Fingerprint Study of Morindae citrifoliae Fructus Extractum by High Performance Thin Layer Chromatography (HPTLC)

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ABSTRACT

Research on fingerprint/chromatogram profile of *Morindae citrifoliae* Fructus (Noni fruit) extractum was done to develop a fingerprint of Noni fruit as one of the methods for quality control and standardization of herbal medicine. Samples were collected from three (3) areas: Bogor, Bandung, Yogyakarta, and its fingerprints were developed by HPTLC. The Stationary phase was HPTLC plates of silica gel 60 F254 and mobile phases was Ethyl acetate-methanol-water (100: 4: 4). TLC Photo Documentary System was used as detector at λ 366 nm and TLC Scanner at λ 343 nm to see the spectrum of scopoletin as a marker compound. Validation was done using specificity and precision parameters. The results showed that the scopoletin spectrum of the standard solution and test solution had appropriate acceptance criteria of specificity, as well as for precision and intermediate precision. In conclusion, the method was valid for the quality control and standardization of product based on the Noni fruit.

Key words : *Morinda citrifolia* L., scopoletin, chromatogram profile, fingerprint, HPTLC

INTRODUCTION

Traditional medicine is defined as ingredient or ingredients in the form of plant, animal, mineral, galenic, or mixtures of these materials, which has been hereditary used for treatment, and can be applied in accordance with the norms prevailing in society (health Law No.36 / 2009). In Indonesia, traditional medicine known as Jamu is the ancestral heritage that has been used for generations. According to the Head of BPOM Regulation No. HK.00.05.41.1384, Indonesian herbal medicine is herbal medicine produced in Indonesia.

Morindae citrifoliae Fructus (Noni fruit) is one of Indonesian tradional medicine. It has been reported to have a broad range of health benefits for cancer, infection, arthritis, diabetes, asthma, hypertension, and pain. The chemical constituents of Noni fruit are scopoletin, octanoic acid, potassium, vitamin C, iridoids, terpenoid, alkaloid, anthraquinone, morindone, rubiadine and rubiadine-1-methyl ether, but the marker compound of Noni is scopoletin (BPOM RI, 2004). The scopoletin has anti inflamatory and anti alergenic effect.

There are some methodes to control the quality of herb, extract, or natural medicine products, i.e.: fingerprint or chromatogram profile of medicinal plant extracts; dentification and determination of spesific chemical compound (marker) contained in a medicinal plant.

Nowadays, availability of the marker compounds is still difficult to obtain and the price is quite expensive. Therefore the fingerprint method is relatively more practical. Fingerprint is a unique profile, which can distinguish a medicinal plant with others. Fingerprint can be made by some methods such as liquid chromatography, gas chromatography, or a combination of these methods. With the fingerprint database of medicinal plants, it can facilitate the quality control of herbal medicine.

This study aims to obtain a fingerprint/chromatogram profile of *Morindae citrifoliae* Fructus as a basic for standardization of *Morindae citrifoliae* Fructus extractum.

MATERIALS AND METHODS

Material

Morindae citrifoliae Fructus/Noni fruits were collected from 3 (three) areas: Bogor, Bandung and Yogyakarta. It was determined in Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Science, Bogor, Indonesia. The reference standard used in this research was scopoletin (Sigma-Aldrich). The solvent and consumable were n-hexane, chloroform, ethanol, ethyl acetate,

methanol, chloroform, diethylether, toluene, acetic acid, and HPTLC Silica gel glass plates F_{254} (Merck Co.)

Equipment

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

Methods

1) *Method Development*

a) *Standard Solution*

Weighed 5 mg of scopoletin, then dissolved with 2.5 mL of ethanol.

b) *Test Solution*

The dried Noni fruits sample was weighed 1 g each, transfered into 10 mL centrifuge tube, added 10 mL of ethanol, then sonicated at 50 °C for 5 minutes. After that, centrifuged the mixture at 4000 rpm for 5 minutes. Supernatant was used as test solution. This procedure was repeated 6 times for each sample from Bogor (test solution A1-F1), Bandung (test solution A2-F2) and Yogyakarta (test solution A3-F3).

c) *Application and Development*

Each of 30 µL test solutions and 10 µL standard solution were spotted on the plate using Linomat V as a band width of 8.0 mm on an HPTLC silica gel glass plate F_{254} (20x10 cm). The following conditions were employed: distance from bottom plate 15 mm, x-potion from 1st track 20 mm. The plate was eluated in Ethylacetate-methanol-water (100:4:4). 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. The length of each chromatogram run was 75 mm. This procedure was applied for each sample from Bogor (test solution A1-F1), Bandung (test solution A2-F2) and Yogyakarta (test solution A3-F3)..

