Journal of
Microbial & Biochemical Technology

Dewi et al., J Microbial Biochem Technol 2012, 4:1
DOI: 10.4172/1948-5948.1000065

Research Article Open Access

Isolation of Antioxidant Compounds from *Aspergillus Terreus* LS01

Rizna Triana Dewi[1], Sanro Tachibana[2]*, Kazutaka Itoh[2] and Muhammad Ilyas[3]

[1]*The United Graduate School of Agriculture Science, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime 790-8566 Japan*
[2]*Department of Applied Biosciences, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime 790-8566 Japan*
[3]*Research Center for Biology - Indonesian Institute of Sciences, Cibinong Science Centre,Jl. Raya Jakarta Bogor KM. 46, Cibinong 16911, Indonesia*

Abstract

The antioxidative activity of an ethyl acetate extract from *Aspergillus terreus* LS01was evaluated using various in vitro assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen-peroxide-scavenging free radical scavenging, and β-caroten-linoleat model assay and the antioxidants in the extract were isolated and identified. Silica gel column chromatography was used to separate the extract into five fractions (F1-F5). Fraction 3 possessed significant antioxidative activity with an IC_{50} of 19.91µg/ml. Fraction 3 was further separated by column chromatography and yielded the crystalline compounds 1 and 2, which were identified as terreic acid and terremutin, respectively, on the basis of UV-vis spectra, MS and NMR analyses. Terreic acid and terremutin exhibited the highest level of DPPH free radical scavenging activity with IC_{50} values of 0.115±4.02 and 0.114±2.19 mM, respectively. These compounds also showed scavenging activity of hydrogen peroxide radicals which terreic acid exhibited higher than terremutin with 74.07±1.48% and 33.74±2.81% respectively. In the β-caroten-linoleat model assay, the inhibition of terreic acid retained 26.01±1.14% and terremutin retained 32.29±2.23%. This is the first report on antioxidative activity of terreic acid and terremutin from *A.terreus*. The findings indicated that *A. terreus* could be considered as potential source of natural antioxidant products.

Keywords: *Aspergillus terreus* LS01; terreic acid; terremutin; antioxidantive activity

Introduction

Free radicals and oxidants play a dual role, since they can be either harmful or helpful to the body. They are produced continuously in all cells as part of normal cell metabolism in situ or from environmental sources (pollution, cigarette smoke, radiation, medications, etc). Overproduction of free radicals in the body can generate oxidative stress, a deleterious process that plays a major part in the development of chronic and degenerative diseases, such as arteriosclerosis, diabetes, and cancer initiation, and has also been implicated in the aging process [1-3]. Therefore, antioxidant therapy represents a promising treatment for the effects of oxidative stress. Antioxidants can prevent the oxidation of substrates when present at low concentrations [4], act as radical scavengers, and inhibit lipid per-oxidation and other free radical mediated processes; and so are able to protect the body from several diseases attributed to the reactions of radicals [2]. Therefore, attention has been directed toward the isolation of natural antioxidants to substitute for synthetic counterparts.

A number of plants and mushrooms (fruiting bodies) are known to produce antioxidants but there are few reports on lower fungi [5]. Filamentous fungi produce a diverse array of secondary metabolites, which have a tremendous impact on society and are exploited for their antibiotic and pharmaceutical activities such as anticancer, antitumor, and antioxidant effects [6]. Fungi belonging to the *Aspergillus* genera are among the major contributors to secondary metabolites of fungal origin [7]. Several antioxidants such as 3,3-di-OH terphenyllin, 3-OH terphenyllin, and candidusin B obtained from the extracts of *Aspergillus candidus* have been reported to have scavenging effects on DPPH radicals [8,9]. Exopolysaccharide has also been reported as an antioxidant, produced by the mangrove entophytic fungus *Aspergillus* sp [10].

