Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 202118(SI-2):104-114 **OPEN ACCESS**

Quantification and characterization of goniothalamin from *Goniothalamus andersonii* using HPLC

Nor Hadirah Iskandar[1], Siti Mariam Yaacob[1], Zalilawati Mat Rashid[1*], Noor Aida Aini Nawawi[2], Intan Safinar Ismail[3]

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, **Malaysia**
[2]Pusat Pengurusan Makmal Berpusat, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, **Malaysia**
[3]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, **Malaysia**

***Correspondence:** zalilawati@unisza.edu.my **Received 05-07-2021, Revised: 12-08-2021, Accepted: 15-08-2021 e-Published: 18-08-2021**

Goniothalamin (GTN) is a styryl lactone compound commonly found in genus *Goniothalamus*. It has been reported to possess antiproliferative activity against leukemia *in vitro* and *in vivo*. This study objectives were to optimize the extraction and HPLC quantification methods as well as to characterize GTN isolated from the dried stem bark of *Goniothalamus andersonii*. The powdered stem bark was extracted separately in four solvents including hexane, petroleum ether, ethyl acetate and methanol. GTN was analyzed by high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC), while characterization was done using nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GCMS). The HPLC method was optimized at a flow rate 1.0 mL/min using ultrapure water (A)/methanol (B) as the mobile phase with gradient elution 0–50% B (10 min), 50–100% B (30 min), 100–60% B (10 min). GTN signal was detected at min 24.1. This method was found to be precise, accurate, and linear over the analysis of several GTN concentrations. The limit of detection (LOD) and limit of quantification (LOQ) of GTN were 1.55 and 5.16 µg/mL, respectively. HPLC quantification indicated the highest amount of GTN in hexane extract (41.6±3.5%), compared to other extracts. 1D-NMR spectrum showed the common GTN signals and GCMS analysis revealed the molecular formula $C_{13}H_{12}O_2$ ($[M]^+$ m/z 200.1) with fragment ions *m/z* (rel. int.): 104, 68, 200, 131, 91, and 172. In conclusion, the findings recorded optimized quantitative analytical methods to perform quality control while promote further researches on goniothalamin.

Keywords: Stem Bark Extraction, Goniothalamin, HPLC Quantification, *Goniothalamus andersonii*

INTRODUCTION

Medicinal plants are commonly applied to treat various diseases and are known to be great sources of bioactive secondary metabolites. Some of the metabolites have been extensively studied for potential use as drugs in the pharmaceutical industry (Jain et al. 2019). Goniothalamus belongs to the *Annonaceae* family and consists of 115 species, which distribute worldwide throughout tropics and subtropics including Southeast Asia. Some of the Goniothalamus species been recognized are medicinal, which are traditionally used to treat asthma, cholera, fever, malaria, rheumatism, stomachache, postpartum protective remedy, abortifacient, and insect repellent (Li et al. 2016; Seyed et al. 2014). Various classes of compounds were isolated from Goniothalamus, including, isoquinoline alkaloid derivatives, lactonic pharmacophores, quinolines, phenanthrene lactones, terpenes, acetogenins, phenolics and

styryl lactones derivatives (Pilli et al. 2018).

Goniothalamin (GTN) is a styryl lactone compound commonly found in the genus Goniothalamus. Previous studies revealed that GTN showed cytotoxicity against several cancer cell lines including cervical (Hela) (Ali et al. 1997), gastric (HGC-27) (Ali et al. 1997), breast (MCF7) (Ali et al. 1997), human oral squamous cell carcinoma (H400) (Li et al. 2016), colon (HT29) carcinomas, and leukemia (HL60) (Chien and Pihie 2003), Jurkat T-cells (Inayat-Hussain et al. 1999), hepatoblastoma HepG2 (Manaf et al. 2011) and CEM-SS (Rajab et al. 2005). *In vivo* study also reported potential antiproliferative activity against leukemia by GTN (Inayat-Hussain et al. 2010). Previous studies reported the usage of methanol and petroleum ether as extractant solvents for phytochemicals extraction from Goniothalamus sp. In many cases, GTN was obtained or purified using nonpolar solvents (Humeirah et al. 2010; Takemura et al. 2012; Wasano et al. 2015). However, there are few reports on analytical measurement to quantify the purified GTN.

Thus, this study mainly aimed to optimize the high performance liquid chromatography (HPLC) method and validate the method by means of the linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to the International Conference of Harmonization (ICH) guideline (Huber, 1999; Shabir, 2003; Suresh et al. 2010). Besides, the structural analysis of GTN isolated from the dried stem bark of *Goniothalamus andersonii* using nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GCMS) was also reported.

MATERIALS AND METHODS

General

Silica gel 60 F_{254}aluminum sheets and Whatman® Grade 1 qualitative filter papers (125 mm) were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). Deuterated methanol, potassium dihydrogen phosphate, sodium 3-trimethylsilyl-(2,2,3,3-D_4) propionate (TSP), sodium deuterium oxide (NaOD), methanol, ethyl acetate, petroleum ether, hexane (analytical grade) and chloroform (liquid chromatography grade) bought from Merck (Darmstadt, Germany). Ultrapure water (UPW) were used in related experiments. Absorbances of colorimetric tests were read using an ELISA microplate reader (TECAN, US). Gas chromatography-mass spectrometry (GCMS) analysis was performed using GCMS Agilent 7890B system. Nuclear magnetic resonance (NMR) spectra were recorded on 500MHz Varian Unity INOVA NMR spectrometer (Varian Inc., USA).

