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ORIGINAL RESEARCH PAPER

Straightforward thin-layer chromatography–densitometric method for the determination of phyllanthin in *Phyllanthus niruri* from different phytogeographical zones

Kartini Kartini[1] · Alfi Syahr Wijayati[1] · Nikmatul Ikhrom Eka Jayani[1] · Finna Setiawan[1] · Ryanto Budiono[2]

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Abstract
Phyllanthus niruri contains various lignan compounds, whose concentrations vary depending on several factors. This study was intended to determine the phyllanthin content of *P. niruri* obtained from various locations in Indonesia by using thin-layer chromatography (TLC)−densitometry to evaluate the effect of geographical factors on their quality. The TLC system comprised silica gel 60 F_{254} for the stationary phase, toluene−ethyl acetate−formic acid (15:10.5:1.5, *V/V*) for the mobile phase, and documentation under ultraviolet (UV) 254 nm light without chemical reagents. This developed method meets the specificity requirement, as marked by the identical UV spectrum between the phyllanthin sample and the standard (λ_{max} = 279 and 230 nm). Further, it shows good linearity for phyllanthin concentrations in the range of 2.36−11.8 µg/band (r = 0.9924), with LOD 0.532 µg/band and LOQ 1.612 µg/band. It also has good intraday and interday precision, as indicated by RSD of 8.87–9.43 and 6.94%, respectively. Eight of the 15 analyzed samples (collected from Batu, Blitar, Kediri, Nganjuk, Jember, Mojokerto, Banyuwangi, and Surabaya) contained only a trace amount of phyllanthin. In contrast, the other seven had varying levels of phyllanthin (1.376−4.130 mg/g dried herbs). Using the Tawangmangu sample as the reference, these seven samples can be grouped into two: significantly lower phyllanthin contents (Tulungagung) and very significantly lower phyllanthin contents (Lumajang, Bangkalan, Pasuruan, Sidoarjo, and Gresik). It can be concluded that TLC−densitometry designed in this research is a straightforward method that, at the same time, meets the validation parameters. Therefore, it can be repeated to analyze phyllanthin in *P. niruri* of different phytogeographical origins.

Keywords *Meniran* · *Phyllanthus niruri* · Phyllanthin · Phytogeographical origins · Thin-layer chromatography−densitometry

1 Introduction

Phyllanthus niruri L. (gale of the wind or *meniran* in Indonesia) is commonly found in the tropics, including Southeast Asia, South India, and China [1, 2]. In Indonesia, this plant is a major component of *jamu*, Indonesian traditional herbal drinks, to alleviate gout, arthritis, high blood pressure, hemorrhoids, high cholesterol, and urinary tract stones, and for physical fitness. Different studies revealed the activities of *P. niruri* extract and its chemical compounds as antidiabetic, hypolipidemic, cardioprotective, antivirus, antibacterial, hepatoprotective, and wound healing [3–5]. Also, various clinical trials showed *P. niruri* as an immunomodulator by activating and augmenting the cellular immune system, particularly neutrophils, macrophages or monocytes, and T as well as B lymphocytes [6].

Aside from being safe and efficacious, herbal products should meet quality requirements. For this reason, the crude drugs used as the ingredients are standardized. Several determining factors for crude drug quality include the environment, soil type, and climate of where the source plant grows, seed quality, age at harvest, and postharvest handling (*e.g.,* drying) and storage [7]. The quality of a crude drug can be evaluated from its chemical fingerprint and marker compound using several methods, one of which is thin-layer chromatography (TLC)−densitometry.

✉ Kartini Kartini
kartini@staff.ubaya.ac.id

[1] Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia

[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia

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In previous research, chemical fingerprinting based on TLC effectively discriminated *P. niruri* crude drugs collected from 15 locations in Indonesia [8]. Also, Fourier transform infrared (FTIR)−fingerprinting analysis was used to control the quality based on the spectral profile of each sample and to distinguish *P. niruri* crude drugs from several locations [9]. However, both methods have limitations because they are qualitative. Therefore, complementary quantitative analyses that measure marker compound levels like TLC−densitometry are needed. Even though TLC has been used for a long time, more than 50% of traditional Chinese medicine (TCM) still use this fairly old quality control method today [10]. Besides, TLC has several advantages: ease of use and relatively low operating cost [11]. In the Indonesian Herbal Pharmacopeia II, TLC is still also used in addition to ultraviolet−visible (UV−Vis) spectrophotometry to standardize crude drugs and extracts [12].

A marker compound is a chemical constituent that may contribute to the therapeutic activity of a herbal ingredient. The concentration of a marker compound in plants is one of the factors considered in its selection [13, 14]. Phyllanthin is a major lignan of *P. niruri* with several known pharmacological activities [1, 15]. This research aimed to determine the levels of phyllanthin in *P. niruri* obtained from various locations in Indonesia using TLC−densitometry as a part of evaluating the effect of phytogeographical factors on crude drug quality.