Aspergillus terreus is a ubiquitous fungus isolated from both marine and terrestrial environments [7,11]. This microorganism produces a variety of secondary metabolites that are economically significant, such as the antihypercholesterolemic drug lovastatin and several other metabolites including sulochrin and terrein which have antibiotic activity [12]. There are relatively few studies of antioxidant from *A. terreus* compared to other *Aspergillus* species such as *A. candidus* [8,9], *A. awamori* [13], and *A. fumingatus* [14]. The objective of this study was to isolate and evaluate of the antioxidants activity of compounds in an ethyl acetate extract of *Aspergillus terreus*. Their antioxidant activity was determined by three different assays, namely, 1,1-diphenyl-2picrylhydrazyl (DPPH-radical) scavenging, hydrogen peroxide-scavenging, and β-caroten-linoleat model assay.

Materials and Methods

General experimental procedures

Melting points were recorded in a Micro melting point apparatus and are uncorrected. Optical rotation values were measured with a Jasco P-2100 polarimeter. The UV-Vis absorption spectra of the active compounds in methanol were recorded on a Hitachi U-1600 spectrophotometer. The mass spectra of the compounds were obtained using high resolution FAB-MS. The nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a JEOL 500 with TMS as an internal standard. Heteronuclear multiple quantum coherence spectroscopy (HMQC) and heteronuclear multiple bond

***Corresponding author:** Sanro Tachibana, Departement of Applied Bioscience, Faculty of Agriculture, Ehime University 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566 Japan.Tel: 81-89-9469864; E-mail: tatibana@agr.ehime-u.ac.jp

Received December 06, 2011; **Accepted** January 19, 2012; **Published** January 25, 2012

Citation: Dewi RT, Tachibana S, Itoh K, Ilyas M (2012) Isolation of Antioxidant Compounds from *Aspergillus Terreus* LS01. J Microbial Biochem Technol 4: 010-014. doi:10.4172/1948-5948.1000065

correlation spectroscopy (HMBC) were used to assign correlations between ^{1}H and ^{13}C signals. The chemical shift values (δ) are given in parts per million (ppm), and coupling constant (*J*) in Hz. Silica gel (60-200 mesh Wako gel) was used for column chromatography. Precoated silicagel 60 F_{254} TLC plates (Merck 5554) were used for analytical TLC. A mixture of chloroform and methanol (90:10, v/v) was used as mobile phase for TLC analysis. Compounds were visualized under UV light (at 254 nm) and on spraying with methanolic sulphuric acid (95:5, v/v) followed by heating at 120°C.

Medium and chemicals

Bacto peptone was obtained from Difco. Co (Detroit, MI). Malt extract was obtained from Becton Dickinson Company (Sparks, MD, USA). 1,1-diphenyl-2picrylhydrazyl (DPPH), β-carotene, and hydrogen peroxide were purchased from Wako Chemical Co.Ltd (Osaka, Japan). Tween 40 and quercetin were purchased from Sigma-Aldrich Co. Ltd. (Tokyo, Japan). Solvents used for column chromatography were obtained from Wako Pure Chemicals and distilled prior to use.

Fungal material

The fungus *A. terreus* LS01 was isolated from the Teluk Kodek area, Pemenang, West Lombok, Indonesia. It was identified as *Aspergillus terreus* Thom., according to its morphological characteristics and 28S rDNA sequence. The fungus has been deposited in the LIPI Microbial Collection (LIPIMC) Microbiology Division Research Center for Biology-LIPI. It was prepared on potato dextrose agar plates and stored at 4°C.

Fermentation and extraction

A. terreus LS01 was grown on PDA medium (dehydrated mashed potatoes 2% w/v, glucose 2% w/v, pH 5.5) for seven days at 25°C. Liquid culture experiments were conducted in a 250 ml Erlenmeyer -flask containing 50 ml of PMP medium (2.4% potato dextrose broth, 1% malt extract, and 0.1% peptone). The flasks were incubated at 25°C with shaking at 100 rpm for seven days. Ethyl acetate (EtOAc) (3x1 L) was added into the culture broth (3 L) and extracted for 20 min by vigorously shaking. The EtOAc extract was concentrated with a rotary evaporator under vacuum at 40°C to dryness and a brown paste (830 mg) was obtained.

