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Review

Goniothalamus Species: A Source of Drugs for the Treatment of Cancers and Bacterial Infections?

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Irrespective of the presence of cytotoxic acetogenins and styryl-lactones in the genus *Goniothalamus*, only 22 species in the genus *Goniothalamus*, out of 160 species (13.7%) have so far been investigated. In an effort to promote further research on the genus *Goniothalamus* which could represent a source of drugs for the treatment of cancers and bacterial infections, this work offers a broad analysis of current knowledge on *Goniothalamus* species. Therefore, it includes (i) taxonomy (ii) botanical description (iii) traditional medicinal uses and (iv) phytochemical and pharmacological studies. We discuss the molecular mechanisms of actions of acetogenins and styryl-lactones, with some emphasis on the possible involvement of protein kinase, Bax and TRAIL receptors in the cytotoxic effects of styryl-lactones. We also report (v) the growth inhibition of several nosocomial bacteria by *Goniothalamus. scortechinii*. The crude methanol extract of *G. scortechinii* showed a good and broad spectrum of antibacterial activity against both Gram-negative and Gram-positive bacteria.

Keywords: acetogenins – antibacterial – antifungal – apoptosis – cytotoxic – foodborn bacteria – *Goniothalamus* – *Goniothalamus scortechinii* – nosocomial – styryl-lactones.

Introduction

The genus *Goniothalamus* Hk. f. et Thoms. (Family Annonaceae A.L. de Jussieu 1789 nom. conserv., the Custard-Apple Family) consists of 160 species of archaic shrubs and treelets which grow in the shady primary rainforest of tropical Asia. These plants can be quickly spotted in field collection by their aromatic bark and fusiform leathery flowers (1,2). A number of *Goniothalamus* species have been used for timber, as fiber sources (2), for ornamental and medicinal purposes, especially in relation with post-partum and abortion (3,4). The genus *Goniothalamus* belongs to a primitive taxon of flowering plants: the Annonaceae (Family Annonaceae A.L. de Jussieu 1789 nom. conserv., the Custard-Apple Family) (5). The Annonaceae form a large, generally recognizable family of about 122 genera and 2000 plant species which are widespread chiefly in tropics and subtropics (6–8). In regards to the pharmacological potentials of *Goniothalamus* species, there is a massive body of evidence to suggest that this taxon has the ability to elaborate series of acetogenins and styryl-lactones which are cytotoxic against a broad array of cancer cells including breast, colon, kidney and pancreatic carcinoma cells. Interestingly, both acetogenins and styryl-lactones are completely different in terms of chemical structure but their cellular activities are involving the same organelles in mammals: the mitochondria. An exciting fact about the mode of action of styryl-lactones, which is still an enigma, is their possible action via protein kinase and TRAIL receptors. In an effort to promote further research on the genus *Goniothalamus* which could be a promising source for chemotherapeutic agents, this work offers a broad analysis of current knowledge on *Goniothalamus* species. We also highlight

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the antibacterial activity of *Goniothalamus scortechinii*. This is the first antibacterial study report on *Goniothalamus* species.

Botanical Description

The botanical characteristics of *Goniothalamus* species are homogenous and simple. When searching for *Goniothalamus* species in the rainforest, one is advised to look for few-leaved slender treelets or shrubs with smooth, thin and fibrous and strongly aromatic bark and upright blackish cylindrical trunk. The leaves are few, simple, alternate and exstipulate. The blade is glossy, oblong—lanceolate to obovate and thick. The secondary nerves are oblique, conspicuous, straight and parallel with scalariform reticulations (9). The flowers are axillary and characteristically woody, often dark green and fusiform (Fig. 1). The calyx consists of three sepals which are valvate, membranous, veined, free or connate. The corolla consists of two series of three petals which are veined and coriaceous, the inner smaller and fused in a vault above the androecium. The outer petals are marked with a prominent midrib. The androecium comprises several stamens which are linear and oblong. The gynaecium consists of several free carpels grooved at the anterior side. The fruits are stalked or sessile one to two seeded ripe carpels (6). The geographical pattern of distribution of this genus suggests the genus *Goniothalamus* to have been among the flowering plants to have colonized hearth during the post-Permian early Cretaceous time.

Traditional Medicinal Uses

Out of 160 species, five *Goniothalamus* species are medicinal. These have been used in traditional medicinal Asian system, and since a long period of time most of these in connection with abortion, childbirth and fever (10). The leaves of *G. macrophyllus* Hook.f & Thoms. are used to allay fever and a decoction of the roots is given as a post-partum remedy and to cause abortion (3). In Malaysia, a decoction of leaves is used externally to allay fever (3). The roots of *G. giganteus* Hook.f & Thoms. are used to abort and treat colds and the heated leaves are applied onto swellings (10). A decoction of *G. scortechinii* is given as a post-partum protective remedy (3). The roots of *G. tapis* Miq. are used as abortifacient during early months of pregnancy (3). In Java, Indonesia, an infusion of the roots is used to treat typhoid fever (11). In Taiwan, the seeds of *G. amuyon* Merr. are used to treat scabies (12). In the Philippines, the seeds are used to treat rheumatism and tympanites, and the fruit is stomachic (4). None of the traditional uses previously mentioned has been substantiated yet via strict pharmacological experimentation. However, these species have been studied for their chemical constituents.