2 Experimental

2.1 Chemicals and plant samples

Phyllanthin standards were acquired from Sigma Aldrich Co. (St. Louis, MO, USA), and precoated silica gel 60 F_{254} plates sized 20 × 20 cm and analytical-grade solvents (methanol, toluene, ethyl acetate, formic acid, *n*-hexane, acetone, chloroform, and dichloromethane) were from Merck KGaA (Darmstadt, Germany). *Phyllanthus niruri* samples were collected from June 2019 to September 2020 from 15 locations in Indonesia. *P. niruri* plants were manually uprooted, and the whole plant was used, except the root. These herbs were washed with running water, drained, and air-dried under indirect sun light at room temperature (31 ± 2 °C). The dried herbs were put into a blender, ground into powder, and then passed through a 45-mesh sieve. The moisture content was then analyzed using moisture analyzer Ohaus MB 120 (Parsippany, NJ, USA). The phytogeographical details of the sample locations are presented in Table 1. All samples were authenticated by the Center for Information and Development of Traditional Medicines (PIPOT), University of Surabaya, Indonesia.

Table 1 Phytogeographical profiles of the *Phyllanthus niruri* samples

No.	Location	Latitude, longitude	Elevation (meters above sea level)	MC (%)*
1	Tawangmangu	7° 42′ S; 111° 08′ E	1200	6.72 ± 0.38
2	Batu	7° 52′ S; 112° 31′ E	831	5.72 ± 0.35
3	Blitar	8° 05′ S; 112° 09′ E	167	6.74 ± 0.60
4	Tulungagung	8° 03′ S; 111° 54′ E	85	5.02 ± 0.43
5	Jember	8° 11′ S; 113° 40′ E	83	5.71 ± 0.18
6	Kediri	7° 50′ S; 112° 01′ E	60	6.93 ± 0.53
7	Nganjuk	7° 36′ S; 111° 53′ E	56	6.90 ± 0.13
8	Lumajang	8° 50′ S; 113° 14′ E	51	5.84 ± 0.55
9	Bangkalan	7° 01′ S; 112° 45′ E	47	6.39 ± 0.32
10	Mojokerto	7° 28′ S; 112° 26′ E	30	6.69 ± 0.33
11	Banyuwangi	8° 13′ S; 114° 22′ E	25	5.25 ± 0.18
12	Pasuruan	7° 38′ S; 112° 53′ E	5	6.73 ± 0.08
13	Sidoarjo	7° 28′ S; 112° 40′ E	3	5.23 ± 0.44
14	Gresik	7° 09′ S; 112° 39′ E	3	5.52 ± 0.19
15	Surabaya	7° 15′ S; 112° 45′ E	2	6.64 ± 0.11

*Mean ± SD ($n = 3$)

2.2 Extraction

The *P. niruri* extract was prepared following the technique described in a previous study, with slight modifications [8]. One gram of the herb sample was extracted with absolute methanol (7 mL) using ultrasound-assisted extraction (UAE) for 15 min at room temperature. UAE was carried out using an ultrasonic cleaner, Branson 1510 (Brookfield, CT, USA) with an ultrasonic power (P) of 150 W at 42 kHz. The derived extract was filtered into a 10-mL volumetric flask; then, the remaining residue was re-extracted with methanol and filtered into the same volumetric flask. Afterward, methanol was added up to the mark.

2.3 TLC system

The phyllanthin standard and *P. niruri* extract were each applied onto a TLC silica gel 60 F_{254} plate (Merck KGaA) as 6-mm bands with a 100-µL Hamilton sample syringe (Bonaduz, Switzerland). TLC plate is more cost-effective than HPTLC plate. However, in some cases its performance is comparable to HPTLC plate [16]. Spotting was carried out automatically in a stream of N_2 gas with Linomat 5 TLC applicator (CAMAG, Muttenz, Switzerland). Afterward, ascending development of the TLC plate was conducted with an elution distance of 80 mm in a twin-trough chamber (CAMAG) presaturated with the mobile phase for 30 min. Three different mobile phases from previous researches [17–20] were tested: toluene−ethyl acetate−formic acid (15:10.5:1.5, *V/V*), *n*-hexane−acetone−ethyl acetate (7:2:1, *V/V*), and chloroform−dichloromethane (9:1, *V/V*). Separated spots on the plate were documented under UV light (254 and 366 nm) using UV Cabinet 4 (CAMAG) without derivatization reagents. The TLC plate was later scanned with a TLC Scanner 4 (CAMAG) at a speed of 100 nm/s with a slit width of 4 mm × 0.3 mm and data step resolution of 1 nm. The resulting densitogram was analyzed in the winCATS program (CAMAG).

2.4 Standard solution preparation

In this step, 1.18 mg of the phyllanthin standard was dissolved in 1.0 mL of methanol to obtain a stock solution with a concentration of 1180 µg/mL. Then, a standard curve was made by spotting different volumes of the stock solution onto the plate.

2.5 Analytical method validation

2.5.1 Specificity analysis

Phyllanthin standard solution (8 µL) and *P. niruri* extract (8 µL) were applied to the TLC plate and then eluted with the optimized mobile phase. Phyllanthin bands of the standard and the samples were then scanned at 200–400 nm wavelengths using a densitometer. The specificity of the method designed in the current study was determined by comparing the R_F values, spectral profiles, and λ_{max} of the phyllanthin standard and the samples. The sample's phyllanthin band was further tested for purity by reading the UV spectrum at the starting point, apex, and end point of the peak purity [21].

