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ScienceDirect
Food Science and Human Wellness xxx (2017) xxx–xxx
Food Science and Human Wellness
www.elsevier.com/locate/fshw

Original Research Paper

α-Glucosidase inhibitor produced by an endophytic fungus, *Xylariaceae* sp. QGS 01 from *Quercus gilva* Blume

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Received 6 January 2017; received in revised form 18 April 2017; accepted 8 May 2017

Abstract

Xylariaceae sp. QGS 01, an endophytic fungus isolated from the stem of *Quercus gilva* Blume showed high α-glucosidase inhibitory activity. α-Glucosidase inhibitor have the role as one of carbohydrate-hydrolyzing enzymes to postpone absorption of glucose in the digestive organs. The α-glucosidase inhibitor constituents were isolated from the ethyl acetate extract of the mycellium of endophytic fungi *Xylariaceae* sp. QGS 01 using a bioassay-guided fractionation technique. Further separation and purification of the active fraction led to the isolation of constituents with strong inhibitory activities against α-glucosidase: 8-hydroxy-6,7-dimethoxy-3-methylisocoumarine (**1**) with inhibitory concentration (IC_{50}) values against α-glucosidase from *Saccharomyces cerevisiae* of 41.75 μg/mL, while quercetin as the standard had an IC_{50} value of 4.80 μg/mL. The results of the present study showed that the endophytic fungus *Xylariaceae* sp. QGS 01 is potentially a rich source of antidiabetic medicine.

Keywords: Endophytic fungi; *Quercus qilva* Blume; α-Glucosidase inhibitory activity; *Xylariaceae* sp.; Isocoumarine derivative

1. Introduction

Diabetes mellitus (DM) is a serious global health program which is characterized by high blood glucose levels, which lead to complications such as retinopathy, hypertension, neuropathy and diabetic foot ulcers [1]. Type 2 DM caused by defects in insulin secretion or insulin resistance [2] is the most frequently encountered form of DM, accounting for more than 80% of all cases [3]. The α-glucosidase enzyme in the intestine is essential for carbohydrate degradation so that the resulted monosaccharides can be absorbed. The inhibition of α-glucosidase enzyme leads to a delay in the digestion of ingested carbohydrates [4]. Thus α-glucosidase inhibitors exhibit high promise as therapeutic agents for the treatment of type 2 of DM [5]. Most of DM treatments are based on the use of synthetic drugs, which are associated with several side effects [6]. Therefore, the development of natural compounds as alternatives pharmaceuticals for the treatment of DM without any side effects is urgently needed. Another advantage is that natural compounds may be safely consumed in the daily diet, thereby reducing the risk of DM [7].

Endophytic fungi are the microorganisms that spend all or part of their life cycles within plant tissue without causing harmful effects on the plant [8]. Endophytic fungi usually get nutrition and protection from their host plant and promote the growth of the plant by producing certain bioactive substances [9]. Endophytic fungi in plants are promising sources of bioactive metabolites. Recent studies show that endophytic fungi have an ability to produce many novel chemicals that could be directly use as drugs or source of bioactive natural products [10].

Several research indicate endophytic fungi have bioactive compounds that could potentially be applied in various applications such as antioxidant [11], antifungal [12], antiviral [13], antibacterial [14] and cyotoxic [15]. In our previous study, we found that several bioactive compounds such as catechin, epicatechin, tiliroside, β-sitosterol glucoside and condensed tannins were isolated from *Quercus gilva* Blume and *Quercus phillyraeoides* A. Gray [16,17]. Catechin and epicatechin

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http://dx.doi.org/10.1016/j.fshw.2017.05.001

Please cite this article in press as: A.W. Indrianingsih, S. Tachibana, α-Glucosidase inhibitor produced by an endophytic fungus, *Xylariaceae* sp. QGS 01 from *Quercus gilva* Blume, Food Sci. Hum. Wellness (2017), http://dx.doi.org/10.1016/j.fshw.2017.05.001

isolated from *Q. gilva* Blume were having good antioxidant properties while tiliroside had strong ability as α-glucosidase inhibitor activity; therefore, in this study we conducted isolation of endophytic fungi from *Quercus gilva* Blume (*Q. gilva*). Endophytic fungus QGS 01 from *Q. gilva* was found to have a strong α-glucosidase inhibitory activity. This QGS 01 fungus was identified as a *Xylariaceae* sp. This present work highlighted the α-glucosidase inhibitory activity of constituents isolated from the mycelium extract of *Xylariaceae* sp. QGS 01. Extract of *Xylariaceae* sp. QGS 01 mycelium were obtained using ethyl acetate and the isolation of active constituent was conducted using bioassay-guided fractionation technique. An *in vitro* assay of α-glucosidase inhibitory activity was conducted using α-glucosidase enzyme obtained from *Saccharomyces cerevisiae* (*S. cerevisiae*) yeast. This assay may be used for preliminary observations in the evaluation of pharmalogical activities and also to verify the medicinal effects of these active constituents isolated from endophytic fungus.

