 2.28 (dd, *J* = 12.6, 4.6 Hz, H-14), 1.88 (dt, *J* = 12.6, 2.9 Hz, H-15*α*), 1.93* (m, H-15*β*), 4.78 (brd, *J* = 2.9 Hz, H-16), 1.50 (s, H_3-18), 1.07 (s, H_3-19), 1.84 (s, H_3-20), 3.58 (s, 2-OCH_3), 3.64 (s, 12-OCH_3), 3.37 (s, 16-OCH_3); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 198.4 (C-1), 149.4 (C-2), 109.5 (C-3), 27.4 (C-4), 36.8 (C-5), 29.2 (C-6), 69.4 (C-7), 38.9 (C-8), 46.0 (C-9), 46.1 (C-10), 193.0 (C-11), 148.4 (C-12), 139.5 (C-13), 44.1 (C-14), 31.1 (C-15), 97.7 (C-16), 11.5 (C-18), 22.2 (C-19), 15.4 (C-20), 55.0 (2-OCH_3), 59.2 (12-OCH_3), 54.7 (16-OCH_3); HR-ESI-MS: *m/z* 391.2070 $[M+H]^+$ (calcd. for $C_{22}H_{31}O_6$, 391.2115) (Prema *et al*., 2019)

Javanicinol A (3): white amorphous powder; $[\alpha]_D^{22}$ +14.8° (*c* 0.1, MeOH); CD (*c* 0.01, MeOH): 230 (Δ*ε* −12.13), 280 (Δ*ε* 7.29), 332 (Δ*ε* −4.39) nm; UV (MeOH) λ_{max}: 219, 250 nm; IR (KBr) ν_{max}: 3428, 2945, 2844, 1720, 1640, 1452, 1384, 1250, 1034 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.79 (dd, *J* = 12.6, 2.9 Hz, H-3), 1.79* (m, H-4*α*), 1.87 (dt, *J* = 12.6, 2.9 Hz, H-4*β*), 1.58 (tt, *J* = 12.6, 2.9 Hz, H-5), 1.76* (m, H-6*α*), 1.98 (ddd, *J* = 14.3, 12.6, 2.9 Hz, H-6*β*), 4.24 (t, *J* = 2.9 Hz, H-7), 3.04 (s, H-9), 2.39 (dd, *J* = 12.0, 6.9 Hz, H-14), 2.57 (dd, *J* = 18.3, 12.0 Hz, H-15*α*), 2.98 (dd, *J* = 18.3, 6.9 Hz, H-15*β*), 1.61 (s, H_3-18), 1.19 (s, H_3-19), 1.89 (s, H_3-20), 3.47 (s, 2*α* -OCH_3), 3.41 (s, 2*β*-OCH_3), 3.63 (s, 12-OCH_3); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 204.2 (C-1), 101.1 (C-2), 74.9 (C-3), 33.8 (C-4), 33.0 (C-5), 28.8 (C-6), 82.1 (C-7), 37.1 (C-8), 47.6 (C-9), 49.0 (C-10), 190.8 (C-11), 148.4 (C-12), 139.7 (C-13), 47.4 (C-14), 31.6 (C-15), 168.8 (C-16), 11.4 (C-18), 23.0 (C-19), 15.8 (C-20), 51.8 (2*α*-OCH_3), 51.9 (2*β*-OCH_3), 59.7 (12-OCH_3); HR-ESI-MS: *m/z* 445.1850 $[M+Na]^+$ (calcd. for $C_{22}H_{30}O_8Na$, 445.1833) (Prema *et al*., 2020)

Javanicinol B (4): white amorphous powder; $[\alpha]_D^{22}$ −15.9° (*c* 0.1, MeOH); CD (*c* 0.01, MeOH): 234 (Δ*ε* −3.26), 264 (Δ*ε* 7.09), 330 (Δ*ε* −4.47) nm; UV (MeOH) λ_{max}: 217, 249 nm; IR (KBr) ν_{max}: 3378, 2940, 2842, 1722, 1638, 1451, 1380, 1249, 1035 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 4.18 (t, *J* = 2.9 Hz, H-3), 1.59 (dt, *J* = 14.3, 2.9 Hz, H-4*α*), 2.15 (td, *J* = 14.3, 2.9 Hz, H-4*β*), 2.32 (tt, *J* = 14.3, 2.9 Hz, H-5), 1.73 (dt, *J* = 14.9, 2.9 Hz, H-6*α*), 2.00 (ddd, *J* = 14.9, 14.3, 2.9 Hz, H-6*β*), 4.23 (t, *J* = 2.9 Hz, H-7), 3.14 (s, H-9), 2.39 (dd, *J* = 12.6, 6.9 Hz, H-14), 2.60 (dd, *J* = 18.3, 12.6 Hz, H-15*α*), 2.95 (dd, *J* = 18.3, 6.9 Hz, H-15*β*), 1.62 (s, H_3-18), 1.19 (s, H_3-19), 1.88 (s, H_3-20), 3.32 (s, 2*α* -OCH_3), 3.34 (s, 2*β*-OCH_3), 3.62 (s, 12-OCH_3); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 204.9 (C-1), 101.9 (C-2), 70.6 (C-3), 29.9 (C-4), 34.2 (C-5), 28.8 (C-6), 82.4 (C-7), 37.0 (C-8), 47.8 (C-9), 50.5 (C-10), 191.1 (C-11), 148.4 (C-12), 139.7 (C-13), 47.7 (C-14), 31.6 (C-15), 168.9 (C-16), 11.3 (C-18), 23.5 (C-19), 15.7 (C-20), 52.2 (2*α*-OCH_3), 48.2 (2*β*-OCH_3), 59.7 (12-OCH_3); HR-ESI-MS: *m/z* 445.1811 $[M+Na]^+$ (calcd. for $C_{22}H_{30}O_8Na$, 445.1833) (Prema *et al*., 2020)

