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Analysis of Curcumin in Ethanolic Extract of *Curcuma longa* Linn. and *Curcuma xanthorrhiza* Roxb. Using High Performance Liquid Chromatography with UV-Detection

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ABSTRACT

A simple and isocratic liquid chromatographic using UV detection at wavelength of 425 nm have been validated and used for quantitative analysis of curcumin in ethanolic extract of turmeric (*Curcuma longa* Linn.) and *Curcuma xanthorrhiza* (Zingiberaceae), indigenous to Java region, Indonesia. The method was optimized for separation of three curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin using Waters Xterra MS C18 column (4.6×250 mm, 5 µm). The mobile phase used consisted of aquabidestilata and acetonitrile (65:35 v/v) containing 1% acetic acid. The analytical method was validated in terms of linearity, sensitivity, precision and accuracy. The developed method was linear over the curcumin concentration range of 10-60 µg mL⁻¹ with correlation coefficient value of 0.999. The precision of the developed method expressed as Relative Standard Deviation (RSD) value was in the range of 0.17-1.17% for three different levels of sample. The recoveries obtained for the accuracy assessment were 8.50-101.23%. The sensitivity of analytical method was expressed with Limit of Detection (LoD) and Limit of Quantification (LoQ). The values of LoD and LoQ were 1.13 and 3.34 µg mL⁻¹, respectively. The method was successfully used for quantitative analysis of curcumin in the rhizomes of *Curcuma longa* and *Curcuma xanthorrhiza*. The levels of curcumin found in those rhizomes are in the range of 3.03±0.01-7.31±0.02 (*C. longa*) and in the range of 1.69±0.02-4.92±0.01 (*C. xanthorrhiza*).

Key words: Validation, curcumin, HPLC, *Curcuma longa* Linn., *Curcuma xanthorrhiza*

INTRODUCTION

About 100 *Curcuma* species are native to South-East Asia, India and China. In Indonesia, turmeric (*Curcuma longa* L.) is known under about 80 different local names, of which kunir and kunyit are most commonly used, while *Curcuma xanthorrhiza* is known with local name of Temulawak (De Padua *et al.*, 1999; Bos *et al.*, 2007). *Curcuma longa* and *C. xanthorrhiza* are belonging to Zingiberaceae family and widely cultivated in the regions of tropical and subtropical, especially in India, Southeast Asia and China. Today, the species cultivation has also widely distributed to some African countries (Parthasarathy *et al.*, 2008).

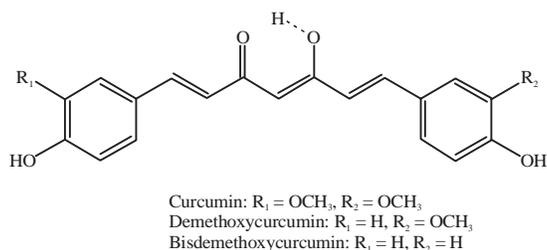


Fig. 1: Chemical structures of curcumin, demethoxycurcumin and bisdemethoxycurcumin

The main components of *C. longa* and *C. xanthorrhiza* are curcuminoids which refer to the group of phenolic substances namely curcumin, demethoxycurcumin and bisdemethoxycurcumin (Wichitnithad *et al.*, 2009; Rohman, 2012). Figure 1 depicted the chemical structures of these curcuminoids. The contents of curcuminoids especially curcumin were used as one of the parameters in quality control of *C. longa* and *C. xanthorrhiza* and other drugs derived from plant-based *Curcuma* (Ministry of Health, Republic of Indonesia, 2009; Cheng *et al.*, 2010). Some biological activities have been reported in *C. longa* and *C. xanthorrhiza* such as antioxidant (Kalpravidh *et al.*, 2010; Samsudin and Panigoro, 2013), anti-inflammatory (Ozaki, 1990; Skrzypczak-Jankun *et al.*, 2000), anti-atherogenic (Ramirez-Bosca *et al.*, 2000) and anticancer effects as reviewed by Aggarwal *et al.* (2003). These effects are associated with the levels of curcuminoid, especially curcumin, therefore, the determination of curcumin is very important to assure the standardization of *C. longa* and *C. xanthorrhiza* used in traditional medicine.

