doi.org/10.36721/PJPS.2020.33.3.REG.969-975.1

Rat alpha glucosidase inhibitor and phytochemicals activities of endophytic actinobacteria from *Ficus deltoidea*

Isra Janatiningrum[1], Dedy Duryadi Solihin[2], Anja Meryandini[2] and Yulin Lestari[2,3]

[1]Microbiology Doctoral Student, School of Graduates, Bogor Agricultural University. Jl Raya Dramaga Bogor, West Java, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University. Jl Raya Dramaga Bogor, West Java, Indonesia
[3]Tropical Biopharmaca Research Center, Bogor Agricultural University, Jl. Taman Kencana No.3 Bogor, West Java, Indonesia

Abstract: *Ficus deltoidea* is a medicinal plant that has high endophytic actinobacteria diversity. Endophytic actinobacteria play an important role in producing various types of bioactive compounds including α-glucosidase inhibitor. Screening of 40 endophytic actinobacteria isolates from *F. deltoidea* showed that 77% of them had inhibitory activity against rat α-glucosidase. The 64% of isolates that have rat α-glucosidase inhibitor activity were derived from leaves. TBL 7, TBL 24, TBS 3, TBS 17 and TBR 20 have high activity. Based on the molecular identification of the 16S rRNA gene, five selected isolates have similarity with *Streptomyces* spp. The aqueous and n-hexane extracts of TBL 7 isolates had the lowest IC_{50} values of 159.25 µg/ml and 118.52 µg/ml, respectively. Qualitative phytochemical analysis showed that aqueous and n-hexane extracts of TBL 7 contained flavonoids, phenols, alkaloids, triterpenoids, tannins, and saponins. These results showed that endophytic actinobacteria from *F. detoidea* have the potential to be developed as α-glucosidase inhibitor.

Keyword: Actinobacteria, endophytes, *Ficus deltoidea,* rat α-glucosidase inhibitor.

INTRODUCTION

Diabetes mellitus is the most common metabolic disorder in the world and its prevalence continues to increase in recent decades. Diabetes cannot be cured, but can be controlled. The α-glucosidase inhibitor is one of the treatments to control glucose level in blood of diabetic patient. The α-glucosidase inhibitor acts by inhibiting α-glucosidase enzymes in small intestine (Suarsana, 2008). The α-glucosidase enzyme serves to break down carbohydrates into glucose in human small intestine. Inhibition of this enzyme leads to inhibition of glucose absorption into the blood, thus lowering blood glucose level. The α-glucosidase inhibitors are widely produced by plants and microbes, including actinobacteria.

Ficus deltoidea is a medicinal plant spread in the Southeast Asia (Corner, 1969). This plant is known to have α-glucosidase inhibitory activity. In Indonesia, *F. deltoidea* is most commonly found in Borneo Island and usually used as traditional medicine. Scientifically, *F. deltoidea* can be used as anticancer, antidiabetes, antihypertensive, and uterus strengthening medicine after childbird (Abdulla *et al.,* 2010; Akhir *et al*., 2011; Bunawan *et al.,* 2014; Misbah *et al*., 2013; Sulaiman *et al.,* 2008).

Bioactive compounds that produced by plant cannot be separated from the existence of microbes that live on plant tissue or known as endophytes. Microbial endophytes live on host tissues without harming their host and may provide benefits, such as being able to produce a variety of secondary metabolite compounds that are useful to its host (Kado *et al.,* 1992). Endophytic microbes, including actinobacteria, in this decade have been the world's attention. This is because of their ability to produce a wide variety of secondary metabolites and sometimes, their ability is higher than that of their hosts (Prado *et al.,* 2013; Pujiyanto *et al.,* 2012).

Recent study has showed that culturable endophytic actinobacteria from *F. deltoidea* are dominated by *Streptomyces*. There were 40 isolates of endophytic actinobacteria; 60% were obtained from leaves, 20% were from stems, 15% were from roots, and 5% were from fruits (Janatiningrum *et al*., 2018). However, the ability of isolates to produce α glucosidase inhibitor has not been explored any further. Therefore, this study aimed to examine the ability of endophytic actinobacteria from *F. deltoidea* in producing α-glucosidase inhibitor and its phytochemical activities. The results obtained from this study can be used as an important information on the potency of endophytic actinobacteria from *F. deltoidea*, thus it can be further developed as antidiabetic agent.

MATERIALS AND METHODS

The study was conducted in 2018 at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB) and Tropical Biopharmaca Research Center, IPB.

