

Fingerprint Study of *Curcuma xanthorrhiza* Rhizome by High Performance Thin Layer Chromatography (HPTLC)

Penulis

Tina Wikara*, Anny Sulistiowaty, Sri Murhandini and Tepy Usia

Afiliasi

Research Center for Drug and Food-BPOM RI

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Tina Wikara
Research Center for Drug and
Food-BPOM RI
Jl. Percetakan Negara No. 23
Jakarta Pusat 10560
Email: tina_wikara@yahoo.com

Abstract

The rhizome of *Curcuma xanthorrhiza* Roxb is intensively used in Indonesia as traditional medicine. It is widely used for hepatoprotective and anti inflammatory activities. To ensure the quality of its extract, we have studied the fingerprint or phytochemical analysis. This research was aimed to produce a chromatogram profile of the rhizome by HPTLC. The HPTLC fingerprint chromatogram of *C. xanthorrhiza* rhizome was performed using HPTLC plate of silica gel 60 F₂₅₄ as the stationary phase and chloroform-methanol (97:3) as the mobile phase. Spot detection was carried out by TLC photo documentary system at 254 and 366 nm and TLC scanner at 427 nm. The developed method was validated according to ICH guidelines by determination of specificity and precision. We found that the specificity and precision of the method were met the acceptance criteria. In conclusion, the developed method is valid and could be used for quality control and standardization of herbal medicine containing *C. xanthorrhiza* rhizome.

Introduction

Indonesia is an endowed country with a variety of medicinal plants with strong potential for a therapeutic application. *Curcuma xanthorrhiza* is one of the most popular medicinal herbs where the rhizome is intensively used in Indonesian traditional medicine for hepatoprotective and anti inflammation activities (Li *et al.* 1995). Two major groups of compounds that have been known to be responsible for the pharmacological activities of *Curcuma* are curcuminoid and volatile oil (Syu *et al.* 1998).

The efficiency of drug therapy is highly dependent on the use of proper and originality of raw materials. Therefore, the guarantee of safety, quality and efficacy of medicinal plants and herbal products has now become a major and important issue so that the plant material required standardization. In recent years, progress in chromatographic and spectral fingerprint has played an important role especially in the quality control of complex herbal medicines (Yamunadevi *et al.* 2011).

Fingerprint analysis by high performance thin layer chromatography (HPTLC) has become an effective and powerful tool for the estimation of chemical and biochemical markers. HPTLC is becoming a routine analytical technique because of some advantage

including the small amount of mobile phase required the speed of the method, and the possibility of analysis of several samples simultaneously (i.e. on the same plate), unlike HPLC. It thus reduces analysis time and cost per analysis. Cloudy samples and suspensions can also be analyzed directly by HPTLC (Ali *et al.* 2007). Automatic sample application is possible and repeated scanning can be performed on the same plate, so scanning conditions can be changed.

The rhizomes of *C. xanthorrhiza* and turmeric (*Curcuma longa*) are generally having a yellow or orange color due to the present of curcuminoids. This study aims to obtain a fingerprint/ chromatogram profile of *C. xanthorrhiza* as a basic for standardization to ensure the safety and quality of *C. xanthorrhiza* using HPTLC technique. This method could be used for identification, differentiation and authentication of raw and extract of *C. xanthorrhiza*.

Methodology

Material

The rhizomes of *C. xanthorrhiza* were collected from 3 (three) different locations: Bandung, Solo and Yogyakarta. Samples of turmeric were collected from Bandung. It were determined in Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Science, Bogor, Indonesia. The reference standard used in this research was curcuminoid (Sigma-Aldrich, Singapore, Singapore) and the solvent were chloroform and methanol (Merck. Darmstadt, Germany). High Performance Thin Layer Chromatography (HPTLC) silica gel glass plate F₂₅₄ (20x10 cm) were used for HPTLC fingerprint analysis.

Equipment

HPTLC fingerprint was carried out using Linomat 5 TLC Spotter (Camag, Muttenez, Switzerland), twin through glass chamber 20x10 cm, TLC Scanner 3 (Camag, Muttenez, Switzerland), TLC Documentation System Reprostar3 (Camag, Muttenez, Switzerland).

