Effect of Liquid Media Composition on α -Glucosidase Inhibitory Activity from *Aspergillus elegans* SweF9

Hani Mulyani, Rizna Triana Dewi, Euis Filailla, and Nina Artanti

Research Center for Chemistry – Indonesian Institute of Sciences, Kawasan PUSPITEK, Serpong, Tangerang Selatan, Banten 15314, Indonesia

*Corresponding author : hmulyani@yahoo.com

ARTICLE INFO	Abstract
<p>Article history:</p> <p>Received date : 5 November 2019</p> <p>Revised date : 24 February 2020</p> <p>Accepted date: 26 February 2020</p> <p>Available online at : https://doi.org/10.14203/jkti.v21i2.426</p>	<p>The aim of this study is to determine the effect of liquid media composition on the inhibitory activity of α-glucosidase from endophytic fungus <i>Aspergillus elegans</i> SweF9 isolated from seaweed (<i>Macroalgae eucheuma</i>). Fermentation was carried out in three types of liquid media, namely: potato dextrose broth (PDB), potato malt peptone (PMP), and Czapek-dox broth (CDB), which was incubated for 10 days at room temperature with static conditions. Ethyl acetate were used to extract active metabolites from fungal biomass and filtrate from each media. Antidiabetic activity was measured based on inhibition of enzyme α-glucosidase. The results showed that filtrate extract of <i>A. elegans</i> SweF9 which was cultured on the media PDB showed the highest inhibitor activity to the α-glucosidase enzyme with an IC_{50} value of 1.74 μg / mL. Based on these results, the PDB media is an appropriate medium for culturing <i>A. elegans</i> SweF9 to produces secondary metabolites that can be used as a new source of antidiabetic agents.</p>
<p>Keywords:</p> <p>α-glucosidase inhibitor, <i>Aspergillus elegans</i> SweF9, endophytic fungus</p>	<p>© 2020 Indonesian Journal of Applied Chemistry. This is an open access article under the CC BY-NC-SA license (https://creativecommons.org/licenses/by-nc-sa/4.0/).</p>

1. INTRODUCTION

The number of people with diabetes mellitus (DM) is increasing rapidly every year throughout the world including Indonesia. The last estimation from the International Diabetes

Federation (IDF) were 382 million people in the world living with DM in 2013. Based on basic health research (Riskesdas) in 2013, people with DM in Indonesia reached 6.8% and impaired

glucose tolerance (TGT) in urban areas reaches 29.9% [1].

Postprandial hyperglycemia plays an important role in the development of type 2 DM and its complications. Therefore patients with type 2 DM have to face therapy throughout their lives to control hyperglycemia and prevent complications. The tendency of the increasing number of people with type 2 DM has become a medical concern of the world which seriously encourages various efforts to examine new therapeutic agents [2].

The α -glucosidase is an enzyme that plays a role in the conversion of carbohydrates to glucose. The digestive process of carbohydrates includes the release of pancreatic enzymes into the intestine which will digest carbohydrates into oligosaccharides. The resulting oligosaccharides will be converted into glucose by the α -glucosidase which released by the cell which is then finally absorbed by the body. By inhibiting the action of the α -glucosidase, glucose levels in the blood can be maintained within normal limits [3].

Some drugs currently used clinically are acarbose and miglitol which inhibit glycosidases such as α -glucosidase and α -amylase, but some hypoglycemic agents have limitations, which cause side effects and increase diabetes complications. The main side effects of α -glucosidase inhibitors in the gastrointestinal tract include bloating, nausea and diarrhea. Natural oral α -glucosidase inhibitors derived from natural ingredients can be used as a therapeutic approach to treat postprandial hyperglycemia because they are thought to have low side effects and prices are more affordable than synthetic drugs [4]. Therefore we need research and development of new α -glucosidase inhibitors from natural sources. With the uniqueness of its ecosystem, marine biota and marine microorganisms (*endophytic microbes*) have proven to have a lot of potential as a source of raw materials for the development of new

medicines and cosmetics with unique molecular structures and new pharmacological mechanisms [5].

