α-Glucosidase Inhibitor Activity of Some Indonesian Syzygium Extracts

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ABSTRAK
Daun dan buah dari tumbuhan Indonesia spesies *Syzygium* spp diantaranya pakoba putih, pakoba merah dan bombongan telah diekstraksi dengan etanol. Etanol ekstrak dipartisi dengan gradient kepolaran pelarut menggunakan n-heksana, etil asetat dan n-butanol. Setiap fraksi diuji antidiabetes dan memberikan variasi tingkat aktivitas ketika diuji menggunakan metode α-glukosidase. Uji antidibetes mengindikasikan bahwa fraksi etil asetat dan butanol dari daun pakoba putih, pakoba merah, dan bombongan berpotensi sebagai sumber senyawa aktif antidiabetes.

Kata Kunci: *Syzygium*; antidiabetes; inhibitor α-glucosidase

ABSTRACT
The leaves and fruits of Indonesian Syzygium spp such as white pakoba, red pakoba and bombongan have been extracted by ethanol. The ethanolic extract was partitioned with polar solvent gradient using n-hexane, ethyl acetate and n-butanol repeatedly. Each fraction was tested for antidiabetic activity and exhibited varying degrees of antidiabetic activity when tested with α-glucosidase method. Antidiabetic test indicated that the ethyl acetate and butanol fractions of the leaves of pakoba white, red pakoba and bombongan has potential as a source of active compounds.

Keywords: *Syzygium; antidiabetic; α-glucosidase inhibitor*

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INTRODUCTION

Diabetes Mellitus (DM) is one of the most prevalent chronic diseases in the world [Kumar *et al*., 2008]. Its major manifestations include disordered metabolism and inappropriate hyperglycemia [Soon & Tan, 2002]. DM is a chronic metabolic disorder affecting approximately five percent of the population of industrialized nations [Zhang *et al*., 2008]. Complications are the major cause of morbidity and mortality in DM [Grover *et al*., 2002], and there are two types of DM in humans. Type 1 DM is due to pancreatic β-cell destruction leading to insulin deficiency and is generally characterized by notable symptoms (weight loss, polyuria, and polydipsia), abrupt onset at a young age but usually after puberty, immune-mediated loss of β-cells by anti-islet cell antibodies, and a need for exogenous insulin therapy [Bellinger *et al*., 2006]. Type 2 DM is characterized by insulin resistance (IR1) and hyperglycemia and differs from type 1 DM in that patients are generally overweight and asymptomatic in the early stages [Bellinger *et al*., 2006]. Type 2 DM usually has a slow onset and, until recently, most often occurred in adults

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[Kaufman, 2005]. Some drugs have been developed for DM, and the best way to control postprandial plasma glucose level is with medication in combination with dietary restriction and an exercise programme. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolysing enzymes, for example á-amylase and á-glucosidase, in the digestive organs. Hypoglycemic drugs could have side effects, therefore the management of diabetes without any side effects is still a challenge to the medical system [Kameswararao *et al*., 2003]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential [Arayne *et al*., 2007]. One of the medicinal plants in Indonesia is *pakoba* plant group that growed in North Sulawesi Province [Lee *et al*., 1999]. This plants used traditionally as diabetic herbal medicine [Kahiking *et al*., 2020] but the information of antidiabetic compounds is surprisingly still limited.

MATERIALS AND METHODS

General

For the purposes of antidiabetic activity assay used potassium phosphate buffer (pH 7.0) 100 mM, p-nitrophenyl-α-D-glukopiranosida 20 mM, the enzyme α-glucosidase, bovine serum albumin, and 200 mM Na_2CO_3 solution, and quersetin as comparator compounds . The equipment used in the form of glass tools commonly used in organic chemistry labs, and Rotavapor Buchi evaporation.

Plant

Plant materials used were the leaves and fruit of white pakoba, red pakoba and bombongan which collected from Laikit village, Dimembe district, North Minahasa regency, North Sulawesi Province, Indonesia. The plant samples were determined in the Department of Biology, Institute of Technology Bandung.

Extraction and Fractionation

Samples of fresh leaves of white pakoba (2000 g), fruit white pakoba (1000 g), red pakoba leaves (2000 g), fruit red pakoba (2500 g), leaf bombongan (3000 g), and fruit bombongan (2500 g) each each blended and macerated with 70% ethanol for 3 x 24 hours and filtered. The filtrate was evaporated to obtain the ethanolic extracts of white pakoba leaves (75 g), white pakoba fruit (78 g), red pakoba leaves (120 g), red pakoba fruit (132.5 g), bombongan leaves (200 g), and bombongan fruit (192 g). The ethanol extract of white pakoba leaves (50 g), white pakoba fruit (35 g), red pakoba leaves (50 g), red pakoba fruit (10 g), bombongan leaves (40 g), and bombongan fruit (50 g), were respectively dissolved in 100 mL distilled water and then partitioned with polar solvent gradient using n-hexane, ethyl acetate and n-butanol repeatedly. Each fraction was evaporated and then each fraction tested antidiabetic activity [Lee & Lee, 2001].

