ISSN 2462-2052
DOI: https://doi.org/10.37134/jsml.vol11.2.12.2023

Journal of Science and Mathematics Letters
Volume 11, Issue 2, 104-109, 2023

RESEARCH ARTICLE

Recycling HPLC for the Isolation of Styryl Lactone from *Goniothalamus lanceolatus* Miq

Seri Arisha Yaakop[1,2], Nur Vicky Bihud[1,2*]

[1]Faculty of Applied Sciences, Universiti Teknologi MARA,
40450 Shah Alam, Selangor, Malaysia
[2]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA,
Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
*Corresponding author: vicky@uitm.edu.my

Received: 5 June 2023; **Accepted:** 6 August 2023; **Published:** 17 August 2023

ABSTRACT

A unique plant from the rainforest of Sarawak, Malaysia, *Goniothalamus lanceolatus* Miq., was chosen for a phytochemical study based on its ethnomedicinal characteristics amongst the locals. Isolation and purification of a minor compound exhibiting similar retention characteristics was made possible by using preparative Recycling HPLC. Due to a recycle valve in the HPLC system, this method enables the unresolved peaks to recirculate into the column repeatedly until the peaks were successfully separated as pure entity. During the recycling process, solvent consumption is not needed. Recycling HPLC expediates the isolation and purification of complex mixtures in such a sustainable way. One styryl lactone was successfully isolated and identified as 6*S*,7*S*,8*S*-(-)-goniodiol.

Keywords: Recycling HPLC, *Goniothalamus*, *Goniothalamus lanceolatus*

1. INTRODUCTION

The genus *Goniothalamus* is one of the most extensive genera within its family, encompassing a total of 165 species comprising of shrubs and small to large trees (Bihud et al., 2019). The genus exhibits a wide distribution in the lowland and submontane forests of tropical South-east Asia, with a notable concentration of the species in Malaysia, particularly in Peninsular Malaysia, Borneo, Indonesia, and Thailand (Bihud et al., 2019). *Goniothalamus* species is commonly used by the natives to treat conditions such as anti-aging, typhoid fever, tympanites, stomach discomfort, rheumatism, and skin issues, and it also serves as a mosquito repellent (Aslam et al., 2016; Alkofahi et al., 1989; Prawat et al., 2012). Two definite classes of secondary metabolites commonly isolated from this genus are styryl lactones and acetogenins, which have been reported to have anticancer activity (Wiart, 2007).

Goniothalamus lanceolatus Miq. is locally known as '*gertimang*' or '*sekulai*' in Sarawak and reported to be useful to treat fever, skin infections, and cancer (Rasol et al., 2018a). Previous studies done on the phytochemicals and pharmacological activities of the dichloromethane crude extract from the stembark and roots of *G. lanceolatus* resulted in the isolation of eight new bis-styryl lactones, fifteen styryl lactones, and four alkaloids with excellent cytotoxic activities against colon and colorectal cancer cell lines (Bihud et al., 2019;

Rasol et al., 2018a, 2018b). Due to the discovery of compounds with promising anticancer properties from the dichloromethane extract, this current study, which focuses on the hexane crude extract, may lead to a new set of bioactive compounds. Recycling HPLC has been used to separate and purify a wide variety of natural products, including enantiomers, diastereomers, positional isomers, and structurally related or unrelated compounds with comparable retention properties (Sidana & Joshi, 2013). Unlike conventional HPLC, recycling HPLC is superior at separating compounds due to the recycle valve in the system that recirculates unresolved peaks back to the column, requiring no fresh solvent, making it economical and sustainable. The recycling method also allows for multiple separation cycles, which efficiently eliminate impurities and contaminants, yielding highly pure compounds.

In this study, the closed-loop recycling technique was used. The injected fraction was recycled in an isocratic mode, using reversed phase silica support, to achieve the desired separation of the components. One compound was successfully purified using recycling HPLC, namely, 6*S*,7*S*,8*S*-(-)-goniodiol. The structure of the isolate and its absolute stereochemistry is shown in Figure 1. This study outlines the set-up of recycling HPLC and its ability to separate mixtures into a single pure entity.

