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Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*

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Abstract

The objective of this study was to evaluate the antioxidant activity of cultivated fruiting bodies of an endophytic *Xylaria* sp. (strain number YX-28), from *Ginkgo biloba*. The results indicated that the methanol extract exhibited strong antioxidant capacity in both 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis and β-carotene–linoleic acid model system. Total phenolic and flavonoid contents extracted by different solvents were determined using Folin–Ciocalteu procedure and the flavonoid–aluminium method. The results showed that total phenolic and flavonoid contents were the highest in methanol extract (54.51 ± 1.05 mg gallic acid equivalent/g dry weight and 86.76 ± 0.58 mg rutin equivalent/g dry weight), while the hexane extract was the lowest (9.71 ± 0.57 mg GAE/g dw and 10.14 ± 0.76 mg RE/g dw, respectively). The correlation coefficients from regression analysis showed a positive relationship between total phenolic content in the extracts and DPPH activity ($R^2 = 0.7336$), as well as between total flavonoid content and DPPH activity ($R^2 = 0.9392$). Furthermore, GC/MS method was used to confirm the presence of phenolics with antioxidant activity in the methanol extract and resulted in the identification of 41 compounds, esters, phenolics, alkanes, carboxylates and alcohols being the main components. In conclusion, cultivated fruiting bodies of *Xylaria* sp.YX-28 may have potential as natural antioxidant.

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Keywords: Endophyte; *Xylaria* sp.; Phenolic; Flavanoid; Antioxidant activity

1. Introduction

Lipid oxidation is a complex free radical chain process involving a variety of radicals. In foods, oxidation occurs during processing and storage, leading to their deterioration. Due to undesirable effects of oxidized lipids on the human health, it seems to be essential to decrease contact with products of lipid oxidation in food (Karpinska, Borowski, & Danowska-Oziewicz, 2001). In order to prolong the storage life of foods, synthetic antioxidants are widely used for industrial processing. According to toxicologists and nutritionists, the side effects of some synthetic food antioxidants have already been documented. For example, two synthetic food antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have carcinogenic effects in living organisms (Baardseth, 1989). In recent years, increasing attention has been paid on the exploration of naturally-occurring antioxidants, because of the growing consumer demand for food products free of "synthetic" additives (Gould, 1995; Reische, Lillard, & Eitenmiller, 1998). An enormous variety of plants, animals and microorganisms have been studied for new sources of natural antioxidants (Essawi & Srour, 2000), especially phenolic and flavonoid compounds derived from plants, which were proved to be potent antioxidants and free radical scavengers (Kähkonen et al., 1999; Sugihara, Arakawa, Ohnishi, & Furuno, 1999).

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; RE, rutin equivalent; Dw, dry weight; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; Asc A, ascorbic acid; Rt, retention time.

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As some microorganisms "hidden" within healthy host plants, endophytes are a poorly investigated group of microorganisms,but they represent an abundant and dependable source of novel bioactive compounds with huge potential for exploitation in a wide variety of medical, agricultural, and industrial areas (Tan & Zou, 2001). Although there have been a number of studies on the biological activities of endophytes, such as antiviral, anticancer, antidiabetic and antimicrobial effects (Guo et al., 2000; Strobel et al., 1996; Zhang et al., 1999), only a few reports about antioxidant activities of endophytic fungi have been published up to date (Strobel et al., 2002). We isolated an endophytic fungus *Xylaria* sp. (strain number YX-28) from the stem of *Ginkgo biloba* tree with the age of more than 1000 years old and developed a new process for cultivating fruiting bodies using solid fermentation. The objective of the present study was to evaluate antioxidant activities of the endophyte, and to determine the chemical composition, total phenolic and flavonoid contents. In addition, the relationships between the phenolic and flavonoid contents of the extracts and their antioxidant activity were studied.

2. Materials and methods

2.1. Source of *Xylaria sp.* YX-28

Xylaria sp. (Strain number YX-28) is an endophytic fungus, isolated from fresh stems of an apparently healthy *G. biloba*, collected in November 2003, in the suburb of Xuzhou city, Jiangsu province, China, according to the method detailed elsewhere (Strobel et al., 1996). Based on its microscopic morphology and ITS sequences analysis (Accession Number DQ022415 in GenBank), the fungus YX-28 was identified as *Xylaria* sp. (These results will be published in a separate paper). A reference living culture was kept at College of Food Science and Technology, Nanjing Agricultural University, Jiangsu province, China. The stock culture was maintained on a potato dextrose agar (PDA) (Sigma, St. Louis, USA) slant and subcultured every month. Slants were incubated at 25 °C for 5 days and subsequently stored at 4 °C.

