ISOLATION OF BIOACTIVE COMPOUNDS FROM *Aspergillus terreus* LS07

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ABSTRACT

*This study aims to search for the active compounds from Aspergillus terreus LS07 which isolated from an Indonesian soil. Bioassay-guided fractionations of the ethyl acetate (EtOAc) extract against α-glucosidase and DPPH free radical to give four isolated compounds: oleic acid (**1**), ergosterol (**2**), butyrolactone I (**3**), and butyrolactone II (**4**). The structures of these metabolites were assigned on the basis of detailed spectroscopic analysis. Oleic acid (**1**) was showed significant activity toward α-glucosidase with IC_{50} value of 8.54 μM, but not for antioxidant. Butyrolactone I (**3**) and II (**4**) were showed significant activities against the α-glucosidase with their IC_{50} values at 52.17 and 96.01 μM, and those against DPPH free radicals at 51.39 and 17.64 μM, respectively. On the other hand, ergosterol (**2**) did not show any activities.*

Keywords: *Aspergillus terreus LS07; α-glucosidase inhibitor; unsaturated fatty acid*

ABSTRAK

*Penelitian ini bertujuan untuk mencari senyawa aktif dari Aspergillus terreus LS07 yang diisolasi dari tanah Indonesia. Pemisahan ekstrak etil asetat (EtOAc) berdasarkan pengujian bioaktivitas terhadap enzim α-glukosidase dan radikal bebas DPPH menghasilkan empat senyawa terisolasi: asam oleat (**1**), ergosterol (**2**), butirolakton I (**3**), dan butirolakton II (**4**). Struktur metabolit tersebut dielusidasi berdasarkan metode analisis spektroskopi. Asam oleat (**1**) menunjukkan aktivitas yang signifikan terhadap α-glukosidase dengan nilai IC_{50} 8,54 μM, tetapi tidak untuk antioksidan. Butirolakton I (**3**) dan II (**4**) menunjukkan aktivitas yang signifikan terhadap α-glukosidase dengan nilai IC_{50} pada 52,17 dan 96,01 μM, dan peredaman radikal bebas DPPH pada 51,39 dan 17,64 μM, masing-masing. Di sisi lain, ergosterol (**2**) tidak menunjukkan aktivitas apapun.*

Kata Kunci: *Aspergillus terreus LS07; penghambat α-glukosidase, asam lemak tidak jenuh*

INTRODUCTION

α-Glucosidase (EC 3.2.1.20) is an enzyme that plays a central role in carbohydrate metabolism by hydrolyzing the terminal glycosidic bonds at the non-reducing end of saccharide polymers to release α-glucose [1]. Recently there had been widespread interest in these enzymes because of their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases. Their potential as therapeutic targets, especially, the inhibition of α-glucosidase had been found to help control postprandial blood glucose levels in diabetic patients because they slow the uptake of dietary carbohydrates [2]. Clinical trials showed that the α-glucosidase inhibitor improved long-term glycemic control as measured by decreased hemoglobin A1c(HbA1c) in patients with type 2 diabetes and delay the development of type 2 diabetes in patients with impaired glucose tolerance [3-4].

Generally, the α-glucosidase inhibitor can be isolated naturally from plants or food products [2]. However, they can also be synthesized chemically or produced by microorganism. It has been reported that some microorganism, including species of *Streptomyces*, *Actinoplanes*, and *Flavobacterium saccharophilium*, were able to produce α-glucosidase inhibitor [5]. Acarbose, a pseudo tetrasacharide isolated from the fermentation broth of *Actinoplanes utahanensis* has been utilized as medicine for treatment of type 2 diabetes mellitus [6]. Some

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α-glucosidase inhibitor were isolated from microorganism such as: validamycin A was isolated from *Streptomyces hygroscopicus* var. *limoneus,* broth of *Bacillus subtilis* B2 also possessed strong α-glucosidase activity [5], the new *N*-containing maltooligosaccharide GIB-638 was isolated from a culture filtrate of *Streptomyces fradiae* PWH638 [7] and Aspergillusol A was isolated from marine-derived fungus *Aspergillus aculeatus* [8]. Interest in the isolation of α-glucosidase inhibitors from certain microorganisms has increased due to fast growing characteristic of microorganisms.