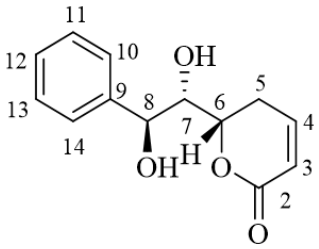

Figure 1. The structure of purified styryl lactone, 6*S*,7*S*,8*S*-(-)-goniodiol.

2. MATERIALS AND METHODS

2.1. Plant Material and Chemicals

G. lanceolatus (stem bark) was collected from Sarawak, Malaysia, and the voucher specimen (FBAUMS 108) was stored at the herbarium of UNIMAS, Sarawak. The stem bark samples were dried, crushed into pieces, and ground into a powder. The material was extracted using hexane, dichloromethane, and methanol (Sigma Aldrich, USA), which led to crude extracts. The hexane crude extract was the focus of this study. The crude extract was fractionated using conventional column chromatography, utilizing analytical grade dichloromethane and methanol, and yielded a series of fractions. Fraction 7 revealed intriguing spots on Thin Layer Chromatography (TLC aluminium sheets 20×20 cm, RP-18 F_{254}, Merck, Germany) and showed a promising profile on proton NMR (Nuclear Magnetic Resonance), hence becoming the subject of this study. For NMR analysis, the fraction and compound were dissolved and recorded in deuterated chloroform without TMS, while for recycling HPLC, methanol was of HPLC grade (Sigma Aldrich, USA). Ultrapure water (18 MΩ/cm) was obtained from a PURELAB® Option water purification system (ELGA), from Veolia Water Technologies, Paris, France.

2.2. Instruments

Isolation of compounds were done using JAI (Japan Analytical Industry) Recycling HPLC (LC-9103) paired with Diode Array and using Jaigel-ODS-AP, SP-120-15 (20×250 mm)

column. The NMR spectra were measured on Bruker Avance 600 FT-NMR (Billerica, USA) in deuterated chloroform without TMS.

2.3. TLC Profiling

The TLC plates were spotted with moderate amount of eluate using a piece of fine glass capillary tube and placed in a saturated chromatography tank. A series of solvent systems of methanol: H_2O (70:30, 60:40, and 50:50 v/v) were conducted on Fraction 7 to find the most suitable mobile phase for isocratic system in recycling HPLC. The spots were then visualized with UV lamp emitting wavelengths of 254 nm and 365 nm. Solvent system of methanol: H_2O (50:50 v/v) exhibited the most optimal separation, making it an ideal mobile phase for recycling HPLC.

2.4. Preparative Recycling HPLC

The isolation and purification procedures were performed on a JAIGEL-LC-9103 preparative column (20×250 mm) eluted at 4 mL/min and detected at 210 nm. The fraction was dissolved in methanol and injected into a partial loop with a volume of 2 mL. In order to enhance the separation process, the recycle valve was utilised to recycle and elute the analytes through the same column. During the process, the drain valve was used to remove impurities from the eluent. The selector valves either remove contaminants or collect the desired chemical substances. Figure 2 shows a schematic diagram of the recycling HPLC system (Ramli et al., 2017).

