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Antioxidant and alpha-amylase inhibitory study of *Sansevieria trifasciata* Prain. leaves extract

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ABSTRACT

Diabetes is one of the chronic diseases whose prevalence is increasing all over the world, including in Indonesia. Diabetes mellitus is characterized by high blood glucose levels that can be overcome by, for instance, inhibiting the alpha-amylase enzymes that play a role in carbohydrate hydrolysis. Several studies have proved that active antioxidant substances can also support the treatment of diabetic patients. Snake plant (*Sansevieria trifasciata* Prain.), or locally known as *lidah mertua* in Indonesia, is a traditional herbal used to treat diabetes. This study investigated the activity of the ethanol extract of *S. trifasciata* leaves in inhibiting α-amylase. The leaves were extracted by maceration with ethanol 70%, followed by phytochemical screening. The antioxidant activity was determined using the DPPH method, while the inhibition of α-amylase was measured using UV-Vis spectrophotometer at 524nm wavelength. The results showed that IC_{50} of the antioxidant activity was 1527.55 ppm, five times greater than the positive control, i.e., vitamin C. In the case of α-amylase enzyme, the IC_{50} of the ethanol extract of *S. trifasciata* leaves was 158.31 μg/ml and 0.26 times more potential than acarbose. The study has proved that the crude extract of *S. trifasciata* leaves has the potential as one of the antidiabetics and antioxidants that can be studied further.

Keywords: *Sansevieria trifasciata* Prain., α-amylase, antidiabetic

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INTRODUCTION

Diabetes mellitus is one of the major chronic diseases and predominant health problems worldwide, including Indonesia. The data from the Indonesian Endocrinology Society states that the number of people with diabetes mellitus in Indonesia reached 9.1 million people in 2015 (PERKENI, 2015). Among countries with the largest population size in the world, Indonesia has moved up from the seventh rank to the top 5 countries with high diabetes mellitus incidence. Such a high prevalence proves that it is a severe public health problem, and diabetic patients are therefore in need of proper treatment.

Diabetes mellitus is characterized by high blood glucose levels that can be overcome by, one of which, inhibiting digestive enzymes such as α-amylase and α-glucosidase that play a role in the hydrolysis of carbohydrates. Digested carbohydrates are absorbed by the wall of the small intestines in the form of monosaccharides (Lee *et al.*, 2010). Diabetes is differentiated based on the cause of the disease. Type 1 is usually attributable to the failure to produce insulin as a result of the destruction of beta cells or abnormal secretion of some hormones in the blood that act as an antagonist to insulin. Hence, the study of antidiabetic through their ability to inhibit the alpha-amylase activity has been increasingly conducted by many researchers. Wickramaratne *et al*. (2016) have reported that *Adenanthera pavonina*, a plant used as a diabetic treatment in Ayurvedic Medical system in Sri Lanka, has inhibitory properties against alpha-amylase. The leaf extract of *Tithonia diversifolia*, one of the plants used as antidiabetic herbal tea in Japan, has been proven to reduce blood glucose levels in diabetic mice and impede α-amylase activity (Miura *et al*., 2005; Fitrianingsih *et al*., 2016).

Several studies have revealed the correlation between antioxidant and antidiabetic activities of numerous natural products. Antioxidants act as scavengers because they prevent cell and tissue damage by stopping or retarding oxidation (Findik *et al*., 2011). For instance, the ethanol extract of *Elaeocarpus ganitrus* bark has not only α-amylase inhibitory properties but also antioxidant potential (Talukdar *et al.*, 2017). Furthermore, the aqueous extract of *Cassia occidentalis* has been documented to exhibit significant antihyperglycemic effect in alloxan-induced diabetic rats through regenerating pancreatic β-cells with 84.23% DPPH inhibition.

Sansevieria trifasciata, or locally known as *lidah mertua*, is widely spread in various regions in Indonesia. *S. trifasciata* is a common perennial that wildly grows and is commonly found in residential areas, parks, and woodlands predominantly as ornamental plants (Rwawiire and Blanka, 2015). It is extensively used in the traditional remedies of various diseases, for instance, diabetes. Several studies about its antidiabetic activity have reported the effect of its leaves in lowering blood glucose levels. The ethanolic extract of *S. trifasciata* can decrease blood glucose levels in sucrose-induced diabetes in rats at a dose of 0.083 g/kg BW (Laimeheriwa *et al.*, 2014). The decoction of the leaves can reduce blood glucose levels of alloxan-induced rats at a dose of 150 mg/kg BW (Qomariyah, 2012), but its mechanism to decrease the glucose levels has not been examined. This study reported the antioxidant activities and the α-amylase inhibitory properties of the ethanol extract of *S. trifasciata* Prain. leaves. The antioxidant activity was examined with the DPPH method (Molyneux, 2004), while the α-amylase inhibition was identified using a DNS (dinitrosalicylate) reagent (Thalapaneni *et al*., 2008).

