Inhibitory activities of *Moringa oleifera* leaf extract against α-glucosidase enzyme in vitro

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Abstract. Alpha-glucosidase is a key enzyme in the final process of breaking carbohydrates into glucose. Inhibition of α-glucosidase affected more absorption of glucose, so it can reduce hyperglycemia condition. The aims of this study is to determine the effectiveness of inhibition wet and dried *Moringa oleifera* leaf extract through α-glucosidase activity in vitro. The effectiveness study of inhibition on the activity of α-glucosidase enzyme obtained from white glutinous rice (*Oryza sativa* glutinosa) was carried out using wet and dried kelor leaf extract of 13% (w/v) with 10 mM α-D-glucopyranoside (PNPG) substrate. A positive control used 1% acarbose and substrate without addition of extract was a negative control. Inhibitory activity was measured using spectrophotometers at a wavelength of 400 nm. The result showed that the inhibition activity against α-glucosidase enzyme of dried leaf extract, wet leaf extract and acarbose was 81.39%, 83.94%, and 95.4%, respectively on pH 7.0. The effectiveness inhibition of the wet Moringa leaf extract was greater than the dried leaf extract. The findings suggest that *M. oleifera* leaf has the potential to be developed as an alternative food therapy for diabetics.

1. Introduction

Glucosidase enzyme is an enzyme that plays a role in the process of complex polysaccharide hydrolysis into glucose that will be distributed into the blood circulation [1]. Reduction in the absorption of carbohydrates from food by the intestines is a therapeutic approach for postprandial hyperglycemia [2,3]. One of the agents that can be used to minimize the increased contents of sugar in the blood is an inhibitor of α-glucosidase (alpha glucosidase inhibitor, AGI). AGI is one of the antidiabetic agents that works in inhibiting α-glucosidase enzymes [4]. During this time, synthetic inhibitors for α-glucosidase such as acarbose have been widely used for the treatment of type II diabetics but reportedly this drug causes various side effects [5,6]. Therefore, a lot of efforts have been made to find the AGI from a natural source to treat diabetes. According to Shibano *et al.* [7], combination of AGI and antioxidant will be more effective in the prophylaxis of type II diabetics.
*Moringa oleifera* is a plant of the Moringaceae, a family of angiosperms consisting of 14 species [8]. In Indonesia, this plant is easy to cultivate because the climate suitability, so it can grow easily. Currently, *Moringa* leaves become interested subject of study for researchers because the nutritional content is very high. In composition, the leaves of *Moringa oleifera* contains a varied chemical compound and very complete. Several studies report that leaves *M. oleifera* contains vitamins A and E, complete amino acids such as hydroxyproline, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, hydroxylysine, ornithine, lysine, arginine, tryptophan, histidine [9-11]. A study on phytochemical analysis by HPLC analysis on *M. oleifera* showed that there were polyphenols such as quercetin glycosides, kaempferol glycoside, and chlorogenic acid in the powder leaf [12,13]. An ethanol extract of *M. oleifera* leaf contained flavonoids, tannins, anthraquinones, cardiac glycosides, alkaloids, saponins, triterpenoid, and reducing sugars [12,14].

Various studies on the application of moringa plants have been conducted, among others, Sashidhara et al. [15] reported antiinflammatory and anti nociceptive effects of the root part of Moringa plant. *Moringa* was also reported to have activity as hepatoprotective and antibiotic. The ethanol extract of *Moringa* leaf combined with 5-fluorouracil was used in chemotherapy of colon cancer cells. *Moringa* was also reported to have chemopreventive activity, antiinflammatory, antispasmodic, antiuretic, cholesterol-lowering, antioxidant and antifungal [14-18]. The potential of *Moringa* leaf as AGI has a very big chance to be developed, referring to the content of antioxidant compounds, amino acids and some other secondary metabolite compounds contained therein. This study was conducted to test the inhibitory activity of moringa leaf extract on the activity of the α-glucosidase enzyme in vitro.