Methods

Method Development

Standard Solution

Standard solution was prepared by dissolving curcuminoid (1 mg) in 1 mL of methanol.

Test Solution

All powder sample were extracted using methanol as solvent. Approximately 0.1 g dried powder

of *C. xanthorrhiza* (Bandung, Solo and Yogyakarta) and turmeric was put into 2 mL centrifuge tube, added 1 mL methanol then sonicated at 60 °C for 5 minutes. The mixture was centrifuged at 4000 rpm for 5 minutes. Supernatant was used as a test solution. This procedure was repeated 6 times.

HPTLC fingerprint Analysis

Test and standard solution were applied to plates using Linomat 5 TLC Spotter. Five microliters of each test solution were spotted as a band width of 7.0 mm on an HPTLC silica gel glass plate F₂₅₄ (20x10 cm). The following conditions were employed: distance from bottom plate 10 mm, x-potion from 1st track 12 mm. HPTLC plate was eluted in chloroform-methanol (97:3). Linear ascending development was carried out in 20x10 cm twin trough glass chamber (CAMAG) 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 85 mm. After developing, the TLC plate was dried using an air dryer. Densitometry scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode at 427 nm. Densitometry of the TLC chromatogram was carried out on Camag reprostar 3.

Detection

The dried plate was observed under UV 254 and 366 nm to visualize the chromatograms, and it were documented by Photo Documentary System (Camag, Muttenez, Switzerland) under UV 254 and 366 nm and scanned by TLC Scanner (Camag, Muttenez, Switzerland) at 427 nm.

Validation Parameters

Suitability System Test

Standard solution and test solutions were made, then applicated and developed as in 1.c. After developed, the plate was detected as i 1.d. Retention Factor (Rf) and average Rf of curcumin spots were specified by TLC Scanner (Camag, Muttenez, Switzerland). Relative Standard Deviation (RSD) of 6 curcumin spots was Calculated. The acceptance criteria was RSD ≤ 2%

Specificity

Standard solution and test solutions were made, then applicated and eluted as in 1.c. After developed, the plate was detected as in 1.d. The spectrum of curcumin in the standard solution and test solution were observed. The acceptance criteria of the curcumin



spectrums in the standard solution and test solution must be the same.

Precision

Standard solution and test solutions made, then applied and developed as in 1.c. After developed, the plate was detected as in 1.d. Rf, average Rf, and RSD of curcumin spots were specified. The acceptance criteria of precision was $RSD \leq 2\%$. And the acceptance criteria of intermediate precision was $RSD \leq 5\%$.

Result

TLC fingerprint analysis is one of the techniques frequently used for evaluation the quality of herbal medicines (Li *et al.* 1995). Chromatogram/fingerprint profile of *C. xanthorrhiza* had been made using three samples from three locations: Bandung, Solo and Yogyakarta. The rhizome had been authenticated at Research Center for Biology - Indonesian Institute of Sciences. The results showed that the plant used was *C. xanthorrhiza*. This study used curcuminoid as reference standard which was a major compound of *C. xanthorrhiza* (Syu *et al.* 1998).

C. xanthorrhiza and turmeric is closely related species belongs to the Zingiberaceae family, so they have similarity on the appearance especially in powdered form and chemicals composition but possesses different pharmacological activities, so it can be sources for adulteration in the herbal medicines product containing one of two rhizomes (Rafi *et al.* 2011). TLC fingerprint method for identification, differentiation and authentication of two rhizomes *C. xanthorrhiza* and *Curcuma longa* have been developed using HPTLC technique.

Chromatographic condition such as, mobile phase have been optimized firstly before applied to

validation. Extraction was done in methanol. Methanol is a polar solvent that could attract curcumin and another polar compound in the rhizome of *C. xanthorrhiza* and turmeric. Fingerprint analysis was conducted using HPTLC silica F₂₅₄ as a stationary phase and chloroform-methanol (97:3) as a mobile phase which was recorded by photo documentary system at UV 254 and 366 nm (can be seen on .). The fingerprint of *C. xanthorrhiza* under UV 254 nm showed spot at a range 0.07-0.78. All samples display a green, blue, red and yellow spot at an approximate Rf value between 0.02-0.85 under UV 366 nm. The Rf values of Curcuminoid were investigated as shown in fig 1. By HPTLC, the Rf value of curcuminoid was confirmed to be curcumin, demethoxycurcumin, and bis-demethoxycurcumin. The average Rf values of curcumin, demethoxycurcumin, and bis-demethoxycurcumin on mobile phase chloroform-methanol (97:3) was 0.47; 0.23 and 0.19 viewed under UV 366 nm.