Phytochemical and Pharmacological Studies

General Concept

Twenty-two species (13.7%) in the genus *Goniothalamus*, out of 160 species, have so far been phytochemically investigated namely: *G. amuyon*, *G. andersonii* J.Sincl., *G. arvensis* Scheff, *G. borneensis* Mat-Salleh, *G. cardiopetalus*, *G. cheliensis*. Hu, *G. donnaiensis* Finet & Gagnep., *G. gardneri* Hook. f. & Thoms., *G. giganteus* Hook. f. & Thoms., *G. griffithii* Hook. f. & Thoms., *G. howii* Merr., *G. leiocarpus* (W.T.) Wang P.T. Li, *G. malayanus* Hook. f. & Thoms., *G. marcanii* Craib, *G. montanus* J. Sincl., *G. scortechinii*, *G. sesquipedalis* Hook. f. & Thoms., *G. tapis* Miq., *G. thwaitesii* Hook. f. & Thoms., *G. umbrosus* J. Sincl., *G. uvaroides* King and *G. velutinus* Airy Shaw. These phytochemical studies have resulted so far in the isolation of two very distinct classes of lipophilic secondary metabolites: acetogenins and styryl-lactones, both of them possessing complex stereochemistry and existing in different stereoisomeric forms (13). Testing of these chemicals for cytotoxicity showed that both acetogenins and styryl-lactones are toxic for several human tumors cell lines. Note that both acetogenins and styryl-lactones are cytotoxic for mammalian cells as the result of distinct biochemical pathways which however take both their molecular origin near or in the mitochondrial membrane and or mitochondrial respiratory system (14). To date some evidence clearly demonstrate that acetogenins have beneficial effects against the growth of tumors, including ovarian tumors (15), gastric tumors (16) and multidrug-resistant cancerous xenografts (17) via the activation of caspases enzymatic cascades (18). Most phytochemical reports found so far on *Goniothalamus* species deal with the chemical constituents of a medicinal species: *G. giganteus* which abounds with cytotoxic acetogenins (Table 1) as mentioned further.

Acetogenins: Unusual Polyketides

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*, G. *donnaiensis* and *G. gardneri* (9,10). In the genus *Goniothalamus*, acetogenins were first characterized as the active principles responsible for shrimp lethality from the bark of *G. giganteus* collected from Thailand. Extract of the bark showed toxicity in the brine shrimp test and showed murine cytotoxicity in the 3PS (P388) leukemia bioassay. The cytotoxicity of this extract compelled a series of phytochemical studies which

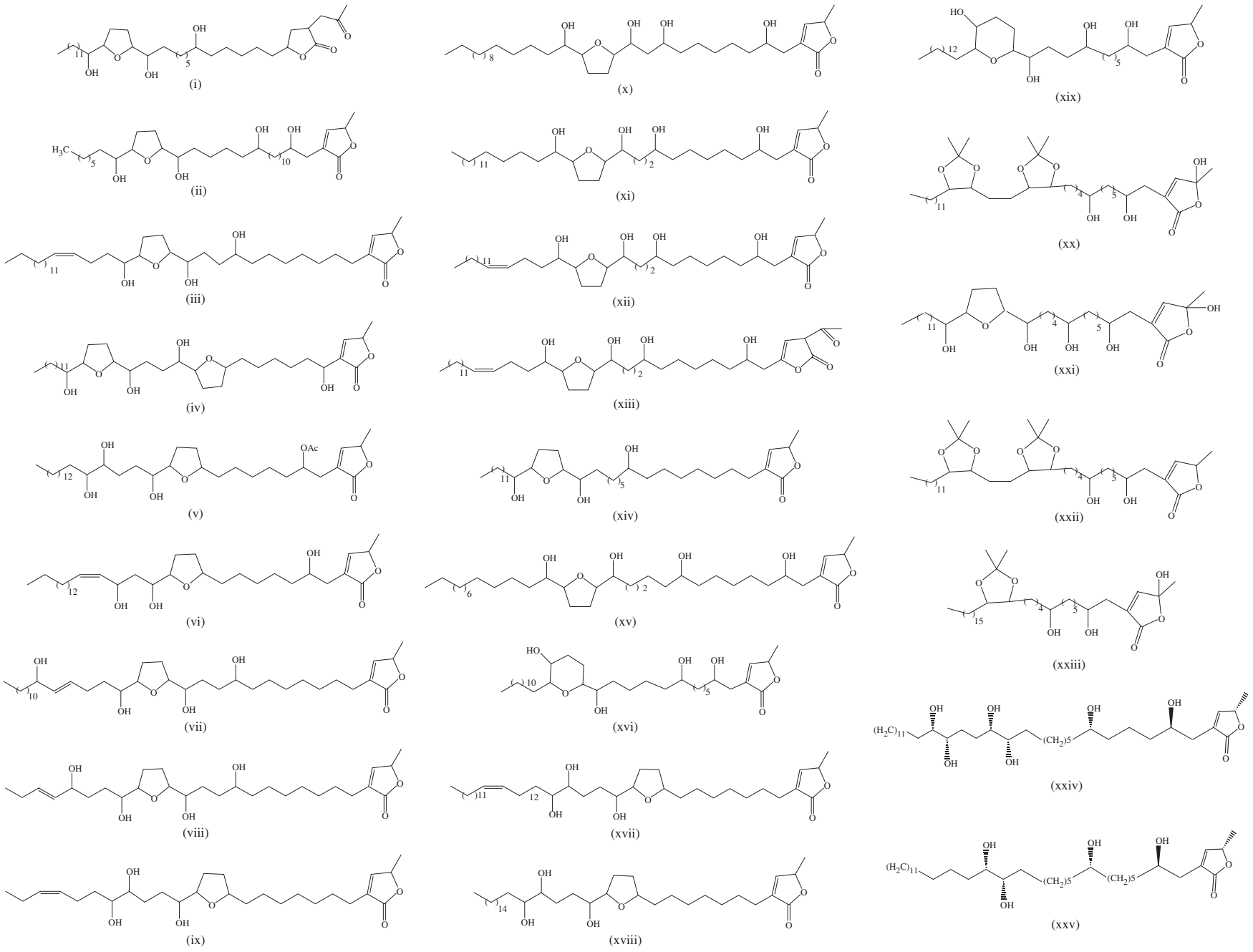


Figure 1. Acetogenins from *Goniothalamus* species.

