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RESEARCH ARTICLE

α-Glucosidase Inhibitory Activity of Ethanol Extract Obtained from *Dillenia suffruticosa* and *Pycnarrhena cauliflora*

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Abstract

The objective of the study was to find out the inhibitory of α-glucosidase activity and the antioxidant properties of an ethanol extract derived from leaves and stems of *Dillenia suffruticosa* and *Pycnarrhena cauliflora*. Dried powder of them was macerated with 96% ethanol solvents. α- Glucosidase inhibitory property was significantly demonstrated by the leaves and stems of *D. suffruticosa*. Antioxidant activity was found high in *D. suffruticosa* leaves and stems. The results indicate that *D. suffruticosa* stem may potential being antioxidant and antidiabetic compounds.

Keywords: *Dillenia suffruticosa, Pycnarrhena cauliflora, diabetes mellitus, α-glucosidase, ethanol extract.*

Introduction

Diabetes mellitus (DM) is known well as one of metabolic disorder characterized mainly by an increase in blood glucose. Diabetes mellitus is closely related to various health risk factors. Postprandial hyperglycemia takes a vital role in type II DM development and becomes a major cause of diabetic complications, i.e., neuropathy, nephropathy, retinopathy, and cardiovascular disorders [1].

One approach used in postprandial hyperglycemia control is by using the α-glucosidase inhibitor to prevent the breakdown of disaccharides into glucose. α-Glucosidase is described as an enzyme that is available in the digestive cell membrane and catalyzes the hydrolysis of α-1,4 glycosidic bonds from oligos and disaccharides into glucose which is then to be absorbed by organisms [2].

α- Glucosidase inhibitors that work antagonists with the α-glucosidase enzyme lead to a reduction of postprandial hyperglycemia as a prevention of the development of type II DM and diabetic complications. Several α-glucosidase inhibitors, i.e., Acarbose (glucobay®), miglitol (glyset®), and voglibose (basen®, volix®) have been used clinically [3]. However, side effects in the digestive tract such as diarrhea and flatulent cause restrictions on the use of those drugs. Therefore, the discovery and development of new α-glucosidase inhibitors with minimal side effects is still needed [4]. Around 2-5% of O_2 consumed by mitochondria in aerobic respiration and metabolism might produce some reactive oxygen species (ROS) as a by-product [5].

Excessive production of ROS might trigger oxidative stress at the cellular level lead to protein, lipids, and even cellular DNA damaged [6]. Plants as a potential source of natural antioxidants have become a concern due to some natural antioxidants such as vitamin C, α-tocopherol, and phenolic compounds in it [7].

Aside from being a source of antioxidants, the use of natural ingredients as a potential source of α-glucosidase inhibitors continues to be studied because natural materials often appear to be more effective and less toxic. The *Dillenia suffruticosa* (Family: Dellinaceae) is one of the medicinal plants

found abundantly in secondary forests and swampy soils in Indonesia, including Kalimantan Barat. Traditionally, it has been used for the treatment of cancer [8], wound healing [9], abdominal pain, and rheumatism [10]. Besides, it has been reported to have antiviral [11], antimicrobial activity [12], cytotoxic and antioxidant [13].

The *Pycnarrhena cauliflora* (Family: Menispermaceae) belongs to a liana group that is found in Kalimantan Barat. The leaves have been used as a flavoring by Dayak and Malay people [14]. *Pycnarrhena genera,* such as *P. ozanta* have been known to contain Bisbenzylisoquinoline compounds that have antitumor activity. In addition, ethanol extracts of *P. cauliflora* leaves showed antioxidant effects with IC_{50} 0.634 mg/mL [15].

Based on the chemo-taxonomic approach and the presence of antioxidant properties possessed by both plants, it is probable that these plants also possess the ability in the α-glucosidase enzyme inhibition. The study aims to determine the antioxidant and α-glucosidase inhibitors of the ethanol extracts of *D. suffruticosa* and *P. cauliflora*.

