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Original Article

Validating and developing TLC-based fingerprinting for *Curcuma longa* L.

[Validación y desarrollo de huellas dactilares basadas en TLC para *Curcuma longa* L.]

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Abstract

Context: *Curcuma longa* (turmeric) is extensively cultivated as spices and herbal medicines in tropical and sub-tropical countries. Geographical origin is known to significantly determine the quality of the herbs used and, consequently, the safety and efficacy of their products.

Aims: To validate and develop TLC-fingerprint combined with chemometrics to differentiate *C. longa* collected from various origins.

Methods: Thin Layer Chromatography (TLC) was employed together with chemometric methods, i.e., Principal Component Analysis (PCA) and Cluster Analysis (CA), to evaluate the quality of *C. longa* rhizomes collected from nine origins in Indonesia.

Results: Chloroform, dichloromethane, and ethanol (64:64:1) were a suitable mobile phase for *C. longa*. The method used met the requirements for a stable and precise TLC system. As analyzed by the chemometric techniques, the TLC-fingerprints could discriminate *C. longa* from various origins. The PCA score plot of the first two principal components (PCs) and CA clearly distinguished two clusters of simples.

Conclusions: When combined with PCA and CA, TLC-fingerprinting can discern the rhizomes of *C. longa* sourced from various locations. TLC-fingerprints that are analyzed with chemometrics can be used as an alternative marker-oriented method for evaluating the quality of *C. longa*.

Keywords: cluster analysis; geographical origin; herbal medicine; principal component analysis; quality.

Resumen

Contexto: *Curcuma longa* (cúrcuma) se cultiva ampliamente como especias y hierbas medicinales en países tropicales y subtropicales. Se sabe que el origen geográfico determina significativamente la calidad de las hierbas utilizadas y, en consecuencia, la seguridad y eficacia de sus productos.

Objetivos: Validar y desarrollar TLC-huella dactilar combinada con quimiometría para diferenciar *C. longa* recolectada de diversos orígenes.

Métodos: Se empleó cromatografía de capa fina (TLC) junto con métodos quimiométricos, es decir, análisis de componentes principales (PCA) y análisis de conglomerados (CA), para evaluar la calidad de los rizomas de *C. longa* recolectados de nueve orígenes en Indonesia.

Resultados: El cloroformo, el diclorometano y el etanol (64: 64: 1) fueron una fase móvil adecuada para *C. longa*. El método utilizado cumplió con los requisitos para un sistema de TLC estable y preciso. Según lo analizado por las técnicas quimiométricas, las huellas dactilares de TLC podrían discriminar *C. longa* de varios orígenes. La gráfica de puntuación de PCA de los dos primeros componentes principales (PC) y CA distinguió claramente dos grupos de simples.

Conclusiones: Cuando se combina con PCA y CA, las huellas dactilares de TLC pueden discernir los rizomas de *C. longa* procedentes de varios lugares. Las huellas dactilares de TLC que se analizan con quimiometría se pueden utilizar como un método alternativo orientado a marcadores para evaluar la calidad de *C. longa*.

Palabras Clave: análisis de componentes principales; análisis de conglomerados; calidad; medicina herbaria; origen geográfico.

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INTRODUCTION

Curcuma longa L. (turmeric) is extensively cultivated and widely used as spices and for herbal medicines in many tropical and sub-tropical countries, such as India, Pakistan, Bangladesh, China, Thailand, Malaysia, and Indonesia (Niranjan and Prakash, 2008; Wichitnithad et al., 2009; Rohaeti et al., 2015). Together with other *Zingiberaceae* family members, it has a long history in Ayurvedic, traditional Indonesian (*Jamu*), and traditional Chinese medicines (TCM) (Rohaeti et al., 2015; Daily et al., 2016). Turmeric has been studied for its various biological activities, including anti-inflammatory, antioxidant, anti-tumor, anti-cancer, anti-HIV, anti-mutagenic, anti-bacterial, anti-fungal, anti-protozoal, antidiabetic, anti-fibrinogen, wound-healing, lipid-lowering, radioprotective, and immunomodulatory activities (Niranjan and Prakash, 2008; Akram et al., 2010; Amalraj et al., 2017).

C. longa is harvested for its rhizome as it contains various chemical components. Terpenoids, essential oils, and phenolic compounds are its major constituents (Rohaeti et al., 2015). The most important phenolic compounds in turmeric are curcumin (60-80%), desmethoxycurcumin (15-30%), and bisdemethoxycurcumin (2-6%), which are collectively called curcuminoids (Wichitnithad et al., 2009). These three compounds are yellow; therefore, curcuminoids and turmeric extracts are used not only as drugs but also as flavoring and coloring agents in the food industry, confectionery, and cosmetics (Niranjan and Prakash, 2008; Gupta et al., 2013).

Besides *C. longa*, there are two other yellow rhizomes from the *Zingiberaceae* family, i.e., *Curcuma xanthorrhiza* and *Zingiber cassumunar*. Due to their similar organoleptic features, the last two rhizomes can be substitutes for *C. longa*. Mistaking one species for the incorrect one can lead to a serious problem in the traditional medicine practices, such as inconsistent effects and, in worse scenarios, toxicity. Turmeric and its products can have consistent quality depending on geographical origins, solvent, and method of extraction (Sogi et al., 2010; Wakte et al., 2011; Paulucci et al., 2013). The geographical origin of plants needs to be recognized in the production of herbal medicines because it affects the levels of bioactive compounds, a general criterion in the selection of raw materials. A simple yet accurate analytical method to identify the geographical origin of *C. longa* is thereby necessary.

