*SHORT REPORT*

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# Antitubercular and Cytotoxic Constituents from *Goniothalamus gitingensis*

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**Abstract:** Phytochemical studies on the leaves of the Philippine endemic Annonaceae plant, *Goniothalamus gitingensis* enabled the isolation and identification of four secondary metabolites corresponding to three styryllactones, isoaltholactone (**1**), altholactone (**2**) and goniopypyrone (**3**), and the alkaloid liriodenine (**4**). Structure identification was facilitated through various spectroscopic experiments such as NMR ($^{1}$H, $^{13}$C, COSY, HSQC, HMBC and NOESY), LR-EIMS, X-ray and through comparison with literature values. Our study accounts the first report of all compounds in this plant species. The extracts together with the isolated compounds (**1-4**) were tested for antituberculosis activity using MABA and cytotoxic activity using the CellTiter-Blue1 assay. The majority of the samples tested indicated good inhibitory activity against *Mycobacterium tuberculosis* $H_{37}$Rv (MIC up to 16 μg/mL). Liriodenine (**4**) showed the most excellent antimycobacterial activity (MIC=16 μg/mL) followed by **1** and the 5:4 mixture of **1** and **2**. Their cytotoxicity against three human cancer cell lines, human umbilical vein endothelial cell line (HUVEC), human leukemia cell line (K-562) and HeLa cells were also assessed. The crude extract, alkaloid extract, petroleum ether sub-extract and EtOAc sub-extract showed moderate cytotoxicity against the cancer cell-lines. In addition, the results showed that compounds **1**, **4** and the mixture of **1** and **2** exhibited highest cytotoxicity against HUVEC, K-562 and HeLa cell lines with $GI_{50}$ values up to 4 μg/mL (vs. HUVEC and/or K-562) and $CC_{50}$ values up to 25.1 μg/mL (vs. HeLa).

**Keywords:** Styryllactones; oxoaphorphine alkaloid; *Goniothalamus gitingensis*; antitubercular; cytotoxic.


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## 1. Plant Source

The Annonaceous genus *Goniothalamus* Hook.f. & Thomas is comprised of about 160 species of shrubs and small to large trees. These species are widely distributed in lowland and tropical forests in Southeast Asia [1-3]. Only six species of *Goniothalamus* have been used as ethnomedicines in Asia. As part of our continuing efforts to investigate the bioactive compounds of Philippine endemic plants [4-12], we explored the leaves of *Goniothalamus gitingensis* Elmer for its antituberculosis and cytotoxic constituents. In this paper, the identification of three styryllactones (**1-3**) and an alkaloid (**4**), and their activity against *Mycobacterium tuberculosis* $H_{37}$Rv and cancer cell lines are reported.

The leaves of *G. gitingensis* were collected in Mt. Guiting-Guiting in the province of Sibuyan Islands, Romblon, Philippines in May 2011 and were identified by one of the authors (G. J. D. Alejandro). A voucher specimen (No. USTH11-013) was deposited at the UST Herbarium of the Research Center for the Natural and Applied Sciences (Manila, Philippines).

## 2. Previous Studies

No previous phytochemical investigations have been reported for *Goniothalamus gitingensis*.

## 3. Present Study

The ground, air-dried leaves of *G. gitingensis* (2.1 kg) were extracted with MeOH-$CH_2Cl_2$ (1:1, 16.5 L). The crude extract (Gs, 150.0 g) obtained after concentration *in vacuo* was subjected to conventional acid-base extraction to yield the alkaloid extract (GsA, 9.3 g). The non-alkaloid extract (EtOAc, 20.0 g) was further fractionated by solvent partitioning to yield three sub-extracts namely the petroleum ether sub-extract (GsPE, 2.4 g), EtOAc sub-extract (GsE, 10.1 g) and n-butanol sub-extract (GsB, 2.1 g). Chromatographic purification of extracts GsA and GsE yielded compounds **1-4** (Figure 1). Detailed isolation procedures for compounds **1-4** are shown in the supplementary material. Structure characterization was aided by various spectroscopic experiments such as EIMS, $^{1}$H and $^{13}$C NMR. The structure elucidation and stereochemistry of **3** was also facilitated by X-ray experiments (see supporting data). Furthermore, their identity was verified through comparison with literature spectral data [13-16].

