รายงานวิจัยฉบับสมบูรณ์

โครงการการหาสารประกอบที่มีฤทธิ์ทางชีวภาพจาก *Goniothalamus repevensis* Pierre ex Finet & Gagnep

โดย ดร.วราพร ชนะกุล

มิถุนายน 2563

สัญญาเลขที่MRG5980036

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มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าพระนครเหนือ

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

Project Code : MRG5980036

Project Title : การหาสารประกอบที่มีฤทธิ์ทางชีวภาพจาก *Goniothalamus repevensis* Pierre ex Finet & Gagnep

Investigator : วราพร ชนะกุล

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Project Period : 2 ปี

Abstract:

Goniothalamus repevensis Pierre Finet & Gagnep (ANNONACEAE) หรือแสดสยาม เป็นพืชเฉพาะถิ่น ในประเทศไทยพบการกระจายอยู่ในป่าดิบชื้น สูงกว่าระดับน้ำทะเล 600 – 900 เมตรในจังหวัดจันทบุรี พืชในจีนัส Goniothalamus หลายชนิดถูกใช้เป็นยาพื้นบ้านโดยชาวบ้านในเอเชียตะวันออกเฉียงใต้ รวมถึงประเทศไทยด้วย งานวิจัยนี้เป็นรายงานแรกที่แสดงการแยกสารประกอบที่มีฤทธิ์ทางชีวภาพจากต้นแสดสยาม แยกสารได้ styryl lactone 2 ชนิด คือ Goniodiol-7-monoacetate (**1**) goniodiol diacetate (**2**) และ aristolactam alkaloids 3 ชนิด piperolactam C (**3**) aristolactam A III (**4**) และ aristolactam B III (**5**) ด้วยเทคนิคโครมาโตกราฟี โครงสร้างของสารเหล่านี้ยืนยันโดยข้อมูลทางสเปคโตรสโคปี และเปรียบเทียบกับข้อมูลที่เคยรายงานมาก่อน จากการทดสอบพบว่า สาร **1**-**4** มีความเป็นพิษต่อเซลล์มะเร็งในหลอดทดลองด้วยค่า IC_{50} value ในช่วง 1.02 – 18.79 µg/mL.

Keywords (3-5 words): *Goniothalamus repevensis*, cytotoxic, alkaloids, styryl lactone

Abstract

Project Code : MRG5980036

Project Title : The investigation of bioactive compounds from *Goniothalamus repevensis* Pierre ex Finet & Gagnep.

Investigator : Waraporn Chanakul

E-mail Address : Waraporn.c@sciee.kmutnb.ac.th

Project Period : 2 years

Abstract:

Goniothalamus repevensis Pierre Finet & Gagnep (ANNONACEAE) is the endemic species, in Thailand it was found distribute in evergreen forest, sea level 600- 900 m in Chanthaburi province. The plants in this genus were used as ethnomedical by people in Sount-East Asia including Thailand. The isolated compounds of this genus have potential to treat cancers. The project of investigation of chemicals from *Goniothalamus repevensis* Pierre Finet & Gagnep should be the first report of bioactive compounds from this species. Two styryl lactone Goniodiol-7-monoacetate (**1**) goniodiol diacetate (**2**) and three aristolactam alkaloids piperolactam C (**3**) aristolactam A III (**4**) and aristolactam B III (**5**). were isolated from methanolic extract of *G. repevensis* by chromatography techniques. The structures of these compounds were elucidated based on their spectroscopic data and compared with literatures. Compound **1**-**4** showed cytotoxic activities against panel of cell lines with IC_{50} value ranged 1.02 – 18.79 µg/mL.

Keywords (3-5 words): *Goniothalamus repevensis*, cytotoxic, alkaloids, styryl lactone

Executive summary

Cancer continues to be one of the major causes of death worldwide, accounting for 8.2 million deaths in 2012 worldwide; these numbers are expected to double by 2030, of which 62% arise in developing countries. Medicinal plants are widely used by majority of populations as primary healthcare to cure various diseases and illnesses and have high an economic impact on the world economy. This research aimed to investigate the phytochemistry from *Goniothalamus repevensis* Pierre Finet & Gagnep and evaluate their cytotoxicity against cancer cell lines.

Leaves twigs and stems of *G. repevensis* Pierre Finet & Gagnep were collected from evergreen forest, sea level 600-900 m in Chanthaburi province, Thailand. The potent cytotoxicities of methanolic extract and fractions led to isolation of five cytotoxic compounds, goniodiol-7-monoacetate (**1**) goniodiol diacetate (**2**) piperolactam C (**3**) aristolactam A III (**4**) and aristolactam B III (**5**). Compound **1**-**4** were subjected to evaluate the cytotoxic activities against panel of cell lines. The results were express as IC_{50} value ranged 1.02 – 18.79 µg/mL.

Literature review

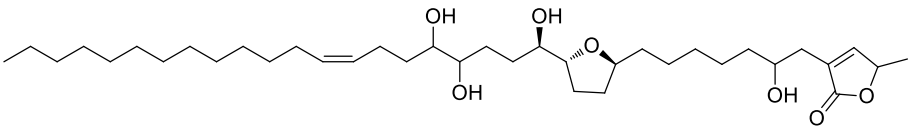
Goniothalamus (Blume) Hook. f. & Thomson is one of the largest genera in the Annonaceae (with over 130 species).[1] Twenty-five species was discovered in Thailand.[2] Among a hundred of species in this genus only thirty seven plants have been phytochemicals investigated. The plants which have been phytochemicals and bioactivities reported are *G. amuyon*, *G. andersonii*, *G. arvensis*, *G. aurantiacus*, *G. borneensis*, *G. cardio-petalus*, *G. cheliensis*, *G. dolichocharpus*, *G. donnaiensis*, *G. dumontetii*, *G. gardneri*, *G. giganteus*, *G. gigantifolius*, *G. grandiflourus*, *G. griffithii*, *G. howii*, *G. laoticus*, *G. leiocarpus*, *G. macrophyllus*, *G. maewongenesis*, *G. malayanus*, *G. marcanii*, *G. montanus*, *G. ridleyi*, *G. rongklanus*, *G. scortechinii*, *G. sesquipedalis*, *G. sinclairinius*, *G. tamirensis*, *G. tapis*, *G. tenui-folius*, *G. thwaitesii*, *G. umbrosus*, *G. undulatus*, *G. uvaroides*, *G. velutinus* and *G. wighti* .[1]

