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By ICOS UnHas 2017 Batch 2

WORD COUNT	2681	TIME SUBMITTED	05-DEC-2017 06:22PM
		PAPER ID	33309995

INHIBITORY ACTIVITIES OF *Moringa oleifera* LEAF EXTRACT AGAINTS α-GLUKOSIDASE ENZYME AN IN VITRO

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ABSTRACT

Alpha-glucosidase is a key of the enzyme in the final catalytic process carbohydrates into glucose. Inhibition α-glucosidase affected more absorption of glucose, so it can reduce hyperglycemia condition. The aims of this study to determine effectiveness inhibition wet and dried Moringa oleifera leaf extract through α-glucosidase activity an in vitro. Inhibition method used wet and dried leaf extract 15% (w/v) against α-glucosidase enzyme activity from Oryza sativa glutinosa 10 mM with para nitrophenyl α-D-glucopyranoside (PNPG) substrate. Positive control used 1% acarbose and negative without an addition of extract. Inhibitory activity measured using spectrophotometers at wavelength 400 nm. The result showed inhibition activity against α-glucosidase enzyme of dried leaf extract 81,39%, wet leaf extract 83,94% and acarbose 95,4% on pH 7,0. The effectiveness inhibition of wet leaf Moringa extracts greater than dried leaf extract. The findings suggest that Moringa leaf has the potential to be developed as an alternative food therapy for diabetics.

Keywords: Moringa oleifera, α-glucosidase, leaf extract, inhibition, hyperglycemia

Introduction

Glucosidase enzyme is an enzyme that plays a role in the process of complex polysaccharide hydrolysis into glucose that will be distributed on a goes into the blood circulation [1]. Reduction in absorption of carbohydrates from food by the intestines is a therapeutic approach for postpadrial hyperglycemia [2][3]. One of the agents that can be used to minimize the increased levels of sugar in the blood is an Inhibitor of α-glucosidase (alpha inhibitor glicosidase, AIG). AIG is one of the antidiabetic agent that works by inhibiting α-glucosidase enzymes [4]. During this time, an inhibitor of α-glucosidase for synthesis such as acarbose has plenty to do for the handling of type II diabetics, however these drugs reported to cause various side effects [5][6]. In connection with such a lot of effort has been done to find the AIG from a natural source to treat diabetes. According to Shibano et al. 2008 combination of AIG and antioxidant will be more effective in the prophylaxis of type 2 diabetes [7].

Moringa oleifera is a plant of the Moringa, a family of flowering plants consisting of 14 of the other species [8]. In Indonesia, this plant is easy to cultivate because the climate suitability, so it can grow easily. Currently, Moringa leaves become interest 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 of vitamins A and E, completely amino acid such us hidroksiprolin, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanin, valin, cysteine, methionine, isoleucine, leusin, tyrosine, fenilalanin, hidroksilisin, ornitin, lysine, arginine, tryptophan, histidin [9][10][11]. Another study about phytochemicals analysis of *M.oleifera* showed that there are polyphenols such as quercetin glycosides, kaempferol glycoside, and chlorogenic acid in M.oleifera flour through HPLC analysis [12][13]. Extraction of M.oleifera leaf used 70% ethanol solution by maceration method, showed there are flavonoids, tannins, anthquinone, cardiac glycosides, alkaloids, saponins, triterpenoid, and reducing sugars [14][12].

Various studies on application of moringa plants have been conducted, among others, Sashidhara et al. 2009 reported antiinflammatory and antinosiseptic effects of the root part of Moringa plant. Moringa is also reported to provide activity as hepatoprotective and antibiotic [15]. Extraction of Moringa leaf with ethanol combined 5-fluorouracil was reported as chemotherapy in colon cancer cells. Moringa is also reported to have chemopreventive activity, anti-inflammatory, antispasmodic, antidiuretic, cholesterol-lowering, antioxidant and antifungal [14][16][17][18][15]. 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 α-glucosidase enzyme in vitro.