The genus *Aspergillus* represents a diverse group of fungi that are among the most abundant fungi in the world [9]. *Aspergillus* is a filamentous, cosmopolitan and ubiquitous fungus commonly found in soil, plant debris and indoor air environment. The genus *Aspergillus* includes over 185 species and is famous for the production of bioactive secondary metabolites (e.g. antibiotic, mycotoxin, antifungal compounds, etc) [10]. *Aspergillus terreus* is ubiquitous fungus isolated from both marine and terrestrial environments, however common in tropical or sub-tropical areas. The compounds isolated from *A. terreus* mostly posses' pharmacological and commercial values. Lovastatin was one of the antihyperlipidemic drugs, which inhibits the cholesterol biosynthesis and was a major drug agent in the treatment of heart disease and atherosclerosis [11]. However, there have been relative few studies on α-glucosidase inhibitors and antioxidants from species of *Aspergillus*.

Previously study, we reported that the EtOAc extract of *A. terreus* showed potential inhibitory activity toward α-glucosidase [12] and produced antioxidants [13]. As continuous of the research on this species, α-glucosidase inhibitors and antioxidant compounds from *A. terreus* LS07 are reported herein.

EXPERIMENTAL SECTION

Materials

α-Glucosidase [(EC 3.2.1.20)] Type I: from *Saccharomyces cereviseae*, *p*-nitrophenyl α-D-glucopyranoside (*p-NPG*), DMSO, 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin dehydrate, linolenic acid ($(CH_3(CH_2CH{:}CH_3)_3(CH_2)_7COOH$), and Silica gel (60-200 mesh Wako gel) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Stearic acid ($C_{18}H_{36}O_2$) and linoleic acid ($C_{18}H_{32}O_2$) were purchased from TCI, Tokyo Chemical Industry. Co. Ltd. All the solvent used in this study were purchased from Wako Pure Chemicals and distilled prior to use.

The fungus *A. terreus* LS07 was isolated from a sea shore in Teluk Kodek, Pamenang area, West Nusa Tenggara Province, Indonesia, in April 2009. This fungus was identified as *A. terreus* based on the sequence data of ITS rDNA. This fungus was deposited in the LIPI Microbial Culture Collection (LIPI MC), Research Center for Biology, Indonesian Institute of Sciences.

Instrumentation

Optical rotation values were measured with a Jasco P-2100 polarimeter. UV-Vis absorption spectra of the active compound in MeOH were recorded on a Hitachi U-1600 spectrophotometer. The mass spectra of the compound were measured with high-resolution FAB-MS. The nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for ^1H and 125 MHz for ^{13}C on a JEOL JNM-ECA 500 with TMS as the internal standard. HMQC and HMBC techniques were used to assign correlations between ^1H and ^{13}C signals. The chemical shift values (δ) are given in parts per million (ppm), and coupling constant (*J*) in Hz. The GC-MS analysis for TMS derivatizations sample were analyzed on the GC-MS QP-2010 gas chromatographed equipped with a silica capillary SPBTM-50 (30 m, id x 0.25 mm x 0.2 μm). Helium was used as carrier gas, sample were injected an oven temperature of 60 °C then oven was heated at 10 °C/min to 280 °C where it was maintained for 10 min. Injected volume was 1 μL was split ratio 100. TLC was run on silica gel 60 F_{254} pre-coated plates (Merck 5554) and spots were detected by UV light.

Procedure

Fermentation, extraction and isolation

The stock culture of *A. terreus* was grown on PDA, incubated at 25 °C for seven days. Two discs (8 mm) of fungal mycelia were used to inoculate 150 mL of CzY(3% sucrose, 0.2% sodium nitrate, 0.1% K_2HPO_4, 0.05% magnesium sulphate, 0.05% potassium chloride, 0.001% ferrous sulphate, and 0.5% yeast extract) in Erlenmeyer flask 500 mL and incubated at 25 °C on shaking condition (60 rpm) for seven days. After harvesting, fermentation broth (10 L) was extracted with EtOAc (10 x 2 L), followed by concentration in vacuo to afford 2.7 g as oily brown gummy. The EtOAc extract (2.7 g) was fractionated on silica gel column and eluted by *n*-hexane: $CHCl_3$ gradient to give eleven fractions (F1-F11). Fraction F2 (100 mg) was further fractionated using Sephadex LH-20 column (*n*-hexane: $CHCl_3$: MeOH 1:5:1) to afford colorless oil F2.1 (**1,** 30 mg). Fraction F8.1 (**2,** 20 mg) was isolated as a colorless crystal from fraction F8 (150 mg) by recrystallization from $CHCl_3$: MeOH. Fraction F10 (500 mg) was re-column chromatography

