Malaysian Journal of Chemistry, 2021, Vol. 23(4), 103-114
(Special Issue: 17th Asian Symposium on Medicinal Plants and Spices (ASOMPS XVII)
August 17-19th 2021 – Virtual Conference)

Dereplication of a Dichloromethane Extract of *Goniothalamus lanceolatus* Leaves using UHPLC-ESI-ORBITRAP HRMS

Nor Nadirah Abdullah[1,2*], Adlin Afzan[3], Nur Hana Jelas[4], Mohd Ridzuan Mohd Abdul Razak[4], Nur Syaidatul[1,2,3], Nurulfazlina Edayah Rasol[1,2], Nur Vicky Bihud[1,2], Murizal Zainol[4], Fasihuddin Badruddin Ahmad[5], Fauziah Abdullah[6] and Nor Hadiani Ismail[1,2]

[1]Atta-ur-Rahman Institute for Natural Products Discovery, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[3]Phytochemistry Unit, Herbal Medicine Research Centre, Institute Medical Research, National Institutes of Health, No.1 Jalan Setia Murni U13/52, Seksyen U13, 40170 Shah Alam, Selangor, Malaysia
[4]Bioassay Unit, Herbal Medicine Research Centre, Institutes Medical Research, National Institute of Health, No. 1 Jalan Setia Murni U13/52, Seksyen U13, 40170 Shah Alam, Selangor, Malaysia
[5]Department of Chemistry, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[6]Herbal Product Development Programme, Natural Products Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia
*Correspondence author (e-mail: nornadirah89@gmail.com; norhadiani@uitm.edu.my)

In our continuing search for bioactive compounds in *Goniothalamus lanceolatus*, we scrutinized the chemical constituents present in the leaves of this plant. As these leaves are traditionally used to repel mosquitoes, we were inspired to examine the plant for potential anti-dengue activity. Our preliminary screening showed that at a concentration of 50 µg/ml, the dichloromethane extract of these leaves was able to inhibit 90.9% of Dengue Virus Type-2 (DENV-2). Dose-dependent plaque assays gave an IC_{50} of 4.16 µg/ml with a selectivity index (SI) of 5.82. Thus, this extract was selected for in-depth phytochemical analysis. This paper reports an efficient chemical profiling of the *G. lanceolatus* leaf dichloromethane extract using high-resolution mass spectrometry (UHPLC-ESI-Orbitrap), via data-dependent MS/MS experiments. We used MZmine2 software version 2.50 (MZmine2) for data processing and peak deconvolution. The dereplication strategy began with the determination of molecular formula from accurate mass measurements (*m/z*). Data mining using MZmine2 followed by cross-search filters against the Dictionary of Natural Products (DNP) produced several hits consisting of styryl-lactones, alkaloids, and acetogenins. A further search of the molecular formula of isolated compounds from different parts of *G. lanceolatus* using an in-house library unambiguously identified seven compounds including goniolanceolatin A**,** goniolanceolatin E, (*6S,7S,8S*)-goniodiol, 1*S*,5*S*,7*R*,8*S*,3-*exo*,7-*endo*-(+)-8-epi-9-deoxygoniopypyrone, goniofupyrone B, parvistone D, and deoxygoniopypyrone B.

Key words: Dereplication; UHPLC-ESI-Orbitrap; MZMine2; *Goniothalamus lanceolatus*

Received: September 2021; Accepted: November 2021

Dengue continues to be a serious threat to public health in tropical countries. The four serotypes of the dengue virus, DENV-1, DENV-2, DENV-3, and DENV-4 cause undifferentiated fevers including dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (Gubler, 1998). At present, there is no specific treatment or vaccine to combat the ravages of this disease (Rajapakse et al., 2012). Tropical plants present an under-explored but promising natural resource for finding compounds with potential anti-dengue properties.

Based on various pharmacological studies, styryl-lactones, acetogenins, alkaloids, and flavonoid derivatives found in many *Goniothalamus sp*. plants are known to be potent against kidney, breast, colon, and pancreatic carcinomas (Cao et al., 1998). Several studies on *G. australis Jessup* (Claire et al., 2013), *G. marcanii Craib* (Ichino et al., 2006), and *G. scortechinii King* (Prawat et al., 2012) have also reported the significant activity of these plant species against malarial parasites. Further, there have been reports that some naphthoquinones (naphthazarins and shikonin) and 9,10-anthraquinones (emodin and doxorubicin derivatives) exhibited antiviral activity (Xiong et al., 2011; Kaptein et al., 2010; Li et al., 2012). Zarina et al. (2020) claimed that the styryl-pyrone derivative from *G. umbrosus* had good

†Paper presented at the 17th Asian Symposium on Medicinal Plants and Spices (ASOMPS XVII) August 17-19th 2021 – Virtual Conference

inhibition potential against DENV-2. In our previous study, we focused on the isolation of secondary metabolites from *G. lanceolatus* extracts for cytotoxicity and anti-cancer properties. This study reports the presence of styryl-lactones, acetogenins, and alkaloid derivatives (Rasol et al., 2018; Bihud et al., 2019). There is no published data available to date on the possible anti-dengue activity of *G. lanceolatus*. Having established the potential anti-dengue activity of the dichloromethane leaf extracts through preliminary screening, we embarked on the chemical profiling of the extracts using a dereplication strategy based on LCMS-ESI-Orbitrap technology. Dereplication is a modern alternative to laborious phytochemical isolation to rapidly annotate known secondary metabolites in crude extracts (Yuliana et al., 2011). In general, rapid annotation can be performed by matching the accurate mass information (MS1) obtained from the HR-MS acquisition with available compound databases. These databases can be obtained commercially such as from an online open-source DNP, built from literature reviews or by using in-house compound libraries generated from isolated compounds.