*Corresponding author: e-mail: yulinlestari@gmail.com

Preparation of endophytic actinobacteria isolates

Collection of endophytic actinobacteria isolates from *F. deltoidea* was obtained from previous study. The number of endophytic actinobacteria isolates from *F. deltoidea* were 40 isolates. Amongst them, 24 isolates (60%), 8 isolates (20%), 6 isolates (15 %) and 2 isolates (5%) were obtained from the leaf, stem, root and fruit, respectively (Janatiningrum *et al.,* 2018).

All isolates were cultured using International *Streptomyces* Project (ISP) 4 agar medium (Soluble starch 1%, K_2HPO_4 0.1%, $MgSO_4$. $7H_2O$ 0.1%, NaCl 0.1%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, $FeSO_4.7H_2O$ 0.001%, $MnCl_2.7H_2O$ 0.001%, $ZnSO_4.7H_2O$ 0.001%) and 1.8% agar as solidifing agent. The isolates were cultured for 10 days at room temperature (±25 °C).

Screening α-glucosidase inhibitor activity was done using supernatant from forty endophytic actinobacteria examined. Each of them was cultured using ISP 2 medium (Malt extract 1%, Yeast extract 0.4%, Dextrose 0.4%) for 10 days at room temperature (25 °C) on a rotary shaker 110 rpm. The endophytic actinobacteria culture was centrifugated at 5000 rpm at 4°C for 30 minutes to separate supernatant and biomass. The supernatant was then ready to be tested.

Screening of endophytic actinobacteria producing α-glucosidase inhibitor

The activity of α-glucosidase inhibitor was tested using the method of Shihabudeen *et al.* (2011) in which mammalian (rat) α-glucosidase was used. Rat α-glucosidase was extracted from rat intestinal acetone powder (Sigma, St. Louis) (200 mg) which was dissolved in 4 ml of cold phosphate buffer (50 mM). The mixture is sonicated for 15 minutes at 4 °C. After that the mixture vigorous vortexing for 20 minutes, then suspension was centrifuged (10.000 rpm, 30 minutes, 4°C). Then supernatant and biomass were separated, supernatant was used for the assay. Substrate used in this experiment was p-Nitrophenyl α-D-glucopyranoside (PNPG) (Sigma, St. Louis). Reaction mixture containing 10 µl of rat α-glucosidase (0.5 U/ml), 50 µl of phosphate buffer (50 mM; pH 7) and 20 µl of sample was pre-incubated for 5 minutes at 37 °C and then 20 µl PNPG (1 mM) was added to the mixture as a substrate. Reaction was stopped by adding 50 µl Na_2CO_3 (200 mM) and the resulting p-nitrophenol was measured in Elisa reader at 410 nm wavelength. Acarbose was used as a positive control in three replications. Inhibition of α-glucosidase enzyme activity was determined by this formula:

$$\text{Inhibition}\,(\%) = \frac{(\text{AC} - (\text{AS1} - \text{AS0}))}{\text{AC}} \times 100\%$$

AC: Absorbance Control, AS0: Absorbance of sample without enzyme, AS1: Absorbance of Sample

16S rRNA gene identification

Actinobacteria endophytes were identified with molecular analysis using 16S rRNA gene. The spores and mycelium of actinobacteria endophytes were collected in 1.5 ml microtube and extracted using Presto Mini gDNA Bacteria Kit in accordance with the protocol. The concentration and purity of the DNA genome were quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). Total genomic DNA was amplified using the Polymerase Chain Reaction (PCR) method with 16S specific primers for actinobacteria 27F 5'-AGAGTTTGATCCTGGCTCAG-3' (Bruce *et al*., 1992) and 16Sact1114R 5'-GAGTTGACCCCGGCRGT-3' (Martina *et al.,* 2008). The PCR conditions were pre-denatured at 94 ^oC for 5 minutes, denaturation at 92 ^oC for 1 minute, annealing at 53 ^oC for 30 seconds, elongation at 72 ^oC for 30 seconds, post elongation at 72 ^oC for 3 minutes, followed by a 30 cycles amplification. The amplification results were visualized using gel electrophoresis with EtBr dyes on UV transilluminator.

The amplified product was sent to the sequencing service company. The sequencing results were processed using Seqtrace software, then identified using EzBioCloud web by entering primary data. Afterward, the phylogenetic tree was constructed using MEGA 7 software with neighbor-joining method approach.