Species from the *Goniothalamus* genus have been used in traditional medicine in many parts of the Thailand. People in Narathiwat province, Southern part of Thailand used flowers of *G. giganteus* and *G. malayanus* as cardiotonic. In Yasothon, Ubon Ratchathani and chaiyaphum, flowers of *G. marcanii* and wood of *G. laoticus* were used for infectious disease in early childhood (under 5 years old).[3]

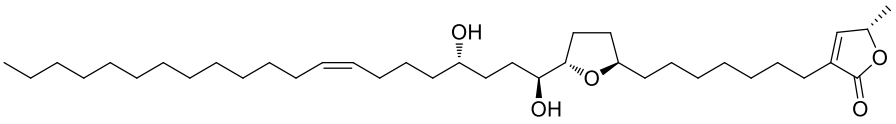
Chemical constituents isolated from *Goniothalamus* species can be grouped into four main classes, namely acetogenins, styryl lactones, alkaloids and flavonoids.[1] Acetogenins and styryl-lactones from *Goniothalamus* species have shown to be cytotoxic to different human tumor cell lines. Other reported biological properties of some compounds are antifungal, antiplasmodial, antimycobacterial, insecticidal, antimalarial, anti-inflammatory, immuno-suppressive, and inhibitor of platelet-activating factor (PAF) receptor binding activities.[4]

Acetogenins are unusual series of polyketides which have so far only been characterized from members of the family Annonaceae including in the genus *Goniothalamus* particularly *G. giganteus*[5-8] with very few acetogenins from *G. donnaiensis*,[9] *G. gardneri*,[10] and *G. undulatus*.[11] Acetogenins are the most cytotoxic group of compounds found in the species from the *Goniothalamus* genus. Acetogenins are also synthetized by the Annonaceae family.

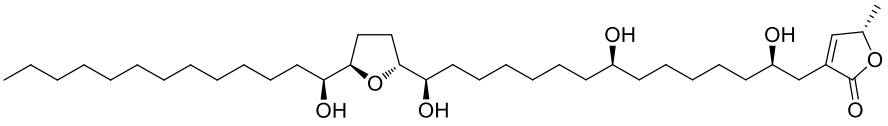
Acetogenins (ACGs) have common skeleton as a linear C 32 or C 34 fatty acid ending in a c-lactone. Several oxygenated functions, such as hydroxyl, ketone, epoxide, tetrahydro-furan (THF) and tetahydropyran (THP), may be present, as well as double and triple bonds. Thus several types of ACG have been characterized, based on the nature of the functional groups which are present. ACGs exhibit a broad range of biological properties such as cytotoxic, antitumoral, antiparasitic, pesticidal, anti-microbial and immunosuppresive activities.[12]