MATERIALS AND METHODS

1. Plant Material

In June 2012, *G. lanceolatus* leaves were collected from the outskirts of the small town of Sematan in Lundu, Sarawak, Malaysia (GPS Coordinates: 1.6714°N. 109.8520°E) by the late Prof Dr Kamaruddin Mat Salleh (Botany Department, Faculty of Science and Technology, Universiti Kebangsaan Malaysia). The specimen was identified by Prof Dr. Fasihuddin Badruddin Ahmad and the voucher specimen (FBAUMS 108) was deposited in the Herbarium Department of Universiti Malaysia Sarawak.

2. Plant Extraction and Sample Preparation

Dried ground leaves (2.04 kg) were defatted with hexane, dichloromethane (DCM), and methanol (MeOH) successively for 48 hours each, at room temperature. This step was repeated three times with fresh solvents. The volume of solvent used for each extraction was approximately 10-15 L. The extracts were concentrated with a rotary evaporator at 45 °C to give hexane (25.30 g), DCM (49.30 g), and MeOH (54.58 g) crude extracts. All the solvents used were analytical grade (Merck, USA).

The DCM extract (10 mg) of *G. lanceolatus* was pre-treated using a solid phase extraction method (DPA-6S, polyamide, 50 mg/1 mL, Supelco, USA) to remove tannins with methanol: water (7:3). The eluent was dried on a rotary evaporator at 45 °C. The stock solution (1000 µg/ml) of the samples and the reference standards (goniolanceolatin A**,** goniolanceolatin E, (*6S,7S,8S*)-goniodiol, 1*S*,5*S*,7*R*,8*S*,3-*exo*,7-*endo*-(+)-8-epi-9-deoxygoniopypyrone, goniofupyrone B, parvistone D, and deoxygoniopypyrone B) were prepared in HPLC grade methanol: milli-Q water (H_2O) (7:3). The isolated compounds were further diluted to a concentration of 10 µg/ml.

3. UHPLC-ESI-Orbitrap analysis

DCM extracts of 1000 µg/mL in MeOH: H_2O (7:3) were analysed with a Thermo Scientific Vanquish Horizon UHPLC coupled to a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ Mass Spectrometer (H-ESI) and controlled by Freestyle software version 1.6 (Thermo Fisher Scientific, Waltham, MA). The Accucore™ Vanquish™ C18 UHPLC column (2.1 x 100 mm, 1.5 µm, Thermo Scientific, USA) was maintained at 40 ºC throughout the analysis. Positive ion mode (ESI+) was preferred as more compounds were expected to ionize in this mode. The gradient was as follows: 5% B for 2 min; 5% B - 100% B from 2-30 min; 100% B from 30-35 min; 100% B to 5% B from 35-35.5 min; and finally, 4.5 min column equilibration with 5% B. The flow rate was 0.3 mL/min throughout the analysis time of 40 min. The injection volume was 1 µL (1000 µg/ml) and the sample tray temperature was maintained at 10 °C.

The high-resolution mass spectrometer (HRMS) was operated at 60,000 orbitrap resolutions in full scan and MS/MS scan mode. The source of parameters was optimized with a static spray voltage of 3.50 kV, a capillary temperature of 350 °C, sheath gas flow of 50 L/min, auxiliary gas flow of 10 L/min, spare gas flow of 1.0 L/min, vaporizer temperature of 350 °C and S-lens RF level of 60%. The mass range was obtained from *m*/*z* 100 to 1000.

4. Dereplication Strategy Procedure

The raw data were converted into mzML format using the MassConvert tool from ProteoWizard. The high-resolution mass spectral (MS) data were processed and imported into MZmine 2.50, a framework for differential analysis of mass spectrometry data. The MS data set was deconvoluted and deisotoped, while mass adducts were primarily identified and sorted with MZmine 2.50. Then, a centroid detector was utilized to isolate the mass ion peaks. The noise level threshold was set to 1.0E5 and an MS level of 1 was selected. For an MS level of 2, the noise level was set as 1.0E0. The chromatogram builder was used with a minimum time of 0 min, and a minimum highest intensity and *m*/*z* tolerance of 5.0E5 and 0.01 *m*/*z* or 5.0 ppm, respectively. To detect individual peaks, the chromatogram deconvolution parameters were set as follows: peak duration range of 0-2 min and minimum feature height of 1.0E5. Isotopes were identified using the isotopic peak grouper with the following parameters: *m*/*z* tolerance: 0.01 *m*/*z* or 5.0 ppm, retention time tolerance: 0.1 absolute (min), maximum

charge: 2, and representative isotope: most intense.