Extraction and determination of IC_{50} Value

Supernatant from selected isolate was extracted using various solvents to obtain the active compound. The solvents used were chloroform, n-hexane, ethyl acetate, butanol, and aqueous solvent. The extraction was done by adding solvent to the supernatant at ratio 1: 6. Extraction was done for 2 hours, until two separate layers were formed. The process was repeated in three-time replications. The resulting organic phase was separated and concentrated with a rotary evaporator. The same procedure was applied for all solvent. The α-glucosidase inhibitor measured using same assay as the procedure above. Crude extract from various fractions was diluted with DMSO 1% to several concentrations, i.e., 1000, 500, 250, 125 and 62.5 µg/ml. The inhibition value of various types of concentrations was then used to determine the IC_{50} value. *F. deletoidea* leaf extract was also calculated for IC_{50} value to compare the activity α-glucosidase inhibitor of isolates and its hosts.

Qualitative phytochemical analysis of selected isolates

The selected endophytic actinobacteria extract was tested for the presence of bioactive compounds using following phytochemical methods (Harborne, 1987)

Alkaloids test

Sample extract was dissolved with 2 ml of 1% HCl in a test tube and heated up. Mayer's, Dragendorf's,and Wagner's reagents were then added to the mixture. The test results are stated positive if with reagent Meyer formed a yellowish white precipitate, with reagent

Wagner formed brown precipitate,and with Dragendorff reagent formed orange-red precipitate.

Flavonoids and phenolic compounds test

Sample extracts were added together with methanol until the sample was submerged, then simmered and filtered. The filtrate waspiped into 2 test tubes. Sufficient 10% NaOH was then added to the first tube .A red color indicates the presence of flavonoid. Concentrated $H_2SO_4$was added to the second tube. A change in color to red is an indication for the presence of hydroquinone.

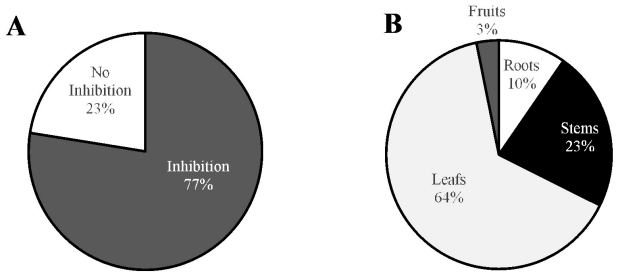

Fig. 1: (A) The percentage of isolates that have α-glucosidase inhibitor activity. (B) Distribution of isolates that potentially produce α-glucosidase inhibitor in *F. Deltoidea.*

Terpenoids and steroid test

Sample extract was mixed with 2ml of chloroform and 5 acetic acid anhydride, evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and mixed. Orange to purple colour indicated the presence of terpenoids. The development of a greenish color indicated the presence of steroids.

Saponins and tannins test

Sample extract was dissolved in 5ml of distilled water in a test tube and then shake until it foam form. The formation of stable foam was taken as an indication for the presence of saponins.Test for tannis, sample extract was mixed with distillated water. Then 2 ml of $FeCl_3$ solution (2%) was added. A black color or blue-green indicated the presence of tannins.

STATISTICAL ANALYSIS

Statistical analysis was performed as means ± SD from three independent replicates. The analysis was performed using Microsoft Excel 2016.

RESULTS

Diversity of α-Glucosidase Inhibitor Activity of Endophytic Actinobacteria from Ficus deltoidea

Screening of 40 endophytic actinobacteria isolates from *F. deltoidea* showed that 31 isolates or 77% of isolates had α-glucosidase inhibitor activity (fig. 1A). The

Tabel 1: Molecular identification of selected endophytic actinobacteria isolates based on 16S rRNA

No	Code	Species	Strain	Similarity (%)	Accession number
1	TBL 7	*Streptomyces tendae*	ATCC 19812	99.91	D63873
		Streptomyces tritolerans	LMG 20318	99.81	AJ781373
2	TBL 24	*Streptomyces tendae*	ATCC 19812	99.91	D63873
		Streptomyces tritolerans	LMG 20318	99.81	AJ781373
3	TBS 17	*Streptomyces tritolerans*	LMG 20318	99.81	AJ781373
		Streptomyces lienomycini	LMG 20091	99.62	AJ781353
4	TBS 3	*Streptomyces tritolerans*	LMG 20318	99.81	AJ781373
		Streptomyces violaceorubidus	LMG 20319	99.81	AJ781374
5	TBR 20	*Streptomyces tritolerans*	LMG 20318	99.81	AJ781373
		Streptomyces rubrogriseus	DAS 165	99.63	DQ345779

Table 2: Phytochemical content of actinobacteria TBL 7 extract and *F. deltoidea* leaves extract

Component	TBL 7		*F. deltoidea* leaves.	
	n-hexane	Aqueous	n-hexane	Aqueous
Alkaloids	+++	+++	+	+++
Flavonoids	+	++	+	++
Phenol hydroquinone	+	++	+	++
Steroids	-	-	+++	-
Triterpenoids	+	-	-	+
Tannins	++	++	+++	+++
Saponins	-	+	-	-

+++ : shows the presence in abundance
++ : shows the presence in moderate quantity
\+ : shows the presence in small amount
\- : shows complete absence of the compound.