2. Materials and methods

2.1. General instrumentation and chemicals

An analysis using gas chromatography (GC) was conducted on a GC-FID 2014 model (Shimadzu, Japan). The electron ionization mass spectra (EI-MS) of isolated constituents were recorded on a GC Mass Spectrometer (GC–MS QP 2010 Plus, Shimadzu, Japan). Single crystal analysis of isolated constituent was recorded on Rigaku Saturn 724 X-Ray Diffractometer. TLC was run on silica gel 60 F_{254} pre-coated plates (Merck 5554) and spots were detected using UV light.

α-Glucosidase [(EC 3.2.1.20)] type I from *S. cerevisiae*, *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) and bis(trimethylsilyl) acetamide (BSA) were purchased from Wako Pure Chemicals, Ltd. (Osaka, Japan). Quercetin, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid were purchased from Sigma-Aldrich Co., Ltd. (Tokyo, Japan). All solvents used in this study (methanol, ethanol, toluene, pyridine, ethyl acetate, chloroform, hexane, and acetone) were purchased from Wako Pure Chemicals, Ltd.

2.2. Isolation and culture of the endophytic fungus

The stem of *Quercus gilva* Blume was collected from Ehime University Garden, Ehime Prefecture, Japan, in October 2014. Samples were cleaned in tap water and sterilized by consecutive washes in 75% EtOH (1 min), 1% NaOCl (2 min), 75% EtOH (30 s) and rinsed with sterile distilled H_2O three times. The surface sterilized material was cut into 0.4 × 0.4 cm pieces and the tissues were deposited on a Petri dish containing potato dextrose agar (PDA) medium and incubated at 25 °C. The hyphal of was transferred to fresh PDA medium and purified for three times.

2.3. Molecular identification of fungus

Fungal strains were maintained on PDA and incubated for 7 days. DNA was extracted from these fungi based on the Doyle and Doyle [18] method with a slight modification. The extracted DNA was used as a template for PCR to amplify the ITS1-F and ITS4-B regions. Products were then sequenced using two PCR primers and an automated ABI Prism DNA sequence. The result of sequencing was compared with the National Centre for Biotechnology Information (NCBI) GenBank database. A phylogenetic tree was constructed using MEGA software (version 5.2.2). The endophytic fungus was identified as *Xylariaceae* sp. QGS 01 (GenBank accession number: KU764517).

Table 1
α-Glucosidase inhibitory activity of Fraction 1 to Fraction 6 of mycelium extract from *Xylariaceae* sp. QGS 01.

Fraction	α-Glucosidase inhibitory activity (IC_{50}), μg/mL	Yield (g)
F1	9.33[a]	1.86
F2	24.97[c]	0.10
F3	11.35[b]	0.08
F4	9.56[a]	0.14
F5	22.38[c]	1.02
F6	9.67[a]	0.86

Different letters in the same column indicate significant differences ($P < 0.05$).

2.4. Fermentation and extraction

The endophytic fungus *Xylariaceae* sp. QGS 01 was inoculated into the Erlenmeyer flasks (500 ml) at 25 °C for 3 weeks, each flask containing 200 ml potato dextrose broth (PDB). Mycelium and culture broth were separated and extracted with equal volume of ethyl acetate at room temperature. The extract of endophytic fungus was condensed in a rotating evaporator under reduced pressure. The obtained crude extracts of the endophytic fungus (mycelium and culture broth extracts) were screened for the α-glucosidase activity and had the inhibitory concentration (IC_{50}) of 12.50 and 21.70 μg/mL, respectively. The results indicated that the mycelium extract of *Xylariaceae* sp. QGS 01 had higher activity; therefore further research was carried out on the fungal strain *Xylariaceae* sp. QGS 01. Large scale fermentation of *Xylariaceae* sp. QGS 01 was conducted with total volume of 30 l which were resulting of 4.12 g of mycelium extract and 1.92 g of culture broth extract.

2.5. Isolation procedures of active constituents

The ethyl acetate extract (4.12 g) was separated using silica column chromatography using solvents with increasing polarity from *n*-hexane, chloroform, ethyl acetate (EtOAc), and methanol (MeOH) to obtain 6 fractions (F1-F6). All fractions (F1-F6) were screened for α-glucosidase inhibitory activity (Table 1), with the F1 fraction exhibiting the highest activity. The active F1 fraction was separated again by silica column chromatography to get 4 fractions (F1.1-F1.4). Compound **1** (5.1 mg) was isolated from F1.1 as a colorless needle crystal by using eluent hexane-ethyl acetate (4:1) and further purified by recrystallization using chloroform.

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