2.2. Cultivation and sampling

For solid cultivation, mycelia discs were inoculated on the sterilized medium containing 75% cotton seedcoats and 25% bran (Moist 56%, W/W) and incubated at 25 °C for 30 days, until pen-like fruiting bodies with white heads formed. The fruiting bodies were harvested, freeze-dried and finely ground in liquid nitrogen. The material (1 g) was extracted with hexane, chloroform, ethyl acetate, acetone and methanol respectively, in a ratio of 1:50 (w/v) for 4 h under continuous stirring. The resulting extracts were evaporated. The residues were dissolved in 5 ml methanol, and then stored at 4 °C prior to further analysis.

2.3. Antioxidant activity

2.3.1. Chemicals

β-Carotene, linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and ascorbic acid (Asc A) were purchased from Sigma (Sigma, Aldrich). Gallic acid, rutin and Folin–Ciocalteu's reagent were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were purchased locally and were of analytical grade.

2.3.2. DPPH assay

The scavenging effects of samples for DPPH radicals were monitored according to the method described by Duan, Zhang, Li, and Wang (2006). Hundred microliters of various concentration of the methanol extract was mixed with 2900 μl DPPH solution (120 μM) in methanol and incubated in darkness at 37 °C for 30 min. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage ($I\%$) was calculated with the following equation:

$$I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the extract from fruiting bodies of *Xylaria* sp. YX-28. BHT and ascorbic acid (Asc A) were used as positive controls, and all tests were carried out in triplicate.

2.3.3. β-Carotene–linoleic acid assay

The antioxidant activity of the extract was determined according to the β-carotene bleaching method described by Dapkervicus, Venskutonis, Van Beek, and Linssen (1998). A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml chloroform (HPLC grade) and 25 μl of linoleic acid and 200 mg of Tween-40 were added. Chloroform was completely evaporated, using a vacuum evaporator. Then, 100 ml of distilled water, saturated with oxygen (30 min, 100 ml/min), were added with vigorous shaking. 4 ml of this reaction mixture were dispensed into test tubes and 200 μl methanol extract, prepared at different concentrations, were added and the emulsion system was incubated for 3 h at 50 °C. The same procedure was repeated with BHT and Asc A as positive controls, and a blank as a negative control. After this incubation period, the absorbance of the mixtures was measured at 490 nm. Antioxidant activity in β-carotene bleaching model in percentage ($A\%$) was calculated with the following equation:

$$A\% = [1 - (A_0 - A_t)/(A'_0 - A'_t)] \times 100$$

where A_0 and A'_0 are the absorbance of the sample and the blank, respectively, measured at zero time, and A_t and A'_t are the absorbance of the sample and the blank, respectively, measured after 3 h. All tests were carried out in triplicate.

2.4. Determination of total phenolic content

The amount of total phenolic was determined using the Folin–Ciocalteu reagent (Djeridane et al., 2006). One ml sample was dissolved in 1.5 ml distilled water and 0.5 ml Folin–Ciocalteu's reagent. After 1 min, 1 ml 20% sodium carbonate solution was added. The final mixture was shaken three times and incubated for 2 h in the dark at 25 °C. The absorbance of the mixture was measured at 760 nm. A standard curve was first plotted using gallic acid (0.5–20 μg/ml) as a standard, giving an equation as

$$\text{Absorbance} = 0.0216 \text{ gallic acid } (\mu\text{g/ml}) + 0.008 \ (R^2 = 0.9964).$$

All tests were carried out in triplicate and the results were expressed as gallic acid equivalents (mg GAE/g dry weight of the fruiting bodies).

2.5. Determination of total flavonoid content

The flavonoid content was determined based on the formation of flavonoid–aluminium (Djeridane et al., 2006). One ml sample was mixed with 1 ml 2% aluminium chloride solution. After incubation for 15 min at room temperature, the absorbance of the reaction mixture was measured at 430 nm. Rutin was used as a standard to make the calibration curve, giving an equation as

$$\text{Absorbance} = 0.0089 \text{ rutin } (\mu\text{g/ml}) + 0.0053 \ (R^2 = 0.9982).$$

The amount of flavonoids was expressed as rutin equivalents (mg RE/g dry weight of the fruiting bodies). All tests were carried out in triplicate.