4-Keto-(16*S*)-methoxyjavanicin B (5): white amorphous powder; $[\alpha]_D^{22}$ +20.8° (*c* 0.1, MeOH); CD (*c* 0.01, MeOH): 215 (Δ*ε* 3.35), 249 (Δ*ε* −3.53), 273 (Δ*ε* 9.93), 337 (Δ*ε* −1.99), 370 (Δ*ε* 2.36) nm; UV (MeOH) λ_{max}: 213, 259 nm; IR (KBr) ν_{max}: 2936, 2848, 1723, 1678, 1637, 1608, 1445, 1386, 1264, 1051 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.77 (s, H-3), 3.13 (dd, *J* = 18.3, 5.7 Hz, H-5), 2.06 (dt, *J* = 14.9, 3.4 Hz, H-6*α*), 1.96* (m, H-6*β*), 3.72 (t, *J* = 3.4 Hz, H-7), 3.42 (s, H-9), 2.35 (dd, *J* = 12.6, 5.2 Hz, H-14), 1.87 (ddd, *J* = 14.5, 5.2, 3.4 Hz, H-15*α*), 1.98* (m, H-15*β*), 4.78 (brd, *J* = 3.4 Hz, H-16), 1.49 (s, H_3-18), 1.07 (s, H_3-19), 1.85 (s, H_3-20), 3.77 (s, 2-OCH_3), 3.63 (s, 12-OCH_3), 3.35 (s, 16-OCH_3); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 196.8 (C-1), 162.9 (C-2), 108.9 (C-3), 197.7 (C-4), 50.2 (C-5), 22.6 (C-6), 68.4 (C-7), 38.6 (C-8), 45.9 (C-9), 49.8 (C-10), 192.1 (C-11), 148.2 (C-12), 141.5 (C-13), 44.3 (C-14), 30.9 (C-15), 97.5 (C-16), 16.2 (C-18), 22.0 (C-19), 15.6 (C-20), 56.6 (2-OCH_3), 59.5 (12-OCH_3), 54.8 (16-OCH_3); HR-ESI-MS: *m/z* 405.1900 $[M+H]^+$ (calcd. for $C_{22}H_{29}O_7$, 405.1908) (Prema *et al*., 2020)

Javanicin F (**6**), Javanicin B (**7**), Picrajavanicin B (**8**), Picrajavanicin C (**9**) (Win *et al*., 2015) Picrajavanicin H (**10**), Picrajavanicin M (**11**), Picrasin A (**12**) (Win *et al*., 2016), Canthin-3-one (**13**) (Koike and Ohmoto, 1985), Lanosta-7,24-dien-3-one (**14**) (Nana *et al*., 2012), and Scopoletin (**15**) (Darmawan *et al*., 2012) are shown in Figure 3.

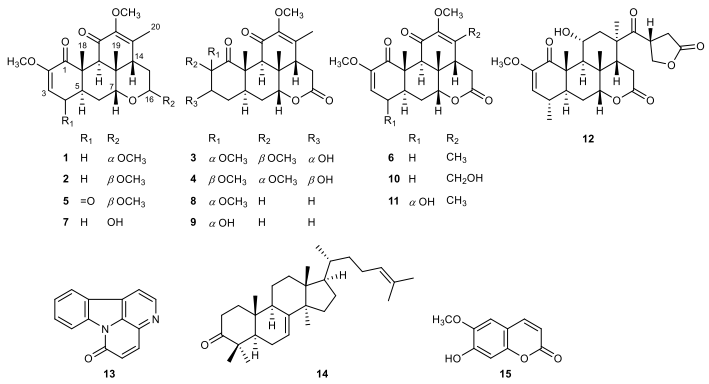

Figure 3. Structures of compounds1-15 isolated from the chloroform extract of P. javanica.

Anti-Vpr Activities of Isolated Compounds from *P. javanica* Wood

The anti-Vpr activities of all isolated compounds (**1-4** and **6-15**) were screened for their inhibition of growing the TREx-HeLa-Vpr cells with the three different concentrations of 1.25, 2.5, and 5 μM (Figure 4). The anti-Vpr activities of the isolated compounds **1**, **2**, **3**, **4**, and **13-15** were investigated for the first time. Compound **5** was not screened for anti-Vpr activity due to the insufficient yield. Among the tested compounds, all isolated compounds showed potent anti-Vpr activities without showing any cytotoxicity at 5 μM. The % cell proliferation of the anti-Vpr activities of all tested compounds and Dam were 165 % (**1**),167 % (**2**), 168 % (**3**), 163 % (**4**), 162 % (**6**), 181 % (**7**), 159 % (**8**), 170 % (**9**), 148 % (**10**), 142 % (**11**), 134 % (**12**), 153 % (**13**), 104 % (**14**), 128 % (**15**), and 176 % (Dam) at the 5 μM concentration, respectively. The previous study reported that quassinoid compounds **6**–**12** exhibited potent anti-Vpr activities on the TREx-HeLa-Vpr cells, which are similar to the assay results in this study.