Table 1. Antitumor activity of *Goniothalamus* species

Species	Chemical component	Cells	Dose	Cell cycle/apoptosis
G. giganteus	4-Deoxyanomontacin (27)	A-549[a]	6.45×10^{-7} μg/ml	
		MCF-7[b]	5.77×10^{-7} μg/ml	
		HT-29[c]	1.41×10^{-1} μg/ml	
		A-498[d]	1.50×10^{-1} μg/ml	
		PC-3[e]	1.73×10^{-1} μg/ml	
		PACA-2[f]	$1. \times 10^{-5}$ μg/ml	
	(2,4-*cis* and *trans*)-annomontacinone (27)	HT-29	2.55×10^{-1} μg/ml	
		PACA-2	6.78×10^{-1} μg/ml	
	cis-Gigantrionenin (32)	A-549	5.99×10^{-2} μg/ml	
		MCF-7	2.68×10^{-1} μg/ml	
		HT-29	6.94×10^{-6} μg/ml	
		A-498	1.39×10^{-2} μg/ml	
		PC-3	1.11×10^{-1} μg/ml	
		PACA-2	1.15×10^{-1} μg/ml	
	4-Acetylgigantetrocin (25)	A-549	$<10^{-2}$ μg/ml	
		MCF-7	8.5×10^{-1} μg/ml	
		HT-29	$<10^{-2}$ μg/ml	
		A-498	1.55×10^{-1} μg/ml	
		PACA-2	$<10^{-2}$ μg/ml	
	Annonacin (19)	PA1[g]	0.452 μg/ml	G1
		SKOV3[h]	0.411 μg/ml	
		HeLa[i]	0.219 μg/ml	
		HeLa S3[j]	0.426 μg/ml	
		MCF-7	0.433 μg/ml	
		T-24[k]	0.324 μg/ml	
		BCC-1[l]	0.427 μg/ml	
	Gigantransenin A (26)	A-549	0.16 μg/ml	
	Gigantransenin B (26)	A-549	0.21 μg/ml	
		MCF-7	2.1×10^{-1} μg/ml	
	Gigantrasenin C (26)	A-549	0.18 μg/ml	
	Goniotetrocin (29)	A-549	$3.9\ 10^{-1}$ μg/ml	
		PC-3	$2.1\ 10^{-1}$ μg/ml	
		PACA-2	$2.6\ 10^{-2}$ μg/ml	
	(2,4-*cis* and *trans*)			
	Gonioneninone (29)	PACA-2	$4.5\ 10^{-2}$ μg/ml	
	Goniothalamicin (24)	A-549	2.8010^{-1} μg/ml	
	Gonionenine (24)	PACA-2	$4.5\ 10^{-1}$ μg/ml	
	Pyranicin (30)	A-549	$2.8\ 10^{-1}$ μg/ml	
		MCF-7	3.6×10^{-1} μg/ml	
		A-498	1.8×10^{-1} μg/ml	
		PACA-2	$1.3\ 10^{-3}$ μg/ml	
	Deoxyannomontacin (27)	A-549	$6.45\ 10^{-7}$ μg/ml	
		MCF-7	1.41×10^{-1} μg/ml	
		A-498	1.50×10^{-1} μg/ml	
		PACA-2	$1.5\ 10^{-5}$ μg/ml	
	Annomontacinone (27)	HT-29	2.55×10^{-1} μg/ml	

(continued)

Table 1. Continued

Species	Chemical component	Cells	Dose	Cell cycle/apoptosis
G. giganteus	Annomontacinone (27)	PACA-2	6.78×10^{-1} μg/ml	
	Pyragonicin (30)	A-549	$<10^{-2}$ μg/ml	
		HT-29	$3.4\ 10^{-1}$ μg/ml	
		A-498	1.55×10^{-1} μg/ml	
		PACA-2	$5.8\ 10^{-3}$ μg/ml	
	Goniotrionin (30)	A-549	$7.7\ 10^{-3}$ μg/ml	
		MCF-7	8.5×10^{-1} μg/ml	
		MCF-7	$5.3\ 10^{-6}$ μg/ml	
		A-498	2.10^{-3} μg/ml	
		PC-3	$3.6\ 10^{-1}$ μg/ml	
		PACA-2	$5.4\ 10^{-3}$ μg/ml	
G. donnaiensis	Goniodonin (34,35)	HCT-8[m]	<10 μg/ml	
	Donhexocin (34,35)	HCT-8	0.82 μg/ml	
	Donbutocin (34,35)	L1210	0.81 μg/ml	
G. gardneri	Gardnerilin A (37)	Bel7402n	3.6 μg/ml	
	Gardnerilin B (37)	Bel7402[n]	8.5 μg/ml	
G. andersonii	Goniothalamin (47)	HL-60[o]		apoptosis
		Jurkat T[p]		apoptosis
G. griffithii	Goniothalamin (82)	HepG2	8.83 μM	G2/apoptosis
		HepG2R	8 μM	
	Altholactone (82)	HepG2	0.7 μM	apoptosis
		HepG2R	6.17 μM	
	Goniodiol (82)	HepG2	10 μM	G2
		HepG2R	8.33 μM	
G. malayanus	Altholactone (74,76)	HL60		apoptosis
G. borneensis	Goniothalamin (49)	P388	0.75 μg/ml	
		WEHI164	1.70 μg/ml	
		MOLT-4	<1 μg/ml	
G. howii	Howiinol (79)	L1210	6.85 μg/ml	G1
G. cheliensis	Goniolactone B (54)	A2780	7.40 μM	
		HCT-8	4.43 μM	
		KB	7.23 μM	
Synthesized	Goniothalamin (66)	MCF-7	10.5 μM	
		HT-29	11.2 μM	

[a]human lung carcinoma; [b]human breast carcinoma; [c]human colon adenocarcinoma; [d]human kidney carcinoma, [e]human prostate adenocarcinoma, [f]human pancreatic carcinoma, [g,h]ovarian cancer cells; [i,j]cervical cancer; [k]bladder cancer; [l]skin cancer, [m]human colon adenocarcinoma, [n]hepatoma cell-line, [o]leukemia cells, [p]promyelocytic leukemia cells; [q]Ehrlich ascites tumor cells.