Curcumin is frequently used as a marker compound by some pharmacopoeias and commercial products (Ri, 2008; Gupta et al., 2013). No less than 65 clinical trials on curcumin have been conducted, and more than 35 others are in progress to further evaluate its efficacy (Gupta et al., 2013). In addition to curcumin, at least 300 chemical constituents have been identified from *C. longa* and found to possess a variety of promising biological activities as well. Previous research proved how curcumin-free turmeric extract could suppress benzo[a]pyrene-induced tumorigenesis in mice (Deshpande et al., 1997). Several other studies also concluded that whole turmeric is more effective than curcumin alone (Deshpande et al., 1998; Nishiyama et al., 2005; Suryanarayana et al., 2005; Kim et al., 2012). These data confirm that curcumin and other compounds must be taken into account in the analysis of the quality of *C. longa*.

Therefore, the quality of *C. longa* crude drugs and products is evaluated more appropriately by measuring all or almost all of *C. longa* constituents. This approach can be performed using chemical fingerprinting. Compared to marker compounds, the application of fingerprints has several superiorities. Fingerprints are a distinctive profile or pattern of samples that chemically reflects the composition of the test plant, presenting as much information as possible (Tistaert et al., 2011; Gad et al., 2013). Therefore, fingerprinting analysis considers nearly all of the compounds in a plant, and this allows a more objective evaluation of the plant quality than using only a specific compound(s). TLC-fingerprint has been widely used in herbal medicines assessment, and it is simpler, faster, and cheaper compared to GC, HPLC, and FT-IR. TLC can process many samples in one running time, and the TLC-chromatograms can be analyzed vis-

ually. However, this technique is subjective and not quantitative. Moreover, fingerprint chromatograms are complex multivariate data sets, due to which the evaluation of very similar chromatograms is challenging unless the analysis incorporates chemometric techniques. This approach, although more difficult, is based on objective mathematical methods and treats each chromatogram as a unique signal without the need to identify and interpret the peaks. Therefore, it provides a good possibility for digging more useful chemical information from original-rich data (Komsta, 2012; Tistaert et al., 2011; Bansal et al., 2014). This research was designed to validate and develop TLC-fingerprint combined with chemometrics to differentiate turmeric collected from various origins. This simple method will be beneficial for herbal industries, especially those in developing countries.

MATERIAL AND METHODS

Chemicals

The following chemicals were procured from Merck (Darmstadt, Germany): TLC plate precoated with silica gel 60 F_{254}, n-hexane, diethyl ether, tetrahydrofuran, acetic acid, 2-propanol, dichloromethane (DCM), ethyl acetate (EA), dioxane, toluene, chloroform ($CHCl_3$), methanol, ethanol, vanillin, and sulfuric acid.

Plant materials

Curcuma longa rhizomes were collected from nine cultivation sites on Java Island, Indonesia (Table 1). The rhizome was harvested by pulling it out, then cleaned with tap water. Authentication of the rhizomes from Batu (sample G) was done by the Materia Medika Batu with accession certificate number: 074/127A/102.7/2018, whereas turmeric rhizomes from other origins were authenticated by the Center for Traditional Medicine Information and Development, University of Surabaya, Surabaya, Indonesia, with accession certificate number: 1401/D.T/V/2019. Rhizomes were then cut into slice and air-dried (Fig. 1), then ground into particles that passed through a 60-mesh screen.

Preparation of extracts

One gram of powdered *C. longa* rhizome was mixed with 10 mL of methanol and extracted with the Ultrasound-Assisted Extraction (UAE) using ultrasonic cleaner (Branson 1510, Branson Ultrasonics Corp., US), power 80 W, frequency 40 kHz, for 15 minutes. The output was then filtrated using Whatman® qualitative filter paper grade 1 (Merck, Darmstadt, Germany) to separate the extract from any residues, and this extract was finally kept in a tightly closed vial.

Table 1. Geographical provenance of *Curcuma longa* from Java Island, Indonesia.

Code	District (City)	Height (m a.s.l.)	Latitude, Longitude	Voucher code
A	Surabaya	6	7°15′ S; 112°42′ E	1401/D.T/V/2019
B	Sidoarjo	10	7°29′ S; 112°43′ E	1401/D.T/V/2019
C	Gresik	12	7°10′ S; 112°40′ E	1401/D.T/V/2019
D	Pacet	600	6°45′ S; 107°03′ E	1401/D.T/V/2019
E	Trawas	700	7°40′ S; 112°36′ E	1401/D.T/V/2019
F	Pasuruan	800	7°41′ S; 112°39′ E	1401/D.T/V/2019
G	Batu	700	7°42′ S; 112°42′ E	074/127A/102.7/2018
H	Tawangmangu	1200	7°42′ S; 111°08′ E	1401/D.T/V/2019
I	Bogor	192	6°37′ S; 106°48′ E	1401/D.T/V/2019
J	Commercial	NA	NA	1401/D.T/V/2019

