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Research Article

Antimicrobial, Anticancer, and Cytotoxicity Activities of A Crude Methanolic Extract from the Bark of *Goniothalamus velutinus* (Airy Shaw) Collected from Brunei Darussalam

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ABSTRACT

Goniothalamus velutinus (Airy Shaw) is a plant used by traditional healers in Brunei Darussalam, Sabah and Sarawak to treat fever, headache, and snakebite-related infections, to cure food poisoning and as a remedy for seizures, to induce abortion, and as a post-partum remedy. This study evaluates the antibacterial and anticancer activities of a methanolic bark extract of *G. velutinus.* The antibacterial activity of the extract was determined against five bacteria strains using the disc diffusion method and their minimum inhibitory concentration (MIC) and minimum bactericidal concentration were also studied. The cytotoxicity activity of the extract was measured by the brine shrimp lethality test (BST) while MTT assay was used to measure the anticancer activity of the extract against five cancer cell lines. The extract had antibacterial activity against the Gram-positive bacteria *Bacillus subtilis, Bacillus spizizenii*, and *Staphylococcus aureus* with MIC values of 0.15, 0.15 and 5 µg/mL, respectively. The LD_{50} for BST of 9.4 µg/mL was calculated by probit analysis. The extract had IC_{50} values of 19.41, 25.34, 28.77, 26.76 and 27.61 µg/mL toward HEK 293, A549, CaSki, Namalwa and Jurkat cells, respectively, as determined by the MTT assay. In conclusion the methanolic bark extract of *G. velutinus* has antibacterial activity against Gram-positive bacteria, cytotoxicity against brine shrimp nauplii and broad anticancer activities against the tested cell lines. This study highlights the potential uses of *G. velutinus* as a folk medicine to treat a multitude of conditions.

Keywords: *Goniothalamus velutinus;* bark extract; antibacterial; cytotoxicity; anticancer; traditional medicine

INTRODUCTION

The tropical rain forest is a promising source of biologically active compounds. According to the World Health Organization (WHO), about 80% of the population uses plants for therapeutic purposes or for their natural health benefits[1]. These plants contain a rich source of organic compounds, many of which are used as agents against several infectious and non-infectious diseases[2]. Brunei Darussalam is situated in the northwest part of Borneo Island. Its tropical climate is characterized by high rainfall, humidity and uniform temperature throughout the year. These environmental conditions contribute to a wide variety of natural vegetation whose therapeutic potential remains largely unexploited. [3,4]

Goniothalamus velutinus belongs to the genus *Goniothalamus* (family Annonaceae), consisting of 160 species of archaic shrub and treelets which grow in the shady primary forest of tropical Asia. Some *Goniothalamus* species are used in traditional medicine for abortion, childbirth, fever and some tumors. The roots of *Goniothalamus giganteus* are used to abort and treat colds, and heated leaves are applied to swollen areas. A decoction of *Goniothalamus scortechinii* is given as a post-partum remedy. The root decoction of *Goniothalamus macrophyllus* and *Goniothalamus tapis* is also used as a post-partum remedy and to induce abortion, and has also been reported to treat some fevers[5].

Goniothalamus velutinus, locally known as "Limpanas hitam, Talipanas hitam," is used as a traditional medicine by herbalists. Its stem is used to alleviate fever while the sap from the stem is used to treat snakebite infections. The leaf can be used to treat fever and is also used as a post-partum remedy and to induce abortion. Its root decoction is used for the treatment of headache and food poisoning. Light smoke of simmering branches (leaves and stems) is recommended as a remedy for seizures. The stem is also believed to have repellent powers against snakes and wild animals and is believed to protect the house from evil spirits[3,6]. Wong (2008), also reported that *G. velutinus* is used as a traditional medicine for the treatment of giddy, injury, diarrhea, aphrodisiac, body pain, cold, stomachache, swollen, headache, food poisoning, to maintain body health and as mosquito repellent.

Chemical components isolated from *Goniothalamus* species have many bioactivities including antibacterial, cytotoxic, antimalarial, analgesic, anti-inflammatory,

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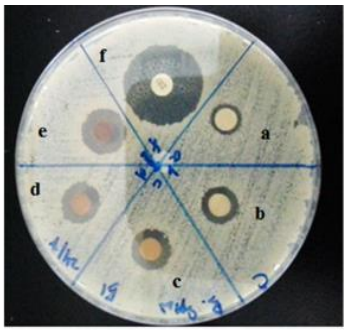
Table 1: Antibiotic used for each bacteria to determine their growth inhibition by the plant extract

Bacterium	Antibiotic
E. coli (EC)	Tetracycline (30 µg)
S. aureus (SA)	Penicillin (10 units)
P. aeruginosa (PA)	Gentamycin (10 µg)
B. subtilis (BS)	Erythromycin (15 µg)
B. spizizenii (BSp)	Vancomycin (30 µg)