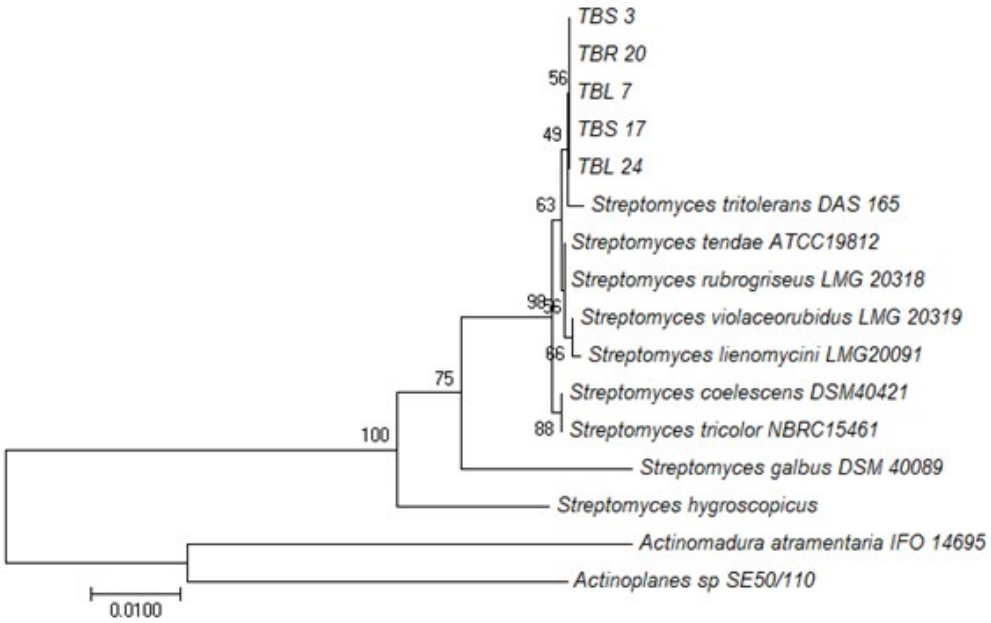

Fig. 2: Neighbor-joining tree of 16S rRNA gene of endophytic actinobacteria. Numbers at each nodes indicate the percentages of branch support of 1,000 bootstrap replicates. Maximum Composite Likelihood model.

inhibitory activity of endophytic actinobacteria isolates was in the range 12.5%-68.3%. Five isolates with high activity, i.e., TBL 7, TBL 24, TBS 3, TBS 17 and TBR 20, were chosen to represent each part of the plant for further testing. The percentage of inhibition activityof the five isolates were 54.5% (TBL 7), 40.4% (TBL 24), 68.3% (TBS 17), 51.6% (TBS 3), and 46.1% (TBR 20). The 64% from 31 isolates that have inhibition activity were originated from leaves (fig. 1B).

Molecular identification selected isolates

The molecular identification results based on the 16S rRNA gene showed that all isolates had similarities with *Streptomyces tritolerans* (99.81%), TBL 7 and TBL 24 were 99.91% similar with *Streptomyces tendae*, TBS 17 was 99.62% similar with *Streptomyces lienomycin*i, TBR 20 had similarities with *Streptomyces rubrogriseus* (99.63%) and TBS 3 had similarities with *Streptomyces violaceorubidus* (99.81%) (table 1). All isolates form into one clade in the phylogenetic tree (fig. 2).