From TLC photo documentary System at λ 254 and 366 nm (Fig. 1) and TLC scanner at λ 427 nm (Fig.2), it were found a major peak in the curcuminoid extracts of all samples curcumin. The other two minor peaks were demethoxycurcumin, and bis-demethoxycurcumin. It was showed that all samples from Bandung, Solo and Yogyakarta contained curcumin as a major compound. When compared to its intensity, the curcumin spot from Yogyakarta was the biggest than two others. It means that the highest curcumin content of the three samples was sample from Yogyakarta. While the third chromatogram profiles were similar but there were a little difference in the number of spots and spot intensity. Wavelength at λ max 427 nm which was used to analyze each curcuminoids content in the samples is the same

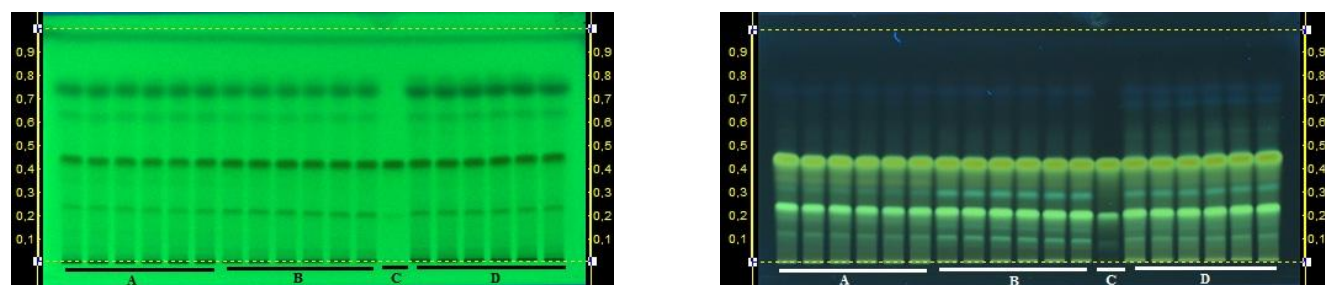


Figure 1. HPTLC fingerprint of *C. Xanthorrhiza* from various locations: A) Bandung; B) Solo; C) reference standard; D) Yogyakarta. Stationary phase: HPTLC silica F₂₅₄; Solvent system: Chloroform-methanol (97:3); detection: (a) UV 254; (b) UV 366 nm

wavelength recommended by Indonesian Herbal Pharmacopoeia (Kementrian Kesehatan RI 2009).

The system suitability test (SST) was carried out by doing 6 replication samples. The result showed that the RSD values of SST test were below 2% so all of the suitability system 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.

Specificity test resulted that the curcumin spectra of standard was same as with the spectra of the spot that suggested as curcumin in test solution from Bandung, Solo and Yogyakarta (Fig.3). It was concluded that these spots were curcumin and meet

