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COMPARISON OF α –GLUCOSIDASE INHIBITORY ACTIVITY OF *Moringa oleifera* L ETHANOLIC EXTRACT ORIGINATED FROM DIFFERENT SOUTHEAST SULAWESI AREAS

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ABSTRACT

Moringa oleifera L. plant that has been traditionally used to treat diabetes mellitus by community in Southeast Sulawesi region. The purpose of this study was to determine the α-glucosidase enzyme inhibitory activity from *Moringa oleifera* L that were originated from three different Southeast Sulawesi areas, i.e. Saragi, Bacuhau and Batumatongka. Moringa leaves were extracted by maceration using ethanol 95%. The α –glucosidase inhibitory activity was performed *in vitro*. The result showed that Moringa ethanolic extract from the Sarangi, Bacuhau, and Batumatongka areas had the IC_{50} value of 18.62, 10.18, 10.58 ppm, respectively. These values were comparable with the IC_{50} of acarbose (11.54 ppm), showing a strong inhibitory towards α–glucosidase activity. It is concluded that Moringa ethanolic extract had a capacity to inhibit α–glucosidase, and the potential was similar to that of acarbose.

Keywords : α –Glucosidase inhibitory, *Moringa oleifera* L , ethanol extract, IC50

1 Introduction

The number of new cases of diabetes mellitus is increasing rapidly worldwide. The prevalence of diabetes mellitus in adults (20–79 years) was 6.4% or 285 million in 2010 and is expected to increase by 7.7% or 439 million in 2030 (1). It is projected that the occurrence of diabetes melitus (DM). in Indonesian may reach 21.3 million people in 2030 (2).

People with diabetes mellitus must take oral antidiabetic drugs for life. One of the pharmacological therapies for the treatment of type 2 diabetes mellitus is oral hypoglycemic drug. One of the most popular choices available is the alpha-glucosidase enzyme inhibitor, since they are associated with lesser adverse effects. The *α* -glucosidase is an enzyme found in the intestine that catalyzes the breakdown of polysaccharide groups to be absorbed in the form of monosaccharides (3). The mechanisms of hypoglycemic action of *α*-glucosidase inhibitors are by reducing the digestive process of complex carbohydrates and their absorption, thereby, futher decreasing the glucose levels in people with diabetes mellitus (4, 5)

The increasing prevalence of diabetes mellitus needs serious attention for the treatment of this disorder. Treatment with synthetic drugs often fails because side effects, the development of insulin resistance, or the high costs for long-term therapy. Many Efforts has been done to search for alternative antidiabetic agents with better efficacy, minimal side effects, controlled blood sugar levels and relatively cheaper cost (6,7).

Indonesia, with its tropical climate, is endowed with abundant natural resources. These natural resources have been impeccable sources of traditional medicines. *Moringa oleifera* L. plant is one of medicinal plants originated from indonesia that has been traditionally used for its antidiabetic effect (8, 9). Moringa, which belongs to the Moringaceae family, is known to contain more than 90 types of nutrients in the form of essential vitamins, minerals, amino acids. Moringa contains 539 compounds known in African and Indian traditional medicine and have been used in traditional medicine, one of which is an antidiabetic (10).

The ethanolic extract of Moringa oleifera leaves contains a range of compounds, such as flavonoids, tannins, anthraquinones, cardiac glycosides alkaloids, triterpenoids, saponins, and reducing sugars. Tende et al. (2011) have shown that flavonoid compounds may have a hypoglycemic effect by stimulating pancreatic β cells and further increasing insulin secretion (11).

2 Material And Methode

2.1 Sample preparation

Moringa oleifera L leaves were harvested from three different regions in Southeast Sulawesi-Indonesia, namely Saragi, Bacuhau and Batumatongka. The collected leaves were cleaned, dried and made into powder.

2.2 Extraction

Moringa leaf powder was extracted by maceration using ethanol 95%. Maceration was carried out for 72 hours. The extract obtained from the maceration process was filtered using Buchner vacuum filter. The liquid extract was then evaporated using rotary evaporator to thicken the solvent, and further dried until crude extract was obtained.

2.3 Thin Layer Chromatography (TLC) profile

The ethanol extracts were analysed with a thin-layer chromatography (TLC) method using toluene : ethyl acetate (7:3) solvent to identify the profile of chemical compounds that potentially act as bioactive substances. The TLC profiles were observed under a visible and UV light at a wavelength of 366 nm. The identification of chemical compounds was performed using spray reagents for alkaloid (Dragendorff) and flavonoid (sitroborat).

2.4 The α-glucosidase inhibitory assay

The inhibition of α-glucosidase activity was tested according to the method previously described in Sancheti's study [12] with some modifications. The α-glucosidase enzyme was dissolved in a phosphate buffer solution (PBS) with pH 7. The enzymatic reaction was initiated by adding the Moringa extract (10 µL) into 25 mM p-NPG substrate (15 µL) in 60 µL PBS in a 96-well plate, then incubated at a temperature of 37°C for 5 minutes. To start the reaction, a

15 µL of α-glucosidase enzyme was added and then incubated for 30 minutes at 37°C. The reaction was stopped by adding 0.2 M Na_2CO_3 solution as much as 100 µL. The absorbance were measured at 405 nm using an ELISA reader. The experiment was triplicates and the %inhibition was calculated.

$$\% \text{ Inhibition} = \frac{Absorbance\ without\ sample - \text{Absorbace with sample}}{\text{Absorbance with sample}} X\ 100\ \%$$

3 Result And Discussion

Extraction process was performed using a maceration method with 95% ethanol solvent. Ethanol solvent was chosen because it has the ability to extract a wide range of chemical substances form from nonpolar to polar compounds (13). The separation. of the compounds may depends on the difference in the solubility of the components to be separated in the solvent (14). Polar compounds will dissolve in polar solvents, and vice versa. Apart from the type of solvent, the size of the sample subjected to any extraction method also affects the yield of extraction. A smaller the sample surface area will promote the contact surface of the sample and thereby, increase its interaction with the solvent (15). The Moringa ethanolic extract from three different areas were analyzed using a TLC method to determine the presence of alkaloid and flavonoid compound class using spray reagents.