2.5.2 Determination of linearity, limit of detection (LOD), and limit of quantification (LOQ)

The phyllanthin standard solution was spotted on five different tracks in different volumes: 2, 4, 6, 8, and 10 µL. Afterward, the TLC plate was eluted, and the phyllanthin area was measured with a TLC scanner at its λ_{max}, which had been identified in the specificity assessment ($\lambda_{max} = 279$ nm). A standard curve ($y = bx + a$) was formed using the linear regression of the mass of phyllanthin per band (µg/band) on the *x*-axis *versus* the area on the *y*-axis. The method's linearity was determined from the correlation coefficient (*r*) of the equation. Linearity testing was also carried out by plotting the residuals, *i.e.,* the distances of the experimentally determined points from the regression line against the quantities of the phyllanthin applied [22]. LOD and LOQ were each calculated as $3.3(SD/\bar{x})$ and $10(SD/\bar{x})$, where SD is the standard deviation of the intercept and $\bar{x}$ denotes the mean slope of the standard curve equation [23].

2.5.3 Precision study

To determine intraday precision, 8 µL of the *P. niruri* extract was spotted on a TLC plate in six replicates. The same extract was also applied onto three plates on three consecutive days to determine interday precision. Afterward, each plate was eluted and analyzed using TLC–densitometry in the designed condition to measure the area of phyllanthin. The relative standard deviation (%RSD) values of the areas from the plate with six replicates and the three plates made on different days represent intraday and interday precision, respectively [21].

2.6 Determination of phyllanthin level and data analysis

The presence of phyllanthin in *P. niruri* plants collected from 15 phytogeographically different locations was determined using the validated TLC–densitometry system and expressed in mg/g dried herbs. The extract samples were spotted in different volumes (15, 20, or 30 µL) depending on the phyllanthin level of each sample. Afterward, the identified phyllanthin concentrations of all *P. niruri* samples were analyzed with one-way analysis of variance (ANOVA) ($\alpha = 0.05$) and then compared with that of the crude drug reference, *i.e.,* the Tawangmangu sample, using Tukey's test. These analyses were performed with the help of GraphPad Prism Version 5.01 (GraphPad Software Inc., San Diego, CA, USA).

3 Results and discussion

Several previous studies have developed a TLC method for the analysis of phyllanthin in the genus *Phyllanthus* either single or simultaneously with other lignan compounds [24–26]. However, the plates used are mostly high-performance thin-layer chromatography (HPTLC) plates, which are twice as expensive as TLC plates. This will certainly

be an obstacle for several traditional medicine industries in low to middle economic level countries. In addition, these studies generally use *Phyllanthus amarus*. While *P. niruri* is an endemic *Phyllanthus* species found in Southeast Asian countries including Indonesia, research analyzing phyllanthin content in this species collected from various locations is still limited. In this work, a simple and cost-effective TLC method using TLC plates was developed for the quantification of phyllanthin in *P. niruri* collected from different phytogeographical zones. The developed method involves the use of TLC plates which are cost-effective compared with HPTLC plates, thus facilitating their application in the routine analysis. The method developed was then validated and used for sample quantification.

3.1 Optimal mobile phase

Phyllanthin is a poorly water-soluble lignan; therefore, in this study *P. niruri* was extracted using absolute methanol. The addition of water to aqueous methanol may decrease the solubility of phyllanthin. Previous scholars have used methanol to extract phyllanthin from *Phyllanthus* species [15, 25, 27–31]. Of the three mobile phases tested for the TLC system, the ratio of toluene, ethyl acetate, and formic acid at 15:10.5:1.5 (*V/V*) produced the best visual separation results (Fig. 1). With this combination, phyllanthin and other compounds in *P. niruri* samples were completely separated. Therefore, this optimized mobile phase was selected for method validation.

3.2 Specificity

Phyllanthus niruri contains numerous compounds with varying physicochemical properties. Two or more different chemical compounds can have the same solubility characteristic in a mobile phase as well as the same adsorption strength onto stationary phase and thus appear as one band on the TLC plate after elution. *Phyllanthus niruri* is composed of several lignans, including phyllanthin, hypophyllanthin, niranthin, lintetralin, phyltetralin, nirtetralin, isolintetralin, 2,3-desmethoxy seco-isolintetralin, 2,3-desmethoxy seco-isolintetralin diacetate, cubebin dimethyl ether, and urinatetralin [1]. These compounds have a common chemical structure, which is responsible for their similar solubility. Therefore, specificity was determined to ensure that the eluted band is of the one compound targeted by the designed analytical method. To evaluate this, first, the color and R_F value of the bands formed by the phyllanthin standard and samples were compared. Second, their UV spectra (200–400 nm) were observed to see any shared similarity.

The TLC chromatogram (Fig. 2) shows suspected phyllanthin on the bands in sample tracks b and c. These bands, parallel to the phyllanthin standard (track a), quenched the fluorescence illuminated by UV 254 nm light. In addition, the phyllanthin standard and the extract samples showed the same spectral pattern: two peaks at λ_{max} 279 nm and 230 nm (Fig. 3). This is consistent with the spectral profile of phyllanthin, which comprises two distinct peaks in the wavelength range of 200–300 nm [17, 32]. Because the absorbance was higher at 279 nm than 230 nm, for the next stage, the area of phyllanthin was measured at 279 nm,

