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COMPARISON OF α –GLUCOSIDASE INHIBITORY ACTIVITY OF *Moringa oleifera* ETHANOLIC EXTRACT

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KEYWORDS

α –Glucosidase inhibitory

Moringa oleifera

Ethanol extract

IC50

Acarbose

ABSTRACT

The purpose of this research was to determine the α-glucosidase enzyme inhibitory activity of *Moringa oleifera* plant samples collected from the three geographical areas viz., Saragi, Bacuhau, and Batumatongka of Southeast Sulawesi Indonesia. Ethanol extract of *Moringa* leaves was prepared by the maceration method using 95% ethanol. The estimation of α –glucosidase inhibitory activity of this extract was performed *in vitro*. The results of the study showed that ethanolic extract of three Moringa samples i.e. Sarangi, Bacuhau, and Batumatongka had the IC_{50}value of 18.62, 10.18, 10.58 ppm, respectively while IC_{50}value for the acarbose positive control was reported 11.54ppm. From the results of this study, it can be concluded that ethanolic extract of *Moringa* could inhibit α –glucosidase and this potential was similar to the commercial α –glucosidase inhibitor acarbose.

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1 Introduction

Now in these days prevalence of diabetes mellitus is rapidly increasing throughout the world. The prevalence of diabetes mellitus in adults (20–79 years) was 6.4% or 285 million in 2010 and is expected to reach 7.7% or 439 million up to 2030 (Shaw et al., 2010). While in the case of Indonesia, the incidence of diabetes mellitus is projected to reach up to 21.3 million by 2030 (Kementerian Kesehatan, 2020). The increasing prevalence of diabetes mellitus needs serious attention for the treatment of this disorder. For a healthy routine diabetes mellitus infected people take insulin or oral antidiabetic drugs piles daily. The oral hypoglycemic drug is one of the most important pharmacological therapies for the treatment of type 2 diabetes mellitus. One of the alternate popular choices is the alpha-glucosidase enzyme inhibitor drugs because these are associated with lesser side effects. The *α*-glucosidase is an enzyme found in the intestine and catalyzes the breakdown of polysaccharide groups to monosaccharides and helps in the glucose absorption by the intestine (Palanuvej et al., 2009). The mechanisms of hypoglycemic action of α-glucosidase inhibitors are the reduction in the digestive process of complex carbohydrates and their reduced intestinal absorption; therefore, it decreases the glucose levels in people with diabetes mellitus (WHO, 1999; Saha et al., 2011).

Treatment with synthetic drugs often fails because these drugs have lots of side effects including the development of insulin resistance, or the huge costs for long-term therapy. Many efforts have been made to search for alternative antidiabetic agents with better efficacy, minimal side effects, controlled blood sugar levels, and relatively cheaper cost. (Fahey, 2005; Rante et al., 2019).

Indonesia has abundant natural resources and among these, medicinal plants as a source of traditional medicine are the most common ones. *M. oleifera* belongs to the family Moringaceae and is widely used by traditional healers for the treatment of diabetes mellitus due to its anti-diabetic properties (Jaiswal et al., 2009; Giridhari et al., 2011). Moringa is well known for its more than 90 types of nutrients in the form of essential vitamins, minerals, amino acids. Further, it contains almost 539 active compounds which have been made this plant a popular choice for the African and Indian traditional healers. Further antidiabetic properties of this plant is also well reported (Toripah et al., 2014). The leaves extract of *M. oleifera* contains various active ingredients including flavonoids, tannins, anthraquinones, cardiac glycosides alkaloids, triterpenoids, and saponins which help in reducing sugars. Tende et al. (2011) reported that the hypoglycemic effect of flavonoids is associated with the stimulation of pancreatic β cells which enhance insulin secretion.

