Antidiabetic and antioxidant potential of *Curcuma mangga* Val extract and fractions

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Abstract

Diabetes is a chronic metabolic disorder and characterized by high blood glucose level that defects in secretion of insulin. Oxidative stress and excess of free radicals have been documented in diabetes occurrence. *Curcuma mangga* Val. is one of traditional medicine that has potency for diabetic treatment. The present study was conducted to evaluate the antidiabetic and antioxidant effects of *Curcuma mangga* Val extract (CME) and fractions. In this study, the antioxidant activity of four fractions of CME (water, hexane, ethyl acetate, and butanol fraction) were measured using nitrite oxide (NO) and H₂O₂-scavenging activity assay, while antidiabetic activity of those fractions were measured by α-glucosidase activity assay. These fractions were also compared to antidiabetic drug, namely acarbose. In the NO-scavenging activity, the butylated hydroxytoluene (BHT) had the highest activity (IC₅₀ 69.75 µg/mL) compared to all fractions of CME and acarbose (ACR). Ethyl acetate fraction of *C. mangga* extract (EACM) showed the highest in H₂O₂-scavenging activity (IC₅₀ 162.78 µg/mL) compared to marker compound (BHT) (IC₅₀ 179.86 µg/mL) and other fractions. Hexane fraction of *C. mangga* (HCM) showed the highest α-glucosidase inhibitory activity (IC₅₀ 182.45 µg/mL).

To conclude, the fractions of *C. mangga* extract could be used as an alternative in the development of antioxidant and antidiabetic medicine.

Keywords: Antioxidant, Antidiabetic, *Curcuma mangga*, Diabetes

Introduction

Hyperglycemia resulted from defects in insulin secretion is the sign of diabetes as a metabolic disease (Ozougwu et al., 2013). Increased oxidative stress has contributed to the progression of diabetes and its complications (Matough et al., 2012). Diabetes is usually accompanied by increased production of free radicals (Matough et al., 2012). The absorption of glucose via inhibition of enzymes, such as α-glucosidase, in the digestive organs can be delayed to treat diabetes, where α-glucosidase in the epithelium of small intestine playing a role in catalyzing the hydrolytic cleavage and facilitating glucose absorption by the small intestine. Inhibiting this enzyme retards the elevation of glucose following a carbohydrate meal (Kumar et al., 2011). Antioxidant could scavenge free radicals which contribute to the pathogenesis of diabetes mellitus (Angel et al., 2013). The antioxidant can be grouped into synthetic and natural antioxidant according to its sources. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl...
gallate (PG) have a good stability on food processing but they have disadvantages by having carcinogenic character and adverse effects in pathological (Taghvaei and Jafari, 2015). Hence, it is necessary to utilize the natural antioxidant materials such as C. mangga and its compounds.

Many researches on natural resources have been performed such as Curcuma mangga Val. (C. mangga) from Zingiberaceae family. C. mangga locally known as ‘temu pauh’ or ‘temu mangga’ is a species of rhizomes plant that has bioactive such as tannin, curcumin, sugar, volatile oil, and flavonoid (Ali et al., 2010), phenolic (Pujimulyani et al., 2010) and Querscetin-3-rutinoside, Quercetin (Pujimulyani et al., 2012).

Curcuminoid in C. mangga has caught scientific attention as a potential therapeutic agent in treating diabetes and its complications (Hasimun, 2016). Antioxidant, anticancer, and antibacterial properties of C. mangga have been reported (Kirana et al., 2003; Abas et al., 2005; Chaisawadi et al., 2006). C. mangga has antioxidant compounds that can suppress oxidative stress (Hendrikos et al., 2014).

Fractionation is a separation process in which a certain amount of mixture (gas, solids, liquids, suspensions, or isotopes) is separated during the phase transition into a small number of parts (fractions), of which the composition varies according to the gradient. Fractions are collected on the basis of differences in the specific properties of each component. The polar compound will get in to polar solvent and the non-polar compound get in to the non-polar solvent (Gorke et al., 2010). This study was used C. mangga extract and its fractions to evaluate the antioxidant and antidiabetic activities through inhibitory of NO and H₂O₂ scavenging activities and also in α-glucosidase activity.

Material and Methods

Preparation C. mangga Extract

C. mangga plants were yielded from the plantation in Bantul, Yogyakarta. The extraction was processed using maceration method. Simplisia of C.mangga rhizomes after dried and mashed were then soaked in 70% (1500 mL) distilled ethanol and filtered until colorless filtrate was gained, every 24 hours. Briefly, the filtrate was evaporated to obtain CME and stored at -20°C (Widowati et al., 2016; Rusmana et al., 2017; Widowati et al., 2017).