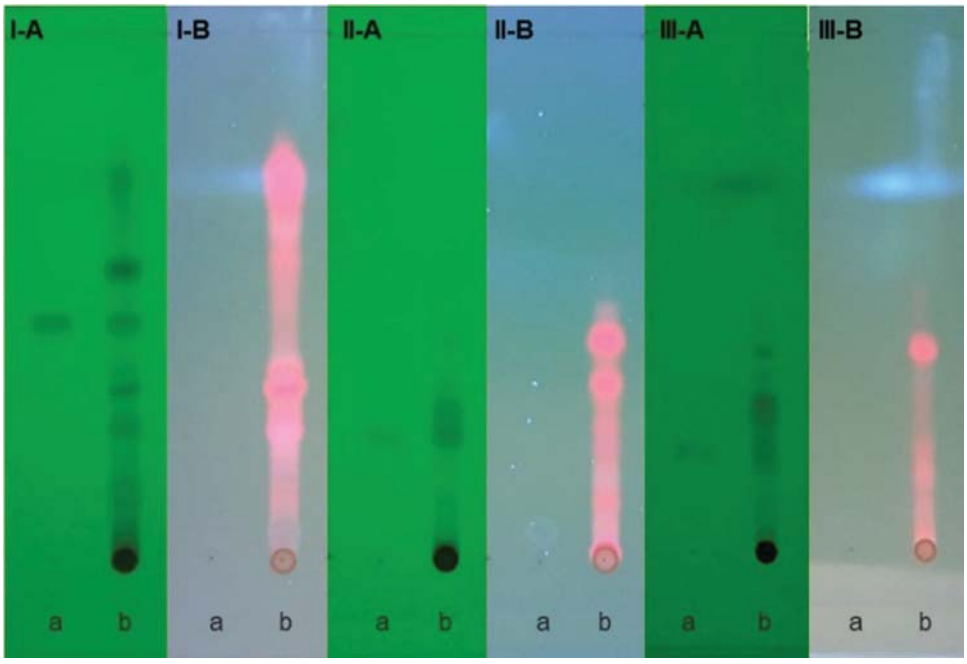

Fig. 1 TLC chromatograms of the phyllanthin standard (**A**) and *Phyllanthus niruri* extract (**B**) with the mobile phase: toluene−ethyl acetate−formic acid (15:10.5:1.5, *V/V*) (I), *n*-hexane−acetone−ethyl acetate (7:2:1, *V/V*) (II), and chloroform−dichloromethane (9:1, *V/V*) (III), observed under UV light at 254 nm (**A**) and 366 nm (**B**)

Fig. 2 TLC chromatogram of the phyllanthin standard (**a**) and *Phyllanthus niruri* extracts (**b**, **c**) under UV 254 nm light

which also corresponds to previous studies that quantified phyllanthin at 282 nm [30, 32]. Other scholars used visible wavelength at 580 nm because the phyllanthin's TLC chromatogram was developed with a staining reagent of 5% concentrated sulfuric acid in ethanol [31].

Band similarity between the phyllanthin standard and samples is also confirmed by the R_F values. Densitograms show that both had the same R_F value of 0.67 (Fig. 4). The peak purity of the sample's phyllanthin (Table 2) is shown by the values of r (s, m) and r (m, e), which were both $0.999 > 0.99$ [23]. These results further confirm that the suspected band of phyllanthin from the *P. niruri* sample is pure phyllanthin, not mixed with other compounds. From these data, it can be inferred that the TLC method designed in this study for determining the phyllanthin levels in *P. niruri* has good specificity.

3.3 Linearity, limit of detection, and limit of quantification

Linearity was defined from the correlation coefficient (r) of the linear regression equation of the standard curve made by spotting ($n = 3$) different concentrations of phyllanthin standard (1.180 µg/µL): 2, 4, 6, 8, and 10 µL or equivalent to 2.36–11.8 µg/band. The TLC chromatogram and 2D densitogram ($\lambda = 279$ nm) of the phyllanthin standard solution (Fig. 5) show that the higher the mass of phyllanthin, the stronger the fluorescence quenching upon its exposure to UV 254 nm light and the wider the peak area. The linear relationship between the mass of phyllanthin per band and the measured area can be determined from the obtained r value, 0.9924 (Fig. 6A). Moreover, Fig. 6B shows that the residuals are randomly distributed around the regression function and do not show any tendency. This indicated that the calibration graph is linear in the tested range [22]. It can

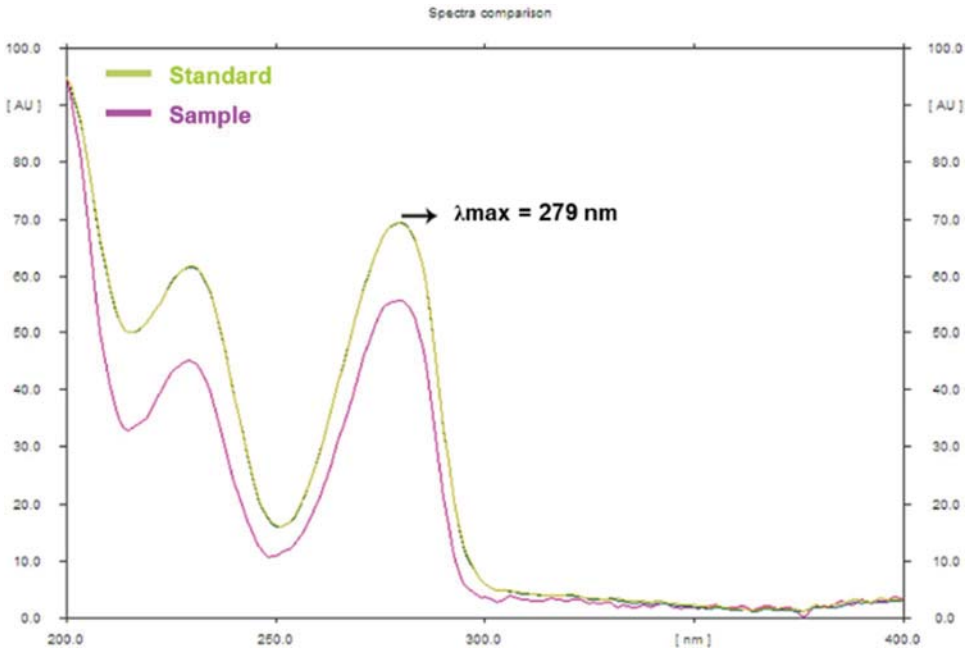

Fig. 3 Overlay between the UV spectra of phyllanthin in the standard and *Phyllanthus niruri* extract samples