Indonesia has abundant natural resources, such as plants, animals, and microorganisms that can produce bioactive compounds as a source of new drug discoveries. One of them is *Aspergillus* fungi which is a type of fungi commonly found in soil but can also be isolated (from marine biota). The *Aspergillus* genus includes more than 185 species and is well known for the production of organic acids, statins, and extracellular enzymes that produce secondary metabolites of active compounds as drugs [6]. *Aspergillus* shows strong activity inhibits the action of the α -glucosidase and free radical DPPH [7]. However, the factors that can influence inhibitor activity are not yet known clearly. Based on this matter, the aim of this study is to determine the effect of liquid media composition on the production of active metabolites and the inhibitory activity of α -glucosidase from the endophytic fungus *Aspergillus elegans* SweF9 which is cultured in three liquid media, namely: potato dextrose broth (PDB), potato malt peptone (PMP), and Czapek-dox broth(CDB).

2. EXPERIMENTAL

2.1. Materials

2.1.1 Microorganism

A. elegans was isolated from marine biota/seaweed (*Macroalgae euchema*) [8] from Pameungpeuk Garut, West Java, which is a collection of Research Center for Chemistry, Indonesian Institute of Sciences (LIPI).

2.1.2 Chemicals

PDB, extra Malt extract, Czapek-dox broth, Pepton, and PDA were obtained from Difco, NaNO₃, K₂HPO₄, MgSO₄.7H₂O, KCl, and FeSO₄.7H₂O, dimethyl sulfoxide oxidase (DMSO) were obtained from Merck. *Saccharomyces cerevisiae* and *p*-nitrophenyl α -

D-glucopyranoside (*p*NPG) were obtained from Wako Pure Chemical Industries Ltd.

2.2. Methods

2.2.1 Media and Fermentation Process

Isolated *A. elegans* SweF9 was inoculated in three types of liquid media, (PDB), (PMP), and (CDB). The nutritional composition of the media used is:

1. PDB : PDB 2.4%
2. PMP : 2.4% PDB added 1.0% malt extract, and 0.1% peptone)
3. CDB: 3% sucrose, 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05 MgSO₄.7H₂O, 0.05% KCl, 0.001% FeSO₄.7H₂O, and 0.5% yeast extract.

Each of the sterilized media (50 mL) was inoculated with *A. elegans* SweF9 which had previously been planted in PDA media for 7 days at 30 °C. Then, all of the media were incubated for 10 days at room temperature under static conditions. After the incubation period is completed, biomass and filtrate are separated and each one is extracted with ethyl acetate, and dried with a rotary evaporator. The dried extract obtained was used to the analysis of α -glucosidase inhibitory activity.

2.2.2. α - Glucosidase inhibitory assay

The enzyme inhibition activity for α -glucosidase was assessed according to the methods reported by Kim et al [9], with minor modifications. The reaction mixture contained 250 μ L of 5 mM *p*-nitrophenyl α -D-glucopyranoside (*p*NPG), 495 μ L of 100 mM phosphate buffer (pH 7.0) adding to flask contain 5 μ L of sample dissolved in DMSO at various concentrations (5 to 50 μ g/mL). The reaction mixture was pre-incubated for 5 min at 37°C, the reaction was start by adding 250 μ L α -

Glucosidase (0.065 Unit/mL) (EC 3.2.1.20 from Wako Pure Chemical Industry) incubation was continued for 15 min. The reaction stopped by adding 1mL of 0.1 M Na₂CO₃. Activity of α -glucosidase was determined by measuring release of *p*-nitrophenol at 400 nm. Individual blanks for test samples were prepared to correct background absorbance where the enzyme was replaced with 250 μ L of phosphate buffer.

The inhibition activity presentation is measured by using the equation:

$$\% \text{ Inhibition} = \frac{(C - S) \times 100}{C}$$

C = blank absorbance (DMSO)

S = sample absorbance (difference between absorbers with and without enzymes).