Isolation of the antioxidative compounds from ethyl acetate extract

The EtOAc extract (800 mg) obtained from the liquid fermentation of *A. terrus* LS01 was subjected to column chromatography on Silica Gel 60 (column length 25 cm; internal diameter 3 cm) using a stepwise gradient from n-hexane: EtOAc 95:5 v/v (Fraction1, 58 mg); n-hexane: EtOAc 85:15 v/v (Fraction 2, 90 mg); n-hexane: EtOAc 75:25 v/v (Fraction 3, 250 mg); n-hexane: EtOAc 1:1 v/v to 100% EtOAc (Fraction 4, 140 mg) and EtOAc: methanol 95:5-90:10 v/v (Fraction 5, 180 mg). The obtained fraction which showed the highest antioxidant activity (F-3) was further purified with column chromatography on Silica Gel 60 (length 15 cm; internal diameter 1.5 cm) with a stepwise gradient from n-hexane: EtOAc 9:1 (Fraction 3.1, 5 mg); n-hexane: EtOAc 8:2-7:3 (Fraction 3.2, 170 mg); n-hexane: EtOAc 1:1 (Fraction 3.3, 65 mg). Fraction F3.2 was recrystallized from ethyl acetate to yield pale yellow needles (compound **1,** 77 mg, mp 126-127°C). Fraction F3.3 was recrystallized from acetone to yield colorless solid (compound **2,** 30 mg, mp 164-166°C). The pure compounds were identified by instrumental analysis.

Compound 1. (1S,6R)-4-hydroxy-3-methyl-7-oxabicyclo[4.1.0] hept-3-ene-2,5-dione *(Terreic acid).* Pale yellow needles, m.p. 126-127°C. UV spectra (MeOH) λ_{max} (log ε) 213 (4.03) and 314 (3.88). $[\alpha]^{28.6}_{D}$ -34 (c, 0.046 in MeOH). Molecular formula: $C_7H_6O_4$. HRFABMS: $[M+H]^+$ m/z 155.0358. Data ^{1}H (500 MHz; $CDCl_3$, δ-values, *J* in Hz): 1.93 (s,3H); 3.86 (d,1H, J=3.9); 3.89 (d,1H, J=3.35); 6.87 (s,1H) and ^{13}C NMR (100 MHz; $CDCl_3$, δ-values): 8.85, 51.63, 53.84, 120.45, 151.92, 187.55, 190.79.

Compound 2**.** (1S, 2S, 6S)-2,5-dihydroxy-4-methyl-7-oxabicyclo[4.1.0]hept-4-en-3-one [(±)-*terremutin*]. Colorless solid, m.p 164-166°C. UV spectra (MeOH) λ_{max} (log ε) 272 (4.05). $[\alpha]^{28.6}_{D}$ -283 (c, 0.16 in MeOH). Molecular formula: $C_7H_8O_4$. HRFABMS: $[M+H]^+$ m/z 157.0498. Data ^{1}H (500 MHz; Acetone-d_6, δ-values, *J* in Hz): 1.65 (s,3H); 3.34 (d,1H, J=2.55); 3.64 (dd,1H, J=1.3); 4.68 and ^{13}C NMR (100 MHz; Acetone-d_6, δ-values) 7.50, 52.35, 55.28, 66.17, 108.87, 168, 191.

Determination of DPPH radical-scavenging activity

The free radicals scavenging activities of extracts were measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Each extract in methanol (4 ml) at a concentration of 10-200 μg/ml was mixed with 1 ml of a methanolic solution containing DPPH radicals at 1 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank [15]. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = [(A0-A1/A0) × 100], where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample. The percentage of scavenging activity obtained was subsequently plotted against the sample concentration. The half maximal inhibitory concentration (IC_{50}) was calculated from the graph of antioxidant activity percentage against sample concentration. The assays were carried out in triplicate and the results expressed as mean values ± standard deviations. Quercetin was used as a reference compound.

