

Cytotoxic effect of compounds isolated from *Goniothalamus marcanii* Craib stem barks

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Abstract

Goniothalamus marcanii Craib (synonym of *Goniothalamus tamirensis* Pierre ex Finet & Gagnep), is locally known as Khao Lam in Thai. The previous study showed that the isolated compounds from stem barks exhibited high cytotoxicity to human tumor cell lines. The objective of the research was to investigate compounds which showed cytotoxic effect. Column chromatography was used for isolation. The structures of the compounds were investigated by spectroscopic methods. The bioactivity of the compounds was determined by SRB assay. The result showed that four compounds, a styryl-lactone: (+)-goniodiol (1); and three alkaloids: ouregidione (2); noraristolodione (3); velutinam (4) were isolated from *G. marcanii* stem barks. Compound 3 possessed the strongest activity (EC_{50} 35.56 and 50.75 μ M), for A549 (lung) cells and HeLa (cervix) cells, respectively. Compound 1-3 displayed cytotoxic activity but less than that of doxorubicin, a conventional chemotherapeutic drug, the EC_{50} levels of which were 0.13 and 0.12 μ M for A549 and HeLa cells, respectively. On the other hand, compound 2 and 4 which could be evaluated from selective index values (SI 1.25 and 1.70, respectively) showed lower toxicity towards normal cells (MRC5) than doxorubicin (SI = 1.07). From this study, *G. marcanii* stem barks found to be a new source of compounds 1-4. In addition, the investigation of compound 3 in cytotoxic activity and the evaluation of compounds 1 and 2 for cytotoxic activity to HeLa cell line have been reported for the first time.

Keyword: *Goniothalamus marcanii*/Cytotoxic/(+)-goniodiol/ouregidione/noraristolodione/velutinam

1. INTRODUCTION

The genus *Goniothalamus* (Blume) Hook. f & Thomson belongs to the Annonaceae family. This genus is particularly interesting in the field of oncology. According to this species have a broad array of secondary metabolites, which is abundant in *annonaceous* acetogenins¹, styryl-lactones², alkaloids³, and flavonoids⁴. *Goniothalamus marcanii* Craib (synonym of *Goniothalamus tamirensis* Pierre ex Finet & Gagnep), locally known as Khao Lam, grows widely in the northern, northeastern and southern parts of Thailand. Its bark has a brown color with a few scattered lenticels. In Thailand, herbalists use this plant for infectious diseases in early childhood (under 5 years old)⁵. The previous studies reported that the extract from stem barks showed a cytotoxicity against human

tumor cell lines, A-549, HT-29, MCF7, RPMI, and U251 with the EC_{50} in the range of 4×10^0 - 1×10^2 μ g/mL. The extract contained 1-azaanthraquinone and naphthoquinone derivatives which possessed the high cytotoxicity against human tumor cell lines in the range of 0.04-3.03 μ M⁶. Moreover, styryl-lactone and alkaloid compounds, isolated from other plants in this family also shown cytotoxic activity^{7,8}. Thus, these finding compounds show a good opportunity to obtain new chemotherapeutic agents from *G. marcanii* stem barks in other fractions which also exhibited high cytotoxicity.

The present study aims to evaluate the *in vitro* cytotoxic activity of the isolated compounds from *G. marcanii* against human carcinoma cell lines, and to identify the components which may be responsible for the cytotoxic activity.

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2. MATERIALS AND METHODS

2.1 Materials

The UV spectra were obtained on a UV-2600 Shimadzu[®] spectrophotometer. The IR spectra were analysed on a Nicolet[™] iS[™]10 infrared spectrometer, using potassium bromide disks and attenuated total reflectance method. High resolution mass spectra, liquid chromatography mass spectra, and Electron Impact mass spectra were examined on a micrOTOF Bruker[®], LCMS-8030 Shimadzu[®], and Polaris Q Thermoquest Finnigan[®], respectively. NMR spectra were recorded on an Avance Bruker[®]. The optical rotation was determined on a Polax-2L[®]. Silica gels 60 were purchased from Merck Co., Ltd, Germany. Dichloromethane, methanol, ethyl acetate, hexane, and acetic acid were purchased from Sigma-Aldrich Co., Ltd, USA. Silica gels P60 were purchased from Silicycle Inc, Canada. All chemicals and reagents were analytical grades. Tris (hydroxymethyl) aminomethane with molecular biology grade was purchased from Pacific Science Co., Ltd, USA. Trichloroacetic acid was purchased from VWR Co., Ltd, USA. Sulforhodamine B and doxorubicin were purchased from Sigma-Aldrich Co., Ltd, USA. Dulbecco's Modified Eagle Medium, 10% v/v foetal bovine serum and RPMI-1640 medium were purchased from Thermo Fisher Scientific Inc, USA. MRC-5, A549, and HeLa cell lines were purchased from American Type Culture Collection (ATCC), USA.