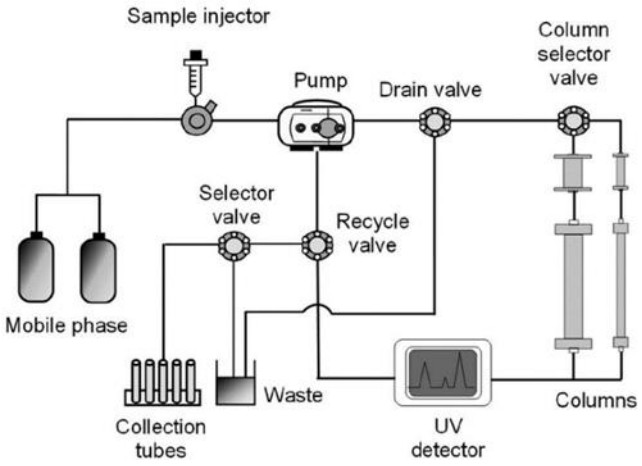

Figure 2. Schematic diagram of recycling HPLC system

3. RESULTS AND DISCUSSION

Prior to the isolation procedure, a profiling approach was done to observe the separation efficiency of the compounds using the preparative recycling HPLC instrument (flow rate 4.0 mL/min; UV detector 210 nm). Solvent system of 50:50 v/v methanol: H_2O was utilized for profiling. The profiling gave crucial information on the separation performance, enabling decisions for the subsequent isolation and characterization steps of the target compounds. Utilization of 50:50 v/v methanol: H_2O for mobile phase in recycling HPLC showed impressive compound separation (Figure 3). The first peak was observed within 10 min. The duration from one cycle to another was appropriate for separation to occur. Therefore, the solvent system of 50:50 v/v methanol: H_2O was chosen for further isolation and purification analysis.

ISSN 2462-2052
DOI: https://doi.org/10.37134/jsml.vol11.2.12.2023
Journal of Science and Mathematics Letters
Volume 11, Issue 2, 104-109, 2023

Approximately 35 mg of sample from Fraction 7 was injected into the recycling HPLC on Jaigel-LC-9103 (flow rate 4.0 mL/min; UV detector 210 nm) with an isocratic solvent system of 50:50 v/v methanol: H_2O. The first peak appeared after 14 min and allowed to be continuously recycled (Figure 4). After 44 min, the peaks reappeared. During this second cycle, the major peak was recycled while the minor peaks were drained. This strategy was intended to provide a better separation for the major peak during the following cycles. The process was continued by repetitively recycling the major peak of the system and draining the impurities until a clean baseline was achieved and the peak showed a single peak (pure compound achieved). On the 10th cycle, which took approximately 238 min, the purified major compound (T7-i) was collected.