resulted in the identification of a series of cytotoxic acetogenins including notably: (2,4-*cis* and *trans*-)-annomontacinones (i), annonacin (ii), giganenin (iii), gigantecin (iv), 4-deoxygigantecin, (2,4-*cis* and *trans*)-giganteciones, 4-acetylgigantetrocin A (v), goniotrionin (vi), gigantransenin A (vii) and C (viii), gigantrionenin (ix), gigantetrocin (x), goniotetrocine (xi), (2,4-*cis* and *trans*)-gigantetrocinones, gonionenin (xii), (2,4-*cis* and *trans*)-gonioneninones (xiii), 4-deoxygigantenin (xiii), 4-deoxyannomontacin (xiv), goniothalamicin (xv), pyranicin (xvi), gigantriocin (xvii), goniotriocin, (2,4-*cis* and *trans*)-isoannonacins, longicoricin, longifolicicin, longimicin C, *cis*-gigantrionenin (xviii), pyragoniocin (xix), xylomaticin, and (2,4-*cis* and *trans*)-xylomaticinones (Fig. 1) (19–33). Gigantransenins A, and C showed selective inhibitory effects on the human breast tumor cell-line (MCF-7) comparable with the potency of adriamycin (26). Both goniotetracin, and 2,4-*cis*- and *trans*-gonioneninone are selectively and significantly cytotoxic to the human pancreatic tumour cell line (PACA-2) (29). Pyranicin exhibited a selective cytotoxic against the pancreatic cell line (PACA-2) in a panel of six human solid tumor cell lines, with pyranicin showing 10 times the potency of adriamycin (30).

Jiang *et al*. isolated donhepocin (xx), goniodin (xxi), donhexocin (xxii) and donbutocin (xxiii), from *G. donnaiensis* Finet & Gagnep. collected from Guangxi Province, China (34–36). Gardnerilins A (xxiv) and B (xxv) from *G. gardneri* Hook.f. & Thoms collected from DiaoLo mount, Hainan Province, China, gave cytotoxic IC_{50} values against Bel 7402 human tumor cell lines of 3.6 and 8.5 μg/ml, respectively (37,38) (Fig. 1). The mode of action of acetogenins is discussed next.

Mode of Action of Acetogenins: Inhibition of NADH-ubiquinone Oxidoreductase

Acetogenins have very potent and diverse biological effects owing to the fact that they inhibit enzymatic activity of a key enzyme in Eukaryotic cells: mitochondrial NADH-ubiquinone oxidoreductase (complex I). To date the most potent existing inhibitor of this enzyme is an acetogenin known as bullatacin (39–41). In regards to the precise molecular mode of action of acetogenins against the enzyme, there is an expanding body of evidences to suggest that the most lipophilic moieties are embedded in the mitochondrial membrane allowing suitable position of the pharmacophore. One might set the hypothesis that the tetrahydrofuran (THF) or tetrahydropyran rings as well as the free alkyl substituent fix the molecule, whereas the lactones maintained by an alkyl spacer acts on the active site of the enzyme as illustrated in Fig. 2 (42,43). The work of Motoyuki *et al*. (13) lends strong support to that hypothesis. They synthesized series of acetogenins and assessed their activity against bovine heart mitochondrial complex I and showed that the length of the alkyl spacer and the polarity of THF surroundings were very important structural factor and that the γ-lactone and THF ring moieties act in a cooperative manner on complex I with the support of some specific conformation of the alkyl spacers as illustrated in Fig. 2.

The cytotoxic activity of acetogenin has prompted further work in an effort to discover synthetic acetogenins (44–46). Oberlies *et al*. (41) studied the cytotoxicity of acetogenins toward cancerous and normal cells and showed that they are selectively cytotoxic against cancerous cells and also effective for drug-resistant cancer cells, while exhibiting only minimal toxicity to 'normal' non-cancerous cells. However, further work is needed to render acetogenins more specific to cancerous cells and very much less active against normal cells or significantly heavy side-effects will preclude clinical trials. A possible approach would perhaps be to use antigen-guided or receptor-guided forms of administrations by associating acetogenins to specific carriers, hemisynthesis could be of value in this instance. More specific cytotoxic principles from *Goniothalamus* species are styryl-lactones reviewed in the next section.

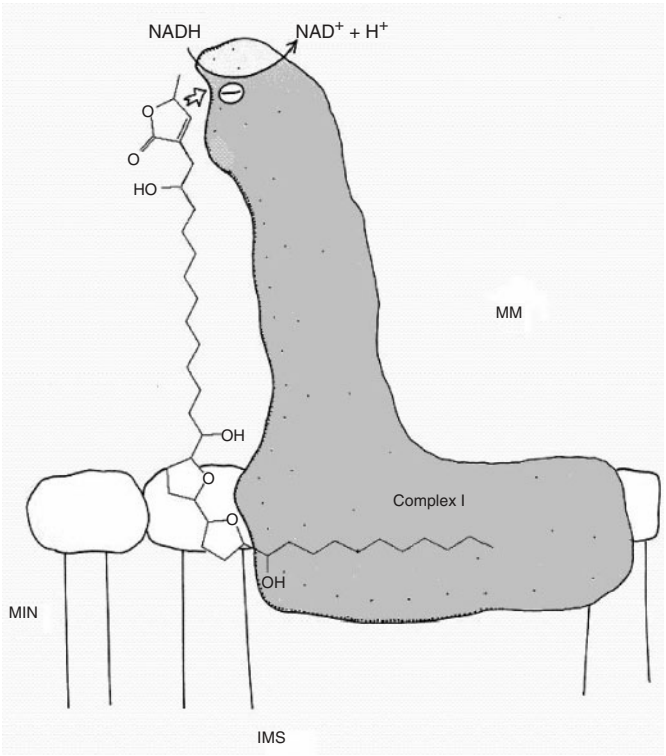


Figure 2. Hypothetical molecular mode of action of bullatacin against Complex I. MM: Mitochondrial Matrix, MIN: Mitochondrial Inner Membrane; IS: Intermembrane Space.

Styryl-lactones: Phenolic Compounds

Styryl-lactones are low molecular weight phenolic compounds, which, like acetogenins are essentially found in members of the Annonaceae family and present a lactonic pharmacophore (9). Examples of styryl-lactones from *Goniothalamus* species are goniothalamin (i), altholactone (ii) and cardiopetalolactone (iii) (Fig. 3).

