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Quantitative analysis of Curcuminoid collected from different location in Indonesia by TLC-Densitometry and its antioxidant capacity

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demethoxycurcumin Curcuminoid, consisting of curcumin, Abstract. and bis demethoxycurcumin, is the active compound in Curcuma longa L. rhizome. It yielded vary depend on the environment and varieties. Curcumin has been known to have a potent antioxidants activity. The present work was conducted to assess the curcuminoid content and antioxidant capacity in the crude extracts of C. longa L. collected from eight different locations in Indonesia. Samples were collected from eight locations including Java island, Sumatera island and Nusa Tenggara island. All samples were extracted using 96% ethanol and analyzed quantitatively using TLC-Densitometry. Antioxidant activity was assessed using diphenylpicrylhydrazyl (DPPH) radical scavenging assay and analyzed at 517 nm using a spectrophotometer. Curcuminoid content in C. longa varied among eight different locations (0.53±0.05 - 5.33±0.12 % w/w). The highest curcumin, demethoxycurcumin and bisdemethoxycurcumin yield were found in the samples from Magetan (5.33±0.12%, 1.54±0.05%, 0.46±0.02% w/w, respectively). In contrast, the lowest curcumin, demethoxycurcumin and bisdemethoxycurcumin yield were found in the sample from Demak (0.53±0.05%, 0.17±0.05%, 0.17±0.05% w/w, respectively). Antioxidant capacity showed similar for all places unless sample from Magetan which exhibited two times lower than other locations. It is apparently curcuminoid content and antioxidant activity varied among places. These results are useful information for curcuminoid standardization method in pharmaceutical products.

1. Introduction

Curcuma longa L. is a rhizomatous small perennial plant belongs to Zingiberaceae family. It is a popular herb used as a household medicine [1], dye [2] and seasoning in East Asian countries for many years. Curcuminoid, i.e. curcumin, demethoxycurcumin and bisdemethoxycurcumin are active constituents in C. longa L. These have been studied as antioxidant [3], antiinflamatory, antimicrobial effects [4].

C. longa L. has been widely cultivated in Indonesia. Total curcuminoids should have more than 5% (w/w) of dried C. longa L. as recommended by Standard of ASEAN Herbal Medicine (1994). Curcuminoid content varied in *C.longa* grown in India [6] and in Thailand [7]. The difference of curcuminoid yield among the various lines of C. longa was due to variation of genotype-phenotype [8]

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and geography [6]. Thus, *C.longa* rhizome which grow in different geography need to be further investigated to identify curcuminoid yield.

Curcumin were previously reported as antioxidant activity [9], [10]. Antioxidant can decrease oxidative stress due to excessive free radicals and reactive oxygen species (ROS). These free radicals could lead to detrimental diseases such as cancer [11]. Antioxidant activity of curcumin has been shown due to either the β -diketone group in its structure (Wright, 2002; Priyadarsini et al., 2003). Curcumin could enhance the activity of many antioxidant enzymes such as heme oxygenase [14], catalase, superoxide dismutase (SOD) and glutathione peroxidase [15]. Curcumin as the major compound present in *C. longa* L. and its analogs showed strong relationships to the structure and antioxidant activity [16]. However, antioxidant activity of curcuminoids in the extract could have either enhance or decrease due to synergistic, additive or antagonistic effect of mixtures. Thus, this study aimed (1) to quantify curcuminoids in the extract of *C. longa* L. and (2) to measure the antioxidant activity of *C. longa* L. extracts. Curcuminoid quantitative analysis used thin layer chromatography (TLC)-Densitometric due to its rapid, simple and reliable method as shown by Pothitirat and Gritsanapan (2005).

2. Materials and methods

2.1. Chemicals and reagents

Curcuminoid standard including curcumin, demethoxycurcumin and bisdemetoxycurcumin obtained from CV. Chemonix Pratama as commercial standard. All chemicals and reagents used in this research were analytical grade.

2.2. Plant material

The rizhomes of *C. longa* were collected from eight different locations in Indonesia i.e. Wonogiri, Sukoharjo, Magetan, Demak, Banten, Nusa Tenggara Timur, Nusa Tenggara Barat and Jambi.