Fractionation of C. mangga Extract

C. mangga ethanol extract (25 g) and aquades (200 mL) were placed into beaker glass and mixed until homogen. The mixture was added into funnel then added each of hexane and water; etil acetate and water; and butanol and water (1:1), shaked until homogen (20-40 min) and then idle until hexane and water separated (replicated 3-4 times) (Widowati et al., 2011a; Tjahjani et al., 2014).

The NO Scavenging Activity Assay

10 μL sample (CME, WCM, EACM, HCM, BCM, BHT, and ACR with level concentration 133.33, 66.67, 33.33, 16.67, 8.33, 4.17, 2.08 μg/mL, respectively) and 40 μL Sodium Nitopruesside 10 mM (SNP) (Merck, 1.06541) were introduced into each well. The mixed solution was incubated for 5 hour at room temperature. Briefly, Greiss reagent (1% Sulphanilamide (Merck, 1.11799), 2% H₃PO₄ (Merck, 1.00573), 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD) (Merck, 1.06237) were added into each well. The absorbance was measured in a microplate reader (MultiSkan Go Thermoscientific) at 546 nm wavelength (Parul et al., 2012). The NO scavenging activity was measured by formula:

\[
\text{NO Scavenging Activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

Abs sample= Sample absorbance
Abs control= Control absorbance

The H₂O₂ Scavenging Activity

The ferrous ammonium sulphate 12 μL, 1 mM (Merck, 1.03792.1000), 60μL sample (CME, WCM, EACM, HCM, BCM, BHT, and ACR with level concentration 400.00, 200.00, 100.00, 50.00, 25.00, 12.50, 6.25 μg/mL, respectively) and H₂O₂ 5 mM (3 μL)(Merck, 1.08597.1000) was added into each well. And then, the mixture solution was incubated for 5 min at the dark room. Briefly, 75 μL 1,10-phenanthroline (Merck, 1.07223.0010) was added into the well and then incubated at temperature room for 10 min. The absorbance of scavenging activity was measured at 510 nm wavelength (Mukhopadhyay et al., 2016; Utami et al., 2017). The formula used to measured H₂O₂ scavenging activity:

\[
\text{H₂O₂ scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]
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Abs sample= Sample absorbance
Abs control= Control absorbance

Alpha-glucosidase Inhibitory Activity Assay
The α-glucosidase inhibitory activity assayed using modification method (Kim et al., 2004; Widowati et al., 2011b; Gondokesumo et al., 2017). The sample (CME, WCM, EACM, HCM, BCM, and ACR with level concentration 250.00, 125.00, 62.50, 31.25, 15.63, 7.81, 3.91 µg/mL, respectively) was diluted in 10% DMSO (Merck, 1029521000), in control also used 10% DMSO. The α-glucosidase from Saccharomyces sp. yeast (25 µL) 20 mM (SIGMA, G5003), sample (5 µL), 25 µL of 20 mM p-nitrophenyl-a-glucopyranoside (SIGMA, N1377), 45 µLphosphate buffer saline (PBS) (pH= 7) (Gibco, 1740576), were added into a microplate and then incubated for 30 min at 37°C. One hundred microlitres of Na₂CO₃ 0.2 M (Merck, A897992.745) was added in microplate, it stopped the reaction. The absorbance was measured at 400 nm by a microplate reader (MultiScanGo Thermoscientific). The α-glucosidase inhibitory activity was calculated using this formula:

\[
\text{Alpha-glucosidase inhibitory activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

Abs sample= Sample absorbance
Abs control= Control absorbance

Results and Discussion

The NO Scavenging Activity
The NO scavenging activity from extract, fractions of CME, BHT, and ACR is presented in Table 1. Based on Table 1, BHT was the highest activity with IC₅₀ value 69.75 ± 1.74 µg/mL compared to other fraction and ACR, meanwhile BCM has the lowest activity with IC₅₀ value 279.63 ± 2.67 µg/mL.

The H₂O₂ Scavenging Activity
The H₂O₂ scavenging activity of extract, fraction of C. mangga, BHT, and ACR presented in Table 2. The present data showed that EACM has the highest H₂O₂ scavenging activity with an IC₅₀ value 162.78 ± 0.98 µg/mL, while the lowest activity is WCM (IC₅₀ value 4468.79 ± 368.27 µg/mL) (Table 2). EACM has highest antioxidant activity compared to fraction of CME and BHT with IC₅₀ value 179.86 ± 1.66 µg/mL.

