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Research Article

CURCUMINOIDS ANALYSIS IN *CURCUMA MANGGA* RHIZOMES

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ABSTRACT

Objective: The current study was conducted to analyze the curcuminoid content in ethanol extract of *Curcuma mangga.*

Methods: The constituent of ethanol extract of *C. mangga* was determined using ultra-performance liquid chromatography (UPLC). The diluted solutions of the extracts and the reference standards were analyzed separately by the reversed-phase UPLC method with mobile phase of acetonitrile: water (40:60) isocratically eluted for 30 min.

Results: The chromatograms of the ethanol extract of *C. mangga* showed the presence of curcumin, bisdemethoxycurcumin, and demethoxycurcumin (DMC). Of all the curcuminoids content, DMC was the major component, corresponding to retention time at 9.96 min as compared to curcuminoid standard at 9.69 min.

Conclusion: The results indicate that ethanol extract of *C. mangga* rhizomes contains DMC at the highest amount as compared to other curcuminoid compounds.

Keywords: *Curcuma mangga*, Curcumin, Bisdemethoxycurcumin, Demethoxycurcumin, Ultra-performance liquid chromatography.

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INTRODUCTION

Curcuma mangga is widely distributed in tropical countries such as Thailand, Malaysia, and Indonesia. The length and width of its leaves is ranging from 15–50 cm to 5–15 cm, respectively. The rhizome is yellow in color and smell like mango [1]. *C. mangga* is a species of *Curcuma* which has been used as traditional medicine. It possesses a wide array of biological effects of *C. mangga* including nitric oxide inhibitory, analgesic, antifungal, anticancer, anti-inflammatory, and antioxidant activities [2-5].

C. mangga was found to contain various organic compounds including polyphenols, flavonoids, triterpenes, and sterols [6,7]. The plant was reported to have curcuminoid as its major components; these include curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Others *Curcuma* species also contain the major curcuminoid. Previous studies reported the presence of curcumin and the absence of BDMC in *Curcuma xanthorrhiza*, while BDMC was found in *C. longa* [8].

The observed pharmacological effects of *Curcuma* species have been attributed to curcuminoid content. Previous studies have shown various pharmacological effects of curcuminoids. Curcumin was reported to have antioxidant, anti-inflammatory, antimutagenic, and immunomodulatory activities [8]. In addition, *C. mangga* was reported to stimulate phagocytosis activity by *in vivo* study [9]. DMC and BDMC were found to inhibit nitric oxide production [3]. However, the precise amount of curcuminoid in *C. mangga* rhizomes was rarely reported.

In the present study, ethanol extract of *C. mangga* was investigated for its curcuminoid content including curcumin, DMC, and BDMC using ultra-performance liquid chromatography (UPLC). The results of this study may provide some insight of the major active component which contribute to the biological activities of *C. mangga* rhizomes.

METHODS

Chemicals and reagents

The chemicals used in this study were ethanol (SmartLab, Indonesia), acetonitrile HPLC grade (Merck, UK), and methanol HPLC grade (Merck, UK). Curcumin ≥94% (curcuminoid content), ≥80% (curcumin) was obtained from Sigma-Aldrich (USA). Constituent determination was performed using ultra-performance liquid chromatography (UPLC) from Agilent Technologies (US), Millipore Millex PTFE membrane (0.45 µm) (Whatman, UK), and C-18 column Eclipse Plus (100 mm × 4.6 mm i.d., 3.5 µm) (Agilent Technologies, US). Detector used was DAD (Agilent 1290).

Plant collection

The *C. mangga* rhizomes were collected from Medan, Sumatera Utara, Indonesia. Then, the plant was authenticated in Herbarium Medanense, Universitas Sumatera Utara, Indonesia.

Extraction procedure

The rhizome materials were allowed to dry under shade at room temperature. The dried material (500 g) was ground and macerated and then subjected to extraction with ethanol at the ratio of 1:10 (w/v). The extraction was repeated twice on the residue. The filtrates were combined and the solvent was removed under reduced pressure to obtain extract of *C. mangga* (38.4 g, 10.95% w/w).