to give F10.3 (**3**, 300 mg). Purification of F11 through column chromatography using stepwise gradient elution from 70% n-hexane in EtOAC to 100% EtOAC and preparative layer chromatography (PLC) with $CHCl_3$: Acetone (3:1) to afford F11.2 (**4**, 100 mg).

Compound **1**: colorless oil, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.33 (2H, m, H-9,10), 2.34 (2H, t, H-2), 2.01 (4H, m, H-8,11), 1.62 (2H, m, H-3), 1.25-1.30 (22H, m, H-4-8, 11-17), 0.88 (3H, t, H-18), MS m/z (%) = 282 ([M], 264 ([M-H_2O]), 97 (64), 83 (72), 69 (80), 55 (84), 41 (13.5); deduced for $C_{18}H_{34}O_2$.

Compound **2**: colorless crystal, mp 166-168 °C, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.57 (dm, 1H, H-6), 5.38 (dm, 1H, H-7), 5.17 (m, 2H, H-22,23), 3.62 (m, 1H, H-3), 2.46 (dm, 1H, H-5), 2.35 (m, 2H, H-20,24), 2.09-1.93 (m, 3H), 1.92-1.89 (m, 4H), 1.88-1.55 (m, 4H), 1.50-1.40 (m, 3H), 1.38-1.16 (m, 3H), 1.02 (d, J=7.2, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.91(d, J=7.2, 3H, CH_3-28), 0.82 (d, J=6.8, 3H, CH_3-27), 0.80 (d, J=6.8, 3H, CH_3-26), 0.61 (s, 3H, CH_3-18); EIMS m/z (%) = 396 ([M], 87), 378 ([M-H_2O]$^+$, 12), 363 ([M-(H_2O+CH_3)]$^+$, 100), 271 (25), 253 (52), 211(33).

Compound **3**: yellowish gum. UV spectra (MeOH) λ_{max} 307 (log ε 4.3). $[\alpha]_D^{22.5}$ +68.333 (c, 0.3 in MeOH). ^{1}H (Acetone-*d6*) δ 1.56 (3H, s), 1.64 (3H, s), 3.10 (2H, d, J=6.8), 3.43 (2H, d, J=14.8), 3.76 (3H, s), 5.51 (1H, br, J=7.3), 6.49 (1H, dd, J=8.0), 6.52 (1H, d, J=8.0), 6.53 (1H, d, J=3.4), 6.95 (2H, d, J=9.0), 7.61 (2H, d, J=9.0). ^{13}C-NMR (Acetone-*d6*) δ 170.97 (C-5), 168.72 (C-1), 158.93 (C-4'), 154.81 (C-4"), 139.12 (C-2), 132.48 (C-9"), 132.36 (C-2"), 130.20 (C-6' and C-3'), 129.60 (C-6"), 122.85 (C-1'), 128.27 (C-3"), 124.94 (C-1"), 123.43 (C-8"), 127.95 (C-3), 116.67 (C-5' and C-2'), 115.05 (C-5"), 86.02 (C-4), 53.76 (OCH_3), 39.32 (C-6), 28.62 (C-7"), 25.95 (C-10"), 17.82 (C-11"). HRFABMS: [M+H]$^+$ m/z =425.1607, calcd for $C_{24}H_{25}O_7$] 13 degrees of unsaturation.