Hydrogen peroxide radical scavenging (H_2O_2) activity

A solution (4 mM) of hydrogen peroxide was prepared in phosphate-buffer saline (PBS, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity of 81 $M^{-1}cm^{-1}$ [15]. Samples (200μg/ml) in 4 ml distilled water were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing samples in PBS without hydrogen peroxide. The percent inhibitory activity was calculated as follows: % Scavenged (H_2O_2) = (A0 - A1/A0) X 100 . Where; A0 is the absorbance of control and A1 is the absorbance of sample. Ascorbic acid was used as positive control.

β-Carotene- linoleate model assay

Antioxidant activity of fractions and isolated compounds were determined using the β-carotene-linoleat model system [16]. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml chloroform. Two milliliters of the solution were transferred into a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40°C for 5 min and, to the residue, 50 ml of distilled water was added, slowly with vigorous agitation, to form an emulsion. 4.8 ml aliquots of the emulsion were transferred into different test tubes containing 0.2

ml of samples in methanol at 0.5 mg/ml. The tubes were shaken and incubated at 50°C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using spectrophotometer. Absorbance readings were then recorded at 15 min interval until the control sample had change color. A blank, devoid of β-carotene, was prepared for background subtractions. Antioxidant activity was calculated using the following equation: % Antioxidant activity (AA)= 100 x [1-{(A_{s0}- A_{se})/(A_{c0}-A_{ce})}], where A_{s0} and A_{se} were absorbance of the sample at 0 and 120 min, and A_{c0} and A_{ce} were absorbance of the control at 0 and 120 min. The assays were carried out in triplicate and results expressed as mean values ± standard deviation. Ascorbic acid was used as standard.

Results and Discussion

Isolation and identification of antioxidative compounds

The major components in ethyl acetate extract of *A. terreus* LS01 were separated on a silica gel column chromatography into five fractions. Antioxidant activities of all the fractions obtained were measured using DPPH method at range concentrations 50-200 µg/ml. For each fraction, the IC_{50} value was calculated and presented in Table 1. The IC_{50} value is the concentration which causes a 50 % inhibition of the DPPH radicals. The lower IC_{50} value, the greater antioxidant activity. The highest scavenging on DPPH free radical activity was shown by fraction F-3 (250 mg) with IC_{50}=19.91µg/ml, and exhibit scavenging of hydrogen peroxide radicals (51.58±1.48%) and hider bleaching β-carotene (30.69±3.05%) at 200 µg/ml of sample solution (Table 1). Fraction 3 also gave a relatively good recovery (31.25%) from ethyl acetate extract than other active fractions; therefore it is more potential for further purification. F-3 was further fractionated by column chromatography (F3.1-F3.3). Fraction F3.1 (5mg) was not tested for antioxidant assays due to the sample limitation. Fraction F3.2 was recrystallized from ethyl acetate to yield pale yellow needles (compound 1,77 mg). Fraction F3.3 was recrystallized from acetone to yield a colorless solid (compound 2,30 mg).

Compound 1 was isolated as pale yellow needles. High resolution FAB-MS [calcd for $C_7H_7O_4$ $[M+H]^+$ 155.0342 found 155.0358] revealed the molecular formula to be $C_7H_6O_4$, indicating five degrees of unsaturation. The ^{1}H NMR spectrum revealed two oxygenated methyne protons [δ_H 3.89 (1H, J=3.7 Hz) and 3.86 (1H, J=3.7 Hz)], and one methyl attached to a quarternary carbon [δ_H 1.93 (3H,s)]. The coupling constants (J = 3.7 Hz) between $\delta_{5\text{-}H}$ 3.89 and $\delta_{6\text{-}H}$ 3.86 and signal at C-5 (51.63) and C-6 (53.84) indicated these carbons to be attached to an oxygen atom forming the *cis*-epoxide, consistent with the findings of others [17,18]. Analysis of the ^{13}C NMR spectrum revealed the presence of two carbonyl ketone groups at δ_C 182.55 (C-4) and 190.79 (C-1), suggesting that 1 is a quinone. In order to determine the connection between the partial structures and assign the NMR signals, 2D NMR spectra (HMQC and HMBC) were measured. In the HMBC spectrum (Figure 1), long range correlations between H-6 and C-1 and C-2 and correlations of H-5 and C-3 and C-4 established the position of the epoxide group [18]. From the data for ^{1}H and ^{13}C, the compound is an epoxy-quinone. Based on UV-vis, MS, ^{1}H NMR and ^{13}C NMR data, 1 was identified as (1S,6R)-4-hydroxy-3-methyl-7-oxabicyclo[4.1.0]hept-3-ene-2,5-dione *(Terreic acid)*. The spectral characteristics of the compound were found to be identical with those in the literature [19-22]. The structure of 1 is shown in Figure 2.