2.2.3. Analysis of fungal metabolite by HPLC

The content of the active compound in the ethyl acetat was analyzed using with HPLC (Water, USA), UV detector at a wavelength (λ) of 300 nm, and reserved – phase C18 column (Type UG 120, 5 μ m, size 4,6 mm I.D. x 250 mm). An isocratic using water : methanol (30:70), at flow rate of 1 mL min⁻¹. The extract was dissolved in methanol at a concentration 1 mg/mL; 10 μ L were injected [10].

3. RESULT AND DISCUSSION

3.1. Selection of optimum media for fungus *A. elegans* SweF9

In the fermentation process, the choice of media composition will greatly affect the metabolites secreted by fungi into the media [11]. Based on this, the present study aimed to investigate the effect of media composition on α -glucosidase inhibitory activity. *Aspergillus* the genus that has the most species and strains found in the marine, terrestrial and plant environments

and is known to have bioactive compounds as antioxidants [12]. Endophytic fungus *A. elegans* SweF9 originated from marine biota is shown in Figure 1.

In this study, three liquid media which were used to culture *A. elegans*, PDB, PMP, and CDB with a fermentation time of 10 days under static conditions is shown in Figure 2.

Fig. 1. *Aspergillus elegans* SweF9

(a)

(b)

Fig. 2. Static liquid fermentation of *A. elegans* SweF9 at room temperature in media PDB, PMP and CDB (a) Day 0th; (b) Day 10th.

After 10 days of incubation, each culture was harvested and separated between fungal biomass (B) and the filtrate (F). The biomass and filtrate obtained were extracted with ethyl acetate and dried with a rotary evaporator. Table 1 shows the weights of each extract of biomass and filtrate.

Table 1. The biomass and filtrate extract weight of *A. elegans* SweEP9 was cultivated in three types of liquid media

Media	Exstract	biomass weight (gram)	Extract weight (gram)
PDB	B	4.44	0.6542
	F	-	0.7878
PMP	B	4.39	0.7245
	F	-	0.8528
CDB	B	1.21	0.2768
	F	-	0.2169

Notes: B = biomass; F = filtrate

Table 1 shows that the biomass weight and extract weight in the PDB and PMP media were relatively similar which indicates that the addition of nitrogen sources in the form of malt and peptone does not have much affect on the biomass weight and the metabolites produced by this fungus. Whereas in CDB medium, the biomass and extracts obtained were significantly less than PDB and PMP media. It was assumed that the medium CDB containing organic salts such as calcium phosphate as a buffer medium magnesium sulfate, potassium chloride, and Fe-sulfate were less suitable for *A. elegans* SweF9 biomass growth hence less extract weight was also obtained. Table 2 shows that the ethyl acetate extracts from biomass and filtrate of *A. elegans* SweF9 grown on 3 different liquid media were able to produce secondary

metabolites that have an inhibitory activity to α -glucosidase so that it has the potential to be developed as antidiabetic agents.

Table 2. α -glucosidase inhibitory activity of extracts

No	Sample name	IC ₅₀ (µg/ml)
1	Quercetin (standard)	1.38
2	PDB (B)	15.96
3	PDB (F)	1.74
4	PMP (B)	38.20
5	PMP (F)	5.49
6	CDB (B)	147.01
7	CDB (F)	81.45

from biomass and filtrate of *A. elegans* SweF9

Notes: B = biomass; F = filtrate

Extract of the filtrate from the three types of media showed better inhibition of the α -glucosidase than the biomass extract. This suggests that the active secondary metabolites that can inhibit the α -glucosidase were more present in the filtrate (extracellular) than in biomass (intracellular). Filtrate extract from the PDB medium showed the best activity as an inhibitor of the α -glucosidase (IC₅₀ 1.74 µg / mL) compared to other extracts. The PDB and PMP filtrate extract showed similar inhibitory activity, whereas the CDB extract showed significantly lower inhibitory activity, on biomass and filtrate extract. This differences can be caused by the influence of the content of secondary metabolites produced in each extract differently due to the influence of the composition of the media used. The order of activity of extracts in inhibiting α -glucosidase is PDB (F) > PMP (F) > PDB (B) > PMP (B) > CDB (F) > CDB (B) respectively.