Compound **4**: colorless gum, UV (MeOH) λ_{max} nm (log ε): 307.5 (4.17). $[\alpha]_D^{28.7}$ +4.78 (c=0.45, Acetone). ^{1}H-NMR (Acetone-*d6*) δ 3.40 (2H, d, J=14.8, H-6), 3.76 (3H, s, 5-OCH_3), 6.59 (2H, d, J=8.0, H-3",5"), 6.69 (2H, d, J=8.0, H-2',6'), 6.98 (2H, d, J=8, H-3',5'), 7.66 (2H, d, J=8.0, H-2",6"). ^{13}C-NMR (Acetone-*d6*) δ 170.94 (C-5), 168.83 (C-1), 158.91 (C-4'), 157.38 (C-4"), 139.28 (C-2), 132.33 (C-2'and C-6'), 130.12 (C-2" and C-6"), 128.06 (C-1"), 124.89 (C-1'), 122.78 (C-3), 115.47 (C-3' and C-5'), 116.66 (C-3" and C-5"), 85.97 (C-4), 53.79 (OCH_3), 39.18 (C-6). FABMS: [M+H]$^+$ m/z 357 for $C_{19}H_{17}O_7$.

GC-MS analysis

The GC-MS analysis for TMS derivatizations sample were analyzed on the gas chromatography coupled with mass spectrometry (GC-MS Shimadzu QP-2010). For MS detection, the electron ionization mode with ionization energy of 70 eV was used, with mass range at m/z 50-550. An SPB-50 column (30 m x 0.25 mm i.d., film thickness of 0.25 µm) was used for GC-MS. Helium was used as carrier gas and the flow rate was maintained at 1 mL/min. Samples were injected on oven temperature of 60 °C then oven was heated at 10 °C/min to 280 °C where it was maintained for 10 min. Injected volume was 1 µL was split ratio 100. The identification of chemicals was performed in comparison with database (NIST08 library) and confirmed using authentic standard samples.

Trimethylsilyl (TMS) derivatize. Compound **1** and **2** were derivatizations with TMS. Sample (100 ppm) was transfer to a GC vial in n-hexane; 25 µL bis (trimethylsilyl) trifluoroacetamide BSTFA add and 25 µL pyridine to the sample. Cap the vial tightly and heat at 65 °C for ~20 min. Sample was cool to room temperature before inject on the GC-MS Shimadzu 2010Q.

Biological activity

α-Glucosidase inhibitory assay. α-Glucosidase inhibitory activity was evaluated according to the method previously reported by Kim et al. [14], with minor modifications. α-Glucosidase (250 µL, 0.065 U/mL), 495 µL of 0.1 M phosphatebuffer (pH 7.0), and 5 µL of various concentrations of sample in DMSO were pre-incubated at 37 °C for 5 min. The reaction was started by the addition of 250 µL of 3 mM *p*NPG. The reaction was incubated at 37 °C for 15 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. α-Glucosidase activity was determined by measuring release of *p*NPG at 410 nm.

Kinetics of inhibition against α-glucosidase. The inhibition type of active compounds against α-glucosidase activity was measured with increasing concentrations of *p*NPG as a substrate in the absence or presence of active compounds at different concentrations. The type of inhibition was determined by Lineweaver-Burk plot analysis.

DPPH free radicals scavenging assay. The antioxidant activities of the isolated compounds were evaluated according to the method of Yen and Chen [15], with minor modification. Aliquots of samples in MeOH (2 mL) at various concentrations (10-200 µg/mL) were each mixed with 0.5 mL of 1 mM DPPH in MeOH. All mixtures were shaken vigorously and left to stand at room temperature for 30 min in the dark. The change in absorbance was measured at 517 nm.