Alpha-glucosidase Inhibitory Activity
The α-glucosidase inhibitory activity of CME, BHT and ACR are presented in Table 3. In IC₅₀ value also showed that the highest activity in inhibition of α-glucosidase is HCM (182.45 ± 7.20 µg/mL), while the lowest activity is ACR with IC₅₀=862.93 ± 87.55 µg/mL (Table 3). This indicated that HCM has the highest in α-glucosidase inhibitory activity compared to ACR and other fractions.

Table 1. The IC₅₀ Value of NO Scavenging Activity of Extract, Fractions of C. mangga, Butylated Hydroxytoluene, and Acarbose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression Equation</th>
<th>R²</th>
<th>Average of IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>y = 0.5027x + 14.935</td>
<td>0.91</td>
<td>69.75 ± 1.74</td>
</tr>
<tr>
<td>CME</td>
<td>y = 0.3202x − 35.604</td>
<td>0.92</td>
<td>267.35 ± 3.55</td>
</tr>
<tr>
<td>WCM</td>
<td>y = 0.3372x − 7.408</td>
<td>0.95</td>
<td>170.33 ± 4.08</td>
</tr>
<tr>
<td>EACM</td>
<td>y = 0.3084x − 22.078</td>
<td>0.92</td>
<td>233.85 ± 6.30</td>
</tr>
<tr>
<td>HCM</td>
<td>y = 0.3848x − 19.382</td>
<td>0.90</td>
<td>180.60 ± 8.71</td>
</tr>
<tr>
<td>BCM</td>
<td>y = 0.2027x − 6.6873</td>
<td>0.91</td>
<td>279.63 ± 2.67</td>
</tr>
<tr>
<td>ACR</td>
<td>y = 0.2493x + 8.6352</td>
<td>0.91</td>
<td>166.00 ± 3.29</td>
</tr>
</tbody>
</table>

*Data consist of linear regression equation, coefficient of determination (R²), the IC₅₀ value were presented as mean ± standard deviation. CME= Curcuma mangga ethanol extracts, WCM= Curcuma mangga water extracts, EACM= Ethyl acetate fraction of C. mangga, BCM= Hexane fraction of C. mangga, BHT= Butanol fraction of C. mangga, ACR= Butylated Hydroxytoluene, ACR= Acarbose.

Table 2. The IC₅₀ Value of H₂O₂ Scavenging Activity of Extract, Fraction of C. mangga, Butylated Hydroxytoluene and Acarbose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression Equation</th>
<th>R²</th>
<th>Average of IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>y = 0.1414x + 24.639</td>
<td>0.93</td>
<td>179.86 ± 1.66</td>
</tr>
<tr>
<td>CME</td>
<td>y = 0.0452x + 3.3725</td>
<td>0.92</td>
<td>1031.32 ± 28.49</td>
</tr>
<tr>
<td>WCM</td>
<td>y = 0.0109x + 1.6813</td>
<td>0.98</td>
<td>4468.79 ± 368.27</td>
</tr>
<tr>
<td>EACM</td>
<td>y = 0.2493x + 9.424</td>
<td>0.99</td>
<td>162.78 ± 0.98</td>
</tr>
<tr>
<td>HCM</td>
<td>y = 0.0809x + 4.9153</td>
<td>0.96</td>
<td>206.48 ± 2.92</td>
</tr>
<tr>
<td>BCM</td>
<td>y = 0.0809x + 4.9153</td>
<td>0.97</td>
<td>566.06 ± 81.30</td>
</tr>
<tr>
<td>ACR</td>
<td>y = 0.011x + 1.5239</td>
<td>0.90</td>
<td>4421.59 ± 91.10</td>
</tr>
</tbody>
</table>
The WCM also has high NO scavenging activity, this result was supported the other result that water extract of *C. mangga* exhibits antioxidant activity using β-carotene bleaching and DPPH scavenging method (Pujimulyani et al., 2004). The higher concentration of *C. mangga* extract will increase the antioxidant activity, it may be due to the curcuminoid content (Pujimulyani et al., 2004). As the previous study, the aqueous extract of *C. mangga* has a good free radical scavenging activity (IC\(_{50}\) = 212.70 mg/L) (Indis and Kurniawan, 2016).