Plant Materials and Extraction

Goniothalamin (GTN) standard and dried *Goniothalamus andersonii* stem barks used in this study were obtained from Department of Chemistry, University Kebangsaan Malaysia (UKM). The stem bark was deposited to Herbarium Laboratory of the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin. The dried barks were ground into fine powder and kept in desiccators before used. The dried powdered sample was extracted separately with methanol, ethyl acetate, petroleum ether, and hexane. The sample (5 g) was macerated in 100 mL solvent for 24 hours (h) in a closed container under dark condition. The mixture was stirred (2 h) at room temperature, sonicated for 20 minutes (min), followed by filtration to separate the residue from the extract. The extraction process using each solvent was done in triplicates. The extracts were pooled and combined into individual extraction solvent. Each extract was concentrated under vacuum using a rotary evaporator (40 °C) and completely dried to crude extract under nitrogen gas flow. Percentages of yields were recorded. The crude extracts were stored at -20 °C before further analysis.

High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC analysis was carried out to detect the presence of GTN in the extracts. The working solution at 100 µg/mL of GTN standard was prepared in chloroform, while *G. andersoniii* extracts were prepared in methanol. The samples were applied on HPTLC plates loaded with silica gel 60 F_{254} (20 cm × 10 cm) and analyzed using the HPTLC CAMAG system controlled by Win-CATS software (Muttenz, Switzerland). 10 µL aliquots were spotted on the plates using Automatic TLC Sampler 4 (ATS4) and dried for 20 min.

In order to determine the best solvent system for GTN detection, different ratios of hexane/ethyl acetate solvent systems (1.1:0.9, 1:1 and 2:3 v:v), were tested as mobile phases. The plates were developed in Automated Developing Chamber 2 (ADC2). The chamber was previously saturated with the solvent system with the relative humidity was adjusted to 38.8%. The plate pre-condition

time was set to 1 min, loading distance to 85 mm, and drying time, after development was 5 min. Images of chemical bands were detected under UV light at a wavelength at 254 nm using TLC Visualizer 2 and bands were profiled using TLC Scanner 4. The retardation factor (R_f) of GTN standard was compared with the R_f of chemicals present in the extracts. The R_f value was measured using the formula below.

$$R_f = \frac{\text{Distance (substance)}}{\text{Distance (solvent)}}$$

High Performance Liquid Chromatography (HPLC) Analysis

HPLC Condition and Optimization

The quantification of GTN composition in each extract was carried out using a Semi-Preparative HPLC system (Shimadzu, Japan) consisted of an LC-6AD binary pump, DGU-20A degassing unit, and SPD-20A UV/Vis detector. The HPLC system fitted with ZORBAX Eclipse Plus C18 column (4.6 mm x 250 mm, 5.0 µm, Agilent Technologies) was operated at 40 °C and the sample injection volume was 1.0 µL.

The sample was separated on the column eluted with gradient elution of ultrapure water (A) and methanol (B) (v/v) at a flow rate of 1.0 mL/min. There were three elution programs tested to obtain optimum resolution chromatogram. The programs were as follow: 1) 0–60% B (10 min), 60–100% B (30 min), 100–60% B (10 min); 2) 0–50% B (10 min), 50–100% B (30 min), 100–60% B (10 min); 3) 0–10% B (10 min), 10–50% B (20 min), 50–100% B (30 min), 100–60% B (15 min). The peak signals were detected at wavelength 254 nm. The elution programs and signal detection were operated using LabSolutions software.

HPLC Method Validation

HPLC method was validated in terms of linearity, recovery, precision, accuracy, the limit of detection (LOD) and limit of quantitation (LOQ) based on ICH Harmonized Tripartite Guidelines using the optimized method (Alquadeib, 2019). The stock solution of standard GTN was prepared at 1.0 mg/mL.

Linearity was used to monitor whether the system used could obtain the results that are concentration-dependent of the analyte tested. For linearity validation, several concentrations of standard solutions (800, 500, 250, 100, 50, and 10 µg/mL) were analyzed. The peak areas against concentrations of GTN were plotted to generate a standard curve graph. The linearity was evaluated by calculating the linear range and correlation coefficient, slope and intercept of the curve.

The accuracy of an analytical method refers to the nearness between the theoretical and the exact values. It is calculated as the percent recovery (%R) of the analyte recovered and the accepted value should be within 90–110%. In this case, successive analyses (n=4) for three different concentrations (100, 250, 500 µg/mL) of standard GTN solutions were performed. Percentage of recovery (%R) was calculated using the formula below.

$$\%\ Recovery = \left(\frac{\text{Recovered conc.}}{\text{Injected conc.}}\right) \times 100$$

The precision of an analytical method refers to the degree of agreement among individual tests when the same technique is applied repetitively on several occasions. The precision validation was carried out by multiple analyses of the three different standard solutions (conc.; 100, 250 and 500 µg/mL) on the same day for intra-day precision and three different days for the inter-day precision. The precision was expressed by comparing the actual weight of each concentration collected with the theoretical amount loaded and percentages of relative standard deviation (%RSD). The %RSD were calculated using the formula below.