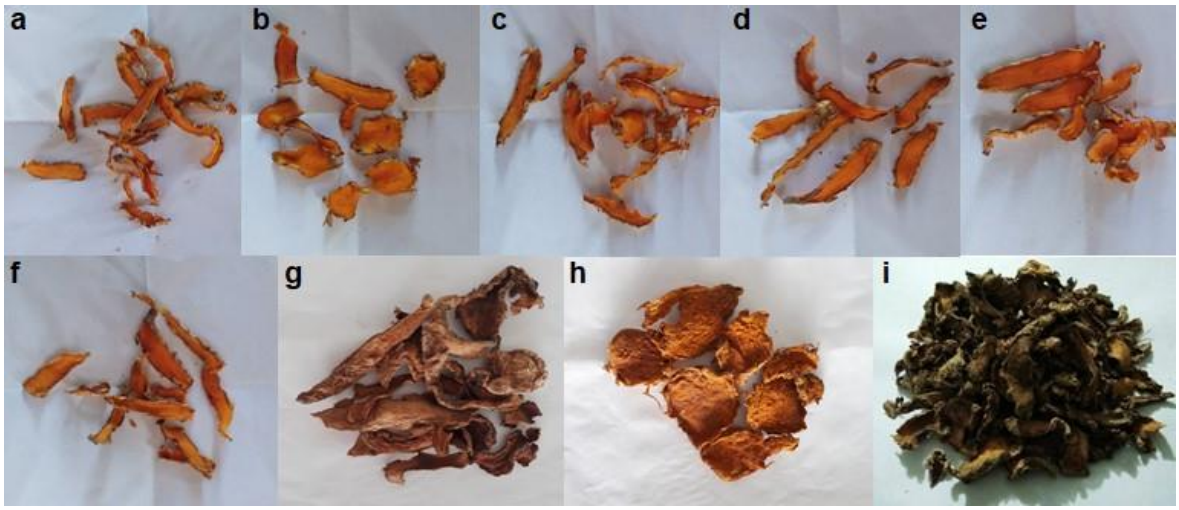


Figure 1. Crude drugs of *C. longa* rhizomes collected from Surabaya **(a)**, Sidoarjo **(b)**, Gresik **(c)**, Pacet **(d)**, Trawas **(e)**, Pasuruan **(f)**, Batu **(g)**, Tawangmangu **(h)**, and Bogor **(i)**.

Method validations

In this research, TLC-fingerprint was used for qualitative analysis. Therefore, validation test was focused on the selection of TLC conditions, selection of the mobile phase, followed by determination of stability and precision.

TLC conditions

The TLC system built consisted of a sample applicator (Linomat 5; Camag, Muttenz, Switzerland), twin-through chamber (Camag), and TLC-Visualizer equipped with a 12-bit CCD camera and Camag VideoScan 1.02 software with the serial number 2503D001 (Camag). Pre-coated silica gel 60 F_{254} (Merck TLC plates, Art. No.: 1.05554.0001, 20 × 20 cm, 175-225 µm layer thickness, aluminum-backed, particle size distribution: 9.5-11.5 µm) was used as the stationary phase. The samples were spotted under a flow of nitrogen (8 mm bands, 10 mm from the left edge, 15 mm from the bottom edge, and 20 mm of track distance) using a 100 µL syringe (Camag). Elution was performed in a chamber that had been previously saturated (for 30 min at room temperature) with the mobile phase (see: *Selection of mobile phase*), and the migration distance was 80 mm. The TLC plates were dried at room temperature, then dipped in a vanillin-sulfuric acid reagent, dried in a fume hood, and heated for 10 min at 100°C. These plates were subsequently illuminated under short-wave UV (254 nm), long-wave UV (366 nm), and white lights using TLC-Visualizer. Afterward, the digitized images were evaluated quantitatively in Camag VideoScan 1.02 program.

Selection of mobile phase

The mobile phase was selected in two steps. In the first step, the twin-through chamber was saturated (30 minutes) with each solvent (single mobile phase), i.e., n-hexane, diethyl ether, 2-propanol, ethanol, tetrahydrofuran, acetic acid, dichloromethane, ethyl acetate, dioxane, toluene, and chloroform. Two µL of *C. longa* extract was applied on the TLC plate and then developed with each mobile phase. Afterward, the plate was removed, dried at room temperature, and visualized (see *TLC conditions*). In the second step, the selection involved a mixture of two or three solvents with various ratios to obtain a chromatogram with the highest number and the best separations of zones.

Stability of chromatogram

The chemical constituents, both in the solution and on the plate, were evaluated for their stability. The extract was left at room temperature and on the plate for 3 hours. A reference extract was freshly prepared and applied just before the elution. A chemical compound was stable in solution or on the plate if the difference between its Rf value and

that of the reference extract was not larger than 0.05. Also, the stability of the chemical compounds during chromatography was evaluated by two-dimensional (2D) elution. They were deemed stable during the chromatographic process if all zones are located on a diagonal line connecting the initial position of the application and the intersection of the two mobile phase fronts. For the stability of the chromatography result, the extract was chromatographed according to the chromatography method used and derivatized with a vanillin-sulfuric acid reagent. The chromatogram was evaluated using TLC-visualizer repeatedly after 5, 10, 30, and 60 minutes. A chromatogram was stable if there were no significant changes in the Rf values (Reich and Schibli, 2007).

Precision of chromatogram

Curcuma longa rhizome was extracted in triplicates. Each extract was chromatographed in duplicates on three different plates. Intraday precision was analyzed by calculating the Rf value of the most prominent zone, its mean value, and relative standard deviation. Acceptable intraday precision occurs if the Rf values of the same compounds did not vary by more than 0.02 from one plate to another. The interday precision was determined by firstly applying the extract twice on three different plates and three different days. Interday precision was acceptable if the Rf values of the same substances did vary not by more than 0.05 between plates from Day 1 to Day 3 (Reich and Schibli, 2007).

TLC-fingerprint analysis

TLC of samples from different origins

Curcuma longa rhizome extracts from nine different locations and one commercial sample were chromatographed on a TLC plate. The TLC plate was eluted as described in *TLC condition section* and subsequently illuminated under 254 nm UV, 366 nm UV, and white lights using TLC-Visualizer 2 (Camag, Switzerland). The videodensitogram of the samples was then analyzed by the chemometric method (Komsta, 2012).

Chemometric analysis

The Rf value, height, and area of each peak (compound) obtained from the videodensitogram were tabulated and then analyzed using Principal Component Analysis (PCA) and Cluster Analysis (CA). PCA is an exploratory data analysis that is based on the information available in the fingerprints only. It reduces the complexity of the multivariate data set by explaining the correlation amongst a large number of variables using a smaller number of underlying factors (principal components or PCs) without losing much information. The projections of the *n* objects from the original data on PCs are called the scores plots. Meanwhile, the contribution of each original variable to the score is presented by its loading, which detects the variables responsible for the clustering (Gad et al., 2013). CA was performed to classify samples based on similarities in their chemical properties. In CA, the samples are grouped in high dimensional space and, thereby, form a dendrogram. In the first step, each sample forms a cluster, and then two nearest objects are grouped. In the next step, either a third sample joins the first two or two other samples join a different cluster. Each of these steps results in one cluster less than the previous step until, eventually, all samples are in one cluster (Yang et al., 2007). PCA and CA were conducted using Minitab v.16 (Minitab Inc., USA).