Antidiabetic procedure

Analysis of α-glucosidase inhibition enzymatically performed as follows: 1.0 mg of enzyme α-glucosidase (Saccharomyces cerevisiae) was dissolved in 100 mL phosphate buffer pH 7.00 containing 200 mg of bovine serum albumin. The enzyme was diluted 10 times using phosphate buffer pH 7.00 before assay. The reaction mixture containing 250 mL 20 mM

p-nitrophenyl-α-D-glucopyranoside, 495 mL 100 mM phosphate buffer, and 5 mL of sample solution. The reaction mixture is then pre incubation for 5 min at 37 ^{0}C for temperature adjustment, then added α-glucosidase enzyme of 250 mL. After that, the reaction was stopped by the addition of 1000 mL solution of 0.02 M Na_2CO_3 The number of p-nitrophenol released was measured with a UV spectrophotometer using the absorbance at λ 400 nm. The percentage inhibition activity was calculated using equation 1.

$$\%inhibition = \frac{(C-S)}{C} x100 \quad (1)$$

C = The absorbance of the enzyme activity without sample, S = The absorbance of enzyme activity addition of the sample.

Concentration (IC_{50}) value was calculated using the straight-line equation y = ax + b of the curve between % inhibition and concentration (ppm) by the following equation 2.

$$IC_{50} = \frac{y-b}{a} \quad (2)$$

y = 50, a = a slope, b = intercept.

RESULTS AND DISCUSSION

Three species of traditional medicine plants, locally name are white pakoba, red pakoba, and bombongan are Syzigium family distributed at North Sulawesi Province, Indonesia, was used as antidiabetic therapy. The selection of these three species is caused by the ease and availability of parts to get the fruit, which coincided with the fruiting season.

Table 1. The type, number of samples, and extracted with ethanol

No	Type Samples	Fresh Samples (gram)	EtOH Extract (gram)	Yield (%)
1	White Pakoba Leaves	2000	75	3,75
2	White Pakoba Fruits	1000	78	7,80
3	Red Pakoba Leaves	2000	120	6,00
4	Red Pakoba Fruits	2500	132,5	5,30
5	Bombongan Leaves	3000	200	6,67
6	Bombongan Fruits	2500	192	7,68

Parts of the plant are used for research is the leaves and fruit. The number of samples collected fresh plant varies between 1000-3000 grams (Table 1). All samples were cleaned and smoothed the way blended with ethanol. Extraction is performed on the leaves and fruit of white pakoba, red pakoba and bombongan using 70% of ethanol to obtain the ethanolic extracts with varying yields ranging from 3.75 to 7.80% as shown in Table 1. Each ethanolic extract was fractionated

by solvent gradient system ranging from non-polar to polar solvents, namely n-hexane, ethyl acetate, and butanol respectivelly. The number of ethanolic extracts partitioned ranging from 10-50 g (Table 2). The results further partition evaporated to obtain the fraction of n-hexane, ethyl acetate, and butanol from each of the ethanol extract of the leaves and fruit of white pakoba, red pakoba, and bombongan.

Table 2. The results of the fractionation by solvent gradient system.

No	EtOH extracts partitioned (gram)	Fractionation Results (gram)			
		n-Heksan	EtOAc	n-BuOH	H_2O
1	White Pakoba Leaves (50)	9	8,6	10,5	15,6
2	White Pakoba Fruits (35)	2,8	0,7	2,5	10,0
3	Red Pakoba Leaves (50)	3,0	3,1	10,0	16,0
4	Red Pakoba Fruits (10)	1,0	0,2	2,0	5,0
5	Bombongan Leaves (40)	8,5	7,5	8	12,5
6	Bombongan Fruits (50)	0,1	5,0	9,5	14,5

Ethanolic extracts of leaves and fruits of white pakoba, red pakoba and bombongan were tested for antidiabetic activity through the inhibition of the enzyme α-glucosidase by comparative quercetin as control positive.

Table 3. IC_{50} values of the ethanol extract and fractions

No	Plant Samples	IC_{50} (ppm)†				
		EtOH	n-Hexane	EtOAc	n-BuOH	H_2O
1	White Pakoba Leaves	15,51*	78,60	11,25*	12,50*	40,56
2	White Pakoba Fruits	84,21	120,25	33,69	35,50	55,60
3	Red Pakoba Leaves	19,39*	80,36	15,50*	15,65*	50,76
4.	Red Pakoba Fruits	158,56	95,65	36,75	34,55	60,56
5.	Bombongan Leaves	16,27*	68,95	12,45*	12,00*	45,50
6.	Bombongan Fruits	61,84	70,56	28,56	32,30	50,55

†Quercetin: 22,40 ppm as control positive

*IC_{50}: Value of the extract and fractions are active antidiabetic.

Fractionation performed for all ethanol extracts to obtain fraction of n-hexane, ethyl acetate, butanol, and water. Antidiabetic test results for all fractions (Table 3) indicated that the ethyl acetate and butanol fractions of the leaves of pakoba white, red pakoba and bombongan has potential as a source of active compounds with antidiabetic indicated IC_{50} values lower by comparison quercetin. As for the ethyl acetate and butanol fractions of the fruit showed an increase in activity compared to ethanol extracts. This indicates that the possibility of such

fractions containing antidiabetic compounds but in small concentrations. The separation and purification of the active compounds still in progress.

CONCLUSION

The ethyl acetate and butanol fractions of the leaves of white pakoba, red pakoba and bombongan has potential as a source of antidiabetic compounds.

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