MATERIALS AND METHOD

Materials

The *S. trifasciata* Prain. leaves were obtained from the Indonesian Institute of Spices and Medicinal Plants Research (BALITRO), Bogor. The plant determination was conducted at Herbarium Bogoriense, LIPI, Bogor. The alpha-amylase enzyme came from *Bacillus licheniformis* (Sigma-Aldrich).

Methods

Extraction

One kg of *S. trifasciata* Prain. leaf powder was macerated with 10 L of ethanol 70% for three days. After filtering the extract, the process was repeated three times. The extracts from this replication were combined and subjected to vacuum evaporation to produce 184.94 g of a concentrated extract.

Phytochemical Screening

The concentrated extract (50 mg) was diluted using ethanol and tested for the presence of alkaloid, flavonoid, tannin, terpene, and steroid (Harborne, 1987).

Antioxidant Properties

Two mg of DPPH was dissolved in 100 ml of MeOH, then 3.8 ml of DPPH solution was added with 0.2 mL of MeOH and left for 30 minutes in the dark. Then, this solution was scanned with a UV-Vis spectrophotometer (400-800 nm wavelengths) to obtain the wavelength of maximum absorbance (λ_{max}). Each sample was diluted with MeOH to prepare extracts with concentrations of 100, 150, 200, 250, and 300 ppm. Using a pipette, 0.2 mL of each extract was taken and mixed with 3.8 mL of DPPH solution. The absorbance of the sample was measured at λ_{max} for 30 minutes. The data obtained from this procedure were processed with the formula below (Molyneux, 2004). Vitamin C was used as the positive control.

$$\% \text{ Inhibition= I (\%)} = [1-(A_{517} \text{ sample}/ A_{517} \text{ control})] \times 100\%$$

The IC_{50} was calculated using the regression equation, then the AAI (*Antioxidant Activity Index*) was calculated to determine the antioxidant level of the sample. Antioxidant properties based on AAI are presented in Table I (Scherer and Godoy, 2009).

$$\text{AAI= Final concentration of DPPH (µg/ml)}/ IC_{50} \text{ (µg/mL)}$$

Table I. Antioxidant properties based on Antioxidant Activity Index (Scherer and Godoy, 2009)

AAI values	Antioxidant properties
> 2.0	Very strong
1.0-2.0	Strong
0.5-1.0	Moderate
< 0.5	Weak

α-amylase inhibition assay

The α-amylase inhibitory activity was determined using a published test method in Thalapaneni *et al*. (2008), with modification. The test samples (90, 150, 260, 450, and 780 ppm) and acarbose (16, 25, 39, 61, 95, and 150 ppm) were combined, amounting to 500 µL, and placed in tubes containing 500 µL of α-amylase solution (0.5 ppm) in 0.02 M phosphate buffer (pH 6.9 with 0.006 M NaCl), then incubated at 25°C for 10 minutes. Each tube was then added with 500 µL of 1% (b/v) of starch solution in 0.02 M phosphate buffer at the time interval, then incubated at 25°C for 10 minutes. After the second incubation, the reaction was stopped with 1000 µL of dinitrosalicylic acid (DNS) reagents. The tubes were then incubated in a boiling water bath for 5 minutes, then cooled to room temperature. Afterward, 10,000 µL of distilled water was added to the reaction mixture and measured at 524 nm.

RESULTS AND DISCUSSION

Phytochemical screening

The results showed that the *S. trifasciata* leaf extract contained alkaloid, flavonoid, tannin, saponin, and steroid.

Antioxidant activity of *S. trifasciata* leaf extract

In-vitro antioxidant activity of *S. trifasciata* was performed with DPPH radical scavenging method, as described by Molyneux (2004). The samples were measured at 517 nm, the λ_{max} of DPPH. DPPH has a strong absorbance, i.e., 0.743, at 517 nm due to its dark violet colors. Free radical scavengers cause electrons to form pairs, which then triggers color removal that is proportional to the number of electrons taken and to the antioxidant activity of the sample (Sunarni *et al.*, 2007). The antioxidant properties of the *S. trifasciata* leaf extract are presented in Table II.