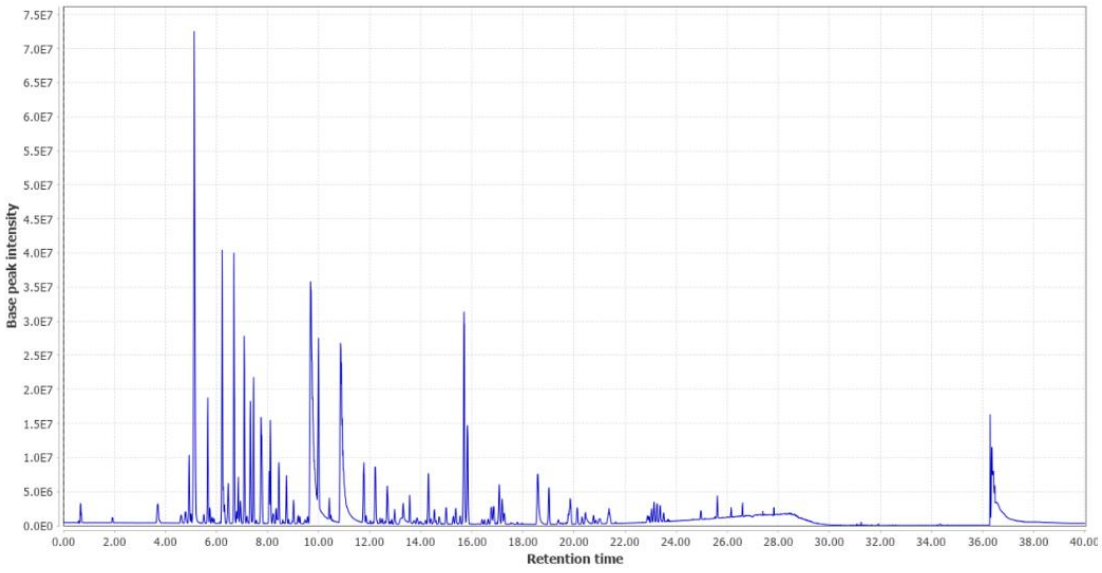
To dereplicate the DCM extract, a specialized compound database was retrieved from the online DNP database. We used the biological source keyword *Goniothalamus* (genus) and this yielded 197 compounds. To supplement this database, an in-house library generated from the previously isolated 24 compounds of *G. lanceolatus* (roots and barks extracts) was added (Rasol et al., 2018; Bihud et al., 2019). All the isolated reference compounds had not yet been reported in the *Goniothalamus* database generated from DNP. Both databases were imported to MZmine 2.50 and employed as the custom database for peak identification and dereplication. Hits were manually cross-checked against the MSMS spectral fragmentation data using Xcalibur 2.1 software.

RESULTS

The metabolite profile of the *G. lanceolatus* DCM extract was optimized with a 40-minute gradient to obtain a comprehensive profile. Pre-processing of the positive ion mode raw data file using MZmine2 resulted in 910 *m/z* features. Annotation of these features was carried out based on accurate mass (MS1) information with the curated DNP and in-house databases (as previously outlined).

In this work, dereplication results were ranked based on the metabolite identification confidence levels (MIC) proposed by the Schrimpe-Rutledge group (Schrimpe-Rutledge et al. 2016). These levels are categorized as follows. Level 1: unambiguous identification by comparison of the retention time and MS/MS fragmentation with reference standards; Level 2: putative identification through MS/MS fragmentation libraries without the presence of standards; Level 3: tentative structure determination by matching MS1 information with the compound database; Level 4: matching with the molecular formula, isotope abundance distribution, adduct ion determination charge state, and ion determination; and Level 5: annotation through unique feature likes mass measurement accuracy (± ppm). From the 910 *m/z* features detected in the metabolite profile, 28 peaks were annotated (Figure 1). The annotations of the peaks are given in Table 1. For all peaks, the proposed metabolites or hits had mass errors of not more than 5 ppm. From the 28 peaks, seven peaks were unambiguously identified using reference standards (Level 1 identification). All other peaks were tentatively identified from the DNP database (*G. lanceolatus* and *Goniothalamus sp*. Database-level 3). For each of the level 3 annotations, more than one compound was proposed due to the presence of isomeric or isobaric compounds.