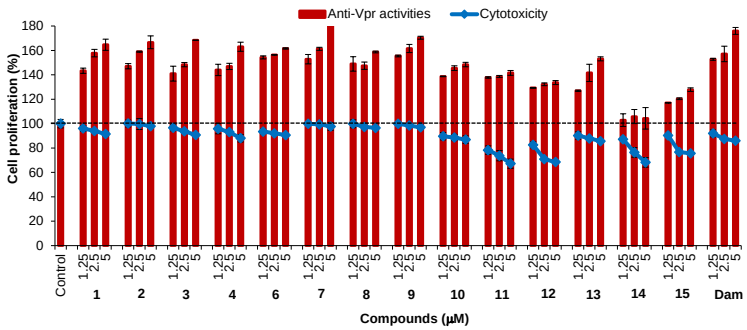

Figure 4. Anti-Vpr activities of 1–15 from *P. javanica*. Data are represented as mean ± SD (n = 3)

In the structure-activity relationship (SAR), the presence of a methyl group at C-13 and a hydroxy group at C-16 of the C-20 type quassinoids was found to be important for their potent anti-Vpr activities, as previously reported. Moreover, comparisons of the structure and activity of **1**, **2**, and **7** with neoquassin type suggested that **1** and **2** have slightly weaker anti-Vpr activities than **7** at the 1.25 μM concentration. These results revealed that the presence of the methoxy group at C-16 was the most essential for Vpr inhibitory activities, as seen by comparing **2** and **7**. In contrast, the anti-Vpr activities of **1** and **2** showed the same value of (%) cell viability, suggesting

that C-16 epimers do not affect their potencies. On the other hand, quassin types **3**, **4**, and **9** revealed that **3** and **4** had slightly weaker activities than **9** at the 1.25 μM concentration. This observation indicated that the absence of the *β*-methoxy group and/or the hydroxy group at C-2 in the quassin type quassinoid may play a critical role in enhancing the anti-Vpr activity.

Structure Elucidation of Isolated Compounds (16-31) from the Rhizomes of *G. sherwoodiana*

Three new compounds (**16-18**), two new naturally occurring compounds (**19-20**), and eleven known compounds (**21-31**) (Figure 5) were isolated from the chloroform extract of the *G. sherwoodiana* rhizomes.

Globbatone A (16): pale yellowish oil; $[\alpha]_D^{22}$ +41.7° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 208, 218 nm; IR (KBr) ν_{max} 3313, 2925, 2855, 1729, 1465, 1372, 1266, 1181, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.71* (m, H-1*α*), 1.00 (td, *J* = 13.2, 4.2 Hz, H-1*β*), 1.58 (dt, *J* = 13.2, 3.4 Hz, H-2*α*), 1.49 (m, H-2*β*), 1.38 (m, H-3*α*), 1.17 (td, *J* = 13.2, 4.2 Hz, H-3*β*), 1.09 (dd, *J* = 12.9, 2.7 Hz, H-5), 1.71* (m, H-6*α*), 1.31 (qd, *J* = 12.9, 4.2 Hz, H-6*β*), 2.38 (ddd, *J* = 12.9, 4.2, 2.4 Hz, H-7*α*), 1.97 (dt, *J* = 12.9, 4.2 Hz, H-7*β*), 1.71* (m, H-9), 1.83 (m, H-11a), 1.71* (m, H-11b), 4.22 (m, H-16a), 3.98 (m, H-16b), 3.86 (t, *J* = 5.5 Hz, H_2-3′), 2.56 (t, *J* = 5.5 Hz, H_2-2′), 4.84 (brs, H-12a), 4.54 (brs, H-12b), 0.88 (s, H_3-13), 0.81 (s, H_3-14), 0.68 (s, H_3-15); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 39.1 (C-1), 19.4 (C-2), 42.1 (C-3), 33.7 (C-4), 55.6 (C-5), 24.4 (C-6), 38.2 (C-7), 148.2 (C-8), 53.1 (C-9), 39.5 (C-10), 23.2 (C-11), 106.7 (C-12), 33.6 (C-13), 21.8 (C-14), 14.4 (C-15), 64.8 (C-16), 173.1 (C-1′), 36.8 (C-2′), 58.4 (C-3′); HR-ESI-MS *m/z* 331.2225 $[M+Na]^+$ (calcd. for $C_{19}H_{32}O_3Na$, 331.2244) (Prema *et al*., 2020)

Globbatone B (17): pale yellowish oil; $[\alpha]_D^{22}$ +20.5° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 208, 218 nm; IR (KBr) ν_{max} 2934, 2830, 1735, 1465, 1378, 1266, 1181, 1035 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.72* (m, H-1*α*), 1.00 (td, *J* = 12.8, 4.2 Hz, H-1*β*), 1.55 (dt, *J* = 13.3, 3.4 Hz, H-2*α*), 1.48 (m, H-2*β*), 1.38 (m, H-3*α*), 1.17 (td, *J* = 13.3, 4.2 Hz, H-3*β*), 1.08 (dd, *J* = 12.8, 2.7 Hz, H-5), 1.72* (m, H-6*α*), 1.30 (qd, *J* = 12.8, 4.2 Hz, H-6*β*), 2.38 (ddd, *J* = 12.8, 4.2, 2.4 Hz, H-7*α*), 1.96 (dt, *J* = 12.8, 4.2 Hz, H-7*β*), 1.72* (m, H-9), 1.83 (m, H-11a), 1.72* (m, H-11b), 4.24 (m, H-16a), 3.95 (m, H-16b), 8.03 (s, 4′-CHO), 4.43 (td, *J* = 6.3, 0.6 Hz, H_2-3′), 2.67 (t, *J* = 6.3 Hz, H_2-2′), 4.83 (brs, H-12a), 4.53 (brs, H-12b), 0.86 (s, H_3-13), 0.79 (s, H_3-14), 0.67 (s, H_3-15); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 39.1 (C-1), 19.4 (C-2), 42.2 (C-3), 33.7 (C-4), 55.6 (C-5), 24.4 (C-6), 38.2 (C-7), 148.1 (C-8), 53.1 (C-9), 39.5 (C-10), 23.0 (C-11), 106.7 (C-12), 33.7 (C-13), 21.8 (C-14), 14.4 (C-15), 64.9 (C-16), 170.5 (C-1′), 33.8 (C-2′), 59.5 (C-3′), 160.7 (C-4′); HR-ESI-MS *m/z* 359.2208 $[M+Na]^+$ (calcd. for $C_{20}H_{32}O_4Na$, 359.2193) (Prema *et al*., 2020)