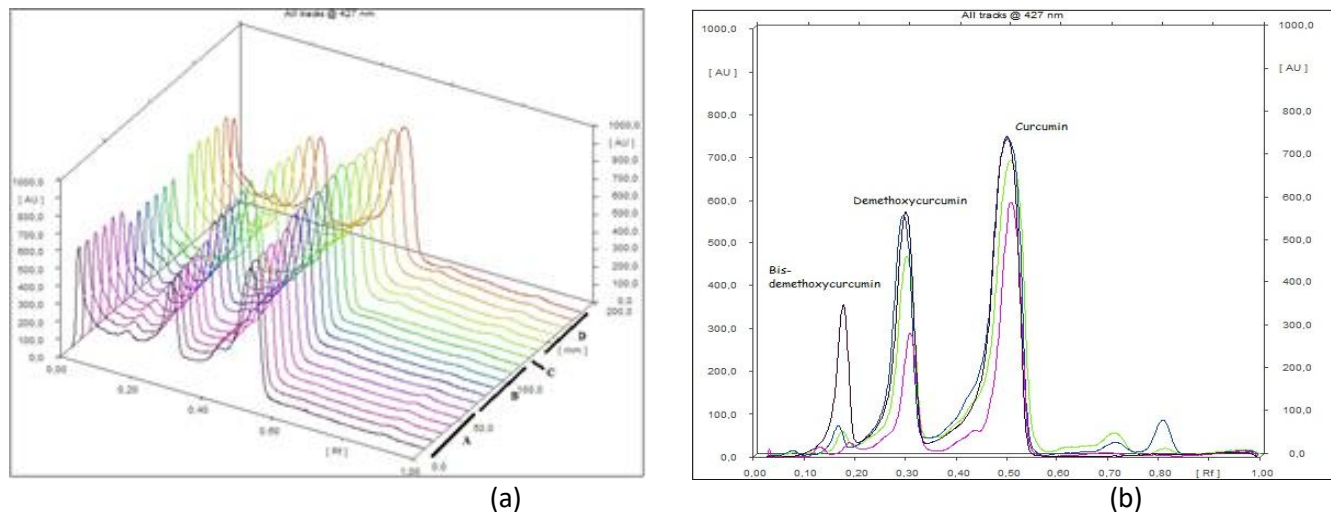


Figure 2. Chromatogram profile of *C. Xanthorrhiza* from various locations: A) Bandung; B) Solo; C) reference standard; D) Yogyakarta. Detection: 427 nm