*a-e represent different concentrations of extract in increasing order and f represents the antibiotic

Figure 1: The antibacterial activity against *B. spizizenii* of a methanol extract of the bark of *G. velutinus*

antitumor, antilarvicidal, and antiviral properties. Some examples of these phytochemical compounds are phenols, alkaloids, styryl lactones, acetogenins, cyanogenic glycoside, tannins, terpenoids and other endogenous metabolites[7,8]. Reports are available on some compounds isolated from *G. velutinus*, namely velutinam, goniothalamin, pinocembrin, naringenin and aristolactam BII. Some Borneo natives use *G. velutinus* for the treatment of tumors and research has shown it is cytotoxic toward various human cell lines. Alkaloids and goniothalamin have been investigated for their cytotoxic activities toward different cancer cell lines[9-12].

The aim of this study was to investigate the antibacterial, cytotoxic and antibacterial activities of a crude methanolic extract of the bark of *G. velutinus* collected from Brunei Darussalam to justify its use as a herbal medicine by the natives. Iqbal et al., (2015) reported that the extracts of leaves and bark contained alkaloids, steroids and cardiac glycosides supporting the antibacterial and cytotoxic activities of the bark extract of *G. velutinus.* To our knowledge, this is the first study into the properties of *G. velutinus* collected from Brunei Darussalam.

MATERIALS AND METHODS

Plant material and sample preparation

Stems of *G. velutinus* were collected from Bukit Panjang in Kampung Kulapis at longitude 4º 51'03.8" N and latitude 114º 49' 00.5 E, in Brunei Darussalam in February 2013. Two voucher specimens of the plant, each with a reference number EI1 were deposited in the National Herbarium of Brunei Darussalam (BRUN) and the Universiti of Brunei Darussalam Herbarium (UBDH). Collected stems of *G. velutinus* were rinsed with tap water followed by distilled water to remove the surface dirt. Bark was removed from stems and cut into small pieces. They were then air dried for 2 days and freeze dried until a constant mass was obtained. Dried samples were ground into a fine powder and stored in desiccators until extracted. The extraction was carried out in a Soxhlet apparatus for 10 hours using absolute methanol. The solvent was then evaporated using a rotary evaporator and the crude extracts were stored in desiccators until testing.

Media and chemicals

For antibacterial and brine shrimp assays:

Ethanol, nutrient agar, nutrient broth, Mueller-Hilton agar, Mueller-Hilton broth, sterile cotton swabs, sterile petri dishes, sterile wire loops, antibiotic discs of tetracycline (30 µg), penicillin (10 units), gentamycin (10 µg), erythromycin (15 µg) were from Oxoid Ltd., UK. The standard pure cultures of *Escherichia coli* (Microbiologics, St. Cloud, MN, ATCC#25922), *Staphylococcus aureus* (BioMérieux, Lyfoculs ATCC#25923), *Pseudomonas aeruginosa* (Microbiologics, ATCC#27853), *Bacillus subtilis* (BioMérieux, Lyfoculs ATCC 6633), *Bacillus spizizenii* (Microbiologics, ATCC#6633) were obtained as indicated. $BaCl_2$ and H_2SO_4, were of analytical grade from Merck. Six-mm-diameter filter paper discs were made from Whatman filter paper #4. For the brine shrimp assays, *Artemia salina* cyst (brine shrimp egg) was obtained from Artemia International LLC, USA and ultra marine synthetic sea salt was obtained from Water Life Research Ltd., Longford, UK.

Cell lines and culture conditions

Table 2: Antibacterial activity of a crude methanolic extract of *G. velutinus*

Extract Concentration		Bacteria Zone of Inhibition (mm)				
Initial Conc. (mg/mL)	Final conc. (µg/disc)	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*B. spizizenii*
250	2500	0.0	0.0	14.66 ± 0.47	14.66 ± 0.47	13.00 ± 0.00
100	1000	0.0	0.0	14.33 ± 0.47	12.00 ± 0.00	12.00 ± 0.00
50	500	0.0	0.0	14.66 ± 0.47	11.66 ± 0.47	12.00 ± 0.00
25	250	0.0	0.0	11.00 ± 0.00	10.33 ± 0.94	10.66 ± 0.47
10	100	0.0	0.0	9.00 ± 0.00	8.66 ± 0.47	8.66 ± 0.47
Control	DMSO	0.0	0.0	0.0	0.0	0.0
Tetracycline	30	19.33 ± 0.47	NT	NT	NT	NT
Gentamycin	10	NT	18.66 ± 0.47	NT	NT	NT
Penicillin	10 units	NT	NT	36.33 ± 1.24	NT	NT
Erythromycin	15	NT	NT	NT	32.33 ± 0.47	NT
Vancomycin	30	NT	NT	NT	NT	23.66 ± 0.47