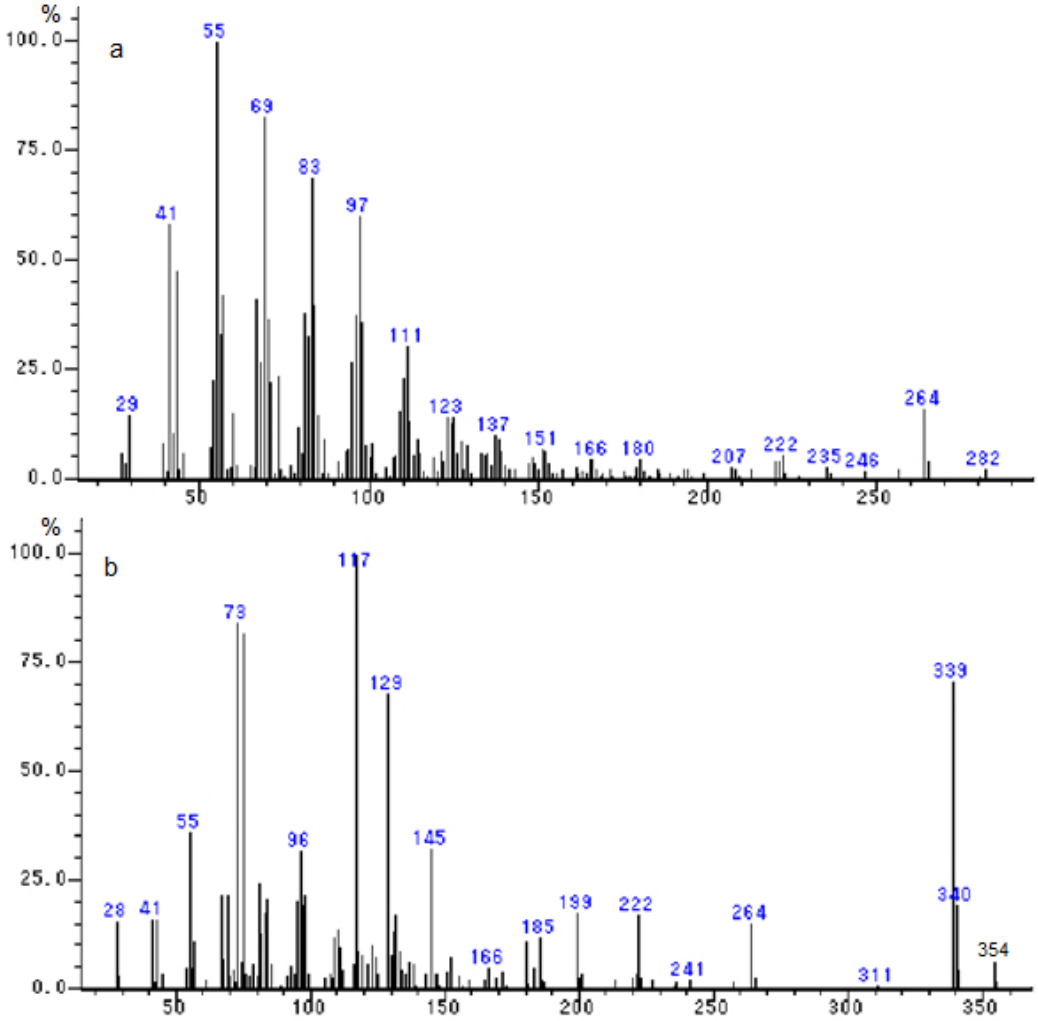

Fig 1. The EIMS of compound **1** (a) and TMS-derivatization of compound **1** (b)

RESULT AND DISCUSSION

Isolation and Characterization of Isolated Compounds

Bioassay-guided fractionation of the EtOAc extract of *A. terreus* LS07 by the general chromatographic techniques give four known isolated compounds. Compound **1** was colorless oil, at TLC analysis turned to yellow color when sprayed by Bromocresol green indicated organic acid group. From ^{1}H-NMR spectrum of compound **1**, two olefinic protons at $_\delta$H 5.33 (2H, m, H-9,10) and four allylic proton at $_\delta$H 2.01 (4H, m, H8,11) indicated a typical unsaturated fatty acid. Moreover, identified based on resemblance with fragmentation in EIMS by GC-MS showed characteristic pattern of unsaturated fatty acid (m/z 41, 55, 69, 83, 97, and 264 for [M-18] indicated lost of H_2O) and molecular ion peak at 282 was deduced to $C_{18}H_{34}O_2$ (Fig. 1a). In order to verify this structure, compound **1** was treated with TMS to derivatize a hydroxyl group (-OH) to GC analysis. Trimethylsilyl (TMS) ethers are a convenient way to derivatize a variety of functional groups prior to GC analysis. Pyridine was added as a basic catalyst to speed reaction with sterically hindered groups. The TMS derivatizations of compound **1** give molecular ion m/z 354 was indicated TMS instead a hydrogen of hydroxyl group (Fig. 1b). Based on those result, compound **1** was identified as oleic acid-9-octadecanoic acid (Z). Oleic acid is unsaturated fatty acid that occurs naturally in microorganism.