2.6. GC/MS analysis

GC/MS was employed for the analysis of major substances present in the methanol extract of *Xylaria* sp. YX-28 (Proestos, Boziaris, Nychas, & Komaitis, 2006). An Agilent-6890 GC system coupled to an Agilent-5873 mass spectrometer was used in the EI mode with the electron energy set at 70 eV, and the mass range at *m/z* 25–700. The capillary column used in GC/MS was DB-5MS 0.32 mm × 30 m × 0.25 μm. GC injector temperature was set at 280 °C and MS transfer line temperature at 290 °C. GC was performed in the splitless mode with 1 min splitless-time. Column temperature was set at 70 °C, gradually increased to 135 °C at 2 °C/min, held for 10 min; then to 220 °C at 4 °C/min, held for 10 min; and finally raised to 270 °C at 3.5 °C /min, held for 20 min. Helium was used as carrier gas with a flow rate of 1.0 ml/min. 1 μl diluted methanol extract of YX-28 was injected after filtration through a 0.22 μm Millipore filters. Compounds were identified based on the comparison of mass spectra from the NIST library.

2.7. Statistical analysis

Values were expressed as means ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with Tukey test using the SAS 8.0 program. Differences at $P < 0.05$ were considered statistically significant. Correlation analysis was carried out using the correlation and regression programme in the EXCEL program.

3. Results and discussion

3.1. Total phenolic and flavonoid contents of Xylaria sp. and their correlation with antioxidant activity

Phenolic and flavonoid compounds seem to have important role in stabilizing lipid oxidation and to be associated with antioxidant activity, which is emphasized in several reports (Duh, Tu, & Yen, 1999; Komali, Zheng, & Shetty, 1999; Yanishlieva-Maslarova, 2001). Therefore, in this work, we determined the total phenolic and flavonoid contents of cultivated fruiting bodies from *Xylaria* sp.YX-28 using different solvents.

As shown in Table 1, there were differences in total phenolic and flavonoid contents of different extracts of the fruiting bodies, depending on solvents. The highest levels of total phenolic and flavonoid contents were found in methanol extract (54.51 ± 1.05 mg GAE/g dw for phenolics and 86.76 ± 0.58 mg RE/g dw for flavonoids), while total phenolic and flavonoid contents of hexane extracts were lowest, 9.71 ± 0.57 mg GAE/g dw and 10.14 ± 0.76 mg RE/g dw, respectively. The results revealed that the methanol extract of YX-28 contains significant more phenolics and flavonoids than those of some plants, commonly used as antioxidants. Proestos et al. (2006) have reported a total phenolic content in the range of 2.9–

Table 1
Total phenolic and flavonoid contents and DPPH scavenging activities of different extracts from *Xylaria* sp. YX-28 obtained by different solvents

	Total phenolics (mg GAE/g dw)	Total flavonoids (mg RE/g dw)	DPPH scavenging activity (%)
Hexane extract	9.71 ± 0.57^{e}	10.14 ± 0.76^{d}	13.75 ± 1.18^{d}
Chloroform extract	12.66 ± 0.92^{d}	20.24 ± 0.68^{b}	20.64 ± 0.75^{c}
Ethyl acetate extract	16.94 ± 0.89^{c}	15.21 ± 0.71^{c}	29.66 ± 0.97^{b}
Acetone extract	37.33 ± 1.50^{b}	19.60 ± 1.18^{b}	23.66 ± 0.59^{c}
Methanol extract	54.51 ± 1.05^{a}	86.76 ± 0.58^{a}	66.29 ± 0.67^{a}

Data expressed as means ± standard deviation. Means within each column with different letters (a–e) differ significantly ($P < 0.05$).

28.2 mg/g in Greek aromatic plants. Djeridane et al. (2006) determined total flavonoid content to be 1.62–13.12 mg/g in some Algerian medicinal plants.

