ANTIOXIDANT ACTIVITIES OF MYCELIA AND BROTH FILTRATE EXTRACTS OF *ASPERGILLUS SPP*

M.A. EBRAHIMZADEH†, G. RAHBAR†,
M. A. TAJICK GHANBARY‡ and M. SOLTANY REZAEE RAD†

† *Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*
‡ *Department of Mycology & Plant Pathology, Faculty of Agronomic Sciences, Sari Agricultural and Natural Resources University, Sari, Iran.*
zadeh20@yahoo.com, gozalrahbar@gmail.com, m.tajick@sanru.ac.ir, mohammad.soltany@gmail.com

***Abstract* - The antioxidant activities of mycelia and broth filtrate ethyl acetate extracts produced from the cultures of 12 *Aspergillus spp.* were investigated. Extracts showed a concentration-dependent antioxidant activity by inhibiting DPPH radicals. Broth filtrate extracts showed higher activity than mycelia filtrate extracts. *A. fumigatus* showed the best activity (with 92.5% inhibition at the concentration of 0.8 mg ml^{-1}). The extracts exhibited potent nitric oxide-scavenging activity. Broth filtrate extract of *A. fumigatus* showed the best activity (with 79.6 % inhibition at 1.6 mg ml^{-1}) followed by the *A. ostianus* and *A. terreus*. Reducing powers of extracts also increased with the increases in their concentrations. Two broth filtrate extracts, *A. terreus* and *A. fumigatus,* have shown potent reducing power. In chelating ability, mycelia filtrate extracts showed higher activity. *A. flavus* showed the best activity (with 49.1% inhibition at 1.6 mg ml^{-1}). The present study demonstrates the potential of soil fungi to have antioxidant activity similar to plants and represent them as new sources of natural antioxidants.**

***Keywords*— Antioxidant activity, *Aspergillus*, Mycelia.**

I. INTRODUCTION

The pathology of numerous chronic diseases, including heart diseases, cancer, inflammatory injuries, and neurodegenerative diseases, involves oxidative injury to cellular components. Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart failure, and many other health problems related to advancing age. Minimizing oxidative injury can well be one of the most important approaches to the primary protection of the body from these aging-associated diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions, and appear to be of primary importance in the prevention of these diseases and health problems (Nabavi *et al.*, 2013a). Antioxidants have been detected in a large number of natural products, including cereal grains, vegetables, fruits, and plant extracts (Tepe and Sokmen, 2007). Antioxidants are usually applied in the food industry to prevent or delay the lipid oxidation that initiates changes in food components. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are usually used as food additives. However, their application has been limited because of possible toxic and carcinogenic components formed during their degradation. In view of these health concerns, finding safer, more effective and economic natural antioxidants is highly desirable (Mathew and Abraham, 2006).

It has been reported that microbial metabolites exhibit antioxidant activity and, therefore, can be used as antioxidants (Ishikawa, 1992). Specifically, fungi are considered to be an abundant source of antioxidant components; for example, *Mortierella* fungi (Hirota *et al*., 1997) and *Aspergillus repens* (Yagi and Doi, 1999). Specific components can be prepared and extracted from both mycelia and broth filtrate (Marchelli and Vining, 1975). Antioxidant components have been extracted from mycelia of *Streptomyces* sp. (Kato *et al.,* 1989). Mycelia pellets have been used in the commercial production of a number of compounds, including citric acid, antibiotics, and enzymes (Braun and Vecht-Lifshitz, 1991). Ethyl acetate extracts from *Aspergillus candidus* CCRC 31543 broth filtrate have been found to be strong antioxidants (Yen and Lee, 1996).

This study was designed to prepare mycelia and broth filtrate ethyl acetate extracts from *Aspergillus spp.* submerged culture, to evaluate their antioxidant properties. Numerous techniques are available to evaluate the antioxidant activities of a compound, and just one procedure cannot identify all possible mechanisms characterizing an antioxidant. The comprehensive evaluation using different tests is important in assessing the antioxidant activity. Therefore, different complementary test systems such as 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) assay, reducing power, ferrous ion chelating activity and nitric oxide scavenging activity were used to assess the antioxidant potential of 12 *Aspergillus spp*.