2. MATERIAL AND METHOD

2.1 Materials

Dried and wet M.oleifera leaves from soppeng district, α-glucosidase enzyme from white glutinous rice, p-nitrophenyl α-D-glucopyranoside (PNPG), 1% acarbose, phosphate buffer, aquades, whatman 42 filter paper.

2.2 Preparation of *Moringa oleifera* leaves

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

2.3 *Moringa oleifera* extraction

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

2.4 *α-Glucosidase* inhibition assay

α-Glucosidase inhibitory activity was measured using modification method an adaptation of the method described in Shim et al. 2003 [19]; Subramanian, Asmawi, and Sadikun 2008 [20]. 0.2 mL enzyme solution was added with a 5 mL buffer of pH 7 into the test tube, and 0.2 ml of Moringa oleifera leaf extract was added with concentrations of 1%, 5%, 10% and 15% (b/v) on a different tube, 0.5 mL of PNPG added to a concentration of 10 mM, incubated at 37 °C for 20 minute and 8 mL of Na_2CO_3 100 mM was added to the mixture. The absorbance was recorded at

400 nm using a spectrophotometer. The optimum concentration results continued on the effect of pH againts α-glucosidase inhibition. Negative control used enzyme solution without the addition of *M.oleifera* leaf extract and positive control is acarbose 1%. The enzyme activity is calculated by the equation:

$$\text{Enzyme activity}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{\text{Abs sample}-\text{Abs blanko x Total volume analyzed x Volume (enzyme+substrat+buffer)}}{\text{Molar extinction coefficients x Incubation time x Mixtured volume x Enzyme volume}}$$

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3.Result and Discussion

In this study, the sample of *M.oleifera* leaves used from soppeng regency. The population of this plant is quite abundant in the area because *M.oleifera* is widely used as a hedge in some houses of the society. *M.oleifera* leaves are sorted then separated from impurities and then washed. *M.oleifera* leaves have been clean and then divided into two parts, one part is a sample of wet *M.oleifera* leaves (WML) which directly extraction. The second sample was used dried *M.oleifera* leaves (DML). Wet Moringa leaves are dried in the pen room with good air circulation and protected from direct sunlight for ± 5 days. The dried moringa leaves are put into the oven at ± 40 ºC for 24 hours. Drying is done to reduce the water content in the sample.

DML and WML have been prepared and then mashed with a blender. The aims of sample refinement to minimize the particle size of *M.oleifera* leaves so the extraction of active compound can be done easily. *M.oleifera* dissolved with buffer pospat pH 7 then stirred for 1 hour to be homogeneous. The aims of buffers as extracts to make the composition of active compounds in the sample do not decompose and the pH stability of the sample was normally.

α-glucosidase inhibitory is useful in treating hyperglycemia in diabetes mellitus patients by reducing the amount of monosaccharides can be absorbed by the intestine. Inhibition of α-glucosidase in this study based on the modified method an adaptation of the method described in [19][20].