*NT: not tested

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of a crude methanolic extract of *G. velutinus*

Bacteria	MIC (mg/mL)	MBC (mg /mL)
S. aureus	5.00 ± 0.00	6.25 ± 0.00
B. subtilis	0.15 ± 0.00	0.62 ± 0.00
B. spizizenii	0.15 ± 0.00	0.31 ± 0.00

Human lung adenocarcinoma epithelial cells (A549) (CCL-185), human embryonic kidney cells (HEK 293) (CRL-1573), epidermoid cervical carcinoma cells (CaSki) (CRL-1550), human Burkitt lymphoma cells (Namalwa) (CRL-1432) and T lymphocyte cells (Jurkat, Clone E6-1) (TIB-152) were obtained from the ATCC (Manassas, VA). Adherent cells were grown in Dulbecco's Modified Eagle Medium (Gibco Life Technologies, USA) and suspension cells were grown in Roswell Park Memorial Institute medium (RPMI) (Gibco, USA), supplemented with 10% fetal bovine serum. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Antibacterial Assay

Disc Diffusion method

The disc diffusion method was used to determine the growth inhibition of bacteria by the plant extract[14]. Briefly, Mueller-Hilton (MH) agar plates were prepared by pouring sterilized MH agar media on sterilized petri plates and allowing it to set. The plates were then inoculated with test microorganisms taken from broth culture prepared from an overnight culture adjusted to 0.5 McFarland standard turbidity (approx. 10^6 CFU/mL) and divided into six regions. Filter paper discs soaked with 10 µL of different concentrations of air-dried extract (10, 25, 50, 100, 250 mg/mL), antibiotic discs and a negative control (dimethyl sulfoxide [DMSO] only) were added to the different regions of the plates and incubated for 24 hours at 37 °C. Table 1 lists the antibiotic used for each bacterium. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition diameter produced by the plant extract compared with the positive control antibiotics. For each test microorganism, three replicates were carried out.

Minimum Inhibitory Concentration (MIC)

The MIC was determined by using the agar twofold dilution method for bacteria against which the extract showed an inhibitory effect in the disc diffusion assay. A stock solution of 250 mg/mL of plant extract was prepared in DMSO and seriously diluted twofold in pure DMSO up to 3.9 mg/mL. Then 1 mL of each dilution was mixed with 19 mL of molten MH agar to give final concentrations in the range of 12.5 to 0.195 mg/mL. Plates were then allowed to solidify and 5 µL of bacterial culture (which was already adjusted to the turbidity equal to 0.5 McFarland standard) was inoculated as a spot on the surface of the agar plates containing different concentrations of extract. Plates were examined after 24 hours of incubation at 37 °C. For each concentration, three replicates were carried out[14,15].

Minimum Bactericidal Concentration (MBC)

The MBC was determined by subculturing cells from Muller Hilton agar well plates that did not show any growth onto a new MH agar plate and incubating it for 24 hours. The highest dilution or concentration of extract that did not allow the growth of a single bacterial colony was taken as the minimum bactericidal concentration (MBC)[16].

Brine shrimp lethality (BST) test

Brine shrimp nauplii (*Artemia salina*) were used to determine the cytotoxicity of the extract. Briefly, 1 g of brine shrimp eggs were soaked in a bleach solution for 1–2 minutes, and then were thoroughly rinsed with distilled water. The eggs were soaked for 30–36 hours in artificial sea water prepared by dissolving 38 g of sea salt in 1 L of distilled water. The pH of the solution was adjusted to 7.6 using sodium hydroxide and hydrochloric acid (1 M). The temperature was maintained at 28 °C under constant aeration[17,18].

After 2 days (when the brine shrimp nauplii had matured), 10–15 swimming nauplii were collected in 1 mL of brine and transferred into a 12-well plate containing 4 mL of fresh brine solution, followed by addition of different concentrations of extract (25 µL) prepared by dissolving the crude bark extract in methanol (to give final concentrations of 1 to 50 µg/mL) and mixing well. Two types of controls were used, the brine solution only and methanol. After 24 h, dead nauplii were counted and the data were analyzed by probit analysis to determine the