$$RSD\ (\%) = \frac{S}{x} \times 100$$

Where;
S = Standard deviation value
x = Mean of the data

Limit of detection (LOD) is defined as the smallest concentration of an analyte in a sample that can be detected by a specific measurement process, while the limit of quantitation (LOQ) is defined as the smallest concentration of an analyte that can be determined with acceptable precision and accuracy according to an individual analytical procedure (European Medicines Agency, 1995). These two parameters were determined using the formula below.

$$LOD = \frac{3}{S} \times \sigma$$

$$LOQ = \frac{10}{} \times \sigma$$

$$S$$

Where;

σ = Standard deviation of intercepts of the calibration curves

S = Mean of slopes of the calibration curves

HPLC Analysis of GTN Composition in Extracts

The optimized HPLC method was used to determine the unknown GTN concentration in each extract. All samples were prepared in chloroform except for methanol crude extract prepared in methanol. Test sample of each extract was prepared at 0.1 mg/mL (100 ppm). GTN standard solution (1 mg/mL) was used to prepare several concentrations of the working solutions (800, 500, 250, 100, 50, and 10 µg/mL) to plot a standard curve graph. The standard curve was generated using the peak areas against concentrations of the GTN standard. The GTN concentration in each extract was determined by plotting the peak area value on the GTN standard curve equation.

Gas Chromatography–Mass Spectrometry (GCMS) Analysis

GCMS analysis was performed using an Agilent GSMS 7890B system (Agilent Technologies, USA), equipped with HP5MS capillary column (30 m x 250 µm x 0.25 µm; p/n 19091S-433UI). Helium was used as a carrier gas at a flow rate of 1 mL/min. Sample (1 µL) was injected in the splitless mode. The injector and detector temperature were set at 250 °C and transfer line temperature was 150 °C. The oven temperature was programmed at 80 °C (2 min), 80 °C to 280 °C at a rate of 10 °C per minute, and 280 (2 min). GTN was identified by comparing GTN mass spectral data with NIST4 Mass Spectral Library and Wiley mass spectral database.

Nuclear Magnetic Resonance (NMR) Analysis

Sample (10 mg in 700 µL) was prepared in deuterated methanol-TSP mixture at pH 6.0. The sample was vortexed, centrifuged and the cleared supernatant was collected before added into the NMR tube. NMR spectra were recorded using 500 MHz ^{1}H-NMR spectroscopy (Bruker, USA) operated at 25 °C, with relaxation delay (RD) 2.0 s and pulse width (PW) 21.0 µs (90°). The spectra were analyzed using MestreNova 14.2.1 software.

Statistical Analysis

All analysis were replicated at least three times. Results are the mean ± standard deviation (SD) of different experiments. The student's *t*-test was used to determine the concentration differences at each day and GTN concentration differences between extracts. The confidence level was 95% with *p*-value of less than 0.05 ($p<0.05$) for the data to be considered as statistically significance. Statistical analysis was conducted by using Statistical Package for the Social Science software (SPSS).

RESULTS AND DISCUSSION

Extraction

The crude extracts collected prior to concentration and drying of extracts were hexane (HE), petroleum ether (PE), ethyl acetate (EE), methanol (ME) with the respective percentage of yields were 0.58%, 0.6%, 1.3% and 2.6% (w/w).

Detection of Goniothalamin (GTN) by HPTLC

TLC was used to detect the presence of GTN in respective crude extracts. It is a simple, fast and versatile tool for qualitative analysis of the targeted compound. The TLC plates were developed using three different ratios of hexane/ethyl acetate mobile phase systems and viewed under 254 nm. TLC analysis profiles indicated that GTN in the extracts and the standard were detected as one clear and single band at the same R_f values (Fig. 1). Among the solvent systems, hexane/ethyl acetate at ratio 1.1:0.9 was determined as the optimized method due to the clear separation of GTN in all extracts at R_f 0.55, with less accumulation of the trace compounds at the upper region of the TLC plate (Fig. 1A). Among all extracts, the most intense GTN signal indicating that the highest amount present, was detected in HE. Other traces of peaks were also detected near the solvent front, revealing the existence of other compounds in the extracts.

HPLC Method Optimization

HPLC mobile phase system was firstly optimized to ensure better separation of chemical components. Three methods were finalized and reported (Fig. 2). As a result, the optimum mobile phase system determined was method 2 which the elution system was ultrapure water (A)/methanol (B) (v/v) with gradient elution 0–50% B (10 min), 50–100% B (30 min), 100–60% B (10 min) and the flow rate at 1.0 mL/min. This method gave good resolution, intense GTN and stable baseline signal (Fig. 2). Previous studies also reported GTN analysis using the same solvents mixture but with varied gradient elution ratios and duration (Takemura et al. 2012; Rasol et al. 2018).

HPLC Method Validation

Selectivity/Specificity

The selectivity, specificity and resolution of HPLC system were determined by calculating the percent relative standard deviation (%RSD) of the retention times and peak areas for three concentrations of GTN (100, 250, 500 µg/mL) (n=4).