The ^{1}H and ^{13}C NMR spectra of 1 and 2 were similar. However a significant difference was found around the quinone moiety of 2, with the appearance of a singlet proton signal at 4.68 ppm and a high field shift of the C4 carbon signal from 182.55 to 66.17 ppm. These results clearly indicated the carbonyl group of 1 to be replaced by a hydroxymethine group in 2. This structure was further supported by the HMBC data (Figure 1). In the ^{1}H NMR spectra of 2, coupling constants ($J_{4,5}$ = 1.4 Hz) indicated a *trans*-didydroquinone epoxide [19,24,25]. This value coincided with that for a trans-dihydroquinone epoxide [18]. The molecular formula of **2** was determined by high resolution FAB-MS [calcd and measured have same value for $C_7H_9O_4$ $[M+H]^+$ 157.0498] as $C_7H_8O_4$, indicating four degrees of unsaturation. From the UV spectra, MS and ^{1}H and ^{13}C NMR data, compounds **2** was identified as (1S, 2S, 6S)-2,5-dihydroxy-4-methyl-7-oxabicyclo[4.1.0] hept-4-en-3-one [(±)-*terremutin*] (Figure 2) [18,20,24-26].

Antioxidative activities of isolated compounds

Terreic acid is a quinone epoxide whereas terremutin is a dihydroquinone epoxide. Both are produced by several members of the genus *A. terreus* and have been reported as antibiotic compounds

Fractions	Free radical scavenging activity		β-Caroten linoleat model system (%)*
	DPPH radicals (IC_{50} µg/ml)	H_2O_2 (%)*	
F-1	NA	NA	NA
F-2	NA	NA	NA
F-3	19.91 ±1.2	64.56±1.48	50.69±3.05
F-3.2	17.881±1.1	51.58±1.55	27.78±1.39
F3.3	17.974±1.0	29.97±0.43	35.43±0.43
F-4	48.64 ±0.8	65.93±0.32	75.78±1.90
F-5	97.43 ±1.1	60.64±1.09	70.74±1.9

Data are presented as the mean ± S.D. of triplicate measurements
*Antioxidant activity determinant at concentration 200µg/ml of samples

Table 1: Antioxidant activity of fractions 1-5 of the ethyl acetate extract from *A. terreus* LS01 obtained by silica gel column chromatography.

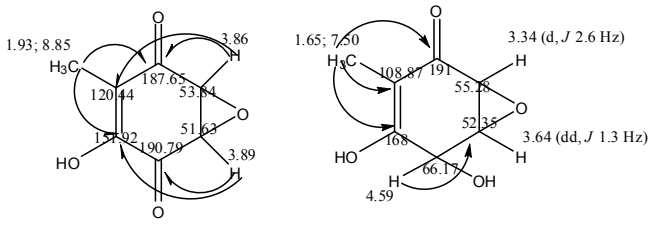

Figure 1: Correlation ^{1}H-^{13}C of compounds 1 and 2.

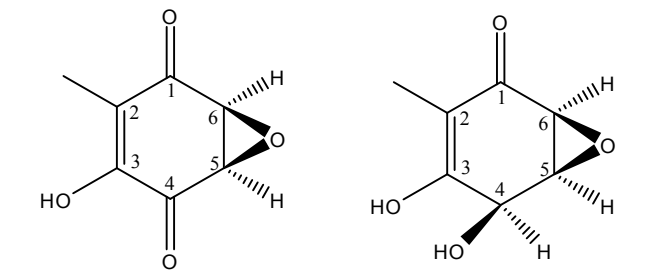

Figure 2: Chemical structure of the antioxidants isolated from *Aspergillus terreus* LS01.