Numerous analytical methods have been validated and used for quantitative analysis of curcumin as well as curcuminoids. Some of the methods are based-spectrophotometric techniques namely UV-Vis spectroscopy based on the intensive absorption intensity at wavelength of 420-430 nm (Jayaprakasha *et al.*, 2005; Scotter, 2009) and near infrared spectroscopy at regions of 1500-2500 and 1850-2040 nm for quantification of individual and total curcuminoids (Tanaka *et al.*, 2008). However, using spectroscopic techniques, it is not possible to separate curcuminoids individually (Jayaprakasha *et al.*, 2002). For this reason, chromatographic based techniques are among the methods of choice for determination of curcumin and curcuminoids attributed to their separation capacities.

Some authors have used chromatographic techniques for quantification of curcumin, namely Thin Layer Chromatography (TLC) for separation and semi-quantitative of individual curcuminoids (Anderson *et al.*, 2000; Zhang *et al.*, 2008), high performance liquid chromatography using UV-vis detector (Jadhav *et al.*, 2007), fluorescence detection at wavelength of 426 nm (excitation) and 539 nm (emission) (Zhang *et al.*, 2009) and mass spectrometer detection (Inoue *et al.*, 2003; Jiang *et al.*, 2006). Some of the reported methods have several disadvantages, including unsatisfactory separation times, poor resolution and/or complicated solvent mixtures in plant extract. In this study, optimize HPLC with UV-Vis detection for analysis of curcumin in ethanolic extract of *C. longa* and *C. xanthorrhiza* using simple mixture of mobile phase consisted of aquabidestilata and acetonitrile.

MATERIALS AND METHODS

The standard of curcumin was obtained from synthesis and is kindly given by Prof. Dr. Sudibyo Martono from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada

University, Indonesia. The mixture of curcuminoids, namely curcumin, demethoxycurcumin and bisdemethoxycurcumin is obtained from Sigma (Aldrich, St. Louis, USA) with the purity of > 99%. Acetonitrile and methanol used were of HPLC grade.

Plant materials: The fresh rhizomes of *Curcuma longa* Linn. and *Curcuma xanthorrhiza* Roxb were obtained from local markets in Yogyakarta (Kulonprogo, Kranggan, Bantul, Godean, Purworwjo) and Central Java (Magelang, Boyolali, Purworejo) in September-October 2013. All rhizome samples were cleaned, cut and air-dried and finally powdered.

HPLC condition: HPLC analysis was performed using Waters HPLC system (Waters Corp., USA), consisting pump (Waters), ACQUITY solvent manager, waters alliance column heater, Vial Amber Glass 12×32 mm 2 mL with Cap and PTFE/silicone Septum, Water 2767 sample manager and operating software of Empower Basic 2 (Waters, USA). Separation of curcuminoids is performed using C₁₈ Waters Xterra MS C18 (5 µm; 4.6×250 mm). The mobile phase used consisted of aquabidestilata and acetonitrile (65:35 v/v) containing 1% acetic acid. The analyte detection was done using UV-Vis detector at λ 425 nm. The injection volume was 20 µL and running time was 40 min.

Sample preparation: Ground *Curcuma longa* Linn and *Curcuma xanthorrhiza* Roxb. samples (100 g) were extracted with ethanol (1 L) using maceration technique for 4 days. The ethanol solution was concentrated using a vacuum rotary evaporator (Büchi Labortechnik AC, Flawil, switzerland). The extract was dried on conventional oven until reach constant weight. The extract was redissolved in mobile phase and an aliquot of 2.0 mL is taken, altered through an HPLC alter and placed in an autosampler vial of 2 mL.

Analytical method validation: The HPLC method was validated according to International Conference Harmonization (ICH, 1996) by assessing several parameters namely: selectivity, linearity and range, sensitivity expressed with Limit of Detection (LoD) and Limit of Quantification (LoQ), precision and accuracy.