Detection

After developing, the TLC plate was dried using an air dryer. Chromatogram was documented by Photo Documentary System under λ 366 nm. Densitometry scanning was performed on TLC scanner 3 in the reflectance-absorbance mode at 343 nm. Densitometry of the TLC chromatogram was carried out on Camag reprostar 3.

2) *Validation Parameters*

a) *System Suitability Test (SST)*

The procedures of SST were as follow: standard and test solutions were prepared as in 2.c.1).a) and 2.c.1).b), then applicated and developed as in 2.c.1).c). The plate was detected as in 2.c.1).d). Rf and average Rf of scopoletin spots were specified. The RSD of 6 scopoletin spots was Calculated. The acceptance criteria was RSD≤ 2%

b) *Specificity*

The procedures of specificity test were as follow: standard and test solutions were prepared as in 2.c.1).a) and 2.c.1).b), then applicated and developed as in 2.c.1).c). The plate was detected as in 2.c.1).d). Observe the spectrum of scopoletin in the standard solution and test solution. For acceptance criteria, the scopoletin spectrums in the standard solution and test solution must be the same.

c) *Precision*

The procedures of precision were as follow: standard and test solutions made as in 2.c.1).a) and 2.c.1).b), then applicated and developed as in 2.c.1).c). The plate was detected as in 2.c.1).d). Rf and average Rf of scopoletin spots were specified. The acceptance criteria of precision was RSD≤ 2% and the acceptance criteria of intermediate precision was RSD≤ 5%.

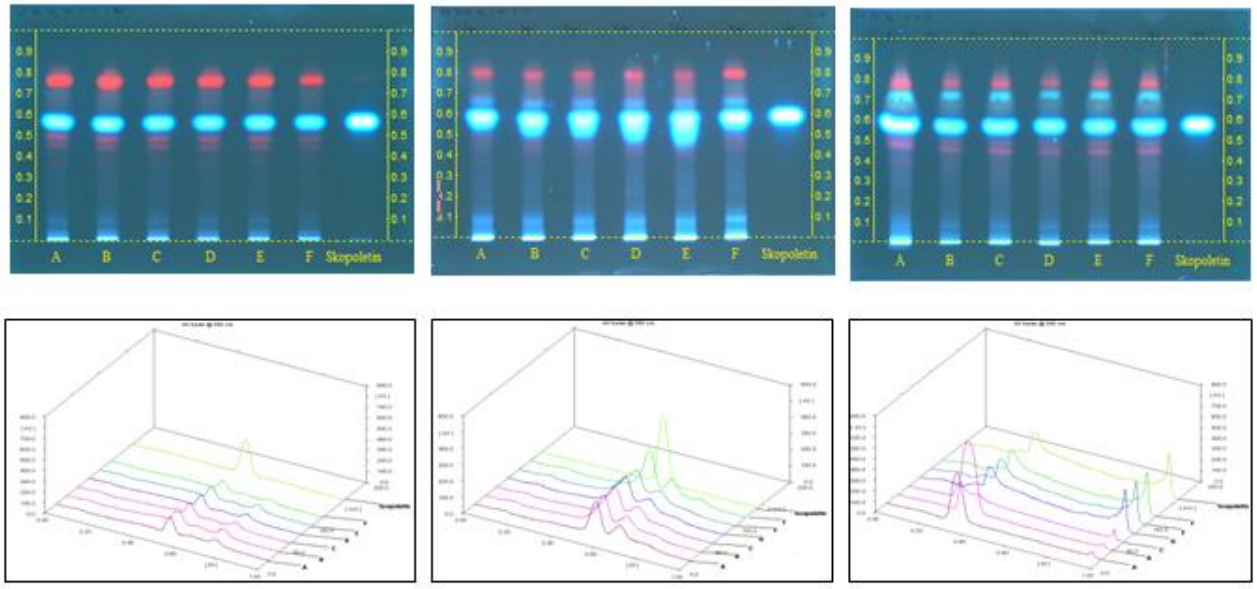
RESULTS AND DISCUSSION

Chromatogram/fingerprint Profile of Noni fruit extract (*Morindae citrifoliae* Fructus extractum) had been made using three samples from three regions: Bogor, Bandung and Yogyakarta. Using scopoletin as a reference standard which is a marker compound of noni fruit (BPOM RI, 2004). Extraction was done using 90% ethanol. Ethanol is a polar solvent that could attract scopoletin and other polar compounds in Noni fruit. While scopoletin had a good solubility in ethanol.