[22,25-27]. However, the antioxidative properties of these compounds are reported here for the first time. This study, we assessed the free radical scavenging activity of compounds by using DPPH radicals and hydrogen peroxide radicals and β-caroten linoleic system assay methods.

The DPPH method is considered to be a model compound of a lipophilic radical. DPPH is a free radical stable at room temperature,and produces a purple color solution in methanol. It is reduced in the presence of antioxidant molecule, giving rise to a yellowish methanol solution. One of the mechanisms involved in antioxidant activity assay is the ability of a molecule to donate a hydrogen atom to a radical, and the propensity of the hydrogen donation is the critical factor involved in free radical scavenging [28]. The DPPH radical scavenging activities of isolated compound are presented in (Figure 3). The compounds were able to scavenge DPPH free radicals in a concentration-dependent manner, with an IC_{50} of 0.115±4.02 and 0.114±2.19 mM, respectively. However, the activity of quercetin with IC_{50} of 0.033±1.02 mM was stronger than these compounds.

The ability of terreic acid and terremutin to effectively scavenge hydrogen peroxide were determined according to the method of Ruch [15], in which those were compared with ascorbic acid as standard (Figure 4). Terreic acid had higher potency of hydrogen

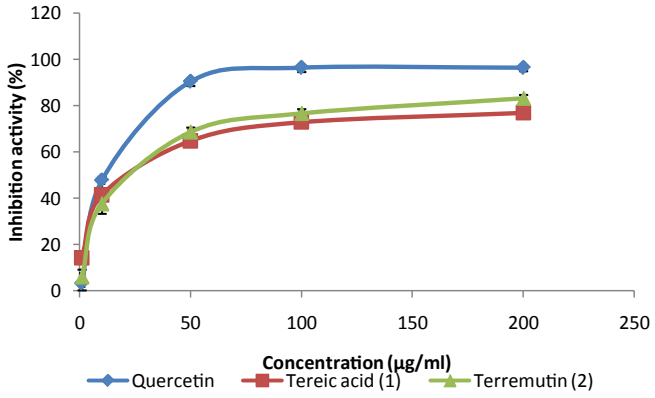

Figure 3: The scavenging effect on DPPH radicals of compounds 1 and 2 from *A.terreus* LS01. Quercetin was used as a positive control. Data are presented as the mean ± S.D. of triplicate measurements.

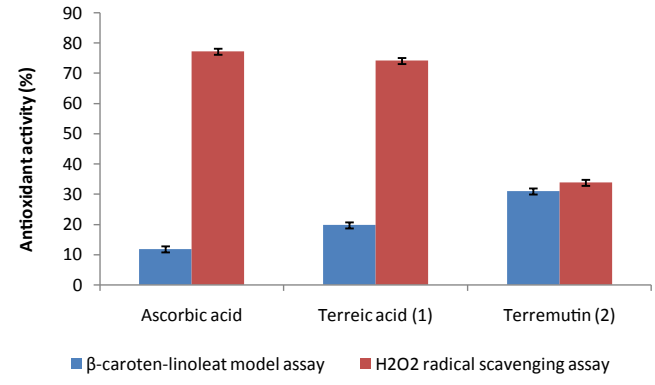

Figure 4: Comparison antioxidant activity of compounds 1 and 2, with ascorbic acid as standard at 200 µg/ml using H_2O_2 radicals scavenging and β-caroten linoleat model system assay. Data are presented as the mean ± S.D. of triplicate measurements.