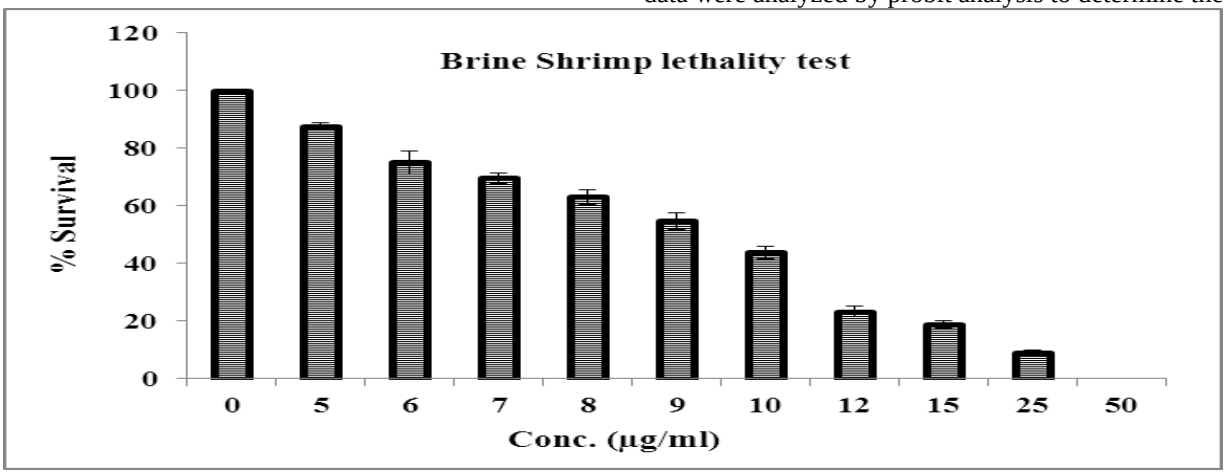


Figure 2: % Survival of brine shrimp nauplii against a methanolic bark extract of *G. velutinus*

Table 4: Brine Shrimp Lethality Test comparison of LD_{50} values

Plant	LD_{50} (µg/mL)	Reference
Goniothalamus velutinus	9.4	This study
Goniothalamus macrophyllus	20	11
Annona purpurea	3.9	28
Friesodielsia obovata	7.6	28
Artabotrys hexapetalus	7.6	29
Annona senegalensis	>1000	30
Annona squamosa	150	31

LD_{50} (lethal dose of the extract at which 50% of the nauplii died).

Anticancer Activity

Morphological assessment of cells by phase-contrast inverted microscopy

A total of 2 mL of cells (five different cell lines) were seeded in six-well tissue culture plates and *G. velutinus* bark extract (25 µL) was added at different concentrations (0–50 µg/mL). The cells were incubated at 37 °C in a tissue culture incubator for 24, 48 and 72 hours. After the incubation period, the cells were observed under phase contrasted inverted microscope at 10× magnification and photographed[19].

A stock solution of bark extract was prepared in DMSO and then diluted with the medium. The final dilution of extract used for treating the cells contained not more than 0.1% DMSO. The control group was treated with 0.1% DMSO in medium.

MTT proliferation assay

The antiproliferative activity of the extract was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay[20,21]. Cells were seeded in 96-well plates at a concentration of 1×10^5 cells/mL (100 µL). After an overnight incubation, different concentrations of the extract (100 µL) was added to give a final concentration of 0 to 100 µg/mL and then the cells were further incubated for 72 hours at 37 °C.

For adherent cells, the medium was removed and MTT (200 µL, 0.5 mg/mL) was added to each well and incubated for 4 hours at 37 °C. After incubation, MTT was removed by inverting the plate and the formazan crystals were solubilized by adding DMSO (150 µL)[22,23].

For the suspension cell lines, 100 µL of the medium was removed followed by addition of MTT (10 µL, 5 mg/mL) to the remaining 100 µL of cells in each well, and incubation for 4 hours at 37 °C. After 4 hours, DMSO (100 µL) was added to dissolve the crystals. The absorbance was measured using a microplate reader at 540 nm and subtracted from background absorbance at 690 nm[4,22,24].

A stock solution of the extract was prepared in DMSO and then diluted with medium. The final dilution of the extract used for treating the cells contained not more than 0.1% DMSO. The control group contained the same amount of DMSO as the medium (0.1 %) and the blank contained only medium. The absorbance reading of the blank was subtracted from the control and the treated cells then the % survival was measured using the following formula.

% survival = [Abs (sample)/Abs (control)] × 100

IC_{50} values were calculated by plotting a graph of the log concentration versus % survival.

DAPI staining

Nuclear morphology was evaluated by staining chromatin with 4′6-diamidino-2-phenylindole (DAPI). A549 cells (1×10^6 cells) were plated in six-well tissue culture plates and incubated for 24 hours at 37 °C in a CO_2 incubator. Cells were treated with different concentrations (30, 40 and 50 µg/mL) of extract (25 µL) for 24 hours. The negative control was treated with 0.1% DMSO. After 24 hours, cells were washed twice with PBS and then fixed by adding 3.7% formaldehyde. After 15 minutes, cells were washed twice with PBS and stained with DAPI solution (1 µg/mL) for 10 minutes. Cells were washed with PBS and observed under a fluorescent microscope[19].