II. METHODS

A. Chemicals

Ferrozine, trichloroacetic acid (TCA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Sodium nitrite, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, ferric chloride and N-(1-naphthyl) ethylenediamine dihydrochloride, ethylenediaminetetraacetic acid (EDTA) were purchased from

Merck (Germany). All other chemicals were of analytical grade or purer.

B. Microorganism

The strains used in this study (Table 1) were isolated from agricultural soils of different regions of Sari, Iran. The strains were maintained on potato dextrose agar (PDA) slants as stock cultures at 4ºC and subcultured monthly. All isolated fungi were exposed to the morphological and microscopic examinations. All species of *Aspergillus* were identified according to Klich (2002).

C. Medium and Preparation of mycelia and culture broth extracts

The medium used for the production of antioxidants was Czapek dox's broth and consisted of sucrose 3%, sodium nitrate 0.2%, K_2HPO_4 0.1%, magnesium sulphate 0.05%, potassium chloride 0.05%, ferrous sulphate 0.001%). 100 ml of medium in Erlenmeyer flasks were incubated with spores (10^6 spores ml^{-1}) of 48 h old culture of the fungi. The cultures were incubated at 25ºC for 14 days with shaking at 100 rpm. The culture broth of each fungus was filtered through Whatman filter paper no.1 and the obtained filtrate was used for different assay procedures. Mycelia and culture broth of *Aspergillus spp.* were extracted with ethyl acetate (solvent: mycelia = 100:1, v/w) for 30 min. The solvent layer was separated from the mycelia or culture broth by passing it through a Whatman No. 1 filter paper, after which it was dried over anhydrous sodium sulfate (1%, w/v), filtered, evaporated to dryness in vacuo, and weighed to determine the yields of soluble solids.

D. DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of the extracts free radical scavenging activity (Dehpour *et al*., 2009). One ml of every concentration of each extract (0.8 and 1.6 mg ml^{-1}) in methanol was added to one ml of methanolic solution of DPPH (100 µM). After 15 min at room temperature, in the dark, the absorbance was recorded at 517 nm. The experiment was repeated three times. The percentage of inhibition was calculated as follows: % inhibition = $[(A_o - A_1)/A_o] \times 100$, where A_o was the absorbance of the control and A_1 the absorbance in the presence of extract or standard. BHA was used as the standard control. IC_{50} values denote the sample concentration required to scavenge 50% of DPPH free radicals.

E. Reducing power determination

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. The reducing power of the extract was determined according to the method described by Yen and Chen (2005). Different concentrations of each extract (0.8 and 1.6 mg ml^{-1}) (2.5 ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium hexacyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min. After incubation, 2.5 ml of TCA (10%) was added to terminate the reaction. The mixture was then centrifuged at 3000 rpm for 10 min. The upper portion of the solution (1 ml) was mixed with 1 ml distilled water and then 0.2 ml of $FeCl_3$ solution (0.1% in water) was added. The absorbance was measured at 700 nm against an appropriate blank. Increased absorbance of the reaction mixture indicated increased reducing power.

F. Iron chelating activity

The chelating of ferrous ions by extracts was estimated according to Ebrahimzadeh *et al.* (2011). 2.8 ml of distilled water was added to 1 ml of different concentrations of extracts (0.8 and 1.6 mg ml^{-1}), which were then mixed with 50 µl of 2 mM $FeCl_2$ and 150 µl of ferrozine (5 mM). The mixture was then shaken vigorously and left to stand at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. The % inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control and A_1 the absorbance of the mixture containing the extract or standard. EDTA was used as a standard.

G. Assay of nitric oxide-scavenging activity

The procedure was performed based on the method by Sreejayan and Rao (1997). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, mixed with different concentrations of each extract (0.8 and 1.6 mg ml^{-1}) were dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without the extracts but with an equivalent amount of water, served as the control. After the incubation period, 0.5 ml of the Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in H_3PO_4 2%) were added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as the positive control.

H. Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The IC_{50} values were calculated using the linear regression analysis.

III. RESULTS AND DISCUSSION

A number of plants and mushrooms (fruiting body) are commonly known to produce antioxidants, but there are few reports on lower fungi. These include *Penicillium roquefortii*, *Aspergillus candidus*, *Mortierella*, *Emericella falconensis*, *Acremonium, Colletotrichum gloeosporioides* (Rios *et al*., 2006), Mycelia sterilia (Moon *et al.*, 2006), *Antrodia camphorata* (Song and Yen, 2002), *Chaetomium* sp., *Cladosporium* sp., *Torula* sp., *Phoma* sp. etc (Rios *et al.*, 2006). A lot more fungi still need to be explored as the production, downstream processing of actual bioactive. Keeping the above in mind, the present study was planned to screen and expand the spectrum of fungi having antioxidant potential. In the present study, 12 soil fungal isolates demonstrated their an-

tioxidant potential to variable levels. In consonance with earlier studies (Yen and Chang, 1999); sucrose proved to be the most promising carbon source to produce bioactive compounds. This explains that a fungal sp may have the ability to utilize a particular carbon source for vegetative growth but may not be able to use it for production of specialized structural molecules. Similarly, sodium nitrate was the best nitrogen source among all organic as well as inorganic nitrogen sources, thus indicating that the availability of easily utilizable carbon and nitrogen sources promote primary metabolism, and feeding with more slowly metabolizable compounds may lead to the formation of secondary products. Nitrogen sources other than sodium nitrate showed a lower activity. This also proves that Czapek dox's medium is the most effective one for metabolite production responsible for antioxidant activity (Arora and Chandra, 2010). In fact, culture media have a major impact on the growth of microbes and the production of microbial products.

A. DPPH radical-scavenging activity

The hydrogen atoms, or the electron donation ability of the extracts, were measured from the bleaching of a purple-colored methanol solution of DPPH, a nitrogen-centered radical converted to 1,1-diphenyl-2-picrylhydrazine. As a very stable organic free radical with a deep violet color, DPPH gives maximum absorption at the range from 515 to 528 nm. Generally, antioxidants will react with DPPH, due to its hydrogen-donating ability at a very rapid rate. The amount of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm. Substances which are able to perform this reaction can be considered as antioxidants and radical scavengers. This method has been used widely to evaluate the radical scavenging ability of antioxidants from different plants due to its advantage of short time and sensibility (Dehpour *et al.*, 2009). The capacity of the extract to scavenge DPPH was measured and the results are shown in Table 1. Substances which are able to perform this reaction can be considered as antioxidants and radical scavengers. Extracts showed a concentration-dependent antioxidant activity by inhibiting DPPH radicals. Most (10 out of 12 *Aspergillus* spp.) broth filtrate extracts (BFEs) showed higher activity than mycelia filtrate extracts (MFEs). In BFEs, *A. fumigatus* showed the best activity (with 93.5 and 92.5% inhibition in 1.6 and 0.8 mg ml^{-1}, respectively) followed by the *A. ustus* and *A. sclerumtiorum*. In MFEs, *A. terreus* showed the highest activity (with 75.1 and 50.5% inhibition in 1.6 and 0.8 mg ml^{-1}, respectively).

B. Metal chelating activity

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry. Therefore, minimizing Fe^{2+} concentration affords protection against oxidative damage. Chelation therapy reduces iron-related complications in humans and thereby improves quality of life and overall survival in some diseases such as Thalassemia, malaria and leukemia (Nabavi *et al.*, 2013a). Because of several adverse effects of clinically useful iron chelators, such as arthritis, nausea and agranulocytosis, cessation of therapy is required in up to 30% of patients. Therefore, natural products have received much attention and many researches focused on them (Nabavi *et al.*, 2013a). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted, resulting in a decrease in the redness of the complexes. In this assay, the absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, i.e. the activity increased with increasing concentrations. In chelating ability, many (7 out of 12 *Aspergillus* spp.) MFEs showed higher activity than BFEs. In MFEs, *A. flavus* showed the best activity (with 49.1 and 38.7% inhibition in 1.6 and 0.8 mg ml^{-1}, respectively) followed by the *A. parasiticus* and *A. awamori*. In BFEs, *A. foetidus* showed the best activity (with 29.1 and 11.8% inhibition in 1.6 and 0.8 mg ml^{-1}, respectively).

