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Assessment of Phenolic Content, Free Radical-Scavenging Capacity and Antimicrobial Activities of 28 Marine Filamentous Fungi

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ABSTRACT

Phenolic contents, free radical scavenging capacity, linoliec acid peroxidation and antimicrobial activities were examined for a total of 28 fungus species isolated along the Red Sea coast of Sharm El Sheikh, Egypt, to determine their potential as a source of natural antioxidants. Total phenolic content were evaluated according to the Folin-Ciocalteu procedure while the free radical scavenging activity was measured by the 2, 2-diphenyl-1-picrylhydrazy (DPPH) method. Most of fungal extracts exhibited strong antioxidant properties. The linoliec acid peroxidation (*I* %) of fungal extracts tested ranged from 44 to 98%. *Aspergillus terrus* EG15 and *Aspergillus terrus* EG14 exhibited the strongest radical scavenging activity with DPPH inhibition of 98 and 93%, respectively. Total phenolic constituents of extracts tested ranged from 1.99 to 26.69 mg/g wet weight. Extracts were also found to possess antimicrobial activity against Gram +ve and Gram -ve bacteria. The results suggest that fungal extracts of some isolates exhibits a potential for use as natural antioxidant. Various factors such as genus, species, cultivation conditions, extraction methods, among others, might be responsible for the observed differences.

Keywords: *Aspergillus terreus*, *Aspergillus niger*, *Penicillium chrysogenum*, phenolic compounds, radical-scavenging activity
Abbreviations: CFU, colony-forming unit; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl; **NA**, nutrient agar; **PDA**, potato dextrose agar; **TBA**, thiobarbituric acid

INTRODUCTION

Oxidative stress imposed by reactive oxygen species (ROS) may be the direct or indirect cause of tissue damage and many human diseases such as aging, cancer, atherosclerosis and inflammation. It is known that ROS levels, in a healthy organism, are controlled by endogenous mechanisms including glutathione and enzymes like catalase or superoxide dismutase (Gerhäuser *et al*. 2003). The oxidative damage of DNA, proteins and other cellular determinants seem inevitable when the concentration of ROS exceeds tolerability of cells. The antioxidants could attenuate this oxidative damage of a tissue indirectly by increasing cells' natural defense (Aruoma 1996; Schinella *et al*. 2002) and/or directly by scavenging the free radical species (Liu and Ng 2000). Antioxidants can be classified as either primary or secondary according to their protective properties at different stages of the oxidation process since they act by different mechanisms. Primary antioxidants can inhibit or retard oxidation by scavenging free radicals by donation of hydrogen atoms or electrons, which converts them to more stable products. Secondary antioxidants function by many mechanisms, including binding of metal ions, scavenging oxygen, converting hydro peroxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen (Gordon 2001). Since consumers are concerned about the use of synthetic antioxidants, such as butylated hydroxylanisole, butylated hydroxyltoluene, and *tert*-butyl hydroquinone, gradually growing attention has been paid to the antioxidant potential of natural products, e.g. ginsenosides from ginseng (Kitts *et al*. 2000), echinacoside from *Echinaceae* root (Hu and Kitts 2000) and dimethylsulphonio propionate from marine algae (Sunda *et al*. 2002).

On the other hand, a large number of biologically active compounds extracted from fungi, such as polysacchriades and polysaccharide-protein complex fractions, are found to have antitumor, immunomodulating and antioxidant properties (Chen *et al*. 2006). Several new compounds have been isolated from fungal endophytes (Gunatilaka 2006; Pongcharoen *et al*. 2008). Several studies have shown that the hot water extract and polysaccharides fractions of *Cordyceps* fungi possess notable antioxidant activities, scavenging radicals and inhibiting lipid peroxidation, and preventing oxidative damage in animal tissue or cells (Leung *et al.* 2009). Chen *et al*. (2006) extracted polysaccharide (PS) from fungus G1 and its antioxidant activity on H22-bearing mice was investigated. The results showed that the H22 tumor growth was significantly inhibited by PS. Moreover, PS significantly enhanced Superoxide dismutase (SOD) activity of liver, brain and serum as well as GSH-Px activity of liver and brain in tumor-bearing mice. PS also significantly reduced the level of malondialdehyde (MDA) in liver and brain of tumor-bearing mice.