With further correlation analysis, it was found that there was positive relationship between the phenolic contents of the different extract and DPPH radical-scavenging capacity, $R^2 = 0.7336$ ($P < 0.05$), whereas a higher correlation between the total flavonoids contents and DPPH radical-scavenging capacity was determined to be $R^2 = 0.9392$ ($P < 0.05$). There is a wide degree of variation between different phenolic compounds in their effectiveness as antioxidant (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). So the unclear relationship between the antioxidant activity and the total phenolics may be explained in various ways, for example, the antioxidant activity of the extract not only depends on the concentration, but also on the structure and the interaction between the phenolics. In this study performed with endophytic *Xylaria* sp., it is thought that the high free radical-scavenging activity and total antioxidant activity may result from the coexistence of phenolic and flavonoid-type compounds. This is in agreement with previous reports that the phenolic compounds and flavonoids contribute significantly to the antioxidant activity (Cakir et al., 2003; Proestos et al., 2006).

3.2. Antioxidant activity

3.2.1. Free radical-scavenging capacity of the methanol extract using DPPH analysis

DPPH method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Shon, Kim, & Sung, 2003). The DPPH radical-scavenging activities of the methanol extract from cultivated fruiting bodies of *Xylaria* sp. YX-28 and of references (BHT and Asc A) are presented in Table 2. It was observed that the methanol extracts from *Xylaria* sp. YX-28 notably reduced the concentration of DPPH free radical. It is clearly demonstrated that free radical scavenging increases with increasing extract concentration. Inhibition values in the concentrations of 25, 50, 100, 200, and 400 μg/ml were 18.4%, 28.0%, 41.8%, 62.3% and 82.4%, respectively. The performance of methanol extracts from *Xylaria* sp. YX-28 was higher than that of BHT and lower than that of Asc A, at the same concentrations. Asc A was observed to have the highest activity in DPPH assay, which is in agreement with previous study (Duan et al., 2006).

3.2.2. Antioxidant capacity in β-carotene–linoleic acid model system

In β-carotene–linoleic acid assay, the linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate radicals formed in the system (Shon et al., 2003). The antioxidant activities of the methanol extract from fruiting bodies of *Xylaria* sp. YX-28 and of two references (BHT and Asc A), as measured by the bleaching of β-carotene, were shown in Table 3. It can be seen that inhibition values of both the methanol extract and the standards increased with concentration. At a low concentration of 25 μg/ml, the methanol extract exhibited the lowest activity. At the concentration of 50, 100 and 200 μg/ml, the extract exhibited weaker activity than BHT and much stronger than Asc A, whereas at 400 μg/ml concentrations, the activity of the extract from *Xylaria* sp.YX-28 increased rapidly, showing the highest inhibition of 72.9%, while BHT 69.8% and Asc A 14.9%. Strobel et al. (2002) obtained an endophytic fungus *Pestalotiopsis microspora* from a combretaceaous plant *Terminalia morobensis*, and isolated one component

Table 2
Free radical-scavenging capacity of methanol extract from *Xylaria* sp. with different concentrations in comparison with those of the references, measured in DPPH assay

	Antioxidant activity ± SD (%)				
	25 (μg/ml)	50 (μg/ml)	100 (μg/ml)	200 (μg/ml)	400 (μg/ml)
Methanol extract	18.34 ± 0.06[b]	27.95 ± 0.07[b]	41.78 ± 0.06[b]	62.31 ± 0.12[b]	82.42 ± 0.04[b]
BHT	14.38 ± 0.18[c]	25.16 ± 0.14[c]	39.34 ± 0.14[c]	54.14 ± 0.12[c]	60.24 ± 0.05[c]
Asc A	24.68 ± 0.06[a]	46.09 ± 0.12[a]	59.10 ± 0.06[a]	73.1 ± 0.06[a]	90.16 ± 0.05[a]

Data expressed as means ± standard deviation. Means within each column with different letters (a–c) differ significantly ($P < 0.05$).

Table 3
Antioxidant activity of methanol extract from *Xylaria* sp. with different concentrations in comparison with those of the references, measured in β-carotene–linoleic acid assay

	Antioxidant activity ± SD (%)				
	25 (μg/ml)	50 (μg/ml)	100 (μg/ml)	200 (μg/ml)	400 (μg/ml)
Methanol extract	4.70 ± 0.08[c]	13.99 ± 0.25[b]	24.27 ± 0.09[b]	52.93 ± 0.12[b]	72.90 ± 0.09[a]
BHT	33.53 ± 0.05[a]	50.10 ± 0.04[a]	62.00 ± 0.05[a]	65.88 ± 0.11[a]	69.77 ± 0.08[b]
Asc A	11.03 ± 0.44[b]	10.73 ± 0.48[c]	12.05 ± 0.08[c]	13.28 ± 0.09[c]	14.85 ± 0.08[c]

Data expressed as means ± standard deviation. Means within each column with different letters (a–c) differ significantly ($P < 0.05$).

of the culture fluid, identified as isopestacin. The ability of isopestacin to scavenge $OH^{\bullet}$ is comparable to that of Asc A, and it has no effect in reducing the lipid free radical. Otherwise, Asc A is at least 14.2 times as effect as isopestacin for scavenging the superoxide radical.