Jewers *et al*. (47) first reported goniothalamin as the active constituent of the bark of *G. andersonii*, *G. macrophyllus* Miq. and *G. malayanus* collected in the peat-swamp of Sarawak. Altholactone was characterized from *G. arvensis* Scheff. collected in the National Park of Varirata in the Central Province of Papua New Guinea and from the *G. borneensis* Mat-Salleh collected in Malaysia (48,49). Cardiopetalolactone was characterized from the stem bark of *G. cardiopetalus* Hook.f. & Thoms. collected from Palaruvi forest in Kerala in India, with altholactone, goniopypyrone, goniothalamin, goniodiol (iv), goniofufurone (v) and goniofupyrone (vi) (50,51). Goniofufurone, goniopypyrone, goniothalamin, goniodiol, goniotriol (vii) and 8-acetylgoniotriol (viii) were isolated from the roots of *G. griffithii* (52,53). An isomer of altholactone, (+)-isoaltholactone (ix), was isolated from stem bark of *G. malayanus*, and from the leaves of *G. montanus* J. Sincl. and the roots of *G. tapis* Miq. (54). Goniolactones A–F were identified from the roots of *G. cheliensis* (55). Digoniodiol, deoxygoniopypyrone A,

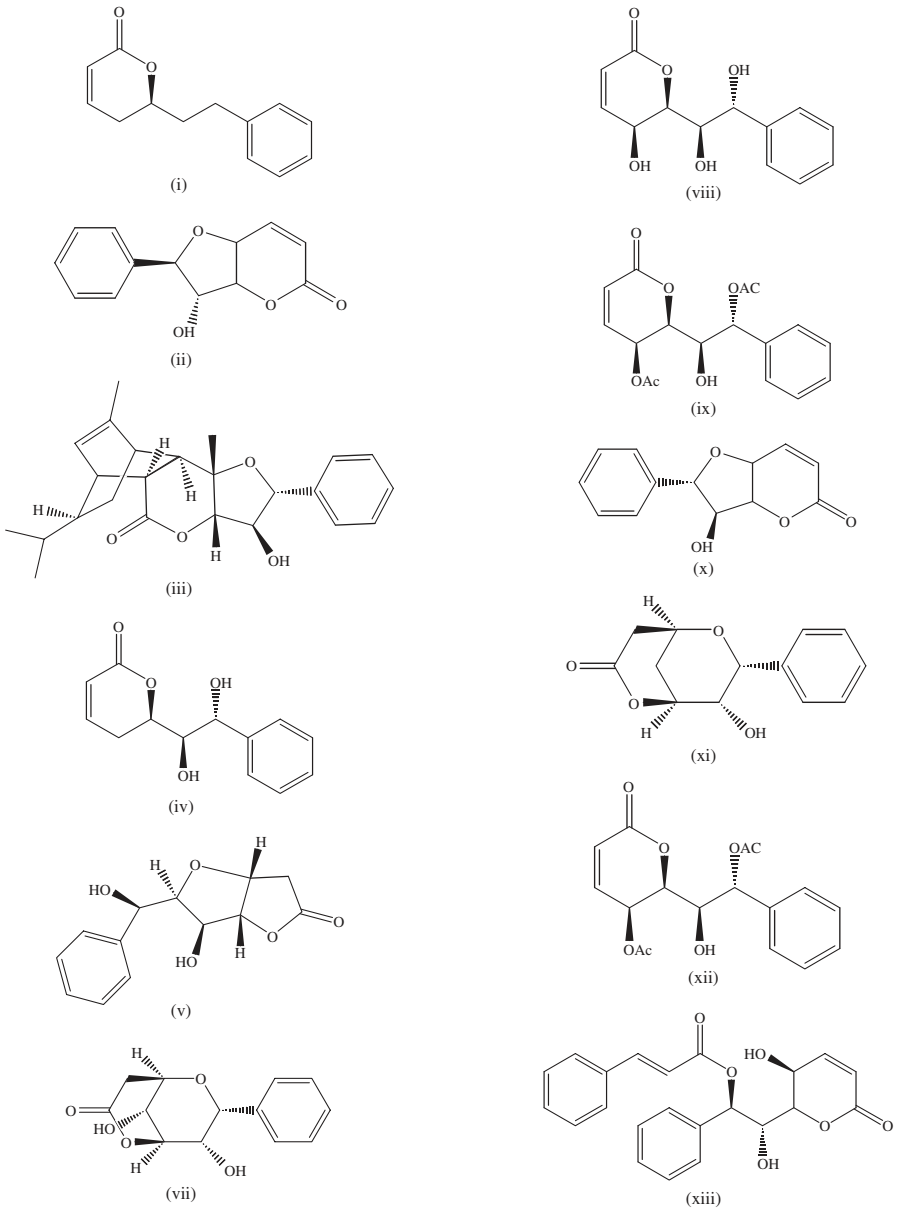


Figure 3. Styryl-lactones from *Goniothalamus* species.

goniofupyrone, goniothalamin, deoxygoniopypyrone A, gonodiol-8-monoacetate and gonotriol (x) and were characterized from the aerial parts of *G. amuyon* collected in the southern part of Taiwan near the coastal regions (56–59). The petroleum ether extract of the stem bark of *G. sesquipedalis* collected in Bangladesh yielded 5-isogoniothalamin oxide (60). 5-Acetyl goniothalamin (xii) was characterized from *G. uvaroides* King collected in Bangladesh (61). Chen *et al*. (62) isolated howiinol A from *G. howii* Merr. (xii). The mode of cytotoxic action of styryl-lactone is described subsequently.

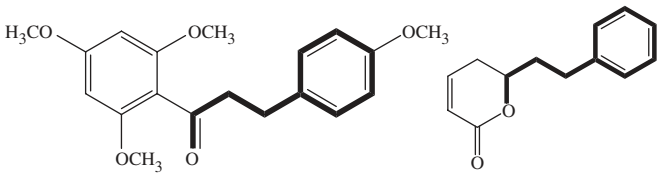
Mode of Cytotoxic Action of Styryl-lactones: Apoptosis