2.2 Plant material

The stem barks of *G. marcanii* were collected from Nakhonphanom, a province in the north-east of Thailand, in October 2014. The specimen was identified by Prof. Wongsatit Chuakul, Ph.D., Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. The voucher specimen (PBM no. 05183) has been deposited at the Pharmaceutical Botany, Mahidol University Herbarium, Faculty of Pharmacy, Mahidol University, Thailand.

2.3 Extraction and Isolation

The ethanolic extract (F001) of the

stem bark (3.3 kg, dry weight) was partitioned using a mixture of CH₂Cl₂-H₂O (1:1). The CH₂Cl₂ fraction (F003, 92.4 g) was further partitioned between hexane-90% aqueous MeOH (1:1). The 90% MeOH fractions (F006, 72.4 g) gave the strongest bioactive fraction in brine shrimp test (LC₅₀ 3.08 µg/ml)⁹. This fraction (F006) was adsorbed onto 150 g of kieselguhr and fractionated by a quick column chromatographic technique using a sintered glass filter column of silica gel (800 g, 20×10 cm). A gradient of hexane/CH₂Cl₂/EtOAc/MeOH was collected and then combined according to their TLC patterns to obtain eleven fractions (F008-018). Then, all fractions were testified by brine shrimp lethality test. The fractions (F008-018) showed cytotoxic activity in different concentration. The fraction F015 (23.5 g) which was the most bioactive fraction in the BST (LC₅₀ 0.98 µg/ml), was subjected to open Si-gel column chromatography (70-230 mesh) and eluted with a gradient of hexane/CH₂Cl₂/EtOAc/MeOH with increasing polarity. All fractions were collected and then combined according to their TLC patterns to obtain eleven fractions (F019-029). Then, all fractions were testified by brine shrimp lethality test and SRB assay. F023-025 showed a highly bioactive fraction in the cytotoxic test. Fraction F024 could be precipitated in EtOAc and F023 could be precipitated in CH₂Cl₂:EtOAc (80:20) which gave an orange and yellow compounds. However, fraction F025 had neither an orange compound nor a yellow compound when examined under UV 365 nm. Fractions F026-027 appeared only tailing dark spot when examined under UV 254 nm. The precipitate of fraction (F024, 0.54 g) was fractionated with a step gradient elution of 2.5% MeOH/CH₂Cl₂ to afford three compounds (compound 1, <1 mg, compound 2, 14.9 mg, compound 3, 11.6 mg). Then, all compounds were testified by SRB assay. The fraction (F023, 0.48 g) was fractionated with a step gradient elution of 2.5% MeOH/CH₂Cl₂ to afford the same compound (compound 1, 16.0 mg) and another compound (compound 4, 42.0 mg). Then, all compounds were testified by SRB assay. The fractions (F023-F024) and eluted compounds showed a high polarity when compared to the 1-azaanthraquinone and

naphthoquinone derivatives in TLC system. Then, all compounds were identified by spectroscopic methods.