Table II. Antioxidant properties of *S. trifasciata* leaf extract (SLE)

Samples	Concentrations (ppm)	Inhibition (%)	IC_{50} (ppm)
SLE	100	6.24	1527.55
	150	6.91	
	200	7.72	
	250	8.80	
	300	13.10	
Vitamin C	2	91.66	308.91
	3	91.80	
	4	91.93	
	5	92.06	
	6	92.20	

The %IC was used to obtain linear regression equations. These equations help to identify at which concentration the *S. trifasciata* leaf extract can effectively reduce DPPH free radicals or IC_{50} values. The IC_{50} is represented by the *x* value, which was obtained by substituting 50 for the *y* value.

The assays showed that the IC_{50} value of the crude extract of *S. trifasciata* leaves was five times greater than that of vitamin C (positive control), and the AAI was 0.0124, indicating weak antioxidant activity. Nevertheless, a further examination of the antioxidant activity of the bioactive compounds in the *S. trifasciata* leaf extract is still possible to perform (Table II).

α–amylase inhibitory activity

Inhibition of postprandial metabolism by impeding α-amylase enzyme is one way to treat hyperglycemia in diabetic patients. In this case, the digestion of carbohydrate can be inhibited, and therefore the blood glucose level can be reduced. This test was conducted to determine the decrease in blood glucose level. Carbohydrates are converted into glucose and maltose that will react with DNS (3,5-dinitrosalicylic) and produce color. The activity of the α-amylase enzyme was measured based on the absorbance of DNS. The more reactions that occur between maltose and glucose with DNS will result in reddish-orange color and higher sample absorbance. On the contrary, if the extract can inhibit the α-amylase enzyme, then the hydrolysis of the starch will be reduced, resulting in lower maltose and absorbance. The alpha-amylase inhibitory properties of *S. trifasciata* leaf (SLE) extract and acarbose are presented in Table III. The results showed that the IC_{50} of the crude extract exhibited α-amylase inhibitory activity with IC_{50}= 158.31 ppm. The IC_{50} value of the standard positive control, acarbose, was 42.7 ppm.

Table III. Alpha-amylase inhibition activities of *S. trifasciata* leaves (SLE) extract and acarbose

Samples	Concentrations (ppm)	Inhibition (%)	IC_{50} (ppm)
SLE	90	36.88	158.31
	150	48.67	
	260	60.46	
	450	74.90	
	780	87.07	
Acarbose	16	21.3	42.7
	25	29.7	
	39	46.4	
	61	57.8	
	95	77.2	

The use of herbal medicine as a source of therapy for various diseases has increased continuously, including for diabetes treatment. Although many antidiabetic drugs have been proven effective, herbal medicines are still in high demand because of their lower prices and relatively fewer side effects.

This study observed the antidiabetic activity of the crude extract by examining its ability as an active antioxidant and inhibitor of the alpha-amylase enzyme activity. The alpha-amylase enzyme contributes to the digestion of carbohydrates into monosaccharides that will be absorbed by the wall of the small intestines (Lee *et al*., 2010). The pathogenesis of diabetes mellitus includes oxidative stress, one of the causes of free radicals and reactive oxygen species (ROS). These two oxygen forms can occur under normal physiological conditions, and unless eliminated appropriately, they can be harmful (Pattanayak *et al*., 2011). Furthermore, a significant increase in endogenous prooxidant activity and a decrease in antioxidant have been shown to contribute to the oxidative stress in diabetes (Nasri and Rafieian-Kopaei, 2013). Diabetic complications are likely to occur as a result of oxidative stress because of the formation of free radicals by glucose oxidation and the subsequent oxidative degradation of the glycated protein (Mehta *et al*., 2006).

By actively searching for plants that have the potential as active antioxidants and inhibitors of *α*-amylase enzymes, cell damage can be prevented, especially pancreatic beta cells that contribute to insulin production and the inhibition of monosaccharides absorption in the gastrointestinal tract. Furthermore, the consumption of antioxidant can support antidiabetic therapy.

CONCLUSION

Although the crude extract of *S. trifasciata* has weak antioxidant activity, active substances that provide better antioxidant activity can still be isolated. Based on the results of the *α*-amylase inhibitory study of *S. trifasciata* leaf extract, *S. trifasciata* leaves can be one of the candidates for the source of antidiabetic treatment in further study.

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