Compound **2** was obtained as colorless solid, give green-blue color when reacted with Liebermann-Burchard reagent indicated the compound is steroid or triterpene group. Structure of **2** was identified as ergosterol (ergosta-5,7,22-triene-3β-ol) confirmed by different spectroscopic means (^{1}H and EI MS), chromatographic, comparison with authentic sample and literature [16-17]. Ergosterol is the primary sterol in the cell membranes of filamentous fungi and is either

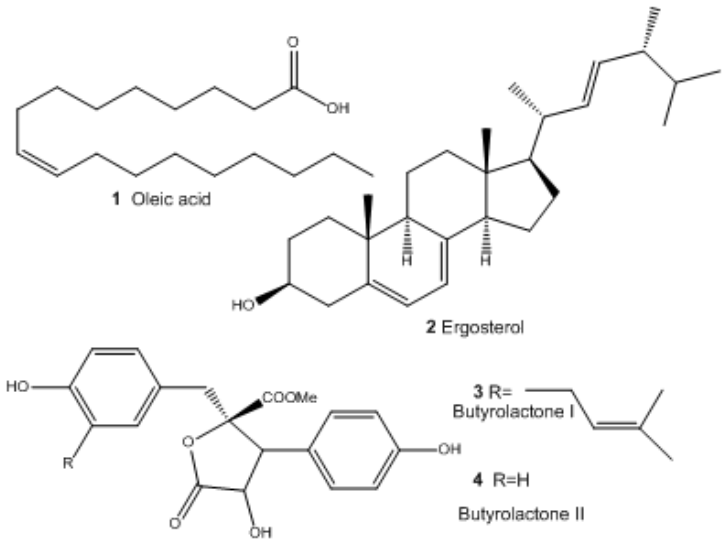

Fig 2. Chemical structures of the isolated active compounds from *A. terreus* LS07

Table 1. Biological activity of isolated compounds of *A. terreus* LS07

Compound	IC_{50} (μM)[a]	
	Inhibitory of α-glucosidase	Antioxidant
1	8.54±0.61	n.d
2	n.d	378.79±3.42
3	52.17±5.68	51.39±3.68
4	96.01±3.70	17.64±6.41
Quercetin	10.92±3.72	39.63±5.21

n.d not detected ; [a]IC_{50} value are shown as mean ± S.D. from three independent experiment

absent or a minor component in higher plants plays an important role as inhibitor of lipid per-oxidation, active against HL-60 cells, MCF-7 cell line [16].

Compound **3** and **4** were the major active compounds in the EtOAc extract. The compound **3** was obtained as colorless crystalline solid, which would eventually turn into a gummy material on storage for long time. The molecular formula **3** was determined to be $C_{24}H_{24}O_7$ from its HRFABMS. It showed a pseudomolecular ion peaks $[M+H]^+$ at 425.1607 (calcd. for $C_{24}H_{25}O_7$), indicating 13 degree of unsaturation. The ^{1}H-NMR spectrum revealed two methyl singlets at δ_H 1.64 and 1.56, and one doublet at δ_H 3.43 and 3.10 representing a prenyl system. One triplet signal δ_H 5.51, indicated an olefinic methine. Three aromatic proton signal at δ_H 6.53, 6.52, and 6.49, being for 1,2,4-trisubtituted phenol along with two doublets at δ_H 7.61 and 6.95 representing 1,4-disubtituted phenolic moiety. The ^{13}C-NMR showed the presence of ten aromatic signals for two aromatic rings, two ester carbonyls at δ_C 171.0 and 168.7, olefenic carbon signals of compound at δ_C 132.5 and 123.4. Two oxygenated carbon at δ_C 154.8 and 158.9 of phenolic system, along with five quertenery carbon (δ_C 139.0-122.9). The methine signals at δ_C 130.2 and 116.7 for 1,4-disubtituted phenol and three methine signals at δ_C 132.4, 128.3, and 115.1 for 1,2,4-trisubtituted benzene ring. In the aliphatic region, signals for oxygenated methine at δ_C 86.0, methoxy at δ_C 53.8, two methylenes at δ_C 39.3 and 28.6, and two methyls 26.0 and 17.80 were assigned. Structure of compound **3** was further deduced on the basis of HMBC experimental data and comparison with literature, which confirms that compound **3**, coincided with butyrolactone I (α-oxo-β-(p-hydroxyphenyl)-γ-(p-hydroxy-m-3.3-dimethylallylbenzy l)-γ-methoxycarbonyl-γ-butyr lactone I) as previous isolated from *A. terreus* MC751 [13]. This compound displays several interesting biological activities, such as antitumor effects (cytotoxicity), allergenic effects, inhibition of microbial and plant growth, and both convulsant and anticonvulsant activity [18]. Butyrolactone I also showed inhibitory activities against soybean lipoxygenese and had DPPH radical-scavenging activity [13,19].