UPLC analysis of the ethanol extract of *C. mangga*

UPLC quantitative analysis of the ethanol extract of *C. mangga* was conducted by the modified method of Ang *et al.* [10]. The ethanol extract and the reference standards (quercetin) were dissolved in methanol to obtain 20 and 1 mg/mL solutions, respectively. The diluted solutions were filtered through Millipore Millex PTFE membrane (0.45 µm) (Whatman, UK). UPLC qualitative and quantitative analyses of the filtered solutions were carried out using a C-18 column (100 mm × 4.6 mm i.d., 3.5 µm).

Table 1: Curcuminoid content of *C. mangga* rhizomes

Curcuminoid	Amount (µg/mL)
Curcumin	12.01
DMC	450.53
BDMC	329.45

C. manga: Curcuma manga, DMC: Demethoxycurcumin, BDMC: Bisdemethoxycurcumin

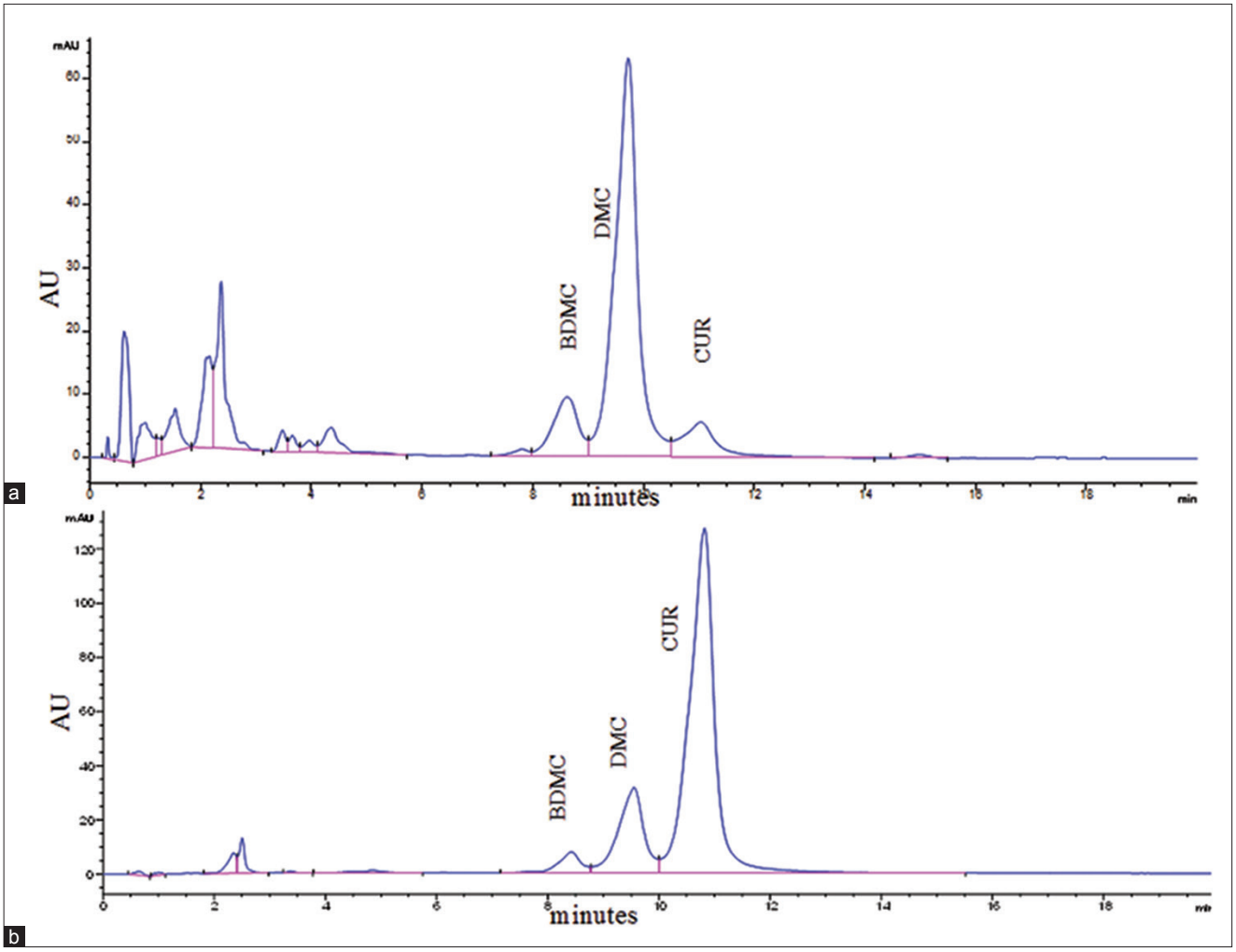

Fig. 1: Representative ultra-performance liquid chromatography chromatograms of (a) *Curcuma mangga* ethanol extract (b) reference standard mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin RT 10.88, 9.62, and 8.50 min, respectively, detected at the wavelength of 370 nm

Table 2: Precisions for interday and intraday repetitions for the quantitative detection of curcumin, DMC, and BDMC

Parameters	Interassay precision						Intra-assay precision					
	CUR		DMC		BDMC		CUR		DMC		BDMC	
	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area
Mean	10.88	4004.8	9.62	994.7	8.5	208.8	10.94	3551	9.68	896.6	8.56	172.7
SD	0.06	453.65	0.06	93.29	0.06	32.19	0.01	17.6	0.01	4.65	0.01	0.91
%RSD	0.56	11.33	0.67	9.38	0.76	15.42	0.09	0.49	0.17	0.52	0.17	0.53

DMC: Demethoxycurcumin, BDMC: Bisdemethoxycurcumin, SD: Standard deviation, RSD: Relative standard deviation, RT: Retention time, CUR: Curcumin

The mobile phase were acetonitrile (40%): water (60%) and eluted isocratically for 5 min at a flow rate of 1.3 mL/min. Detector used was DAD (Agilent 1290), wavelength: 370 nm. Quercetin was identified by comparing the ultraviolet-visible spectra and retention time of the peak and those of the standard. Quantification of the compounds in the extract was carried out by plotting calibration curves of standard solution with four different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) versus the areas under the peaks.

Validation procedure