RESULTS AND DISCUSSION

Antibacterial activity

The plant used in this study has been used in Brunei, Sabah and Sarawak as a traditional medicine for a variety of conditions. The antimicrobial activity of the crude methanolic extract of the bark of *G. velutinus* was evaluated using Gram-positive and Gram-negative bacteria by the disc diffusion method. Fig. 1 is a representative figure showing the zone of inhibition against *Bacillus spizizenii* when treated with different concentrations of the bark extract. The antimicrobial activity was then further evaluated by determination of the MIC and MBC as shown in Tables 2 and 3, respectively.

The results revealed that the bark extract of *G. velutinus* exhibited some activities against Gram-positive bacteria but not against Gram-negative bacteria or fungi, similar to the results reported by Galappathie et al. (2014). However, this report did not mention whether the extract was from bark or leaf.[6]

Hexane, DCM and methanolic extracts of *Goniothalamus umbrosus* leaves also showed antibacterial activities against *S. aureus* (SA) and *P. aeruginosa* (PA) when tested using the disc diffusion method. By comparison, MIC values for a methanolic extract of *G. umbrosus* leaves against PA, SC and BS were 4.5 mg/mL, 4 mg/mL, and 3.5 mg/mL, respectively. The MIC value for the extract of *G. velutinus* bark toward BS (0.15 mg/mL) is much lower than that for the extract of *G. umbrosus* leaves while the MIC for SA was similar (5.0 mg/mL) to that obtained for *G. umbrosus*[25-27]. These results indicate that *G. velutinus* has much higher antibacterial activity against *Bacillus* species than other extracts from other *Goniothalamus* species. Wiart (2007) showed that crude methanolic and DCM extracts of *G. scortechinii* have antibacterial activity against *Bacillus* spp., *E. coli* and many other Gram-positive and Gram-negative bacteria but are inactive against PA. This study and other studies on the

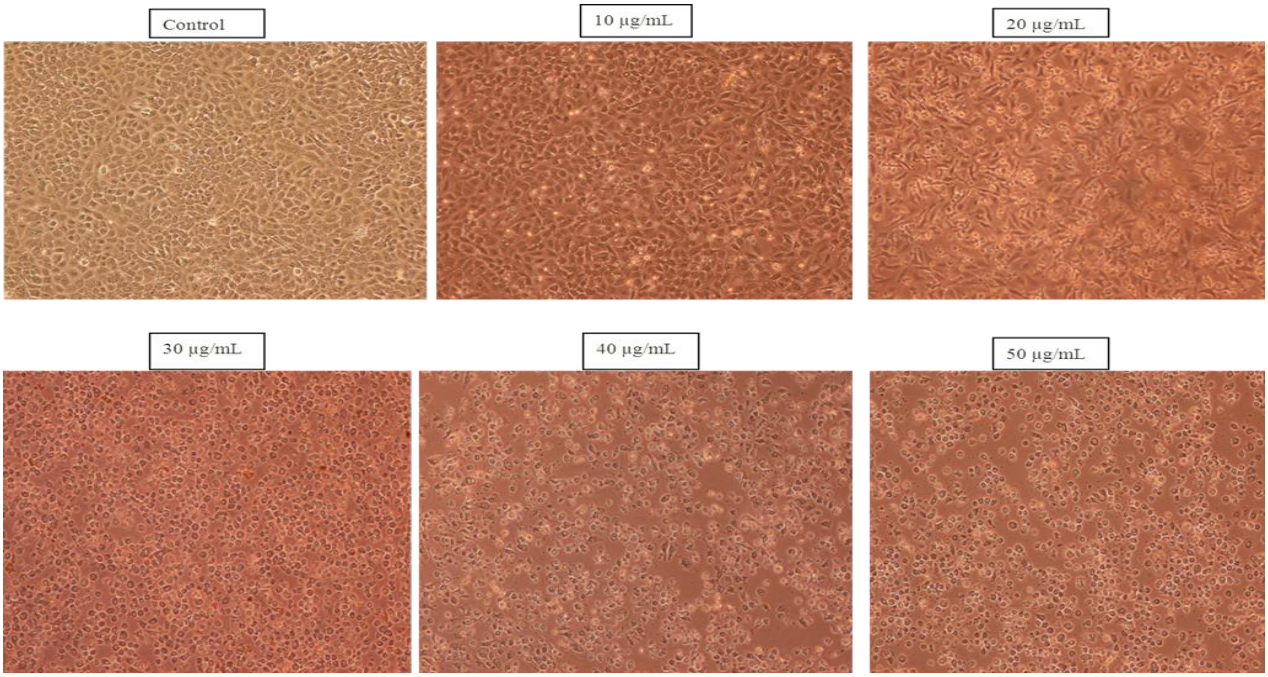


Figure 3: Morphological observation of A549 lung carcinoma cells treated with different concentrations of methanolic bark extract of *G. velutinus* (magnification 10×)

