

Fingerprint Study of *Phyllanthus Niruri* L. By High Performance Thin Layer Chromatography (HPTLC)

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ABSTRACT

Phyllanthus niruri L. (family Euphorbiaceae) has been used as traditional medicine for centuries. It has several pharmacological properties for treating gastric lesion, urolithiasis, urinary infections and diuretics including jaundice and anti-inflammatory property. To ensure the quality and prevent counterfeiting of the products, the standardization of crude materials or extracts should be developed. Standardization can be done by comparing its chromatogram profile or fingerprinting. The research on fingerprinting of *P. niruri* L. from various regions: Solo, Bogor and Yogyakarta was conducted by High Performance Thin Layer Chromatography (HPTLC) using silica gel F₂₅₄ as a stationary phase and mix solution of chloroform : acetonitril : methanol : formic acid (60 : 30:10: 0.5) as a mobile phase. Preparation of the extracts was carried out in methanol. The extracts were eluted after spotting 10 µL each using a Linomat-5 tools, then detected by a photo documentary system and TLC-Scanner on λ 254 nm, 229 nm and 366 nm. All extracts contained phyllanthin as a specific marker of *P. niruri* corresponding with reference standar, however the chromatogram profile of extract from Bogor had a bit different from Solo and Yogyakarta. In conclusion, the chromatogram/fingerprint profile can be used for standardization of *P. niruri* L extract as well as to verify the content of *P. niruri* L based product.

Key words: *Phyllanthus niruri* L, fingerprinting, standardization, HPTLC

INTRODUCTION

Meniran (*Phyllanthus niruri* Linn.) from the family Euphorbiaceae, wild plants originating from tropical Asia, are scattered throughout mainland Asia including Indonesia. This plant is only 30-100 cm high and has leaves that are finned, even every one petiole composed of compound leaves that have a small size and elliptical (Dalimarta, 2000). Meniran is traditionally used for various medications such as nephrolithiasis, jaundice, malaria, epilepsy, fever, cough, excessive menstruation, dysentery, burns exposed to flames or hot water, scabs and sores to treat acne. Chemical constituents in the meniran herb are phyllanthin flavonoids, hipophyllanthin, potassium, resin, and tannin substances. Phyllanthin reported as therapeutic active compounds which has function as a hepatoprotective agent (Khan, 2010).

Finger print is a qualitative analysis of extracts of natural medicines by displaying such chromatogram profile based on the qualitative identification or polarity of the solvent used in High Performance Thin Layer Chromatography (HPTLC). This HPTLC is the preferred analytical technique because of some advantages including the small amount of mobile phase required, the speed of the method, and the possibility of analysis of several samples simultaneously on the same plate. It also reduces analysis time and cost per analysis. In addition, cloudy samples and suspensions can also be analyzed directly. Automatic sample application is also possible and repeated scanning can be performed on the same plate, so scanning conditions can be changed.

This study aimed to obtain a chromatogram profile (fingerprint) of meniran as the basis for standardization and quality control of herbal medicines based on meniran.

MATERIALS AND METHODS

Materials

Dry powder herbs of meniran (*P. niruri* L.) as samples were obtained from 3 (three) different locations: Bogor, Solo and Yogyakarta. It were determined in Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Science, Bogor, Indonesia. The reference standard used in this research was Phyllanthin (Sigma-Aldrich) and the reagent and consumables were chloroform, acetonitrile, methanol, formic acid and HPTLC Silica plates F₂₅₄ (Merck Co.).

Equipment

HPTLC set which consist of Linomat 5 TLC Spotter, TLC Scanner 3, TLC Documentation System Reprostar3 and Twin Trough Glass Chamber (CAMAG).

Method

Test solution: were prepared by extracting 1 g sampel each in 10 mL methanol, sonication at 60°C, then centrifuging at 4000 rpm and the supernatan was used as test solution. Standard solution: were prepared by dissolving 1 mg phyllanthin in 1 mL methanol.

Analysis: Test and standard solution were applied to an HPTLC silica gel glass plate F254 plate using Linomat 5 TLC Spotter with volume of 10 µL for test solution and 20 µL for standard solution each. The following conditions were employed: distance from bottom plate 10 mm, x-potion from 1st track 12 mm. The plate was eluted in chloroform: acetonitrile: methanol: formic acid (60:30:10:0,5). Linear ascending development was carried out in 20x10 cm twin trough glass chamber saturated with the mobile phase (20 mL back side and 10 mL front side) and filter paper at back side for 20 minutes. After drying, the plate was detected by TLC Documentary and TLC- System Scanner.

RESULTS AND DISCUSSION

Chromatogram profile or fingerprint of meniran extracts obtained from 3 different areas and analyzed using photo documentary system and TLC-Scanner can be seen in Figures 1 below.

