ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF ENDOPHYTIC BACTERIA METABOLITES OF DAUN SALAM (Syzygium polyanthum (Wight) Walp.)

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Submission date: 28-Dec-2020 12:10PM (UTC+0700)
Submission ID: 1481600655
File name: Alpha.pdf (231.97K)
Word count: 2459
Character count: 14349

ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF ENDOPHYTIC BACTERIA METABOLITES OF DAUN SALAM (*Syzygium polyanthum* (Wight) Walp.)

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Abstract: Diabetes mellitus is a metabolic disease associated with abnormally high level of the sugar glucose in the blood. One of the treatment to er the level of blood sugar is using the drug which inhibit the activity of α-glucosidase. Daun salam (Syzygium polyanthum (Wight) Walp.) also known as bay leaves is a medicinal plant contains active compound which showed α-glucosidase inhibitory activity. As every vascular plant contains endophytic bacteria, the purpose of this study was to determine the alpha-glucosidase inhibitory activity of endophytic bacteria's metabolites of bay leaves. Isolation of endophytic bacteria was conducted using direct plating method of bay leaves using NA medium. The obtained bacterial isolates then cultivated in a liquid F4 medium, incubated for 5 days, and harvested by c ifugation to obtain endophyte's metabolites. Supernatant was us for enzyme inhibitory assay using p-nitrophenyl-Alpha-D-glucopiranoside (p-NPG) as a substrate. Result showed that the alpha-glucosidase inhibitory activity of the metabolites of 5 endophytic bacteria isolates were 37.67%; 37.77%; 36.21; 45.81%; and 41.90%. respectively. It can be concluded that the metabolites of endophytic bacteria of bay leaves have alpha-glucosidase inhibitory activity.

Keywords: daun salam, endophytic, alpha-glucosidase, p-NPG assay

Introduction

Diabetes mellitus is a metabolic disorder genetically and ically manisfestate of the loss of carbohydrate tolerance. Diabetes mellitus could be divided into two type i.e. diabetes type 1 and diabetes type 2 (Schteingart 2005). Department of Health of Republic of Indonesia reported that there is not less than 150 million people of over the world suffered diabetes mellitus (Depkes RI 2005). Oral antidiabetic drugs that currently available were sulfonylurea, biguanide, glitazon, meglitinid, and acarbose. Acarbose is one of antidiabetic drug derived from microbial metabolites which inhibit alpha-glucosidase activity, that caused retard polysaccharide and disaccharide absorption in intestine (Priyanto 2009).

Inhibition of alpha-glucosidase will lower blood glucose level (Suherman and Nafrialdi 2012). One of the herbs that has an inhibit activity of alpha-glucosidase is daun salam or bay leaf (Dalimartha 2000). Zang *et al*. (2006) reported that almost all classes of vascular plants and grasses examined to date are found to host endophytic organisms. Secondary metabolites can be derived from endophytic microbes that have been isolated from the plant. Endophytic microbes are microsc ic living organisms (bacteria and fungi) that live-in plant tissue, leaves, roots, fruits, and stems (Simarmata *et al*. 2007). Exploration of endophytic microbes is expected to produce secondary metabolites that have properties like metabolites produced by the host plant (Pujiyanto *et al*. 2012). Studiawan and Santosa (2005) reported that the bay leaves extract can lower blood glucose levels in mice induced by alloxan.

According to Mayur *et al*. (2010), alpha-glucosidase inhibitory activity assay can be performed using $_{50}$ spectrophotometric method with the substrate p-nitrophenyl-α-D-glucopiranoside (p-NPG). Handayani *et al*. (2006) reported that extracts of daun salam or bay leaves had inhibitory activity of alpha-glucosidase with IC value 28.0975 ppm. Pujiyanto and Ferniah (2010) reported that acarbose can be used as a comparative drug trials alpha-glucosidase inhibitory activity in vitro. Alpha ucosidase activity can be detected from the breakdown of p-NPG into p-nitrophenol which measured using a microplate absorbance reader at a wavelength of 415 nm. Inhibition of alpha-glucosidase seen from the lower p-nit phenol formed, the higher the alpha-glucosidase inhibition that occurs. The problem of this study was ether the metabolites of bay leaves endophytic bacteria have inhibitory activity of alpha-glucosidase. The purpose of this study was to determine the alpha-glucosidase inhibitory activity of metabolites of bay leaves (*Syzygium polyanthum* (Wight) Walp.) endophytic bacteria.

Material and Method

mple of daun salam. Daun salam or bay leaves (*Syzygium polyanthum* (Wight) Walp.) was collected from Balai Penelitian Tanaman Rempah dan Obat (Balittro) Bogor.