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

Exp: DML: Dried Moringa Leaf extract
WML: Wet Moringa Leaf extract

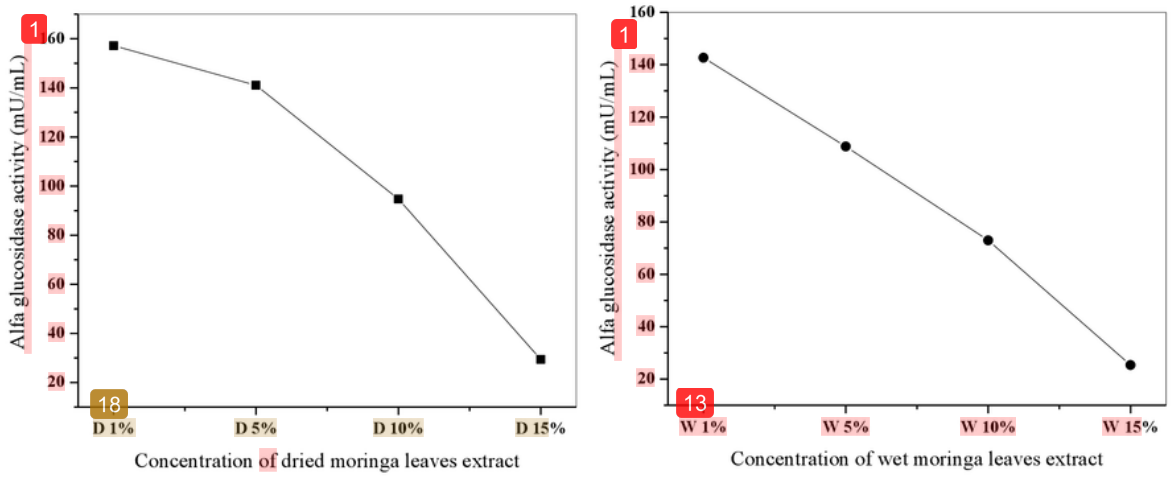

Figure 1. Concentration effect of dried and wet *M.oleifera* leaves extract on the effectiveness inhibition of α-glucosidase enzyme.

As shown in the table 1 and figure 1, based on the inhibition assay showed that the higher concentration of the extract will be increasing the activity of inhibition enzyme. The extract of DML is the lowest activity inhibition there are 157.17 mU/mL with the activity percentage of 99.43% at the concentration of 1%. For WML 142.66 mU/mL with percentage of activity 90.25% at concentration of 1%. For the optimum inhibitory concentration of α-glucosidase enzyme activity at 15% concentration with 29.42 mU/mL (29.42%) on DML and 25.39 mU/mL (16.06%) on wet *M.oleifera* leaves extract (WML).

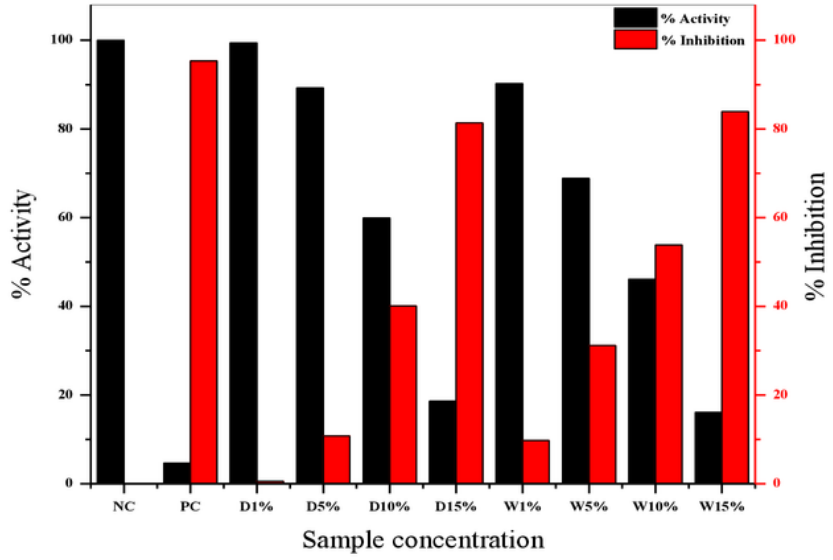

Figure 2. Percentage of activity and inhibition of dried and wet *M.oleifera* leaves extract, positive and negative control (acarbose 1%)

Based on the value of α-glucosidase inhibition obtained different percentage of inhibition between dried and wet *M.oleivera* leaves. At the leaves of wet *M.oleivera* extract with a concentration of 1%, 5%, 10% and 15% obtained the percentage inhibition of 9.75%, 31.16%, 53.85% and 83.94%. It shows that the Moringa oleifera leaf extract has the potential of antihiperglikemik activity by inhibiting the enzyme α-glucosidase in the brush border of intestinal smooth. Inhibition of α-glucosidase enzymes decreased the rate of digestion of carbohydrates into monosaccharides that can be absorbed by the intestine.

The pH effect against activities and inhibition of α-glucosidase by dried and wet *M.oleifera* leaves extract using pH 6.5 and 7.0 buffer phosphate. Based on the table 3 and figure 3 showed that the optimum inhibition on WML 15% with inhibition values of 34.60% and 33.50%.