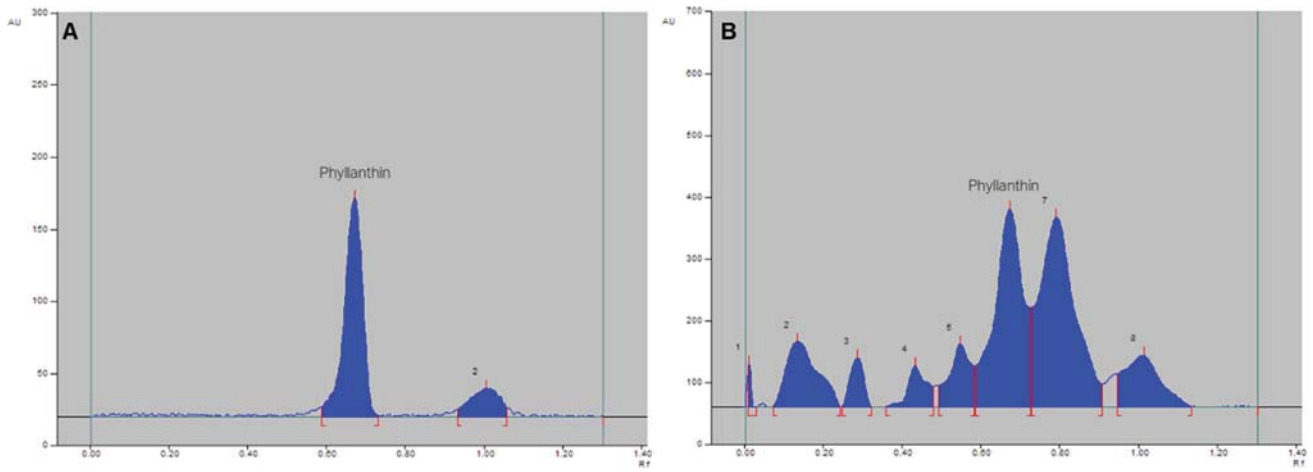

Fig. 4 Densitograms of phyllanthin in the standard (**A**) and the *Phyllanthus niruri* extract (**B**) ($\lambda = 279$ nm)

Table 2 R_F values and peak purity of the phyllanthin standard and sample

Track	Identity	Assigned substance	R_F	r (s, m)	r (m, e)
1	Standard	Phyllanthin	0.67	0.999	0.999
2	Sample	Phyllanthin	0.67	0.999	0.999

be concluded that the method developed in this study has good linearity for phyllanthin concentrations in the range of 2.36–11.8 µg/band [30].

The LOD and LOQ of the method were calculated from the standard curve: 0.532 µg/band and 1.612 µg/band. These figures are close to the LOD (0.48 µg/spot) and LOQ (1.49 µg/spot) of the TLC–densitometric analysis used to determine the concentrations of phyllanthin in *P. amarus* crude drugs and preparations [30]. In contrast, HPTLC for the determination of phyllanthin with the staining reagent of 5% concentrated sulfuric acid in ethanol has smaller LOD and LOQ, 0.18 and 0.59 µg/spot [31]. This shows that the method developed in the current study is more sensitive. LOD and LOQ are defined as the smallest amount of analyte that can be detected and quantified, respectively. One thing that distinguishes HPTLC from TLC is the use of a stationary phase with a smaller particle size and narrower particle size distribution that provides better separation abilities,