2.4 Structural Identification

Compound 1: (+)-goniodiol

Appearance: Orange amorphous

Molecular formula: $C_{13}H_{14}O_4$

UV λ_{max} nm (log ϵ): 242 (3.38), 275 (3.44), 302 (3.48), 314 (3.50), and 440 (3.64)

IR ν_{max} cm^{-1} : 3412, 2924, 1720, 1254, and 1080

LCMS (1%) m/z : 234.30 (100)

Optical rotation: $[\alpha] + 7.2^{\circ}$ ($c = 0.3$, $CHCl_3$)

1H NMR: 400 MHz and ^{13}C NMR: 100 MHz in $CDCl_3$: Data were shown in Table 1 and 2; Figure 1

Compound 2: ouregidione

Appearance: Yellow crystals

Molecular formula: $C_{19}H_{15}NO_5$

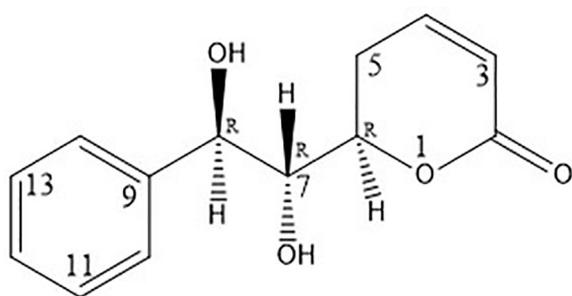
UV λ_{max} nm (log ϵ): 242 (3.38), 272 (3.43), 305 (3.48), 317 (3.50), and 421 (3.62)

IR ν_{max} cm^{-1} : 3438, 2923, 1686, 1621, and 1390

Mass Spectrum EI-MS (rel. int.) m/z : 337 (9.7), 336 (42.0), 322 (26.1), 321 (100), 308 (5.5), and 294 (6.3)

ESI-MS (1%) m/z : 360.0853 $[M+Na]^+$ (100)

1H NMR: 500 MHz and ^{13}C NMR: 125 MHz in $CDCl_3$: Data were shown in Table 1 and 2; Figure 2



1

Compound 3: noraristolodione

Appearance: Orange amorphous

Molecular formula: $C_{17}H_{11}NO_4$

UV λ_{max} nm (log ϵ): 244 (3.39), 274 (3.44), 301 (3.48), 314 (3.50), and 445 (3.65)

IR ν_{max} cm^{-1} : 3368, 2923, 2853, 1684, 1663, and 1379

Mass Spectrum EI-MS (rel. int.) m/z : 294 (3.7), 256 (3.4), 220 (5.6), 185 (9.9), 179 (13.53), 178 (100), 161 (40.2), 149 (30.6), 133 (19.5), 121 (23.83), 97 (17.5), 95 (28.4), 81 (33.5), and 57 (25.9)

ESI-MS (1%) m/z : 316.0582 $[M+Na]^+$ (100)

1H NMR: 500 MHz and ^{13}C NMR: 125 MHz in $DMSO-d_6$: Data were shown in Table 1 and 2; Figure 3

Compound 4: velutinam

Appearance: Brownish-yellow amorphous

Molecular formula: $C_{17}H_{13}NO_4$

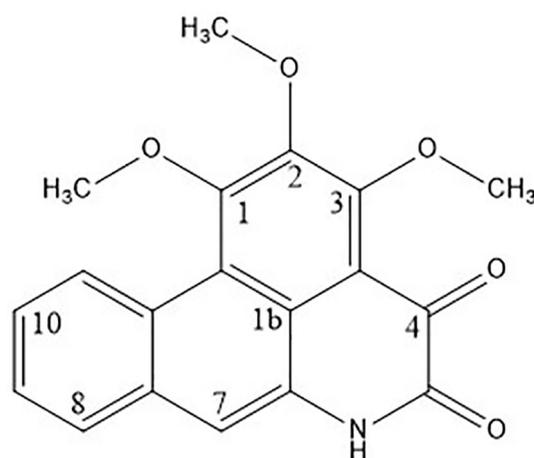
UV λ_{max} nm (log ϵ): 245 (3.39), 298 (3.47), and 405 (3.61)

IR ν_{max} cm^{-1} : 3415, 2923, 1672, and 1649

Mass Spectrum EI-MS m/z : 295 (20.3), 294 (100), 280 (16.1), 252 (57.6), 237 (13.07), and 209 (14.8)

ESI-MS (1%) m/z : 318.0748 $[M+Na]^+$ (77.5)

1H NMR: 500 MHz and ^{13}C NMR: 100 MHz in $DMSO-d_6$: Data were shown in Table 1 and 2; Figure 4