Globbatone C (18): pale yellowish oil; $[\alpha]_D^{22}$ +50.4° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 214, 267 nm; IR (KBr) ν_{max} 3353, 2934, 2840, 1762, 1720, 1649, 1462, 1380, 1279, 1188, 1026 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.70* (m, H-1*α*), 1.05 (td, *J* = 13.6, 3.4 Hz, H-1*β*), 1.60 (m, H-2*α*), 1.50 (m, H-2*β*), 1.37 (m, H-3*α*), 1.17 (td, *J* = 13.3, 4.1 Hz, H-3*β*), 1.03 (dd, *J* = 12.8, 1.9 Hz, H-5), 1.70* (m, H-6*α*), 1.32 (qd, *J* = 12.8, 5.0 Hz, H-6*β*), 2.36 (m, H-7*α*), 1.93 (td, *J* = 12.8, 5.0 Hz, H-7*β*), 1.54 (brd, *J* = 2.3 Hz, H-9), 1.87 (m, H-11a), 1.53 (m, H-11b), 2.56 (dd, *J* = 9.0, 3.6 Hz, H-12a), 2.29 (m, H-12b), 2.62 (dd, *J* = 9.5, 4.8 Hz, H-14), 3.82 (t, *J* = 4.8 Hz, H-15), 4.81 (brs, H-17a), 4.42 (brs, H-17b), 0.85 (s, H_3-18), 0.79 (s, H_3-19), 0.67 (s, H_3-20); ^{13}C NMR (500 MHz, $CDCl_3$) δ_C 39.1 (C-1), 19.4 (C-2), 42.2 (C-3), 33.7 (C-4), 55.6 (C-5), 24.5 (C-6), 38.3 (C-7), 148.4 (C-8), 56.3 (C-9), 39.9 (C-10), 17.4 (C-11), 42.5 (C-12), 212.5 (C-13), 44.5 (C-14), 58.0 (C-15), 106.4 (C-17), 33.7 (C-18), 21.8 (C-19), 14.4 (C-20); HR-ESI-MS *m/z* 315.2293 $[M+Na]^+$ (calcd. for $C_{19}H_{32}O_2Na$, 315.2295) (Prema *et al*., 2020)

(*E*)-Labda-8(17),12-dien-15,16-olide (**19**) (Sheeja and Nair, 2014), γ-Bicyclohomofarnesen-12-ol (**20**) (Boukouvalas and Wang, 1988), 13,14,15,16-tetranor-8(17)-labden-12-al (**21**) (Kumar and

Chein, 2014), Zerumin (**22**) (Xu *et al*., 1995), 16-oxo-(8)17–12-labdadien-15,11-olide (**23**) (Shaaria *et al*., 2009), Amoxanthin A (**24**) (Win *et al*., 2017), Sceptrumlabdalactone A (**25**) (Ali *et al*., 2011), Coronarin D ethyl ether (**26**), Coronarin D methyl ether (**27**) (Chimnoi *et al*., 2008), Pahangensin B (**28**), (*E*)-Labda-8(17),12-dien-15,16-dial (**29**) (Win *et al*., 2017), Calcaratarin A (**30**), and Kravanhin B (**31**) (Yin *et al*., 2013).

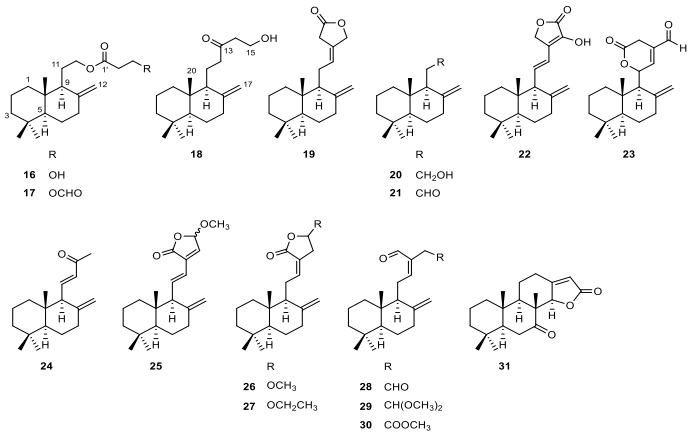

Figure 5. Structures of compounds 16-31 isolated from the chloroform extract of *G. sherwoodiana*