RESULTS AND DISCUSSION

Selected mobile phase

The methanol extracts of the *C. longa* rhizomes collected from nine origins and one commercial sample are presented in Fig. 2. All of the extracts exhibited dark-orange color, except for the samples from Surabaya, Sidoarjo, and Gresik (samples a, b, and c). In this context, color provides preliminary information that the quality of these extracts may differ. One of these extracts was chosen for the selection of the mobile phase. Firstly, chloroform, dichloromethane, and ethanol were the three mobile phases that yielded the highest number of zones with good separation. Secondly, these solvents were combined using simplex centroid with axial design (Brereton, 2003), from which ten com-

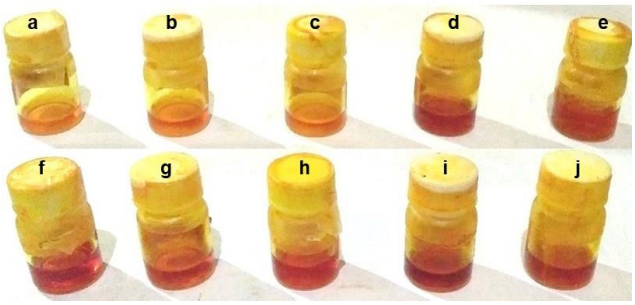


Figure 2. The methanol extracts of *Curcuma longa* rhizomes collected from Surabaya **(a)**, Sidoarjo **(b)**, Gresik **(c)**, Pacet **(d)**, Trawas **(e)**, Pasuruan **(f)**, Batu **(g)**, Tawangmangu **(h)**, Bogor **(i)**, and commercially available product **(j)**.

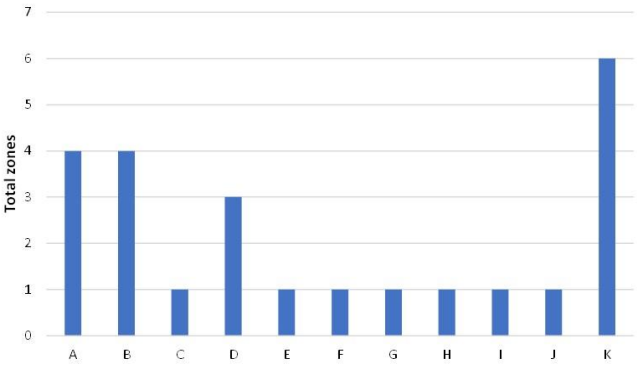


Figure 3. The total zones of the *Curcuma longa* rhizome extract. MP (mobile phase): $CHCL_3$, DCM, and EtOH with different ratios. D (detection): vanillin-sulfuric acid reagent, white light. **A-K** represent tracks with mobile phases of $CHCL_3$, DCM, and EtOH at the ratios as shown in the appropriate column below.

binations with various ratios were deduced (Fig. 3). However, the separation of zones with these solvent ratios was unsatisfactory; therefore, modified ratios were applied. Finally, the mixture of $CHCL_3$, DCM, and EtOH (64:64:1) was selected as the solvent system used in the development of TLC-fingerprinting for *C. longa*. Six zones appeared after derivatization with a vanillin-sulfuric acid reagent and visualization under white light.

Stability

Owing to the off-line nature of TLC systems, the stability of chemical contents before and during chromatography must be established. The stability of the sample before chromatography was determined by preparing two extracts at different times. The Rf values of the prominent zone or marker (*, yellowish-green) on tracks I and II were compared to those on tracks III and IV to evaluate the stability of the sample on the plate. Meanwhile, the zones on tracks V and VI were compared to those on tracks III and IV to assess whether or not the sample in the solution was stable (Reich and Schibli, 2007). The results showed that all tracks exhibited the same pattern (Fig. 4A) and Rf value, with $\Delta Rf < 0.05$ (Table 2), indicating that the samples, both on the plates and in the extract solutions, remain stable for three hours before the chromatography.

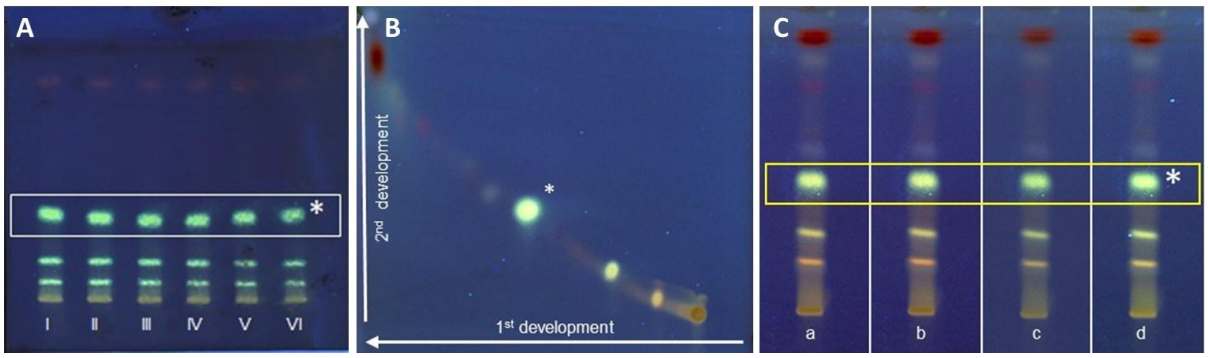


Figure 4. (A) The varying levels of stability of *C. longa* extracts on the plate and in solution. **I, II:** extracts on the plate were stable for 3 hours; **III, IV**: extracts in solution and on the plate were stable for less than 5 minutes; **V, VI**: extracts in solution were stable for 3 hours; **(B)** The stability of the *C. longa* extracts during chromatography; **(C)** The stability of the chromatographic results after 5 **(a)**, 10 **(b)**, 30 **(c)**, and 60 minutes **(d)**. MP: $CHCL_3$, DCM, and EtOH (64:64:1). D: vanillin-sulfuric acid reagent, 366 nm UV light. (*) marker compound.