From the figure, the profile showed that a non-polar to polar compounds indicated well separation and good intensity in the mobile phase of methanol: formic acid (60: 30: 10: 0.5 v/v. All sample extracts from 3 different regions have phyllanthin spot (*R_f* 0.55) which is the marker compound of meniran. It proved that the samples are truly meniran. Detected at λ 254 nm resulted the similar chromatogram profile for all extracts, however when detection was done at λ 366 nm, the extract from Bogor (7-12) had a bit different profile.

It showed at figure 1 ^{c)} where a strange spot (green colour) occurred at *R_f* of \pm 0.8 which was not owned by extracts from Solo and Yogyakarta. It means that the sample from Bogor had an additional compound compare to others.

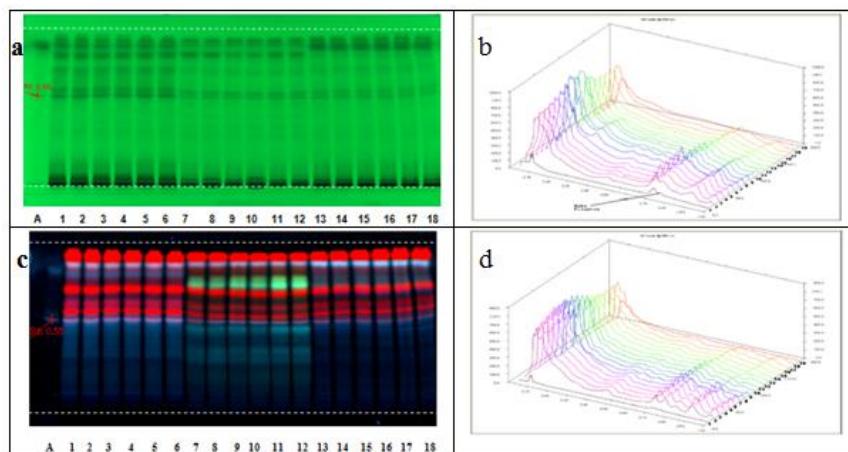


Figure 1. HPTLC Fingerprint of phyllanthin standard solution (A), meniran herbs extract from Solo (1-6), Bogor (7-12) and Yogyakarta (13-18), detected at λ 254nm^{a,b)}, at λ 366 nm^{c,d)}

This may be due to differences in soil nutrients and the weather where it grew, the land in Bogor may have more nutrients than others.

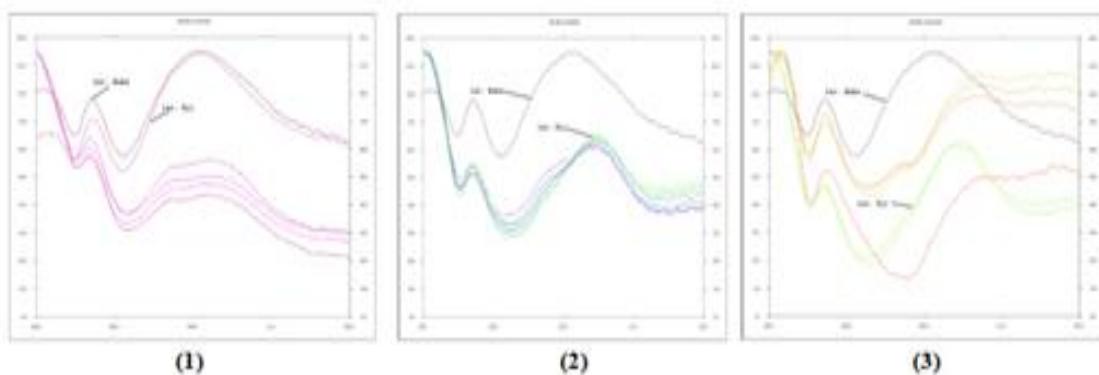


Figure 2. The spectrum of phyllanthin contained in sample extract from Solo (1), Bogor (2) and Yogyakarta (3) Specificity test resulted that all samples from Solo, Bogor and Yogyakarta had similar UV spectras to the phyllanthin standard which mean that these spectra were phyllanthin and meet the specificity test acceptance criteria. The spectra was observed by TLC scanner at a wavelength of 229 nm. The phyllanthin spectra of standard and test solutions can be seen on figure 2.

CONCLUSION

All sample extracts contained phyllanthin that proved truly Meniran (*Phyllanthus niruri* L.).

Chromatogram profile or fingerprint of meniran extract from Bogor had a bit different compare to others when detected at λ 366 nm. This method can be used for quality control and standardization of natural medicine based on meniran.

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