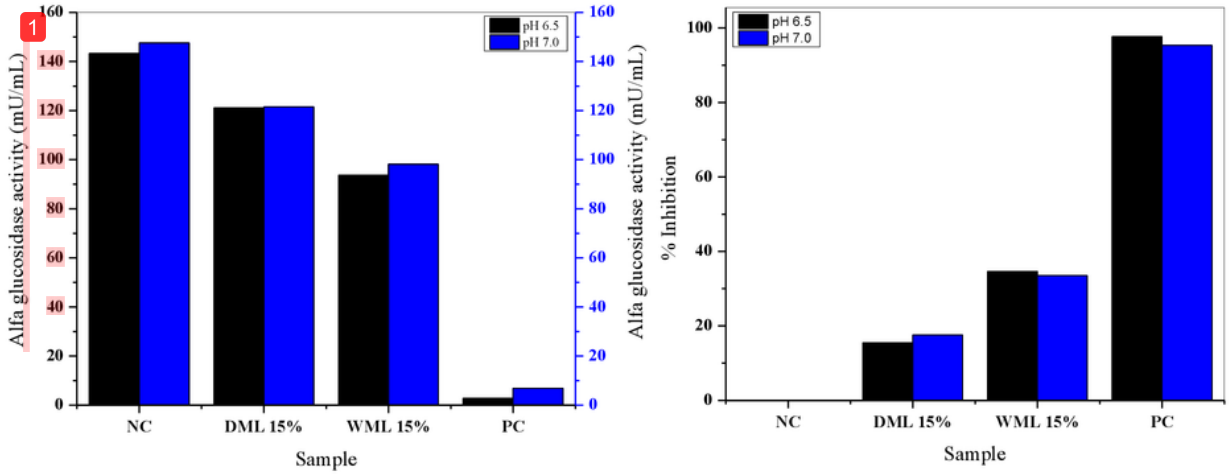

Figure 3. Value of activity and inhibition test result of α-glucosidase enzyme at pH 6.5 and 7.0

Table 3. α-glucosidase activity activity, percentage of activity and percentage of inhibition of α-glucosidase enzyme at pH 6.5 and 7.0

Sample	α-glucosidase activity (mU/mL)	% Activity	% Inhibition
Negative control (NC), 6.5	143.28	100	0
DML 15%, 6.5	121.10	84.52	15.48
WML 15%, 6.5	93.70	65.40	34.60
Acarbose 1% (PC), 6.5	2.82	2.33	97.67
Negative control (NC), 7.0	147.56	100	0
DML 15%, 7.0	121.51	82.35	17.56
WML 15%, 7.0	98.13	66.50	33.50
Acarbose 1% (PC), 7.0	6.85	4.64	95.36

Based on inhibitory test showed that the effectiveness of inhibition of wet *M.oleifera* leaf extract is higher than dried *M.oleifera* leaf extract. This occurs because the drying process on a sample *M.oleifera* leaves with warming causing the occurrence of oxidation or decomposition against active compounds contained in the sample [21][22]. According to Adisakwattana and Chanathong, 2011, inhibition ability of *M.oleifera* leaf against α-glucosidase enzymes showed a

good antihiperglikemik activity, so that it's very potential to be developed. An intake of *Moringa oleifera* leaves may delay glucose absorption to blood circulation in pre-diabetic patients that help to prevent the development of type 2 diabetes [23].

4. Conclution

The optimum inhibition of α-glucosidase enzymes showed that the optimum concentration of *M.oleifera* leaves extract on 15% dried and wet extract with values 81.39% for the dried *M.oleifera* leaves extract and 83.94% for wet *M.oleifera* leaves extract. Effective pH in the process of inhibition of α-glucosidase enzyme at pH 7.0. Inhibition value seen the difference activity between wet leaves 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 α-glucosidase enzyme compared to the wet leaves. The potential of Moringa from the aspect of nutritional composition and the effectiveness of inhibition showed potential as a natural α-glucosidase inhibitor (AIG).

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Acknowledgments

The Author gratefully acknowledges to Hasanuddin University for fully supported in this research through Unggulan UNHAS Research Grant 2017.

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