C. Nitric oxide-scavenging activity

Nitric oxide exhibits a numerous range of beneficial functions in organisms, including regulation of vascular tone, neurotransmission, killing microorganisms and tumor cells, and other homeostatic mechanisms. High levels of nitric oxide have been described in a variety of pathophysiological processes such as various forms of circulatory shock, inflammation, carcinogenesis, muscle diseases, primary headaches, and neurodegenerative disorders such as Alzheimer's disease (Nabavi *et al.*, 2013b). The scavenging of NO is based on the principle that, sodium nitroprusside in aqueous solutions at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using the Griess reagent. Scavengers of NO compete with oxygen, leading to the reduced production of nitrite ions (Nabavi *et al.*, 2013b). In our study, the extracts exhibited potent nitric oxide-scavenging activity. Most (11 out of 12 *Aspergillus spp*.) broth filtrate extracts showed higher activity than mycelia filtrate extracts. In BFEs, *A. fumigatus* showed the best activity (with 79.6 and 54.3% inhibition in 1.6 and 0.8 mg ml^{-1}, respectively) followed by the *A. ostianus* and *A. terreus*. In MFEs, only *A. parasiticus* showed a little more activity than its broth filtrate extract (29.1 vs. 28.6% inhibition in 1.6 mg ml^{-1}, respectively). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation and other pathological conditions (Moncada *et al.*, 1991). Antinociceptive and CNS activities in some plants such as the *Hypericum* genus have been explained by scavenging of NO (Eslami *et al.*, 2011). There is some evidence that strongly suggests the involvement of NO signalling pathways in CNS disorders (Wegener and Volke, 2010).

D. Reducing power

Reducing power has been used as an antioxidant capability indicator of medicinal Herbs. Fe^{3+} reduction is an

important mechanism of antioxidants and often used as an indicator of electron donating activity. In this assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Ebrahimzadeh *et al.*, 2011). Increasing absorbance at 700 nm indicates an increase in the reductive ability. The greater the intensity of the color, the higher is the antioxidant activity of the sample. Table 1 shows the dose- response activity for the reducing powers of *Aspergillus* spp. It was found that the reducing powers of extracts also increased with the increases in their concentrations. Two extracts had shown the potent reducing power (Absorbance > 1.5 at the concentration of 1.6 mg ml^{-1}). These extracts belong to broth filtrate extracts. *A. terreus* showed the best activity (with the absorbance of 2.2 and 1.7 at 1.6 and 0.8 mg ml^{-1}, respectively) followed by the *A. fumigatus* with the absorbance of 1.5 at the concentration of 1.6 mg ml^{-1}.