2.3. Preparation of sample solutions, standard solutions and calibration curves

A sample of 50 mg of dry material was transferred to a microtube to which 500 ul of ethanol 96% were added. The mixture was vortexed at room temperature for 3 min and centrifuged at 30.000 rpm for 20 min. The supernatant (5 ul) was taken for TLC-densitometry. Standard curves of curcuminoid were prepared in ethanol at 2000, 1000, 500, 250 and 125ppm. Five microliters of each curcuminoid solutions were spotted into the precoated silica gel aluminium plate 60GF₂₅₄ (20 x 10 cm; E. Merck, Germany). Plate was developed using saturated mobile phase of dichloromethane : methanol (97:3). The length of each chromatogram was 8 cm. densitometric scanning was performed on Camag TLC Scanner 4 in the reflectance-absorbance mode at 420 nm, perated by CATS software. The amount of each curcuminoid content was calculated using calibration curve. The content of curcumin was expressed as 100 grams of the dried sample.

2.4. Antioxidant activity, DPPH (diphenylpicrylhdrazyl) radical scavenging assay

Antioxidants react with DPPH, a stable fee radical which is reduced to DPPH-H and as a consequence the absorbance is decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

The free radical scavenging capacity of the *C. longa* was determined using DPPH according to the method of Blois. DPPH (0.004% w/v was prepared freshly in 97% ethanol and was added to sample solution (10 ppm) in ethanol. The mixture was allowed to stand at room temperature in dark for 20 mins. Then the mixture was vortexed and the absorbance was read at 517 nm using a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were performed in duplicates. Antioxidant activity (%) was calculated at 100 μ g/mL.

2.5. Data analysis

Differences between curcuminoid, curcumin, demethoxycurcumin, bisdemethoxycurcumin related to antioxidant capacity were analyzed by one-way ANOVA. Relationship between each metabolites and antioxidant capacity were conducted using Pearson correlation.

3. Result and discussion

3.1. Quantification of curcuminoid yield

Thin layer chromatography analysis showed the separation of curcuminoid content, curcumin, demethoxycurcumin and bisdemethoxycurcumin, nicely depicted on Figure 1. Rf value of these curcumin, demethoxycurcumin and bisdemetoxycurcumin were 0.81, 0.5, 0.3, respectively. Curcuminoid content varied among eight different locations $(0.53\pm0.05\% \text{ to } 5.33\pm0.12\% \text{ w/w})$ (Table 1). Curcumin, demethoxycurcumin and bisdemethoxycurcumin were highly significant among eight different location (F = 21.325, df = 7, P = 0.000; F = 4.733, df = 7, P = 0.008; F = 20.010, df = 7, P = 0.000; respectively). The highest curcumin, demethoxycurcumin and bisdemethoxycurcumin will were found in the samples from Magetan ($5.33\pm0.12\%$, $1.54\pm0.05\%$, $0.46\pm0.02\%$ w/w, respectively). In contrast, the lowest curcumin, demethoxycurcumin and bisdemethoxycurcumin yield were found in the sample from Demak ($0.53\pm0.05\%$, $0.17\pm0.05\%$, $0.17\pm0.05\%$ w/w, respectively).

Curcuminoid content in *C. longa* varied as reported by Ashraf et al.(2012) and Pawar et al. (2014) collected in India, Pothitirat and Gritsanapan (2005) collected in Thailand. *C. longa* from Magetan showed the total curcuminoid above 5% which fulfilled the ASEAN standard. Besides, it was the highest curcuminoid among others. Meanwhile, the curcuminoid content of other samples varied below the standards. Anandaraj et al. (2014) reported that 70% and 16% variation of curcuminoid yield in *C. longa* was attributed to environments and genotype effect, respectively. Altitude, temperature, rainfall and soil type were taken into account for curcuminoid yield. Moreover, genotypic factor of variation and heritability were highly parameter for yield and curcumin content [17]. Hayakawa et al. (2011) suggested the hybridization and introgression with other Curcuma species could effect curcuminoid content.