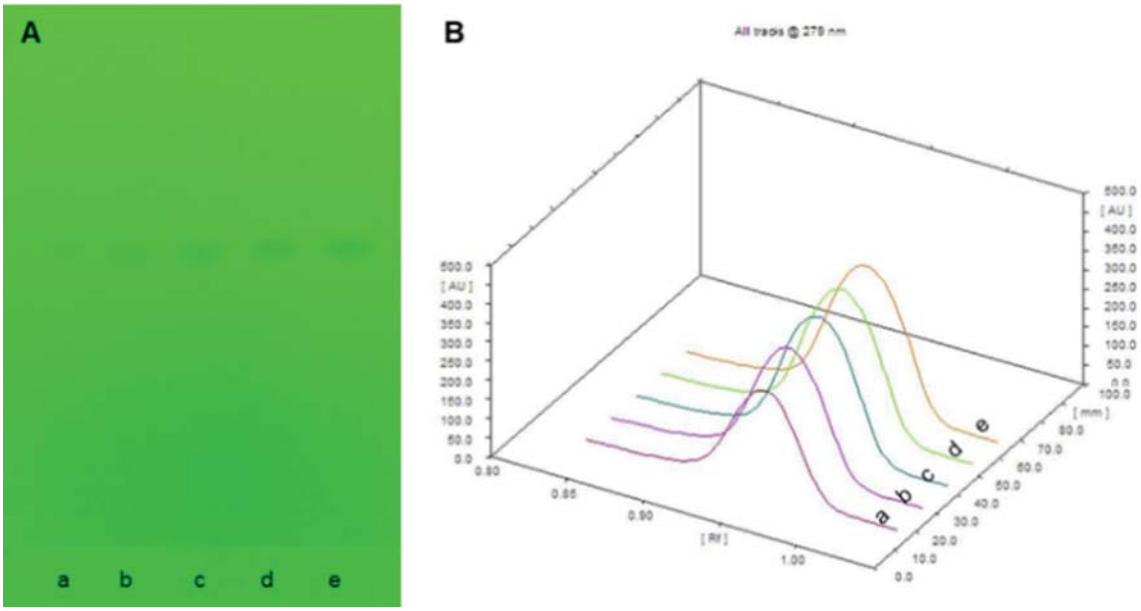

Fig. 5 TLC chromatogram (**A**) and 2D densitogram (**B**) of the phyllanthin standard

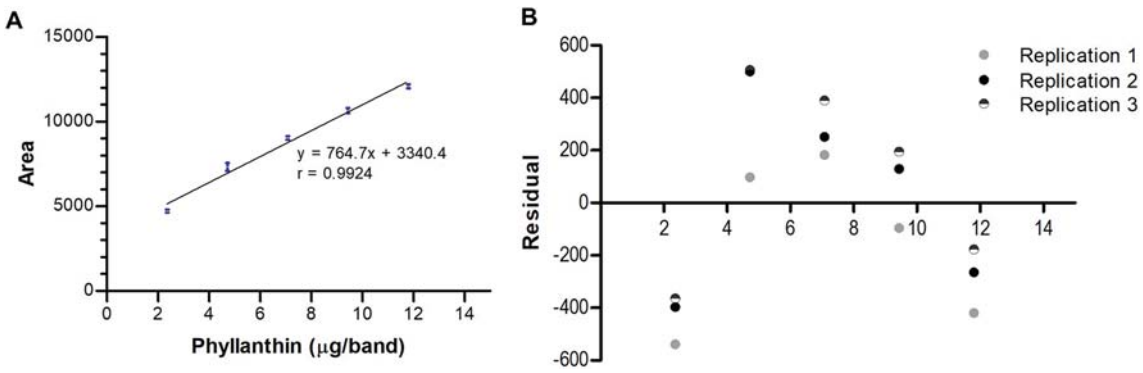

Fig. 6 Linear regression curve linking phyllanthin level (µg/band) to area (AU) (**A**) and plotting residual *versus* phyllanthin level (µg/band) (**B**)

but HPTLC plates cost more than TLC plates [11, 33, 34]. Results indicated that the developed TLC method is sufficiently sensitive for analyzing phyllanthin levels in *P. niruri*.

3.4 Precision

Precision is defined as the closeness between the results of multiple sample replications and random errors of a method. The errors may come from sampling, weighing, sample preparation, dilution, sample application, development, detection, evaluation, and calculation [22]. One of the TLC chromatograms obtained from intraday and interday precision testing in this study is shown in Fig. 7, and the areas of the phyllanthin read from the chromatogram are presented in Table 3. Data in Table 3 indicate that the TLC–densitometry designed to determine the phyllanthin levels in *P. niruri* has good intraday and interday precision, with RSD values of 8.87–9.43 and 6.94%, respectively.

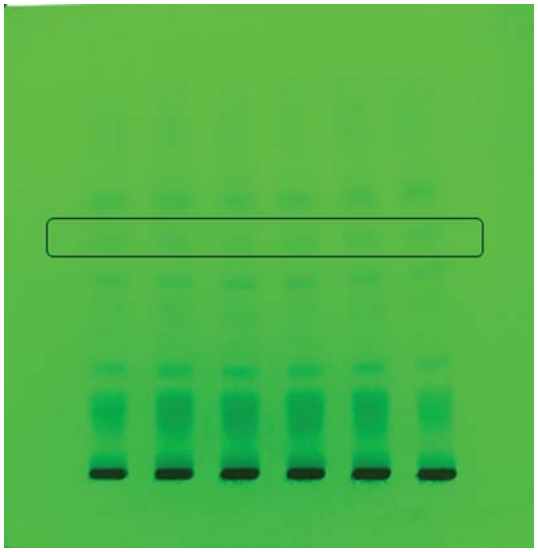

Fig. 7 TLC chromatogram from the precision study, detected under UV 254 nm light

3.5 Phyllanthin levels in *P. niruri* from locations with different phytogeographical profiles

To quantify the phyllanthin levels in *P. niruri* herbs, each extract sample was applied onto a TLC plate with the appropriate volume and determined using the designed and validated method. The TLC chromatograms of 15 *P. niruri* samples and their phyllanthin contents are presented in Fig. 8 and Table 4. Table 4 shows that samples from Batu (a), Blitar (b), Kediri (d), and Nganjuk (e) contained very low level of phyllanthin (below LOD and LOQ), whereas the samples from Jember, Mojokerto, Banyuwangi, and Surabaya contained low level of phyllanthin (below LOQ), but the other seven samples had varying levels of phyllanthin from 1.376 to 4.130 mg/g. The one-way ANOVA showed that the phyllanthin concentrations of the seven samples were significantly different ($p < 0.05$).