Table 1. Antioxidant activities of 12 *Aspergillus spp.*

Fungal species	Extract	Concentration (mg/ml)	DPPH. scavenging activity (% of inhibition)[a]	Iron chelatory activity (% of inhibition)[b]	NO radical scavenging activity (% of inhibition)[c]	Reducing power (Absorbance)
A. ostianus	Mycelial	1.6	21.70 ± 1.12***	33.51 ± 2.30***	14.17 ± 0.32***	0.68 ± 0.03***
		0.8	16.12 ± 0.94***	1.09 ± 0.14***	1.04 ±0.11	0.35 ± 0.01***
	Broth	1.6	48.28 ± 3.07***	8.76 ± 0.43***	68.66 ±3.32***	0.94 ± 0.05***
		0.8	30.98 ± 2.16***	4.67 ± 0.21***	53.65 ±2.16***	0.51 ± 0.04***
A. fumigatus	Mycelial	1.6	0.43 ± 0.00 [ns]	1.12 ± 0.08***	31.98 ±1.67***	0.29 ± 0.03***
		0.8	0.01 ± 0.00	0.02 ± 0.00 [ns]	18.29 ± 1.06***	0.12 ± 0.01***
	Broth	1.6	93.53 ± 3.49***	7.10 ± 0.63***	79.63 ± 3.46***	1.51 ± 0.07***
		0.8	92.58 ± 4.26***	5.69 ± 0.32***	54.30 ± 2.13***	0.73 ± 0.06***
A. terreus	Mycelial	1.6	75.14 ± 3.98***	23.80 ± 1.69***	35.11 ± 1.89***	0.46 ± 0.04***
		0.8	50.57 ± 3.49***	22.58 ± 1.54***	30.54 ± 1.40***	0.22 ± 0.03***
	Broth	1.6	45.27 ± 2.56***	17.72 ± 1.63***	64.74 ± 3.79***	2.22 ± 0.15***
		0.8	39.17 ± 2.17***	7.60 ± 0.04***	55.77 ± 2.89***	1.70 ± 0.09***
A. awamori	Mycelial	1.6	29.66 ± 1.13***	41.84 ± 2.10***	37.72 ± 1.14***	0.13 ± 0.02***
		0.8	5.21 ± 0.41***	37.36 ± 2.65***	29.37 ± 1.85***	0.03 ± 0.00*
	Broth	1.6	27.50 ± 1.92***	24.68 ± 1.94***	57.29 ± 2.62***	0.07 ± 0.00***
		0.8	25.71 ± 1.67***	17.08 ± 0.98***	44.68 ± 1.93***	0.05 ± 0.00***
A. parasiticus	Mycelial	1.6	14.30 ± 1.04***	42.99 ± 1.64***	29.11 ± 1.76***	0.71 ± 0.05***
		0.8	8.23 ± 0.05***	42.24 ± 2.05***	23.10 ± 2.04***	0.31 ± 0.03***
	Broth	1.6	25.76 ± 1.12***	25.08 ± 1.59***	28.59 ± 2.03***	0.54 ± 0.02***
		0.8	23.78 ± 2.13***	19.00 ± 0.93***	9.66 ± 0.61***	0.44 ± 0.02***
A. sclerumtiorum	Mycelial	1.6	39.50 ± 2.30***	37.30 ± 2.54***	36.42 ± 2.86***	0.36 ± 0.02***
		0.8	25.71 ± 1.16***	26.61 ± 2.16***	21.93 ± 1.49***	0.14 ± 0.01***
	Broth	1.6	70.57 ± 3.14***	18.34 ± 0.96***	43.82 ± 3.28***	0.53 ± 0.03***
		0.8	64.93 ±2.38***	17.23 ±0.86***	37.72 ± 2.71***	0.30 ± 0.02***
A. niger	Mycelial	1.6	4.62 ± 0.51***	23.22 ± 1.05***	21.01 ± 1.46***	0.14 ± 0.01***
		0.8	1.58 ± 0.07 [ns]	22.90 ± 1.18***	12.53 ± 0.89***	0.07 ± 0.00***
	Broth	1.6	21.80 ± 0.44***	25.19 ± 1.26***	40.73 ±2.05***	0.15 ± 0.00***
		0.8	18.29 ± 0.93***	24.53 ±1.49***	35.63 ± 2.44***	0.13 ± 0.01***
A. flavus	Mycelial	1.6	2.74 ±0.08*	49.10 ± 2.93***	20.10 ± 1.37***	0.63 ± 0.04***
		0.8	1.73 ± 0.04 [ns]	38.72 ± 2.01***	17.36 ± 1.29***	0.38 ± 0.01***
	Broth	1.6	70.43 ± 3.45***	11.38 ± 0.90***	60.31 ± 3.41***	0.03 ± 0.00*
		0.8	55.65 ± 3.82***	9.59 ± 0.30***	53.39 ± 2.29***	0.02 ± 0.00
A. foetidus	Mycelial	1.6	5.53 ± 0.11***	14.41 ± 0.86***	34.46 ± 2.48***	0.42 ± 0.02***
		0.8	4.04 ± 0.08***	0.20 ±0.02***	25.45 ± 1.54***	0.23 ± 0.09***
	Broth	1.6	27.17 ± 0.13***	29.17 ±1.49***	45.69 ± 2.84***	0.08 ± 0.01***
		0.8	11.83 ± 0.64***	11.83 ± 0.29***	27.93 ± 1.73***	0.02 ± 0.00
A. ustus	Mycelial	1.6	19.96 ± 0.87***	0.52 ± 0.03***	8.35 ± 0.42***	0.16 ± 0.01***
		0.8	8.41 ± 0.011***	0.35 ± 0.02***	7.44 ± 0.36***	0.05 ± 0.00***
	Broth	1.6	74.15 ± 1.03***	15.71 ± 0.81***	36.68 ± 1.23***	0.64 ± 0.05***
		0.8	44.31 ± 1.22***	8.94 ± 0.36***	30.80 ±1.65***	0.46 ± 0.02***
A. corneus	Mycelial	1.6	2.31 ± 0.01*	1.23 ± 0.06***	9.79 ± 0.28***	0.15 ± 0.03***
		0.8	0.82 ± 0.01[ns]	0.61 ± 0.07***	8.87 ±0.17***	0.10 ± 0.02***
	Broth	1.6	19.35 ± 0.10***	8.42 ± 0.26***	18.66 ±0.89***	0.17 ± 0.01***
		0.8	11.22 ± 0.67***	5.93 ± 0.36***	8.74 ± 0.64***	0.12 ± 0.01***
A. niveus	Mycelial	1.6	3.96 ± 0.10***	2.11 ± 0.18***	8.61 ± 0.73***	0.29 ± 0.02***
		0.8	2.47 ± 0.04*	1.23 ± 0.06***	7.96 ± 0.40***	0.26 ± 0.00***
	Broth	1.6	49.34 ± 2.21***	1.01 ± 0.07***	56.65 ± 2.09***	0.60 ± 0.03***
		0.8	31.25 ± 1.09***	0.01 ± 0.00	42.86 ± 2.39***	0.28 ± 0.01***