A



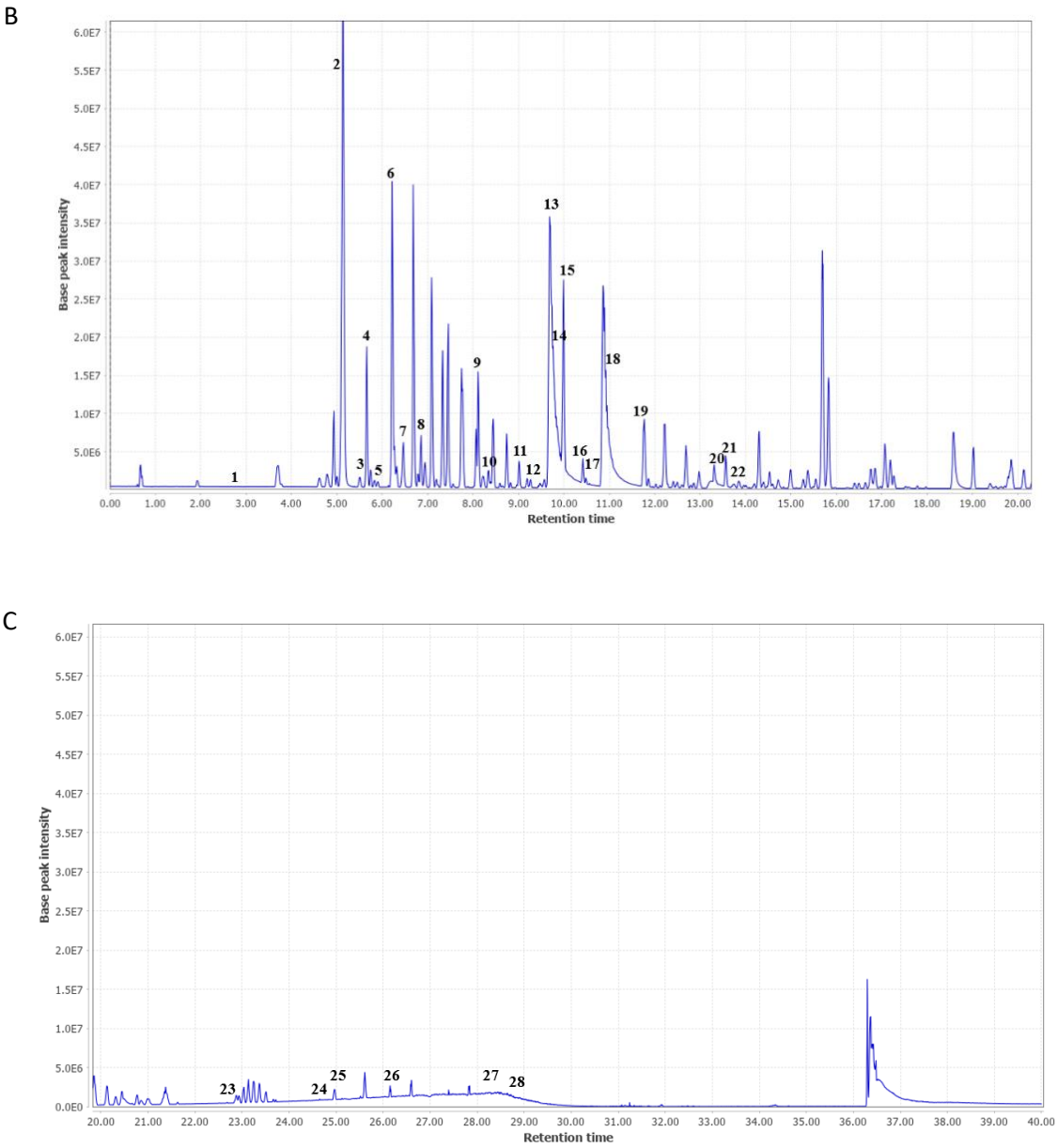


Figure 1. Base peak chromatogram of the dichloromethane extract of *G. lanceolatus* leaves using Orbitrap Fusion™ Tribrid™ Mass Spectrometry. (A) The chromatogram displays a run time of 40 minutes. The same chromatogram (B) at 0-20 min and (C) at 20-40 min. All numbered peaks are identified in Table 1.

Table 1. Dereplication of the dichloromethane extract of *Goniothalamus lanceolatus* leaves

Peak no.	Retention time (min)	Experimental *m/z* [ionisation]	Adduct	Molecular formula (mass error in ppm)	Proposed identification	Chemical class	Taxonomical occurrence (Annonaceae/ *Goniothalamus sp.)*	MIC (Level)
1	2.94	235.0964	$[M+H]^+$	$C_{13}H_{14}O_4$ (0.5)	Altholactone; 6,7-Dihydro 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7R,8R)-form 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7S,8R)-form 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6S,7R,8S)-form 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6S,7S,8S)-form Goniofupyrone A Goniopypyrone; 5,7-Dideoxy, 3ξ-hydroxy 8-Hydroxy-7-phenyl-2,6-dioxabicyclo[3.3.1]nonan-3-one; (4R,6R,7R,8S)-form 8-Hydroxy-7-phenyl-2,6-dioxabicyclo[3.3.1]nonan-3-one; (4R,6R,7S,8R)-form 8-Hydroxy-7-phenyl-2,6-dioxabicyclo[3.3.1]nonan-3-one; (4R,6R,7S,8S)-form 8-Hydroxy-7-phenyl-2,6-dioxabicyclo[3.3.1]nonan-3-one; (4S,6S,7S,8R)-form	Styryl-lactone	*G. arvensis* *G. leiocarpus* *G. leiocarpus* *G. leiocarpus* *G. leiocarpus* *G. amuyon* *G. wightiii* *G. dolichocarpus* *G. leiocarpus* *G. giganteus* *G. tamirensis* *G. tamirensis* *G. lanceolatus* *G. tamirensis*	3
2	5.15	235.0968	$[M+H]^+$	$C_{13}H_{14}O_4$ (1.2)	Deoxygoniopypyrone B	Styryl-lactone	*G. lanceolatus*	1
3	5.50	275.0894	$[M+Na]^+$	$C_{13}H_{16}O_5$ (0.5)	Cardiobutanolide; 3-Deoxy 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7R,8R)-form, 4β-Hydroxy, 3,4-dihydro 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7S,8S)-form, 5S-Hydroxy, 3,4-dihydro	Styryl-lactone	*G. macrocalyx* *G. leiocarpus* *G. arvensis*	3