2

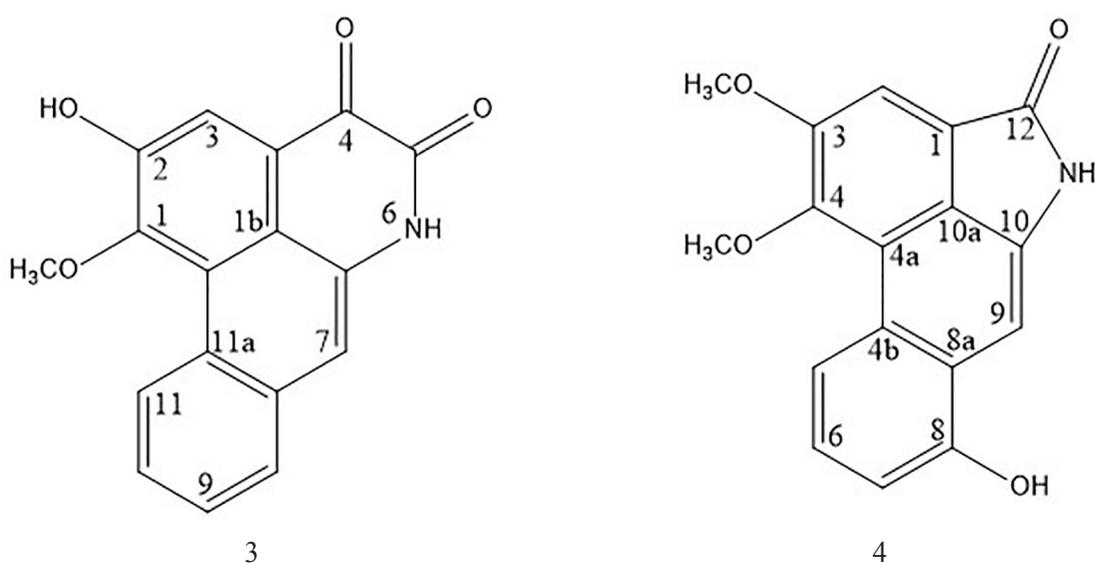


Table 1. ^1H assignments of compounds (+)-goniodiol and ouregidione in CDCl_3 and noraristolodione and velutinam in $\text{DMSO}-d_6$

Position	δ ^1H (ppm) multiplicities (J, Hz)			
	(+)-Goniodiol	Ouregidione	Noraristolodione	Velutinam
1	-	-	-	-
1a	-	-	-	-
1b	-	-	-	-
2	-	-	-	7.85 (1H, s)
3	5.97 (1H, dd, J=10.0, 2.4)	-	8.08 (1H, s)	-
3a	-	-	-	-
4	6.93 (1H, ddd, J=9.6, 6.4, 2.0)	-	-	-
4a	-	-	-	-
4b	-	-	-	-
5	2.19 (1H, ddd, J=18.4, 6.0, 4.0) 2.78 (1H, tdd, J=18.8, 10.8, 2.0)	-	-	8.62 (1H, d, J=7.50)
6	4.78 (1H, ddd, J=11.6, 3.6, 2.4)	11.55 (1H, s)	12.04 (1H, s)	7.36 (1H, t, J=8.0)
6a	-	-	-	-
7	3.71 (1H, dd, J=7.6, 2.4)	7.81 (1H, s)	7.48 (1H, s)	7.08 (1H, d, J=7.7)
7a	-	-	-	-
8	4.93 (1H, d, J=7.2)	7.97 (1H, m)	7.92 (1H, m)	-
8a	-	-	-	-
9	-	7.65 (1H, m)	7.61-7.66 (2H, m)	7.42 (1H, s)
10	7.32-7.43 (5H, m)	7.66 (1H, m)		-
10a		-	-	
11		9.50 (1H, m)	9.45 (1H, m)	10.81 (1H, s)
11a	-	-	-	
12	-	-	-	
1-OCH ₃	-	4.17 (3H, s)	4.05 (3H, s)	-
2-OCH ₃	-	4.10 (3H, s)	-	-
3-OCH ₃	-	4.10 (3H, s)	-	4.05 (3H, s)
4-OCH ₃	-	-	-	4.01 (3H, s)
2-OH	-	-	10.84 (1H, s)	-
8-OH	-	-	-	10.17 (1H, s)
7,8-OH	2.57 (s), 3.05 (s)	-	-	-