[a] IC_{50} of BHA was 53.8 ± 3.7 μg ml^{-1}.

[b] EDTA used as control (IC_{50} = 17.4 ± 0.4 μg ml^{-1}).

[c] IC_{50} for quercetin was 155.0 ± 6.4 μg ml^{-1}.

IV. CONCLUSIONS

The present study demonstrated the potential of soil fungi to have antioxidant activity similar to plants and mushrooms, thus further highlighting their significance as new sources of natural antioxidants. Results obtained in this study clearly demonstrate that some species such as *A. fumigatus*, *A. terreus* and *A. flavus* possesses significant antioxidant activity in some tests. Further investigation of individual compounds, their in *vivo* antioxidant activities, and in different antioxidant mechanisms is needed.

ACKNOWLEDGEMENTS

This research was supported by a grant from Mazandaran University of Medical sciences.

REFERENCES

Arora, D.S. and P. Chandra, "Assay of antioxidant potential of two *aspergillus* isolates by different methods under various physiochemical conditions," *Braz. J. Microb,*. **41**, 765-777 (2010).

Braun, S. and S. E. Vecht-Lifshitz, "Mycelial morphology and metabolite production," *Tibtech*., **9**, 63-68 (1991).

Dehpour, A.A., M.A. Ebrahimzadeh, S.F. Nabavi and S.M. Nabavi, "Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition,"*Grasas Aceites*, **60**, 405-412 (2009).

Ebrahimzadeh, M.A., S.M. Nabavi, S.F. Nabavi and A.A. Dehpour, "Antioxidant activity of hydroalcholic extract of *Ferula gummosa* Boiss roots," *Eur. Rev. Med. Pharmacol. Sci.,* **15**, 658-664 (2011).

Eslami B., S.F. Nabavi, S.M. Nabavi, M.A. Ebrahimzadeh and M. Mahmoudi, "Pharmacological activities of *Hypericum scabrum L*," *Eur. Rev. Med. Pharmacol. Sci.,* **15**, 532-537 (2011).