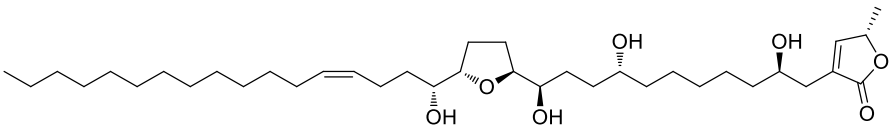
Gigantetronenin **(1)**



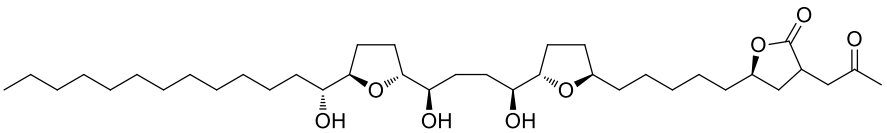
cis-Gigantetronenin **(2)**



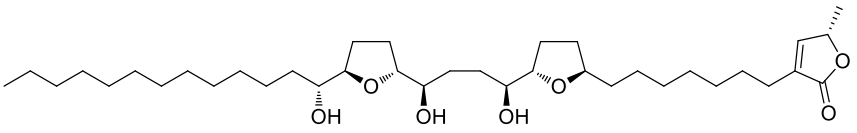
Annomontacin A **(3)**



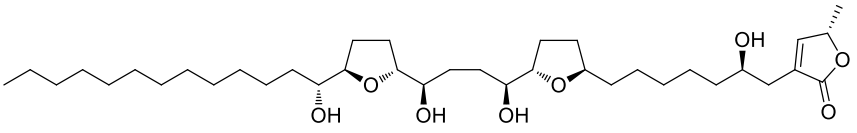
Gonionenin **(4)**



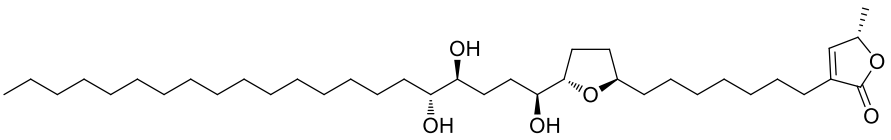
2,4-*cis*-gigantecinone **(5)**



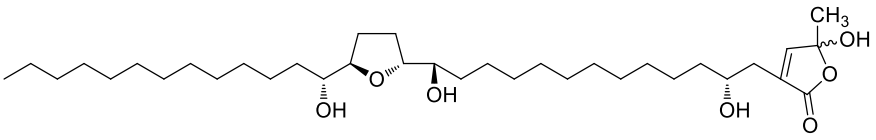
4-Deoxygigantecin **(6)**



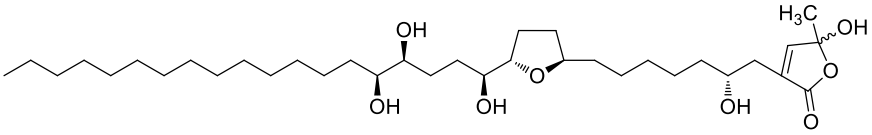
Gigantecin **(7)**



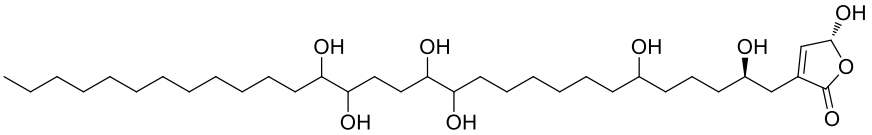
Gigantriocin **(8)**



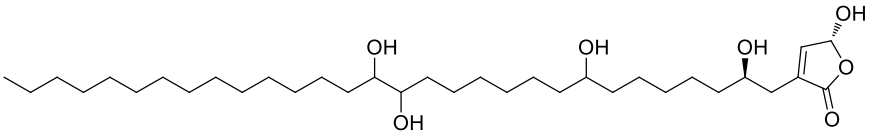
Donnaienin A **(9)**



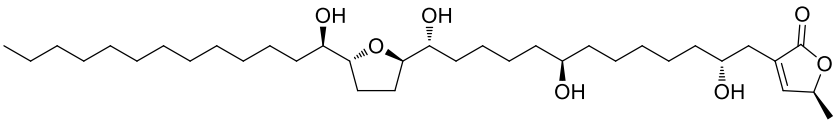
Donnaienin B **(10)**



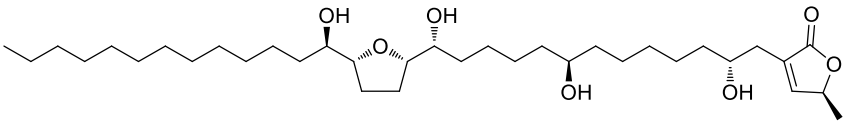
Gardnerilin A **(11)**



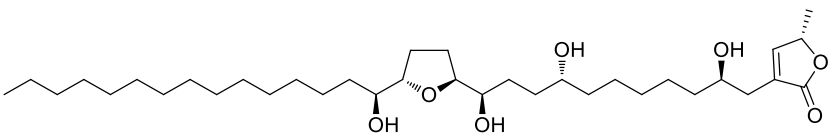
Gardnerilin B **(12)**



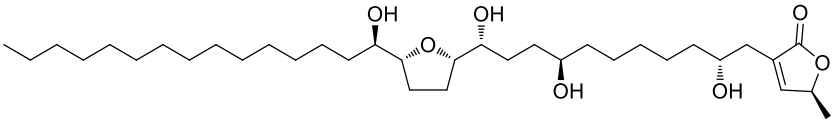
Annonacin **(13)**



cis-Annonacin **(14)**



Goniothalamicin **(15)**



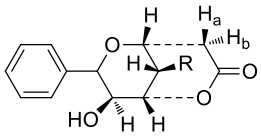
cis-Goniothalamicin **(16)**

Table 1 Acetogenins isolated from the *Goniothalamus* genus.

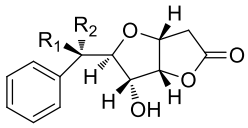
Plant species	part	Chemical constitutes	Inhibition concentration (μg/mL)											Ref.
			KB	HCT-8	Bel-7402	A-549	MCF-7	H-T29	A-498	PC-3	PACA-2	COR-L23	MRC-5	
G. giganteus	bark	Gigantetronenin (**1**)	-	-	-	$4.17x10^{-3}$	$6.03x10^{-1}$	$5.37x10^{-2}$	-	-	-	-	-	5
		Gigantrionenin (**2**)	-	-	-	$3.94x10^{-3}$	8.06	$2.92x10^{-3}$	-	-	-	-	-	
		Annomontacin (**3**)	-	-	-	$7.72x10^{-3}$	$1.65x10^{-1}$	$2.58x10^{-3}$	-	-	-	-	-	
		Gonionenin (**4**)	-	-	-	$1.34x10^{-3}$	$4.54x10^{-3}$	$1.12x10^{-3}$	-	-	-	-	-	6
		2,4-*cis* gigantecinone (**5**)	-	-	-	$2.14x10^{-1}$	>1	>1	$2.12x10^{-1}$	$1.08x10^{-3}$	>1	-	-	7
		4-deoxygigantecin (**6**)	-	-	-	$1.31x10^{-1}$	1.0	$1.43x10^{-1}$	$3.28x10^{-1}$	$1.50x10^{-1}$	$3.93x10^{-1}$	-	-	
		Gigantecin (**7**)	-	-	-	$2.19x10^{-7}$	$4.11x10^{-9}$	$2.68x10^{-4}$	-	-	-	-	-	
		Goniotriocin (**8**)	-	-	-	$3.3x10^{-2}$	$3.3x10^{-5}$	$1.2x10^{-3}$	1.1	$2.6x10^{-1}$	1.4	-	-	8
G. donnaiensis	root	Donnaienin A (**9**)	<16.7	<16.7	-	-	-	-	-	-	-	-	-	9
		Donnaienin B (**10**)	<16.3	-	-	-	-	-	-	-	-	-	-	10
G. gardneri	root	Gardnerilin A (**11**)	>10	>10	3.6	-	-	-	-	-	-	-	-	
		Gardnerilin B (**12**)	5.5	4.2	8.5	-	-	-	-	-	-	-	-	
G. undulatus	root	Annonacin (**13**)	-	-	-	-	-	-	-	-	-	0.34	9.31	11
		cis-Annonacin (**14**)	-	-	-	-	-	-	-	-	-	0.32	7.04	
		Goniothalamicin (**15**)	-	-	-	-	-	-	-	-	-	1.00	10.95	
		cis-Goniothalamicin (**16**)	-	-	-	-	-	-	-	-	-	1.02	18.74	

Abbreviations for cancer cell lines: KB (human nasopharyngeal carcinoma), HCT8 (human colon carcinoma), Bel 7402 (hepatoma cell lines), A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), and PACA-2 (human pancreatic carcinoma), COR-L23 (human large cell lung carcinoma), MRC-5 (normal human fetal fibroblast)

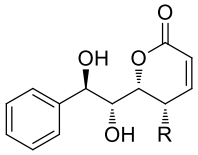
Styryl lactones possess a basic C_6 –C_3 –C_4 skeleton, represented by a phenylpropanoid group connected to four carbons. Styryl lactones with cytotoxic activity were categorized into five-, six- and eight-membered ring molecules or unusual styryl lactones. The five-membered ring styryl lactones exhibit cytotoxic activity.