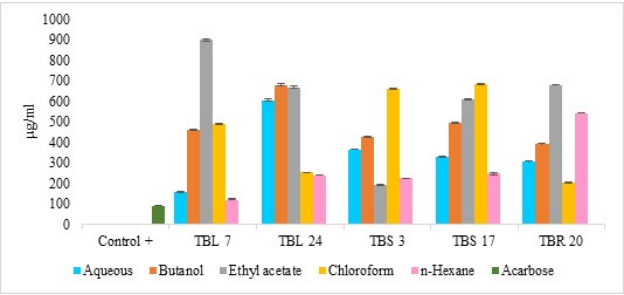

Fig. 3: IC_{50} value of α-glucosidase inhibitor activity from five endophytic actinobacteria selected isolates in various fraction (µg/ml)

IC_{50} value of aqueous and n-hexane extract

Solvents which were used in this study consisted of aqueous, butanol, ethyl acetate, chloroform and n-hexane. Extraction with various solvents are presented in fig. 3. Extract of TBL 7 isolate had the lowest IC_{50} value compared to the extract of other isolates. IC_{50} values for n-hexane extract were 118.52 µg/ml and 159.25 µg/ml for aqueous extract. The values were higher than positive control, acarbose at 90.38 µg/ml.

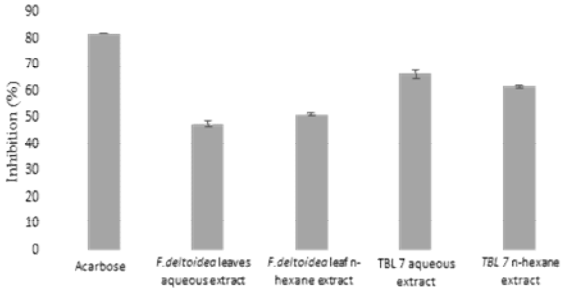

Fig. 4: Comparison of IC_{50} values n-hexane extract and aqueous extract between *F. deltoidea* leaves and TBL7 isolates

This study also compared the activity of α-glucosidase inhibitor produced by *F. deltoidea* leaves and endophytic actinobacteria extracts. The result showed the inhibition ability of n-hexane and aqueous *F. deltoidea* leaf with TBL 7 isolates extract. The leaves aqueous (47%) and n-hexane (51.1%) extracts of *F. deltoidea* plants had lower inhibition compared to endophytic actinobacteria aqueous (61.6%) and n-hexane (66.2%) extracts at 500 µg/ml (fig. 4).

Phytochemical content

The phytochemical test of aqueous and n-hexane of TBL 7 isolates extract was carried out to strengthen the assumption of the main metabolite groups of chemical compounds contained in the extract. Major group of active compounds contained in the extract could be obtained through this analysis. The phytochemical test results showed that the TBL 7 n-hexane extract had a group of alkaloids, flavonoids, triterpenoids and tannins, while the TBL 7 aqueous extract had alkaloids, flavonoids, phenols, tannins and saponins. Phytochemical analysis results showed that TBL 7 aqueous extracts have bioactive compounds like n-hexane extracts, except triterpenoid. Phytochemical contained in TBL 7 extract was not significantly different from their host extract, *F. deletoidea* (table 2).

DISCUSSION

The α-glucosidase is an enzyme in small intestine which can hydrolyze polysacaride into monosacaride such as glucose. Compound which can inhibit this enzyme activity will be potential to be antidiabetic drug since it can decrease blood sugar level or hyperglycemia by slowing the absorption of postprandial glucose. The discovery of endophytic actinobacteria from *F. deltoidea* which produce α-glucosidase inhibitor has been reinforced by the study of Pujiyanto (2012) which also found endophytic actinobacteria that have the same ability. Endophytic actinobacteria from *Tinospora crispa* have the ability to produce α-glucosidase inhibitor. This research has an important meaning in strengthening the opinion of Tan and Zou (2001) which has stated that plants containing endophytic microbes can produce biological compounds or secondary metabolites that are the same as their hosts. The ability of *F. deltoidea* as an antidiabetic agent has been widely reported by several previous researchers (Adam *et al.,* 2012; Farsi *et al.,* 2011; Draman *et al.,* 2012; Misbah *et al.,* 2013; Kalman *et al.,* 2013). The leaves of the *F. deltoidea* have been reported to have potential antidiabetic effects. The n-butanol *F. deltoidea* leaves extract showed to have α-glucosidase and α-amylase inhibition with IC50 values of 15.1 µg/ml and 39.42 µg/ml (Farsi *et al*., 2011).

Leaf is part of the *F. deltoidea* plant which is mostly used as traditional medicine. The ability of leaf to produce α-glucosidase inhibitor compounds may be influenced by the amount of endophytic actinobacteria inside which possesses the ability to produce such compounds. *F. deoidea* leaf has the largest number and diversity of endophytic actinobacteria isolates. Based on previous research, 60% endophytic actinobacteria from *F. deltoidea* are originated from leaves (Janatiningrum *et al*., 2018). In accordance with that report, endophytic actinobacteria from leaves are the most abundant in producing α-glucosidase inhibitor as well. Endophytic actinobacteria from leaves that have glucosidase inhibitor activity were also discovered by Kimura *et al*. (2004) who found out that endophytic actinobacteria from *Morus alba* leaves have the ability of glucosidase inhibitor, revealed with 1-deoxynojirimycin compound.