The results of this study differ from the antidiabetic activity of ethyl acetate extract of *A. terreus* MC751 [11] and RCC1 [13]. *A. terreus* MC751 [11] has the highest antidiabetic activity

when cultured in CDB media compared to PDP, PMP and malt extract media [11]. *A. terreus* RCC1 fungi extract which has the best antidiabetic activity on PMP media [13]. Although the media to produce the best antidiabetic activity of two different types of fungi in *A. terreus* MC751 and RCC1 were different, the compound that has been successfully isolated is the same, namely *butyrolactone I* which has antidiabetic activity [11,13]. These results indicate that fungal species and media composition factors greatly influence on the secondary metabolites produced

and secreted by fungi which has the α -glucosidase inhibitory activity.

1.1. HPLC profiling from biomass and filtrate ethyl acetate extracts

To determine the effect of 3 different media compositions used in the profile of compounds produced by *A. elegans* Swe9, the biomass and filtrate extracts were analyzed using HPLC as shown in Figure 3. It appears that the HPLC profiles were relatively similar but has a different peak intensity.

(i) HPLC chromatogram of PDB (B)

Fig. 3. HPLC chromatogram profiling of ethyl acetate extracts of *A. elegans* SweF9 biomass and filtrate.

(ii) HPLC chromatogram of PDB (F)

(iii) HPLC chromatogram of PMP (B)

Fig. 3 (continued). HPLC chromatogram profiling of ethyl acetate extracts of *A. elegans* SweF9 biomass and filtrate.

(iv) HPLC chromatogram of PMP (F)

(v) HPLC chromatogram of CDB (B)

Fig. 3 (continued). HPLC chromatogram profiling of ethyl acetate extracts of *A. elegans* SweF9 biomass and filtrate.

(vi) HPLC chromatogram of CDB (F)

Fig. 3 (continued). HPLC chromatogram profiling of ethyl acetate extracts of *A. elegans* SweF9 biomass and filtrate.**Table 3.** Effect of media composition on antidiabetic activity and main peak from the HPLC analysis of *A. elegans* SweF9

Fermentation media	Antidiabetic activity rank	Retention time (Peak)	Area of Main Peak
PDB (F)	1	1.946	7723745
PMP (F)	2	1.938	6876414
PDB (B)	3	1.934	1998430
PMP (B)	4	1.930	1395375
CDB (F)	5	-	-
CDB (B)	6	-	-

Notes: B = biomass; F = filtrate.

The results of the antidiabetic activity analysis can be correlated to HPLC results as shown in Table 3. HPLC chromatograms from

PDB and PMP media filtrate extracts which have higher antidiabetic activity have similar main peak with retention times of 1.946 and 1.938 while the area the peaks are 7,723,745 and 6,876,414 respectively. Biomass extracts from PDP and PMP media have the same retention time but the peak area is much smaller which is in line with lower antidiabetic activity. Both filtrate and biomass extracts from CDB media which have significantly lower antidiabetic activities also showed different HPLC chromatogram pattern. These results are similar to the results of the analysis of HPLC *A. terreus* LS07, where ethyl acetate extract which has the best activity antidiabetes also has a high peak [13]. Further research is necessary to isolate the antidiabetic active compound from the fungus *A. elegans* SweF9.

4. CONCLUSION

Based on the results of this study, Potato Dextrose Broth (PDB) is the most suitable medium for increasing the production of secondary metabolites that have α -glucosidase inhibitory activity from endophytic fungus *A. elegans* SweF9. Accordingly, this fungus can be developed as a new source of antidiabetic. Further research is needed to isolate the antidiabetic compound from *A. elegans* SweF9.

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