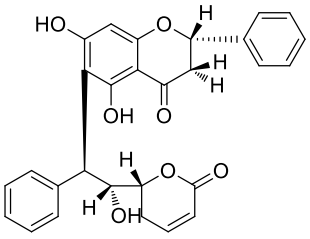
9-deoxygoniopypyrone **(17)** R = H
goniopypyrone **(18)** R = OH



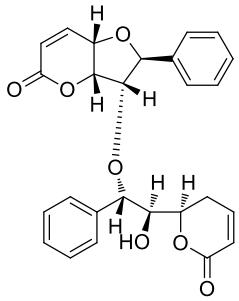
7-epigoniofurfurone **(19)** R_1= OH, R_2 = H
goniofurfurone **(20)** R_1= H, R_2 = OH



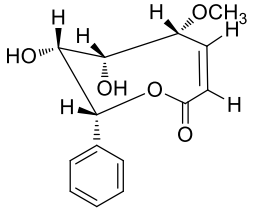
goniodiol **(21)** R = H
goniotriol **(30)** R = OH



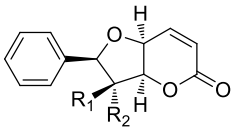
goniolactone B **(22)**



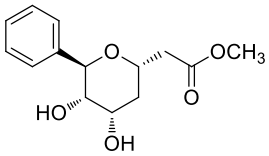
goniodilactone **(23)**



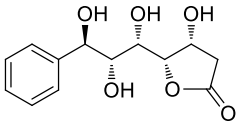
gonioheptenolactone **(24)**



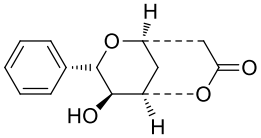
isoaltholactone **(25)** R_1= OH, R_2 = H
altholactone **(28)** R_1= H, R_2 = OH



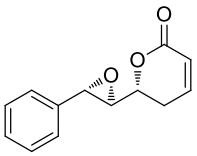
goniothalesdiol **(26)**



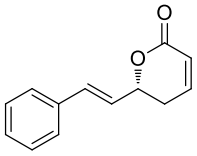
cardiobutanolide **(27)**



(-)-8-epi-9-deoxygoniopypyrone **(29)**



goniothalamin oxide **(31)**



goniothalamin **(32)**

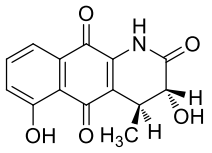
Table 2 Styryl lactones isolated from the *Goniothalamus* genus.

Plant species	part	Chemical constitutes	Inhibition concentration (μg/mL)											Ref.
			KB	KBvin	HCT-8	A-549	MCF-7	H-T29	A-2780	DU-145	PC-3	NCI-187	Vero cell	
G. giganteus	Stem bark	9-deoxygoniopypyrone (**17**)	-	-	-	27.20	25.35	7.38	-	-	-	-	-	13
		Goniopypyrone (**18**)	-	-	-	ND	ND	ND	-	-	-	-	-	
		7-epigoniofurfurone (**19**)	-	-	-	85.49	49.11	>100	-	-	-	-	-	
		Goniofurfurone (**20**)	-	-	-	ND	ND	ND	-	-	-	-	-	
		Goniodiol (**21**)	-	-	-	$1.22x10^{-1}$	8.27	2.45	-	-	-	-	-	
G. cheliensis	root	Goniolactone B (**22**)	7.23	-	4.43	-	-	-	7.40	-	-	-	-	14
	leaves	Goniodiol (**21**)	5.18	4.31	-	5.14	5.46	-	-	4.66	8.40	-	-	15
		Goniodilactone (**23**)	1.82	4.31	-	2.65	1.29	-	-	4.34	3.07	-	-	
		Gonioheptenolactone (**24**)	4.10	4.53	-	4.56	2.64	-	-	3.15	3.78	-	-	
		Isoaltholactone (**25**)	8.14	4.31	-	7.19	8.19	-	-	5.48	7.46	-	-	
G. elegants	bark	Goniothalesdiol (**26**)	IA	-	-	-	IA	-	-	-	-	IA	IA	16
		(+)-cardiobutanolide (**27**)	IA	-	-	-	IA	-	-	-	-	2.17	IA	
		(+)goniofurfurone (**20**)	IA	-	-	-	34.06	-	-	-	-	34.06	IA	
		(+)-altholactone (**28**)	0.96	-	-	-	4.25	-	-	-	-	1.44	6.248	
		(-)-8-epi-9-deoxygoniopypyrone (**29**)	IA	-	-	-	IA	-	-	-	-	13.34	IA	
		(+) goniopypyrone (**18**)	IA	-	-	-	IA	-	-	-	-	17.41	16.27	
		(+)-goniodiol (**21**)	5.27	-	-	-	29.45	-	-	-	-	IA	IA	
		(+)-goniotriol (**30**)	14.57	-	-	-	11.90	-	-	-	-	6.37	15.48	
		(+goniothalamin oxide (**31**)	5.63	-	-	-	8.25	-	-	-	-	7.46	IA	

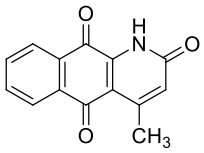
Plant species	part	Chemical constitutes	Inhibition concentration (μg/mL)											Ref.
			KB	KBvin	HCT-8	A-549	MCF-7	H-T29	A-2780	DU-145	PC-3	NCI-187	Vero cell	
		(+)-goniothalamin (**32**)	0.786	-	-	-	3.61	-	-	-	-	0.538	0.675	
G. tapis	leaves	(+)-goniothalamin (**32**)	0.56			0.67	0.56							19

Abbreviations for cancer cell lines: KB (human nasopharyngeal carcinoma), KBvin (multidrug-resistant expression P-glycoprotein), HCT8 (human colon carcinoma), A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), DU145 (human prostate carcinoma), PC-3 (human prostate adenocarcinoma), NCI-187 (small cell lung cancer) and Vero cell (African green monkey kidney). IA (Inactive), ND (not determine).

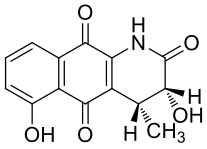
Cytotoxic alkaloids were grouped into three classes, 1-azaanthraquinones, phenanthrene lactams and aporphines. laoticuzanone A, an azanthraquinone alkaloid isolated from stems of *G. laoticus* demonstrated potent cytotoxic activity against both KB and HeLa cells with IC_{50} values of 2.49 and 1.8 μM, respectively.[16] In addition, laoticuzanone A exhibited cytotoxic activity against KB and human cervical carcinoma (HeLa) cells with IC_{50} values of 0.68 and 0.50 μg/mL, respectively.[17] (-)-Nordicentrine exhibited most potent cytotoxic activity against KB, NCI-H187 and MCF-7 cells with IC_{50} values of 0.4, 0.4 and 2.9 μg/mL, respectively.[18]