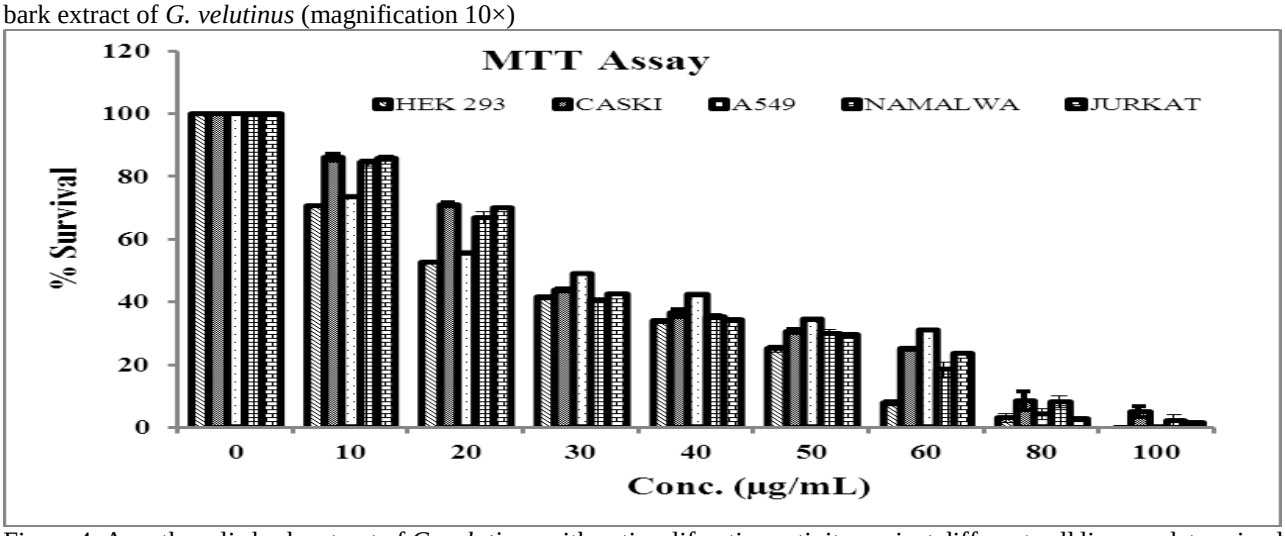


Figure 4: A methanolic bark extract of *G. velutinus* with antiproliferative activity against different cell lines as determined by the MTT assay

Table 5: MTT IC_{50} values for the bark extract of *G. velutinus* against several cell lines

Cell lines	IC50 (□g/mL)	Std. Error
HEK 293	19.41	0.28
CASKI	28.77	0.40
A549	26.34	0.12
NAMALWA	26.76	0.25
JURKAT	27.61	0.11

antimicrobial activity of *Goniothalamus* spp. explain their use as a post-partum remedy. Acetogenin, alkaloids, styryl lactones and other compounds isolated from *Goniothalamus* spp. may synergistically act to achieve the observed antimicrobial and cytotoxic effects, further confirming this plant is likely valuable for its potential medicinal uses.

Brine Shrimp Lethality Test

The brine shrimp lethality test is a simple, easy, inexpensive and rapid method to determine the cytotoxicity of crude plant extracts. The results obtained from this assay generally correlate well with cytotoxic and antitumor properties of compounds. The results from the BST were analyzed by probit analysis to estimate a LD_{50} value at a 95% confidence interval. Fig. 2 shows the relationship of the % survival of brine shrimp nauplii when treated with different concentrations of extract.

The LD_{50} value for a crude methanolic bark extract was found to be between 9.396–10.074 µg/mL which indicates it may be broadly cytotoxic. Table 4 compares the LD_{50} of the bark extract with the LD_{50} values of some known medicinal plants of the same family (Annonaceae). The *G. velutinus* extract is approximately two-fold more cytotoxic that the *G. macrophyllus* stem methanolic extract. Furthermore, when compared with the other plants of the

Table 6: Comparison of the cytotoxicity of *G. velutinus* bark extract with extracts from other *Goniothalamus* species (IC_{50} determined by MTT assay)

Plant	Extraction	IC_{50} (µg/mL)	Cell line	Reference
G. velutinus	Methanolic bark extract	25.34 ± 0.36	A549	This study
G. velutinus	Methanolic bark extract	19.41 ± 0.50	HEK 293	This study
G. velutinus	Methanolic bark extract	28.77 ± 1.21	CaSki	This study
G. velutinus	Methanolic bark extract	26.76 ± 0.75	Namalwa	This study
G. velutinus	Methanolic bark extract	27.61 ± 0.34	Jurkat	This study
G. macrophyllus	Methanolic stem extract	18.38 ± 0.57	Mdbk*	33
G. scortechinii	Methanolic stem extract	25.33 ± 1.15	Mdbk	33
G. umbrosus	Ethyl acetate leaf extract	24.50 ±0.12	MCF-7**	25
G. umbrosus	Hexane leaf extract	20.00 ± 4.46	MCF-7	34
G. umbrosus	Dichloromethane leaf extract	19.5 ± 0.33	MCF-7	35