The evidence currently available clearly indicate that goniothalamin and congeners are toxic for several sorts of cancer cells cultured *in vitro* including HL-60 leukemia cells, breast cancer cell line MCF-7, liver cancer cell line HepG2, PANC-1, HeLa cell lines (63–80) (Table 1). Current paradigms of apoptosis suggest that styryl-lactones from *Goniothalamus* activate in mammalian cells the caspases enzymatic cascades via a loss of mitochondrial transmembrane which results in the release of mitochondrial cytochrome *c* (72). To date, the very precise premitochondrial mechanism involved in this activation remains an enigma, and an exciting fact is that the activation of caspases, 3, 6, 7 and 9 is a sign of TRAIL receptors/Bax activation (65). Other examples of goniothalameous styryl-lactones of possible chemotherapeutic value are altholactone, goniolactone B and howiinol. Altholactone is apoptogenic in HL-60

promyelocytic leukemia cells via oxidative stress and mitochondrial respiratory abrogation (75,76). Goniolactone B exhibited significant cytotoxicity against A2780, HCT-8 and KB cells with IC_{50} values of 7.40, 4.43 and 7.23 μM, respectively (55). Howiinol A showed significant antitumor activities toward human tumor cell *in vitro* and *in vivo* (77–81). A remarkable advance in the pharmacological knowledge of howiinol A has been provided by the work of He *et al*. Using techniques of cell growth curve determination, MTT test, soft agar colony assay and experimental therapy of transplantable tumors in mice, they showed that howiinol exerts potent inhibitory effect on cancer cells including drug-resistant cell line, KB/VCR 2000, whereas normal cells are less affected. Howiinol is active in rodents infected with H22 hepatoma and Lewis lung cancer and ascetic sarcoma 180. In addition to flow cytometry technique, they showed that the cycle of howiinol A block is used to analyze the cell cycle of L1210 cells from G1 phase to S phase with structural damage on DNA molecules. Tian *et al*. (82) showed that *Goniothalamus* styryl-lactones which are cytotoxic against both HepG2 and HepG2-R cell lines show less toxicity on normal mice hepatocytes as the IC_{50} values of them on normal mouse hepatocytes were about 3 times of that on HepG2. They demonstrated that cells treated with goniothalamin and altholactone stopped to multiply at G(2)/M and were apoptotic, whereas cells with chromosomes gathered at the equator were easily found in gonodiol-treated cultures.

Indicating that not all *Goniothalamu*s styryl-lactones are exclusively apoptogenic, Zhong *et al*. (83) investigated the apoptosis-inducing effect of styryl-lactones from *G. cheliensis*, on human promyelocytic leukemia HL-60 and showed the activation of caspase-3, reduced the expression of the anti-apoptotic gene Bcl-2, and increased the expression of the pro-apoptotic gene Box via cAMP-dependent protein kinase mechanism. Taking into consideration the available evidence, one might propose the hypothesis that goniothalamin and congeners induce apoptosis at the TRAIL-BAX system level via protein kinase modulation. Protein kinase has long been known to be involved in cell growth and proliferation. Wang *et al*. showed that protein kinase is involved in apoptosis mediated by TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) (84). An example of styryl-lactone which inhibits kinase is flavokavain A from kava, or *Piper methysticum* in the closely related family Piperaceae (85). The Fig. 4 shows the similitude of chemical structure between flavokavain and goniothalamin.

A possible mechanism of action for *Goniothalamus* styryl-lactones would be a cAMP-dependent protein kinase-mediated TRAIL-induced apoptosis, by stimulating TRAIL-induced translocation of Bax from cytosol to mitochondria, loss of mitochondrial transmembrane potential, and subsequent release of cytochrome *c* from mitochondria and activation of caspases, SMAC/Diablo, endo G and finally chromatin deterioration (Fig. 5). Protein kinase modulators are of immense therapeutic usefulness. Note that flavokavains are present in the genus *Goniothalamus*, as discussed next.



Flavokavain A Goniothalamin

Figure 4. Note the proximity of chemical structure between flavokavain A, an inhibitor of protein kinase, and goniothalamin. What is the precise activity of styryl lactones against protein kinases?

Other Phytochemicals

The aerial parts of *G. gardneri* have yielded the known flavonoids 2′-hydroxy-4,4′,6′-trimethoxychalcone (flavokavain A), 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone, 4,2′,4′-trihydroxy-6′-methoxydihydrochalcone, 5,7,4′-trimethoxyflavanone (naringenin trimethyl ether) and 7-hydroxy-5,4′-dimethoxyflavanone (tsugafolin) together with three novel compounds, the dimer characterized as (*rel*)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β,4α-di-(4-methoxyphenyl)-cyclobutane, 2′,4′-dihydroxy-4,6′-dimethoxychalcone and 2′-hydroxy-4,4′,6′-trimethoxydihydrochalcone (86). A similar study of the aerial parts of *G. thwaitesii* led only to the isolation of the known flavonoids myricetin 4′-*O*-methyl ether-3-*O*-α-L-rhamnopyranoside (mearnsitrin) and myricetin-3-*O*-methyl ether (annulatin), together with a series of triterpenes friedelinol, friedelin and betulinic acid (86).

Isoquinoline alkaloids were characterized from *G. amuyon* (87). Other miscellaneous secondary metabolites isolated from members of this genus include goniopedaline, a phenanthrene lactam, aristololactam A-II and its *N*,*O*-diacetyl derivative, taliscanine, aurantiamide acetate and β-sitosterol and its β-D-glucoside were isolated from the leaves and twigs of *G. sesquipedalis* Hook.f. & Thoms. (88). 3-Amino naphthoquinones were characterized from the stem bark of *G. marcanii* (89). Alkaloids were characterized from G. *griffithii* and essential oils were distilled from *G. malayanus*, *G. uvarioides*, *G. macrophyllus* and *G. andersonii* (90,91). In the genus *Goniothalamus*, 138 species still await to be phytochemically investigated, including *G. scortechinii*, the antibacterial property of which is reported in the next section.