Anti-Vpr Activities of Isolated Compounds from *G. sherwoodiana* Rhizomes

Except for **21**, **22**, **25**, **26**, and **30** with insufficient yields, the anti-Vpr activities of **16**–**20**, **23**, **24**, **27**–**29**, and **31** (2.5, 5, 10 μM) were screened. The results (Figure 6) showed that **16**, **18**-**27**, and **29**-**31** had moderate anti-Vpr activities, although **31** possessed a weak cytotoxic effect at the concentration of 10 μM. In contrast, **17** and **20** were found to slightly inhibit the Vpr activity, without any cytotoxicity. Compared with the other tested compounds, **28** did not inhibit the Vpr activity in the TREx-HeLa-Vpr cells. The order of the anti-Vpr activities of the isolated compounds and positive control Dam at the 10 μM concentration was Dam (141%) > **27** (130 %) =**18** (129 %) > **23** (123 %) = **31** (122 %) > **29** (120 %) = **19** (119 %) = **16** (118 %) > **24** (116 %) > **20** (103 %) > **17** (92 %) > **28** (58 %), respectively. However, the isolated compounds **16**, **18**, **19**, **23**, **24**, **27**, **29**, and **31** had weaker anti-Vpr activities as compared to Dam.

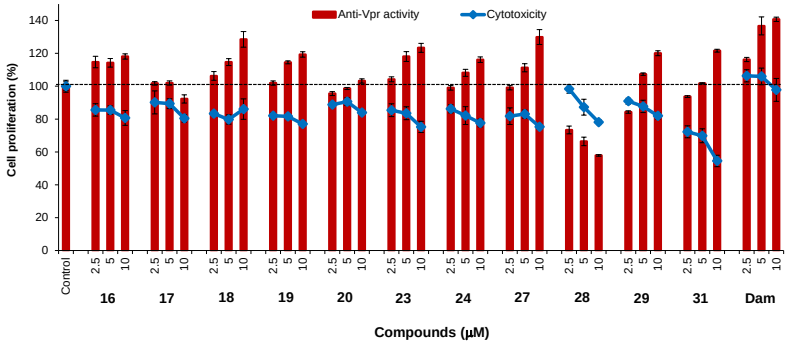

Figure 6. Anti-Vpr activities of 16–31 from *G. sherwoodiana*. Data are represented as mean ± SD (n = 3).

The structure–activity relationship (SAR) study of homodrimane sesquiterpenes revealed that **16** possessed stronger activity than those of **17** and **20**, suggesting that the presence of the hydroxypropionate group at C-12 was important for increasing the activities. Comparisons of the 8(17)-eomethylene labdane diterpenoids, **18**, **19**, **23**, **24**, **27**, and **31** revealed the higher potency of

28, suggesting that the presence of the lactone ring and carbonyl group could be the important functionalities to enhance the activity.

Structure Elucidation of Isolated Compounds (32-39) from the Roots and Rhizomes of *K. candida*

Three new compounds (**32-34**), together with five known compounds (**35**-**39**) (Figure 7) were isolated from the chloroform extract of the *K. candida* roots and rhizomes.

7-Hydroxymustakone (32): colorless oil; $[\alpha]_D^{22}$ –58.6° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 205, 218, 229, 260 nm; IR (KBr) ν_{max} 3440, 2928, 2865, 1725, 1661, 1460, 1380, 1164 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 2.57 (dd, *J* = 6.9, 1.1 Hz, H-1), 5.79 (d, *J* = 1.5 Hz, H-3), 2.62 (dd, *J* = 6.9, 1.5 Hz, H-5), 2.69 (s, H-6), 1.92 (m, H-8*α*), 1.64 (m, H-8*β*), 1.89 (m, H-9*α*), 1.78 (m, H-9*β*), 1.73 (sept, *J* = 6.9 Hz, H-11), 0.92 (d, *J* = 6.9 Hz, H_3-12), 0.88 (d, *J* = 6.9 Hz, H_3-13), 2.04 (s, H_3-14), 0.99 (s, H_3-15); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 57.6 (C-1), 203.1 (C-2), 121.9 (C-3), 170.3 (C-4), 49.8 (C-5), 60.5 (C-6), 76.1 (C-7), 29.3 (C-8), 34.5 (C-9), 57.4 (C-10), 36.7 (C-11), 16.9 (C-12), 16.4 (C-13), 23.9 (C-14), 20.2 (C-15); HR-ESI-MS: *m/z* 257.1515 $[M+Na]^+$ (calcd. for $C_{15}H_{22}O_2Na$, 257.1512) (Prema *et al*., 2020)

15-Hydroxynerolidol (33): colorless oil; $[\alpha]_D^{22}$ +18.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 211, 218, 228, 276 nm; IR (KBr) ν_{max} 3397, 2926, 2871, 1720, 1643, 1448, 1380, 1063 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 5.38 (dd, *J* = 17.3, 2.3 Hz, H-1a), 5.29 (dd, *J* = 10.8, 2.3 Hz, H-1b), 5.82 (dd, *J* = 17.3, 10.8 Hz, H-2), 1.65 (m, H-4a), 1.53 (m, H-4b), 2.12 (m, H-5a), 1.99 (m, H-5b), 5.13 (m, H-6), 1.97 (m, H_2-8), 2.04 (m, H_2-9), 5.06 (m, H-10), 1.59* (s, H_3-12), 1.66 (s, H_3-13), 1.59* (s, H_3-14), 3.48 (dd, *J* = 21.8, 10.8 Hz, H_2-15); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 115.5 (C-1), 140.9 (C-2), 76.5 (C-3), 36.9 (C-4), 22.1 (C-5), 124.4 (C-6), 136.2 (C-7), 39.8 (C-8), 26.8 (C-9), 124.2 (C-10), 131.7 (C-11), 25.9 (C-12), 17.9 (C-13), 16.2 (C-14), 69.1 (C-15); HR-ESI-MS: *m/z* 261. 1814 $[M+Na]^+$ (calcd. for $C_{15}H_{26}O_2Na$, 261. 1825) (Prema *et al*., 2020)