Figure 1. TLC profile of curcuminoids (Curcumin (C), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC)) in the extracts of *Curcuma longa* L. collected from various locations. Stationary phase: 60GF₂₅₄; solvent system: dichloromethane-methanol (97:3). S = standard curcuminoid, 1 = Jambi, Sumatera, 2 = Sukoharjo, Middle Java, 3 = Magetan, Middle Java, 4 = Banten, West Java, 5 = Wonogiri, Middle Java, 6 = Demak, West Java, 7 = Nusa Tenggara Timur and 8 = Nusa Tenggara Barat

Location	Each curcuminoid content (% w/w in dried powder)			Total curcuminoid content (% w/w in dried powder)
	С	DMC	BDMC	
Wonogiri	0.68 ± 0.28	0.59±0.31	$0.16{\pm}0.05$	1.66±0.21
Sukoharjo	1.32 ± 0.02	1.05 ± 0.02	$0.34{\pm}0.02$	3.14±0.09
Magetan	3.26±0.01	$1.54{\pm}0.05$	0.46 ± 0.02	5.33±0.12
Demak	$0.27{\pm}0.08$	$0.17{\pm}0.05$	$0.06{\pm}0.03$	$0.53{\pm}0.05$
Banten	1.78 ± 0.02	$1.52{\pm}0.02$	0.43 ± 0.01	3.83±0.11
NTT	0.81±0.13	$0.74{\pm}0.14$	$0.24{\pm}0.02$	1.89±0.13
NTB	1.10 ± 0.32	$0.84{\pm}0.44$	$0.26{\pm}0.02$	$2.47{\pm}0.28$
Jambi	1.41 ± 0.06	$1.24{\pm}0.03$	0.32 ± 0.01	3.09±0.11

Table 1. The content of curcuminoid in ethanolic extracts of Curcuma longa collected from differentlocation in Indonesia by TLC-densitometry. Analysis was done in triplicate as mean \pm SE

3.2. Antioxidant activity

Antioxidant capacity was determined by the ability of substances to donate a hydrogen atom to a free radical DPPH into a nonradical DPPH [19]. It reduced the violet colour of a free radical DPPH into a residual pale yellow colour from the picryl group

Antioxidant capacities of *C. longa* ethanolic extracts are presented in Table 2. Antioxidant capacity was significantly different (F = 36.440, df = 7, P = 0.000). It showed similar activity for all places unless sample from Magetan which exhibited two times lower than other locations. The more potent antioxidant shows the higher antioxidant capacity value. Reynertson et al. (2007) classified antioxidant into strong, medium and weak activity with IC₅₀ value of <50 µg/mL, 50-100 µg/mL and 100-200 µg/mL, respectively. Present study showed the antioxidant capacity of curcuminoid was 54.76 µg/mL. These exhibited medium antioxidant capacity. In contrast, curcumin showed to have IC₅₀ of 12.88 µg/mL [21].

Curcumin, a major phenolic compounds in *C.longa* L., has been known as a strong antioxidant agent [22]. Antioxidant capacity crude extract *C.longa* L. has been shown by Singh (2010) to have 86-92% inhibition against DPPH radical. Besides, major constituents of essential oil such as aromatic-turmerone, alpha-turmerone, beta-turmerone, oleoresin played a role in antioxidant activity in *C.longa* L. [23].

Location	Concentration (ug/mL)	Antioxidant capacity (%)
Wonogiri	0.1	56.27 ± 0.80
Sukoharjo	0.1	66.82 ± 0.86
Magetan	0.1	35.98 ± 0.96
Demak	0.1	63.30 ± 1.13
Banten	0.1	61.71 ± 0.85
NTT	0.1	56.03 ± 1.53
NTB	0.1	59.57 ± 0.20
Jambi	0.1	64.45 ± 0.70
Curcuminoids	0.1	54.76

Table 2. Antioxidant activity of ethanolic extracts of Curcuma longa using DPPH method.

To understand the correlation between curcuminoid yield and antioxidant activity, we do the correlation statistical analysis. It resulted no correlation between demethoxycurcumin and bisdemethoxycurcumin to antioxidant capacity (R= -0.262, df= 8, P= 0.531 and R= -0.294, df= 8, P= 0.479, respectively). However, curcumin showed little correlation with antioxidant activity (R= -0.715, df= 8, P= 0.046) (Fig. 2). In general, the total curcuminoid content and antioxidant showed no significant correlation (R= -0.562, df= 8, P= 0.147). Curcumin was reported as a strong antioxidant agent [3, 24]. However, *C. longa* L. extract as whole constituent system, the antioxidant activity is influenced by other

component as synergistic or antagonistic actions. Thus, instead of curcumin, other constituents i.e essential oils and oleoresin as reported by Zaeoung et al. [23].

4. Conclusion

These results are useful information for curcuminoid standardization method in pharmaceutical products. It has to fulfill the requirement of total curcuminoid above 5% based on the ASEAN standard. Thus, the standardized cultivation for related herbal medicines needs to be done to produce an acceptable herbal material for pharmaceutical industry.

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