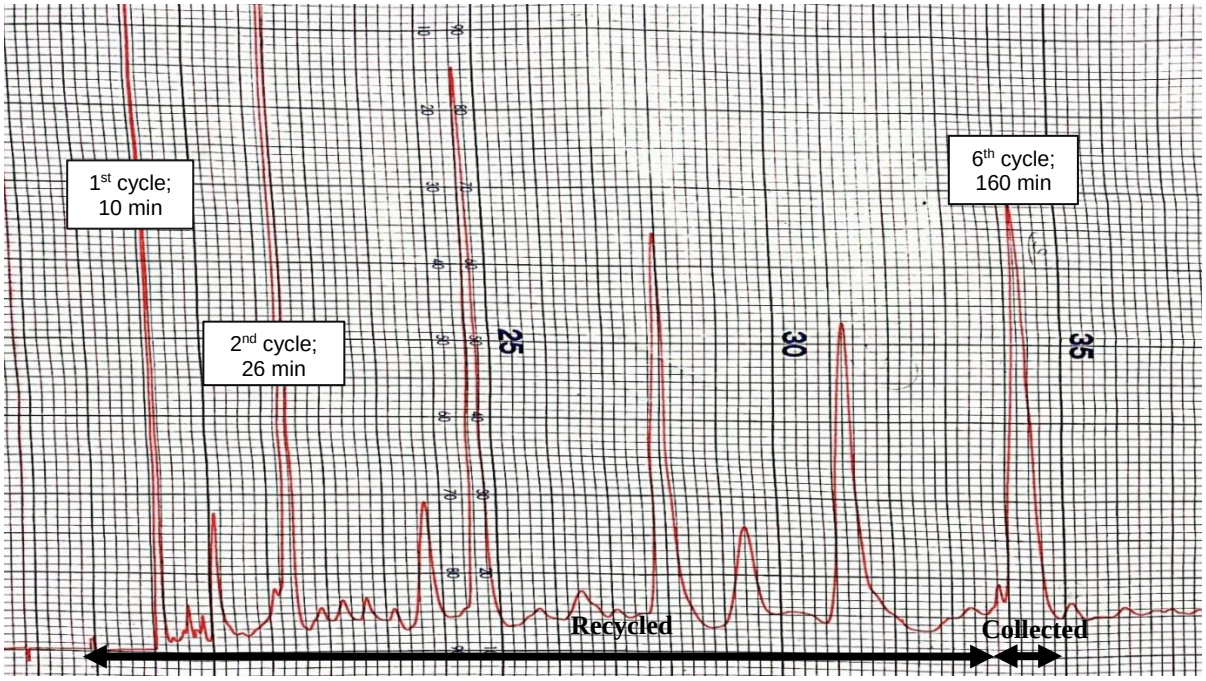

Figure 3. Profiling of Fraction 7 using recycling HPLC

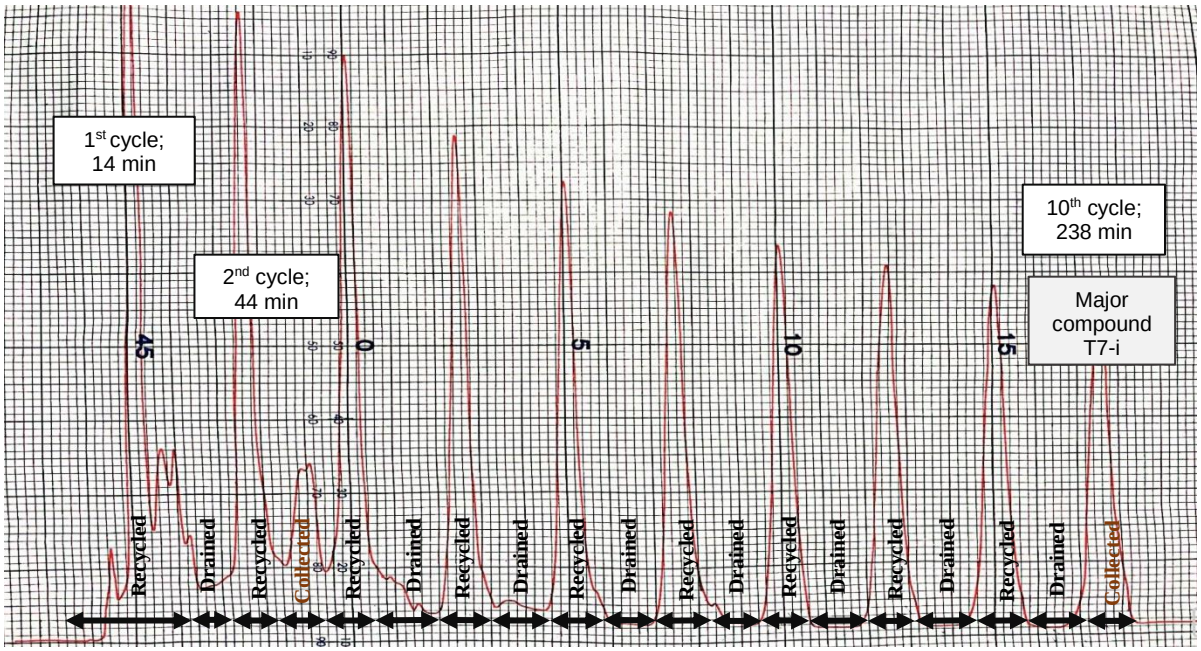

Figure 4. Separation of Fraction 7 using recycling HPLC

Compound T7-i was the only isolate obtained from Fraction 7 of hexane extract from the stem bark of *G. lanceolatus.* The minor isolates were too minute to be analysed. The ^{1}H-NMR spectra of Compound T7-i showed five aromatic signals between δ 7.34 and 7.44, indicating a mono-substituted phenyl moiety. Two olefinic protons resonated at δ 6.01 (ddd, *J* = 9.7 and 2.2 Hz) and 6.95 (ddd, *J* = 9.6, 6.7, and 1.1 Hz) were typical α,β-unsaturated δ-lactone, H-3 and H-4. Three oxygenated methine protons, H-6, H-7 and H-8, resonated at δ 4.82 (ddd, *J* = 14.2, 3.5, and 2.2 Hz), 3.74 (dd, *J* = 7.4 and 2.2 Hz) and 4.96 (d, *J* = 7.4 Hz) respectively. The $J_{7,8}$ coupling constant of 7.4 Hz indicated an *erythro* form between H-7 and H-8, while H-6 and H-7 gave a coupling constant, $J_{6,7}$ of 2.2 Hz, indicated a *threo* form (Talapatra et al.,1997). The methylene germinal protons of H-5a and H-5b resonated at δ 2.20 and 2.80 (ddd, *J* = 18.6, 5.6, and 4.0 Hz). The ^{13}C-NMR spectrum indicating seven sp^2 methine carbons δ 120.6 (C-3), 146.2 (C-4), 126.6 (C-10/C-14), 128.7 (C-11/C-13), 128.3 (C-12), three sp^3 methine carbons (δ 76.8 (C-6), 75.0 (C-7), 73.7 (C-8), and one sp^3 methylene carbon at δ 26.0 (C-5). In addition, the spectrum also showed one sp^2 quaternary carbon belonging to the aromatic ring at δ 140.8 (C-9), and one carbonyl carbon at δ 163.7 (C-2), a characteristic signal for an α,β-unsaturated δ-lactone in the styryl pyrones (Lan et al., 2003). The ^{1}H and ^{13}C-NMR data (Table 1) of compound T7-i agreed with previous assignments for 6*S*,7*S*,8*S*-(-)-goniodiol, isolated from the dichloromethane extract from the stem bark of the same plant (Bihud, 2020). This compound was first isolated from the methanolic extract of *Polyalthia parviflora* leaves (Liou et al., 2014).

Table 1. ^{1}H-NMR (600MHz) and ^{13}C-NMR (150 MHz) spectral data for compound T7-i in $CDCl_3$ and comparison with literature values (δ in ppm and *J* in Hz)

