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Original Research Article

Antioxidant and antidiabetic potential of *Curcuma longa* and its compounds

Wahyu Widowati[1], Teresa Liliana Wargasetia[1], Ervi Afifah[2], Tjandrawati Mozef[3], Hanna Sari Widya Kusuma[2], Hayatun Nufus[2], Seila Arumwardana[2], Annisa Amalia[2], Rizal Rizal[2]

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Abstract

Antioxidant agent can eliminate the free radicals due to oxidative stress that has been reported as the main cause of diabetes mellitus. This study evaluated the effect of *Curcuma longa* rhizomes and its compounds curcumin and bisdemethoxycurcumin as antioxidants and antidiabetic activity.

The phytochemical assay was performed with modified Farnsworth method. Quantitative curcumin, bisdemethoxycurcumin and curcumol of *C. longa* extract (CLE) were evaluated using HPLC. The antioxidant assay was performed with 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzo thiazoline-6-sulfonate acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP) assay. Antidiabetic properties were measured by inhibitory activity of α-, β-glucosidase, and α-amylase. This study suggested that CLE has terpenoids in high level. Based on HPLC test, CLE contained curcumin (16.92%), curcumol (15.51%), and bisdemethoxycurcumin (5.27%). Bisdemethoxycurcumin has DPPH scavenging activity (IC_{50}= 64.94±2.44 µg/ml), curcumin has the highest activity (IC_{50}=0.92±0.03 µg/ml) in ABTS assay, while CLE has the highest activity in FRAP assay (IC_{50}=311.10 µM Fe(II)/µg) in the highest concentration (250.00 µg/ml). In antidiabetic assay, bisdemethoxycurcumin has the highest in α-glucosidase and α-amylase inhibitory activity (IC_{50}=3.76±0.33 µg/ml; 1.79± 0.15 µg/ml), while in β-glucosidase inhibitory activity curcumin has the highest activity (IC_{50}=1.03±0.03 µg/ml). CLE and its compounds possess antioxidants and antidiabetic activities.

Keywords: Antioxidant, Antidiabetic, Bisdemethoxycurcumin, *Curcuma Longa*, Curcumin

Introduction

Diabetes is metabolic diseases characterized by high levels of blood sugar, which is one of the major diseases for morbidity and mortality worldwide (Kumar et al., 2009). Diabetes is resulted from defects in insulin production, and impaired function in the metabolism of carbohydrates, lipids and proteins which lead to long-term complication (Gezginci-Oktayoglu et al., 2009). Oxidative stress caused by Reactive Oxygen Species (ROS) is also usually linked to elevating glucose and other metabolic disorders (Kowluru and Chan, 2007). Several antidiabetic drugs such as acarbose and voglibose can lose their efficacy,

which can cause side effects and trigger diabetic complications (Garhyan et al., 2006; Mukherjee et al., 2006). Medicinal herbs have been suggested as an alternative antidiabetic drug due to their effectiveness, minimal side effects, relatively low costs and more safe (Playford et al., 2013; Sawant and Godghate, 2013).

Zingiberaceae family is the most widely grown crop in the Asia. This plant is important for natural resources for human as sources of food, spices, dyes, food coloring and herbal medicine (Tsai et al., 2011). Turmeric (*C. longa* L.) is one of the most widely species of Zingiberaceae studied. *C. longa* is one of plants that possess medicinal properties as antioxidant and antidiabetic agents (Singh et al., 2010; Sivabalan and Anuradha, 2010). *C. longa* is used for several purposes apart for flavoring and coloring food. Numerous studies have shown the plant contains curcumin and has antioxidant properties (Hsu and Cheng, 2007). *C. longa* ethanol and hexane extract were proved significantly to reduce the blood glucose levels (Nishiyama et al., 2005).

The compounds of *C. longa*, curcumin and bisdemethoxycurcumin have caught scientific attention as a potential therapeutic agent in the treatment of diabetes (Sivabalan and Anuradha, 2010; Perez-Torres et al., 2013). Based on Nishiyama *et al.,* (2005), curcumin showed an effect on glycemia in type-2 diabetic mice models and KK-A (y) mice (Nishiyama et al., 2005). Curcumin has the highest antioxidant activity among the group of curcuminoid that has cytoprotection against oxidative stress (Motterlini et al., 2000; Jayaprakasha et al., 2006). Curcumin is a competitive inhibitor of an α-amylase and it reduce the level of blood glucose in diabetic and normal rats (Ponnusamy et al., 2011).

One of the therapeutic approaches to lower postprandial blood glucose is to inhibit starch breakdown by inhibiting carbohydrate hydrolysis enzymes such as α-glucosidase, β-glucosidase, and α-amylase. Then, the reduction of glucose level in diabetic and normal rats can be occurred via inhibition of hydrolysis enzyme by curcumin (Najafian, 2015). In this study, we evaluated the phytochemical content, quantitative curcumol, bisdemethoxycurcumin and curcumin using High-performance liquid chromatography (HPLC) analysis of CLE, as well as an antioxidant activity through DPPH scavenger, ABTS-reducing, FRAP activitiesof and antidiabetic activities through inhibition of α/β-glucocidase and α-amylase.