Table 2. The varying levels of stability of the samples on the plate and in solution.

Markers (*)	Track I	Track II	Track III	Track IV	Track V	Track VI
Color	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green
Rf values	0.495	0.485	0.478	0.475	0.483	0.493
Δ Rf[1]	0.019	0.009	0	0	0.007	0.017

[1]Compared to the marker compound on tracks III and IV. (*) marker compound.

Table 3. The stability of the chromatographic results.

Marker (*)	Track a	Track b	Track c	Track d
Color	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green
Rf	0.439	0.438	0.452	0.420
Δ Rf[1]	0	0.001	0.013	0.019

[1]Compared to the marker zone on Track a. (*) marker compound.

The two-dimensional (2D) development was carried out to determine the stability of the samples during chromatography. A stable compound will have the same Rf value in the first and second development (Reich and Schibli, 2007). Fig. 4B shows that the chemical compounds were stable during the chromatographic process, as indicated by the appearance of a diagonal line on the 2D chromatogram.

The stability of the chromatographic results was determined by observing the color of the zones for 60 minutes. Fig. 4C and Table 3 show that the number and color of the zones were stable for 60 minutes. The difference between the Rf values of the marker zones was not larger than 0.05.

Precision test results

In the intraday precision test, the extract was chromatographed in duplicates, i.e., on three different plates on the same day. Fig. 5A and Table 4 show that the intraday precision of the method was acceptable since the ΔRf values of the marker zone (*) from one plate to another was not more than 0.05 (Reich and Schibli, 2007).

The interday precision test was carried out similarly to that of the intraday precision test.

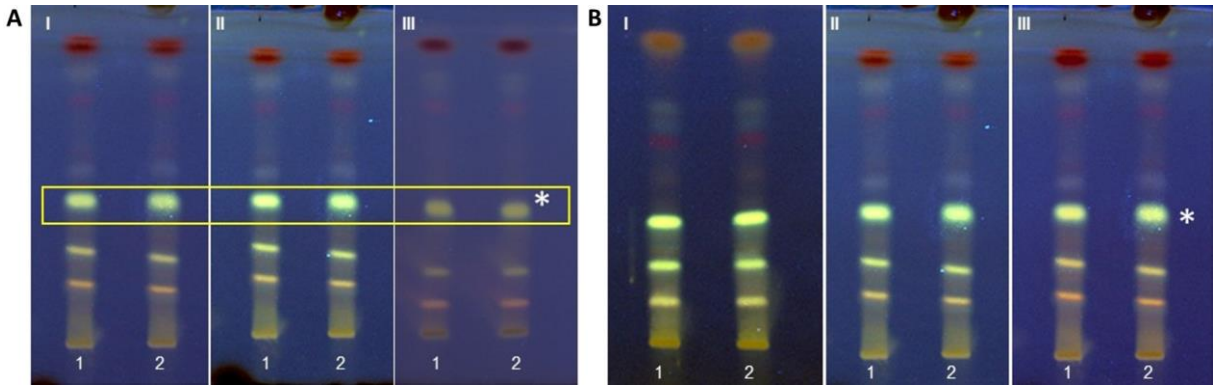


Figure 5. (A) The intraday precision test results. 1, 2 were the *C. longa* extracts applied in duplicates, while **I, II,** and **III** marked the different plates; **(B)** The interday precision test results. 1, 2 were the *C. longa* extracts applied in duplicates, while **I, II**, and **III** marked the plates developed on three different days. MP: $CHCL_3$, DCM, and EtOH (64:64:1). D: vanillin-sulfuric acid reagent, 366 nm UV light. (*) marker compound.

Table 4. The intraday precision test results.

Markers (*)	**Tracks**					
	I.1	**I.2**	**II.1**	**II.2**	**III.1**	**III.2**
Color	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green
Rf values	0.440	0.440	0.421	0.421	0.403	0.403
Mean Rf values	0.440		0.421		0.403	
Δ Rf	0.037					

(*) marker compound.

Table 5. The interday precision test results.

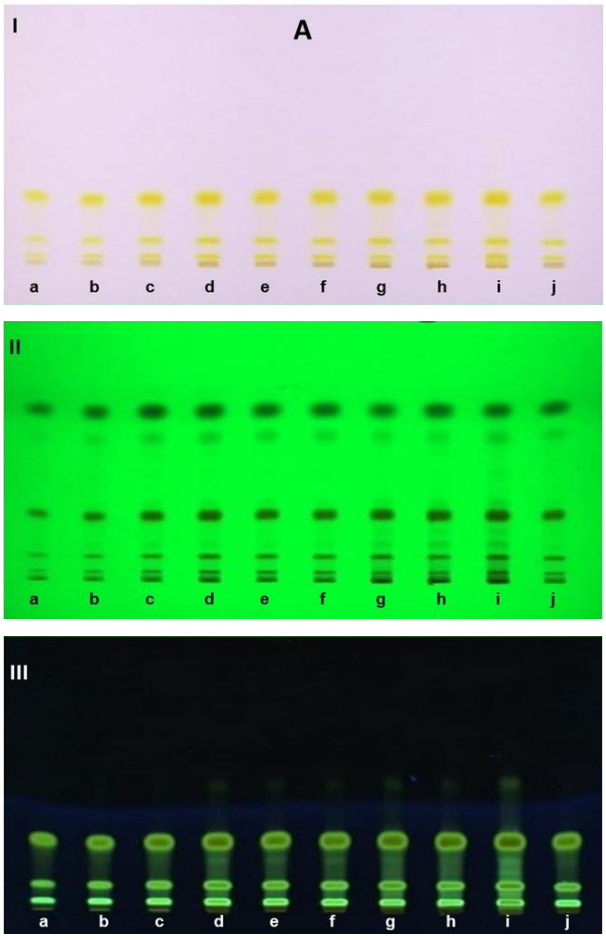
Marker (*)	**Track**					
	I.1	**I.2**	**II.1**	**II.2**	**III.1**	**III.2**
Color	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green	Yellowish-green
Rf values	0.440	0.440	0.427	0.427	0.452	0.452
Mean Rf values	0.440		0.427		0.452	
Δ Rf	0.03					

(*) marker compound.

