Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):600-604

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Scavenging activity of secondary metabolites extracted from food borne fungi

Shimaa R. Hamed[1*], Manal G. Mahamoud[1], El Sayed O. H.[1] and Samer R. Hamed[2]

[1]Microbial Biotechnology Department, National Research Centre, Dokki, Egypt, 12622
[2]October University for Modern Sciences and Arts, 6th October City, Egypt

---

ABSTRACT

Antioxidants are natural compounds known for their high scavenging activity toward free radicals that can damage the healthy cells of human body. Therefore, this study aims to produce anti-oxidants from eight isolates belonging to seven different species of fungi (Aspergillus candidus, Aspergillus terreus, Aspergillus flavus, Aspergillus niger, Penicillium Sp, Penicillium lividum, and Penicillium brevicompactum) isolated from food and feed stuffs. Each individual isolate was cultivated on synthetic medium (MYGP medium) to be extracted by two different methods: aqueous extraction and ethyl acetate extraction. The anti-oxidant activity was measured using DPPH (1,1-diphenyl 1-2-picrylhydrazyl) to determine the isolate that give the highest activity. The isolates that recorded the highest antioxidant activity was selected for further identification studies. The result showed that the highest antioxidant activity was in the ethyl acetate solution after 120 minutes. Interestingly, the Aspergillus species gave the highest antioxidant activity, especially, Aspergillus terreus (93.20%).

Keywords: Antioxidant activity, *Penicillium*, *Aspergillus*, DPPH, Secondary metabolites.

INTRODUCTION

Antioxidants are very important for human health and for maintaining the wellbeing as they protect the body against the free radical damage. Antioxidants are increasingly needed as a result of the current high exposure to free radicals that can damage the healthy cells of human body. Because Oxygen is very reactive atom, it is able to form the harmful free radical molecules that damage the structure and function of body cells. Free radical molecules are characterized by having odd number of electrons, electrically charged, so they try to neutralize themselves by accepting electrons from other molecules. Therefore, they stimulate a chain reaction to start which occurs within second of the starter reaction. The damage of free radical molecules is made when they interact with active cellular component such as DNA, or the cell membrane. Thus, antioxidants are working on stabilizing or deactivating the free radical molecules before interaction with other molecules [1]. Reactive Oxygen Species (ROS) are reactive molecules have derived from active molecular oxygen. Reactive oxygen species and free radicals are produced by all aerobic species as a byproduct during aerobic respiration process or by metal catalyzed oxidation. The normal doses of (ROS) are important for some biological activities such as apoptosis and gene expression. They are, also, proved to be intracellular and intercellular messengers [2]. However, the high or inadequate doses of (ROS) can cause oxidative damage that result in damage to the healthy cells [3]. The free radicals damage the structure and function of important macromolecules, resulting in abnormal gene expression, faults in proliferation mechanism, or cell death. Moreover, it may lead to various human diseases such as cancer, neurodegenerative diseases, and ocular degeneration [4]. Some antioxidants work together in a network to regenerate their original properties; this mechanism is known as “antioxidant network” [5]. Synthetic antioxidants such as butylated hydroxyl anisole

(BHA), butylated hydroxyl toluene (BHT)have recently been found to cause carcinogenesis in rodents, and by analogy also possibly in man [6], highlighting the benefits of antioxidants from natural sources such as fungi. Fungi are proved to be very important microorganisms for the ecosystem and the human life. Some filamentous fungi such as *Penicillium janthinellum, P.commune, P. herquei,* and *Aspergillus niger*have an antioxidative effect [7-11].In regards to feed and food, Hossain, et al.[12], observed the decomposition of lipids and a decrease in oxidized lipids of mackerel meal when fermentation with *A.terreus* was induced. Matsuo[13],also reported the inhibition of autoxidation of vegetable oil mixed with bean-curd refuse. Overall, many studies have shown that natural antioxidants, such as fungal phenolics, may be extracted for use as functional ingredients in food. In our study, antioxidant ability of food-borne mold was studied and an antioxidant-producing fungal strain was identified to species.

EXPERIMENTAL SECTION

Collection of samples

Eight different isolates of filamentous fungi were collected from different five sources of food stuffs. They packed directly into sterilized plastic bags and were kept in refrigerator until use.

Isolation of fungi

Food stuffs samples were subjected to series of washing with sterile distilled water. They were dried between sterile filter paper, cut into equal segments (about 1 cm each). Five segments were placed on the surface of the agar medium in each plate which supplemented with rose- Bengal and chloramphenicol as bacteriostatic agents, then the plates were incubated at 28 $^{\circ}$C for 7 days, and the developed colonies were isolated and grown again for three times until purification; the purified colonies were identified and counted.

Identification of the fungal species

Identification of the isolated fungi during the investigation was carried out using the morphological characteristics and microscopic features were examined by optical light microscope (10×90). The identification is according to the following references; Ainsworth [14] as a dictionary of the fungi, Klich and Pitt [15]for *Aspergillus* species; and Ramirez [16] and Pitt [17,18] for *Penicillium* species.