Material and Methods

Plant extract preparation

C. longa rhizomes was collected from farmer plantation located in Bogor, West Java, Indonesia. Plants of *C. longa* was determined by the herbarium staff of Biology Department, Bandung Institute of Technology, Bandung, Indonesia. One kilogram of dried rhizomes of *C. longa* was extracted with destilled ethanol 70% using maceration method. The ethanol's filtrate was filtered and collected every 24 h until colorless. The collected filtrate was evaporated using rotary evaporator (IKA RV 3 V-C), resulted in 138.51 g extract. The extract of *C. longa* was stored at -20 °C (Widowati et al., 2011; Widowati et al., 2012; Widowati et al., 2016; Widowati et al., 2017)

Qualitative phytochemical analysis

CLE was analyzed using modified Farnsworth method to identify the presence of several compounds, such as phenols, steroids/triterpenoids, saponins, tannins, terpenoids, flavonoids, and alkaloids (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Phenol identification

$FeCl_3$ [Merck 1.03861.0250] in 1% aquades was added into samples in the dropping plate. The presence of phenol indicates by color alteration into green/red/purple/blue/black (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Steroid/triterpenoid identification

Sample extract 10 mg was added in a dropping plate, and then added acetate acid until the sample was submerged. One drop of absolute acid (H_2SO_4) [Merck 109073] was added into the sample after 10-15 min. The presence of steroid indicated by green or blue color formation while the presence of triterpenoid by red/orange sediment (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Saponin identification

Sample extract 10 mg was diluted in the aquades, boiled for 5 min, and then shaked vigorously. The presence of saponin was indicated by persistence of froth on the surface of a solution (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Tannin identification

HCl 2N [Merck 1003171000] 2 ml was added to 10 mg sample and heated in the waterbath for 30 min. The

mixture solution was cooled down and filtered, then added with amyl alcohol [Merck 10979]. The presence of tannin was indicated by the formation of purple color on the surface of amyl alcohol (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Terpenoid identification
Sample extract 10 mg was added with vanillin and H_2SO_4. Color changes to purple indicates positive reaction (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Flavonoid identification
Sample extract 10 mg was added into a test tube which contained Mg [Merck EM105815] and HCl 2N, incubated for 5-10 min. Furthermore, amyl alcohol was added into the filtrate. Formation of red or orange colour indicates presence of flavonoid (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

Alkaloid identification
Ammonia 10% was added to sample and then extracted with chloroform until two layers were formed. The bottom layer was collected and added with HCl 1N whilst the upper layer was collected and added with 1-2 drops of draggendorf solution. A positive reaction was performed by formation of yellow or red (Adnyana et al., 2016; Widowati et al, 2016; Widowati et al., 2017).

HPLC assay
The analysis of chemical profiling of CLE by HPLC. Quantification CLE used the standard curcumol ($C_{15}H_{24}O_2$) [Biopurify Phytochemical Ltd Chengdu 4871-97-0], bisdemethoxycurcumin ($C_{19}H_{16}O_4$) [Biopurify Phytochemical Ltd Chengdu 33171-05-0], and curcumin ($C_{21}H_{20}O_6$) [Sigma-Aldrich C1386]. HPLC analysis used the Hitachi Pump HPLC L-6200, Hitachi L-4000 UV detector and Reverse Phase Column C-18 (Phenosphere ODS-2, Phenomenex, 4.6 mm x 250 mm). Acetonytril 70% [Merck 100030] was used to mobile phase (isocratical) with a flow rate of 1.0 ml/min. The samples were dissolved in methanol 70% (1 mg/ml) and filtered through a 0.22 µm syringe and injected 20 µl. UV absorbance was measured at 254 nm (Ahmad et al., 2012; Widowati et al., 2014a; Widowati et al., 2017).

DPPH scavenging activity assay
Fifty microlitres of various level of samples (curcumin, bisdemethoxycurcumin, CLE) was added to each well in a 96 well-microplate, and then 200 µl of DPPH [Sigma-Aldrich D9132] solution (0.077 mmol/l in methanol) was added into the well, incubated in the dark room for 30 min at room temperature. Afterwards, the absorbance was measured at 517 nm wavelength by microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA)(Widowati et al., 2015; Widowati et al., 2016; Widowati et al., 2017). The DPPH scavenging activity was measured using the following formula:
Scavenging Activity % = (Ac – As) / Ac x 100
Ac: negative control absorbance (without sample)
As: sample absorbance

ABTS-reducing activity assay
Two microlitres of various level samples (curcumin, bisdemethoxycurcumin, CLE) were added to the sample well, then $ABTS^{*+}$ solution (198 µl) was added to each well (96 well-plate), incubated for 6 min at 30 °C. Absorbance was measured at 745 nm wavelengths using Multiskan™ GO Microplate Spectrophotometer (Widowati et al., 2014a; Widowati et al., 2014b; Widowati et al., 2016; Widowati et al., 2017).

FRAP assay
FRAP assay was performed using modified method (Widowati et al., 2014b; Widowati et al., 2016; Widowati et al., 2017). Briefly 7.5 µl of various level samples (curcumin, bisdemethoxycurcumin, CLE) was added with 142.5 µl FRAP reagent into each well in a 96 well-microplate and then incubated at 37 °C for 30 min. Absorbance was measured at 593 nm wavelength using Multiskan™ GO Microplate Spectrophotometer.

α-glucosidase inhibitory activity assay
The α-glucosidase inhibitory activity was tested by the modified method (Kim et al., 2004; Soeng et al., 2015; Widowati et al., 2015; Gondokesumo et al., 2017). Briefly, 5 µl of various level of samples (curcumin, bisdemethoxycurcumin, CLE), 25 µl of 200 mM p-nitrophenyl-a-glucopyranoside, 45 µl phosphate buffer saline (PBS) (pH 7), 25 µl of *Saccharomyces* sp. yeast α-glucosidase was added into each well in a 96 well-microplate, incubated at 37 °C for 5 min.