4	5.61	235.0967	$[M+H]^+$	$C_{13}H_{14}O_4$ (0.8)	(6S,7S,8S)-goniodiol	Styryl-lactone	*G. lanceolatus*	1
5	5.83	289.1051	$[M+Na]^+$	$C_{14}H_{18}O_5$ (0.3)	Goniothalesdiol	Styryl-lactone	*G. borneensis*	3
6	6.27	235.0967	$[M+H]^+$	$C_{13}H_{14}O_4$ (0.8)	1S,5S,7R,8S,3-exo,7-endo-(+)-8-epi-9-deoxygoniopypyrone	Styryl-lactone	*G. lanceolatus*	1
7	6.46	217.0861	$[M+H]^+$	$C_{13}H_{12}O_3$ (0.7)	5,6-Dihydro-5-hydroxy-6-(2-phenylethenyl)-2H-pyran-2-one; (5S,6S,7E)-form Goniothalamin; (R,E)-form, 7R,8R-Epoxide Goniothalamin; (R,E)-form, 7S,8S-Epoxide	Styryl-lactone	*G. dolichocarpus* *G. dolichocarpus and G. macrophyllus* *G. sesquipedalis*	3
8	6.86	305.0999	$[M+Na]^+$	$C_{14}H_{18}O_6$ (0.7)	Gonioheptolide A	Styryl-lactone	*G. giganteus*	3
9	8.09	235.0968	$[M+H]^+$	$C_{13}H_{14}O_4$ (1.2)	Parvistone D	Styryl-lactone	*G. lanceolatus*	1
10	8.24	235.0967	$[M+H]^+$	$C_{13}H_{14}O_4$ (0.8)	Goniofupyrone B	Styryl-lactone	*G. lanceolatus*	1
11	8.96	319.1156	$[M+Na]^+$	$C_{15}H_{20}O_6$ (0.5)	Gonioheptolide A; Et ester analogue	Styryl-lactone	*G. giganteus*	3
12	9.18	299.0896	$[M+Na]^+$	$C_{15}H_{16}O_5$ (0.2)	6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7R,8R)-form, 7-Ac 6-(1,2-Dihydroxy-2-phenylethyl)-5,6-dihydro-2H-pyran-2-one; (6R,7R,8R)-form, 8-Ac	Styryl-lactone	*G. amuyon*	3
13	9.68	276.0660	$[M+H]^+$	$C_{17}H_9O_3N$ (1.8)	Lirodenine	Isoquinoline alkaloid	*G. lanceolatus*	3
14	9.99	451.1760	$[M+H]^+$	$C_{26}H_{26}O_7$ (1.9)	Goniolanceolatin A	Bis-styryl-lactone	*G. lanceolatus*	1
15	10.14	483.2023	$[M+H]^+$	$C_{27}H_{30}O_8$ (1.9)	Goniolanceolatin E	Bis-styryl-lactone	*G. lanceolatus*	1
16	10.39	310.1079	$[M+H]^+$	$C_{18}H_{15}NO_4$ (0.1)	1,2,7-Trihydroxydibenz[cd,f]indol-4(5H)-one; 2,7-Di-Me ether	Aristolactam BI- Alkaloid	*G. marcanii*	3

17	10.59	296.0923	$[M+H]^+$	$C_{17}H_{13}NO_4$ (0.1)	1,2,3-Trihydroxydibenz[cd,f]indol-4(5H)-one; 1,3-Di-Me ether 1,2,7-Trihydroxydibenz[cd,f]indol-4(5H)-one; 2,7-Di-Me ether 1,2,9-Trihydroxydibenz[cd,f]indol-4(5H)-one; 1,2-Di-Me ether	Uvarilactam-Alkaloid	*G. griffithii*	3
18	11.36	258.0763	$[M+H]^+$	$C_{14}H_{11}NO_4$ (1.2)	Goniothaline A; O6-De-Me Griffithazanone A	Benzopyrans alkaloid Quinolines alkaloid	*G. australis* *G. griffithii*	3
19	11.77	451.1760	$[M+H]^+$	$C_{26}H_{26}O_7$ (1.9)	Digoniodiol Goniolanceolatin F Goniolanceolatin G	Bis-styryl-lactone	*G. lanceolatus*	3
20	13.31	493.1867	$[M+H]^+$	$C_{28}H_{28}O_8$ (2.0)	Digoniodiol; Mono-Ac	Bis-styryl-lactone	*G. lanceolatus*	3
21	13.56	433.1653	$[M+H]^+$	$C_{26}H_{24}O_6$ (1.6)	Leiocarpin E	Styryl-lactone	*G. leiocarpus*	3
22	13.86	286.0715	$[M+H]^+$	$C_{15}H_{11}NO_5$ (0.3)	Marcanine A; 3-Methoxy, 6-hydroxy Scorazanone	Quinolines alkaloid	*G. marcanii*	3
23	22.69	613.469	$[M+H]^+$	$C_{35}H_{64}O_8$ (2.6)	3-(8,11-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (10R,13R)-form, 4R/14S,17S18S,34ξ-Pentahydroxy(1) 3-(8,11-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (10R,13R)-form, 4R,14S,17S,18S,34ξ-Pentahydroxy(2) 3-(12,15-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (14R,17R)-form, 4R,10S,13S,15R,18S-Pentahydroxy 3-(12,15-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (14R*,17R*)-form, 4R*,10S*,13S*,18S*,21ξ-Pentahydroxy 3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19R)-form, 4R,10ξ,15R,20S,34ξ-Pentahydroxy(1)	Acetogenins	*G. donnaiensis* *G. donnaiensis* *G. donnaiensis* *G. sawtehii* *G. cheliensis* *G. cheliensis* *G. donnaiensis* *G. donnaiensis* *G. gardneri*	3

					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19R)-form, 4R,10ξ,15R,20S,34ξ-Pentahydroxy(2)		*G. donnaiensis*	
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,8R,10S,15R,20R-Pentahydroxy		*G. donnaiensis*	
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,10ξ,15R,20R,34ξ-Pentahydroxy(1)			
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,10ξ,15R,20R,34ξ-Pentahydroxy(2)			
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,10ξ,15R,20R,34ξ-Pentahydroxy(1)			
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,10ξ,15R,20R,34ξ-Pentahydroxy(2)			
24	24.65	597.4744	$[M+H]^+$	$C_{35}H_{64}O_7$ (3.2)	Annonacin	Acetogenin	*G. lanceolatus*	3
25	25.29	619.4557	$[M+Na]^+$	$C_{35}H_{64}O_7$ (1.2)	3-(8,11-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (10R,13R)-form, 4R,14S,17R,18R-Tetrahydroxy	Acetogenins	*G. giganteus*	3
							G. howii	
					3-(8,11-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (10R,13R)-form, 4R,14S,17S,18S-Tetrahydroxy		*G. howii*	
					3-(10,13-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (12S,15R)-form, 4R,16S,19R,20R-Tetrahydroxy		*G. giganteus, G. donnaiensis, G. gardneri, G. cheliensis*	
					3-(12,15-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (14S,17S)-form, 4R,10R,13R,18R-Tetrahydroxy		*G. giganteus, G. gardneri*	
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,10R,15R,20R-Tetrahydroxy		*G. donnaiensis*	
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,15R,20R,34ξ-Tetrahydroxy (1)		*G. donnaiensis*	
					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16S,19S)-form, 4R,15R,20R,34ξ-Tetrahydroxy (2)		*G. howii*	
							G. giganteus	