Table 2. ^{13}C assignments of compounds (+)-goniodiol and ouregidione in CDCl_3 and noraristolodione and velutinam in $\text{DMSO}-d_6$

Position	$\delta^{13}\text{C}$ (ppm)			
	(+)-Goniodiol	Ouregidione	Noraristolodione	Velutinam
1	-	157.5	153.3	123.8
1a	-	121.2	124.5	-
1b	-	120.2	117.7	-
2	163.8	147.5	151.5	110.3
3	120.5	158.6	117.5	154.6
3a	-	117.6	125.2	-
4	146.2	175.3	177.4	151.0
4a	-	-	-	120.6
4b	-	-	-	127.5
5	26.0	160.4	156.1	118.3
6	76.8	-	-	126.2
6a	-	128.3	130.7	-
7	75.0	115.9	112.5	112.6
7a	-	131.7	132.9	-
8	73.6	128.4	128.8	154.2
8a	-	-	-	124.4
9	140.8	127.5	128.3	99.3
10	128.2	127.4	127.1	134.3
10a	-	-	-	122.0
11	128.7	127.2	127.6	-
11a	-	127.2	126.5	-
12	126.6	-	-	168.8
13	128.7	-	-	-
14	128.2	-	-	-
1-OCH ₃	-	62.0	60.09	-
2-OCH ₃	-	61.7	-	-
3-OCH ₃	-	61.1	-	57.3
4-OCH ₃	-	-	-	60.3

2.5 Bioassay

Brine shrimp lethality assay (BST): This assay was used for cytotoxicity-guided fractionation. The brine shrimp lethality bioassay is an efficient, rapid and inexpensive test that requires only a relatively small amount of sample (2-20 mg). This bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity. About 2 mg

of each sample was accurately weighed and dissolved in 30 μl of dimethyl sulfoxide (DMSO), then 1,570 μl of sea water was added. The solution obtained was diluted with sea water to get 1000, 100, 10 and 1 $\mu\text{g}/\text{mL}$ solutions. The final concentrations of DMSO in each test were 1.82, 0.089, 0.0043, and 0.0002 %v/v. The negative control was DMSO in seawater. The 50 μl -portion of the suspension containing 4-8

shrimps was pipetted and added to each well. The number of surviving was recorded after 24 hours. Using Probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} was determined by plot dose response curve between; Y: The mean of percentage mortality; X: Concentrations⁹

Cytotoxicity assay: Cells are cultured in a microtitre well plate and the rate of multiplication and growth is measured indirectly by the formation of a color, the intensity of which is directly proportional to the number of cells present. The reason for using SRB assay because this assay exhibiting a number of advantages including sensitivity, simplicity, reproducibility and rapidity when compared to formazan-based assays. In addition, a stable end-point that does not require a time-sensitive measurement has set SRB assay more applicable than the MTT or XTT assays¹⁰. The human tumor cell lines utilized as the target cells in this test were shown in Table 3. Human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium. Cells were inoculated into 96-well microtiter plates in 100 μ l. The microtiter plates were incubated at 37°C, 5% CO_2 , 95% air and 100% relative humidity for 24 h. With 10-fold serial dilution, the ranges of final concentrations in the wells were 100 to 0.01

μ g/mL for the extracts and 100 to 0.01 ng/mL for the compounds. Doxorubicin was used as a positive control. Then, each concentrations (100 μ l) of the test sample in culture medium were added to the wells containing the cells. Medium (100 μ l) only was added to the control wells. The cells with the samples were incubated at 37 °C, 5% CO_2 , 95% air and 100% relative humidity for 48 h. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of cold 50% (w/v) TCA (50 μ l) (final concentration, 10% TCA). Sulforhodamine B (SRB) solution (100 μ l) at 0.4% (w/v) in 1% acetic acid was added to each wells, and plates were incubated for 10-30 minutes at room temperature. After staining unbound dye was removed by washing with 1% acetic acid. The bound stain was subsequently solubilized with 10 mM Trizma base (100 μ l) and the absorbance was read on an automated plate reader at a wavelength of 515 nm. The optical density (OD) of SRB in each wells were directly proportional to the cell number so the OD values can be plotted against concentration and the EC_{50} determined by plot dose response curve between Y: Percent of control cell growth = [(mean OD test – mean OD day0) / (mean OD negative count – mean OD day0)] \times 100; X: Concentrations¹¹