The reaction was stopped by adding 100 µl of 200 mM Na_2CO_3 and then measured at 400 nm using a Multiskan™ GO Microplate Spectrophotometer.
The α-glucosidase inhibitory activity was calculated by formula:

$$\text{inhibition \%} = \frac{(C-S) \times 100}{C}$$

C : Enzymatic activity absorbance (without sample)
S : Enzymatic activity sample absorbance

β-glucosidase inhibitory activity assay
The ß-glucosidase inhibitor activity was tested using modified method (Widowati et al., 2015; Gondokesumo et al., 2017). Briefly, 20 µl of various level samples (curcumin, bisdemethoxycurcumin, CLE) was added into each well in a 96 well-microplate, and then 200 µl master mix reaction was added. Initial absorbance was measured at 405 nm. Then the samples were incubated at 37 °C for 20 min. The final absorbance was measured at 405 mn using Multiskan™ GO Microplate Spectrophotometer.

$$\text{Inhibition \%} = \frac{(C-S) \times 100}{C}$$

C : Enzymatic activity absorbance (without sample)
S : Enzymatic activity sample absorbance

α-amylase inhibitory activity assay
The α-amylase inhibitory activity assay using a modified method (Wu et al., 2012; Adnyana et al., 2015; Gondokesumo et al., 2017). Briefly, 30 µl of various level of sample (curcumin, bisdemethoxycurcumin, CLE) was added into each well in a 96 well-microplate. Then each well was added with 10 µl enzyme α-amylase 0.075 mg/ml, then incubated at temperature of 37 °C for 10 min and added with 40 µl PBS. Incubated at 37 °C for 15 minutes, then added with 100 µl acidic iodine solution into each well as enzymatic stop reaction, absorbance was measured by Multiskan™ GO Microplate Spectrophotometer at 565 nm. Quantification of inhibition activities by formula:

$$\text{Inhibition \%} = \frac{(C-S) \times 100}{C}$$

C : Enzymatic activity absorbance (without sample)
S : Enzymatic activity sample absorbance

Statistical analysis
The antioxidant and antidiabetic activities were derived from three independent experiments. Statistical analysis was performed using SPPS software (version 17.0). The significant differences in FRAP activity data were analyzed by analysis of variance (ANOVA) continued with Tukey HSD post hoc test, $p<0.05$ was considered as statistically significant. The median inhibitory concentration (IC_{50}) was measured to determine the DPPH scavenging activity, ABTS-reducing activity and inhibitory activites of α/β-glucosidase, α-amylase, according to linear regression.

Results

Phytochemical analysis of CLE
Phytochemical analysis was performed to determine compounds contained in CLE. Qualitative phytochemical test was performed to detect presence of phenols, saponins, flavonoids, steroids/triterpenoids, and tannins in CLE. Terpenoid was detected in high intensity, whereas saponin and tannin were not detected (Table 1).

Table 1. The Result of Qualitative Phytochemical Screening of CLE (Phenols, Steroids/Triterpenoids, Terpenoids, Saponins, Flavonoids, Tannins and Alkaloids)

Compounds	CLE
Phenols	+
Steroids/Triterpenoids	+/-
Terpenoids	+++
Saponins	-
Flavonoids	+
Tannins	-
Alkaloid	+

*The data of phytochemicals content in CLE are presented in qualitative data, which ++++ (very high content); +++ (high content); ++ (moderate content); + (less content); - (not detected)

HPLC analysis of CLE
HPLC analysis was evaluated to determine content of CLE. The HPLC standards used curcumin, bisdemethoxycurcumin and curcumol. Curcumin has a retention time at 3.85 min, bisdemethoxycurcumin at 3.31 min, and curcumol at 5.94 min. The CLE peaked at 3.32 was assumed as bisdemethoxycurcumin. Meanwhile, retention time at 3.84 and 5.84 was assumed as curcumin and curcumol, respectively (Fig. 1D). CLE contained curcumin,

bisdemethoxycurcumin and curcumol. The compounds of CLE are bisdemethoxycurcumin 5.27%, curcumin 16.92% and curcumol 15.51%. Curcumin showed the highest percentage concentration than other compounds (Table 2).

DPPH scavenging activity

The DPPH scavenging activity based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical 2,2-diphenyl-1-picrylhydrazine (DPPH-H) (Sohn et al., 2003; Widowati et al., 2016; Widowati et al., 2017). Bisdemethoxycurcumin showed DPPH scavenging activity with IC_{50} value of 64.94±2.44 µg/ml (Table 3). These results indicate that antioxidant activity contained in sample reduced 1,1-diphenyl-2-picrylhydrazyl free radical to 1,1,-diphenyl-2-picrylhydrazin. The previous research that DPPH scavenging activities of CLE and curcumin with IC_{50} values 8.33 µg/ml and 7.85 µM (2.89 µg/ml), respectively (Widowati et al., 2011). It showed that curcumin has higher DPPH scavenging activity compared to bisdemethoxycurcumin and CLE.

ABTS-reducing activity

ABTS-reducing activity assay measures the relative ability of antioxidant to scavenge the ABTS generated (Shalaby and Shanab, 2013; Widowati et al., 2016; Widowati et al., 2017). Percentage of ABTS-reducing activity of curcumin and bisdemethoxycurcumin were comparable (99.09±0.48% and 99.63±0.17%, respectively) (Fig. 2A). However, curcumin has the lowest IC_{50} value (0.92±0.03 µg/ml), indicates that curcumin is the highest in reducing ABTS compared to CLE (6.99±0.02µg/ml) and bisdemethoxycurcumin (2.86±0.05 µg/ml) (Table 3).

