HMG-CoA reductase and lipase enzyme inhibition from combination of Gynura procumbens and Curcuma xanthorrhiza aqueous extract

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Abstract

Lifestyle habits related to consuming excessive fatty foods lead to high levels of lipids in the blood. Some drugs are used to reduce lipids by inhibiting the action of the enzyme HMG-CoA reductase and the pancreatic lipase. The Curcuma xanthorrhiza rhizome and Gynura procumbens leaves were empirically used to reduce cholesterol and lipid levels. This study used the combination of aqueous extract of G. procumbens and C. xanthorrhiza rhizomes (4:1) and their single forms as herbs tested. Furthermore, this study aimed to determine the activity of the tested herbs in inhibiting the enzymatic actions of HMG-CoA reductase and pancreatic lipase in vitro, compared with quercetin, curcumin, and positive control. The results showed that the aqueous extract of C. xanthorrhiza with an IC50 value of 127.54 ppm has the highest activity in inhibiting the enzyme HMG-CoA reductase compared to the aqueous extract of G. procumbens and their combination. In the inhibition of lipase enzyme, G. procumbens aqueous extract had the most potent inhibitory activity compared to C. xanthorrhiza and their combination with an IC50 value of 100.08 ppm.

1. Introduction

Fatty foods that contain triglycerides and cholesterol are absorbed by mucosal cells. When food containing fat is consumed in excess, it leads to high levels of fat in the blood (Pebrianty, 2013). Excessive lipids in the body can be reduced by inhibiting the action of the HMG-CoA enzyme (3-hydroxy-3-methylglutaryl coenzyme A) reductase and pancreatic lipase (Last et al., 2011). Basic Health Research in 2018 showed that the Indonesian population used traditional medicine with 48% of herbal products, 31.8% of homemade herbs, and 24.6% of family medicinal plants (Ministry of Health Indonesia, 2018).

Statins are inhibitors of the HMG-CoA reductase, and it effectively inhibits this enzyme up to 95%, similar to Simvastatin, which is effective for reducing blood lipid levels even though it has several side effects, such as inflammation of the muscles (0.5%), myalgia (2-10%), rhabdomyolysis with acute kidney failure (0.1%), and impaired liver function (1-3%) (Varras, 2008; Burg and Espenshade, 2011; Lachenmeier et al., 2012). Lipase is an enzyme that breaks down and converts lipids to fatty acids (Shin et al., 2004). Increased pancreatic lipase activity can increase the absorption of monoglycerides and fatty acids (Joshita et al., 2000), and it causes the accumulation of fat in the body. Orlistat is an effective lipase inhibitor, with several side effects such as gastrointestinal disorders manifesting as oily feces, diarrhea, abdominal pain, and fecal stains (Filippatos et al., 2008). Synthetic drugs such as simvastatin and orlistat have some side effects on the body. Therefore, natural ingredients are needed as alternatives to inhibit the mechanism of HMG-CoA reductase and lipase enzymes. Natural ingredients that were empirically used to reduce cholesterol and lipid levels were Curcuma xanthorrhiza Roxb. (C. xanthorrhiza) and Gynura procumbens (Lour.) Merr (G. procumbens) (Achmad et al., 2009; Niaga Swadaya, 2010).

One of the contents of C. xanthorrhiza is curcumin, which can inhibit acyl-CoA activity in the liver (Aggarwal et al., 2004). Furthermore, it reduces peroxidase enzyme and total cholesterol, as well as increases high-density lipoprotein levels and Apolipoprotein A-1 levels (Yasni et al., 1993; Kertia and Sudarsono, 2005). G. procumbens leaf extract inhibits...
cholesterol synthesis, esterification, triglycerides, and HMG-CoA reductase activity in mice (Metcally et al., 2009). However, there is no in vitro study of the combination of the C. xanthorrhiza rhizome and G. procumbens leaves in inhibiting the enzymatic actions of HMG-CoA reductase and lipase.