Isolation of a broth extract of the endophytic fungus *Corynespora cassiicola* L36 afforded three compounds, corynesidones A (1) and B (3), and corynether A (5). Corynesidone B (3) exhibited potent radical scavenging activity in the DPPH assay, whose activity was comparable to ascorbic acid. Based on the oxygen radical absorbance capacity assay, compounds 1, 3, 5, and 7 showed potent antioxidant activity (Chomcheon *et al*. 2009).

Furthermore, some fungal polysaccharides are potent antioxidants (Hou *et al*. 2002; Kim *et al*. 2002; Qin *et al*. 2002; Katapodis *et al*. 2003). Some antioxidants compounds were isolated from the fermentation medium of *Aspergillus niger* (Gunatilaka 2006; Pongcharoen *et al*. 2008; Rukachaisirikul *et al.* 2008). Some marine microorganisms have demonstrated to be excellent producers of biologically active

Received: 31 January, 2010. Accepted: 10 August, 2010.

Original Research Paper

and/or structurally novel metabolites (Fenical 1993). However, few reports dealing with the structure and function of secondary metabolites synthesized by marine fungi could be found. In this study, 28 marine filamentous fungus isolates inhabiting in the sediment at a 30-m depth under the Red Sea near Sharm El Sheikh, Sinai were isolated, identified and tested for their antioxidants, radical-scavenging, and antimicrobial activity.

MATERIALS AND METHODS

Isolation and identification of fungi

Samples of seawater and sediment (30-m depth) were collected in sterile containers along the Red Sea coast from Sharm El Sheikh, Sinai, Egypt. Fungi were isolated using a serial dilution method described by Liu *et al*. (2002). Samples were inoculated on potato dextrose agar (PDA) medium prepared using sea water, then incubated at 28 ± 2°C for seven days. Fungi isolates were primarily purified on PDA and culture purity was determined from colony morphology. The mycelium and conidiophores were observed after cultured on concavity slide with PDA medium at 28°C for 5 days and then colored by fungus staining solution (20.0 g phenol, 20.0 mL lactic acid, 40.0 mL glycerin, 20.0 mL distilled water, 0.05 g cotton blue). Isolates were routinely maintained at 4°C on PDA slants.

Culturing conditions and sample preparation

Three discs 1 cm diameter of 5 days old cultures grown on PDA medium were inoculated into 250-mL Erlenmeyer flasks containing 50 mL PDA broth medium and then incubated for 7 days at 28°C (stationary condition). At the end of incubation time the fungal cells and broth were separated by filtration through Whatman filter paper (No. 1).

Filtered fungal mats were mixed with 5 g of sea sand and 10 mL of methanol and milled in mortar according to a modified method of Kaminishi *et al*. (1999). The mixtures were left over night at 4°C and then filtered again (Whatman No. 1). The residues were washed with 10 mL of methanol then concentrated at 40°C using a Rotary Evaporator to dryness. The fungal powders were stored at -80°C until further tests. When needed, the fungal residues were redissolved in 10 mL methanol.

Linoleic acid assay

The sample preparation was carried out as described by Kikuzaki and Nakatani (1993). A mixture of 1 mL fungal extract, 4 mL 99.5% ethanol, 4.1 mL 2.5% linoleic acid in 99.5% ethanol, 8.0 mL 0.02 M phosphate buffer (pH 7.0) and 3.9 mL distilled water was placed in an oven at 40°C in the dark. Linoleic acid assay was conducted according to a pilot method described by Ottolenghi (1959) and Kikuzaki and Nakatani (1993). 2.0 mL of prepared sample was added to 1.0 mL 20%. trichloroacetic acid (TCA) and 2.0 ml of 0.2% thiobarbituric acid (TBA), then the mixture was placed in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 3000 rpm for 20 min. The resultant supernatant was incubated up to 7 days at room temperature. The same procedure was repeated with a blank. Along this period absorbance of the mixtures was measured at 532 nm every day and the absorbance value taken one day after the control reached maximum was considered. Antioxidant activity is described by percent inhibition as in Eq (1):

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (1)$$

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 sample.