However, plates I, II, and III were developed on three different days. Fig. 5B and Table 5 show that the interday precision of the method was acceptable because the ΔRf value of the marker (*) was not more than 0.05 (Reich and Schibli, 2007).

TLC-fingerprints of *C. longa* rhizomes collected from different origins

The TLC profiles of *C. longa* from 9 (nine) locations and one commercial sample before and after derivatization are shown in Fig. 6A-B. All samples



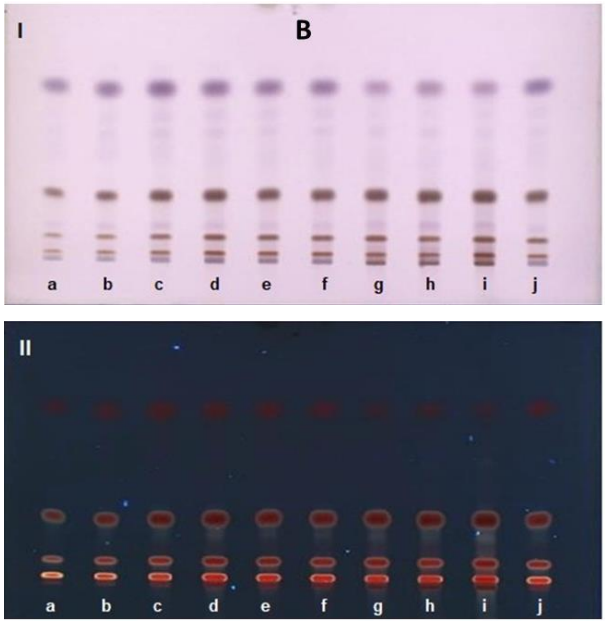


Figure 6. The TLC-fingerprints of the *C. longa* rhizomes collected from Surabaya **(a)**, Sidoarjo **(b)**, Gresik **(c)**, Pacet **(d)**, Trawas **(e)**, Pasuruan **(f)**, Batu **(g)**, Tawangmangu **(h)**, Bogor **(i)**, and commercially available product **(j)**. MP: $CHCL_3$, DCM, and EtOH (64:64:1). D: **(A)** None, white light (I), 254 nm UV light (II), 366 nm UV light (III); **(B)** Vanillin-sulfuric acid reagent, white light (I), 366 nm UV light (II).

show an almost similar pattern. Curcuminoids, as marked by three yellow bands, were detected on all samples. Tracks a and b (samples from Surabaya and Sidoarjo) presented smaller areas of curcuminoid than the others. This result is consistent with the color of the extract, as seen in Fig. 2.

Some purple bands were detected after derivatization with vanillin-sulfuric acid reagent (Fig. 6B.I). These bands were suspected as terpenoids. Tracks c, d, e, and f had more intensive purple bands than the others. Precise comparison of all chromatograms based on merely visual observation is nearly impossible. A more objective technique is required to overcome this issue.

Before the derivatization and visualization under 254 nm UV light, the TLC-fingerprints of the samples were transferred into a videodensitogram (Fig. 7) to show their Rf value, heights, and areas. The total peak number of each sample varied, with averagely 31 peaks, but showed major peaks at the Rf values of 0.1-0.2, 0.2-0.3, and 0.6-0.7. These peaks can be further studied and considered as a marker compound(s) for *C. longa*. Chemometric analysis was performed on these videodensitograms to compare the chemical components of *C. longa* more objectively.

Principal Component Analysis (PCA) results

The videodensitograms of the *C. longa* extracts from nine origins and one commercial sample were analyzed with a chemometric approach, viz., PCA. The height and area of each peak detected on the videodensitogram were then tabulated based on the origin of the sample (table is not shown). PCA with full cross-validation was applied to the data set from the ten fingerprints of *C. longa,* each with three replications. The height and area of the