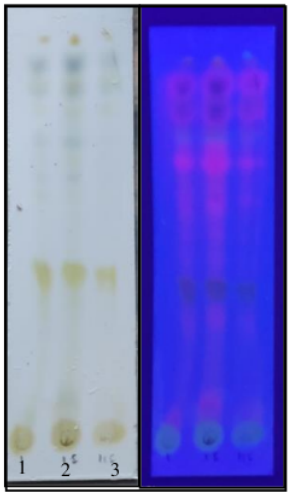

Figure 1. Chromatogram of ethanol extract of Moringa leave using a mobile phase of toluene : ethyl acetate (7:3), under a visible light (A) and 366 nm UV light (B).1: Saragi, 2: Bacuhau, 3: Batumatongka

Table 1. Screening phytochemicals of ethanolic extract from *M. oleifera* leaves from different origins

Compound class	Moringa Extract		
	Saragi	Bacuhua	Batumatongka
Flavonoid	+	+	+
Alkaloid	+	+	+

In this study, the potential antidiabetic effect of Moringa extract was confirmed by an *in vitro* assay by calculating the % inhibition of α-glucosidase activity. Acarbose was used as a comparison since acarbose itself is a competitive and reversible inhibitor of alpha-amylase in pancreatic tissue as well as the inhibitor of membrane-bound intestinal α-glucoside hydrolase. In addition, it has widely used in *in vitro* studies as a standard. By impeding carbohydrate metabolism, acarbose delays glucose absorption, and thereby, it causes a decrease in postprandial glucose serum levels (16).

Indeed, for this reason, acarbose has been widely used as antidiabetic treatment. When acarbose inhibits the action of α -glucosidase hydrolase in small intestine, it inhibits the breakdown of complex carbohydrates (disaccharides, trisaccharide, polysaccharides) to simple carbohydrates (glucose and fructose), leading to a depletion of glucose absorption in the brush border of the small intestine (16).

From the α-glucosidase inhibitory assay, it was revealed that the ethanolic extract of Moringa leaves from the three different areas in Southeast Sulawesi had different levels of α-glucosidase inhibition . Moringa extract from the Saragi area had IC_{50} of 18.6196, from the Bacuhau areawith IC_{50} of 10.1851 and from the Batumatongka area with IC_{50} of 10.5801 (Table 2) . Meanwhile, the α-glucosidase inhibitory activity from acarbose showed the value of IC_{50} of 11.5434.

Table 2. Comparison of the α-glucosidase inhibitory activity of Moringa leaf extracts from three different areas

Sample	Concentration (ppm)	% inhibition	Linear regretion	IC 50
Moringa leaves from Saragi	10 20 30 40 50	6.1313 8.981 10.8409 12.6212 16.773	y = 2,4924x + 3,5924 R^2 = 0,9746	18.6196
Moringa leaves from Bacuhau	10 20 30 40 50	3.2217 7.0945 10.8808 18.2809 21.1904	y = 4,7124x - 2,0035 R^2 = 0,9817	10.1851
Moringa leaves from Batumatongka	10 20 30 40 50	8.0045 9.9575 13.6044 20.4796 25.4949	y = 4,5503x + 1,8573 R^2 = 0,9622	10.5801
Acarbose	10 20 30 40 50	7.5063 12.0965 16.2482 18.1546 24.545	y = 4,0136x + 3,6695 R^2 = 0,9782	11.5434

The active compounds in Moringa leaves that may have hypoglycemic effect are flavonoids (quercetin and kaempherol) and triterpenoids. This has been demonstrated in arat

model of diabetes mellitus using a streptozotocin (STZ) as the inducing agent (17). Flavonoid compounds are shown to be able to regenerate pancreatic β cells in STZ-induced diabetes mellitus rats (17). Moreover, the compound quercetin that is also found in Moringa leave, was found beneficial to stimulate progenitor cells in the pancreatic duct to promote cellular differentiation. This, subsequently, facilitates a formtion of new islet Langerhans cells or endocrine cells in diabetic mice that has been subjected to induced pancreatic damage (18). In addition, Moringa leaves also rich in vitamins, minerals and essential amino acids that will be useful in cell regeneration (19). Therefore, this present study support the use of Moringa leave extract as an antidiabetic agent, mainly from its inhibition on α-glucosidase activity.

4. Conclusion

Moringa oleifera ethanolic extract had α- glucosidase inhibitory activites that may differ based on the sample origin, ie Sarangi, Bacuhau and Batumatongka area. The lowest IC_{50} was found in Moringa extract originated from Bacuhau (IC_{50} = 10.18 ppm), but not much different from the IC_{50}. Of Moringa extract from Batumatongka areas (IC_{50}= 10.58 ppm). The IC_{50} was even lower compared to the acarbose (IC_{50} = 11.54 ppm), suggesting a greater α- glucosidase inhibitory activity of the extract.

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