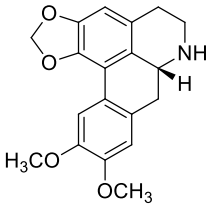
Laoticuzanone A **(33)**



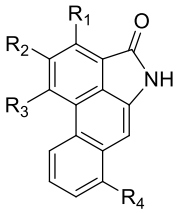
3-Methyl-1H-1-azaanthracene-2,9,10-trione **(34)**



griffithazanone A **(35)**



(-)-Nordicentrine **(36)**



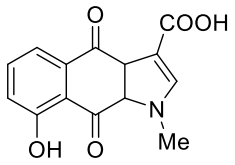
	R_1	R_2	R_3	R_4
Piperolactam B **(37)**	OCH_3	OCH_3	OH	H
Goniopedaline **(38)**	OCH_3	OH	OCH_3	H
Velutinam **(39)**	H	OCH_3	OCH_3	OH
Aristolactam BII **(40)**	H	OCH_3	OCH_3	H
Piperolactam C **(41)**	OCH_3	OCH_3	OCH_3	H

Table 3 Alkaloids isolated from the *Goniothalamus* genus.

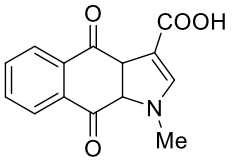
Plant species	part	Chemical constitutes	Inhibition concentration (μg/mL)						Ref.
			KB	HeLa	BC1	MCF-7	NCI-187	Vero cell	
G. laoticus	stems	Laoticuzanone A (**33**)	0.68	0.50	-	-	-	-	17
		3-Methyl-1H-1-azaanthracene-2,9,10-trione (**34**)	5.50	4.00	-	-	-	-	
		Griffthazanone A (**35**)	5.20	3.00	-	-	-	-	
	flowers	(−)-Nordicentrine (**36**)	0.4	-	-	2.9	0.4	-	18
G. elegants	bark	Piperolactam B (**37**)	27.33	-	-	20.54	IA	Non-cytotoxic	16
		Goniopedaline (**38**)	9.39	-	-	13.90	IA	6.14	
		Velutinam (**39**)	6.43	-	-	2.30	7.13	28.17	
		Aristolactam BII (**40**)	IA	-	-	15.4	0.0072	Non-cytotoxic	
		Piperolactam C (**41**)	IA	-	-	IA	IA	Non-cytotoxic	

Abbreviations for cancer cell lines: KB (human nasopharyngeal carcinoma), HeLa (human cervical carcinoma), BC1 (Human breast cancer cells), MCF-7 (human breast carcinoma), NCI-187 (small cell lung cancer) and Vero cell (African green monkey kidney). IA (inactive).

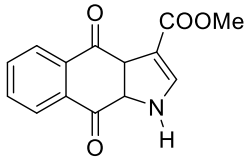
The investigation of compounds from *Goniothalamus* species are still an interesting field. In 2019 Jaidee W. et al. have isolated eight new compounds including three alkaloids (**42**-**44**) and five styryllactones (**46**-**50**) together with 36 known compounds from leaves and twigs extracts of *G. cheliensis* collected from Doi Tung, Chiang Rai Province, Thailand.[20]



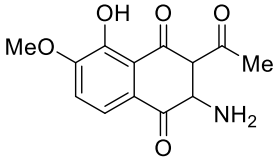
goniochelienic acid A (42)



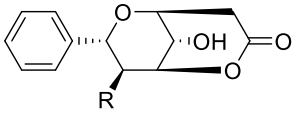
goniochelienic acid B (43)



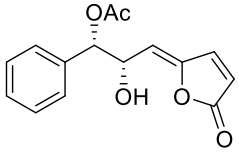
methyl goniochelienate (44)



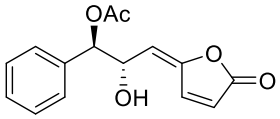
Goniochelieninone (46)



(-)-(*4S,5S,6R,7S,8S*)-goniochelienlactone (47) R = OH
(+)-(*4S,5S,6R,7S,8S*)-7-acetylgoniochelienlactone (48) R = OAc



(+)-(*7S,8S*)-goniochelienbutenolide A (49)

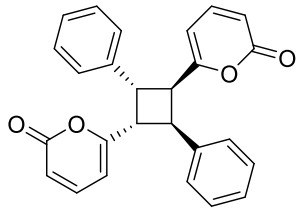


(-)-(*7S,8R*)-goniochelienbutenolide B (50)

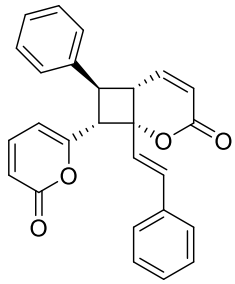
The isolated compounds were found to have no cytotoxicities against the colon cancer cell line HCT-116 at 50 μM except for griffithazanone A (**35**) which had potent cytotoxicities with an IC_{50} value of 2.39 μM.

In 2020, Meesakul et al. reported the isolation of three previously undescribed styryllactones (**51**-**53**) together with 12 known compounds from the twig and leaf extracts of *Goniothalamus tamirensis* which were collected from a plant growing at Mae Fah Luang University, Chiang Rai Province, Thailand.

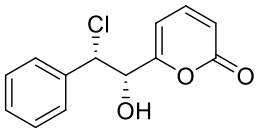
The cytotoxicities of some of the isolated compounds against the colon cancer cell line HCT116 are also reported. Only (–)–5-acetoxygoniothalamin (**54**) and (Z)-6-styryl-5,6-dihydro-2-pyranone (**55**) displayed promising cytotoxicity against a colon cancer cell line (HCT116). Compound **54** had an IC_{50} value of 8.6 μM which was better than standard control (doxorubicin, IC 50 = 9.7 μM), while **55** had an IC_{50} value of 22.2 μM.[21]



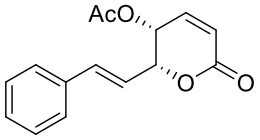
Goniotamirenone A (51)



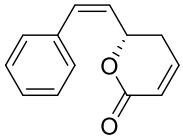
(-)-(*7S,8S,5'S,6'S*)-Goniotamirenone B (52)



(-)-(*7S,8S*)-Goniotamirenone C (53)



(-)-5-acetoxygoniotamin (54)



(*Z*)-6-styryl-5,6-dihydro-2-pyranone (55)

Objectives 1) Isolation of pure natural occurring products from *G. repevensis*

2) Structure elucidation of isolated compounds from *G. repevensis*

3) Evaluation the biological activities of isolated compounds from *G. repevensis* particulary, cytotoxicities and anti-bacterial activities.

