Alpha-Glucosidase Inhibitor Activity of Momordica Charantia L After Inoculated by Endophytic Bacteria

by Sunarno Sunarno

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Alpha-Glucosidase Inhibitor Activity of *Momordica Charantia* L After Inoculated by Endophytic Bacteria

Sri Pujiyanto[1,*], Sunarno[2], and Rejeki Siti Ferniah[3]

[1]*Laboratory Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang, 50275, Indonesia*
[2]*Laboratory Animal Structure and Function, Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang, 50275, Indonesia*
[3]*Laboratory Genetics, Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang, 50275, Indonesia*

1. INTRODUCTION

The number of patients with diabetes mellitus (DM) throughout the world in 2025 is estimated to reach 300 million people. More than 95% of diabetics are type 2 diabetics often called non-insulin dependent diabetes.[1] DM is the leading cause of death among other chronic diseases. Diabetes mellitus can cause complications, such as cardiovascular disease, kidney failure, blindness, impotence and gangrene. DM can not be cured, but can be controlled. Treatment of DM in principle is to keep blood glucose levels can be maintained in normal conditions (80–120 mg/dl). Wide choice of antidiabetic drugs both modern and traditional has been known in the community.

Traditionally treatment of diabetes in general is by utilizing various types of plants that contain active ingredients that can lowering blood sugar levels. Various medicinal plants that are empirically known to have efficacy as an agent of hypoglycaemia include: brotowali (*Tinospora crispa*), bitter melon (*Momordica charantia*), aloe vera (*Alloe vera*), garlic (*Allium sativum*), onion (*Allium cepa*), stevia (*Stevia rebaudiana*), bitter (*Andrographis paniculata*), crown god (*Phaleria macrocarpha*) and red fruit (*Pandanus conoideus*).[2,3]

*Author to whom correspondence should be addressed.

Some typical Indonesian medicinal plants such as: brotowali, bitter melon, bitter, empirically also have been known have antidiabetes properties.[4] Antidiabetic plants are a potential source of producing α-glucosidase inhibitors.[5] Allmost of plants contains any endophytes in their tissues. The endophyte can produce the same compound with the compound produced its host plant. Thus the presence of endophyte on medicinal plants diabetes can contribute antidiabetes production of bioactive compounds in plants.[6] Isolates potential of the medicinal plant, expected to be used to produce α-glucosidase inhibitor compound with a number of more and better quality. Introducing the endophytic microbes on antidiabetic medicinal plants is expected to increase the content of secondary metabolites important, especially bioactive compounds that have a high activity of alpha-glucosidase inhibitors.

2. EXPERIMENTAL DETAILS

Selected isolates endophytic bacteria[7] was grown in 100 ml medium[8] containing 1% maltose, 0.5% peptone and 0.1% yeast extract (pH 7) in a 250 ml Erlenmeyer for 48 hours. This culture was used as inoculum. A total of 1% starter then inoculated into 500 ml medium with the same composition and incubated

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at room temperature with agitation speed of 120 rpm for two days. This culture was used as inoculum was applied to plants. The application endophytic bacteria to increase the production of α-glucosidase inhibitor compounds in plants performed using completely randomized design. As the treatment is the way of the application is to sprinkling and spraying with inoculum suspension and the control without inoculation. As an experimental unit is polybag with sterile soil as much as 2 kg. Each treatment was replicated four times. Responses were observed α-glucoisade inhibitor compounds in plants.

Part of the plant is extracted using a solvents to obtain the active compound. The solvent used is ethyl acetate. Extraction is done by maceration the plant parts that have been cut into small pieces and dried in the ratio 1:1, for 48 hours. Solvent fraction was then separated and done concentrated by rotary evaporator to obtain a concentrated fraction. Concentrated fraction obtained is then dried, weighed and evaluated their α-glucosidase inhibitor activity.

Alpha-glucosidase inhibitor activity was tested by Moon procedure with modifications.[9] Evaluation of enzyme inhibition is based on solving a substrate to produce a colored product, which measured an absorbance during a certain set time period. The enzyme α-glucosidase (Sigma) was dissolved in 0.1 M phosphate buffer pH 7 at a concentration of 0.2 units/ml. As a substrate *p*-Nitrophenyl α-D-glucopyranoside (Sigma) 2.5 mM dissolved in 0.1 M phosphate buffer pH 7. The reaction mixture consisting of 50 μl sample, μl 0.1 M phosphate buffer pH 7 and 50 μl substrate. After incubated at 37 °C for 5 minutes, 50 μl of enzyme was added and further incubated for 15 min at 37 °C. The reaction was stopped by adding 800 μl 200 mM Na_2CO_3 solution. *P*-nitrophenol compound produced from this reaction is measured absorbance at 405 nm. Acarbose solution is used as a comparison. Inhibition of α-glucosidase enzyme activity is determined by the formula:

$$\text{Inhibition } (\%) = (Ac - (A - Ab)/Ac \times 100\%$$

wherein, *Ac*: absorbance of control, *Ab*: absorbance of background, *A*: the absorbance of the sample.