To determine the quality profiles of *P. niruri* herbs from different locations, the statistical analysis was followed

Table 3 Phyllanthin areas ($\lambda = 279$ nm) from the intraday and interday precision testing

Replicate	Area		
	Day 1	Day 2	Day 3
1	6452.96	6219.92	6159.57
2	6531.03	6414.09	5480.16
3	5778.52	6102.68	5185.18
4	5416.17	6241.72	5059.14
5	5320.55	6416.53	4971.08
6	5631.08	4913.18	4856.03
Mean ± SD	5855.05 ± 519.38	6051.35 ± 570.55	5285.19 ± 478.85
RSD (%)	8.87	9.43	9.06
Intraday precision (%RSD, $n = 6$) = 8.87–9.43			
Interday precision (%RSD, $n = 3$) = 6.94			

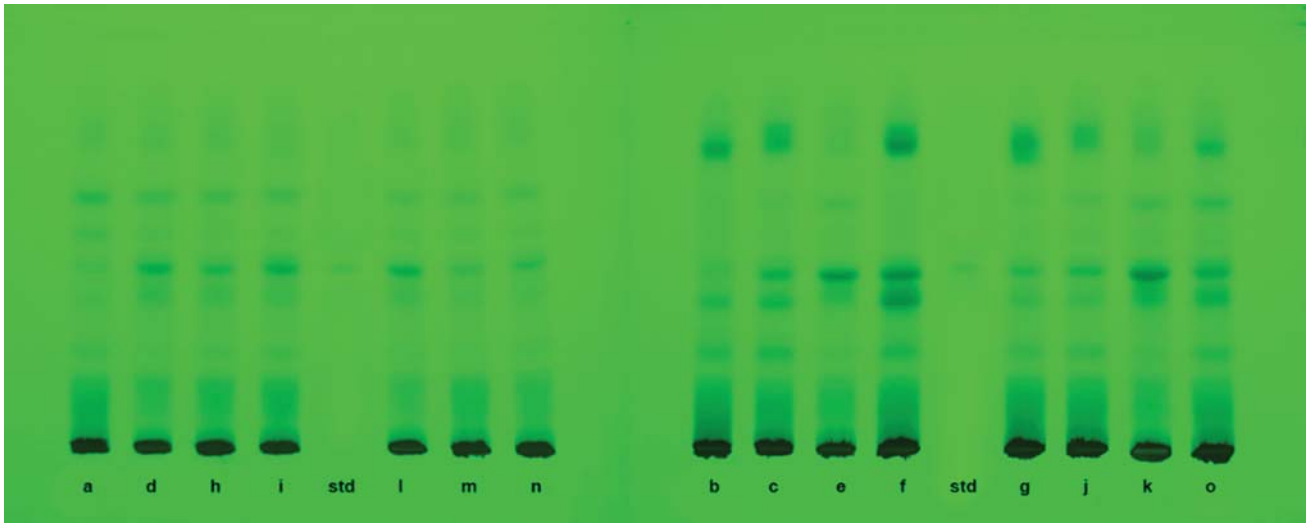

Fig. 8 TLC chromatograms of the phyllanthin standard (std) and *Phyllanthus niruri* samples from 15 locations (a−o), detected under UV 254 nm light

Table 4 Phyllanthin levels of *Phyllanthus niruri* from 15 phytogeographical locations

Sample code	Sample origin	Phyllanthin level (mg/g)
a	Batu	NA
b	Blitar	NA
c	Jember	NA*
d	Kediri	NA
e	Nganjuk	NA
f	Mojokerto	NA*
g	Banyuwangi	NA*
h	Surabaya	NA*
i	Tawangmangu	4.130 ± 0.084
j	Tulungagung	3.287 ± 0.060
k	Lumajang	2.107 ± 0.173
l	Bangkalan	2.317 ± 0.121
m	Pasuruan	2.011 ± 0.313
n	Sidoarjo	2.382 ± 0.023
o	Gresik	1.376 ± 0.107

Mean ± SD ($n = 3$), NA: undetectable (< LOD and LOQ); NA*: undetectable (< LOQ)

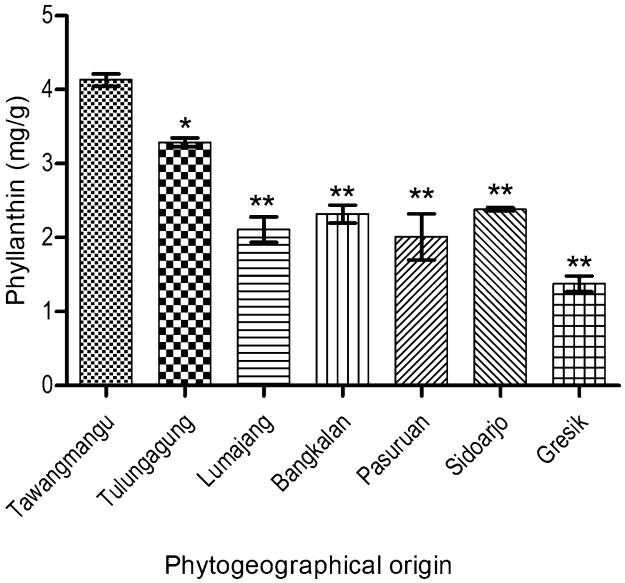

Fig. 9 Comparison between the phyllanthin contents of *Phyllanthus niruri* samples from Tawangmangu and other locations. *Significantly different ($p < 0.05$) and **significantly different ($p < 0.0001$) from the Tawangmangu sample