RESULTS AND DISCUSSION

Figure 2 exhibited the chromatogram obtained during the separation of curcuminoid (curcumin, demethoxycurcumin and bisdemethoxycurcumin) using the optimized condition as in HPLC condition. System suitability test revealed that % Relative Standard Deviation (RSD) values for retention time and peak area is lower than 2%, indicating low variation of the measured values (Wichitnithad *et al.*, 2009). The tailing factors (T) for bisdemethoxycurcumin, demethoxycurcumin and curcumin were 0.9797, 0.964 and 0.982, respectively, demonstrating symmetry of all peaks (T<2). The resolution (R) between bisdemethoxycurcumin, demethoxycurcumin and curcumin was 2.960 and 3.002, respectively, indicating a high degree of peak separation (R>2), as compiled in Table 1. The efficiency of the column, as expressed by the number of theoretical plates, was more than 2000. These results indicate the suitability of the HPLC system and conditions, which were subsequently used for analytical validation and sample analysis.

The developed method was validated in terms of selectivity, linearity, sensitivity, precision and accuracy. Based on Fig. 2, it can be stated that the optimized HPLC condition is selective enough capable of separating three curcuminoids with resolution (R) of > 2.0. The linear dynamic range

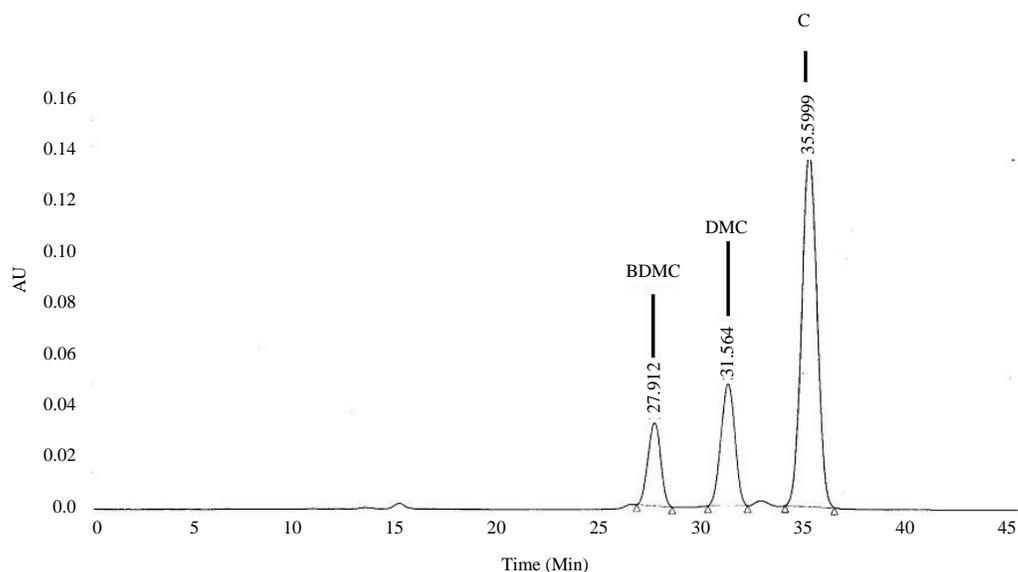


Fig. 2: Chromatogram obtained during the separation of bisdemethoxycurcumin (RT = 27.912), demethoxycurcumin (RT = 31.56) and curcumin (RT = 35.599), using the optimized condition, RT = Retention time

Table 1: System suitability test for the separation of curcuminoids (n = 5)

Analyte(s)	Retention time	Asymmetric factor	Resolution
Bisdemethoxycurcumin	27.91	0.9797	2.960
Demethoxycurcumin	31.56	0.964	2.960
Curcumin	35.59	0.982	3.002

was evaluated at the concentration of curcumin at level of $10\text{-}60\ \mu\text{g mL}^{-1}$. A good correlation was observed for the relationship between concentration of curcumin and peak area with coefficient of correlation (r) of 0.999 (Fig. 3). Limit of Detection (LoD) and Limit of Quantification (LoQ) were computed based on the regression equation, as in Miller and Miller (2005). The LoD and LoQ values obtained were 1.13 and $3.34\ \mu\text{g mL}^{-1}$, respectively.