Compound **4** was isolated as colorless gum. It gave spot on TLC more polar, but having color and appearance like compound **3**, indicating it to have similar structure. The molecular formula $C_{19}H_{16}O_7$ was revealed by FAB-MS, which showed $[M+H]^+$ at m/z 357, and was 68 units lesser compared to compound **3**, suggesting absence of prenyl chain. The absence of prenyl chain was also evidence from the absence of signal for vynilic methyl, allylic methylene, and olefinic methin in its NMR and complemented by the presence of signals for two pairs of *p*-disubstituted benzene ring in ^{1}H-NMR and 15 signals accounting for 19 carbons in ^{13}C-NMR. Therefore, compound **4** was identified as deprenyl derivative of compound **3** by comparison its NMR data. The comparison of the above

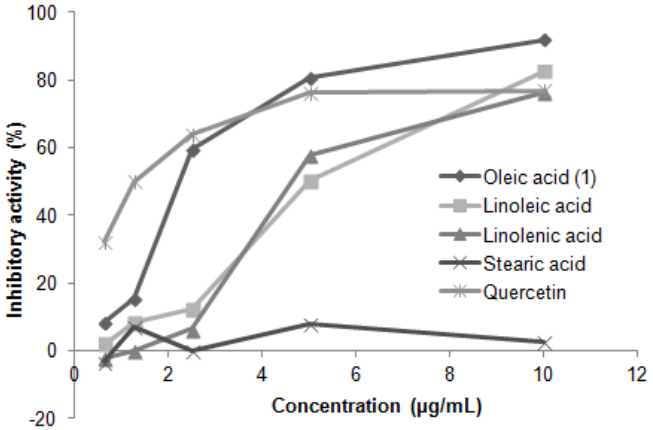

Fig 3. α-Glucosidase inhibitory activity of unsaturated and saturated fatty acid

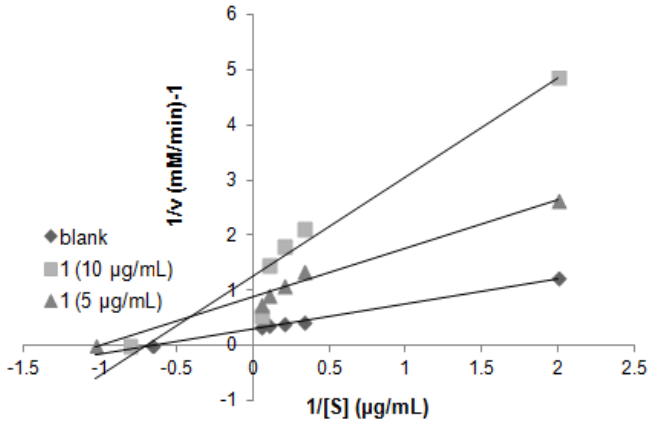

Fig 4. Lineweaver-Burk plot for the inhibition of α-glucosidase by compound **1**

were coincided with butyrolactone II [methyl-4-hydroxy-2-(4-hydroxybenzyl)-3-(4-hydroxyphenyl)-5-oxo- 2,5-dihydro furan-2-carboxylate], which was isolated for the first time in 1982 from *A. terreus* IFO 4100 [20]. All the isolated compounds from EtOAc extract of *A. terreus* LS07 were showed in Fig. 2.