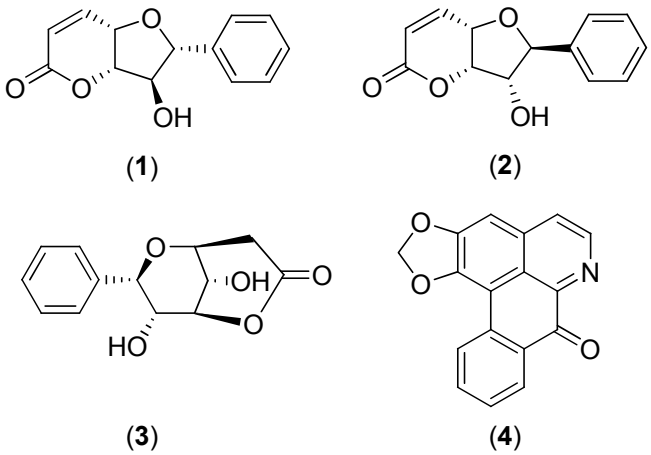


**Figure 1**. Secondary metabolites isolated from *G. gitingensis.*

The compounds were spectroscopically identified as isoaltholactone (**1**), a mixture of **1** and its diastereomer altholactone (**2**), goniopypyrone (**3**) and liriodenine (**4**). All compounds are reported for the first time in this *Goniothalamus* species. While the presence of highly oxidized styrene derivatives has been indicated in previous studies, the report of oxoaphorphine alkaloids is less documented in the genus *Goniothalamus* [17]. Phytochemical literature survey indicates that this is the second reported

isolation of liriodenine (**4**) from this genus, which presence has been previously reported in *G. amuyon* [18].

*Microplate Alamar Blue assay (MABA):* The method given in reference [19] was used for testing *Mycobacterium tuberculosis* $H_{37}Rv$ susceptibility. For reference purposes, the standard TB drugs rifampin (RMP), isoniazid (INH) and streptomycin (SM) were used as positive drug controls. The MIC was defined as the minimum concentration inhibiting fluorescence by 90% relative to bacteria-only controls. Results of the antitubercular assay are reflected in Table 1.

*CellTiter-Blue1 Assay*. The method indicated in reference [20] was used for the determination of inhibition of cell proliferation specifically against leukemia (K-562), human umbilical vein endothelial cord (HUVEC) and HeLa cell lines. The $GI_{50}$ and $CC_{50}$ values were defined as the value at the intersection of the dose curve with the 50% line, compared to untreated control. For comparison, the standard anticancer drugs imatinib and doxorubicin were used as positive drug compounds.

The crude extract (Gs) indicated moderately strong inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC= 47 μg/mL) (Table 1). Among the sub-extracts, the petroleum ether (GsP) and EtOAc (GsE) sub-extracts showed strong antimycobacterial activity with MIC's of 17 and 64 μg/mL, respectively, along with the alkaloid extract showing appreciable inhibitory activity (MIC=128 μg/mL). While strong activity was observed for the GsP sub-extract, further efforts to isolate the bioactive compounds were discontinued due to low extract yields. Interestingly, the styrylpyrone isoaltholactone (**1**) exhibited moderately strong antitubercular activity (MIC=32 μg/mL) while a synergy with its diastereomer, altholactone (**2**), resulted in depressed MIC value (128 μg/mL). Meanwhile, the oxoaphorphine alkaloid liriodenine (**4**) showed the most potent inhibitory activity against the TB organism (MIC=16 μg/mL). The pyranyl-annulated lactone goniopypyrone (**3**) did not exhibit antitubercular activity. With reference to other tested antimicrobial drugs against *M. tb* $H_{37}Rv$ [19], sub-extract GsP and compound **4** show comparable inhibitory activity with fusidic acid (MIC=12 μg/mL) and clarithromycin (MIC=10 μg/mL), and better inhibition compared to bacitracin (MIC=256 μg/mL), piperacillin/tazobactam (MIC=18.5 μg/mL), ampicillin/sulbactam (MIC=128 μg/mL), cloxacillin (MIC=256 μg/mL), cepotaxime (MIC=192 μg/mL), cycloserine (MIC=28.1 μg/mL), lincomycin HCl (MIC=>256 μg/mL) and azithromycin (MIC=>128 μg/mL). With activity comparable to isoaltholactone (**1**), extracts GsE and GS have better antimycobacterial activity than bacitracin, piperacillin/tazobactam, ampicillin/sulbactam, cloxacillin, cepotaxime, cycloserine, lincomycin HCl and azithromycin. Moreover, goniopypyrone (**3**) and sub-extracts GsA and GsB showed comparable inhibitory effects with ampicillin/sulbactam, cepotaxime, and azithromycin.