					3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16ξ,19ξ)-form, 4ξ,10ξ,15ξ,20ξ-Tetrahydroxy Pyragonicin Pyranicin			
26	26.11	617.4404	[M+Na] $^{+}$	$C_{35}H_{62}O_7$ (1.7)	3-(8,11:12,15-Diepoxytriacontyl)-5-methyl-2(5H)-furanone; (10S,13R,14R,17S)-form, 4R,9R,18R-Trihydroxy	Acetogenin	*G. giganteus*	3
27	28.23	603.4606	[M+Na] $^{+}$	$C_{35}H_{64}O_6$ (0.8)	3-(8,11-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (10R,13R)-form, 14S,17R,18R-Trihydroxy 3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16R*,19R*)-form, 10ξ,15S*,20S*-Trihydroxy 3-(14,17-Epoxytriacontyl)-5-methyl-2(5H)-furanone; (16ξ,19ξ)-form, 4ξ,15ξ,20ξ-Trihydroxy 5-Methyl-3-triacontyl-2(5H)-furanone; 4R,10R,17R,18R-Tetrahydroxy, 13,14Z-didehydro	Acetogenin	*G. giganteus and G. howii* *G. howii* *G. howii* *G. giganteus*	3
28	28.82	601.4443	[M+Na] $^{+}$	$C_{35}H_{62}O_6$ (0.2)	3-(8,11:12,15-Diepoxytriacontyl)-5-methyl-2(5H)-furanone; (10S,13R,14R,17S)-form, 4R,18R-Dihydroxy Goniotrionin	Acetogenins	*G. giganteus*	3

*MIC: Metabolite identification confidence. [1] Unambiguous identification by comparison of the retention time and MS/MS fragmentation with reference standards (isolated compound - *G. lanceolatus*); [3] Tentative structures determined by matching MS1 information with a compound database (DNP; biological source- *Goniothalamus sp*.)

DISCUSSION

In this preliminary study, we focused mostly on MS1 dereplication (Level 3) for rapid analysis of the *G. lanceolatus* leaf DCM extract. Higher confidence in annotation was achieved by comparison with seven reference standards (Level 1). These standards were the styryl-lactones (Figure 2) isolated by our team from the barks and roots part of *G. lanceolatus* (Rasol et al. 2018; Bihud et al. 2019). The structural elucidations of these standards were confirmed from their MS/MS, UV and NMR data.

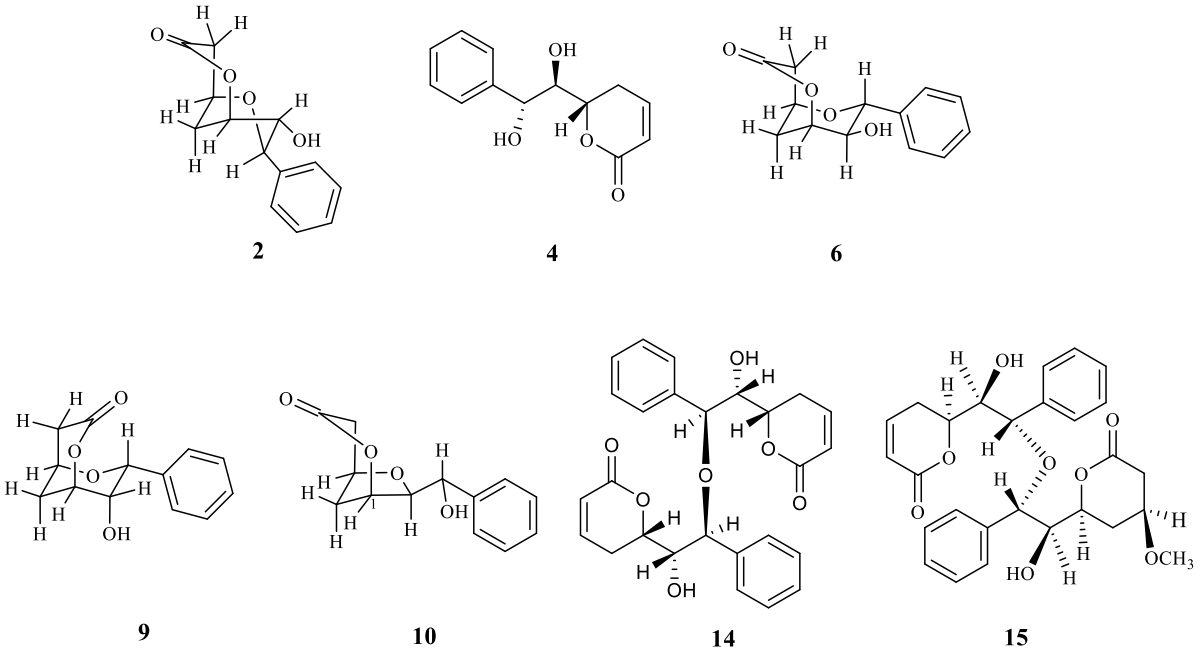


Figure 2. 2D chemical structures of seven styryl-lactones identified from *G. lanceolatus* leaves dichloromethane extract