Biological Activity of Isolated Compounds

The α-glucosidase inhibitory and antioxidant activity of isolated compounds were investigated and compared with quercetin as referent standard. In thisstudy we used quercetin as standard due to several reports that quercetin have stronger inhibition of α-glucosidase from yeast *S. cerevisiae* than acarbose [21]. The activities of isolated compounds against α-glucosidase were shown in Table 1. In particular, oleic acid (**1**) showed excellent inhibition on yeast α-glucosidase with IC_{50} value of 8.54 µM which is lower than that of quercetin (IC_{50} value of 14.6 µM), however no activity on DPPH radicals.

To our best knowledge, this is the first report on the inhibitory activity against α-glucosidase of oleic acid which isolated from *A. terreus*. However, other unsaturated fatty acid were reported have potential activity toward α-glucosidase such as 7(Z)-octadecanoic acid and 7(Z),10(Z)-octadecanoic acid with IC50 were 1.81 and 2.86 µM, respectively. These compounds were purified from the body wall of *Stichopus japonicas* [22] and 10-hydroxy-8(E)-octadecanoic acid, an intermediate of bioconversion of oleic acid [23].

In addition, we evaluated the inhibitory activities of saturated and unsaturated fatty acid to clarify whether inhibitory activity of compound **1** due to double bond in fatty acid or not. The result was presented in Fig. 3, the inhibitory activity of oleic acid (**1**), linoleic acid, and linolenic acid exhibited high inhibition at 10 µg, with 91.92 ± 0.85%, 82.84 ± 1.51%, and 76.26 ± 2.41%, respectively. On the other hand, stearic acid (C18:0) exhibited poor inhibitory activity (8 ± 2.01% at 10 µg/mL).

The inhibitory activity against α-glucosidase of the unsaturated fatty acid were ranked as follows; oleic acid > linoleic acid ≥ linolenic acid, while stearic acid, saturated fatty acid, did not show significant activity. Therefore, we consider that a double bond in fatty acid is crucial for the activity; however increasing of the double bond number will decrease the inhibitory activity on α-glucosidase. The investigation of α-glucosidase activity of stearic acid compared to unsaturated fatty acid has not been reported.

This result accorded with previous studies that the presence of the double bond in the fatty acid affects the inhibitory potency [22-23]. The activity of unsaturated fatty acid on α-glucosidase was assumed that the binding of fatty acid may affect the secondary and tertiary structure of proteins because of their detergent effects alone, which was suggested for the effect of fatty alcohol sulfate and palmitic acid binding to bovine serum albumin [24]. The resultant alteration in the conformation of the protein molecule may effects its biological activity, which may be one of early effects involved in the inhibition of enzymes by fatty acids [22]. Moreover, Takahashi & Miyazawa also suggested that the olefin in serotonin derivatives is crucial for the inhibition of α-glucosidase [25].

To determine the inhibition mode of compound **1** on α-glucosidase, series of experiments were carried out in which the substrate concentration was varied, and several different concentration of the compound **1** were used. Compound **1** showed a mixed type inhibition against α-glucosidase (Figure 4) with inhibition constant (*Ki*) was 0.56 µg/mL. In mixed inhibition the inhibitor, oleic acid (**1**) was capable of binding to both the free enzyme and to the enzyme-substrate complex.

In the contras, ergosterol (**2**) shows weak activities both against of DPPH radicals and α-glucosidase. This results similar with previous reported by Fatmawati et al. [17] that ergosterol did not active against α-glucosidase. On the other hand, butyrolactone I (**3**) and II (**4**) were showed significant activities against α-glucosidase and DPPH radical. The presence of a phenolic hydroxyl group in those compounds was assumed to contribute to the α-glucosidase inhibitory effect and scavenging DPPH radicals [26, 27].

CONCLUSION

The results presented here showed that butyrolactone I (**3**) and butyrolactone II (**4**) are the main active compounds as α-glucosidase inhibitor and antioxidant of *A. terreus* LS07, while oleic acid (**1**) was displayed the strongest activity against α-glucosidase but not active against DPPH free radical. Double bond in oleic acid (unsaturated fatty acid) is crucial for the α-glucosidase inhibitory activity; however increasing of the double bond number will decrease the α-glucosidase inhibitory activity.

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