2 Material and Methods

2.1 Sample preparation

Leaves samples of *M.oleifera* were collected from the three different regions namely Saragi, Bacuhau, and Batumatongka of the Southeast Sulawesi, Indonesia. The collected leaves were cleaned, shaded dried and a fine powder was made with the help of an electronic mixture & grinders.

2.2 Extraction

Ethanolic extract of *Moringa* leaf powder was extracted by using the maceration method with 95% ethanol. The obtained extracts were filtered by using a Buchner vacuum filter and evaporated using a rotary evaporator to thicken the solvent, and this drying procedure was continued until the crude extract was obtained.

2.3 Thin Layer Chromatography (TLC) profile

The ethanol extract was analyzed by using thin-layer chromatography (TLC), and for this, toluene: ethyl acetate (7:3) mixture was used as a mobile phase to qualitatively identify the presence of bioactive substances. The TLC profiles were observed under visible light and UV light at a wavelength of 366 nm. The identification of chemical compounds was performed using spray reagents Dragendorff and sitroborat for alkaloid and flavonoid respectively.

2.4 α-Glucosidase Inhibitory Activity Test

The activity of the α-glucosidase enzyme was analyzed using the Sancheti et al. (2009) method with some necessary modifications. The enzyme stock solution was prepared in a phosphate buffer solution (pH 7). The enzymatic reaction was performed in 96 well plate by mixing 15 µL of 25 mM p-NPG as substrate, 60 µL phosphate buffer solutions, and 10 µL of *M. oleifera* ethanol extract, this was followed by the 5 minutes incubation of plate at 37^{o}C and mixing of 15 µL of α-glucosidase. This was followed by the incubation of the reaction mixture at 37°C for 30 minutes, the reaction was stopped by adding 100 µL of 0.2 M Na_2CO_3 solution. The resulting P-nitrophenol was measured at λ = 405 nm using an Elisa reader. The experiment was conducted with 3 replications and % inhibition was calculated using the following formula:

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

3 Results and Discussion

The extraction process was carried out using a maceration method with 95% ethanol solvent. From all three sampling sites, a similar yield of extract was obtained. Ethanol solvent was chosen because

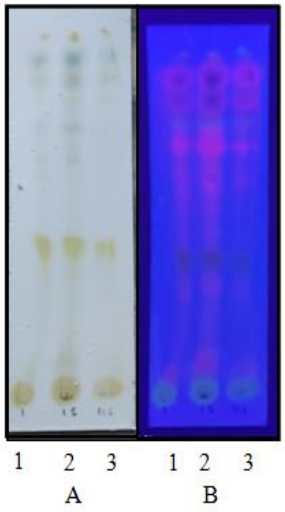

Figure 1 Chromatogram of ethanol extract of *Moringa* leaves using mobile phase toluene : ethyl acetate (7:3), under visible light (A) and 366 nm UV light (B), 1: Saragi, 2: Bacuhau, 3: Batumatongka study sites

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

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

it can dissolve and extract a wide range of nonpolar to polar active ingredients (Saifuddin et al., 2011). According to Suryanto & Wehantouw (2009), the separation of the compounds depends on the solubility of the components to be separated in the solvent. Apart from the type of solvent, the size of the sample subjected to any extraction method also affects the yield of extraction. A smaller sample surface area will promote the contact surface of the sample substance which increases its interaction with the solvent (Sineke et al., 2016). The *Moringa* ethanolic extract from three different areas was analyzed using a TLC method to determine the presence of alkaloid and flavonoid compound class using Dragendorff and sitroborat spray reagents respectively (Figure 1; Table1).