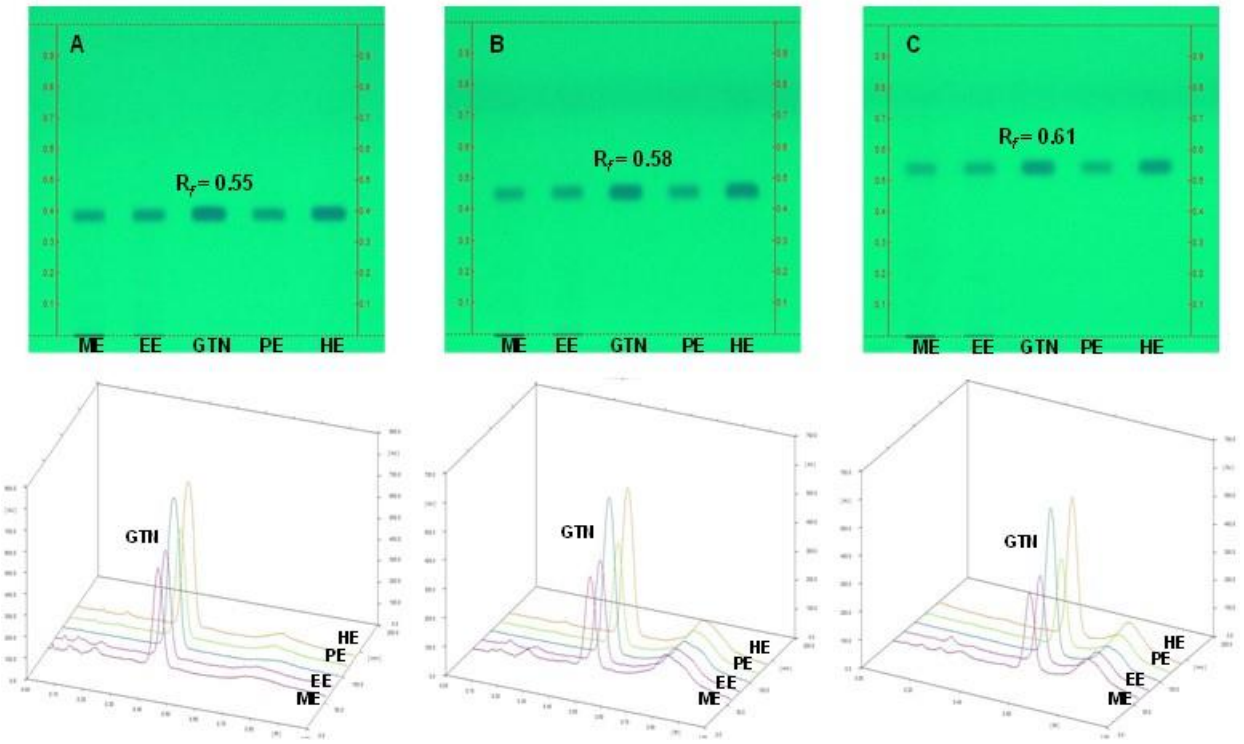

Figure 1: HPTLC analysis of GTN standard and *Goniothalamus andersonii* extracts. HPTLC plates (upper panel) and profiles (lower panel) developed with different mobile phases (MP). A) MP hexane/ethyl acetate (1.1:0.9, v/v), B) MP hexane/ethyl acetate (1:1, v/v), C) MP hexane/ethyl acetate (2:3, v/v). HPTLC plates were visualized under UV 254 nm. GTN = goniothalamin, ME = methanol extract, EE = ethyl acetate extract, PE = petroleum ether extract, HE = hexane extract.

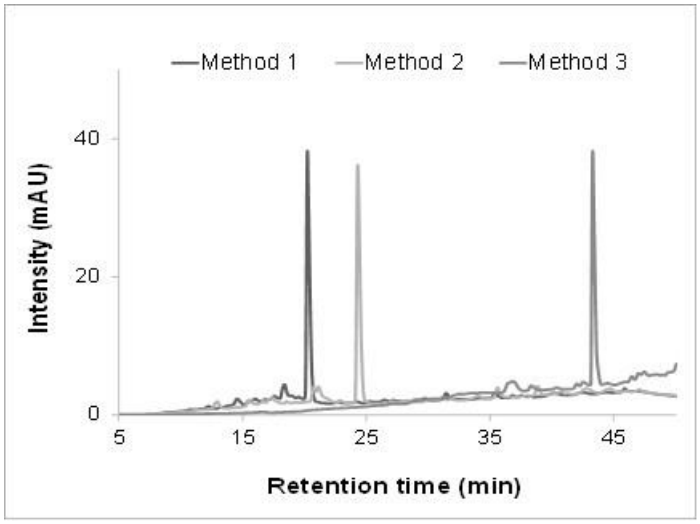

Figure 2: Representative chromatogram of a sample analyzed using three HPLC methods.

GTN was detected at min 24.09±0.07 under UV/Vis 254 nm which corresponded to the previous study by Takemura et al. (2012). %RSD of RT was in the range of 0.1 to 0.2%, while %RSD of peak areas varied between 1.7 to 4.4% (Table 1). The acceptance criterion was ±2% (Shabir, 2003; Suresh et al. 2010; Alquadeib, 2019). Thus, it was concluded that the system was selective in the detection of GTN. Compared to the previous study, GTN peak was detected at a shorter retention time due to different solvents and HPLC method used (Zohdi et al. 2017).

Linearity

The chromatograms of a series of GTN standard solutions revealed peaks at retention time (RT) min 24.17±0.07. The slope and the intercept resolved from the peak areas and the nominal GTN concentrations were used to determine the HPLC linearity. The linear regression equation obtained from the standard curves was; y=7862x-74487, where y is the between area under the peak (AUP) of GTN signal and x is the concentration of GTN. A linear relationship was revealed between the AUP of GTN and the corresponding concentrations (range: 10-800 µg/mL), with a correlation coefficient (R^2) is approximately 0.9992 (Fig. 3). According to the ICH guideline, an R^2 value of more than 0.999 is considered as evidence of acceptable linearity in the regression line (Shabir, 2003; Suresh et al. 2010).