FRAP activity

FRAP assay is considered as the ability of the antioxidants present in the samples to scavenge radical species and to reduce Fe(III)/tripyridyltriazine complex (Benzie and Strain, 1996). The antioxidants present in samples reduced colorless FeIII-TPTZ complex to FeII-TPTZ, a blue colored compound in FRAP method (Katalinic et al., 2004). Increasing concentration of CLE generated greater FRAP activity. CLE has the highest FRAP activity compared to bisdemethoxycurcumin and curcumin with value 311.10±4.60 µM Fe(II)/µg sample in the highest concentration (250.00 µg/ml) (Fig. 2B). The data based on statistical analysis showed that CLE, curcumin, and bisdemethoxycurcumin have significant differences in FRAP activity among concentrations in each sample ($p<0.05$) (Table 4).

α-glucosidase inhibitory activity assay

Alpha-glucosidase is a key enzyme in carbohydrate digestion. Inhibition of α-glucosidase can delay the intestinal carbohydrate absorption and slow the gaining of blood glucose levels (Hanhineva et al., 2010). The results of α-glucosidase activity of CLE, bisdemethoxycurcumin and curcumin showed α-glucosidase inhibitory activity in concentration dependent manner. In the highest concentration (37.50 µM), the α-glucosidase inhibitory activity of curcumin was comparable to bisdemethoxycurcumin with value 70.74± 0.92% and 69.33±4.60%, while CLE has value 64.63±2.08% (Fig. 3A). However, bisdemethoxycurcumin has the highest α-glucosidase inhibitory activity. These were supported by results of IC_{50} value, bisdemethoxycurcumin has lower IC_{50} value (3.76±0.33 µg/ml) compared to curcumin (5.33±0.16 µg/ml), while CLE has the highest IC_{50} value (17.18±0.56 µg/ml). It indicates that bisdemethoxycurcumin has the highest α-glucosidase inhibitory activity compared to CLE and curcumin (Table 5).

β-glucosidase inhibitory activity assay

In general, β-glucosidases cleave the beta-1,4-glucosidicbonds in a variety of glucosides. Two carboxylic acids are involved in catalysis at the active site (Sorensen et al., 2013). The result of β-glucosidase enzyme activity in CLE, curcumin and bisdemethoxycurcumin showed that increased extract concentrations caused inhibition of the enzyme β-glucosidase greater. CLE had the highest inhibitory activity compared to curcumin and bisdemethoxycurcumin. The highest value of CLE was 73.01±4.13% (Fig. 3B). Based on IC_{50} value showed that β-glucosidase inhibitory activity CLE has the lowest activity with value 2.72±0.40 µg/ml compared to curcumin (1.03±0.03 µg/ml) and bisdemethoxycurcumin (1.47± 0.57 µg/ml) (Table 5).

α-amylase inhibitory activity assay

α-amylase catalyzes the hydrolysis of α-(1,4)-D-glycosidic linkages of starch and other glucose polymers. Inhibition of α-amylase leads to inhibition of starch breakdown that results in lower levels of blood glucose (Wu et al., 2012).

The result of α-amylase inhibitory activity in CLE, bisdemethoxycurcumin and curcumin showed inhibitory activity in concentration-dependent manner. At the highest concentration, bisdemethoxycurcumin had the highest α-amylase inhibitory activity (83.33%), while CLE and curcumin has inhibition percentage around 75.69% and 66.67% respectively (Fig. 3C). These data were supported by IC_{50} value, bisdemethoxycurcumin had the highest α-amylase inhibitory activity with IC_{50} value 1.79±0.15 µg/ml compared to CLE (IC_{50}= 13.25 ± 0.02 µg/ml) and curcumin (6.99 ± 0.92 µg/ml) (Table 5)

Table 2. The Compounds Concentration in CLE Based on HPLC Analysis Using Bisdemethoxycurcumin, Curcumin and Curcumol as Standard

Marker Compounds	Area (replication)			Concentration (replication)			Average Concentration (ppm)
	Area 1 (µV.s)	Area 2 (µV.s)	Area 3 (µV.s)	C 1 (ppm)	C 2 (ppm)	C 3 (ppm)	
Bisdemethoxy-curcumin	479111.50	465882.05	492935.43	52.64	51.19	54.16	52.66
Curcumin	2319946.01	2333589.11	2379328.81	167.41	168.39	171.67	169.16
Curcumol	271433.75	286045.58	300750.63	147.18	155.10	163.07	155.11

*The data concentration compounds in CLE are presented in ppm = part per million. The HPLC analysis were performed in triplicate.

Table 3. The IC_{50} Value of DPPH Scavenging and ABTS-reducing Activity of CLE, Curcumin and Bisdemethoxycurcumin as Antioxidant

Samples	Antioxidant Activity			
	DPPH Scavenging Activity		ABTS-reducing Activity	
	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)
CLE	-	-	-	6.99 ± 0.02
Curcumin	-	-	2.49 ± 0.09	0.92 ± 0.03
Bisdemethoxycurcumin	210.63 ± 7.94	64.94 ± 2.44	9.27 ± 0.17	2.86 ± 0.05

*The data of IC50 value are presented as mean ± standard deviation. The IC50 value of CLE was presented in µg/ml, while curcumin, and bisdemethoxycurcumin were presented in µg/ml and µM. The DPPH scavenging and ABTS-reducing activity assay were performed in triplicate.