The performance in β-Carotene-linoleic acid assay of methanol extracts from *Xylaria* sp. YX-28 was better than that of Asc A and lower than that of BHT, which was on the contrary with the result provided by the DPPH radical-scavenging assay. There was no significant association between the DPPH radical scavenging capacity and the antioxidant activity with β-carotene–linoleic acid assay. The antioxidant activity has been attributed to various mechanisms (Cakir et al., 2003). The difference is probably as a consequence of a higher specificity of the assay for lipophilic compounds.

3.3. GC/MS analysis of the methanol extract from Xylaria sp. YX-28

The presence of complex components in the extract makes it difficult to quantify each antioxidant component separately. Therefore, the chemical composition of methanol extract from cultured fruiting bodies of *Xylaria* sp. YX-28 was analysed by GC/MS, leading to comparison of the relative retention times (Rt) and mass spectra of the extract components with those of authentic samples and mass spectra from data library. As shown in Table 4, GC/MS analysis resulted in the identification of 41 compounds,

Table 4
Chemical composition of the methanol extract from *Xylaria* sp. YX-28

No.	Compounds[a]	Rt[b]	% Composition
1	1-Acetyl-1,2,3,4-tetrahydropyridine	11.084	0.82
2	Dodecane	11.562	0.39
3	*Z*,*Z*-7-11-Hexadecadien-1-ol	12.290	6.40
4	Phosphonic acid, methyl-, bis(trimethylsilyl) ester	14.504	0.39
5	Isosorbide	16.172	0.76
6	Tridecane	16.680	1.33
7	DL-Proline, 5-oxo-, methyl ester	21.116	4.67
8	Tetradecane	22.329	0.23
9	3,4-Dimethoxy-phenol	24.490	0.31
10	1-Hydroxymethyl-1,2,3,4-tetrahydro-naphthalen-2-ol	25.968	2.61
11	(1,4-Dimethylpent-2-enyl) benzene	27.689	1.73
12	Pentadecane	28.031	0.41
13	2,4-Bis(1,1-dimethylethyl)-phenol	28.235	0.30
14	3-Phenyl-4-methyl-isoxazol-5(4)-one	28.455	1.29
15	3,4-Dihydro-8-hydroxy-3-methyl-isocoumarin	29.464	0.70
16	[1-(3-Butenylthio)-2-nitroethyl]-benzene	33.194	0.67
17	d-Ribopyranose 1-O-benzoate	41.148	0.31
18	Benzoic acid, 4-methyl, 3-methylbutyl ester	49.466	0.70
19	Pentadecanoic acid, methyl ester	49.746	0.47
20	Dibutyl phthalate	52.643	0.20
21	14-Octadecenal	53.378	0.17
22	E-11,13-Dimethyl-12-tetradecen-1-ol acetate	53.492	0.52
23	Hexadecanoic acid, methyl ester	53.674	1.41
24	*n*-Hexadecanoic acid	54.948	1.03
25	2-Undecenal	55.349	0.37
26	Hexadecanoic acid, 14-methyl-, methyl ester	56.790	0.22
27	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester	58.526	6.44
28	9-Octadecenoic acid (*Z*)-, methyl ester	58.731	0.91
29	3,7,11-trimethyl-2,6,10-Dodecatrien-1-ol	59.042	0.40
30	9,12-Octadecadienoic acid (*Z*,*Z*)	59.618	2.98
31	9-Octadecenamide, (*Z*)	64.759	0.58
32	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	66.018	1.07
33	Ferruginol	66.427	0.35
34	*N*-[2-(Dimethylamino)ethyl]-2-Propenamide	67.375	3.12
35	9,12-Octadecadienoic acid (*Z*,*Z*)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	67.822	0.51
36	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	69.286	2.77
37	Bis(2-ethylhexyl) phthalate	69.938	0.68
38	9,12-Octadecadienoic acid (*Z*,*Z*)-, 2,3-dihydroxypropyl ester	76.656	11.90
39	5,6,8,9,10,11-Hexahydrobenz[A]anthracene	85.459	1.06
40	1,2,3,4-tetrahydro-Triphenylene	86.331	1.11
41	2-Hydrazino-8-hydroxy-4-phenylquinoline	88.529	2.35
	Total		64.64