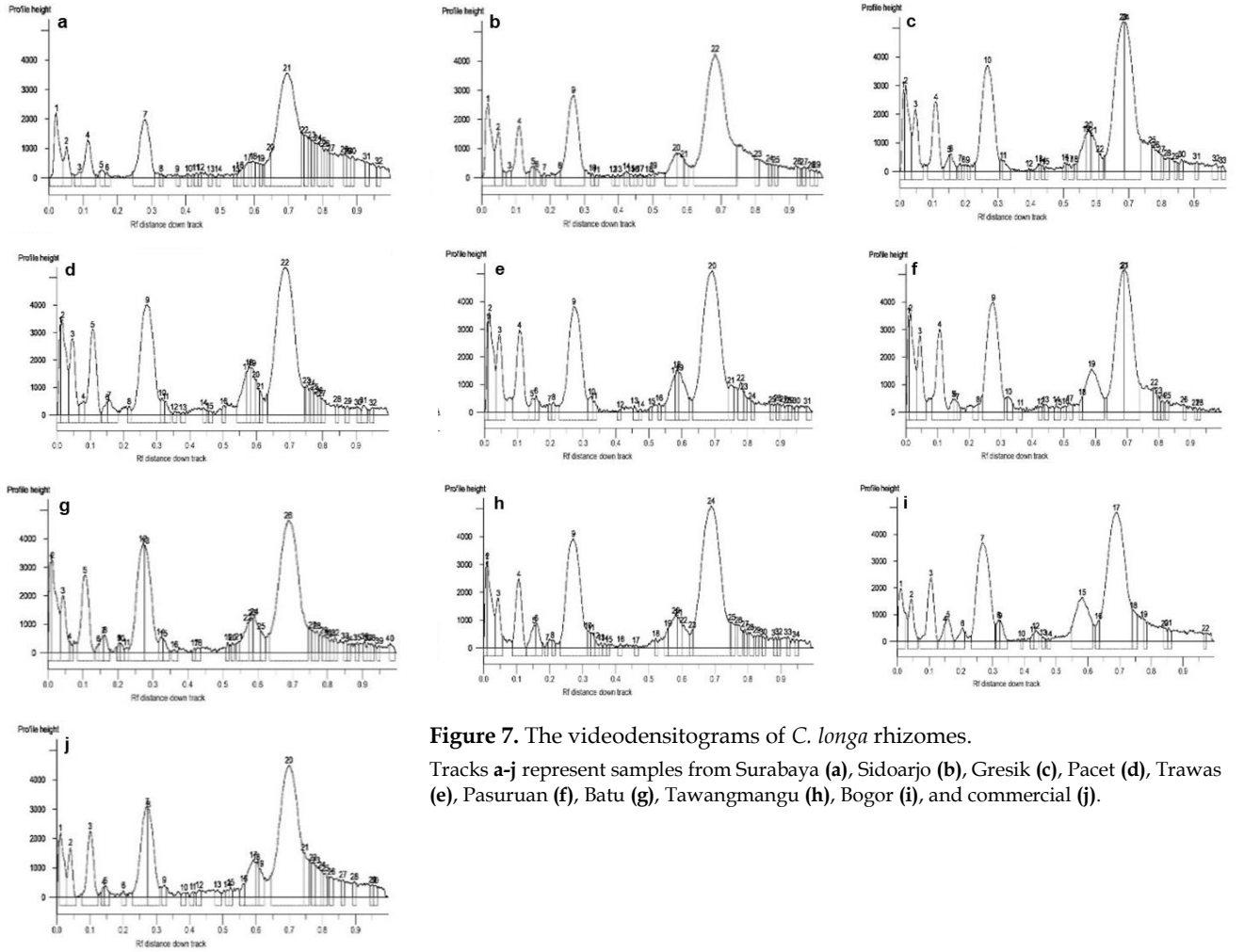


Figure 7. The videodensitograms of *C. longa* rhizomes.

Tracks **a-j** represent samples from Surabaya **(a)**, Sidoarjo **(b)**, Gresik **(c)**, Pacet **(d)**, Trawas **(e)**, Pasuruan **(f)**, Batu **(g)**, Tawangmangu **(h)**, Bogor **(i)**, and commercial **(j)**.

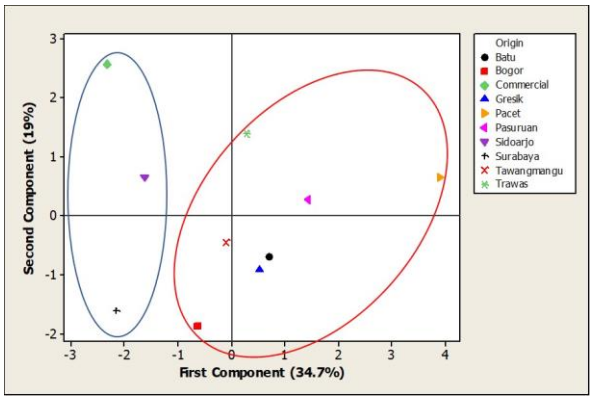


Figure 8. The PCA score plots of *C. longa* from different origins on the first two principal components.

peak in the full fingerprints were analyzed without any preprocessing. The score plot of the first two PCs (Fig. 8) clearly distinguished two groups of samples. The first group consisted of *C. longa* from Surabaya, Sidoarjo, and the commercial sample, whereas the second group included samples from Trawas, Tawangmangu, Bogor, Gresik, Batu, Pasuruan, and Pacet. Samples in Cluster 2 showed that the compounds with the Rf values of 0.6-0.7 were higher than those in Cluster 1. The *C. longa* samples from Surabaya, Sidoarjo, and Gresik represent the population in low-lying areas (0-100 m a.s.l.), while the others typify *C. longa* from moderately elevated altitudes to highlands (>700 m a.s.l.). These results indicate that the grouping of *C. longa* is apparently related to the height of the original location. Two from the three samples col-

lected from low-lying areas were grouped into Cluster 1, but one of them belonged to Cluster 2, the majority of the cluster members was the samples from highland. Because of this finding, further study is needed to investigate other contributing factors. Soil type, rainfall, sunlight, and fertilizer application are predicted to be responsible for the samples grouping (Kartini and Azminah, 2012).

Clustering Analysis (CA) results

Cluster analysis was made as an attempt to derive groups from the autoscaled dataset and to compare them with the previous ones from the PCA. Two main components were taken as a basis for classification. The clusters formed (Fig. 9) showed different characteristics in *C. longa*. In this study, the CA used a complete linkage with the Euclidean distance and found that *C. longa* could be divided into two clusters, as in the PCA. The first cluster consisted of crude drugs originating in Surabaya (1), Sidoarjo (2), and commercial sample (10), while the second cluster included samples from Gresik (3), Pacet (4), Trawas (5), Pasuruan (6), Batu (7), Tawangmangu (8), and Bogor (9). A high similarity was observed between the results of the PCA and CA, confirming that the fingerprint and PCA analysis methods established in this paper are reliable for assessing the quality of *C. longa*.