Based on molecular identification of 16S rRNA gene (1087 bp), TBL 7 and TBL 24 isolates have similarities with *S. tendae*. Vertesy *et al*., (1984) have reported that *S. tendae*4158 (ATCC 31210) contained a novel of polypeptide α-amylase inhibitor tendamistat (HOE 467). All isolates had similarities with *S. tritolerans. S. tritolerans* was first isolated from soil in Kartanaka, India (Syed *et al.,* 2007). Isolate of *Streptomyces* sp. VITMSS05 isolated from the sea has α-amylase inhibitor and α-glucosidase inhibitor. Based on the 16S rRNA gene, it is similar to *S. tritolerans* (Revathy *et al.,* 2013). The α-amylase is an enzyme that plays an important role in the regulation of digestive starch degradation. The role of α-amylase inhibitor as well as α-glucosidase inhibitor is to inhibit the degradation of starch into glucose which will cause hyperglycemia in diabetic patients. TBL 17, TBR 20, and TBL 24 isolates have similarities with *S. violaceorubidus, S. rubrogriseus*, and *S. lienomycini.* The three types of actinobacteria are reported to have antibacterial activity (Hozzein *et al.,* 2011; Song *et al.,* 2015; Lee and Song 2018). All isolates form into one clade in the phylogenetic tree. This clade is different from actinobacteria which has been known to have α-glucosidase inhibitory activities such as *Streptomyces hygroscopicus* and *Actinoplanes* sp. This indicates, perhaps the α-glucosidase inhibitory compounds produced by endophytic actinobacterial isolates from *F. deltoidea* are new and different from pre-existing compounds

Based on the IC_{50} value of all isolate samples, the n-hexane was the best solvent to extract α-glucosidase inhibitor compounds. Aqueous and chloroform solvent also have ability to extract α-glucosidase inhibitor compounds. This result showed that the α-glucosidase inhibitor compounds are present in the polar and non-polar phases. Reddy *et al.* (2009) have reported that n-hexane extract from *Hedychium spicatum* that was observed α-glucosidase inhibitory and antihyperglycemic activity. The inhibitor compound was identified as labdane diterpenoids, a novel of mammalian intestinal α-glucosidase inhibitor. Some discoveries also found that α-glucosidase inhibitor are exist in aqueous extracts. Aqueous extract of Qingzhuan dark tea has α-glucosidase inhibitor activity of up to 2.47 µg/ml (Liu *et al.,* 2016). Onal *et al.,* (2005) have reported that some medicinal herbs have α-glucosidase inhibitor activity in aqueous extract.

For this endophytic actinobacteria extract, in vitro test was conducted using α-glucosidase from rat intestine. It was because we speculated that it would be better to

reflect thein *In Vivo* state. Extract of TBL 7 isolate had the lowest IC_{50} value compared to the extract of other isolates. Analysis of the data showed that maximum inhibition in α-glucosidase was noted by n-hexane extract at the highest concentrations, compared to other samples. Aqueous extract was also effective to inhibit enzyme with an IC_{50} value that was not significantly different from n-hexane extract. The activity of TBL 7 n-hexane and aqueous extract was not significantly different from the positive control, i.e., acarbose. On the other hand, report of endophytic actinobacteria derived from *Datura stramonium* L. shows α-glucosidase rat intestinal inhibitor activity (730.21 µg/ml) (Christhudas *et al.,* 2013). This value was not better than n-hexane extract of TBL 7 (118.52 µg/ml). If inhibition activity of endophytic actinobacteria extracts TBL 7 is compared to *F. deltoidea* leaves extract, it has higher inhibitory value than its host does. These data indicate that endophytic actinobacteria in the *F. deltoidea* plant might contribute to the production of α-glucosidase inhibitor.

Phytochemical screening of different samples extract showed the presence of several bioactive compounds, including alkaloids, phenol, triterpenoids, tannins, flavonoids, and saponins. The content of these bioactive compounds in endophytic actinobacteria extract is responsible for their α-glucosidase inhibitor activity. The phytochemical compounds in endophytic actinobacteria extracts are not much different from the compounds contained in the host extract, *F. deltoidea*. Some researchers have previously reported the existence of a group of bioactive compounds, such as phenols, tannins, saponins, terpenoids, and flavonoids in *F. deltoidea* plant extract (Shafaei *et al.,* 2011). Acarbose, miglitol, and voglibose are commercial α-glucosidase inhibitors and are widely used as controllers of hyperglycemia. Acarbose is an oligosaccharide, voglibose is a derivative of valiolamine, and miglitol is a derivative of deoxynojirimycin. Acarbose and voglibose are produced from actinobacteria, whereas miglitol is synthetic compound (Lebovitz *et al*., 1997).