Accuracy and Precision

The accuracy and precision of HPLC method were determined by calculating the respective recovery percentage (%R) and relative standard deviation (%RSD) for three concentrations of GTN (100, 250, 500 µg/mL) (n=4). As a result, the recoveries of the standard drug were found to be accurate which was exhibited by the average %R of 98.14±1.52% for intraday and 98.11±3.62% for inter-day analysis (Table 2). The system intra-day and inter-day precision were proven from the respective average %RSD, 1.55% and 3.69% (Table 2). %RSD of inter-day and intra-day precision obtained in this study less than 7% which follow the recommendation by ICH guidelines for the drugs (Alquadeib, 2019).

Limit of detection (LOD) and Limit of quantitation (LOQ)

The GTN LOD value using HPLC optimized method were calculated and identified based on the intercepts standard deviation (SD) and the slope's mean of GTN standard curves (n=4). SD for the curves intercepts was 15088.35 and slope's mean was 7792.48. LOD was determined to be 1.25 µg/mL, while the first point which fits into LOQ value was 5.16 µg/mL.

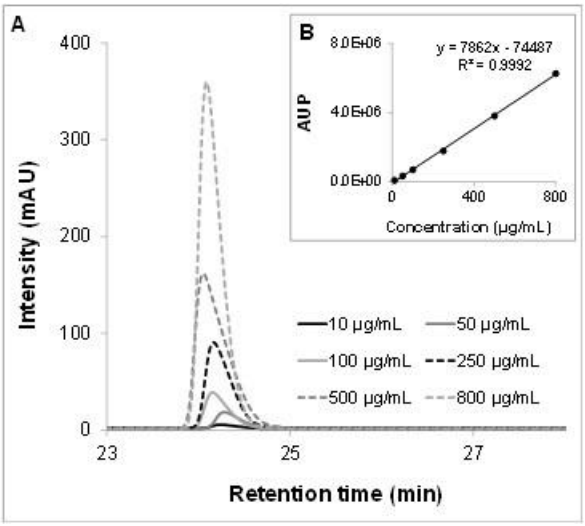

Figure 3: Representative HPLC chromatograms and standard curve of a series Goniothalamin (GTN). A) Chromatograms of GTN standard at different concentrations, B) Standard curve plotted using the area under peaks (AUP) vs concentrations of GTN.

GTN Composition in Extracts

HPLC analysis of all extracts revealed that the intensity and peak area of GTN in HE was found to be the highest, followed by PE, EE and ME (Fig. 4). The unknown GTN concentration in each extract was calculated by plotting the respective AUP into the linear regression equation obtained (y=7862x-74487). The concentration of GTN in HE was found to be 80.75 µg/mL which accounted for 41.6±3.5% of compounds in HE. Meanwhile, GTN content in PE was 37.73 µg/mL (24.2±0.9%), in EE was 32.09 µg/mL (20.7±4.5%), and the least amount was in ME which has 18.10 µg/mL (13.7±3.3%). HE was further purified using HPTLC using solvent system hexane/ethyl acetate (1.1:0.9) and afforded goniothalamin at R_f 0.55.

This study indicated the application of different polarities of solvent influenced the yield of GTN obtained. The nonpolar solvents, (hexane and petroleum ether) retained a larger amount of GTN compared to other solvents (ethyl acetate and methanol).