Methods

Materials

D. suffruticosa stems and leaves were collected from Pontianak, Kalimantan Barat while *P. cauliflora* stems and leaves were gathered from Kapuas Hulu, Kalimantan Barat. Plant identification was carried out at the Bogoriense Herbarium, Biology Research Center, Indonesia. After selection and sorting, the samples were cleaned with running water, cut into small sizes, and dried. Dry samples were then pulverized by a grinding machine, stored in a plastic bag until extraction.

Sample Extraction

Dry powder of leaves and stems of *D. suffruticosa* and *P. cauliflora* were macerated with ethanol 96% for 7 days. The ethanol extract obtained was concentrated with a rotary vacuum evaporator at 45 ºC and dried.

α- Glucosidase Inhibitory Activity Analysis

The measurement of α-glucosidase inhibitory activity was referred to Sancheti et al. (2009) with a slight modification [16]. The reaction mixture consisted of 50 µL 0.1 M phosphate buffer (pH 7.0), 25 µL 4-nitrophenyl α-D-glucopyranoside 0.5 mM (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 µL test sample with a concentration range of 15.125-1000 µg/mL and 25 µL α-glucosidase solution (stock solution 1 mg/mL in 0.01 M phosphate buffer, pH 7, diluted 0.04 unit/mL with the same buffer, pH was measured before analyzes).

The incubation of the reaction mixture was at 37 °C for 30 minutes. The reaction was stopped by adding 100 µL of 0.2 M sodium carbonate solution. Enzymatic hydrolysis of the substrate was monitored based on the amount of p-nitrophenol released in the reaction mixture by using a microplate reader at 410 nm. Acarbose was used as a positive control. The inhibitory activity was expressed as a percentage of inhibitory enzyme activity.

Antioxidant Activity Analysis

DPPH free radical inhibition activity by spectrophotometry was carried out based on Salazar-Aranda et al. (2009) with a slight modification [17]. A total of 500 µL from each extract with a concentration range of 15.125-1000 µg/mL was mixed with 500 µl of 125 µM DPPH solution in ethanol. The mixture was shaken and left in the dark at room temperature for 30 minutes. Absorbance was measured with a microplate reader at 517 nm. Vitamin C was used as a positive control. DPPH free radical inhibition capacity was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(A-B)}{A} \times 100\%$$

A: Negative absorbance control (DPPH and ethanol (without plant extract))

B: Sample absorbance (DPPH, ethanol, and plant extract).

Data Analysis

All analysis results were expressed as an average of three times measurements. Data were presented as means ± standard deviations. IC_{50} was determined from the

linear regression equation of percent inhibition curves.

Result and Discussion

Phytochemical Contains

Phytochemical screening was determined by using colors generated chemical reagent to detect substances in the ethanolic extract of *D. suffruticosa* dan *P. cauliflora*. The result showed in Table 1.

Table 1: Phytochemical of etanolic extracts

Samples	Test	Reagent	Result
D. suffruticosa			
Leaves	Alkaloids	Dragendroff	+
		Mayer	+
		Wagner	+
	Flavonoids	HCl and Mg	+
	Phenols	$FeCl_3$	+
	Saponins	Foam test	-
	Steroids	Anhydrous Acetat and concentrated H_2SO_4	+
	Terpenoids	Liebermen-Bauchard	+
Stem	Alkaloids	Dragendroff	+
		Mayer	+
		Wagner	+
	Flavonoids	HCl and Mg	+
	Phenols	$FeCl_3$	+
	Saponins	Foam Test	-
	Steroids	Anhydrous Acetat and concentrated H_2SO_4	+
	Terpenoids	Liebermen-Bauchard	+
P. cauliflora			
Leaves	Alkaloids	Dragendroff	+
		Mayer	+
		Wagner	+
	Flavonoids	HCl and Mg	+
	Phenols	$FeCl_3$	+
	Saponins	Foam Test	+
	Steroids	Anhydrous Acetat and concentrated H_2SO_4	+
	Terpenoids	Liebermen-Bauchard	+
Stem	Alkaloids	Dragendroff	+
		Mayer	+
		Wagner	+
	Flavonoids	HCl and Mg	+
	Phenols	$FeCl_3$	+
	Saponins	Foam Test	-
	Steroids	Anhydrous Acetat and concentrated H_2SO_4	+
	Terpenoids	Liebermen-Bauchard	+