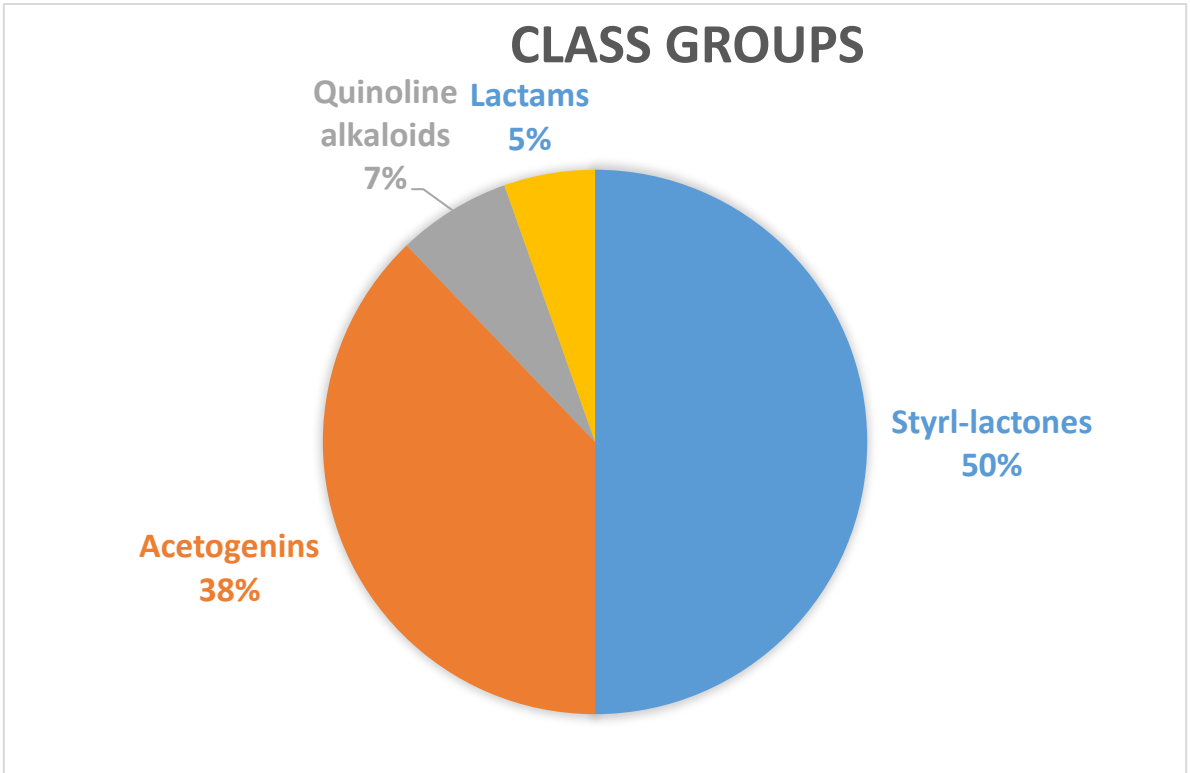


Figure 3. Signal intensities of compounds in *G. lanceolatus* leaf dichloromethane extracts, grouped into styryl-lactones, acetogenins, quinoline alkaloids, and lactams.

From the results (Table 1), a total of 28 peaks were annotated. 17 peaks were annotated as styryl-lactones, 6 peaks as acetogenins and another 5 as alkaloids. Three out of five peaks were annotated as quinoline alkaloids and another two peaks as lactams.

The most dominant group of compounds detected was styryl-lactones (50%) and acetogenins (35%). Apart from these, some alkaloid groups such as quinolines (7%) and lactams (5%) were also detected (Figure 3). Acetogenins were observed at retention times (t_R) greater than 20 minutes, while the styryl-lactones and alkaloids were detected earlier. It should be noted that annotation based on MS1 is a rapid dereplication method for an overview of the phytochemical composition of the extract of interest, but this strategy alone often results in a large number of predicted metabolites. To reduce these hits and dereplicate all the detected *m/z* features, MS/MS spectral information can be used. Dereplication by MS/MS fragmentation patterns can be done automatically with public domain software such as SIRIUS (https://bio.informatik.uni-jena.de/sirius/) and Global Natural Products Social molecular networking (GNPS) (Wang et al. 2016). MS/MS dereplication is often aided by generating a molecular network (MN). MNs have recently been developed to organize and visualize relationships between all MS/MS features of single or multiple samples. This approach has been applied concurrently with bioassay-guided fractionation for prioritizing important and interesting metabolites (Nothias et al. 2018).

CONCLUSION

Our preliminary work unambiguously identified seven known compounds in the chemical profile of a dichloromethane extract of *G. lanceolatus* leaves, while another 21 peaks were tentatively identified by MS1 dereplication. Styryl-lactones and acetogenins were the major compound groups present. For wider annotation coverage and greater confidence, a combination of spectral MS/MS comparisons and molecular networking appears to be the way forward.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Malaysian Ministry Higher of Education for funding through a research grant (FRGS), [UiTM600-RMC/FRGS 5/3 (106/2021)]. Nor Nadirah Abdullah would also like to thank UiTM and AuRIns for the financial support and excellent research facilities.