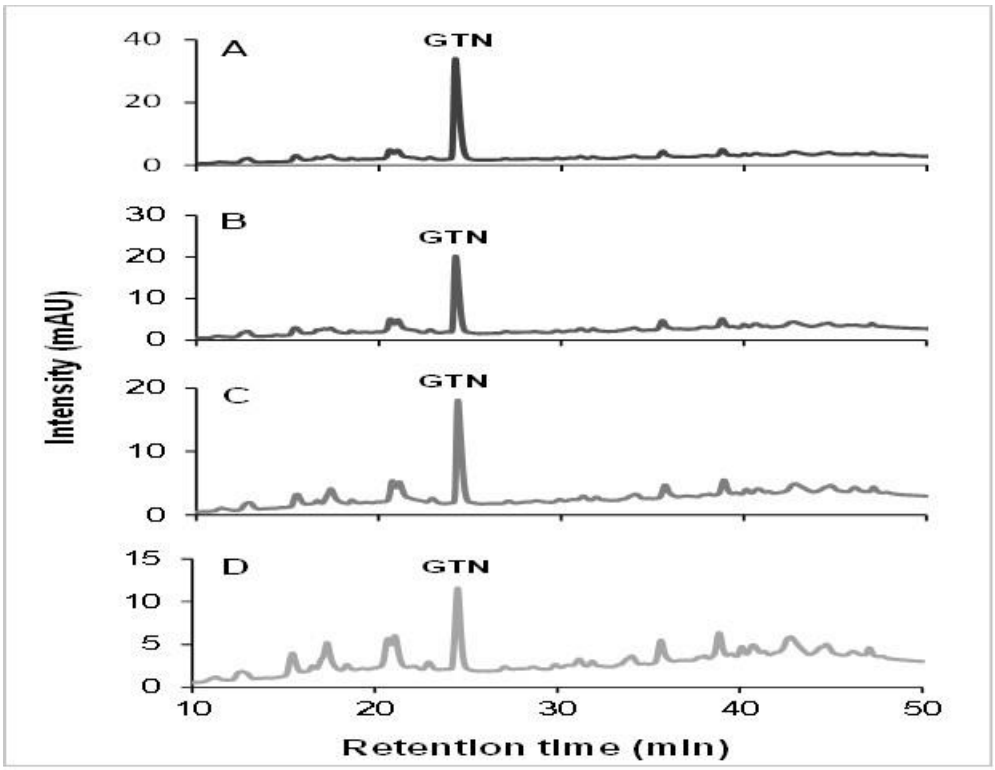

Figure 4: HPLC chromatograms of extracts of different solvents revealing the presence of GTN at minute 24.1. A) Hexane extract, B) Petroleum ether extract, C) Ethyl acetate extract, D) Methanol extract.

Table 1: Selectivity/specificity test of HPLC system analyzed by precision (%RSD) of retention time (RT) and peak area of goniothalamin detected.

Conc. (µg/mL) (n=4)	100		250		500	
	RT (min)	Peak area	RT (min)	Peak area	RT (min)	Peak area
Mean	24.13	733193	24.13	1791328	24.01	3876562
SD	0.04	32126	0.03	33004	0.05	65921
%RSD	0.16	4.38	0.10	1.84	0.20	1.70
Average RT	24.09 ± 0.07					

Table 2: Robustness test of HPLC proposed method in terms of precision (%RSD) and accuracy (%Recovery) analyzed by the intra-day and inter-day statistical.

Conc. (µg/mL) (n=4)	Intra-day analysis			Inter-day analysis		
	100	250	500	100	250	500
% Recovery (R)	100.30	93.61	100.51	105.16	94.46	94.71
SD	1.83	1.06	1.68	3.79	2.04	5.03
%RSD	1.82	1.13	1.67	3.60	2.16	5.31
Average %R	98.14			98.11		
Average SD	1.52			3.62		
Average %RSD	1.55			3.69		

*RT = retention time; RSD = relative standard deviation

The nonpolar property of GTN tends to dissolve in nonpolar solvent following the 'like-dissolves-like' theory. The theory states that compounds of similar chemical characteristics and dielectric constant tend to attract and dissolve each other (Visht and Chaturvedi, 2012; Mandal et al. 2015; Zhuang et al. 2021). As polarity of the extractant solution used would affect the composition of chemical constituents retained (Rashid et al. 2018), thus in this case, using hexane as extractant solvent was found to be the most suitable to retain the optimum amount of GTN.

Goniothalamin Structural Analysis

Goniothalamin was afforded as a pale-yellow solid and analyzed for structural conformation using GCMS and NMR as well as by comparison with the published data. GTN molecular formula $C_{13}H_{12}O_2$ was deduced from MS data that showed a strong a prominent molecular ion $[M]^+$ at *m/z* 200.1 and base peak at *m/z* 68 (cyclopentenyl ion, $[C_5H_7]^+$). Other fragment ions generated were *m/z* (rel. int.): 104 (styrenyl ion,$[C_8H_8]^+$), 91 (tropilium ion, $[C_7H_7]^+$), 131 (cinnamyl ion, $[C_9H_9]^+$), 172 [M-CO]$^+$ and 77 (phenyl ion) $[C_6H_5]^+$ (Fig. 5) (Jusoh et al. 2015).

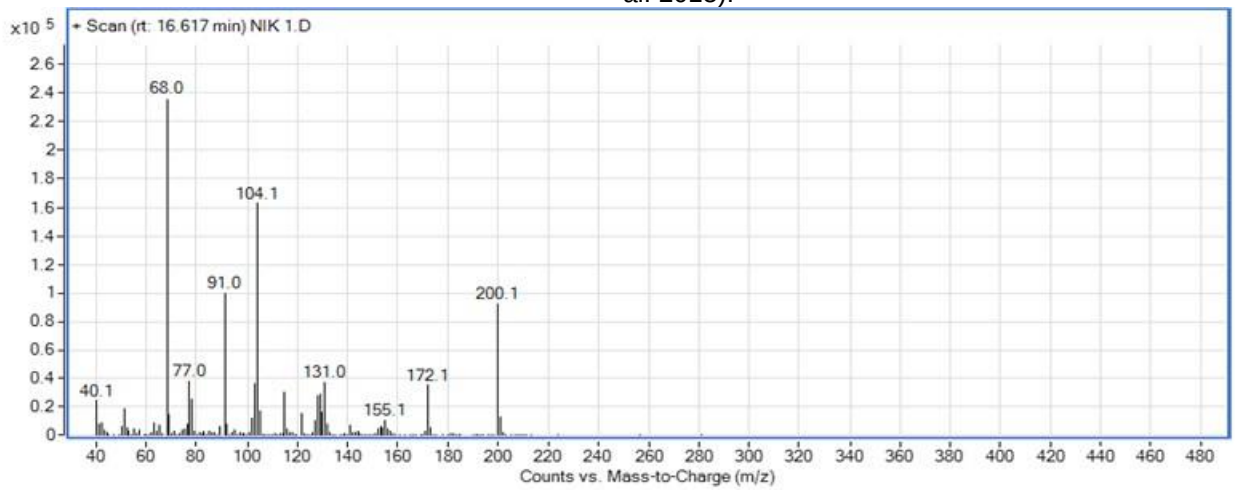

Figure 5: Mass spectrum of goniothalamin (GTN).