**Table 1**. MIC values of extracts, **1-4** and TB drug compounds in MABA

| Extract/Fraction | MABA MIC (μg/mL) vs. *M. tb.* $H_{37}Rv$ |
|---|---|
| Gs | 47 |
| GsA | 128 |
| GsP | 17 |
| GsE | 64 |
| GsB | >128 |
| **1** | 32 |
| **1&2** | 128 |
| **3** | >128 |
| **4** | 16 |
| RMP | 0.15 |
| INH | 0.68 |
| SM | 0.83 |

As shown in Table 2, the extracts and compounds **1-4** were evaluated for their cytotoxicity against three human cancer cell lines, human leukemia cell line (K-562), human umbilical vein endothelial cell line (HUVEC) and HeLa cells. The results showed the petroleum ether sub-extract to be most cytotoxic followed by the crude extract (Gs) and EtOAc sub-extract while no activity was observed for the alkaloid extract (GsA) and n-butanol crude sub-extract (GsB). Against HUVEC cells, alkaloid **4** ($GI_{50}$=8.2 μg/mL) exhibited the most antiproliferative effect followed by compounds **1** ($GI_{50}$=15.4

μg/mL) and the mixture of **1** and **2** ($GI_{50}$=20.5 μg/mL). Against K-562 cells, isoaltholactone **1** ($GI_{50}$=4.3 μg/mL) exhibited the most antiproliferative activity followed by compounds **4** ($GI_{50}$=6.1 μg/mL) and the mixture of **1** and **2** ($GI_{50}$=10.2 μg/mL). Cytotoxicity against HeLa cells revealed liriodenine **4** to be the most cytotoxic ($CC_{50}$=24.8 μg/mL) followed by **1** ($GI_{50}$=34.3 μg/mL) and the mixture of **1** and **2** ($CC_{50}$=35.2 μg/mL).

**Table 2**. Cytotoxicity of extracts, **1-4** and anti-cancer drug controls.

| Test Samples | Antiproliferative Effect | | Cytotoxicity |
|---|---|---|---|
| | HUVEC | K-562 | HeLa |
| | $GI_{50}$ [μg/mL] | $GI_{50}$ [μg/mL] | $CC_{50}$ [μg/mL] |
| GsD | 38.7 (±0.7) | 22.4 (±1.1) | 36.1 (±0.1) |
| GsP | 22.1 (±0.7) | 10.2 (±0.3) | 38.0 (±0.2) |
| GsE | 44.7 (±2.0) | 19.8 (±2.3) | 37.8 (±1.7) |
| GsB | >50 | >50 | >50 |
| GsA | >50 | >50 | >50 |
| **1** | 15.4 (±0.8) | 4.3 (±0.2) | 34.3 (±2.4) |
| **1&2** | 20.5 (±0.8) | 10.2 (±0.3) | 35.2 (±1.4) |
| **3** | >50 | >50 | >50 |
| **4** | 8.2 (±0.3) | 6.1 (±0.8) | 24.8 (±1.8) |
| imatinib | 10.9 (±1.2) | 0.1 ($\pm 6.7 \times 10^{-3}$) | 38.8 (±1.4) |
| doxorubicin | 0.1 | 1.0±0.6 | 2.0±0.8 |

*Cytotoxicity runs per sample concentration were done in four replicate measurements.

This study reported the antituberculosis and cytotoxic activities of *G. gitingensis* on the basis of its extracts and isolated constituents for the first time. The results of biological activities observed further indicate the phytomedicinal impact of this Philippine endemic medicinal plant as potential source of anti-TB and anti-cancer agents.

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