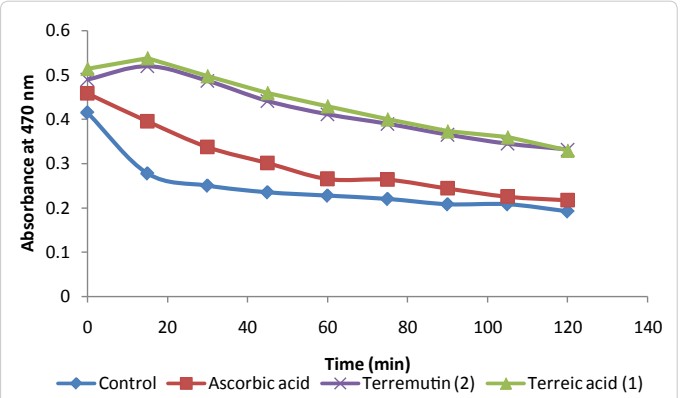

Figure 5: Antioxidant activity of compounds 1 and 2 from *A.terreus* LS01 at 200 µg/ml in β-caroten linoleat model system. Ascorbic acid was used as a reference. Data are presented as the mean ± S.D. of triplicate measurements.

peroxide radical-scavenging activity (74.07±1.48%) than terremutin (33.74±2.81%) and similar with ascorbic acid (77.13±1.40%) at 200 µg/ml concentration.

The study assessed the lipid peroxidation inhibitory activity of the isolated compounds by the β-caroten-linoleic model assay (Figure 4). The system containing 200 µg/ml of samples, terreic acid retained 26.01±1.14% and terremutin retained 32.29±2.23% of the initial β-carotene after 120 min of the assay whereas ascorbic acid as standard retained only 11.8±0.5%. The mechanism of bleaching of β-caroten is a free-radicals-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-caroten models. The presence of different antioxidants can hinder the extent of β-caroten-bleaching by neutralizing the linoleat-free radical and other free radicals formed in the system [16]. Accordingly, the absorbance decreased rapidly in sample without antioxidant whereas in the presence of an antioxidant, the colour could be retained for a longer time (Figure 5).

In this study, terreic acid and terremutin showed potential scavenging activity toward free radicals. The antioxidative activity of these compounds is due to a quinine group which serves as an effective single electron acceptor, acts as a free radical chain-breaker by reacting with the alkyl radical [29]. This mechanism is similar to that of α-tocopherolhyroquinone [30].

Conclusion

The obtained result o in this study clearly demonstrated that terreic acid and terremutin from ethyl acetate extract of *A. terreus* exhibited significant antioxidant activity. To our knowledge, this is the first report on antioxidative activity of terreic acid and terremutin isolated from *A.terreus.* The findings indicated that *A. terreus* could be considered as potential source of natural antioxidant products. Further investigations to explore the antioxidant compound from *A.terreus* are underway in our laboratories.

Acknowledgment

We thank to Dr. Muhammad Hanafi and Mr. Ahmad Darmawan for valuable discussions and their excellent technical assistance.

References

1. Pham-Huy L, Hua He, Pham-huy C (2008) Free radicals antioxidants in disease and health. Int J Biomedical Science 4: 89-96.

2. Young IS, Woodside JV (2001) Antioxidant in health and disease. J Clin Pathol 35: 176-186.

3. Valko M, Leibfritiz D, Moncola J, Cronin MT, Mazur M (2007) Free radicals and antioxidant in normal physiological functions and human disease. Int J Biochem Cell Biol 39: 44-84.

4. Helliwell B (1995) Antioxidant characterization: methodology and mechanism. Biochem Pharmacol 49: 1341-1348.

5. Rios MF, Pajan CMG, Galan RH, Sanchez AJM, Callado IG (2006) Synthesis and free radicals scavenging activity of a novel metabolite from the fungus Colletotrichum gloeosporides. Bioorg Med Chem Lett 16: 5836-5839.

6. Fox EM, Howlett BJ (2008) Secondary metabolism: regulation and role in fungal biology. Current opinion in Microbiol 11: 481-487.

7. Parvatcar RR, D'Souza C, Tripathi A, Naik CG (2009) Aspernolides A and b butenolides from marine-derived fungus *Aspergillus terreus.* Phytochem 70: 128-132.

8. Yen GC, Chang YC, Sheu F, Chiang HC (2001) Isolation and characterization of antioxidants compound from *Aspergillus candidus* broth filtrate. J Agri Food Chem 49: 1426-1431.