2. Materials and Methods

2.1 Materials

Materials used were dried and wet *M. oleifera* leaves from Soppeng district, a α-glucosidase enzyme from white glutinous rice, p-nitrophenyl α-D-glucopyranoside (PNPG), 1% acarbose, phosphate buffer, distilled water, Whatman 42 filter paper.

2.2 Preparation of *M. oleifera* leaves

*M. oleivera* leaves were selected from young and fresh leaves from the plantation area. Samples were processed by leaf sorting (fresh young leaf separated from the dirt). The sorted samples were divided into two parts of the wet *M. oleivera* leaf (WML) and dried *M. oleivera* leaf (DML).

2.3 *M. oleivera* extraction

Dried and wet *M. oleivera* leaves were mashed with a blender, weighed as many as 15 g and then added with 100 mL of phosphate buffer. The mixed solution stirred for 1 hour then the extract of *M. oleivera* was filtered with a filter paper. Extracts will be used in an inhibitory test against α-glucosidase enzyme activity.

2.4 α-Glucosidase inhibition assay

The α-Glucosidase inhibitory activity was measured using a modification method and adaptation of the method described in Shim et al. [19]; Subramanian, Asmawi, and Sadikun [20]. 0.2 mL of enzyme solution was added with a 5 mL buffer of pH 7 into the test tube, and 0.2 mL of *M. oleivera* leaf extract was added with concentrations of 1%, 5%, 10% and 15% (v/v) on a different tube. 0.5 mL of PNPG was added to a concentration of 10 mM, incubated at 37 °C for 20 min and 8 mL of 100 mM Na2CO3 was
added to the mixture. The absorbance was recorded at 400 nm using a spectrophotometer. The optimum concentration was used in the effect study of pH against α-glucosidase inhibition. The negative control used an enzyme solution without the addition of *M. oleifera* leaf extract and positive control was 1% acarbose. The enzyme activity was calculated by equation 1:

\[
\text{Enzyme activity} \left[ \frac{\text{U}}{\text{mL}} \right] = \frac{\text{Abs sample} - \text{Abs blanko} \times \text{Total volume analyzed} \times \text{Volume} (\text{enzyme + substrat + buffer})}{\text{Molar extinction coefficient} \times \text{Incubation time} \times \text{Mixedtured volume} \times \text{Enzyme volume}}
\]

Abs = absorption

3. Result and discussion

The process of extracting wet and dried moringa leaves using buffer pospat pH 7 to prevent decomposition. The inhibitory test against α-glucosidase enzyme is done by variation of concentration of extract of wet and dried moringa leaves. The concentration variations used were 1%, 5%, 10% and 15%. This is intended to determine the effect of wet and dried leaves on the inhibition of α-glucosidase enzyme activity. Table 1 shows the activity percentage and the inhibition percentage of M. oleifera on α-glucosidase.

**Table 1.** Value of α-glucosidase activity, percentage of activity and percentage of inhibition on α-glucosidase enzyme.

| Sample        | α-glucosidase activity (mU/mL) | % Activity | % Inhibition |
|---------------|-------------------------------|------------|-------------|
| Negative Control (NC) | 158.07                        | 100        | 0           |
| Acarbose 1% (PC)     | 6.85                          | 4.64       | 95.36       |
| DML 1%             | 157.17                        | 99.43      | 0.57        |
| DML 5%             | 141.05                        | 89.23      | 10.77       |
| DML 10%            | 94.71                         | 59.91      | 40.09       |
| DML 15%            | 29.42                         | 18.61      | 81.39       |
| WML 1%             | 142.66                        | 90.25      | 9.75        |
| WML 5%             | 108.81                        | 68.84      | 31.16       |
| WML 10%            | 72.94                         | 46.15      | 53.85       |
| WML 15%            | 25.39                         | 16.06      | 83.94       |

The effects of the dried and wet *M. oleifera* extract on the inhibition of the α-glucosidase enzyme is given in figure 1.