Table 3: 1H-NMR spectrum assignment (400 MHz) data of goniothalamin ($CDCl_3$, δ in ppm, *J* in Hz)

#H	δH	δH of Reference*
3	6.09 (dd, J = 9.8, 1.1 Hz)	6.09 (tt, *J*=10.8, 1.2 Hz)
4	7.34 (t, J = 7.3 Hz, 4H)	6.92 (ddd, J=4.0; 5.0; 9.5 Hz)
5 6	2.45 – 2.69 (m) 5.19 (m)	2.53 (m) 5.10 (1H, ddd, J=1.0; 6.5; 15.5 Hz)
7	6.41 (dd, J = 16.0, 6.7 Hz)	6.28 (dd, J=15.9, 6.6 Hz)
8	6.78 (d, J = 16.0 Hz)	6.73(d, J=15.6 Hz)
11,13	7.34 (ddd, J = 9.6, 5.6, 2.9 Hz)	7.19–7.34 (2H, m)
10, 14	7.50 (d, J = 7.3 Hz)	7.40 (d, J=7.8 Hz)
12	7.18 (ddd, J = 9.6, 5.6, 2.9 Hz, 16H)	7.27 (m, J=7.8 Hz)

*Kabir et al. (2003), Chang et al. (2005), Kim et al. (2013), Jusoh et al. (2015) and Duc et al. (2016).

The 1H-NMR spectrum showed CH_2 signal at δ2.53 (2H, m, H-5) while CH signal could be detected at 2.64 (m). Meanwhile, benzene rings were detected at δ7.27 (m, J=7.8 Hz) and δ7.40 (d, J=7.8 Hz). The spectrum indicated upfield signals at δ2.58, 2.69 and 2.71 which were assigned to H-1, H-4α and H-5. The downfield signals consisted of δ6.09, 6.41, 6.78, 7.18, 7.37 and 7.50 were assigned to H-6, H-3, H-7, H-8, H-12, H-11, H-13, H-10 and H-14. H-7 and H-8 are in trans-configuration forms, with a *J* coupling value of 16.0 Hz (Kim et al. 2013). The *J* coupling value for H-7 and H-8 is the same as the *J*-coupling value from previous study (Jusoh et al. 2015). Thus, this compound was characterized as Goniothalamin. It could be yielded at 41.6% of total hexane extract.

CONCLUSION

In conclusion, the findings recorded optimized quantitative methods to perform quality control of extraction and detection of goniothalamin using HPTLC and HPLC. This optimization was required for systematic researches and development of goniothalamin as a potential drug as it has already been proven to be a potential anti-leukemic agent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This project was funded by the Ministry of Higher Education, Malaysia under Fundamental Research Grant Scheme (FRGS/1/2018/STG04/UNISZA/02). The authors would like to thank Prof. Emeritus Dato' Dr. Laily Din from the Department of Chemistry, Universiti Kebangsaan Malaysia for kindly providing the goniothalamin standard. The authors also thank Prof. Dr. Khozirah Shaari and Prof. Dr. Abdul Manaf Ali for their guidance and advice.

AUTHOR CONTRIBUTIONS

NHI, SMY and ZMR wrote the manuscript. ZMR and ISI supervised and designed the study and provided all the materials. NHI and SMY performed the experiments, and NAAN assists the experiment. All authors approved the final version.

REFERENCES

Ali, A. M. et al. 1997. “Cytotoxicity and Electron Microscopy of Cell Death Induced by Goniothalamin.” *Planta Medica* 63(1): 81–83.

Ali, A. M., Mothanna, A. Q., Rosli, R., & Swee-Keong, Y. 2011. “Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells”. *Molecules*.

Alquadeib, Bushra Tuwfeeq. 2019. “Development and Validation of a New HPLC Analytical Method for the Determination of Diclofenac in Tablets.” *Saudi Pharmaceutical Journal* 27(1): 66–70. https://doi.org/10.1016/j.jsps.2018.07.020.

Chien, Alvin Lee Teck, and Azimahtol Hawariah Lope Pihie. 2003. “Styrylpyrone Derivative Induces Apoptosis through the Up-Regulation of Bax in the Human Breast Cancer Cell Line MCF-7.” *Journal of Biochemistry and Molecular Biology* 36(3): 269–74. https://pubmed.ncbi.nlm.nih.gov/12787481/ (March 15, 2021).

Duc Loi Vu, Thanh Tung Boi, Thanh Hai Nguyen andTienVung Nguyen. 2016. "Chemical constituents and cytotoxic effect from the barks of Goniothalamus chinensis Merr. & Chun. growing in Vietnam" Journal of Applied Pharmaceutical Science Vol. 6(04): 001-005. (April, 2016).

F. R. Chang, Y. H. Lan, C. C. Liaw, C.C. Wu, M. Y. Chiang and Y. C. Wu. (2005). "Digoniodiol, Deoxygoniopypyrone A, and Goniofupyrone A: A Three New Styrllactones from Goniothalamus amuyon" *Planta Medica* 71: 152-159.

Huber, L. (1999). Validation of HPLC methods. *BioPharm*, *12*(3), 64-66.

Humeirah, A. S., Azah, M. N., Mastura, M., Mailina, J., Saiful, J. A., Muhajir, H., & Puad, A. M. (2010). Chemical constituents and antimicrobial activity of Goniothalamus macrophyllus (Annonaceae) from Pasoh Forest Reserve, Malaysia. *African Journal of Biotechnology*, *9*(34).