Table 3. The plating densities of individual cell lines

MRC5	Normal lung cells	20,000 cells/well
A549	Human lung carcinoma cells	7,500 cells/well
HeLa	Human cervical cancer cells	5,000 cells/well

Selectivity index (SI) = EC_{50} of pure compound in normal cell line/ EC_{50} of pure compound in cancer cell line

As the value of SI demonstrates the differential activity of a compound, the greater the index value, the better it was. An SI value less than 2.0 indicates the general toxicity of the compound¹².

2.6 Statistical analysis

All calculations have been done using

Microsoft Excel and SPSS statistics 17.0 analyze program. The comparisons have been done using One-Way ANOVA. All values were expressed as mean \pm SD. Statistical significances were tested. Differences were considered statistically significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

The structures of the isolated compounds were identified by spectroscopic methods. Comparisons of compound 1-4 with the literature

data¹³⁻¹⁶ were performed. *G. marcanii* stem barks found to be a new source of compounds 1-4. Compound 1 had a styryl-lactone structure showing cytotoxicity against human tumor cell lines HL-60 (human promyelocytic leukemia cells)¹⁷ and P-388 (murine leukemia cells)¹⁸ and exhibited strong *in vitro* antimalarial activity against *Plasmodium falciparum* with EC₅₀ values of 3.28 µg/mL¹⁹. This compound was found in *Goniothalamus giganteus*¹¹ and *Goniothalamus maewongensis*²⁰. Compound 2, one of aporphine alkaloids, possessed a wide array of biological activities such as the reduction of the oxidative stress burden²¹, cytotoxic activity toward hepatocellular carcinoma cells, breast cancer cells, and small cell lung cancer with being able to stimulate lymphocyte proliferation^{22, 23} and antiproliferative activity in erythroleukemic, promonocytic, and promyelocytic leukemic cell lines. This compound was isolated from *Pseuduvaria monticola*²¹ and *Pseuduvaria rugosa*²⁴.

Compound 3 is also a type of aporphine alkaloids. This compound was isolated from *Fissistigma balansae* and *Fissistigma oldhamii*. It was reported to have antiplatelet aggregation property. Compound 4 was also isolated from

Fissistigma balansae and *Fissistigma oldhamii*. The compound belonged to aristololactams, showed strong inhibition of platelet aggregation induced by collagen²⁵ and exhibited cytotoxicity against human epidermoid carcinoma (KB), human breast adenocarcinoma (MCF7), human small cell lung cancer (NCI-H187) and Vero cells. The compound exhibited strong *in vitro* antimalarial activity against *P. falciparum* with EC₅₀ values of 5.89 µg/mL but inactive in antimycobacterial activity against *Mycobacterium tuberculosis*¹⁹. Moreover, the previous study showed selectively active against Gram-positive bacteria, but was weakly cytotoxic against HeLa (human cervical carcinoma), L1210 (murine leukaemia) cell lines¹⁶. Compound 4 showed the strongest inhibition of CDK1/cyclin B activity with EC₅₀ values of 1.5 µM and inhibition of DYRK1A activity with EC₅₀ values of 0.6 µM²⁶. The isolated compounds from *G. marcanii* were evaluated for cytotoxic activity against normal lung fibroblast (MRC5), adenocarcinoma non-small cell lung epithelial (A549), and epitheloid cervix carcinoma (HeLa) cell lines. The results of bioactivity tests on compounds were shown in Table 4.