DPPH free radical and scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts was measured from the bleaching of purple colored methanol solution of DPPH (Cuendet *et al.* 1997; Burits and Bucar 2000). Fifty µL of methanolic fungal extract were added to 5 mL of 0.004% methanolic solution of DPPH. After a 50 min of incubation at dark, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated as in Eq (2):

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (2)$$

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 sample.

Determination of total phenolics content

The total phenolic content in the fungal methanolic extracts was determined by using Folin–Ciocalteu reagent as described by Gulluce *et al*. (2005). One mL extract was added to 10 mL deionized water and 2.0 mL Folin–Ciocalteu reagent. The mixture was then allowed to stand at room temperature for 5 min and 1.5 mL sodium carbonate (2%, w/v) was added. Reaction mixture was further left at room temperature for 2 h with intermittent shaking, then the absorbance was measured at 765 nm using a spectrophotometer (UV-Vis model SP – 2000 UV Spectrum). The concentration of total phenolic compounds in all fungal extracts was expressed as milligram of gallic acid equivalents per gram wet weights of fungal cells, using the linear Eq. (4) derived from Eq. (3), which was determined from known concentration of gallic acid standard similarity. Data were reported as a mean ± standard deviation for three replicates.

Absorbance (at 765 nm) constant × (gallic acid concentration) (3)

Gallic acid equivalents = Absorbance × (at 765 nm)/0.0508 (4)

Antimicrobial activity

Fungal extracts were individually tested against a panel of gram positive and negative bacterial pathogens (**Table 2**). Antimicrobial tests were then carried out by the agar well diffusion method (Perez *et al*. 1990) using 100 µL of suspension containing 10^8 CFU/mL of pathological tested bacteria spread on nutrient agar. After the media had cooled and solidified, wells (10 mm in diameter) were made in the agar and 100 µL of fungal culture filtrate were loaded. The inculcated plates were then incubated for 24 h at 37°C. Negative controls were prepared using the same solvents employed to dissolve the fungal extracts. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. The distinct zone of inhibition surrounding the disc was measured. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) as follows: N.A. (no activity) ≤ 4 mm; + (weak) = 5-9 mm; ++ (moderate) = 10-15 mm; +++ (strong) = 16-20 mm and ++++ (very strong) ≥ 21 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Statistical analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD). One-way analysis of variance (ANOVA) was used to comparison the data. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant. Correlations were obtained by Pearson's correlation coefficient (r) using Statistical software SPSS (version 16.0).

RESULTS AND DISCUSSION

Linoleic acid assay

In the linoleic acid assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydro peroxides arising from linoleic acid oxidation. Only a few reports concerning the antioxidative nature of fungi are available. Methanolic extracts of all fungal isolates tested exhibit

Table 1 Antioxidant activities of the marine fungal isolates.[a]