Isolation of endophytic bacteria of daun salam (*Syzygium polyanthum* (Wight) Walp.)

Bay leaves samples as much as 2 pieces, cleaned with running water for 10 minutes and leaf size was reduced by approximately 2-3 cm pieces. Then do the surface sterilization at bay leaf samples in a graduated manner samples were stored in 75% ethanol, and then shaken gently for 1 minute, then leaves soaked in a solution of 5.3% NaOCl for 5 minutes, and soaked again in 75% ethanol for 30 sec. The sterilization process was carried out in a laminar air flow (Khan 2007). Leaves that have dried sterile and sterile wipes allowed to stand over until the ethanol evaporates. Samples were dried and cut into pieces, one leaves diced into 4 small pieces and then grown in a Petri dish containing Nutrient Agar (Difco) media containing nystatin for the growth of endophytic bacteria. Incubation was carried out at 27-30^{o}C (room temperature) for 5-7 days (Desriani *et al*. 2014).

Metabolite production of endophytic bacteria of daun salam

The obtained pure isolates of endophytic bacteria were further fermented in a fermentation medium. The medium was sterilized in an autoclave at 121^{o}C for 15 minutes. Isolates of endophytic bacteria were taken and put into 10 ml medium contained test tube, and then incubated on a rotary shaker (Eyela Multi Shaker) at 120 rpm for 5 days. Microbial cells were then separated by centrifugation (Bio Lion XC-HR20) at 4000 rpm for 20 minutes. The obtained supernatant will be used as a test sample for testing the inhibition of the alpha-glucosidase enzyme (Agustina 2003).

Alpha-glucosidase inhibitory assay procedure

Alpha-glucosidase inhibitory$_2$ activity$_3$, assay (Table 1) was conducted by testing the blank solution, control solution, and test solution. The solutions were incubated for 30 minutes and the enzyme activity was stopped by the addition of 100 Na CO then the absorbance was read using a microplate reader 96 wells (Bio-Rad iMark) at a wavelength of 415 nm (Yuniarsih 2012; Mayur *et al*. 2010).

Table 1: Alpha-glucosidase Inhibitory Assay Preparation Scheme

Materials	Blank µ	Control µ	Assay Sample µ	Acarbose µ
Endophyte Metabolite	-	-	10	-
Acarbose				
Buffer	50	50	50	50
Substrate p-NPG	25	25	25	25
DMSO	10	10	-	-
Buffer	25	-	-	-
Enzyme α- glucosidase	-	25	25	25
Na CO	100	100	100	100

The percentage of inhibition of α-glucosidase enzyme activity can be calculated by the formula:

$$\% \text{ Inhibition of metabolites of endophytic} = \frac{A\ C\ \_A\ X}{A\ C} \times 100\% \quad \text{..... (1)}$$

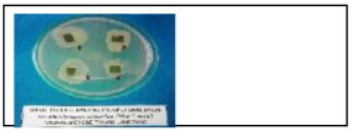
$$\% \text{ Inhibition acarbose} = \frac{A\ C - A\ A}{A\ C} \times 100\% \quad \text{..... (2)}$$

Note:
Abs C = Absorbance of control – Absorbance of Blank Abs X = Absorbance of sample Abs A = Absorbance of Acarbose

RESULT AND DISCUSSION

Isolation of endophytic bacteria of daun salam (*Syzygium polyanthum* (Wight) Walp.)

Figure 1: Isolation of Endophytic Bacteria of Daun Salam Which Obtained Six Bacterial Isolates



Isolation of endophytic bacteria was conducted to obtain pure cultures of endophytic bacteria, using Nutrient Agar medium. Giving nystatin into the medium as an antifungal were intended so there is no mold growing at the time of isolation (Desriani dkk. 2014). Isolation of bacteria was conducted using direct planting. Observation of the isolation was performed for 5 – 7 days. on day 5 the observation had been shown to result in isolates of endophytic bacterial. In one plate that contained 4 isolates which were successfully isolated seen from the growth of endophytic bacteria that grow on the different leaf pieces. Isolates 1 and 2 were taken from the top leaf pieces. Isolates 3 and 4 were taken from the bottom leaf pieces. On the other plate, there were 2 isolates which were successfully isolated and taken from the upper leaves (Figure 2).

Metabolite production of daun salam endophytic bacteria

Metabolite of endophytic bacteria was produced by isolates endophytic bacteria through sub-merged fermentation for 5 days. According Margino (2008) sub-merged fermentation aims to produce secondary metabolites of endophytic bacteria. Supernatant of fermentation product was used for alpha-glucosidase inhibitory activity assay represents of endophytic metabolites. The supernatants, were prepared by centrifugation at 4000 rpm for 20 minutes (Margino 2008).