α-Glucosidase Inhibitory Activity

The α-glucosidase enzyme in the digestive tract catalyzes the hydrolysis of α-1, 4 glycosidic bonds in carbohydrates by releasing α-glucose and triggering an increase in blood glucose levels after eating. α-glucosidase inhibitors work antagonists with the α-glucosidase enzyme, thereby reducing postprandial hyperglycemia and preventing glucose absorption [18, 19]. Therefore, a synthetic α-glucosidase inhibitor such as acarbose being used clinically as an oral antihyperglycemic agent [20, 21]. Due to some side effect of synthetic α-glucosidase inhibitors, medication by using natural ingredients are an attractive choice for the treatment of hyperglycemia [22].

Table 2: IC_{50} (µg/mL) of α-glucosidase inhibitory activities

Samples	IC_{50} (µg/mL)
D. suffruticosa	
Leaves	103.98
Stems	41.13
P. cauliflora	
Leaves	Not Detected
Stems	>1000
Positive control	
Acarbose (glucobay®)	0.25

Tabel 3: IC_{50} (µg/mL) of free radical inhibitory activities

Samples	IC_{50} (µg/mL)
D. suffruticosa	
Leaves	<15.63

Stems	<15.63
P. cauliflora	
Leaves	99.18
Stems	55.68
Positive control	
Vitamin C	5.69

The α-glucosidase inhibitory activity of ethanol extracts obtained from leaves and stems of both plants native Kalimantan Barat, *D. suffruticosa* and *P. cauliflora* has been carried out with a 4-nitrophenyl α-D-glucopiranoside (4-NPG) substrate.

The measurement of catalytic activity product of the α-glucosidation enzyme, called p-nitrophenyl, was executed by a microplate reader at 410 nm. The α-glucosidase inhibitory activity of the samples was determined in the concentration range of 15.125-1000 µg/mL. Acarbose was used as a standard inhibitor. Acarbose may decrease the 4-nitrophenyl α-D-glucopyranoside hydrolysis by the action of α-glucosidase inhibition. The results showed that all extracts, except *P. cauliflora* leaves, exhibit an α-glucosidase inhibitory activity (Fig.1). Inhibitory percentage of both plants at a concentration of 15.125-1000 µg/mL indicates a positive correlation with extract concentration. The higher concentration results in higher inhibitory activity. The highest α-glucosidase inhibitory percentage was demonstrated by the ethanol extract of the *D. suffruticosa* stem, which was about 98.5%.

The rate of inhibition varied from 0.3 to 98.5 (Fig. 1). Overall, the α-glucosidase inhibitory activity obtained from *D. suffruticosa* plant was stronger than *P. cauliflora*. It means that α-glucosidase is more sensitive to *D. suffruticosa* than *P. cauliflora*, particularly stem extract. Only about 40µg/mL was needed to inhibit 50% of the α-glucosidase enzyme activity (IC_{50}) (Table 1).