Fungal isolates	% Inhibition of linoleic acid peroxidation	% Inhibition of DPPH	Total phenolic content (mg/g wet wt)
Aspergillus niger EG1	71 ± 3.5	67.24 ± 2.6	3.89 ± 0.4
A. niger EG2	57 ± 1.4	44.83 ± 1.5	4.66 ±. 02
A. niger EG3	66 ± 2.1	56.16 ± 0.8	14.64 ± 0.2
A. niger EG4	53 ± 3.5	42.24 ± 1.2	12.02 ± 0.6
A. niger EG5	79 ± 2.8	70.45 ± 1.7	16.87 ± 0.7
A. niger EG6	56 ± 2.8	40.55 ± 1.0	11.95 ± 0.0
A. niger EG7	85 ± 2.8	73.31 ± 1.3	26.69 ± 1.6
A. niger EG8	66 ± 2.1	54.78 ± 0.1	15.20 ± 0.5
Aspergillus terreus EG9	49 ±1.4	39.09 ± 1.3	2.22 ± 0.1
A. terreus EG10	44 ± 2.1	36.34 ± 1.1	1.97 ±.0.0
A. terreus EG11	64 ± 1.4	53.49 ± 1.0	11.83 ± 0.8
A. terreus EG12	78 ± 3.5	68.05 ± 1.3	14.99 ± 0.7
A. terreus EG13	64 ± 1.4	59.23 ± 2.6	15.06 ± 0.6
A. terreus EG14	97 ± 3.5	93.05 ± 1.3	20.97 ± 0.7
A. terreus EG15	98 ± 1.7	98.56 ± 0.3	26.05 ± 1.3
A. terreus EG16	77 ± 2.1	63.12 ± 1.3	14.29 ± 1.2
A. terreus EG17	52 ± 2.1	48.47 ± 1.0	15.05 ± 0.6
Aspergillus flavus EG18	50 ± 2.8	39.49 ± 0.3	2.63 ± 0.2
Aspergillus versicolor EG19	44 ± 2.1	37.88 ± 1.4	11.04 ± 0.7
Aspergillus wentii EG20	53 ± 1.4	42.65 ± 0.9	12.64 ± 0.9
Emericella nodulans EG21	50 ± 3.5	44.19 ± 0.5	15.14 ± 0.6
E. nodulans EG22	70 ± 4.2	55.03 ± 0.7	13.32 ± 0.4
E. nodulans EG23	52 ± 4.9	44.02 ± 2.1	13.57 ± 1.0
Penicillium chrysogenum EG24	75 ±1.4	59.43 ± 0.3	8.21 ± 0.5
P. chrysogenum EG25	54 ± 2.1	49.44 ± 1.8	15.45 ± 0.3
P. chrysogenum EG26	55 ± 2.8	47.02 ± 2.1	5.17 ± 0.5
Penicillium corylophilum EG27	59 ± 1.4	46.21 ± 0.5	4.30 ± 0.2
P. corylophilum EG28	77 ± 2.1	64.33 ± 0.4	12.60 ± 0.2
F value	155	362	248
P value[b]	≤0.001***	≤0.001***	≤0.001***

[a] $n = 3$, mean values are shown, SD was always < 20% .
[b]: *P ≤ 0.05,** P ≤ 0.01, ***P ≤ 0.001

notable free radical scavenging activity and were effective in the inhibition of linoleic acid oxidation (**Table 1**). The linoliec acid peroxidation (*I*%) of fungal extracts tested ranged from 44 to 98%. The most effective fungal isolate was *Aspergillus terreus* EG15 (98%) followed by *A. terreus* EG14 (98%), *Aspergillus niger* EG7 (85%) and *A. niger* EG5 (79%). These results were in agreement with the findings of Kaminishi *et al*. (1999) who investigated the ability of five xerophilous fungi for producing effective antioxidant and they found that *Erotium herbariorum* NE-1 had the stronger activity as indicator for peroxide value, while *A. ruber* and *A. repens* gave excellent results due to high quantity. The considerable linoliec acid peroxidation activities of most of the tested fungal isolates indicate that they contain antioxidant constituents such as anthraqunones and coumarins as well as arbutin or phenolic compound (Permana *et al*. 1999; Ahmed *et al* 2005).