CONCLUSION

Endophytic actinobacteria derived from *F. deltoidea* have the potential to produce α-glucosidase inhibitor. There are 31 isolates from 40 isolates having α-glucosidase inhibitor. Most of the isolates are from leaves. TBL 7 is an isolate that has the highest inhibition of α-glucosidase activity. Based on 16S rRNA gene, TBL 7 has similarities with *Streptomyces tendae*. TBL 7 aqueous and n-hexane extract have IC_{50} value of 158.25 µg/ml and 118.52 µg/ml, respectively. Phytochemichal analysis found out that TBL 7 aqueous and n-hexane extract contain some bioactive compounds which may be related to an α-glucosidase inhibitor agent.

REFERENCES

Abdulla MA, Ahmed KA, Abu-Luhoom FM and Muhanid M (2010). Role of *Ficus deltoidea* extract in the enhancement of wound healing in experimental rats. *Biomed. Res*., **21**(3): 241-245.

Adam Z, Khamis S, Ismail A and Hamid M (2012). *Ficus deltoidea*: A potential alternative medicine for diabetes mellitus. *eCAM*., **201**(2): 1-12.

Akhir NAM, Chua LS, Majid FAA and Sarmidi MR (2011). Cytotoxicity of aqueous and ethanolic extracts of *Ficus deltoidea* on human ovarian carcinoma cell line. *Br. J. Medic. Med. Res.,* **1**(4): 397-409.

Bruce KD, Hiorns WD, Hobman JL, Osborn AM, Strike P and Ritchie DA (1992). Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. *Appl. Environ. Microb.,* **58**(10): 3413-3416.

Bunawan H, Amin NA, Bunawan SN, Baharum SN and Noor NM (2014). *Ficus deltoidea* Jack: A review on its phytochemical and pharmacological importance. *eCAM.,* pp.1-8.

Christhudas IVS, Kumar PP and Agastian (2013). *In vitro* α-glucosidase inhibition and antioxidative potential of an endophyte species (*Streptomyces* sp. Loyola UGC) isolated from *Datura stramonium* L. *Curr. Microbiol.,* **67**(1): 69-76.

Corner EJH (1969). The complex of *Ficus deltoidea*; a recent Invasion of the Sunda Shelf. *PTRSB*., **256**(808): 281-317.

Draman S, Aris MAM, Razman, Akter SFU, Azlina H, Nor Azlina A, Muzaffar R, Norazlanshah H and Azian (2012). Mas cotek *(Ficus deltoidea)*: A possible supplement for type II diabetes: (a pilot study). *Pertanika J. Trop. Agric. Sci.,* **35**(1): 93-102.

Farsi E, Shafaei A, Hor SY, Ahamed MBK, Yam MF, Attitalla IH, Asmawi MZ and Ismail Z (2011). Correlation between enzymes inhibitory effects and antioxidant activities of standardized fractions of methanolic extract obtained from *Ficus deltoidea* leaves. *Afr. J. Biotechnol*., **10**(67): 15184-15194.

Harborne JB. 1987. Metode Fitokimia. ITB Press., Bandung. pp. 20-21.

Hozzein WN, Rabie W and Ali MIA (2018). Screening the egyptian desert actinomycetes as candidates for new antimicrobial compounds and identification of a new desert *Streptomyces* strain. *Afr. J. Biotechnol.,* **10**(12): 2295-2301.

Janatiningrum I, Solihin DD, Meryandini A and Lestari Y (2018). Comparative study on the diversity of endophytic actinobacteria communities from *Ficus deltoidea* using metagenomic and culture dependent approaches. *Biodiversitas*., **19**(4): 1514-1520.

Kado CI (1992). Plant pathogenic bacteria. In:Ballows A, Truper GG, Dworkin M, Harden W, Schleifer KH, editors. The prokaryotes. Springerverlag, New York, USA, pp.660-662.

Kalman DS, Schwartz HI, Feldman S and Krieger DR (2013). Efficacy and safety of *Elaeis guineensis* and *Ficus deltoidea* leaf extracts in adults with pre-diabetes. *Nutr. J*., **12**(1): 36.