The HPTLC analysis profiles of each samples on HPTLC silica gel F254 using mobile phase ethyl acetate-methanol-water (100 : 4 : 4) which were recorded by photo documentary system at λ 366 nm can be seen on Figure 1. The mobile phase had a medium polarity index or classified as a semi polar eluent which could separate the compounds contained in the extract clearly. Therefore it can be inferred that Noni fruit extracts had the best separation result in a semi polar eluent/mobile phase. The average Rf value of scopoletin was 0.56 (Bogor and Bandung) and 0.57 (Yogyakarta).

Detection using TLC photo documentary sistem at λ 366 nm and TLC scanner at λ 343 nm resulted that all of contained scopoletin as a marker copound. When compared to its intensity, the scopoletin spot from Bandung samples was the biggest than two others. It means that the highest scopoletin content was sample from Bandung. All chromatogram profiles were similar but there were a little differences in the number of spots and spot intensity.

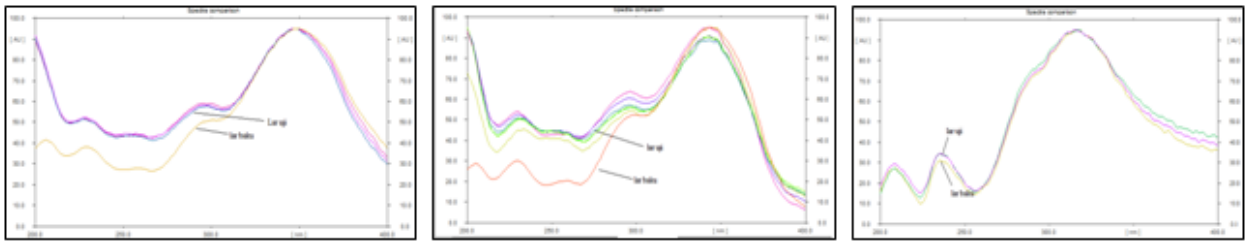
The system suitability test (SST) was carried out by doing 6 replicated of samples. The result showed that the RSD values of SST test was 0% for all samples from three areas. The system suitability test meets the acceptance criteria, i.e RSD values of 6 sample replicates is less than 2%, therefore system suitability test results met the requirements.



(A) (B) (C)

Figure 1. Chromatogram profile (λ = 366 nm) on HPTLC silica plates of Morindae citrifoliae extractum fructus after elution with ethyl acetate-methanol-water (100 : 4 : 4): A) Bogor; B) Bandung; C) Yogyakarta. Note: A-F: Test solution A-F

Specificity test result showed that the scopoletin spectra of standard was same as with the spectra of the spot that suggested as scopoletin in test solution from Bandung, Bogor and Yogyakarta. It can be concluded that these spots was scopoletin and meet the specificity test acceptance criteria. The spectra was observed by TLC scanner at λ 343 nm. The scopoletin spectra of standard and test solution can be seen in Figure 2.



(A) (B) (C)

Figure 2. The Scopoletin Spectrum of Standard and Test Solution with mobile phase: Ethyl acetate-methanol-water (100 : 4 : 4): A) Bogor; B) Bandung; C) Yogyakarta.

The intermediate precision was carried out by doing each 6 replicated of samples on 2 diffrent plates. The acceptance criterias of intermediate precision were RSD of 6 replication on a plate ≤ 2% and RSD between 2 plates ≤ 5%. The result showed that the RSD values of all plates tested were less than 2%. It met the acceptance criteria of precision. While the intermediate precision of samples from Bogor, Bandung and Yogyakarta for mobile phase ethyl acetate-methanol-water (100: 4: 4) met the acceptance criteria, with the RSD values: 0% (Bogor), 2.97% (Bandung) and 0.68% (Yogyakarta). For the overall validation parameters tested in this study, it can be concluded that the method of determining the profile chromatogram/ fingerprint Noni (*Morinda citrifolia* fructus) was valid.

CONCLUSION

Chromatogram/fingerprint Profile of Noni fruit (*Morindae citrifoliae* Fructus extractum) can be made using HPTLC under conditions: HPTLC Plates Silica Gel GF254 as stationary phase and ethyl acetate-methanol-water (100: 4: 4) as mobile phase. Detection was done by UV light at λ 366 nm and TLC scanner at λ 343 nm. Based on the results, the method met all acceptance criterias of validation testing. In conclusion, the method was valid for the quality control and standardization of product based on the Noni fruit.

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