DPPH free radical scavenging activity

Since the main mechanism of antioxidant action in foods is radical scavenging, many methods have been developed in which the antioxidant activity is evaluated by the scavenging of synthetic radicals in polar organic solvents such as methanol at room temperature. In this study, the DPPH method was selected to evaluate the antioxidant activity of fungal isolates because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials active by a chain-breaking mechanism (Niki 1987). The DPPH radical is a stable free radical and the DPPH radical-scavenging activity was determined by the decrease in absorbance at 517 nm, due to reduction by the antioxidant (AH) or reaction with a radical species, as shown in Eqs. (5) and (6) (Gordon 2001):

$$DPPH^{\cdot} + AH \rightarrow DPPH - H + A^{\cdot} \quad (5)$$

$$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH - R \quad (6)$$

We found that most fungal extracts showed considerable free radical scavenging activities (**Table 1**). *Aspergillus terreus* EG15 was the strongest radical scavenger among fungal isolates with 98.6% DPPH inhibition, followed by *A. terreus* EG14 (93.1%), *Aspergillus niger* EG7 (73.31%), *A. niger* EG5 (70.45%), *A. terreus* EG12 (68%), and finally *A. niger* EG1 (67.24%).

However, *Penicillium chrysogenum* EG28, *A. terreus* EG16, *P. chrysogenum* EG24, and *A. niger* EG3 were moderate radical scavengers with DPPH inhibition of 64.33, 63.12, 59.43, and 56.16%. Earlier studies of Gunatilaka (2006), Pongcharoen *et al*. (2008), Rukachaisirikul *et al.* (2008) and Chomcheon *et al.* (2009) showed that *A. niger* produced antioxidant compounds in their fermentation medium.

Total phenolic content

Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro-peroxides into free radicals. The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic content but it is known that different phenolic compounds have different responses in the Folin-Ciocalteu method (Kähkonen *et al*. 1999). Therefore, in this work, we calculated the total phenolic contents in marine fungal isolates in units of mg gallic acid equivalent of phenolic compounds as shown in **Table 1**. The total phenolic content differed among the different species of fungal isolates and ranged from 1.97 to 26.69 mg gallic acid/g wet weight. The total [henolic content of *A. terreus* EG15, *A. niger* EG7 and *A. terreus* EG15 were very high (20.97-26.69 mg gallic acid/g) compared to that obtained from *A. terreus* EG10, *A. flavus* EG18 and *A. niger* EG1 (1.97-3.89 mg gallic acid/g).

We could observe that fungal extracts with higher phenolic content had higher antioxidative capacities. As reported elsewhere, this activity increases with the presence of polyphenols in particular (Behera *et al*. 2005; Culluce *et al*.

Table 2 Antimicrobial activity of marine fungal isolates extract against the pathological strains based on well diffusion assay.[a]

Fungal isolates	Gram-positive bacteria			Gram-negative bacteria			
	B. megaterium ATCC9885	*B. subtilis ATCC6633*	*S. aureus* ATCC29213	*K. peneumoniae* ATCC13883	*S. typhii* ATCC700931	*P. aeruginosa* ATCC27953	*E. coli*
Aspergillus niger EG1	N.A.	N.A.	N.A.	+	N.A.	N.A.	N.A.
A. niger EG2	+++	N.A.	N.A.	N.A.	N.A.	N.A.	++
A. niger EG3	+++	N.A.	N.A.	++	N.A.	N.A.	++
A. niger EG4	N.A.	+++	++++	++++	+++	+++	++++
A. niger EG5	++	++	N.A.	+++	N.A.	++	++
A. niger EG6	N.A.	+++	N.A.	N.A.	++	N.A.	N.A.
A. niger EG7	+++	+++	+++	+++	+++	+++	++++
A. niger EG8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Aspergillus terreus EG9	++++	+++	+++	+++	+++	+++	+++
A. terreus EG10	+++	++	N.A.	++	N.A.	++	++
A. terreus EG11	++++	++	+++	++++	+++	+++	+++
A. terreus EG12	+++	++	++	++	++	++	++
A. terreus EG13	++++	+++	++++	++++	++++	++++	+++
A. terreus EG14	++	+++	+++	+++	+++	N.A.	+++
A. terreus EG15	++	+++	++	++	++	+++	+++
A. terreus EG16	++	+++	++	+++	++++	+++	+++
A. terreus EG17	++	N.A.	++++	++	+++	+++	+++
Aspergillus flavus EG18	+++	+++	N.A.	++	N.A.	++	++
Aspergillus versicolor EG19	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Aspergillus wentii EG20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Emericella nodulans EG21	+++	+++	+++	++++	+++	++++	+++
E. nodulans EG22	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
E. nodulans EG23	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Penicillium chrysogenum EG24	++	+++	++	++	N.A.	++	++
P. chrysogenum EG25	+++	+++	++	++	N.A.	N.A.	++
P. chrysogenum EG26	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Penicillium corylophilum EG27	N.A.	++	N.A.	++	N.A.	N.A.	N.A.
P. corylophilum EG28	+++	+++	N.A.	+++	N.A.	+++	++