### Table 3. IC\(_{50}\) Value of α-Glucosidase Inhibitory Activity of Extract, Fraction of *C. mangga* and Acrabose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression Equation</th>
<th>(R^2)</th>
<th>Average of IC(_{50}) (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CME</td>
<td>(y = 0.1231x - 7.1573)</td>
<td>0.91</td>
<td>469.69 ± 58.49</td>
</tr>
<tr>
<td>WCM</td>
<td>(y = 0.0667x - 1.9402)</td>
<td>0.87</td>
<td>778.72 ± 1.79</td>
</tr>
<tr>
<td>EACM</td>
<td>(y = 0.3064x - 8.618)</td>
<td>0.96</td>
<td>191.54 ± 6.73</td>
</tr>
<tr>
<td>HCM</td>
<td>(y = 0.273x + 0.2305)</td>
<td>0.98</td>
<td>182.45 ± 7.20</td>
</tr>
<tr>
<td>BCM</td>
<td>(y = 0.0846x - 0.0729)</td>
<td>0.96</td>
<td>595.50 ± 5.08</td>
</tr>
<tr>
<td>ACR</td>
<td>(y = 0.0586x - 0.1829)</td>
<td>0.91</td>
<td>862.93 ± 87.55</td>
</tr>
</tbody>
</table>

*Data consist of linear regression equation, coefficient of determination (\(R^2\)), the IC\(_{50}\) value were presented as mean ± standard deviation. CME= Curcuma mangga ethanol extracts, WCM= Curcuma mangga water extracts, EACM= Ethyl acetate fraction of *C. mangga*, HCM= Hexane fraction of *C. mangga*, BCM= Butanol fraction of *C. mangga*, BHT= Butylated Hydroxytoluene, ACR= Acarbose.

In \(H_2O_2\) scavenging activity, EACM has the highest activity compared to other fraction and marker compound (BHT and ACR). Ethyl acetate fractions of *C. mangga* has curcuminoid and zerumin A as phenolic compounds (Malek et al., 2011). In Widowati et al. (2010) study, ethyl acetate fraction show the highest \(H_2O_2\) scavenging activities because of its phenolic compounds. Curcumin as phenolic compound in the fraction of *C. mangga* extract has strong antioxidant activity and can protect biological systems against the oxidative stress that is found to be an important pathophysiological event in a variety of diseases including aging, cancer, diabetes (Borra et al., 2013). *C. mangga* ethanol extract in antioxidant activity not significantly differences compared to Butylated Hydroxy Anisole (BHA) because has curcuminoid (Pujimulyani et al., 2004), condensed tannin (Pujimulyani et al., 2010), and catechin, epigallocatechingallat (Pujimulyani et al., 2013). Alpha-glucosidase inhibitors (AGIs) can be used as monotherapy, combination therapy with other oral drugs and insulin, and as fixed dose combinations, that is suitable diabetes (van de Laar, 2008; Gondokesumo et al., 2017). In the α-glucosidase inhibitory activity, HCM exhibited the highest activity compared to other fraction and acarbose. In other study showed tannin and flavonoid compounds in plants have antidiabetic activity (Velayutham et al., 2012; Babu et al., 2013). This result validated with previous research that *C. mangga* can decrease glucose level in blood and repair histology of mice pancreas glands (*Mus musculus L.*) that induced with 400 mg/kg bb alloxan. *C. mangga* has antidiabetic activity through decreasing β-cell necrosis at dose (200 mg/kg bb) (Madihah and Gani, 2016). In other study, *C. longa* (turmeric), Zingiberaceae plants, is similar to *C. mangga* which contained curcuminoid and ar-turmerone that has antidiabetic properties due to inhibitory activity of α-amylase (IC\(_{50}\)= 31.0 μg/mL) and α-glucosidase (IC\(_{50}\)= 192 μg/mL) (Lekshmi et al., 2012a; Lekshmi et al., 2012b). *C. longa* extracts has α-amylase inhibitory activity with IC\(_{50}\)= 24.5 μg/mL, while α-glucosidase inhibitory activity has value IC\(_{50}\)= 0.28 μg/mL. This data indicated that *C. longa* extract has higher inhibitory activity of α-glucosidase (Lekshmi et al., 2012b). *Curcuma* extracts may control diabetic-dyslipidemia more effectively because of synergistic therapy with other plant extracts such as *Z. officinale* (Hussain et al., 2018). Curcumin has potential as antihyperglycemic that can induces Hsp70 and improves pancreatic β-cells recovery (Kanitkar et al., 2008). *C. longa* and *Z. officinale* extracts combined has some bioactive compounds that have strong intrinsic antidiabetic and anti dyslipidemic therapeutic potentials (Hussain et al., 2015; Gulfraz et al., 2011).
Conclusions

In summary, fractions of *C. mangga* ethanol extract has potential as antioxidant and antidiabetic agent through scavenging of NO and H$_2$O$_2$ and inhibitory of α-glucosidase.

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Conflict of Interest

All contributing authors declare no conflict of interests.

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