Kaempcandiol (34): white amorphous solid; $[\alpha]_D^{22}$ +46.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max}: 209, 219, 223 nm; IR (KBr) ν_{max} 3440, 2934, 2865, 1702, 1652, 1542, 1523, 1457, 1396, 1161, 1067 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.69 (m, H-1*α*), 1.06 (td, *J* = 12.9, 3.7 Hz, H-1*β*), 1.55 (m, H-2*α*), 1.48 (m, H-2*β*), 1.38 (m, H-3*α*), 1.18 (td, *J* = 12.9, 3.7 Hz, H-3*β*), 1.16 (dd, *J* = 12.9, 2.7 Hz, H-5), 1.72 (m, H-6*α*), 1.31 (qd, *J* = 12.9, 4.2 Hz, H-6*β*), 2.39 (ddd, *J* = 12.9, 4.2, 2.7 Hz, H-7*α*), 2.03 (td, *J* = 12.9, 5.6 Hz, H-7*β*), 2.08 (m, H-9), 1.70 (m, H_2-11), 4.45 (dd, *J* = 10.0, 4.9 Hz, H-12), 6.32 (s, H-16a), 5.89 (s, H-16b), 4.85 (s, H-17a), 4.59 (s, H-17b), 0.87 (s, H_3-18), 0.79 (s, H_3-19), 0.66 (s, H_3-20); ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C 39.1 (C-1), 19.5 (C-2), 42.2 (C-3), 33.8 (C-4), 55.6 (C-5), 24.5 (C-6), 38.4 (C-7), 148.9 (C-8), 52.4 (C-9), 39.4 (C-10), 31.3 (C-11), 70.1 (C-12), 142.9 (C-13), 170.3 (C-14), 126.6 (C-16), 106.2 (C-17), 33.8 (C-18), 21.9 (C-19), 14.8 (C-20); HR-ESI-MS: *m/z* 305.2122 $[M-H]^-$ (calcd. for $C_{15}H_{25}O_2$, 305.2122) (Prema *et al*., 2020)

Mustakone (**35**) (Nyasse *et al*., 1988), Coronadiene (**36**) (Win *et al*., 2017), Aromaticane J (**37**) (Dong *et al*., 2017), Longpene A (**38**) (Xu *et al*., 2015), and Docosyl ferulate (**39**) (Nishiyama *et al*., 2019).

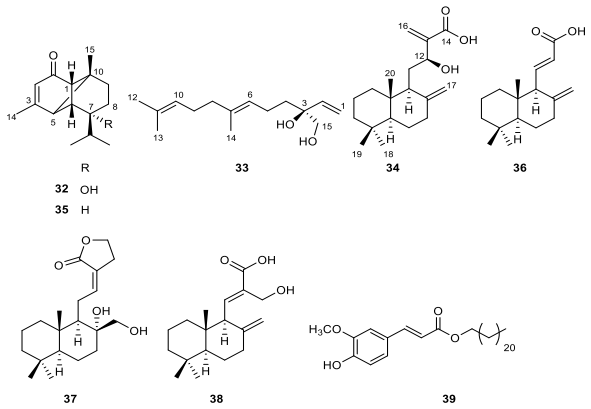

Figure 7. Structures of compounds 32-39 isolated from the chloroform extract of *K. candida*

Anti-Vpr Activities of Isolated Compounds from *K. candida* Roots and Rhizomes

All isolated compounds **32**-**39** and positive control Dam (Figure 8) were screened for their anti-Vpr activity in TREx-HeLa-Vpr cells. Compounds **32** and **34**-**39** possessed potent anti-Vpr activities at the 5 μM concentration, without showing any cytotoxicity. Compound **33** exhibited moderate anti-Vpr activities at the 5 μM concentration, although this compound possessed a weak cytotoxic effect. The anti-Vpr activities of tested compounds and Dam were 159 % (Dam), 136 % (**32**), 113 % (**33**), 131 % (**34**), 137 % (**35**), 129 % (**36**), 135 % (**37**), 134 % (**38**), and 120 % (**39**) at the 5 μM concentration, respectively.

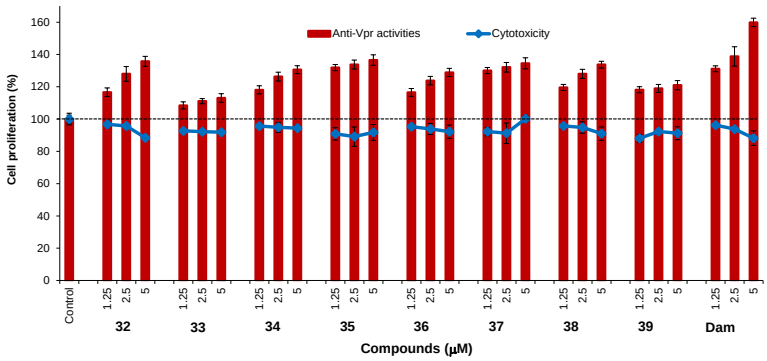

Figure 8. Anti-Vpr activities of 32–39 from *K. candida*. Data are represented as mean ± SD (n = 3).

SAR of sesquiterpenes **32** and **35** showed stronger anti-Vpr activities than **33**, suggesting that tricyclic sesquiterpenes with *α*, *β*-unsaturated ketone group at C-2 could be potential candidates for the anti-Vpr activities. On the other hand, the labdane diterpenes **34** and **36**-**38** possessed potent anti-Vpr activities, revealing that the presence of tertiary carboxylic acid and 8(17)-exomethylene and/or hemiacetal groups may play essential roles for anti-Vpr activities.