Table 2: Supernatant Contained Metabolite of Endophytic Bacteria of Daun Salam After Sub-Merged Fermented for 5 Days

Isolate code	Volume of supernatant from replication (ml)			Average volume of supernatant (ml)	SD
	1	2	3		
Isolate DA1	8,2	7,9	8,6	8,23	0,35
Isolate DA2	7,6	8,5	8,2	8,10	0,46
Isolate DA3	8,1	9,2	8,0	8,73	0,57
Isolate DA4	8,3	8,2	8,0	8,17	0,15
Isolate DA5	9,0	8,3	8,8	8,7	0,36

Alpha-glucosidase inhibitory assay

Alpha-glucosidase inhibitory assay was conducted using the enzyme alpha-glucosidase (*Sigma-Aldrich USA*) with a concentration of 0.2033 units/ml and the substrate p-nitrophenyl-α-D-glucopiranoside (p-NPG) (*Sigma-Aldrich USA*) with a concentration of 5 mM. Enzyme inhibitory assay was conducted by measuring the absorbance of p-nitrophenol as the enzymatic reaction product (Pujiyanto and Ferniah 2010). Result of absorbance and inhibition percent of each isolate can be seen in the Table 3.

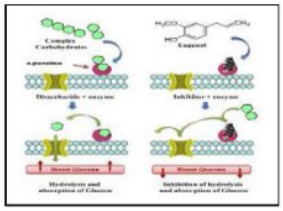
The lowest inhibition of enzyme activity caused by endophytic metabolite was 36.21% which produced by isolates DA3, and the highest was 45.81% which produced by isolates DA4 (Table 3). Difference in the percentage of inhibition at each metabolite of endophytic might cause by the content of secondary metabolites were different in each isolate. As Radji (2005) mentioned that the endophytic bacteria could produce biological compounds or secondary metabolites that are like the host plant. Result of this assay proved that endophytic bacteria of bay leaves or daun salam could produce secondary metabolites, which have alpha-glucosidase inhibitory activity that were like the host plant (leaves) extract. As has been reported Handayani *et al*. (2006) that active compound of daun salam or bay leaf extract, which inhibit alpha-glucosidase was eugenol.

Table 3: Inhibitory Activity of Alpha-Glucosidase of Endophytic Bacteria of Bay Leaves Metabolites and Acarbose Using Microplate Reader at 415 nm

Sample Test	Absorbance	Percentage of enzyme inhibition (%)
Blank	0.05	
Control	2.52	
Isolate DA1	1.56	37.67
Isolate DA2	1.54	37.77
Isolate DA3	1.58	36.21
Isolate DA4	1.34	45.81
Isolate DA5	1.44	41.90
Acarbose	0.42	82.99

Metabolite of endophytic bacteria that works as an inhibitor was suspected of having a reversible competitive mechanism. Endophytic metabolites compete with p-NPG to occupy active site of enzyme, forming an enzyme – inhibitor complex, thus causing enzyme inhibition. Eugenol was Eugenol is thought to be secondary metabolites produced by endophytic bacteria of bay leaf that acts as an inhibitor of alpha-glucosidase. Singh *et al*. (2016) reported that eugenol has two mechanisms work in treating diabetes that lowers blood glucose by inhibiting α-glucosidase (Figure 2) and prevents the formation of AGE by binding to ε-amine groups on lysine, cluster 4 'OH of compound eugenol which may potentially capable of binding to amine groups of lysine residues in the protein molecule, and competitively inhibits the binding of glucose.

Figure 2: Alpha-glucosidase Inhibitory of Eugenol (Singh *et al.* 2016)



Inhibitory percentage of acarbose greater than metabolites of bay leaves endophyte. Acarbose more effective in inhibiting the activity of alpha-glucosidase compared to metabolites of endophytic bacteria, because acarbose is a microbial oligosaccharide that have been commercialized as oral antidiabetics. In inhibiting alpha-glucosidase, acarbose works irreversible competitively by occupying the active site of enzyme, forming an enzyme – inhibitor complex (Poedjiadi 1994; Tjay dan Rahadja 2007). Inhibition of alpha-glucosidase activity causes the inhibition of breakdown of polysaccharides into monosaccharides, which ultimately lowering glucose levels in the blood.

Conclusion

Based on this study, it can be concluded that it has acquired five isolates of endophytic bacteria from daun salam or bay leaves (*Syzygium polyanthum* (Wight) Walp.) and metabolites produced by the five isolates of endophytic bacteria have inhibitory activity of alpha-glucosidase.

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