Research Methodology

1) The extraction of dried plant sample which has deposit the voucher specimen in Herbarium.

2) Screening of bioactivities of crude extract in variety of assays.

3) Purification of compounds by using the chromatographic techniques.

4) Structure elucidation of pure compounds by using ^{1}H-NMR and 2D-NMR and other spectroscopic techniques such as IR, UV, CD spectra.

5) Evaluate the biological activities of isolated pure compounds. Explaining the results for structure activity relation (SAR) studies.

5) Submit the isolated compounds to evaluate the cytotoxicities in human cancer cell lines and anti-bacterial activities.

Experimental

1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter, UV spectra were recorded using a Shimadzu 2800 UV-visible spectrophotometer. IR spectra were obtained using a Perkin-Elmer Frontier FT-IR spectrophotometer. EIMS were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe). The HRMS were recorded on a Micromass model VQ-TOF-2 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer and

a Bruker Avance-500 spectrometer in $CDCl_3$ or CD_3OD using TMS or residual non-deuterated solvent peak as an internal standard. Chromatographic methods were performed by using silica gel 60 (Merck, 70–230 mesh) for column chromatography and silica gel plates (Merck, Silica gel 60 PF_{254}) for preparative TLC and sephadex LH 20 (GE Healthcare) for gel filtration. All solvents used for extraction and isolation were distilled at their boiling point ranges prior to use.

2. Plant Materials

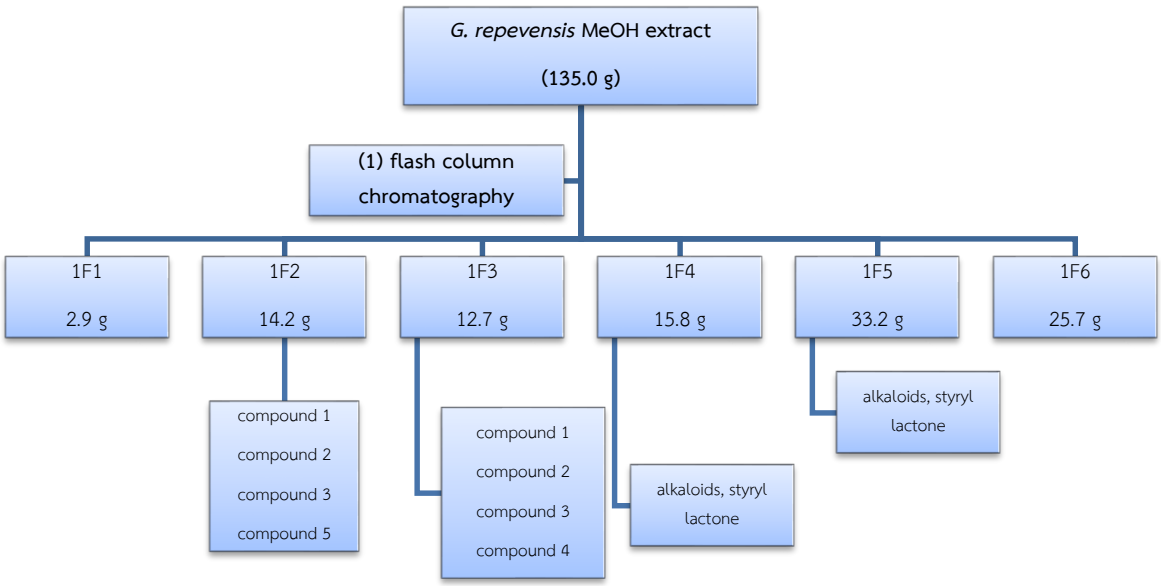
Leaves twigs and stem of *G. repevensis* Pierre ex Finet & Gagnep were collected from Khao Khitchakut National Park, Chanthaburi Province, Thailand. It was identified and the voucher specimen (BKF 192543) was deposited at the Forest Herbarium, Royal Forestry Department, Bangkok, Thailand.

3. Extraction and isolation

Air-dried, powdered leaves twigs and stems (1.88 kg) of *G. repevensis* were extracted with methanol (6 × 4 L) at room temperature. After filtration and removal of the solvents by rotary evaporator followed by vacuum desiccation, the crude methanol extract (181.5 g) was obtained. The methanol extracts was evaluated for in vitro cytotoxic activities against seven cell lines, the results as shown in Table 1.

The methanol extract (135.0 g) was subjected to flash column chromatography over silica gel (400 g), eluting with a gradient solvent system. Elution was initially conducted with 100% hexane, gradually enriched with EtOAc (10–100%), followed by increasing the amount of MeOH in EtOAc (10–80%) and finally with 100% MeOH. The fractions (500 mL each) were collected, monitored by TLC and combined on the basis of their TLC characteristics to afford six fractions (F1–F6). All fractions were subjected for testing *in vitro* cytotoxic screening assays. Goniodiol-7-monoacetate (**1**, 0.53 g) was filtered from F2, goniodiol diacetate (**2**, 20 mg), piperolactam C (**3**, 40 mg) and

aritolactam B III (5, 21.6 mg) were isolated from F2 and F3. Aristolactam A III (**4**, 20 mg) was isolated from F3.



Scheme 1. Chart of isolation compounds from *G. repevensis* methanol extract.

4. Results and Discussions

The cytotoxic activities of methanol extract of *G. repevensis* and six fractions after flash column chromatography as shown in Table 4. The investigation of bioactive compounds was start from fraction 2 which active against P-388, KB and MCF 7 cell lines. Goniodiol-7-monoacetate (**1**) and goniodiol diacetate (**2**) were isolated from F2.

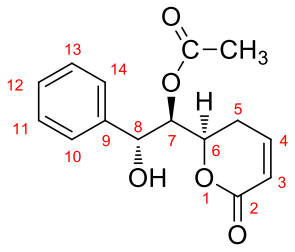
Table 4. Cytotoxic activities of *G. repevensis* methanol extract and fractions F1-F6.