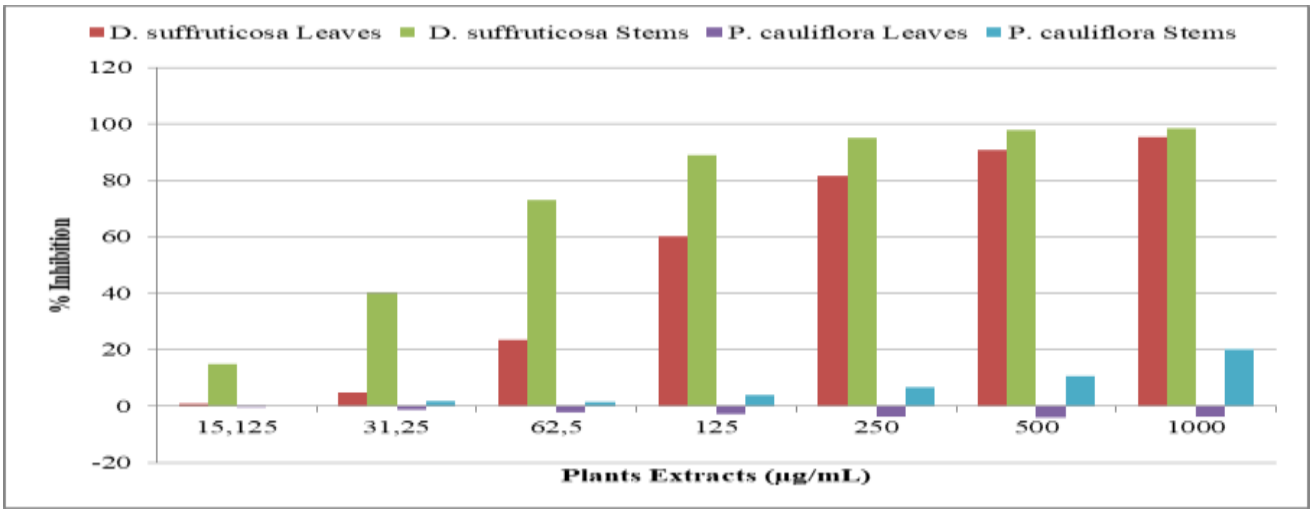

Fig. 1: α-Glucosidase Inhibitory Activities

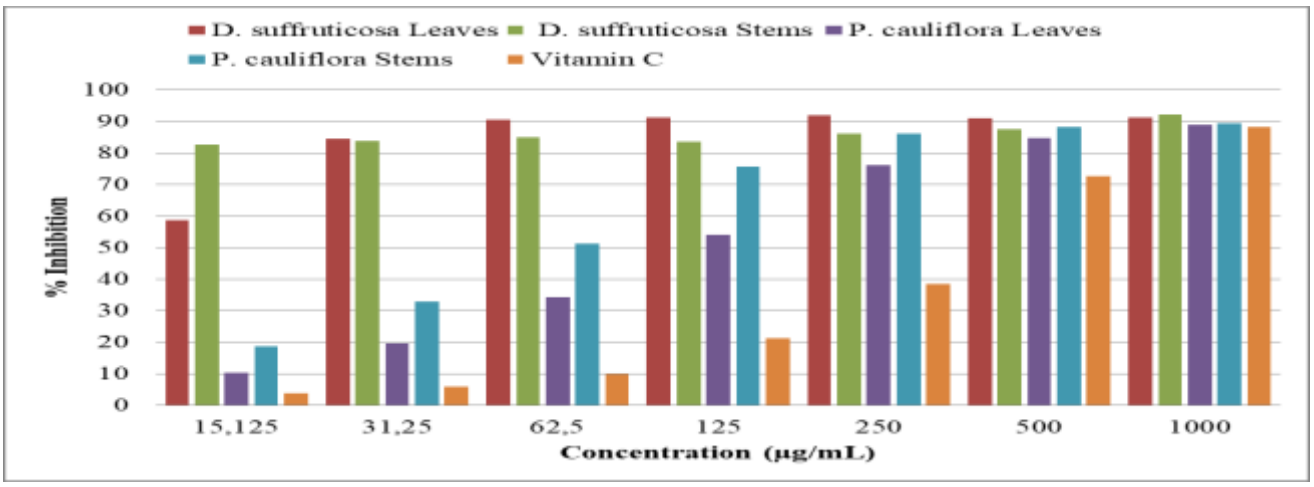

Fig. 2: Free Radical Inhibitory Activities

As regards the IC_{50} value, the highest α-glucosidase inhibitory activity indicated by the lowest IC_{50} value, which belonging to *D. suffruticosa* stem extract, was about 40 µg/mL. In contrast, the most moderate activity was shown by the *P. cauliflora* stem. Due to the IC_{50} value < 200 µg/mL, the ethanolic extract of *D. suffruticosa* leaves, and stem were dedicated as a potential source of α-glucosidase inhibitors active compounds. Otherwise, ethanol extract of leaves and stems of *P. cauliflora* shown the low potential source of α-glucosidase inhibitor compounds (IC_{50} > 200 µg / mL) [23]. Acarbose, as the standard of IC_{50} for the α-glucosidase inhibitor, was much higher compared to the IC_{50} of all samples due to its purity compound.