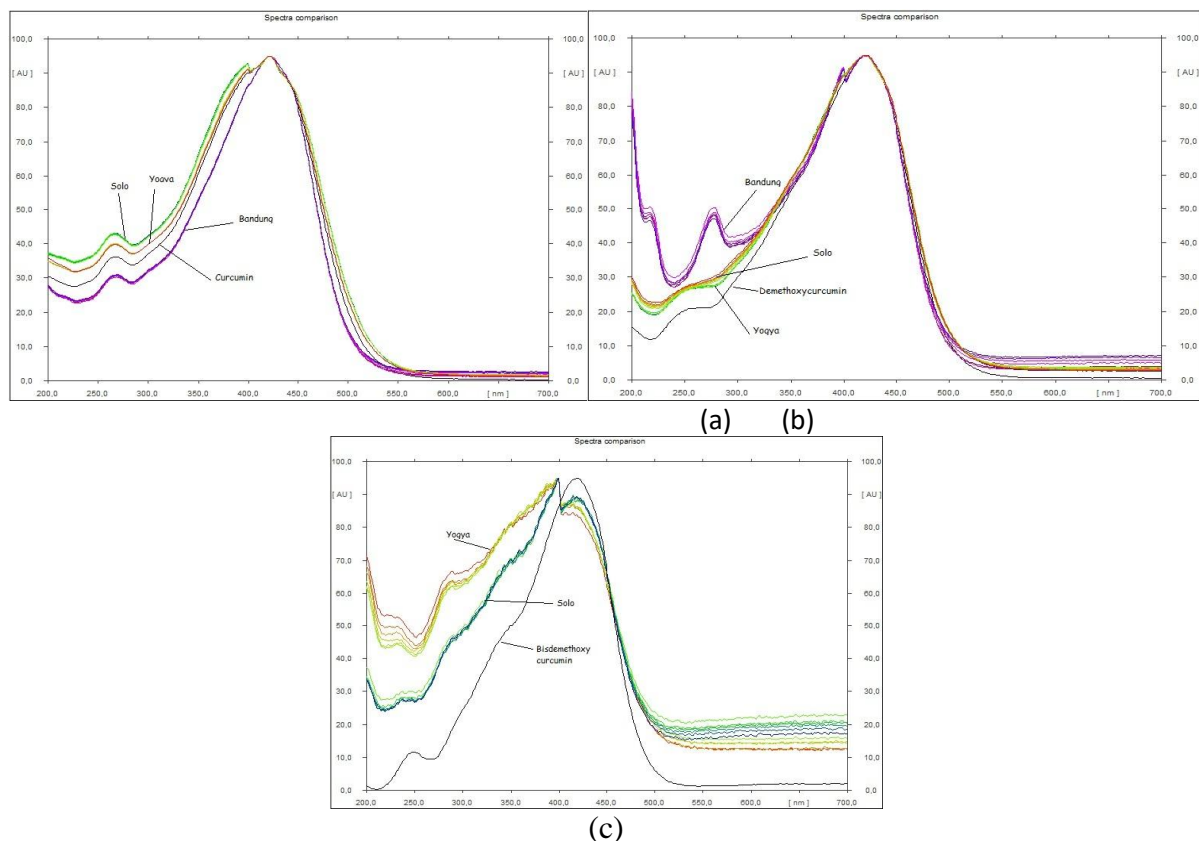


Figure 3. Spectrum of Standard and Test Solution with mobile phase chloroform-methanol (97:3): (a). Curcumin; (b). Demethoxycurcumin; (c). Bis-demethoxycurcumin



the specificity test acceptance criteria. The spectra was observed by TLC scanner at λ 427 nm. The curcumin spectra of standard and test solution can be seen in Figure 3.

The intermediate precision was carried out by doing each of 6 replication samples on 2 different plates. The acceptance criterias of intermediate precision were RSD of 6 replication on a plate $\leq 2\%$ and RSD between 2 plates $\leq 5\%$. The result showed that the RSD values of all plates tested were less than 2%.

Figure 4 displays fingerprint chromatograms for *C. xanthorrhiza* and turmeric viewed under UV (254 and 366 nm). The fingerprints of turmeric under UV 254 and 366 nm clearly showed 4 strong spot at a range 0.19-0.80, three yellow spot represent curcumin, demethoxycurcumin and bisdemethoxycurcumin. The R_f values corresponding to 0.47; 0.29 and 0.19, respectively. The HPTLC fingerprints of *C. xanthorrhiza* give 2 strong spot at R_f 0.48 and 0.82 and 4 weak absorbed weak spot at range R_f 0.12-0.69 under UV

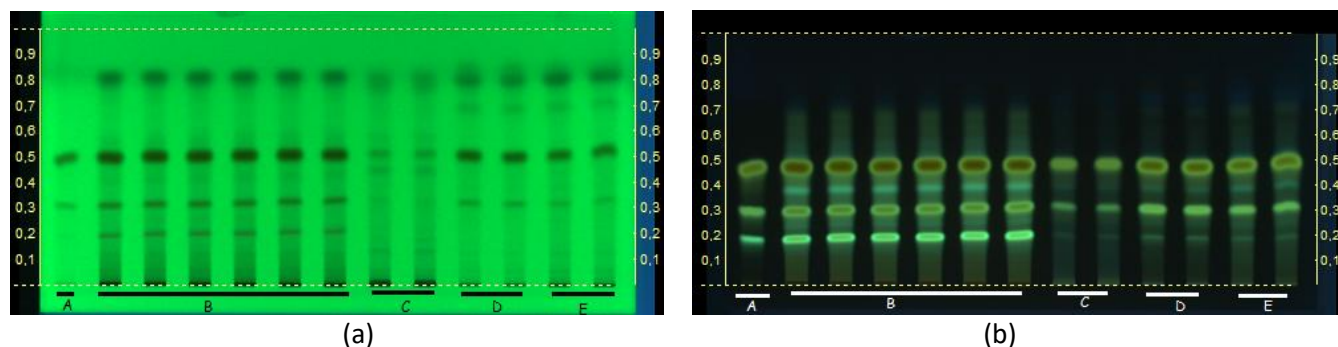


Figure 4. HPTLC fingerprint of *C. Xanthorrhiza* from various locations and turmeric: A) Curcuminoid; B) Turmeric; C) Bandung; D) Solo; E) Yogyakarta. Stationary phase: HPTLC silica F254; Solvent system: Chloroform-methanol (97:3); detection: (a) UV 254; (b) UV 366 nm