Table 4. The FRAP Activity of CLE, Curcumin, and Bisdemethoxycurcumin as Antioxidant

Concentration (µg/ml for CLE) (µM for Curcumin, Bisdemethoxycurcumin)	FRAP Activity (µM Fe (II)/µg sample)		
	CLE	Curcumin	Bisdemethoxycurcumin
3.13	13.54 ± 0.35^{a}	7.08 ± 0.22^{a}	4.22 ± 1.18^{a}
6.25	22.24 ± 1.85^{b}	10.28 ± 0.37^{a}	6.42 ± 0.58^{a}
12.50	34.11 ± 0.48^{c}	18.30 ± 0.21^{a}	7.61 ± 0.28^{b}
25.00	61.85 ± 1.19^{d}	34.45 ± 1.55^{b}	14.54 ± 0.29^{c}
50.00	107.97 ± 2.28^{e}	62.86 ± 1.28^{c}	23.60 ± 0.72^{d}
75.00	173.57 ± 3.28^{f}	103.84 ± 0.48^{d}	43.51 ± 1.05^{e}
125.00	242.45 ± 5.22^{g}	176.67 ± 2.72^{e}	71.93 ± 2.86^{f}
250.00	311.10 ± 4.60^{h}	238.26 ± 1.48^{f}	109.53 ± 2.39^{g}

* The data are presented as mean ± standard deviation. Different letters in the same coloumn were among treatment concentrations of CLE, curcumin, bisdemethoxycurcumin are significant at $P < 0.05$ (Tukey post hoc test). (The concentrations of CLE in µg/ml; curcumin and bisdemethoxycurcumin in µM). The FRAP activity assay were performed in triplicate.

Table 5. The IC_{50} value of α-Glucosidase, β-Glucosidase, and α-Amylase Inhibitory Activity of CLE, Curcumin, and Bisdemethoxycurcumin as Antidiabetic

Samples	Antidiabetic Activity					
	α-glucosidase Inhibitory Activity		β-glucosidase Inhibitory Activity		α-amylase Inhibitory Activity	
	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)
CLE	-	17.18 ± 0.56	-	2.72 ± 2.40	-	13.25 ± 0.02
Curcumin	14.46 ± 0.44	5.33 ± 0.16	2.79 ± 0.09	1.03 ± 0.03	18.96 ± 2.50	6.99 ± 0.92
Bisdemethoxy-curcumin	12.20 ± 1.06	3.76 ± 0.33	4.76 ± 1.86	1.47 ± 0.57	5.82 ± 0.49	1.79 ± 0.15

*The data are presented as mean ± standard deviation. The IC50 value of CLE was presented in µg/ml, while curcumin, and bisdemethoxycurcumin were presented µg/ml and µM. The α-glucosidase, β-glucosidase, and α-amylase inhibitory activity assay were performed in triplicate.

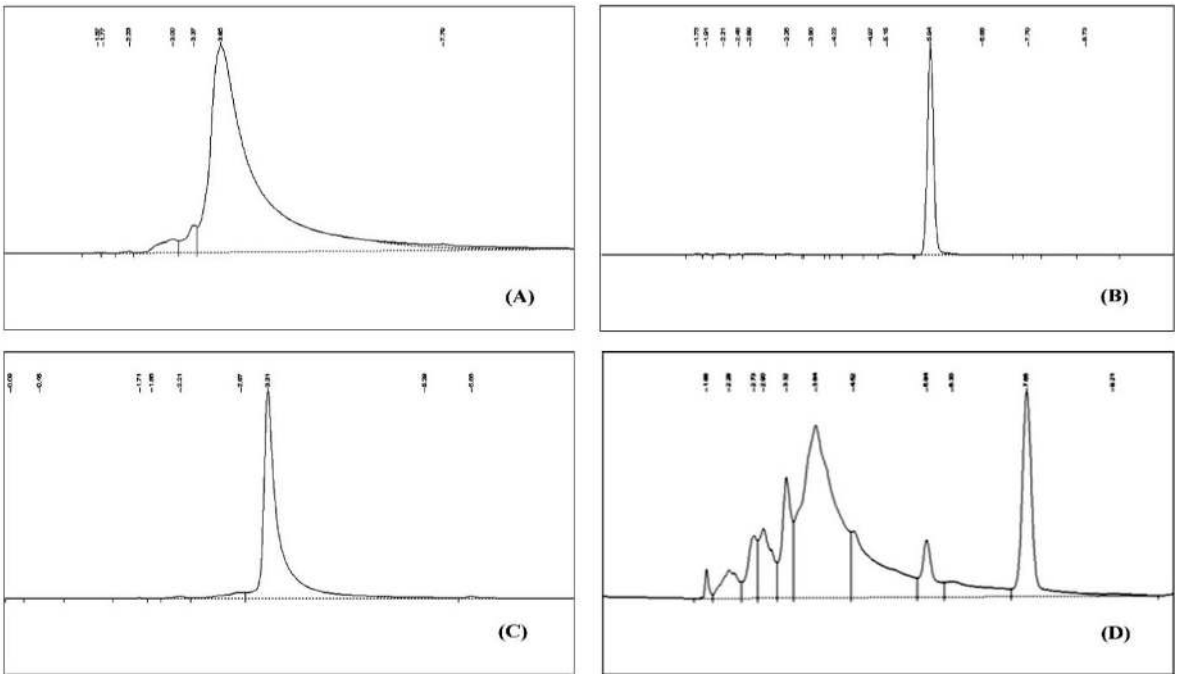

Figure 1. Standard Chromatogram HPLC using Methanol as Solvent at 254 nm Absorbance
(A) Curcumin; (B) Bisdemethoxycurcumin; (C) Curcumol; (D) *C. longa* extract (CLE).