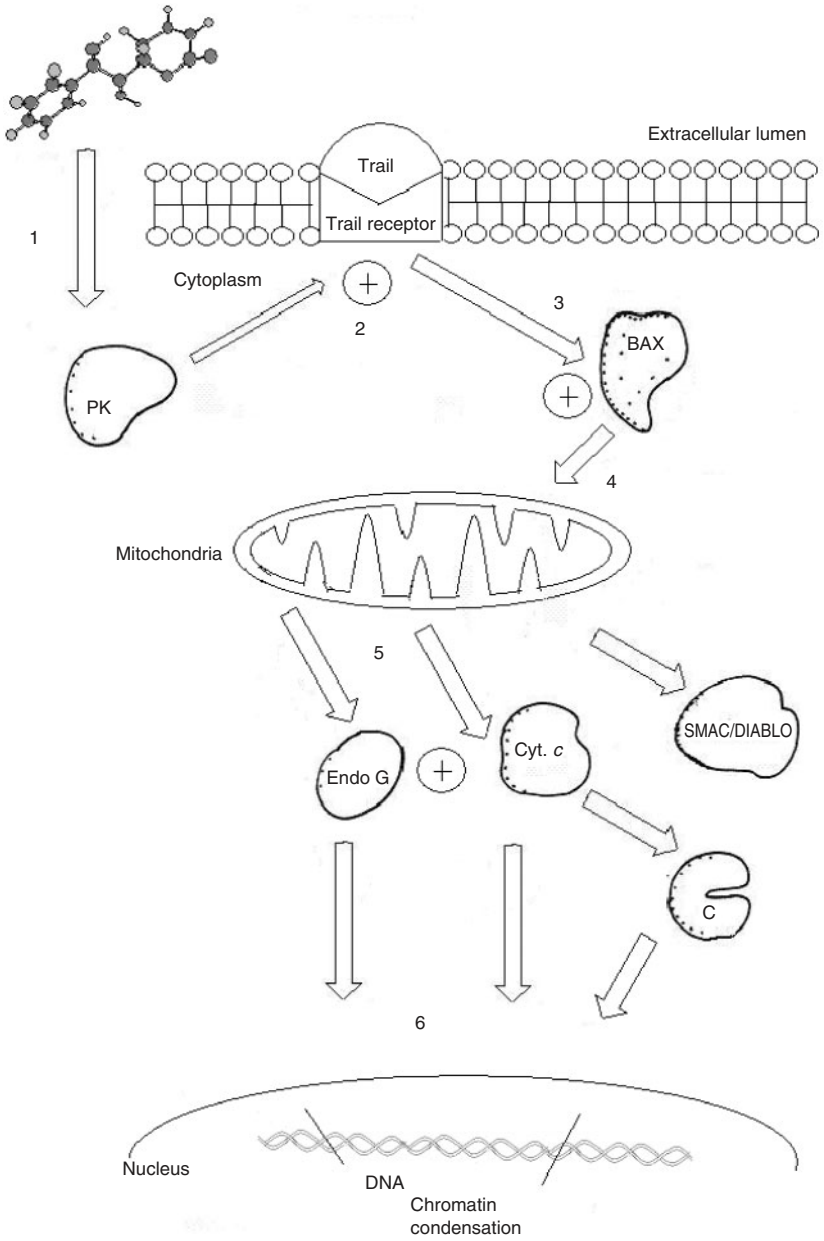


Figure 5. Putative mechanism of action of *Goniothalamus* styryl-lactones in apoptosis. TRAIL R: TRAIL receptor, Cyt. *c*: Cytochrome *c*; C: caspases. 1: styryl-lactone interacts with cellular kinase, 2: kinase mediation of TRAIL induction of apoptosis, 3: TRAIL induced translocation of Bax to mitochondria, 4, 5: release of cytochrome C and activation of caspases, SMAC/Diablo, EndoG, 6: chromatin condensation and cellular death.

Antibacterial Activity of *G. Scortechinii*

G. scortechinii or in Malay *akar gajah beranak* (climber of the elephant bringing forth) is a small tree found from Penang to Selangor and Pahang used by Malays apparently freely, either alone or with other substances after childbirth, and taken internally to prevent bacterial infection (3). The plant is known to exhibit potent schizonticidal activity *in vitro* (92). We report the first evaluation of the antibacterial activity of hexane, dichloromethane and aqueous fractions of *G. scortechinii*. The plant was collected from 6° North and 98° East, near Kuala Kangsar, State of Perak, Malaysia in August 2004, 300 m above sea level. The plant material was identified on comparison with specimens available at the Herbarium of the 'Forest Research Institute of Malaysia', Kepong, Malaysia. A voucher specimen (number W1332) has been deposited in our Herbarium collection for future reference. Finely powdered, air-dried leaves of *G. scortechinii* (800 g) were extracted with methanol (2 l) using a soxhlet apparatus. Hexane (250 ml), dichloromethane (250 ml), and water fractions (250 ml) were obtained by the partitioning of liquid methanol extract (250 ml) (yield: 5.52, 8.43, 64.5).

The different fractions obtained were concentrated with a rotary evaporator and brought to complete dryness over water bath to yield the crude extracts. Hexane fraction (yield: 5.52) gave a positive chemical test for steroids, dichloromethane fraction (yield: 8.43) gave a positive chemical test for steroids and terpenes, and aqueous fraction (yield: 64.5) gave a positive chemical test for tannins (93). These extracts were screened for antibacterial activity using the following antibacterial assay.

The crude methanol extract of *G. scortechinii* and fractions were subjected to antimicrobial assay using the disc diffusion method of Bauer *et al*. (94). Both Gram-positive and Gram-negative bacteria (Table 2) were obtained from the stock cultures of the Department of Medical Microbiology at the University of Malaya. The organisms were of the American Typed Culture Collections (ATCC) and some nosocomial isolates. The organisms included *Bacillus* sp., *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 24922, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae, Shigella sonnei*, *Shigella flexneri* and a yeast *Candida albicans* (ATCC 90028). The organisms selected for testing in this experiment are commonly responsible for foodborn and nosocomial bacterial infections (95). Mueller–Hinton agar was prepared according to the manufacturer's instruction. It was dispensed into sterile plates in 20 ml aliquots. After gelling and drying, the plates were seeded with appropriate organisms by streaking evenly in three planes onto the surface of the medium with cotton swabs. The inoculum was dried for 5 min. Sterile filter paper disks (6 mm diameter) soaked with 50 µl of extract (100 mg/ml) were placed onto the agar with flamed forceps and gently pressed down to ensure contact. Streptomycin (10 µg/disc) and nystatin (100 IU) were used as a positive standard against bacteria and fungi as they are both inexpensive and broad spectrum antimicrobials. The plates were incubated at 37°C for 24 h. The zones of inhibition were measured with a ruler. The experiment was carried out in triplicate. Results obtained for antibacterial activity of the crude methanol extract of *G. scortechinii* and fractions are reported in Table 2. Methanol, hexane, dichloromethane and water used for reconstitution of the extracts showed no activity. Analysis of the data revealed that among the tested fractions, the dichloromethane fraction exhibited the highest rates of antibacterial activity. It showed antibacterial activity against *S. aureus* ATCC 25923: 23 mm, *S. aureus* ATCC 29213: 27 mm, *E. faecalis* ATCC 24922: 20 mm, *Escherichia coli* ATCC 25922: 19 mm, *Bacillus* sp.: 20 mm, *K. pneumoniae*: 13 mm (Fig. 6), *S. sonnei*: 28 mm, *S. flexneri*: 13 mm, and *Proteus* sp. 17 mm. The extract inhibited the growth of *C. albicans* ATCC 90028. It was inactive against *P. aeruginosa* ATCC 27853.