**Figure 1.** The effect of the concentration of dried and wet *M. oleifera* leaves extract on the inhibition effectiveness of the α-glucosidase enzyme.

It is clear that the higher the concentration of the extract the higher the activity of inhibition enzyme will
be. The extract of DML has the lowest activity inhibition i.e., 0.57% with the activity of 157.17 mU/mL and the activity percentage of 99.43% at the concentration of 1%. For WML, the lowest activity inhibition is 9.7% with the activity of 142.66 mU/mL and the activity percentage of 90.25% at the leaf concentration of 1%. The optimum activity of dried and wet M. oleifera leaves on the \( \alpha \)-glucosidase enzyme was 29.42 mU/mL (18.61%) and 25.39 mU/mL (16.06%), respectively at the concentration of the extract of 15%. Based on the value of \( \alpha \)-glucosidase inhibition obtained at a different percentage, it is clear that the M. oleifera leaf extract has the potential of anti-hyperglycemia activity by inhibiting the enzyme \( \alpha \)-glucosidase in the brush border of intestinal smooth. Inhibition of the \( \alpha \)-glucosidase enzyme decreased the rate of digestion of carbohydrates into monosaccharides that can be absorbed by the intestine.

The pH effect on the activation and inhibition of \( \alpha \)-glucosidase by dried and wet M. oleifera leaves extract was studied using buffer phosphate solutions of pH 6.5 and 7.0. Table 2 shows the \( \alpha \)-glucosidase activity, the activity percentage and the inhibition percentage on the \( \alpha \)-glucosidase enzyme at the used pH.

**Table 2. \( \alpha \)-glucosidase activity, percentage of activity and percentage of inhibition of \( \alpha \)-glucosidase enzyme at pH 6.5 and 7.0.**

| Sample                  | \( \alpha \)-glucosidase activity (mU/mL) | % Activity | % Inhibition |
|-------------------------|------------------------------------------|------------|-------------|
| Negative control (NC), 6.5 | 153.79                                   | 100        | 0           |
| DML 15%, 6.5            | 45.94                                    | 29.87      | 70.13       |
| WML 15%, 6.5            | 29.42                                    | 19.13      | 80.17       |
| Acarbose 1% (PC), 6.5  | 7.25                                     | 7.89       | 92.11       |
| Negative control (NC), 7.0 | 157.30                                   | 100        | 0           |
| DML 15%, 7.0            | 29.47                                    | 18.70      | 81.30       |
| WML 15%, 7.0            | 25.79                                    | 16.40      | 83.60       |
| Acarbose 1% (PC), 7.0  | 1.61                                     | 5.48       | 94.52       |

The effectiveness of inhibition of wet M. oleifera leaf extract is higher than that of dried M. oleifera leaf extract. This is occurred because the heat used for drying process of M. oleifera leaves caused oxidation or decomposition of active compounds contained in the sample [21][22]. According to Adisakwattana and Chanathong [23], the inhibition ability of M. oleifera leaf against \( \alpha \)-glucosidase enzymes showed a good anti-hyperglycemia activity, so that it is very potential to be developed. An intake of M. oleifera leaf may delay glucose absorption to blood circulation in pre-diabetic patients that help to prevent the increase of type 2 diabetes [23].

**4. Conclusion**

The optimum concentration of the dried and wet M. oleifera leaves extract in inhibiting the activity of the \( \alpha \)-glucosidase enzyme was 15% with the inhibition percentage of 81.39% and 83.94%, respectively. The effective pH in the process of inhibition was 7.0. There was quantitatively the difference activity between wet and dried leaves. The degradation factor of the chemical compound composition due to sample drying has a significant effect on the decrease of inhibition activity on the \( \alpha \)-glucosidase enzyme. Besides of the nutritional composition of the M. oleifera leaf, the effectiveness of inhibition on the \( \alpha \)-glucosidase enzyme showed that M. oleifera is potential to be used as a natural \( \alpha \)-glucosidase inhibitor (AGI).

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