[a] Compounds listed in order of retention time.
[b] Rt: Retention time (as min).

with the similarity more than 95% between the standard mass spectra in the library, representing 64.64% of the relative area in the methanol extract. The methanol extract of the endophyte is characterized by the presence of phenolics, including 2-hydrazino-8-hydroxy-4-phenylquinoline (2.349%), 3,4-dimethoxy-phenol (0.307%), 2,4-bis(1,1-dimethylethyl)-phenol (0.302%), 3,4-dihydro-8-hydroxy-3-methyl-isocoumarin (0.702%) and ferruginol (0.350%), commonly used as antioxidants previously. These may confirm and contribute to the strong antioxidant capacity of the methanol extract from *Xylaria* sp. YX-28. Kitagawa, Raddi, Khalil, Vilegas, and Fonseca (2003) isolated a natural isocoumarin from the capitula of *Paepalanthus bromelioides*, which inhibited luminal oxidation in both myeloperoxidase/H_2O_2 and myeloperoxidase/H_2O_2/Cl^- systems. Wu (2001) identified 5.8-dihydroxy-3-methyl-3,4-dihydero-icocoumarin from the fungal Chinese medicine "Wulingshen" (*Xylaria nigripes*), which has DPPH radical scanvenging activity as 1.67 times as that of ascorbic acid and 2.10 times as that of α-tocopherol. Wang, Wu, Shyur, Kuo, and Chang (2002) found that ferruginol exhibited the strongest antioxidant activity among the diterpenes isolated from the heartwood of *Taiwania cryptomerioides*. The compound 2-hydrazino-8-hydroxy-4-phenylquinoline has the quinoline nucleus and a hydroxy on benzene cycle, and Bickoff, Livingston, Guggolz, and Thompson (1954) proved that certain related quinoline derivatives had an effective antioxidant activity. Jeong et al. (2004) found that 3,4-dimethoxy phenol newly formed in the sesame meal with antioxidant activity, as the compound identified in our study. 2,4-bis(1,1-dimethylethyl)-phenol, also named as 2,4-Di-*tert*-butylphenol, has been previously isolated from the dried body of *Scolopendra subspinipes*, and exhibited antioxidant activities on copper-mediated, AAPH-mediated oxidation, and SIN-1-mediated oxidation in the TBARS assay (Yoon et al., 2006). Moreover, the major compounds are *Z*,*Z*-7-11-hexadecadien-1-ol (6.396%), DL-proline, 5-oxo-, methyl ester (4.665%), *N*-[2-(dimethylamino)ethyl]- 2-propenamide (3.117%), 9,12-Octadecadienoic acid (*Z*,*Z*)-,2,3-dihydroxypropyl ester (11.895%), forming the specific composition and the pleasant mushroom-like flavour of YX-28.

4. Conclusion

The data presented in this study have demonstrated that the extracts from cultivated fruiting bodies of endophytic fungus *Xylaria* sp. YX-28, especially the methanol extract, have rich phenolics and flavonoids. Both the total phenolic and flavonoid contents showed positive correlations with DPPH radical-scavenging activity. The methanol extract, with the highest phenolic and flavonoid contents, showed excellent activity by the DPPH assay and β-carotene–linoleic acid assay. The activity of the methanol extract was comparable to that of the positive controls, BHT and Asc A, and was significantly higher than that of BHT at the same concentration in DPPH assay. In β-carotene–linoleic acid assay, YX-28 exhibited much higher activity than Asc A, and was more effective than BHT at high concentration. The results of GC/MS proved the presence of the phenolics with antioxidant activity in the methanol extract of YX-28.

These results suggest that endophytic fungus *Xylaria* sp. YX-28 is a potential source of natural antioxidants. This is the first time to report that an endophytic *Xylaria* sp. from *G. biloba* has the antioxidant activity using DPPH radical and β-carotene–linoleic acid models. However, the toxicity of the endophyte extracts with high antioxidant activity should be tested, to confirm their safety for use as food additives. In addition, the characteristics of the phytochemicals and the antioxidant mechanisms of the extract should be further studied, to gain more understanding of their antioxidant activity in food systems. These experiments are in progress.

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