[a]Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) as follows: N.A. (no activity) ≤ 4 mm; + (weak) = 5-9 mm; ++ (moderate) = 10-15 mm; +++ (strong) = 16-20 mm and ++++ (very strong) ≥ 21 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated

2005). The antioxidant activity of phenolics was mainly due to their redox properties, hydrogen donators and single oxygen quenchers (Rice-Evans *et al*. 1995). Jayaprakasha and Rao (2000) emphasized the correlation between antioxidative capacity and phenolic constituents, particularly methyl orsenillate, orsenillic acid, atranorin and lecanoric acid. In our study, regression analysis between antioxidant power of fungal extracts tested and total phenolic content showed a linear correlation ($P < 0.01$). The high correlation coefficient ($r = 0.993$) indicates that phenolic compounds are the main components responsible for the antiradical activity of fungal extracts. This statistically significant correlation was in agreement with the findings of Turkmen *et al*. (2006), Baltrusaityte *et al*. (2007), Buratti *et al*. (2007), and Silici *et al*. (2010) who also confirmed a strong relationship between antiradical capacity and total phenolic content.

Antibacterial activity

The antimicrobial activities of marine fungal isolate extracts against target pathogens were examined qualitatively and quantitatively by the presence or absence of inhibition zones and zone diameter. Results given in **Table 2** showed that most fungal isolates have antibacterial activity against the tested pathogenic bacteria. Extracts with the strongest antibacterial activity were obtained from *A. terreus* EG13, *Emercilla nodulans* EG 21, *A. terreus* EG11, *A. niger* EG7, *A. niger* EG9 and *A. terreus* EG9. In addition, *Penicillium chrysogenum* EG24, *P. chrysogenum* EG25 and *P. corylophilum* EG28 also showed notable antimicrobial activity. *A. niger* EG1, *A. niger* EG2, *A. niger* EG6, *P. corylophilum* EG27 exhibited weak antibacterial activities. Finally, the extracts of *A. niger* EG1, *A. niger* EG8, *E. nodulans* EG22, *E. nodulans* EG23, and *P. chrysogenum* EG26 did not exhibit any antibacterial activity. Cazar *et al.* (2005) stated that *A. terreus* produces butyrolactone derivatives which have a strong antimicrobial activity, while Marx *et al*. (2008) showed that *P. chrysogenum* produces an antifungal protein. Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nuclide acids (Oyaizu *et al*. 2003). Taking into consideration the properties of the organic solvent used for the extraction, the extract seams to contain diverse substances, ranging from non-polar to polar compounds.

CONCLUDING REMARKS

According to the evaluation of phenolic constitunnts and free radical-scanvenging capacity from ethanolic extracts of 28 fungal isolates, *Aspergillus terreus* EG15 and *Aspergillus niger* EG14 were found to have a higher radical scavenging and *in vitro* antioxidant activity than other isolates. Various factors such as genus, species, cultivation conditions, extraction methods, among others, might be responsible for the observed differences. Our work has also shown that *A. terreus* EG13, *Emercilla nodulans* EG21, *A. terreus* EG11, *A. niger* EG7 and *A. niger* EG9 exhibited strong antimicrobial activities against the tested pathogenic bacteria. The present study suggests that methanolic extracts of these fungal isolates are a potential source of natural antioxidants. However, the toxicity of the fungal 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 extracts should be further studied, to gain more understanding of their antioxidant activity.

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