Kimura T, Nakagawa K, Saito Y (2004). Simple and rapid determination of 1-deoxynojirimycin in mulberry leaves. *Biofactors*., **22**(4): 341-345.

Lebovitz HE (1997). Alpha-Glucosidase Inhibitor. *Curr Therap Diabet.,* **26**(3): 539-551.

Lee DS and Song HG (2011). Antibacterial activity of isolated bacteria against *Propionibacterium acnes* causing acne vulgaris. *Kor. J. Microbiol.,* **54**(3): 272-279.

Liu S, Yu Z, Zhu H, Zhang W and Chen Y (2016). In vitro α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea. *eCAM*., **16**(1): 378.

Martina K, Jan K, Tamas F, Ladislav C, Marek O and Genevieve LG (2008). Development of a 16S rRNA gene-based prototype microarray for the detection of selected actinomycetes genera. *Antonie Leeuwenhoek.,* **94**(3): 439-453.

Misbah H, Abdul Aziz A and Aminudin N (2013). Antidiabetic and antioxidant properties of *Ficus deltoidea* fruits extracts and fractions. *eCAM*., **13**(1): 2-12.

Onal S, Timur S, Okutucu B and Zihnioglu F (2010). Inhibition of α-glucosidase by aqueous extracts of some potent antidiabetic medicinal herbs. *Prep. Biochem. Biotechnol.,* **36**(1): 29-36.

Prado S, Buisson D, Ndoye I, Vallet M and Nay B (2013). One-step enantioselective synthesis of (4S)-isosclerone through biotransformation of juglone by an endophytic fungus. *Tetrahedron. Lett.,* **54**(10): 1189-1191

Pujiyanto S, Lestari Y, Suwanto A, Budiarti S and Darusman LK (2012). Alpha-glucosidase inhibitor activity and characterization of endophytic actinomycetes isolated from some Indonesia diabetic medicinal plants. *Int. J. Pharm. Pharm. Sci*., **4**(1): 327-333.

Reddy PP, Tiwari AK, Rao RR, Madhusudhana K, Rao VRS, Ali AZ, Babu KS and Rao JM (2009). New Labdane diterpenes as intestinal α-glucosidase inhibitor from antihyperglycemic extract of *Hedychium spicatum* (Ham. Ex Smith) rhizomes. *Bioorganic. Med. Chem. Lett.,* **19**(9): 2562-2565.

Revathy T, Jayasri MA and Suthindhiran K (2013). Anti-oxidant and enzyme-inhibitory potential of marine *Streptomyces*. *AJBB.,* **9**(3): 282-290.

Shafaei A, Farsi E, Ahamed BMK, Siddiqui MJA, Attitalla IH, Zhari I and Asmawi MZ (2011). Evaluation of toxicological and standardization parameters and phytochemical investigation of *Ficus deltoidea* leaves. *J. Biochem. Mol. Biol.,* **1**(3): 237-243.

Shihabudeen HMS, Priscilla DH and Thirumurugan K (2011). Cinnamon extract inhibits α-glucosidase activity and dampers postprandial glucose excursion in diabetic rats. *Nutr. Metab*., **8**: 46-57.

Song Y, Liu G, Li J, Huang H, Zhang X, Zhang H and Ju J (2015). Cytotoxic and antibacterial angucycline and prodigiosin analogues from the deep sea derived *Streptomyces* sp. SCSIO 11594. *Mar. Drugs.,* **13**(3): 1304-1316.

Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS and Israf DA (2008). Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. *Fitoterapia.,* **79**(7): 557-561.

Suarsana (2008). Aktivitas daya hambat enzim α-glukosidase dan efek hipoglikemik ekstrak tempe pada tikus diabetes. *Veteriner. J.,* **9**(3): 122-127.

Syed DG, Agasar D, Kim C, Li W, Lee J, Park D, Xu L, Tian X and Jiang C (2007). *Streptomyces tritolerans* sp. nov., a novel actinomycete isolated from soil in Karnataka, India. *Antonie Leeuwenhoek.,* **92**(4): 391-397.

Tan RX and Zou WX (2001). Endophytes: A rich source of functional metabolites. *Nat. Prod. Rep.,* **18**(4): 448-459.

Vertesy L, Oeding V. Bender R, Zepf K and Nesemann G (1984).Tendamistat (HOE 467), a tight-binding α-amylase inhibitor from *Streptomyces tendae* 4158 Isolation, biochemical properties. *Eur. J. Biochem*., **141**(3): 505-512.