Table 4. Cytotoxic activity of compounds

Compound	Cytotoxicity (EC ₅₀ in µM) in tumor cell lines			
	MRC5	A549	HeLa	Selectivity index (SI)
(+)-Goniodiol (1)	37.96±2.42	159.16±8.16	60.57±2.34	0.63
Ouregidione (2)	73.09±7.12	58.58±3.35*	67.77±1.86	1.25
Noraristolodione (3)	37.54±4.91	35.56±0.99	50.75±2.18	1.06
Velutinam (4)	306.98±5.12	180.64±19.72*	455.12±7.29	1.70
Doxorubicin	0.14	0.13	0.12	1.07

A pure compound was considered inactive when EC₅₀ is over than 100 µM²⁸.

*, EC₅₀ is significantly difference when compared with normal cells (MRC5) / only lower than MRC5; *p*-value < 0.05.

Compound 1 exhibited weakly cytotoxic activity against HeLa with EC₅₀ value 60.57 µM and exhibited moderately cytotoxic activity against MRC5 but was inactive in A549 cells. Compound 2 was weakly cytotoxic against MRC5, A549, and HeLa (EC₅₀: 58.58-73.09 µM) but exhibited significant difference when

compared tumor cells (A549) with normal cells (MRC5). Compound 3 exhibited the strongest cytotoxic activity against A549 and HeLa with EC₅₀ values 35.56 and 50.75 µM, respectively. The compound 3 exhibited a moderate cytotoxic activity against MRC5. Compound 4 was inactive toward all cell lines but exhibited

significant difference when compared tumor cells (A549) with normal cells (MRC5).

Compound 1-3 displayed cytotoxic activity but less than that of doxorubicin; a conventional chemotherapeutic drug, the EC_{50} levels of which were 0.14, 0.13, and 0.12 μ M for MRC5, A549, and HeLa cells, respectively. On the other hand, compound 2 showed a selective index value (SI = 1.25) greater than doxorubicin (SI = 1.07). Besides, the fractionated extracts displayed better cytotoxic activity than the pure compounds, as shown in Table 5. The fractionated extract (F024), having EC_{50} values

in the range of 12.40-13.37 μ g/mL, could be classified as highly cytotoxic activity against the selected human tumor cell lines and moderate effect against normal cell line MRC5 (EC_{50} values of 24.85 μ g/mL). Based on the above findings, some synergistic or antagonistic effects needed to be clarified. Combinations of cytotoxic agents require lower doses of each agents to obtain better therapeutic effects with less side-effect and toxicity²⁷. Moreover, the active pure compound may not be eluted yet. Thus, the study may be worth further investigation with a view for isolating the active compounds.

Table 5. Cytotoxic activity of fractions

Fraction	Cytotoxicity (EC_{50} in μ g/ml) in tumor cell lines			
	MRC5	A549	HeLa	Selectivity index (SI)
F023	30.2±2.77	7.82±0.14*	35.84±3.89	3.86
F024	24.85±1.96	12.40±2.09*	13.37±0.83*	2.00

A fitted dose-response curve were plotted to enable the calculation of the concentrations that kill 50% of the cells (EC_{50}). The criteria used were as follows: EC_{50} < 20 μ g/ml (high cytotoxic activity), EC_{50} : 20-100 μ g/ml (moderate cytotoxic activity), EC_{50} : 201-500 μ g/ml (weak cytotoxic activity), EC_{50} > 500 μ g/ml (no cytotoxic activity)²⁹.

*, EC_{50} is significantly difference when compared with normal cells (MRC5) / only lower than MRC5; p -value < 0.05.

4. CONCLUSION

From the previous study, the stem bark extracts of *Goniothalamus marcanii* Craib were highly cytotoxic to the human tumor cell lines. Large-scale extraction of stem barks by solvent partitioning, and fractionation by chromatography on silica yielded three groups of cytotoxic compounds. One styryl-lactone, (+)-goniodiol (1), was obtained together with two known aporphine alkaloids, ouregidione (2), noraristolodione (3), and one known aristolactam velutinam (4). The isolated compounds showed the cytotoxic activity against human tumor cell lines in the range of 35.56-73.09 μ M. *G. marcanii* stem barks found to be a new source of compounds 1-4. In addition, the investigation of compound 3 in cytotoxic activity and the evaluation of compound 1 and 2 for cytotoxic activity to HeLa cell line have been reported for the first time.

5. ACKNOWLEDGEMENT

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