Position	**Compound T7-i**		**6*S*,7*S*,8*S*-(-)-Goniodiol***	
	δ_H	**δC**	**δ_H***	**δC***
2	-	163.8	-	163.7
3	6.01, ddd (9.7, 2.2)	120.6	6.00, dd (9.6, 2.4)	120.6
4	6.95, ddd (9.6, 6.7, 1.1)	146.2	6.93, ddd (9.6, 6.0, 1.8)	146.2
5	2.20, ddd (18.6, 5.6, 4.0)	26.1	2.17, ddd (18.6, 6.6, 4.2)	26.0
	2.81, dddd (18.5, 13.7, 12.9, 2.0)		2.78, dddd (18.6, 13.2, 5.2, 2.4)	
6	4.82, ddd (14.2, 3.5, 2.2)	76.8	4.80, ddd (13.2, 3.6, 2.4)	76.8
7	3.74, dd (7.4, 2.2)	75.1	3.72, dd (7.2, 2.4)	75.0
8	4.96, d (7.4)	73.7	4.95, d (7.2)	73.7
9	-	140.8	-	140.8
10, 14		126.6		126.6
11, 13	7.34-7.44, m	128.8	7.33-7.42, m	128.7
12		128.3		128.3

*Bihud 2020 recorded in $CDCl_3$ at 600 MHz

4. CONCLUSION

The utilization of preparative recycling HPLC has proven to be remarkably effective in isolating and purifying complex mixtures. This advanced method involves recycling unresolved peaks back to the column through a dedicated valve in the HPLC system, significantly enhancing the separation efficiency and minimizing peak dispersion. One of the notable advantages of recycling HPLC is the solvent-saving quality, as fresh solvent is not required during the recycling process. This innovative approach is particularly valuable in the field of natural product chemistry, as it overcomes the challenges of conventional phytochemical isolation. Recycling HPLC proves to be cost-effective and time-efficient, maximizing the discovery potential of natural products and optimizing the use of valuable resources. In this research, one compound was successfully isolated and purified from a fraction of hexane crude extract of the stembark of *G. lanceolatus* within 238 min. Upon comparison with literature data

from a previous study, the compound was identified as 6*S*,7*S*,*8S*-(-)-goniodiol, a styrylpyrone. The occurrence of 6*S*,7*S*,8*S*-(-)-goniodiol reported from nature was very limited if compared to its enantiomer 6*R*,7*R*,8*R*-(+)-goniodiol, which was abundant in *Goniothalamus* species. This plant reported the second isolation of 6*S*,7*S*,8*S*-(-)-goniodiol from nature and the first in *Goniothalamus* species.