In preclinical study showed that the combination of G. procumbens and C. xanthorrhiza aqueous extract (4:1) more effective decreases total cholesterol, triglycerides, and low-density lipoprotein, and then any other combination ratio (Damanik and Ikawati, 2015; Luthfia and Ikawati, 2015). Therefore, this study aimed to determine the inhibitory activity of aqueous extracts of G. procumbens leaves and C. xanthorrhiza rhizomes either in a combination (4:1) or in single form on lipase enzymes and HMG-CoA reductase through in vitro study.

2. Materials and methods
2.1 Materials

C. xanthorrhiza rhizomes and G. procumbens leaves were obtained from Nanggulan, Kulon Progo, and Gamagiri, Mangunan, Yogyakarta respectively. Conversely, curcumin was purchased from Nacalai, while quercetin, a lipid porcine from pancreas L3126-25G, orlistat PH 1445-1G, simvastatin pharmaceutical secondary standard was obtained from Sigma Aldrich®. Furthermore, lipase activity was measured with a colorimetric assay kit K-722 (Biovision®), while the enzymatic actions of HMG-CoA were measured using the screening kit Colorimetric K-588 (Biovision®).

2.2 Preparation of G. procumbens leaf and C. xanthorrhiza rhizome water extract

G. procumbens dried leaves (40 g) was poured in 400 mL distilled water and boiled in an infusion pan. The dried rhizome of the C. xanthorrhiza weighed at 80 g was poured in 800 mL distilled water and boiled in separate infusion pan. Subsequently the aqueous extract of G. procumbens leaves and C. xanthorrhiza rhizomes were freeze-dried (Virtis Bench Top Pro 3 ES USA).

2.3 Inhibition assay of HMG-CoA Reductase enzyme

The HMG-CoA reductase inhibition assay followed the HMG-CoA reductase activity/inhibitor screening kit Colorimetric (Biovision®) guidelines, while Simvastatin, quercetin, and curcumin were weighed using ultramicro scales (Radwag, sensitivity 0.1 μg). The concentration used of simvastatin, quercetin, and curcumin were 0.82-13.16 μg/mL, 2.63-42.11 μg/mL, and 2.63-42.11 μg/mL of ethanol, respectively. Samples of G. procumbens leaves, C. xanthorrhiza rhizome, and their aqueous combinations were weighed with Mettler Toledo scales (sensitivity 0.0001 g) at a concentration of 105.26-263.16 μg/mL, 52.63-157.89 μg/mL, and 52.63-157.89 μg/mL, respectively.

The test solution consisted of simvastatin, curcumin, quercetin, C. procumbens leaves, C. xanthorrhiza rhizome, and a combination of their aqueous extract in the ratio of 4:1. The test solution was made into 6 concentration series, and their reaction mixture contained 190 μL, consisting HMG-CoA of 12 μL substrate, 164 μL buffer, and 5 μL reductase enzyme, NADPH (Nicotinamide adenosine dinospotide hydrogen phosphate) 4 μL, and 5 μL test solution. In addition, it contains a mixture of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in duplicate, incubated at 37°C for 10 mins, and the absorbance was measured at 340 nm wavelength.

2.4 Inhibition of lipase enzyme

The method used was based on the lipase activity colorimetric assay kit (Biovision®) guidelines that were modified by McDougall et al. (2009). Modifications made were the replacement of the enzyme mix with lipase and the absorbance was measured at a maximum wavelength of 400 nm. The lipase enzyme used was obtained from porcine pancreas (Sigma Aldrich®). Meanwhile, the concentration of orlistat was 0.39-6.25 μg/mL dimethyl sulfoxide. The sample used to confirm the obtained value was similar to the one used in the HMG-CoA reductase inhibitory test.

The reaction mixture of the test solution contained 100 μL of 88 μL buffer, 2 μL oxired probe, 2 μL lipase enzyme, 3 μL substrate, and 5 μL test solution. In addition, the mixture consists of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in duplicate and incubated at 37°C for 60 mins, and its absorbance was measured at a wavelength of 400 nm.