Hirota, A., Y. Morimitsu and H. Hojo, "New antioxidative indophenol-reducing phenol compounds isolated from the Moretierella sp. Fungus," *Biosci. Biotechnol. Biochem.,* **61**, 647-650 (1997).

Ishikawa, Y., "Development of new types of antioxidants from microbial origin." *J. Japan. Oil. Chem. Soc*., **41**, 762-767(1992).

Kato, S., H. Kawai, T. Kawasaki, Y. Toda, T. Urada and Y. Hayagawa, "Studies on free radical scavenging substances from microorganisms," *J. Antibiotics*, **42**, 1879-1881(1989).

Klich, M.A., *Identification of common Aspergillus species*, CBS, the Netherlands(2002).

Mathew, S. and T.E. Abraham, "Studies on the antioxidant activities of Cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models," *Food Chem.,* **94**, 520-528 (2006).

Marchelli, R. and L.C. Vining, "Terphenyllin, a novel p-terphenyl metabolite from *Aspergillus candidus*," *J. Antibiotics*, **28**, 328-331 (1975).

Moncada, A., R.M.J. Palmer and E.A. Higgs, "Nitric oxide: physiology, pathophysiology and pharmacology," *Pharmacol. Rev.,* **43**, 109-142 (1991).

Moon, B.S., I.J. Ryoo, B.S. Yun, K.S. Bae, K.D. Lee, I. Yoo and J.P. Kim, "Glyscavins A, B and C, new phenolic glycoside antioxidants produced by a fungus *Mycelia sterilia* F020054," *J. Antibiot.,* **59**, 735-739 (2006).

Nabavi, S.F., S.M. Nabavi, M.A. Ebrahimzadeh, B. Eslami and N. Jafari, "*In vitro* antioxidant and antihemolytic activities of hydroalcoholic extracts of *Allium scabriscapum* Boiss. & Ky. aerial parts and bulbs," *Int. J. Food Prop.*, **16**,713-722 (2013a).

Nabavi, S.F., S.M. Nabavi, M.A. Ebrahimzadeh, N. Jafari and S. Yazdanpanah, "Biological Activities of Freshwater Algae, *Spirogyra singularis Nordstedt*," *J. Aquat. Food Prod., T.* **22,** 58-65 (2013b).

Rios, M.F., C.M.G. Pajan, R.H. Galan, A.J.M. Sanchez and I. G. Callado, "Synthesis and free radical scavenging activity of a novel metabolite from the fungus *Colletotrichum gloeosporioides*," *Bioorg. Med. Chem. Lett.*, **16**, 5836-5839 (2006).

Sreejayan N. and M.N.A. Rao, "Nitric oxide scavenging by curcuminoids," *J. Pharm. Pharmacol.,* **49**, 105-107 (1997).

Song, T.Y. and G.C. Yen, "Antioxidant properties of *Antrodia camphorata* in submerged culture," *J. Agri. Food Chem.,* **50**, 3322-3327 (2002).

Tepe, B and A. Sokmen, "Screening of the antioxidative properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora," *Bioresource Technol.,* **98**, 3076-3079 (2007).

Wegener, G. and V. Volke, "Nitric oxide synthase inhibitors as antidepressants," *Pharmaceuticals*, **3**, 273-299 (2010).

Yagi, R. and M. Doi, "Isolation of an antioxidative substance produced by *Aspergillus repens*," *Biosci. Biotechnol. Biochem*., **63**, 932-933(1999).

Yen, G.C. and H.Y. Chen, "Antioxidant activity of various tea extracts in relation to their antimutagenicity," *J. Agri. Food Chem.,* **43**, 27-32 (1995).

Yen, G.C. and Y.C. Chang, "Medium optimization for the production of antioxidants from *Aspergillus candidus*," *J. Food Prot.,* **62**, 657-661 (1999).

Yen, G.C. and C.A. Lee, "Antioxidant activity of extracts from molds," *J. Food Prot.,* **59**, 1327-1330 (1996).

Received: June 19, 2015.
Sent to Subject Editor: December 2, 2015.
Accepted: January 10, 2017.
Recommended by Subject Editor: Octavio Furlong.