*Mdbk: Madin-Darby Bovine Kidney cells, **MCF-7: Human Breast Carcinoma cell line

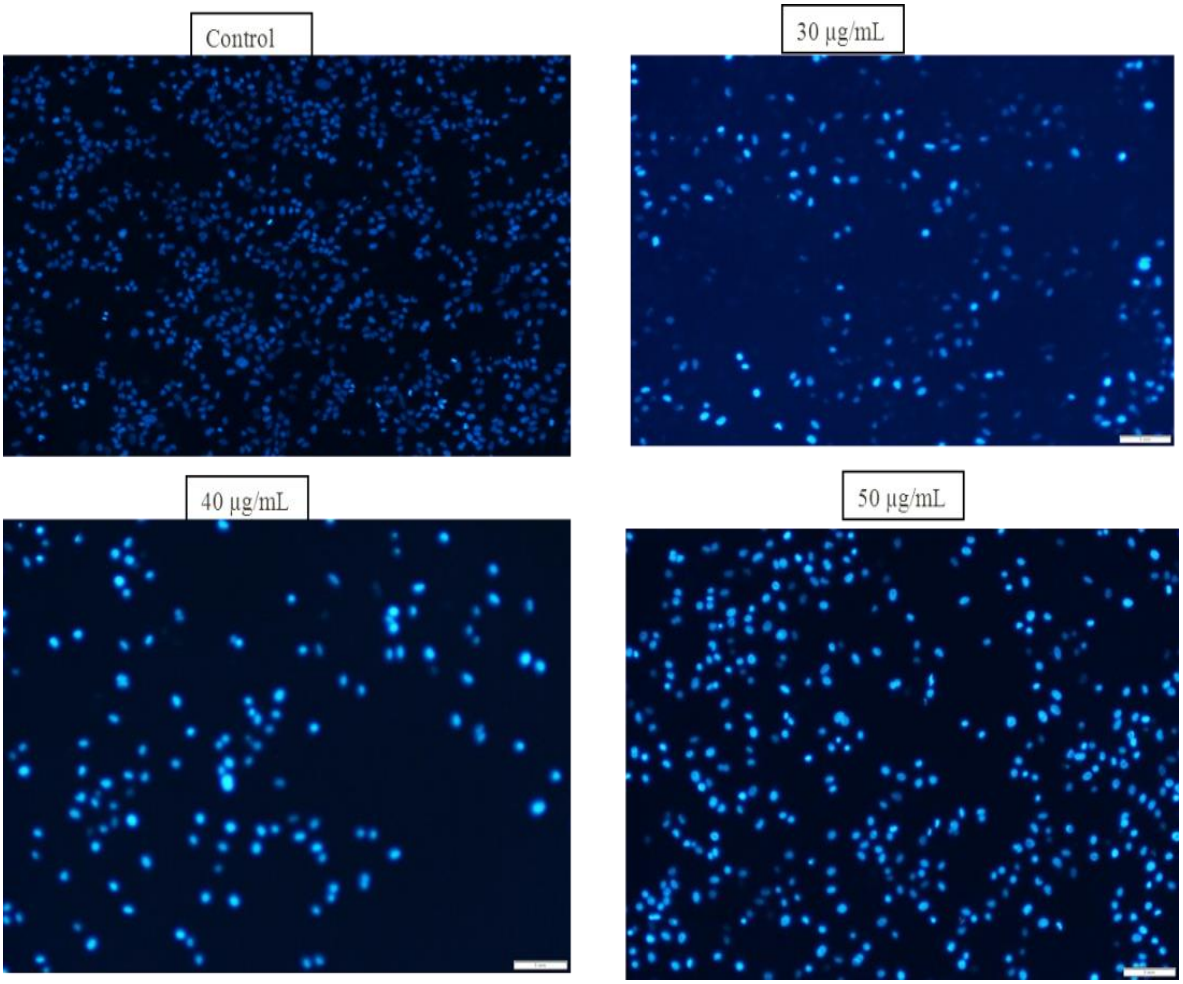


Figure 5: Morphological observation by fluorescence microscopy with DAPI staining for A549 cells 24 hours after treatment with a methanolic extract from the bark of *G. velutinus* (magnification 10×)

same family, *G. velutinus* possesses comparable cytotoxicity. This result suggests that biocidal compounds are present in *G. velutinus* as it belongs to Annonaceae family which is known to contain acetogenins with potential pesticidal and antitumor activities[28].