	Inhibition concentration (ED_{50}, μg/mL)						
	P-388	**KB**	**HT-29**	**MCF 7**	**A549**	**ASK**	**Hek293**
Ellipthicine (1)	0.42	0.51	0.64	0.50	0.54	0.51	0.60
MeOH extract	4	1.58	3.81	3.89	3.16	3.17	2.01
Ellipthicine (2)	0.45	0.58	0.56	0.53	0.53	0.63	0.53
F1	>20	>20	>20	>20	>20	>20	>20
F2	9.38	6.40	>20	9.70	>20	>20	11.56
F3	3.66	4.73	13.17	10.90	12.85	12.84	4.79
F4	0.75	0.75	13.37	2.42	4.01	8.99	0.54
F5	0.63	0.24	7.89	0.53	2.51	0.19	4.10
F6	0.65	0.29	8.43	0.59	1.70	5.32	0.42

ED50 ≤ 20 μg/mL is considered active; P-388, murine lymphocytic leukemia; KB, human oral nasopharyngeal carcinoma; Col-2, human colon cancer; MCF-7, human breast cancer; Lu-1, human lung cancer; ASK, rat glioma cell, Hek293, human embryonic kidney cell.

Ellipticine was used as a positive control for cytotoxicity assay.

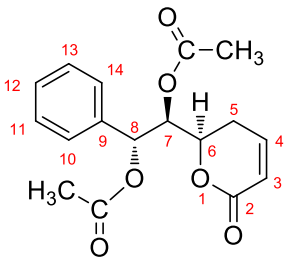
Data from MNR spectrum of compound **1** and **2** compared with those report in literature are shown in Table 5 and 6.



(-)-Goniodiol-7-monoacetate

Table 5. NMR spectroscopic data of isolated (-)-goniodiol-7-monoacetate in $CDCl_3$ compared with literature.[22]

position	Experiment		Literature (Wu, 1991)	
	δ_C	δ_H	δ_C	δ_H
2	164.04	-	170.48	-
3	120.94	6.01 (ddd, J= 9.76, 1.57, 0.84)	121.32	6.02 (ddd, J = 9.6, 2.2, 1.0)
4	145.48	6.91 (ddd, J = 9.76,5.66. 2.88)	146.50	6.90 (ddd, J = 9.6, 4.8, 2.2)
5	26.14	2.32 (m, 2H)	26.40	2.34 (2H, m)
6	75.09	5.13 (dd, 1.5, 4.9)	70.64	5.08 (ddd, J = 9.6, 4.8, 1.0)
7	75.22	5.10 (dd, 9.1, 1.5)	75.62	5.16 (dd, J = 8.0, 4.8)
8	70.61	5.18 (d, 9.1)	75.49	5.12 (d, J = 8.0)
9	140.60	-	141.44	-
10, 14	128.26	7.xx (m, 5H)	128.70	7.37 (m, 5H)
11, 13	126.90		127.54	
12	128.21		128.70	
7-OCOMe	169.76	-	165.07	-
7-OCOMe	20.35	1.81 (s, 3H)	20.55	1.82 (s, 3H)
8-OH	-		-	3.12 (br. s., 1H)

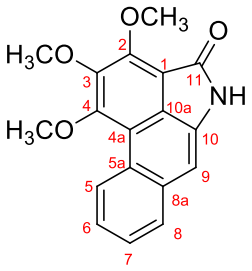


Goniodiol diacetate

Table 6. NMR spectroscopic data of isolated goniodiol diacetate in $CDCl_3$ compared with literature.[23]

position	Experiment		Literature (Wu, 1992)	
	δ_C	δ_H	δ_C	δ_H
2	162.98	-	163.6	-
3	121.37	6.04 (ddd, J = 9.8, 2.4, 1.3)	121.9	6.07 (ddd, J = 9.8, 2.6, 1.4)
4	144.36	6.88 (ddd, J = 9.8, 5.3, 3.1)	145.0	6.87 (ddd, J = 9.8, 5.0, 3.4)
5	26.05	2.35 (m, 2H)	26.3	2.37 (m, 2H)
6	74.61	4.78 (ddd, J = 10.4, 5.6, 2.6)	72.6	7.48 (ddd, J = 9.4, 4.0, 2.6)
7	73.44	5.35 (dd, J = 8.5, 2.6)	73.8	5.36 (dd, J = 8.6, 2.6)
8	72.30	6.04 (d, J = 8.5)	74.9	6.02 (d, J = 8.6)
9	136.48	-	137.0	-
10, 14	127.43	7.37 (m, 5H)	127.9	7.35 (m, 5H)
11, 13	128.43		128.9	
12	128.70		129.2	
7-OCOMe	169.77	-	169.7	-
7-OCOMe	20.33	1.82 (s, 3H)	20.6	1.83 (s, 3H)
8-OCOMe	169.03	-	170.4	-
8-OCOMe	21.04	2.09 (s, 3H)	21.7	2.09 (s, 3H)

NMR spectroscopic data of piperolactam C (**3**)[24], aristolactam A III (**4**)[25,26] and aristolactam B III (**5**)[25,26] are shown in Table 7 - 9, respectively.

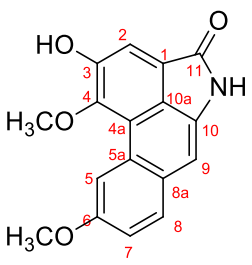


Piperolactam C

Table 7. NMR spectroscopic data of isolated piperolactam C in $CDCl_3$

Position	Experiment		Literature (Desai, 1988)	
	δ_C	δ_H	δ_C	δ_H
1	109.17	-		-
2	154.10	-		-
3	146.33	-		-
4	157.27	-		-
4a	109.17	-		-
5a	116.39	-		-
5	126.51	9.19 (s, 1H)		9.10 (d)
6	125.72	7.53 (m, 2H)		7.57 (m)
7	125.72	7.53 (m, 2H)		7.57 (m)
8	128.76	7.82 (m, 1H)		7.98 (d)
8a	133.57	-		-
9	106.47	7.22 (s, 1H)		7.27 (s)
10	133.40	-		-
10a	125.87	-		-
11	167.89	-		-
NH	-	9.29 (s)		11.00 (s)

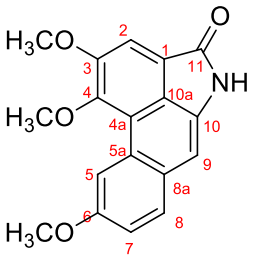
2-O<u>C</u>H_3	63.04	4.55 (s, 3H)		3.93 (s, 3H)
3-O<u>C</u>H_3	61.65	4.00 (s, 3H)		4.12 (s, 3H)
4-O<u>C</u>H_3	60.87	4.19 (s, 3H)		4.39 (s, 3H)