Conclusion

In this study, the chemical investigation of the active chloroform extracts of the *P. javanica* wood, *G. sherwoodiana* rhizomes, and *K. candida* roots and rhizomes led to the isolation of 39 compounds, including eleven new ones, namely (16*R*)-methoxy javanicin B (**1**), (16*S*)-methoxyjavanicin B (**2**), javanicinols A and B (**3** and **4**), 4-keto-(16*S*)-methoxyjavanicin B (**5**), globbatones A-C (**16**-**18**), 7-hydroxymustakone (**32**), 15-hydroxynerolidol (**33**), kaempcandiol

(**34**), two new naturally occurring (*E*)-labda-8(17),12-dien-15,16-olide (**19**) and γ-bicyclohomofarnesen-12-ol (**20**), and 26 known compounds **6**–**15**, **21-31**, **35-39**. The biological screening suggested that most of the quassinoids, bicyclic and/or tricyclic sesquiterpenes, and labdane diterpenes showed potent anti-Vpr activities. In addition, except for triterpenoid **14**, three minor constituents (**13**, **15**, and **39)** had anti-Vpr activities, with the less cytotoxic effect on the TREx-HeLa-Vpr cells. These results suggested that the three skeletons could be crucial for the development of anti-HIV-1 drug. The findings will also contribute to the scientific development of Myanmar's traditional medicine, especially in areas concerned with the disease of AIDS.

Acknowledgements

The authors would like to thank the Department of Higher Education, the Ministry of Education and the University of Yangon for some support in doing this research. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. We would like to acknowledge Professor Dr Hiroyuki Morita (Takahashi Industrial and Economic Research Foundation), Institute of Natural Medicine, University of Toyama. Finally, one of the authors, the corresponding author, is also thankful to the Japanese Government (Monnukagakusho: MEXT Scholarship), for financial support for her PhD study.

References

Ali, Z. C., T. Okpekon, F. Roblot, C. Bories, M. Cardao, J. C. Jullian, E. Poupon and P. Champy. (2011). "Labdane Diterpenoids from *Aframomum sceptrum*: NMR Study and Antiparasitic Activities". *Phytochemistry Letters*, vol. 4, pp. 240-244

Boukouvalas, J., and J. X. Wang. (1988). "Structure Revision and Synthesis of a Novel Labdane Diterpenoid from *Zingiber ottensii*". *Phytotherapy Research*, vol. 2, pp. 33-36

Chawengrum, P., J. Boonsombat, P. Kittakoop, C. Mahidol, S. Ruchirawat, and S. Thongnest. (2018). "Cytotoxic and Antimicrobial Labdane and Clerodane Diterpenoids from *Kaempferia elegans* and *Kaempferia pulchra*". *Phytochemistry Letters*, vol. 24, pp. 140–144

Chimnoi, N., S. Pisutjaroenpong, L. Ngiwsara, D. Dechtrirat, D. Chokchaichamnankit, N. Khunawutmanotham, C. Mahidol, and S. Techasakul. (2008). "Labdane Diterpenes from the Rhizomes of *Hedychium coronarium*". *Natural Product Research*, vol. 22(14), pp. 1249-1256

Darmawan, A., S. Kosela, L. B. S. Kardono, and Y. M. Syah. (2012). "Scopoletin, a Coumarin Derivative Compound Isolated from *Macaranga gigantifolia* Merr". *Journal of applied pharmaceutical science*, vol. 2, pp. 175-177

Dong, S., B. Li, W. Dai, D. Wang, Y. Qin, and Y. Zhang. (2017). "Sesqui- and Diterpenoids from the Radix of *Curcuma aromatic*". *Journal of Natural Products*, vol. 80, pp. 3093–3102

Jenjittikul, T. and K. Larsen. (2000). "*Kaempferia candida* Wall. (Zingiberaceae), a New Record for Thailand". *Thai Forest Bulletin (Botany)*, vol. 28, pp. 45-49

Koike, K., and T. Ohmoto. (1985). "Carbon-13 Nuclear Magnetic Resonance Study of Canthin-6-one Alkaloids". *Chemical and Pharmaceutical Bulletin*, vol. 33, pp. 5239-5244

Kress, W. J., R. A. DeFilipps, E. Farr and D. Y. Y. Kyi. (2003). "*A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar* (revised from the original works by J. H. Lace, R. Rodger, H. G. Hundley and U Chit Ko Ko on the "*List of Trees, Shrubs, Herbs and Principal Climbers etc. Recorded from Burma*")". Contributions from the United States National Herbarium, vol. 45, pp. 1–590

Kumar, C. N. S. S. P. ,and R. J. Chein. (2014). "Synthesis of Labdane Diterpenes Galanal A and B from (+)-Sclareolide". *Organic Letters*,vol. 16, pp. 2990-2992

Morellet, N., S. Bouaziz, P. Petitjean, and B. P. Roques. (2003). "NMR Structure of the HIV-1 Regulatory Protein VPR". *Journal of molecular biology*, vol. 327, pp.215-227

Nana, F., L. P. Sandjo, F. Keumedjo, P. Ambassa, R. Malik, V. Kuete, V. Rincheval, M. I. Choudhary and B. T. Ngadjui. (2012). "Ceramide and Cytotoxic Constituents from *Ficus glumosa* Del. (Moraceae)". *Journal of the Brazilian Chemical Society*, vol. 23, pp. S1–S9