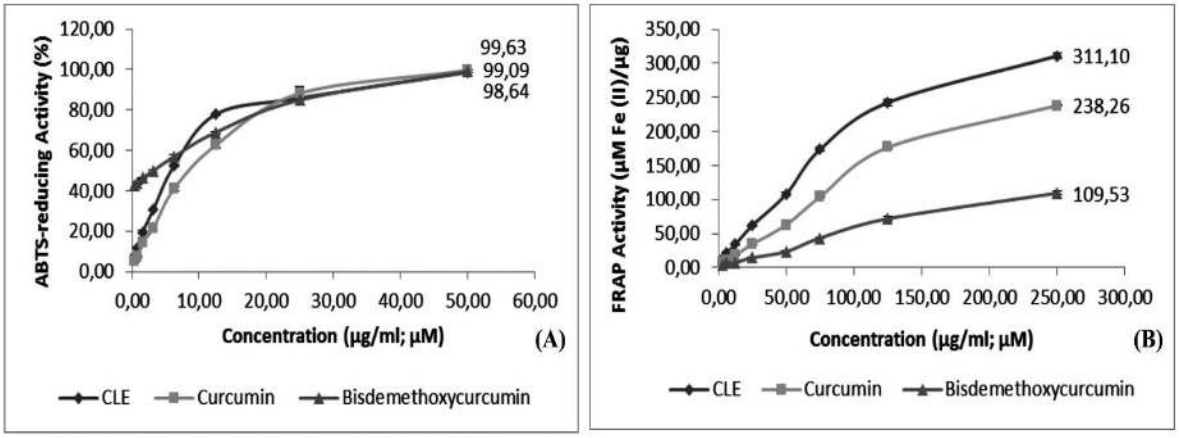

Figure 2. Antioxidant Activity of CLE, Curcumin and Bisdemethoxycurcumin.

(A) ABTS-reducing activity, and (B) FRAP activity.
*Each sample in ABTS-reducing activity was diluted in PBS to reach the final concentration of 50.00; 25.00; 12.50; 6.25; 3.13; 1.56; 0.78; 0.39 µg/ml; µM, and FRAP activity assay in concentration of 250.00; 125.00; 75.00; 50.00; 25.00; 12.50; 6.25; 3.13 µg/ml; µM, respectively. (CLE in µg/ml; curcumin and bisdemethoxycurcumin in µM).

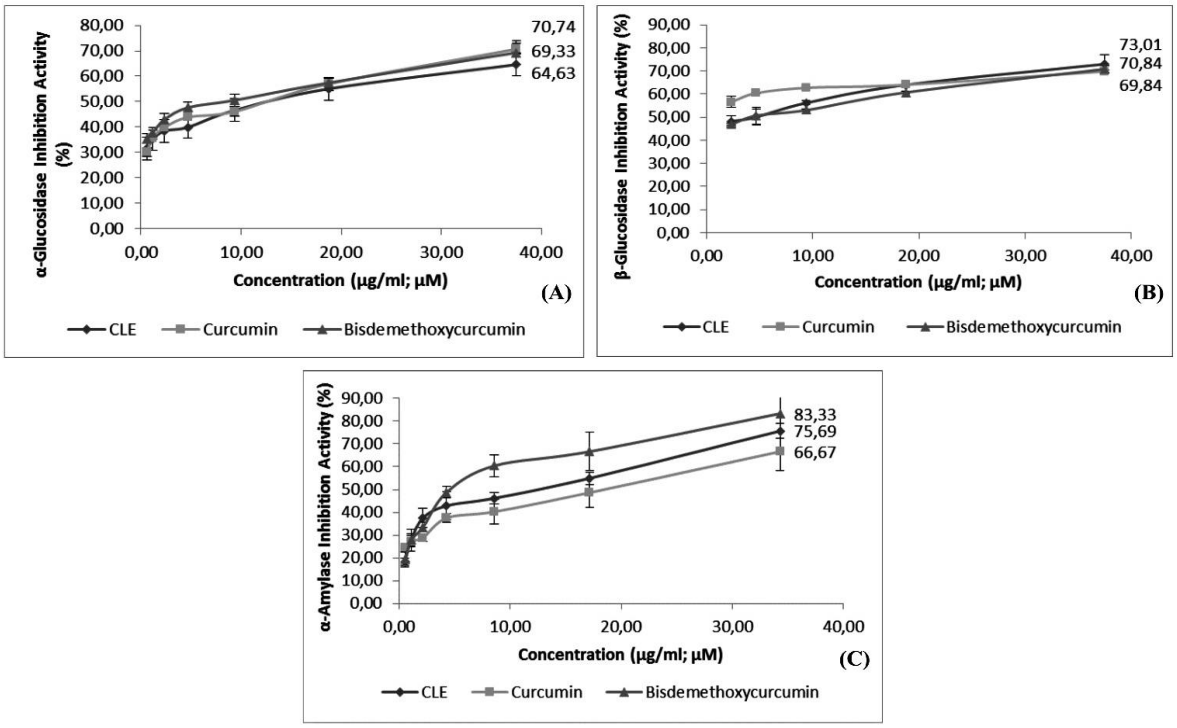

Figure 3. Antidiabetic Activity of CLE, Curcumin and Bisdemethoxycurcumin

(A) α-glucosidase inhibitory activity, (B) β-glucosidase inhibitory activity and (C) α-amylase inhibitory activity.
*Each sample of α-, β-glucosidase activity assay was diluted in DMSO to reach the final concentration of 37.50; 18.75; 9.38; 4.69; 2.34; 1.17; 0.59 µg/ml; µM, while α-amylase activity assay in concentration of 34.29; 17.14; 8.57; 4.29; 2.14; 1.07; 0.54 µg/ml; µM (CLE in µg/ml; curcumin and bisdemethoxycurcumin in µM).