The reaction mixture on inhibition of HMG CoA reductase and lipase was measured on the Corona SH-1000 Microplate Reader (Ibaraki-Ken, Japan) and the percentage of inhibition was calculated using the following formula where the difference between the absorbance delta of enzyme and the absorbance delta of the sample divided by the absorbance delta of enzyme than multiplied by 100%. The absorbance delta was the absorbance minus control. The IC50 value was obtained from the grade series linear regression with the percentage inhibition.
3. Results

Curcumin is the important ingredient in *C. xanthorrhiza* while *G. procumbens* is quercetin. Therefore, a comparison of the inhibition test of the enzyme HMG CoA reductase and lipase between curcumin and quercetin were conducted. Furthermore, the result was compared with the standard drug for each assay, i.e. simvastatin for HMG-CoA reductase, and orlistat for lipase enzymes assays. Enzyme inhibition is considered strong when the inhibitory activity is 34.49%, at a maximum concentration of 100 ppm, or with a percentage less or maximum of 2.89. While the inhibition of 10 - <34.49% and less than 10% are considered moderate and weak, respectively (Ong et al., 2014).

3.1 Inhibition assay of HMG-CoA reductase enzyme

This study used a combination of *G. procumbens* and *C. xanthorrhiza* aqueous extracts (4:1) because previous studies had shown that this combination was effective in lowering lipid profiles in rats (Damanik and Ikawati, 2015; Luthfia and Ikawati, 2015). The results of the IC<sub>50</sub> inhibitory assay of the compounds on HMG-CoA reductase enzyme are shown in Figure 1. *C. xanthorrhiza*, *G. procumbens*, and their combined aqueous extract (4:1) water had 50% inhibitory potency at a concentration of 127.54 ppm, 180.86 ppm, and 150.13 ppm, respectively. The most potent of the samples, which can inhibit 50% of the HMG-CoA reductase enzyme is *C. xanthorrhiza* compared with *G. procumbens* and a combination of *G. procumbens* and *C. xanthorrhiza* (4:1) aqueous extract.

Table 1 shows positive inhibitory potential, comparative and sample controls on HMG-CoA Reductase enzyme. The ratio is obtained from the division between concentration and percentage inhibition. *C. xanthorrhiza* extract has strong potential, while the mixture of *G. procumbens* and combination of *G. procumbens* and *C. xanthorrhiza* (4:1) extracts have moderate potential. Furthermore, IC<sub>50</sub> was obtained from a sample divided by a comparison. The comparison of simvastatin with *G. procumbens*, *C. xanthorrhiza*, and the combination of their aqueous extract (4:1) were 20.62, 14.54, and 17.12, respectively.

3.2 Inhibition assay of lipase enzyme

The results of the IC<sub>50</sub> inhibitory assay of the compounds on lipase are shown in Figure 2. *G. procumbens*, *C. xanthorrhiza*, and the combination of their aqueous extract (4:1) at respective concentrations of 100.08 ppm, 208.12 ppm, and 112.06 ppm can inhibit the activity of the lipase enzyme in hydrolyzing oleic acid by 50%. The most potent of the sample solution that can inhibit 50% of the lipase enzyme is *G. procumbens*. Lipase inhibitors inhibit enzymes that cause the lipolysis process to become inhibited and when reacted, the substrate will bind to a calibrator, which has high competition with the sample (Hidayat et al., 2014). In this study, orlistat has a 50% inhibitory potency at a concentration of 2.59 ppm. Several studies indicate that IC<sub>50</sub> value of orlistat was 0.05–2 ppm (Hadváry et al., 1988; Lewis and Liu, 2012; Dechakhamphu and Wongchum, 2015; Padilla-Cambreros et al., 2015).