Anticancer activity

The anticancer activity of the crude extract was determined by treating different cancer cell lines with varying concentrations of extract. After 24, 48 and 72 hours, the cells were observed under the microscope to observe any morphological changes attributed to the extract (Fig 3). When A549 cells were treated with 0 to 50 □g/mL of extract and observed after 72 hours, cells became rounded, the number of cells declined and many were no longer attached. This may be a result of active compounds present in extract which cause cellular necrosis or apoptosis,

blocking pathways essential for proliferating cells[32]. Further investigation of the cytotoxicity of the extract was evaluated using the MTT proliferation assay.

MTT assay

The cytotoxicity effects of the extract toward HEK293, CaSKi, A549, Namalwa and Jurkat cell lines were evaluated by measuring cell viability using the MTT assay following 72 hours treatment with different concentrations of extract. The IC_{50} values were obtained by plotting the % cell survival vs the log concentration. As shown in Table 5, the *G. velutinus* methanolic bark extract inhibited the growth of all tested cell lines to a similar degree.

The extract was also investigated for its anti-proliferative activities towards the cancer cell lines. As shown in Fig. 4, the extract inhibits cell growth in a time and dose dependent manner. According to the American National Cancer Institute (NCI), the criteria for a crude extract to possess cytotoxic activity is an $IC_{50} < 30$ µg/mL[21].

The methanolic crude extract of the bark of *G. velutinus* exhibits strong antiproliferative activity against all cell lines under study ($IC_{50} < 30$ µg/mL), with the IC_{50} for HEK 293 the lowest at 19.41 µg/mL. By the NCI criteria, they are considered to be promising potential anticancer agents. Although compounds which have anticancer activities have been isolated from this plant, they have not been individually tested against these cell lines. One of the compounds, goniothalamin, isolated from this plant and other species of *Goniothalamus*, was found to have anticancer activity against colon cancer cells (LS-174T and HT-29), lung carcinoma cells (COR-L23 and NCI 460), breast cancer cells (MCF 7), kidney tumor cells (786-0) with IC_{50} of 3.41 µg/mL, 23.33 µg/mL, 12.62 µg/mL and 4 nM respectively. It also shows cytotoxicity against CEM-ss T-lymphoblastic cells with an IC_{50} value of 2.4 µg/mL and promyelocytic leukemia cells with an IC_{50} value of 4.5 µg/mL[12].

Upon comparing the IC_{50} values from the MTT assay on *G. velutinus* bark extract with the available reports (Table 6) on other *Goniothalamus* spp., it was evident that all species from the genus *Goniothalamus* had similar cytotoxicities toward the cells tested. Wiart (2007) postulated that the cytotoxicity of *Goniothalamus* spp. could be attributed to the presence of styryl lactones that activate caspases in cells through the loss of mitochondrial transmembrane potential leading to the release of cytochrome c and initiation of apoptosis. Furthermore, Abdel-Wahab et al. (2011) reported that the cytotoxic activity of *Annonaceous* plant species is due to the presence of goniothalamin and other styryl lactones that initiate inhibition of superoxide dismutase activity in malignant cells, causing free radical mediated damage to the mitochondrial membrane, and ultimately resulting in cellular apoptosis.

DAPI nuclear staining

DAPI is a fluorescent dye used to stain nuclei and investigate cellular morphological changes using fluorescence microscopy. Apoptosis is a primary pathway responsible for morphological changes when cells are exposed to environmental stress. Apoptosis is initially characterized by chromatin condensation, nuclear fragmentation and membrane blebbing. Based on the MTT assay results, DAPI staining was conducted to examine whether the extract shows any apoptotic activity. A549 cells were treated with 30, 40 and 50 µg/mL of extract and their DAPI staining was compared with the control. As shown in Fig. 5, in the untreated control well, cells maintained their original shape and nuclei were homogenously stained a dull blue color. Treated cells displayed more intense blue fluorescence compared with the untreated cells. This more intense fluorescent staining may be a result of the presence of highly condensed chromatin or because of cell cycle arrest at the G2/mitosis phase of the cell cycle[19].

CONCLUSION

This study provides some preliminary results on the antibacterial and anticancer activities of a bark extract of a previously unexplored plant, *G. velutinus*, collected from Brunei. Its antibacterial activity supports the traditional use by the local herbal practitioners to alleviate fevers with bark decoction. Similar to other *Goniothalamus* species, the extract possessed anticancer activities against all tested cell lines. The antibacterial and anticancer activities of the extract may be the result of the presence of different chemical constituents that work in synergy. Some of the compounds known to have antibacterial and anticancer properties have been isolated and identified from *Goniothalamus* species, but further work is needed to identify the components responsible for the biological activities of *G. velutinus* and its use as a traditional medicine by the natives of Brunei.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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