Table 2. Antibacterial activity of extractives from *G. scortechinii*

	H	D	A	M	S	N
Gram-positive bacteria						
Bacillus sp.	13	20	–	20	18	
Staphylococcus aureus ATCC 25923	14	27	–	21	16	
Staphylococcus aureus ATCC 29213	14	23	–	20	16	
Enterococcus faecalis ATCC 24922	–	20	–	14	14	
Streptococcus pneumoniae ATCC 49619	–	21	–	13	10	
Gram-negative bacteria						
Pseudomonas aeruginosa ATCC 27853	–	–	–	–	17	
Escherichia coli ATCC 25922	–	19	10	13	20	
Klebsiella pneumoniae	–	11	–	–	20	
Shigella sonnei	16	28	–	18	20	
Shigella flexneri	–	13	7	–	13	
Proteus sp.	–	17	12	10	20	
Yeast						
Candida albicans ATCC 90028	13	23	–	9		17

Average zone of inhibition (in mm) of triplicate including the diameter of the filter paper disc (6 mm). H = hexane fraction (5 mg/disc) D = dichloromethane fraction (5 mg/disc) A = aqueous fraction (5 mg/disc) M = methanol fraction (5 mg/disc) S = streptomycin (10 µg/disc) N = Nystatin (100 IU).

This report is the first data available on the antibacterial activity of *Goniothalamus species* and lends support the traditional use of *Goniothalamus species* as post-partum remedy. An interesting development from these results would be first to identify the active constituents and next to study their precise molecular activity against bacteria. Note that mitochondria in eukaryotic cells take their origin in pro-bacterial ancestors from which they inherited NADH:ubiquinone oxidoreductase (96). One can perhaps envisage a new antibacterial pathway that would encompass a 'bacterial apoptosis'. One wonders.

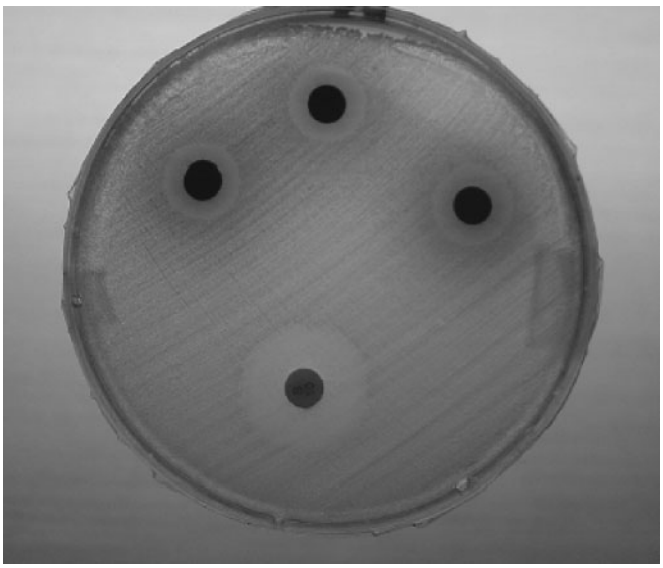


Figure 6. Antibacterial activity of *G. scortechinii* against *Klebsiella pneumoniae*.

Conclusion

G. scortechinii was investigated as part of our study on the medicinal plants of Asia-Pacific (9,10, 97–100) A critical factor for Goniothalamus' use as a medicinal herb is its content of styryl-lactones, which promote apoptosis in mammalian cells. One might propose the hypothesis that the abortifacient and/or post-natal and anti-inflammatory reported traditional uses of *Goniothalamus* species might involve styryl-lactones since apoptosis is known to play a crucial role in trophoblasts of patients with recurrent spontaneous abortion of unidentified cause, and in T cells in the human decidua as defense mechanism against rejection of fetal allograft by the maternal immune system (101,102). In addition, goniothalamin induces apoptosis in vascular smooth muscle cells, the growth of which is required to allow embryo implantation and the development of the blood supply for fetal survival and inhibit the cell surface expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 on the surface of murine endothelial cells (103,104)

In regards to the result obtained for antibacterial activity of the crude methanol extract of *G. scortechinii* and fractions, it can be concluded that the dichloromethane extract of *G. scortechinii* is very active against both Gram-positive and Gram-negative bacteria. and the results obtained tend to answer positively the question of Chinnok *et al.* (105). This work illustrates the fact that the careful study of the biochemical architecture of medicinal plants represents a fascinating and fruitful aspect of pharmaceutical research (106). In regards *G. scortechinii*, it will be interesting to know whether further studies on this plant disclose any molecules the treatment of nosocomial urinary, respiratory and wound nosocomial infections (*S. aureus*, *E. coli* and *K. pneumoniae*) which are developed by hospital patients.

In summary the evidence for the existence of anticancer, antibacterial and antiviral agents in the genus *Goniothalamus* is strong and it seems likely that further consistent and systematic research on this genus of flowering plants will lead to the discovery of antineoplastic and antimicrobial agents. If enough botanical, phytochemical and pharmacological work is dedicated to this discrete tropical genus of flowering plants, a couple of drugs for the treatment of tumors and/or bacterial and even viral infections should be developed in the relatively close future.

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