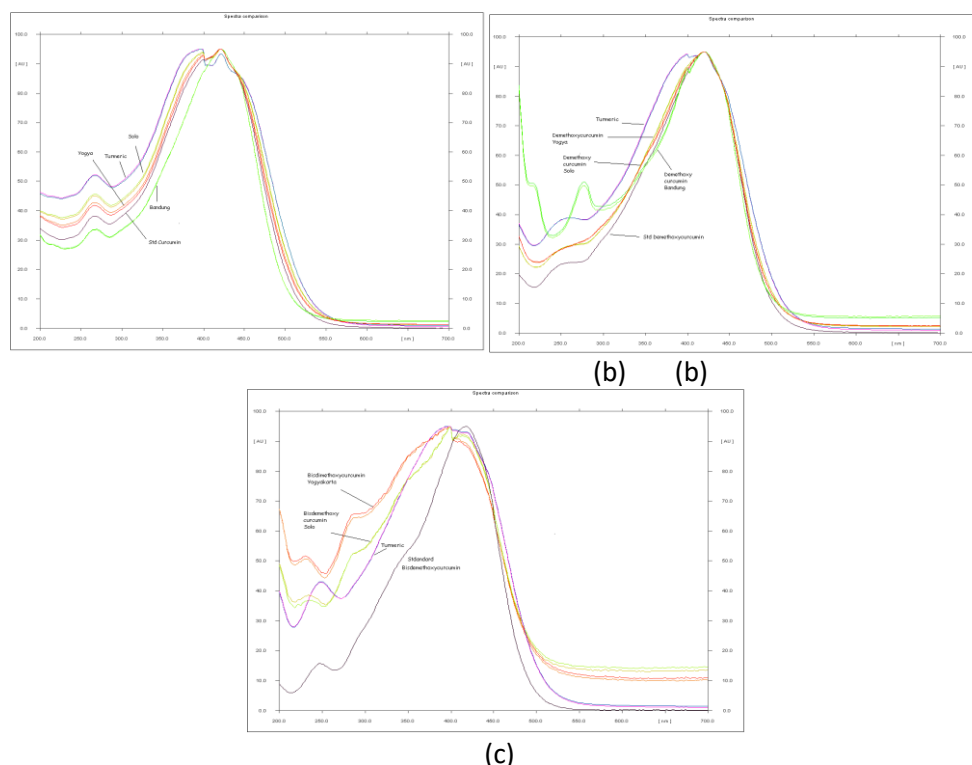


Figure 5. Spectrum of Standard of Curcuminoid and Test Solution (*C.xanthorrhiza* and turmeric) with mobile phase chloroform-methanol (97:3): (a). Curcumin; (b). Demethoxycurcumin; (c). Bisdemethoxycurcumin

254 nm. The Rf values corresponding to 0.29 and 0.47 represent demethoxycurcumin and curcumin under 366 nm and a weak absorbed spot at 0.19 represent bisdemethoxycurcumin. Turmeric and *C. xanthorrhiza* can be differentiating with a 2 marker strong spot at Rf 0.29 and 0.47 respectively. *C. xanthorrhiza* has a spot at Rf 0.19 but only a weak spot.

Specificity test for *C. xanthorrhiza* and turmeric resulted that the curcumin spectra of standard was same as with the spectra of the spot that suggested as curcumin in test solution (*C. xanthorrhiza* and turmeric) (Fig.5). It was concluded that these spots were curcumin and meet the specificity test acceptance criteria. The spectra was observed by TLC scanner at λ 427 nm. The curcumin spectra of standard and test solution can be seen in Figure 5. Overall, validation parameters tested in this study had met the requirements so that the method of determining the profile chromatogram profiles/fingerprints *C. xanthorrhiza* was valid.

Conclusion

C. xanthorrhiza grown in different parts of Indonesian contains different amounts of various curcuminoid. Therefore, for obtaining the high yield of certain curcuminoid, the area for plant collection should be considered. Based on the results, analytical method validation for the determination of fingerprint of *C. xanthorrhiza* was valid. The results showed that the curcumin 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 and it

would provide a useful guidance for the quality control and standardization of curcuminoid extracts on the *C. Xanthorrhiza* used in pharmaceutical products and cosmetics.

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