REFERENCES

1. Gubler, D. J. (1998) Dengue and dengue hemorrhagic fever. *Clinical Microbiology Review*, **11(3)**, 480–496.

2. Rajapakse, S., Rodrigo, C. & Rajapakse (2012) Treatment of dengue fever. *Infection and Drug Resistance*, **103**, doi:10.2147/idr.s22613.

3. Kwon, H. J., Kim, H. H., Yoon, S. Y., Ryu, Y. B., Chang, J. S., Cho, K. O., Rho, M. C., Park, S. J., Lee, W. S. (2010) In vitro inhibitory Activity of Alpinia katsumadai extracts against influenza virus infection and hemagglutination. *Virol J*., **7,** 307.

4. Yasuhara-Bell, J, Yang, Y., Barlow, R., Trapido-Rosenthal, H., Lu, Y. (2010) In vitro evaluation of marine-microorganism extracts for anti-viral activity. *Virol J*., **7**, 182.

5. Zandi, K., Taherzadeh, M., Yaghoubi, R., Tajbakhsh, S., Rastian, Z., Sartavi, K. (2009) Antiviral activity of Avicennia marina against herpes simplex virus type 1 and vaccine strain of poliovirus (An in vitro study). *J. Med. Plant Res*., **3**, 771-775.

6. Cao, S. G., Wu, X. H., Sim, K. Y., Tan, B. K. H., Pereira, J. T., Goh, S. H. (1998) Styryl-lactone derivatives and alkaloids from *Goniothalamus borneensis* (Annonaceae). *Tetrahedron*, **54**, 2143–2148.

7. Claire, L., Melodie, B., Karren, D. B., Anthony, R. C., Frederic, M., Vanida, C., Rohan, A. D. (2013) Pyridocoumarine, aristolactam and aporphine alkaloids from the Australian rainforest plant *Goniothalamus australis. Phytochemistry*, **86**, 121-126.

8. Ichino, C., Soonthornchareonnon, N., Chuakal, W., Kiyohara, H., Ishiyama, A., Sekiguchi, H., Namatame, M., Otoguro, K., Omura, S., Yamada, H. (2006) Screening of Thai medicinal plant extracts and their active constituents for *in vitro* antimalarial activity. *Phytotherapy Research*, **20**, 307-309.

9. Prawat, U., Chaimanee, S., Butsuri, A., Salae, A. W., Tuntiwachwuttikul, P. (2012) Bioactive styryl lactone, two new naphthoquinones, and one new styryl lactone, and other constituents from *Goniothalamus scortechinii*. *Phytochemistry Letters*, **5**, 529-534.

10. Xiong, H. R., Luo, J., Hou, W., Xiao, H., Yang, Z. Q. (2011) The effect of emodin, an anthraquinone derivative extracted from the roots of Rheum tanguticum, against herpes simplex virus in vitro and in vivo. *J. Ethnopharmacol*, **133**, 718–723.

11. Kaptein, S. J., De Burghgraeve, T., Froeyen, M., Pastorino, B., Alen, M. M., Mondotte, J. A.,

Herdewijn, P., Jacobs, M., de Lamballerie, X., Schols, D. (2010) A derivate of the antibiotic doxorubicin is a selective inhibitor of dengue and yellow fever virus replication in vitro. *Antimicrob. Agents Chemother*, **54**, 5269–5280.

12. Li, Y. H., Lu, Q. N., Wang, H. Q., Tao, P. Z.; Jiang, J. D. (2012) Geldanamycin, a ligand of heat shock protein 90, inhibits herpes simplex virus type 2 replication both in vitro and in vivo. *J. Antibiot.*, **65**, 509–512.

13. Noor Zarina Abd Wahab and Nazlina Ibrahim (2020) *In vitro* study, Antiviral Activity of Styrylpyrone Derivative Against Dengue Virus Type 2. *Asian Journal of Plant Sciences*, **19(4)**, 438-442. DOI:10.3923/ajps.2020.438.442

14. Rasol, N. E., Ahmad, F. B., Mai, C. W., Bihud, N. V., Abdullah, F., Awang, K. and Ismail, N. H. (2018) *Natural Product Communication*, **13(12)**,1575-1578.

15. Bihud, N. V., Rasol, N. E., Imran, S., Awang, K., Ahmad, F. B., Mai, C. -W., Ismail, N. H. (2019) Goniolanceolatins A–H, Cytotoxic Bis-styryl lactones from *Goniothalamus lanceolatus*. *Journal of Natural Products*, **27**, 82(9), 2430-2442

16. Yuliana, N. D., Khatib, A., Choi, Y. H. (2011) Verpoorte, R. Metabolomics for bioactivity assessment of natural products. Phytother. Res., 25, 157–169.

17. Corley D. G. & Durley R. C. (1994) Strategies for database dereplication of natural products. *J. Nat. Prod.*, **57**, 1484–1490.

18. Schrimpe-Rutledge, A. C., Codreanu, S. G., Sherrod, S. D. & McLean, J. A. (2016) Untargeted Metabolomics Strategies—Challenges and Emerging Directions. *Journal of The American Society for Mass Spectrometry*, **27(12)**, 1897–1905.

19. Wang, M., Carver, J. J., Phelan, V. V., Sanchez, L. M., Garg, N., Peng, Y., Luzzatto-Knaan, T. (2016) Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology*, **34(8)**, 828–837.

20. Nothias, L. -F., Nothias-Esposito, M., da Silva, R., Wang, M., Protsyuk, I., Zhang, Z., Dorrestein, P. C. (2018) Bioactivity-Based Molecular Networking for the Discovery of Drug Leads in Natural Product Bioassay-Guided Fractionation. *Journal of Natural Products*, **81(4)**, 758–767.