3. RESULTS AND DISCUSSION

The application effects of endophytic bacteria on *Momordica charantia* plants can be seen in Figure 1. *Momordica charantia* extracts were tested for inhibitory activity against α-glucosidase enzyme. In this evaluation, α-glucosidase enzymes hydrolyze the substrate *p*-nitrophenyl α-D-glucopyranoside into *p*-nitrophenol which is yellow colours and glucose. The enzyme activity was measured by the absorbance of *p*-nitrophenol which is yellow. The *Momordica charantia* extract acts as an inhibitor of α-glucosidase, the *p*-nitrophenol produced is reduced which is characterized by reduced intensity of the yellow color. Alpha glucosidase inhibitory activity *Momordica charantia* extract can be seen in Table I.

Table I. Alpha glucosidase inhibitor activity of *Momordica charantia* ethyl acetate extract.

Cont. extract (ppm)	Rep.		S1	S0	S1–S0	Inhibition (%)	IC_{50}
	1	2					
1000	0,486	0,462	0,474	0,040	0,434	71,20	526,4
500	0,747	0,748	0,747	0,022	0,725	51,86	
250	0,927	0,934	0,930	0,012	0,918	39,05	
125	1,167	1,096	1,131	0,005	1,126	25,25	
62,5	1,264	1,164	1,214	0,004	1,210	19,71	

Ethyl acetate extracts are used as unfounded previous studies provide the highest inhibitory activity compared with other extracts. At a concentration of 1000 ug/ml of ethyl acetate extract provides inhibition of 71.20%. The lower the concentration of the extract to produce the lower the inhibition against α-glucosidase enzyme activity. Based on preliminary test results[10] that the use of ethyl acetate extract to produce the highest yield and the activity of α-glucosidase inhibitor highest compared to other solvents and ethyl acetate compound known not toxic, then for the purposes of further extraction of selected compounds ethyl acetate.

The use of ethyl acetate to extract the bioactive compounds in particular α-glucosidase inhibitor compounds have been done by some previous researchers. Extraction of *Glycyrrhiza uralensis* with some kind of solvent. The results of investigation showed that the extract of the ethyl acetate extract of the plants produce α-glucosidase inhibitors to a high of 83.2% compared with extracts from another solvent.[11] Other reseacher also reported having *Crossostephium chinense* plant extract with ethyl acetate to obtain compound α-glucosidase inhibitor. Results fractionation of the ethyl acetate extract is finally obtained compounds scopoletin, tanacetin, hispidulin, quercetagetin, celagin which showed strong inhibitory activity against α-glucosidase enzyme *in vitro*.[12]

In this study, the IC_{50} value of the extract was determined. IC_{50} is a value that indicates the concentration of extract that caused 50% inhibition of the enzyme. The smaller the IC_{50} value means stronger inhibitory inhibitor compounds against the

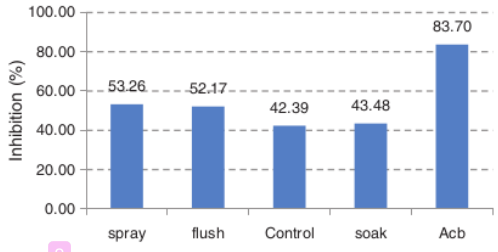

Fig. 1. Alpha glucosidase inhibitor activity of *Momordica charantia* plant treated by endophytic bacteria.

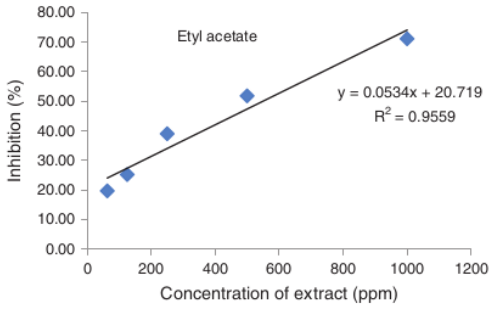

Fig. 2. Determination of IC_{50} values of ethyl acetate extract of *Momordica charantia* plant.

enzyme α-glucosidase. This value is obtained by testing the inhibitory activity of an extract in a wide range of concentrations. Having obtained inhibition values of each extract concentration then made equation which is a function of the concentration of the extract and the amount of inhibition produced. In this relation, the extract concentration as abscission (variable X) and the amount of inhibition as coordinate (Y). The test results showed that the ethyl acetate extracts have IC_{50} value of 526.4 μg/ml. (Fig. 2).

4. CONCLUSION

The application of endophytic bacteria on *Momordica charantia* plant can be increased up to 25.6% of the activity of α-gluosidase inhibitor. The IC_{50} value of the ethyl acetate extract of the *Momordica charantia* plant is 526.4 μg/ml.

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