Discussion

Methanolic extract of *C. longa* rhizome has the compound of steroids, alkaloids and flavonoids (Prashanth and Bhavani, 2013). The result of phytochemical screening showed that CLE contains phenol, steroids, terpenoids, flavonoids, and alkaloids. Terpenoid is one of polyphenol abundantly present in the CLE (+++). This result was in line with previous research that phytochemical screening of two different *C. longa* varieties, that they have steroids, alkaloids, flavonoids (Prashanth and Bhavani, 2013). Phenol is not present in the ethanol and chloroform extract of *C. longa* (Sawant and Godghate, 2013). However *C. caesia* contained several compounds such as curcuminoids, phenolics, flavonoids, volatile oils, protein, amino acids and alkaloids were reported in the rhizomes of Indian *C. caesia* (Sarangthem and Haokip, 2010).

HPLC analysis displayed that CLE contained curcumin, bisdemethoxycurcumin and curcumol. The concentration of curcumin was the highest than curcumol and bisdemethoxycurcumin. These results were comparable with previous research by Osorio-Tobon et al. (2016) that extract of turmeric (*C. longa*) contained curcumin (17.48%) and bisdemethoxycurcumin (7.65%) (Osorio-Tobon et al., 2016).

The antioxidant from plants can control blood glucose level and prevent diabetic complications. Reactive Oxygen Species (ROS) plays an important role in the development of type 2 diabetes (Kaneto et al., 2010). The most widely compounds properties of plants is antioxidant activities through capability in scavenging free radical (Yang and Landau, 2000). The present study evaluated antioxidant activity of bisdemethoxycurcumin in DPPH assay (IC_{50}=64.94±2.44 µg/ml), antioxidant activity of *C. longa* and curcumin has been evaluated in our previous study, curcumin had DPPH scavenging activity with IC_{50} value of 7.85 µM or 2.89 µg/ml, while *C. longa* had IC_{50} value 8.33 µg/ml (Widowati et al., 2011). These results indicate that curcumin shows the highest antioxidant activity than bisdemethoxycurcumin and CLE. Referring to Borra et al. (2013), curcumin has the higher scavenging activity than ascorbic acid in DPPH assay (55.60 to 71.64%) with IC_{50} value 1.08 µM and 1.34 µM, respectively at the concentration of 5 µg/ml (Borra et al., 2013). The DPPH scavenging activity of curcumin (83.00%) was higher than ascorbic acid (77.00%) at the concentration of 0.2 mM (Asouri et al., 2013).

ABTS assay presents as percentage of ABTS-reducing activity of curcumin, bisdemethoxycurcumin, and CLE. It was comparable but based on IC_{50} value, curcumin showed the highest activity to reduce ABTS activity. These results were in line with another study that relatively low concentrations of curcumin exhibits remarkable antioxidant effects meaning curcumin has potential as an antioxidant, antitumor and anti-inflammatory by the cytoprotective effect against oxidative stress (Motterlini et al., 2000). CLE has low antioxidants activity and this result was comparable to other study showed that CLE also has lower antioxidant activity (IC_{50}= 7.61 µg/ml) compared to Epigallo-catechin-3-gallate (EGCG), the phytochemical of tea plants that have strong antioxidants (IC_{50}=0.42 µM) (Widowati et al., 2012). Bisdemethoxycurcumin also has antioxidant properties which is quite high compared to other flavonoid compounds (Cikrikci et al., 2008).

Based on this study showed that CLE has the highest value in FRAP activity, this data indicates that CLE was more effective in the antioxidant activity compared to bisdemethoxycurcumin and curcumin. Cikrikci et al. (2008) reported that all of the isolated curcumin showed very good antioxidant activity by Cupric ion Reducing Antioxidant Capacity (CUPRAC) method with total antioxidant capacity 0.8 compared to standard compounds, α-tocopherol (0.95) and hydroquinone (0.97) (Cikrikci et al., 2008). Besides curcuminoid, *C. longa* has other compounds such as flavonoids, phenols, steroids, and saponins which also may scavenge free radicals and potent as antioxidants (Zhu et al., 2002). The terpenoids and other compounds of Curcuma species contribute toward antioxidants effect that may act alone or have synergistic activity with curcuminoids (Widowati et al., 2012). Therefore, CLE may be more effective as antioxidant compared to curcumin and bisdemethoxycurcumin.

Alpha and beta glucosidase are carbohydrate hydrolyzing enzymes that related to a metabolic disorder such as diabetes. Inhibition of carbohydrate hydrolyzing enzymes are a therapeutic approach to decrease hyperglycemia (Kim et al., 2004; Soeng et al., 2015). Inhibition of glucosidase activity regulates blood sugar level by postponing sugar breakdown (Yin et al., 2014). Three curcuminoids isolated from C. *longa* showed strong inhibitory activity on α-glucosidase (Du et al., 2006). In the result of this study,

the inhibition percentage of curcumin showed the highest activity to inhibit α-glucosidase which is comparable to inhibition percentage of bisdemethoxycurcumin. Turmeric volatile oils from dried rhizomes can inhibit glucosidase enzymes more effectively (IC_{50}= 0.38 µM) than the antidiabetic drug, acarbose (IC_{50}=18.12 µg/ml) (Lekshmi et al., 2012; Curcumin has potential treatment for diabetes and its complications by lowering a blood glucose (Zhang et al., 2013). CLE has the lowest inhibition percentage of α-glucosidase (64.63%). These result was in line with previous study that *C. longa* had no significant effect on the glucosidase response but it had effect on insulin secretion (Wickenberg et al., 2010). There are no studies on the ability of CLE to β-glucosidase inhibitory activity, thereby inhibitory activity of CLE in the present study is considered active. In the present study showed that CLE has the highest β-glucosidase inhibitory activity compared to curcumin and bisdemethoxycurcumin. CLE has several compounds such as of terpenes, alkaloids, flavonoids, phenols, steroids, and compounds with other structural and functional isolated showed potent inhibitory activity toward α-/β-glucosidase (Yin et al., 2014). Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles. Fractions such as flavonoids, phenols, steroids, and saponins also may scavenge free radicals by alloxan in diabetic rats. Flavonoid in some plants reported has hypoglycemic effects (Rauter et al., 2009; Widowati et al., 2012).