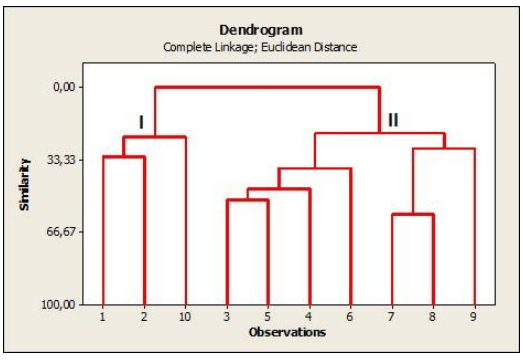


Figure 9. The dendrogram resulted from a complete linkage in the cluster analysis.

The number represents the origin of the sample: Surabaya **(1)**, Sidoarjo **(2)**, Gresik **(3)**, Pacet **(4)**, Trawas **(5)**, Pasuruan **(6)**, Batu **(7)**, Tawangmangu **(8)**, Bogor **(9)**, and commercial **(10)**.

CONCLUSIONS

The TLC method developed in this study fulfills the validation parameters for TLC-fingerprint, especially in terms of stability and precision. When combined with PCA and CA, TLC-fingerprinting can discriminate between the rhizomes of *Curcuma longa* originated in various locations. The development of a simple analytical method for the geographical provenance of *C. longa* will positively affect the quality control of this herbal material, which will ultimately guarantee the safety and efficacy of the product.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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REFERENCES

Akram M, Shahab-Uddin AA, Usmanghani K, Hannan A, Mohiuddin E, Asif M (2010) *Curcuma longa* and curcumin: a review article. Rom J Biol Plant Biol 55: 65–70.

Amalraj A, Pius A, Gopi S, Gopi S (2017) Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–A review. J Tradit Complement Med 7: 205–233.

Bansal A, Chhabra V, Rawal RK, Sharma S (2014) Chemometrics: a new scenario in herbal drug standardization. J Pharm Anal 4: 223–233.

Brereton RG (2003) Chemometrics: data analysis for the laboratory and chemical plant: John Wiley & Sons.

Daily JW, Yang M, Park S (2016) Efficacy of turmeric extracts and curcumin for alleviating the symptoms of joint arthritis: a systematic review and meta-analysis of randomized clinical trials. J Med Food 19: 717–729.

Deshpande SS, Ingle AD, Maru G (1997) Inhibitory effects of curcumin-free aqueous turmeric extract on benzo [a] pyrene-induced forestomach papillomas in mice. Cancer Lett 118: 79–85.

Deshpande SS, Ingle AD, Maru GB (1998) Chemopreventive efficacy of curcumin-free aqueous turmeric extract in 7, 12-dimethylbenz [a] anthracene-induced rat mammary tumorigenesis. Cancer Lett 123: 35–40.

Gad HA, El-Ahmady SH, Abou-Shoer MI, Al-Azizi MM (2013) Application of chemometrics in authentication of herbal medicines: a review. Phytochem Anal 24: 1–24.

Gupta SC, Kismali G, Aggarwal BB (2013) Curcumin, a component of turmeric: from farm to pharmacy. Biofactors 39: 2–13.

Kartini, Azminah (2012) Chromatographic fingerprinting and clustering of *Plantago major* L. from different areas in Indonesia. Asian J Pharm Clin Res 5: 191–195.

Kim JH, Gupta SC, Park B, Yadav VR, Aggarwal BB (2012) Turmeric (*Curcuma longa*) inhibits inflammatory nuclear factor (NF)-κB and NF-κB-regulated gene products and induces death receptors leading to suppressed proliferation, induced chemosensitization, and suppressed osteoclastogenesis. Mol Nutr Food Res 56: 454–465.

Komsta Ł (2012) Chemometrics in fingerprinting by means of thin layer chromatography. Chromatography Research International 2012: 893246.

Niranjan A, Prakash D (2008) Chemical constituents and biological activities of turmeric (*Curcuma longa* L.)-a review. J Food Sci Technol 45: 109–116.

Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K (2005) Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. J Agric Food Chem 53: 959–963.

Paulucci VP, Couto RO, Teixeira CC, Freitas LaP (2013) Optimization of the extraction of curcumin from *Curcuma longa* rhizomes. Rev Bras Farmacogn 23: 94–100.

Reich E, Schibli A (2007) High-performance thin-layer chromatography for the analysis of medicinal plants. New York: Thieme, pp. 280.

Ri D (2008) Farmakope Herbal Indonesia Edisi I. Jakarta: Departemen Kesehatan Republik Indonesia.

Rohaeti E, Rafi M, Syafitri UD, Heryanto R (2015) Fourier transform infrared spectroscopy combined with chemometrics for discrimination of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar*. Spectrochim Acta A Mol Biomol Spectrosc 137: 1244–1249.

Sogi D, Sharma S, Oberoi D, Wani I (2010) Effect of extraction parameters on curcumin yield from turmeric. J Food Sci Technol 47: 300–304.

Suryanarayana P, Saraswat M, Mrudula T, Krishna TP, Krishnaswamy K, Reddy GB (2005) Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 46: 2092–2099.

Tistaert C, Dejaegher B, Heyden YV (2011) Chromatographic separation techniques and data handling methods for herbal fingerprints: a review. Anal Chim Acta 690: 148–161.

Wakte PS, Sachin B, Patil A, Mohato D, Band T, Shinde D (2011) Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Sep Purif Technol 79: 50–55.

Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P (2009) A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochem Anal 20: 314–319.

Yang J, Chen LH, Zhang Q, Lai MX, Wang Q (2007) Quality assessment of *Cortex cinnamomi* by HPLC chemical fingerprint, principle component analysis and cluster analysis. J Sep Sci 30: 1276–1283.

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Contribution	Kartini K	Andriani YA	Priambodo W	Jayani NIE	Hadiyat MA
Concepts or ideas	x			x	x
Design	x			x	x
Definition of intellectual content	x				
Literature search	x	x	x	x	x
Experimental studies	x	x	x	x	
Data acquisition	x	x	x	x	
Data analysis	x			x	x
Statistical analysis					x
Manuscript preparation	x			x	
Manuscript editing	x				x
Manuscript review	x	x	x	x	x

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