Inayat-Hussain, S. H. et al. 2010. “Goniothalamin-Induced Oxidative Stress, DNA Damage and

Apoptosis via Caspase-2 Independent and Bcl-2 Independent Pathways in Jurkat T-Cells." *Toxicology Letters* 193(1): 108–14. http://dx.doi.org/10.1016/j.toxlet.2009.12.010.

Inayat-Hussain, Salmaan H et al. 1999. "Caspases-3 and -7 Are Activated in Goniothalamin-Induced Apoptosis in Human Jurkat T-Cells." *FEBS Letters* 456(3): 379–83.

Jain, Chitra, Shivani Khatana, and Rekha Vijayvergia. 2019. "Bioactivity of Secondary Metabolites of Various Plants: A Review." *International Journal of Pharmaceutical Sciences and Research* 10(2): 494–504.

Jusoh, Samsiah, Zuriati Zakaria, Fasihuddin Ahmad, and Laily Din. 2015. 44 Sains Malaysiana *Isolation and Characterization of Styryllactone of Goniothalamus Ridleyi*.

K. E. Kabir, A. R. Khan and M. A. Mosaddik. 2003."Goniothalamin – a potent mosquito larvicide from Bryonopsis laciniosa L." J. Appl. Ent. 127: 112-115.

Kim, Rosalind Pei Theng et al. 2013. "Cytotoxic and Antioxidant Compounds from the Stem Bark of Goniothalamus Tapisoides Mat Salleh." *Molecules* 18: 128–39. www.mdpi.com/journal/moleculesArticle (April 15, 2021).

Li, L. K., Rola, A. S., Kaid, F. A., Ali, A. M., & Alabsi, A. M. (2016). Goniothalamin induces cell cycle arrest and apoptosis in H400 human oral squamous cell carcinoma: A caspase-dependent mitochondrial-mediated pathway with downregulation of NF-κβ. *Archives of oral biology*, *64*, 28-38.

Mandal, S. C., Mandal, V., & Das, A. K. (2015). Profiling crude extracts for rapid identification of bioactive compounds. In: Essentials of botanical extraction: Principles and applications. *Academic press, Elsevier Inc*. 188-201.

Pilli, Ronaldo Aloise, Ian de Toledo, Matheus Andrade Meirelles, and Thiago Augusto Grigolo. 2018. "Goniothalamin-Related Styryl Lactones: Isolation, Synthesis, Biological Activity and Mode of Action." *Current Medicinal Chemistry* 26(41): 7372–7451.

Rajab, Nor Fadilah et al. 2005. "Evaluation of the Cytotoxic and Genotoxic Effects of Goniothalamin in Leukemic Cell Lines." *Environmental Mutagen Research* 27(3): 161–64.

Rashid, Zalilawati Mat, Natasha Aziz, and Nashriyah Mat. 2018. 7 International Journal of Engineering & Technology *Drying and Extraction Methods Effect on Biochemical and An-Tioxidant Capacity of Malaysian Wild Edible Vegetables*. www.sciencepubco.com/index.php/IJET (March 16, 2021).

Rasol, N. E., Ahmad, F. B., Mai, C. W., Bihud, N. V., Abdullah, F., Awang, K., & Ismail, N. H. (2018). Styryl lactones from roots and barks goniothalamus lanceolatus. *Natural Product Communications*, *13*(12),1934578X1801301 203.

Seyed, Mohamed Ali, Ibrahim Jantan, and Syed Nasir Abbas Bukhari. 2014. "Emerging Anticancer Potentials of Goniothalamin and Its Molecular Mechanisms." *BioMed Research International* 2014.

Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, *987*(1-2), 57-66.

Suresh, R., Sree, V. J., Manavalan, R., & Vallippan, K. (2010). Aspect of validation in HPLC method development for pharmaceutical analysis-comparison of validation requirements by FDA, USP And ICH. Inter. *J. Pharm. Science*, *1*(12), 123-132.

Takemura, T., Kamo, T., Ismil, R., Bakar, B., Wasano, N., Hiradate, S., & Fujii, Y. (2012). Plant growth inhibitor from the Malaysian medicinal plant Goniothalamus andersonii and related species. *Natural product communications*, *7*(9), 1934578X1200700924.

Visht, S., & Chaturvedi, S. (2012). Isolation of natural products. *Journal of Current Pharma Research*, *2*(3), 584.

Wasano, N., Takemura, T., Ismil, R., Bakar, B., & Fujii, Y. (2015). Transcriptomic evaluation of plant growth inhibitory activity of Goniothalamin from the Malaysian medicinal plant Goniothalamus andersonii. *Natural product communications*, *10*(5), 1934578X1501000507.

Zhuang, B., Ramanauskaite, G., Koa, Z. Y., & Wang, Z. G. (2021). Like dissolves like: A first-principles theory for predicting liquid miscibility and mixture dielectric constant. *Science Advances*, *7*(7), eabe7275.

Zohdi, R. M., Mukhtar, S. M., Bihud, N. V., Rasol, N. E., Ahmad, F. B., Awang, K., & Ismail, N. H. (2017). In vivo antiplasmodial and toxicological effects of Goniothalamus lanceolatus crude extracts. *Natural Product Communications*, *12*(8), 1934578X1701200826.