The result of the present study showed that CLE, bisdemethoxycurcumin and curcumin have α-amylase inhibitory activity. These results were supported by the previous study that bisdemethoxycurcumin inactivated human pancreatic α-amylase, as oral hypoglycemic agents in type 2 diabetes (Ponnusamy et al., 2012). Bioactive compound, that possesses α-amylase inhibitory activity, has highly lipophilic structure that may easily cross the membrane and exert its pharmacological effects. Inhibition of α-amylase delay carbohydrate breakdown and decrease the postprandial blood glucose in diabetes (Wulan et al., 2015). Common α-amylase inhibitors are betulinic acid, curcumin, and bisdemethoxycurcumin (Karthic et al., 2008; Najafian, 2015; Wulan et al., 2015). Based on previous study showed that bisdemethoxycurcumin (ki 45.86 µM; Ebinding −5.92 kcal/mol) was the third place in binding ability towards α-amylase enzyme after betulin (ki 13.12μM; Ebinding −6.66 kcal/mol) and betulinic acid (ki 75.66 µM; Ebinding −5.62 kcal/mol) (Wulan et al., 2015). Volatile oils from dried rhizomes of turmeric (IC_{50}=34.30 µM) showed higher α-amylase inhibitory activity than acarbose (IC_{50}=296.3 µg/ml) (Lekshmi et al., 2012).

Conclusion

The present study shows that CLE contain curcumol, curcumin, bisdemethoxycurcumin and its compounds have a potency as antioxidant and antidiabetic agents. However, *in vivo* test in an animal model is still needed to confirm the antioxidants and antidiabetic activity of the CLE, curcumin, and bisdemethoxycurcumin.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Judul Karya Ilmiah (Artikel)	:	Antioxidant and antidiabetic potential of Curcuma longa and its compounds
Jumlah Penulis	:	9 orang
Nama-nama Penulis	:	**Wahyu Widowati,** Teresa Liliana Wargasetia, Ervi Afifah, Tjandrawati Mozef, Hanna Sari Widya Kusuma, Hayatun Nufus, Seila Arumwardana, Annisa Amalia, Rizal Rizal
Status Penulis	:	Penulis Pertama / ~~Penulis ke ....~~ / Penulis Korespondensi **)
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Penelitian tentang ekstrak Curcuma longa sebagai antioksidan, antidiabetes

c. Kecukupan & kemutakhiran data serta metodologi.....
Sumber pustaka mutakhir, hasil penelitian membahas ekstrak C. longa dengan metode HPLC, Antioksidan dengan metode DPPH, ABTS dan FRAP juga antidiabetes dengan metode inhibisi α-TG glukosidase dan α-amilase

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LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Antioxidant and antidiabetic potential of Curcuma longa and its compounds
Jumlah Penulis	:	9 orang
Nama-nama Penulis	:	**Wahyu Widowati,** Teresa Liliana Wargasetia, Ervi Afifah, Tjandrawati Mozef, Hanna Sari Widya Kusuma, Hayatun Nufus, Seila Arumwardana, Annisa Amalia, Rizal Rizal
Status Penulis	:	Penulis Pertama / ~~Penulis ke ....~~ / Penulis Korespondensi **)
Identitas Jurnal Ilmiah	: a.	Nama jurnal : Asian Journal of Agriculture and Biology
	b.	Nomor ISSN : 23078553
	c.	Vol., No., Bulan, Tahun : Vol.6 No.2; 2018
	d.	Penerbit : Asian Journal of Agriculture and Biology
	e.	DOI Artikel (jika ada) : -
	f.	Alamat Web Jurnal : https://www.asianjab.com/
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c. Kecukupan & kemutakhiran data serta metodologi
Kemutakhiran data sudah terpenuhi dan metode yang dipakai sudah sesuai dengan tujuan penelitian

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REVIEWER 2

(Prof. Dr. Ermi Girsang, M. Kes)
NIK : 0117057501
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KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Antioxidant and antidiabetic potential of Curcuma longa and its compounds
Jumlah Penulis	:	9 orang
Nama-nama Penulis	:	**Wahyu Widowati,** Teresa Liliana Wargasetia, Ervi Afifah, Tjandrawati Mozef, Hanna Sari Widya Kusuma, Hayatun Nufus, Seila Arumwardana, Annisa Amalia, Rizal Rizal
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a.	Kelengkapan unsur isi karya	(10%)	4			3,65
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a. Kelengkapan dan kesesuaian unsur.....
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Jurnal ini sudah sesuai kaidah dan unsur ilmiah juga sudah terpenuhi

b. Ruang lingkup & kedalaman pembahasan .....
Penelitian tentang ekstrak curcuma longa sebagai antioksidan, antidiabetes
Cakupan pembahasan sudah cukup mendalam dengan tambahan teori dan materi yang

Jelas dan sesuai

c. Kecukupan & kemutakhiran data serta metodologi Sumber pustaka mutakhir, hasil penelitian membahas ekstrak curcuma longa dengan metoda HPLC antioksidan dengan metode DPPH, ABTS dan FRAP juga anti diabetes dengan metode Inhibisi α/β glukosidase dan α-amilase Kemutakhiran data sudah terpenuhi dan metode yang dipakai sudah sesuai dengan tujuan penelitian

d. Kelengkapan unsur dan kualitas penerbit

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Medan,
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