by Tukey's test to compare their phyllanthin contents with those of the reference crude drug, the Tawangmangu sample. Based on Tukey's analysis results (Fig. 9), samples from Tulungagung, Lumajang, Bangkalan, Pasuruan, Sidoarjo, and Gresik had significantly lower phyllanthin concentrations than the reference sample. In a previous research, TLC–fingerprinting coupled with chemometrics found that *P. niruri* herbs from the same 15 locations could be categorized into five groups. The first group consisted of samples from Tawangmangu, Batu, and Blitar; the second group from Lumajang, Jember, and Sidoarjo; the third group from Nganjuk; the fourth group from Kediri, Mojokerto, Pasuruan, Tulungagung, Banyuwangi, and Gresik; and the fifth group from Surabaya and Bangkalan [8]. This previous research shows different results from the current study because the analytical parameters used were the chemical fingerprints of *P. niruri*, which included not only phyllanthin but also other compounds present in the herb.

A previous study found that the concentration of phyllanthin and hypophyllanthin in *P. amarus*, another species of *Phyllanthus*, varied from season to season. Among the samples collected in July, August, September, and October, *P. amarus* collected in August showed the highest phyllanthin and hypophyllanthin content [24]. Results of the current study proved that the phyllanthin contents in *P. niruri* are shaped by the phytogeographical location of where the herb grows. However, further research is needed to decide whether the variation in the content is due to elevation or other contributing variables. In addition, further research is also needed to evaluate the effect of the harvest season on the phyllanthin content in *P. niruri*.

This study gives scientific evidence that, in cultivating *P. niruri*, geographical conditions need to be considered in order to produce *P. niruri* with consistent phyllanthin content (*i.e.,* standardization). In addition, different analytical methods can also be used together to provide comprehensive information on the geographical influence.

4 Conclusion

The TLC–densitometry system developed for determining phyllanthin levels in this study can be categorized as simple, but it still meets the validation parameters. Therefore, it is suitable for analyzing the phyllanthin content of *Phyllanthus niruri* herbs both qualitatively and quantitatively. This study shows variations in the phyllanthin levels of *P. niruri* herbs collected from different phytogeographical locations in Indonesia. In the next stage, research can be developed to gather data from less explored phytogeographical locations in Indonesia and other countries.

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Data availability All data generated or analyzed during this study are included in this published article. Any further data can be requested from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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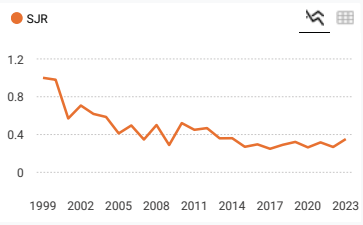

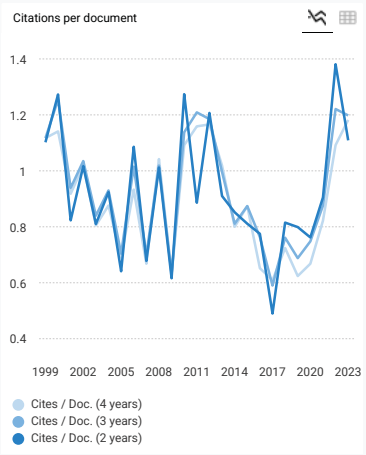

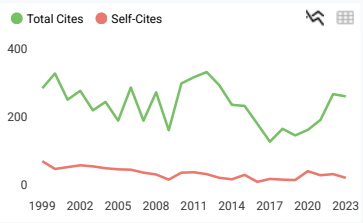

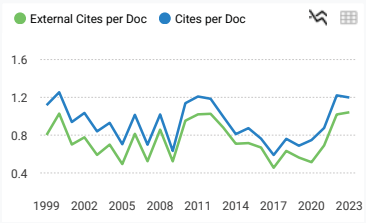

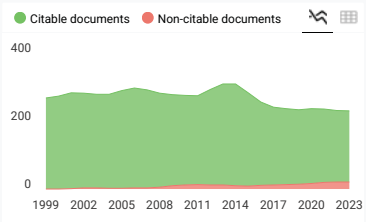

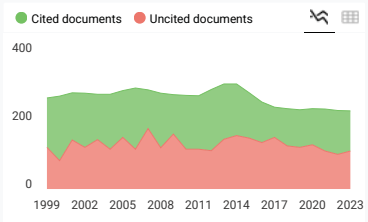

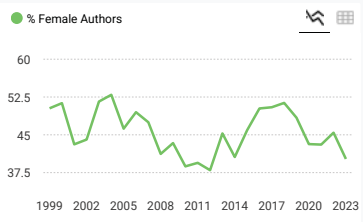

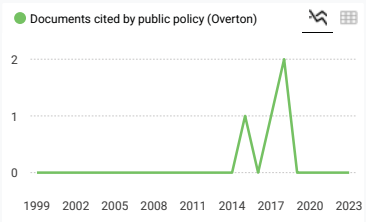

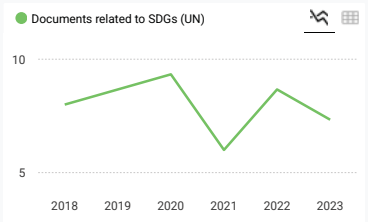

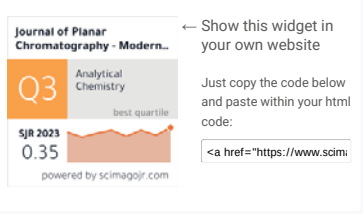

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