Declaration of Interest
The authors declare that there is no conflict of interest.

Acknowledgement
The authors would like to acknowledge Japan Analytical Industry Co Ltd for their assistance while using preparative Recycling HPLC (LC-9103).

REFERENCES

Alkofahi A, Ma WW, McKenzie AT, Byrn SR, McLaughlin JL. (1989). Goniotriol from *Goniothalamus giganteus*. *Journal of Natural Products*, 52, 1371-1373.

Aslam MS, Ahmad MS, Mamat AS, Ahmad MZ, Salam F. (2016). *Goniothalamus*: phytochemical and eEthnobotanical review. *Recent Advances in Biology and Medicine*, 2, 34-47.

Bihud NV, Rasol NE, Imran S, Awang K, Ahmad FB, Mai CW, Leong CO, Cordell GA, Ismail NH. (2019). Goniolanceolatins A-H, cytotoxic bisstyryllactones from *Goniothalamus lanceolatus. Journal of Natural Products*, 82(9), 2430-2442.

Bihud NV. (2020). Styryllactones and bis-styryllactones from the stembark of *Goniothalamus lanceolatus* Miq. and their cytotoxic activity. PhD Thesis, Universiti Teknologi MARA (UiTM).

Lan YH, Chang FR, Yu JH, Yang YL, Chang YL, Lee SJ, Wu YC. (2003). Cytotoxic styrylpyrones from *Goniothalamus amuyon. Journal of Natural Products*, 66, 487-490.

Liou JR, Wu TY, Thang TD, Hwang TL, Wu CC, Cheng YB, Chiang MY, Lan YH, El-Shazly M, Wu SL, Beerhues L, Yuan SS, Hou MF, Chen SL, Chang FR, Wu YC. (2014). Bioactive 6*S*-styryllactone constituents of *Polyalthia parviflora*. *Journal of Natural Products*, 77, 2626-2632.

Prawat U, Chaimanee S, Butsuri A, Salae AW, Tuntiwachwuttikul P. (2012). Bioactive styryl lactone, two new naphthoquinones, and one new styryl lactone, and other constituents from *Goniothalamus scortechinii*. *Phytochemistry Letters*, 5(3), 529-534.

Ramli R, Ismail NH, Manshoor N. (2017). Recycling HPLC for the purification of oligostilbenes from *Dipterocarpus semivestitus* and *Neobalanocarpus heimii* (Dipterocarpaceae). *Journal of Liquid Chromatography & Related Technologies*, 40(18), 943-949.

Rasol NE, Ahmad FB, Lim XY, Chung FFL, Leong CO, Mai CW, Bihud NV, Zaki HM, Ismail NH. (2018b). Cytotoxic lactam and naphthoquinone alkaloids from roots of *Goniothalamus lanceolatus* Miq. *Phytochemistry Letters*, 24, 51-55.

Rasol NE, Ahmad FB, Mai CW, Bihud NV, Abdullah F, Awang K, Ismail NH. (2018a). Styryl lactones from roots and barks of *Goniothalamus lanceolatus*. *Natural Product Communication*, 13(12), 1575-1578.

Sidana J, Joshi LK. (2013). Recycle HPLC: a powerful tool for the purification of natural products. *Chromatography Research International*, 7(2), 1-7.

Talapatra B, Porel A, Biswas K, Talapatra SK. (1997). Absolute configuration of goniodiol, goniodiol monoacetate and other related dihydropyrones from synthetic, circular dichroism and X-ray crystallographic evidence. *Journal of the Indian Chemical Society*, 74, 896-903.

Wiart C. (2007). *Goniothalamus* species: a source of drugs for the treatment of cancers and bacterial infections? *Evidence-Based Complementary and Alternative Medicine*, 4(3), 299-311.