Table 1. Inhibitory potential and comparison of positive control, comparators and samples on HMG-CoA reductase enzyme

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio Content ≤ 100 with</th>
<th>Potency</th>
<th>Comparison IC&lt;sub&gt;50&lt;/sub&gt; with</th>
<th>Comparison IC&lt;sub&gt;50&lt;/sub&gt; with</th>
<th>Comparison IC&lt;sub&gt;50&lt;/sub&gt; with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>0.18</td>
<td>Strong</td>
<td>-</td>
<td>0.61</td>
<td>0.50</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.59</td>
<td>Strong</td>
<td>1.64</td>
<td>-</td>
<td>0.83</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.62</td>
<td>Strong</td>
<td>1.99</td>
<td>1.21</td>
<td>-</td>
</tr>
<tr>
<td><em>C. xanthorrhiza</em></td>
<td>2.49</td>
<td>Strong</td>
<td>14.54</td>
<td>8.83</td>
<td>7.31</td>
</tr>
<tr>
<td><em>G. procumbens</em></td>
<td>3.22</td>
<td>Moderate</td>
<td>20.62</td>
<td>12.52</td>
<td>10.36</td>
</tr>
<tr>
<td>Combination of <em>G. procumbens</em> and <em>C. xanthorrhiza</em> (4:1)</td>
<td>3.17</td>
<td>Moderate</td>
<td>17.12</td>
<td>10.39</td>
<td>8.60</td>
</tr>
</tbody>
</table>
Table 2. Inhibitory potential and comparison of positive control, comparators and samples on lipase enzyme

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio Content ≤ 100 with percent of inhibition</th>
<th>Potency</th>
<th>Comparison IC₅₀ with Orlistat (times)</th>
<th>Comparison IC₅₀ with curcumin (times)</th>
<th>Comparison IC₅₀ with quercetin (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orlistat</td>
<td>0.80</td>
<td>Strong</td>
<td>-</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.55</td>
<td>Strong</td>
<td>6.49</td>
<td>1.39</td>
<td>0.72</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.52</td>
<td>Strong</td>
<td>4.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. xanthorrhiza</td>
<td>3.67</td>
<td>Moderate</td>
<td>37.48</td>
<td>16.75</td>
<td>12.05</td>
</tr>
<tr>
<td>G. procumbens</td>
<td>2.07</td>
<td>Strong</td>
<td>78.15</td>
<td>8.03</td>
<td>5.78</td>
</tr>
<tr>
<td>Combination of G. procumbens and C. xanthorrhiza (4:1)</td>
<td>2.24</td>
<td>Strong</td>
<td>41.97</td>
<td>8.99</td>
<td>6.47</td>
</tr>
</tbody>
</table>

Table 2 shows the inhibitory potential of positive control, comparative, and sample controls on lipase enzyme G. procumbens and its combination with C. xanthorrhiza extracts have strong potential, while C. xanthorrhiza extract has moderate potential. Meanwhile, the comparison of orlistat with G. procumbens, C. xanthorrhiza, and their aqueous combination (4:1) were 37.48, 78.15, and 41.97, respectively. G. procumbens have strong potency inhibition compared to other samples because the amount of secondary metabolite compound content is more than C. xanthorrhiza and combination G. procumbens and C. xanthorrhiza (4:1) aqueous extract (Pradono et al., 2011). Furthermore, the content of alkaloids in the leaves of G. procumbens results in decreased inhibition potency of extracts, while the content of flavonoids, saponins, and tannins result in increased inhibition potency of extracts (Pradono et al., 2011). Orlistat, curcumin, and quercetin have a strong inhibitory effect on the lipase enzyme.

4. Discussion

Acetyl CoA is an intermediate product from carbohydrates, fats, and some amino acids from proteins that cause animals and humans to convert food substrate to cholesterol. The HMG-CoA reductase enzyme can catalyze the process of converting acetate to mevalonate. Inhibition of cholesterol biosynthesis in the liver is performed by inhibiting the action of the HMG-CoA reductase enzyme. It is a unique enzyme that plays a role at the beginning of irreversible cholesterol biosynthesis (Burg and Espenshade, 2011). Cholesterol is synthesized from acetate under the influence of the HMG-CoA reductase enzyme. It becomes active when there is a deficiency of endogenous cholesterol. In the chain of reaction to produce cholesterol, Acetyl CoA is converted to HMG-CoA then converted to mevalonate. Furthermore, the mevalonate is converted to pyrophosphate, after that it becomes isopentenyl pyrophosphate, then converted to pyrophosphate geranyl and pyrophosphate farenil into squalent which eventually becomes cholesterol (Harikumar et al., 2013).