Aristolactam AIII

Table 8. NMR spectroscopic data of isolated aristolactam A III in DMSO-d_6

Position	Experiment		Literature (Priestep 1985, Mix 1982)	
	δ_C	δ_H	δ_C	δ_H
1	122.35	-		
2	113.98	7.62 (s, 1H)		7.65 (s)
3	152.25	-	151.9	
4	149.26	-	148.9	
4a	120.49	-		
5a	127.55	-		
5	109.71	8.65 (d, J = 2.6)		8.67 (d, J = 3.5)
6	157.34	-	157.0	
7	116.57	7.25 (dd, J = 8.8, 2.6)		7.25 (dd, J = 9, 3.5)
8	130.47	7.87 (d, J = 8.8)		7.89 (d, J = 9)
8a	129.27	-		
9	104.34	7.06 (s, 1H)		7.08 (s)
10	133.79	-		
10a	122.98	-		
11	168.74	-		
NH	-	10.72 (s)		
3-OH	-	10.29 (br. s.)		10.22 (s)
4-OCH_3	59.96	4.04 (s, 3H)		4.08 (s)
6-OCH_3	55.59	3.92 (s, 3H)		3.95 (s)



Aristolactam BIII

Table 9. NMR spectroscopic data of isolated aristolactam B III in $CDCl_3$

Position	Experiment		Literature (Priestep 1985, Mix 1982)	
	δ_C	δ_H	δ_C	δ_H
1		-		
2	109.75	7.81 (s, 1H)		7.81, s
3	154.20	-	154.0	
4	151.63	-	150.6	
4a		-		
5a		-		
5	109.9	8.79 (d, J = 2.6)		8.63 (d, J= 2.5)
6	157.7	-	157.1	
7	116.5	7.21 (dd, J = 8.8, 2.6)		7.20 (dd, J = 9, 2.5)
8	129.9	7.71 (d, J = 8.8)		7.84 (d, J = 9)
8a	124.6	-		
9	105.5	7.06 (s, 1H)		7.07 (s)
10		-		
10a		-		
11	168.9	-		
NH	-	8.65 (br. s)		10.70 (s)
3-OCH_3	56.95	4.13 (s, 3H)	57.1	4.05 (s)

4-OCH_3	60.03	4.06 (s, 3H)	60.0	4.05 (s)
6-OCH_3	55.42	3.99 (s, 3H)	55.2	3.92 (s)

Cytotoxic activity

Results for cytotoxic activity against a panel of cancer cell lines are shown in Table 10.

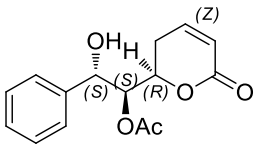
Table 10. Results of cytotoxic activities of compound **1**-**4**

compound	Cell line					
	KB	HT 29	MCF-7	A 549	ASK	CL
Ellipthicine	0.50	0.57	0.48	0.49	0.48	0.50
1	6.63	9.70	15.36	10.40	2.84	8.95
2	9.25	8.81	11.24	9.01	4.23	8.32
3	2.62	> 20	9.65	3.68	1.02	2.54
4	3.02	13.50	18.79	13.60	2.18	12.14

ED50 ≤ 4 μg/mL is considered active; P-388, murine lymphocytic leukemia; KB, human oral nasopharyngeal carcinoma; HT-29, human colorectal adenocarcinoma; MCF-7, human breast carcinoma; A 549, human lung carcinoma; ASK, rat glioma cell, CL, human normal liver hepatic cells. Ellipticine was used as a positive control for cytotoxicity assay.

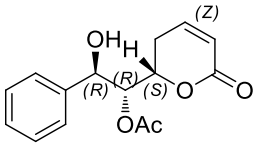
Goniodiol-7-monoacetate (**1**) and goniodiol diacetate (**2**) have been isolated from *Goniothalamus amuyon*.[22] They showed potent cytotoxicity against human epidermoid carcinoma of the nasopharynx (KB), murine lymphocytic leukemia (P-388), human melanoma (RPMI) and central nervous system carcinoma (TE671) with ED_{50} values of < 0.1 μg/mL.[23]

(+)-Goniodiol-7-monoacetate (**+1**) isolated from *G. amuyon* showed more potent cytotoxic activity than its diastereomer (-)-7-O-acetylgoniodiol (**-1**) isolated from *G. griffithii*.[27]



(+)-7-O-acetylgoniodiol

Wu et al. 1992



(-)-7-O-acetylgoniodiol

Kampong et al. 2013

Compound	Cell lines (ED_{50}, μg/mL)											
	P-388	KB	RPMI	TE671	Col2	MCF7	Lu1	A549	HCT8	T24	ASK	Hek293
(+) **1**	<0.1	<0.1	<0.1	<0.1	-	-	-	>4	>4	-	-	-
(-) **1**	3.31	3.26	-	-	9.64	6.24	7.74	8.95	-	8.55	9.41	1.89

The structure - activity relationship (SAR) of six-membered ring styryl lactone from *Goniothalamus* species, goniodiol with two hydroxyls in C- 7 and C-8 was most toxic againsts A-549 cells with an IC_{50} value of 0.5 μM. The presence of an acetoxy instead of a hydroxyl moiety reduced the cytotoxic activity against A-549 cells by 30-fold compared to goniodiol. Nonetheless, it exhibited strong cytotoxic against KB and P-388 cell lines with IC_{50} less than 0.4 μM. [1]

Aristolactam is one type of compounds have been found in Annonaceae family. The substituents (methoxyl and hydroxyl) on rings B and D of compounds are probably the major influence on the cytotoxic activity. [25] It have been reported that the cytotoxicities of aristolactams are less active than styryl lactones which isolated from the same plant as seen in the activities of compounds from *G. elegants*. [26] But from this research, aristolactams are more active than isolated styryl lactones. It might due to the styryl lactone are in acetylated form which will reduce their cytotoxic activities.

Conclusion

Five known compounds including, (-)-goniodiol-7-monoacetate **1**, goniodiol acetate **2**, piperolactam C **3**, aristolactam AIII **4** and aristolactam BIII **5** were isolated from methanolic extract of leaves twigs and stem of *Goniothalamus repevensis*. The structures of isolated compounds were elucidated and compared with literatures. Compound **1**-**4** were subject to test cytotoxic activities against panel of cancer cell lines and express the results as IC_{50} value ranged 1.02 – 18.79 µg/mL.

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