The in vitro antidiabetic properties of *Moringa* extract was estimated by measuring the α-glucosidase enzyme inhibition capacity. Acarbose is a competitive, reversible inhibitor of pancreatic alpha-amylase and membrane-bound intestinal *α* -glucoside hydrolase, and because of these characteristics, it had been used as a standard antidiabetic positive control in this study. Results of the α-glucosidase inhibitory assay revealed that the ethanolic extract of *Moringa* leaves collected from the three different areas of Southeast Sulawesi had different levels of α-glucosidase inhibition (Table 2). *Moringa* extracts prepared from the leaves collected from the Saragi area had an IC_{50} value of 18.62, while this was reported 10.19 and 10.58 for the leaves samples collected from Bacuhau and Batumatongka localities of the southeast Sulawesi (Table 2). In the case of standard control acarbose, the IC_{50} value for the α-glucosidase inhibitory activity was reported 11.54. Results of the study revealed that *M.oleifera*

Table 2 Comparison of α-glucosidase inhibitory activity of *Moringa* leaf extracts prepared from the leaves collected from three different localities of Southeast Sulawesi

Moringa leaves Sample localities	Concentration (ppm)	Percentage inhibition	Linear regression	IC_{50}
Saragi	10	6.13	y = 2,4924x + 3,5924 R² = 0,9746	18.62
	20	8.98		
	30	10.84		
	40	12.62		
	50	16.77		
Bacuhau	10	3.22	y = 4,7124x - 2,0035 R² = 0,9817	10.19
	20	7.09		
	30	10.88		
	40	18.28		
	50	21.19		
Batumatongka	10	8.01	y = 4,5503x + 1,8573 R² = 0,9622	10.58
	20	9.96		
	30	13.60		
	40	20.48		
	50	25.49		
Acarbose	10	7.51	y = 4,0136x + 3,6695 R² = 0,9782	11.54
	20	12.09		
	30	16.25		
	40	18.15		
	50	24.55		

leaves extract to have significant α-glucosidase inhibitory activity and this was at par the standard positive control.

Acarbose inhibits the action of α -glucosidase hydrolase enzyme in the small intestine which significantly inhibit the breaks down of oligosaccharides, trisaccharides, and disaccharides (sucrose, maltose) to monosaccharides (glucose, fructose), which leading to a depletion of carbohydrate absorption by the brush border cell of the small intestine (Schnell et al., 2016). By making a delay in the digestion of carbohydrates, acarbose slows down the glucose absorption, which resulted in a reduction of postprandial blood glucose concentration (Schnell et al., 2016).

According to Adewole et al. (2006) α -glucosidase hydrolase inhibitory activity of the *Moringa* leaves extract might be due to the presence of flavonoids (quercetin and kaempherol) and triterpenoids. This has been already established in a rat model of diabetes mellitus using a streptozotocin (STZ) as the diabetes-inducing agent (Adewole et al., 2006). Flavonoid compounds can regenerate pancreatic β cells in STZ-induced diabetes mellitus rats. Further, the presence of the quercetin was also reported from the *Moringa* leaves, and it was found beneficial to stimulate progenitor cells in the pancreatic duct to promote cellular differentiation. This, subsequently, facilitates the formation of the new islet of Langerhans cells or endocrine cells in diabetic mice that has been subjected to induced pancreatic damage (Rifaai et al., 2012). In addition, *Moringa* leaves are also rich in vitamins, minerals, and essential amino acids that will be useful in cell regeneration (Farooq et al., 2012). Therefore, the results of the present study supported the use of *Moringa* leaves extract as an antidiabetic agent, mainly for its inhibition of α-glucosidase activity.

Conclusion

Ethanolic extract of *M. oleifera* had α- glucosidase inhibitory activities that may differ based on the origin of the sample. The lowest IC_{50} value was found for the *Moringa* extract originated from Bacuhau (IC_{50}= 10.18 ppm), but this is not significantly different from the IC_{50} value of *Moringa* extract of Batumatongka areas leaves (IC_{50}= 10.58 ppm). The IC_{50} value was even lower compared to the positive control acarbose (IC50= 11.54 ppm), that suggesting a greater α- glucosidase inhibitory activity of the *Moringa* leaves extract.

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Conflict of Interest

The authors declare that they have no conflict of interest

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