While the sample tested was a crude extract with many various compounds in it. Also, those other compounds might be antagonistic with desired active compounds and cause a decrease in α-glucosidase inhibitory activity. However, there is still an opportunity to obtain pure active α-glucosidase inhibitory compounds through the process of isolation and purification of extracts to gain a pure compound with more potent activity.

The inhibition of the enzymatic reaction depends on the level of substrate and the enzyme concentrations, temperature, pH, and incubation time with the enzyme. So, the inhibitory effect of α-glucosidase from the same compound is likely to be different. It can be seen in the acarbose activity in this study has IC_{50} was about 0. 248 µg/mL.

The α-glucosidase inhibitory activity of leaves and stems might be due to the chemical compounds, i.e., terpenoids, phenolics, flavonoids, and alkaloids (Table 1). The α-Glucosidase inhibitory activity could be caused by a high phenolic compound and antioxidant activity. According to Zhang et al. (2014), phenolic acids, biphenyl, and flavonoids or proanthocyanins have been evinced in blood glucose reduction through the mechanism of carbohydrate digestive enzymes inhibition, mainly α-glucosidase [24].

Other studies indicated that the substitution of hydroxyl groups in flavonoids might reduce α-glucosidase inhibitory activity. Therefore, the rise of free phenolic groups leads to the rise of the α-glucosidase inhibitory ability [25]. Some studies revealed that α-glucosidase inhibitory activity of phenolic was evident against α-glucosidase of yeast and mammalian digestive (maltase, sucrose, and isomaltase) *In vitro*. In addition, phenolic also showed a high inhibitory activity in several studies [2]. In contrast, The research of Zhang et al. (2014) resulted that the inhibition of phenolic compounds in vivo to α-glucosidase enzymes of the rat was not significant [24].

Antioxidant Activity

Free radicals of oxidative stress have been known to be involved in various degenerative diseases, including diabetes mellitus. Due to its activity as free radical scavenging, antioxidants are beneficial to prevent the diseases. The antioxidant activity of ethanolic extracts obtained from leaves and stems of *D. suffruticosa* and *P. cauliflora* was measured by the DPPH free radical scavenging method and compared with standard antioxidants, ascorbic acid. The four test samples showed a concentration-dependent activity. At the lowest concentration (15.125 µg / mL), the highest percentage of inhibition was shown by the ethanol extract of the *D. suffruticosa* stem, while the lowest was the *P. cauliflora* leaves. The inhibitory percentage of *D. suffruticosa* stem even exceeds the standard antioxidant; vitamin C (Figure 2).

Antioxidant activity is expressed as the number of antioxidants needed to reduce 50 percent of DPPH radicals (IC_{50}). The resulting IC_{50} values were about 5.69 - 99.18 µg/mL (Table 2). Ascorbic acid as the control shows the most potent antioxidant activity, while the lowest was *P. cauliflora* leaves. Ethanol extract derived from *D. suffruticosa* leaves and stems showed strong antioxidant activity, while *P. cauliflora* stem and leaves had moderate antioxidants.

The mechanism of antioxidants and DPPH depends on the conformational structure of the antioxidant. Generally, the more hydroxyl groups (-OH) or groups that donate H such as -SH or -NH in the molecular structure in a compound, the antioxidant activity will also increase [26]. Antioxidant activity of both plants, possibly caused by the phenolic compounds of *D. suffruticosa* and

Bisbenzylisoquinoline alkaloids of *P. cauliflora*.

Conclusion

The ethanolic extract of *Dillenia suffruticosa* and *Pycnarrhena cauliflora* leaves and stems contain an alkaloid, terpenoid, steroid, flavonoid, and phenol. The ethanolic extract derived from *D. suffruticosa* leaves and stem showed inhibitory activity of α-glucosidase, and the highest activity is found in *D. suffruticosa* stems. The ethanolic extract derived from *D. suffruticosa* and *P. cauliflora* showed antioxidant activity, and the highest is found in *D. suffruticosa* leaves and stem.

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