Linearity, precision, limits of quantification (LOQ), and limits of detection (LOD) were determined to validate the reversed-phase UPLC method. The precision of the method was determined by interassay and intra-assay validation. Linearity was determined by linear calibration analysis while the calibration curves were used to calculate the correlation coefficient (r2). LOQ and LOD were calculated from the RSD and slope (S) of the calibration curves using equations,

$$LOQ = 3.3 \times (RSD/S)$$

$$LOQ = 10 \times (RSD/S)$$

RESULTS AND DISCUSSION

Quantitative analysis of the ethanol extract of *C. mangga* by UPLC

Curcumin was in a mixture with DMC and BDMC as compared with previous studies [10]. The chromatogram of the ethanol extract of *C. mangga* showed major peak of curcuminoid, which include curcumin, DMC, and BDMC with retention times at min 11.19, 9.96, and

8.44 min, respectively. The peak was compared to reference standards curcuminoid with retention times at 10.88, 9.62, and 8.50 min, respectively (Fig. 1). Table 1 summarizes that DMC (450 µg/mL) was the most abundant compound as compared to other curcuminoid. The result was in agreement with previous study which was able to isolate DMC from *C. mangga* rhizomes, indicating the high amount of DMC in *C. mangga* [11].

Method validation

Calibration curves plotted were linear over the concentration range 100–6.25 µg/mL with a correlation coefficient (r^2) of 0.998, 0.996, and 0.980 for curcumin, DMC, and BDMC, respectively. These %RSD values of intra-assay precision of peak area and retention time demonstrated the reproducibility of the results (Table 2). LOD of curcumin, DMC, and BDMC were found to be 1.14, 20.24, and 103.29 µg/mL, respectively. Meanwhile, LOQ of curcumin, DMC, and BDMC were found to be 3.44, 59.12, and 312.99 µg/mL, respectively.

CONCLUSION

The HPLC analysis methods proposed in this study enable identification and quantification of major curcuminoids in the ethanol extracts of *C. mangga*. These include curcumin, BDMC, and DMC. Of all the curcuminoid identified, DMC presents in highest amount in *C. mangga* rhizomes. However, further studies using various techniques are required to elucidate others constituent.

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