One of the contents of C. xanthorrhiza is curcumin, and it helps to maintain cholesterol homeostasis through the expression of the mRNA receptor (messenger ribonucleic acid) gene that codes for the enzyme biosynthesis of HMG-CoA reductase and farnesyl diphyosphate. The expression of these genes causes increased protein-binding sterol elements due to synthesis and cellular absorption resulting in a reduction of protein-bound fatty acids translocation and mRNAs from alpha peroxisome proliferator receptors (Peschel et al., 2007).

Human pancreatic lipase is the main enzyme responsible for breaking down fat in the human digestive tract, and it converts the triglyceride substrate in food into monoglycerides and free fatty acids (Svendsen, 2000). Absorption of dietary fat in the body can be prevented by orlistat since it reduces triglycerides. Therefore, free fat can be reduced due to the orlistat mechanism in inhibiting lipase activity because of the binding ability of its catalytic site. These bonds show that the lipase enzyme cannot catalyze the hydrolysis reaction of triglycerides, which leads to a reduction in the absorption of the amount of free fatty acids by the intestine (Pebranyt, 2013).

Pancreatic lipase enzymes play a role in digestion and absorption of triglycerides from 90 to 95% of the digested fat (Ros, 2000). The ethanol extract fraction of G. procumbens leaves contain polyphenols and flavonoids which are used as anti-dyslipidemia by inhibiting the activity of lipase enzymes and play a role in lipid absorption (Setiawan, 2012). Furthermore, Dechakhamphu and Wongchum (2015) showed that flavonoid, phenolic, and alkaloid compounds are important in inhibiting lipase activity in vitro. However, phenolic compounds inhibit the lipase enzyme in the pancreas (Tiss et al., 2004) and it plays a role in catalyzing the hydrolysis of triglycerides to be absorbed by the body in order to reduce cholesterol levels (Sreerama et al., 2012; Onakpoya et al., 2015). Polyphenol compounds can inhibit enzymes involved in fat metabolisms, such as lipase and glycerophosphate dehydrogenase. Extracts containing polyphenols can reduce triglyceride and LDL levels, increase energy...
The combination of G. procumbens and C. xanthorrhiza (4:1) aqueous extract on inhibition of HMG-CoA reductase enzyme showed smaller IC₅₀ value than the single G. procumbens extract, as well as smaller value when compared with single C. xanthorrhiza extract. This study showed that G. procumbens extract can increase the effect of C. xanthorrhiza extract on inhibition of lipase enzyme, while C. xanthorrhiza extract can increase the effect of G. procumbens extract on inhibition of HMG-CoA reductase. Therefore, a combination of Gynura procumbens and Curcuma xanthorrhiza (4:1) extract is needed to obtain a synergistic effect as a dyslipidemia therapy. The results showed that the combination of G. procumbens and C. xanthorrhiza (4:1) aqueous extract was effective in the reduction of cholesterol levels by inhibiting the HMG-CoA and reducing triglyceride through inhibition of the lipase enzyme. Therefore, the combination of G. procumbens and C. xanthorrhiza (4:1) aqueous extract was effective as a therapy for dyslipidemia to reduce total cholesterol and triglyceride in vitro assay.

5. Conclusion
The results of the inhibitory analysis of HMG-CoA reductase showed that aqueous extract of C. xanthorrhiza has the most potent IC₅₀ value than G. procumbens and a combination G. procumbens and C. xanthorrhiza (4:1), i.e 127.54 ppm. While the results of the lipase enzyme inhibition assay demonstrated that G. procumbens have the most potent IC₅₀ value than C. xanthorrhiza and a combination G. procumbens and C. xanthorrhiza, i.e 100.08 ppm. Therefore, it is advisable to conduct in vivo to the efficacy and safety of lipid profiles. Suggestions for further study is that a combination of G. procumbens and C. xanthorrhiza (4:1) aqueous extract should be made for preclinical study of these products on the effectiveness of lipid profiles.

Conflict of interest
The authors declare no conflict of interest.

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