9. Yen GC, Chang YC, Su SW (2003) Antioxidant activity and active compounds of rice koji fermented with *Aspergillus candidus.* Food Chem 83: 49-54.

10. Chen Y, Mao W, Tao H, Zhu W, Qi X (2011) Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus Aspergillus sp Y16. Bioresource Technology 102: 8179-8184.

11. Balajee SA (2009) *Aspergillus terreus* complex. Med Mycology 47: S42-S46.

12. Maacedo FC, Porto ALM, Marsaioli AJ, Fernando C, Anita J (2004) Terreinol-a novel metabolite from Aspergillus terreus: structure and 13C labeling. Tetrahedron Letters 45: 53-55.

13. Lee IH, Hung YH, Chou CC (2007) Total phenolic and anthocyanin contents as well as antioxidant activity of black bean koji fermented by Aspergillus awamori under different culture conditions. Food Chemistry 104: 936-942.

14. Arora DS, Chandra P (2011) Antioxidant activity of *Aspergillus fumigates.* ISRN Pharmacology 2011: 1-11.

15. Yen GC, Chen HY (1995) Antioxidant Activity of Various Tea Extracts in Relation to Their Antimut agenicity. J Agric Food Chem 43: 27-32.

16. Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. Food Chemistry 73: 285-290.

17. Koizumi F, Takahashi Y, Ishiguro H, Tanaka R, Ohtaki S (2004) Structure elucidation of EI-1941-1 and -2, novel interleukin-1β converting enzyme inhibitor produced by *Forrowira* sp. E-1941. Tetrahedron Letters 45: 7419-7422.

18. Shiono Y, Murayama T, Takahashi K, Okada K, Katohida S, et al. (2005) Three oxygenated cyclohexanone derivatives produced by an endophytic fungus. Biosci Biotechnol Biochem 69: 287-292.

19. Sheehan JC, Lawson WB, Gaul RJ (1958) The structure of Terreic Acid. J Am Chem Soc 80 : 5536-5538.

20. Kiriyama N, Higuchi Y, Yamamoto Y (1977) Studies on the metabolic products of *Aspergillus terreus* II Structure and biosynthesis of the metabolites of the strain ATCC 12238. Chem Pharm Bull 25: 1265-1272.

21. Ehsen A, Karabeles K, Haerding J, Moore H (1990) Synthesis of hydroquinones and related compounds: semisquaric acids (±)- terreic acid (±)- perezone and (±)- isoprezone. J Org Chem 55: 1177-1185.

22. Cazar ME, Hirschman SG, Astudillo L (2005) Antimicrobial butyrolactone I derivates from the Ecuadorian soil fungus *Aspergillus terreus* Thorn var terreus. J Micrb & Biotech 21: 1067-1075.

23. Read G, Ruiz VM (1970) Quinone epoxides Part IV The synthesis and stereochemistry of terremutin. J Chem Soc (Section) C: Organic 14: 1945-1948.

24. Sekiguchi J, Gaucher GM (1979) Isoepoxydon, a new metabolite of the patulin pathway in *Penicillium urticae.* Biochem J 182: 445-453

25. Miller MW (1968) The structure of terremutin. Tetrahedron 24: 4839-4851.

26. Read G, Weslake DWS, Vining LC (1969) Quinone epoxideV The biosynthesis of terreic acid. Can J Biochem 47: 1968-1079.

27. Kawakami Y, Hartman S, Kinoshita E, Suzuki H, Kitaura I, et al. (1999) Terreic acid a quinine epoxide inhibitor of Bruton's tyrosine kinase. Proc Natl Acad Sci USA 96: 2227-2232.

28. Bondet V, Williams WB, Berset C (1997) Kinetics and Mechanisms of Antioxidant Activity using the DPPH• Free Radical Method. Lebensm-Wiss u-Technol 30: 609-615.

29. Liebler DC, Burr JA (2000) Antioxidant reactions of α-tocopherollhydroquinone. Lipids 35: 1045-1047.

30. Flora SJS (2009) Structural, chemical and biological aspects of antioxidant for strategies against metal and metalloid exposure (Review) Oxidative medicine and cellular longevity 2: 191-206.