Nishiyama, Y., Y. Noda, N. Nakatani, N. Shitan, T. Sudo, A. Kato and P. B. Chalomutiso. (2019). "Structure of Constituents Isolated from the Bark of *Cassipourea malosana* and their Cytotoxicity against a Human Ovarian Cell Line". *Journal of Natural Medicines*, vol. 73, pp. 289–296

Nyasse, B., R. Ghogomu, T. B. L. Sondengam, M. T. Martin and B. Bodo. (1988). "Mandassidione and Other Sesquiterpenic Ketones from *Cyperus articulates*". *Phytochemistry*, vol. 27, pp. 3319–3321

Prema, T. Kodama, H. H. W. Nyunt, H. Ngwe, I. Abe, and H. Morita. (2020). "Anti-Vpr Activities of Sesqui- and Diterpenoids from the Aerial Parts of *Kaempferia candida*. *Journal of Natural Medicines*, vol. 75(3), pp. 489-498

Prema, T. Kodama, C. P. Wong, A. H. El-Desoky, H. H. W. Nyunt, H. Ngwe, I. Abe, and H. Morita. (2020). "Anti-Vpr Activities of Homodrimane Sesquiterpenoids and Labdane Diterpenoids from *Globba sherwoodiana* Rhizomes". *Fitoterapia*, ,vol. 146, pp. 104705

Prema, C. P. Wong, T. Kodama, A. E. Nugroho, A. H. El-Desoky, M. D. Awouafack, Y. Y. Win, H. Ngwe, I. Abe, and H. Morita. (2020). "Three New Quassinoids Isolated from the Wood of *Picrasma javanica* and their Anti-Vpr Activities". *Journal of Natural Medicines*, vol. 74, pp. 571-578

Prema, C. P. Wong, A. E. Nugroho, M. D. Awouafack, Y. Y. Win, N. N. Win, H. Ngwe, H. Morita, and H. Morita. (2019). "Two New Quassinoids and Other Constituents from *Picrasma javanica* Wood and their Biological Activities". *Journal of Natural Medicines*, vol. 73, pp. 1-8

Saiin, C., and B. Sirithunyalug. (2017). "Review of the Chemical Structures and Antimalarial Activities of Indole Alkaloid Isolated from *Picrasma javanica* Bl". *Advancement in Medicinal Plant Research*, vol. 5, pp. 29-36

Shaaria, K., Maulidiania, C. Paetzb, J. Stanslasa, F. Abasa, and N. H. Lajisa. (2009). "Naturally Occurring Labdane Diterpene and Benzofuran from *Globba pendula*". *Natural Product Communications*, vol. 4, pp. 1031-1036

Sheeja, A. D. B., and M. S. Nair. (2014). "Facile Isolation of (*E*)-Labda-8(17),12-dien-15,16-dial from *Curcuma amada* and its Conversion to Other Biologically Active Compounds". *Indian Journal of Chemistry*, vol. 53, pp. 319-324

Thongam, B., B. Konsam, and N. Sarangthem. (2013). "*Globba sherwoodiana* (Zingiberaceae) – A New Record for India from Manipur". *Rheedea*, vol. 23, pp. 34-36

Win, N. N., T. Ito, Ismail, T. Kodama, Y. Y. Win, M. Tanaka, H. Ngwe, Y. Asakawa, I. Abe, and H. Morita. (2015). "Picrajavanicins A-G, Quassinoids from *Picrasma javanica* Collected in Myanmar". *Journal of Natural Products*, vol. 78, pp. 3024-3030

Win, N. N., T. Ito, Ismail, T. Kodama, Y. Y. Win, M. Tanaka, H. Okamoto, H. Imagawa, H. Ngwe, Y. Asakawa, I. Abe, and H. Morita. (2016). "Picrajavanicins H-M, New Quassinoids from *Picrasma javanica* Collected in Myanmar and their Antiproliferative Activities". *Tetrahedron*, vol. 72, pp. 746-752

Win, N. N., T. Ito, H. Ngwe, Y. Y. Win, Prema, Y. Okamoto, M. Tanaka, Y. Asakawa, I. Abe, and H. Morita. (2017). "Labdane Diterpoids from Curcuma amada Rhizomes Collected in Myanmar and their Antiproliferative Activities". *Fitoterapia*, vol. 122, pp. 34-39

Win, N. N., T. Ito, Y. Y. Win, H. Ngwe, T. Kodama, I. Abe and H. Morita. (2016). "Quassinoids: Viral Protein R Inhibitors from *Picrasma javanica* Bark Collected in Myanmar for HIV infection". *Bioorganic and Medicinal Chemistry Letters*, vol. 26, pp. 4620-4624

Xu, H. X., D. Hui, and K. Y. Sim. (1995). "The Isolation of a New Labdane Diterpene from the Seeds of *Alpinia zerumbet*". *Natural Product Letters*, vol. 7, pp. 29-34

Xu, J., F. Ji, J. Kang, H. Wang, S. Li, D. Q. Jin, Q. Zhang, H. Sun, and Y. Guo. (2015). "Absolute Configurations and NO Inhibitory Activities of Terpenoids from *Curcuma longa*". *Journal of Agricultural and Food Chemistry*, vol. 63, pp. 5805−5812

Yin, H., J. G. Luo, and L. Y. Kong. (2013). "Tetracyclic Diterpenoids with Isomerized Isospongian Skeleton and Labdane Diterpenoids from the Fruits of *Amomum kravanh*". *Journal of Natural Products*, vol. 76, pp. 237-242