The precision of the method was evaluated using repeatability assay based on the RSD values peak area from three different levels. The results showed that RSD values were in the range of 0.17-1.17%. These results suggested that the proposed method indicates good precision with RSD values $< 8\%$ (Gonzalez and Herrador, 2007). Accuracy was performed by determining the recovery percentage using standard addition method by spiking 80, 100 and 120% of the target analytes. The mean recoveries obtained were in the range of 98.50-101.23%. These values were in agreement with those required by AOAC PVM. It can be stated that the developed method is accurate enough for quantitative analysis of curcumin in ethanolic extract of *C. longa* and *C. xanthorrhiza*.

The validated method was subsequently used for quantification of curcumin in the samples of ethanolic extract of *C. longa* and *C. xanthorrhiza*. Table 2 compiled the analytical results of curcumin in both ethanolic extracts. The levels of curcumin found in those rhizomes are in the range of $3.03\pm 0.01\text{-}7.31\pm 0.02$ (*C. longa*) and in the range of $1.69\pm 0.02\text{-}4.92\pm 0.01$ (*C. xanthorrhiza*). In general, the curcumin level in *C. longa* is higher than that in *C. xanthorrhiza* as reported by several investigators.

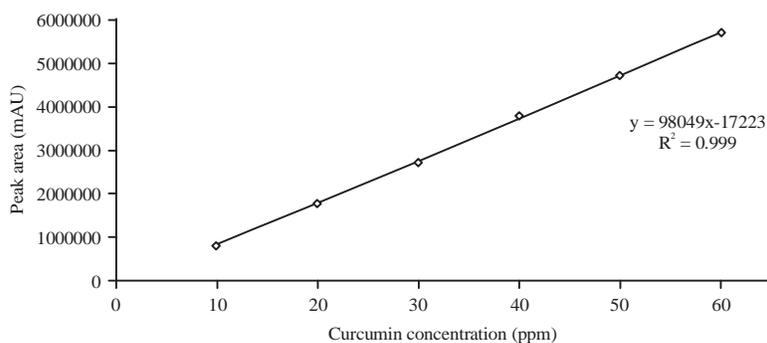


Fig. 3: Calibration curve for the relationship between curcumin concentration and its peak area showing high correlation coefficient

Table 2: Levels of curcumin in ethanolic extracts of *Curcuma longa* L. and *Curcuma xanthorrhiza* Roxb. from Yogyakarta and Central Java Regions

Regions	Kandungan kurkumin (% b/b)*
<i>C. longa</i> Kulonprogo, Yogyakarta	4.82±0.03
<i>C. longa</i> Bantul, Yogyakarta	4.71±0.03
<i>C. longa</i> Kranggan, Yogyakarta	3.03±0.01
<i>C. longa</i> Godean, Yogyakarta	6.02±0.05
<i>C. longa</i> Bringharjo, Yogyakarta	4.41±0.03
<i>C. longa</i> Purworejo, Central Java	6.95±0.09
<i>C. longa</i> Magelang, Central Java	7.31±0.02
<i>C. longa</i> Boyolali, Central Java	5.63±0.04
<i>C. xanthorrhiza</i> Bantul, Yogyakarta	2.96±0.02
<i>C. xanthorrhiza</i> Kranggan, Yogyakarta	4.92±0.01
<i>C. xanthorrhiza</i> Godean, Yogyakarta	2.88±0.03
<i>C. xanthorrhiza</i> Bringharjo, Yogyakarta	2.67±0.07
<i>C. xanthorrhiza</i> Purworejo, Central Java	1.69±0.02

*Expressed as Mean±SD from three replicates

CONCLUSION

In this study, a simple reversed phase liquid chromatographic using UV detection at wavelength of 425 nm is used for quantitative analysis of curcumin in ethanolic extract of *Curcuma longa* Linn. and *Curcuma xanthorrhiza* Roxb. The validation parameters revealed that the used methods comply with those required by International Conference on Harmonisation (ICH). The levels of curcumin found in those rhizomes are in the range of 3.03±0.01 to 7.31±0.02 (*C. longa*) and in the range of 1.69±0.02-4.92±0.01 (*C. xanthorrhiza*).

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