Fungal culture for isolation of secondary metabolites

The spores of 4 days' culture of tested fungi were collected by addition 5 ml of sterile saline to slant and the suspension was inoculated to 100 ml of Czapek's agar medium in 250 ml Erlenmeyer flask. After incubation for 4 days, the cultures were filtered and the mats were collected, washed by distilled water**.** Each individual isolate was cultivated on synthetic medium (MYGP medium) [19]. The cultures were grown in 250 ml Erlenmeyer flasks, each containing 50 ml of the synthetic medium. The flasks with the synthetic media were inoculated with 2ml of (7-10) days' old cultures. The inoculum spore suspensions were prepared by adding sterile distilled water to the slant culture, followed by gentle agitation. The cultures were incubated at 28°C on rotary shaker (120 rpm) for 7 days.

Fungal extraction

Aqueous extraction

At the end of fermentation period, the content of each flask (supernatant and mycelium) for eight isolates were homogenized in a blender (16000 rpm), the mixture was filtrated. The combined filtrate was collected for further bioassay processes (antioxidant activity.

Extraction with ethyl acetate

After the cultivation, the fungal biomass, including the medium, was homogenized in a blender (16000 rpm), and the mixture was extracted with ethyl acetate. The mixture was shaken and then left to stand overnight in a separating funnel. The ethyl acetate layer obtained was then dried with anhydrous sodium sulfate and evaporated using a rotary evaporator to remove the solvent in *vacuo* as described by Serizawa *et al*. [20]. The MYPG medium without the inoculation of fungi was, also, processed as above.

Measuring the anti-oxidant activity

The free radical scavenging activity (RSA) of the isolated active secondary metabolite was assessed by the discoloration of an ethanol solution or ethyl acetate solution of DPPH (1,1-diphenyl 1-2-picrylhydrazyl) radical

(violet color) according to Brand-Williams et al. [21]. The scavenging activity of free radical by active compound was evaluated spectrophotometrically at 517 nm. The scavenging activity was calculated as follows:

Scavenging ability (%) = (A517 of control – A517 of sample / A517 of control) * 100

Biomass Determination

After the fermentation process, the cultures were centrifuged at 5,000 rpm for 10 min at 4°C followed by filtration through Whatman® no. 1 filter paper. The cell pellets were washed with distilled water and dried at 80°C until a constant weight was achieved. The dry biomass was expressed as grams per liter of fermentation medium.

RESULTS

Isolation and Identification of the fungal species

Identification of the isolated fungi during our investigation was carried out using the morphological characteristics as colony diameter, the color of conidia, extracellular exudates, pigmentation and the color of reverse mycelium and microscopic features were examined also as conidial heads, fruiting bodies, degree of sporulation and the homogeneity characters of conidiogenous cells by optical light microscope (10×90) Olympus CH40. The data in Table (1) show the identification of the different fungal isolates.

Table 1: Identification of different isolated fungal strains

Code number	Scientific Name
1 NRC	*Aspergillus candidus* Link
2 NRC	*Aspergillus terreus* Thom
3 NRC	*Aspergillus flavus* Link
4 NRC	*Penicilliumsp* Pitt
5 NRC	*Aspergillus niger* Van Tieghem
6 NRC	*Penicillium lividum* Westling
7 NRC	*Aspergillus niger* Van Tieghem
8 NRC	*Penicillium brevicompactum* Dierckx

The antioxidant activity in aqueous and ethyl acetate solution

Eight different isolates of filamentous fungi were analyzed at different times using spectrophotometer to measure the highest antioxidant activity at certain time. The results showed that the increase in time causes continuous increase in the scavenging activity of isolates. Therefore, the highest antioxidant activity was after 120 minutes. Since the scavenging activity depends on the solvent type as well the results were measured in the aqueous solution and in ethylacetate solution. Table (2) presents the scavenging activity of different species of *Aspergillus* and *Penicillium* in aqueous solution at certain times. Table (3) presents the scavenging activity of different species of *Aspergillus* and *Penicillium* in ethyl acetate solution at certain times. *Penicillium brevicompactum* showed the highest antioxidant activity in aqueous solution after 120 minutes (44.20%).

Table 2: Scavenging activity of different isolates in aqueous solution at certain times

Scientific name	Scavenging ability (%) Time (min)			
	30	60	90	120
Aspergillus candidus Link	11.20	11.5	15.70	24.55
Aspergillus terreus Thom	2.60	13.00	18.00	20.00
Aspergillus flavus Link	21.52	25.00	30.00	33.90
Penicilliumsp Pitt	24.21	27.5	40.00	41.20
Aspergillus niger Van Tieghem	16.14	17.5	20.99	23.50
Penicillium lividum Westling	10.80	13.45	18.70	23.40
Aspergillus niger Van Tieghem	13.90	14	17.89	21.30
Penicillium brevicompactum Dierckx	19.30	20.00	25.87	44.20

The tested isolates showed the best results in ethyl acetate solution and after 120 minutes. However, *Aspergillus* species showed higher antioxidant activity in comparison to *Penicillium* species, especially, *Aspergillus terreus* that had the best ability to scavenge free radical molecules in ethyl acetate solution and after 120 minutes (93.20%).

Table 3: Scavenging activity of different isolates in ethyl acetate solution at certain times

Scientific name	Scavenging ability (%)			
	Time (min)			
	30	**60**	**90**	**120**
Aspergillus candidus Link	62.73	66.54	78.00	80.20
Aspergillus terreus Thom	87.07	87.83	91.21	93.20
Aspergillus flavus Link	33.46	34.98	40.20	41.02
Penicillium. Sp Pitt	7.60	9.12	15.70	31.01
Aspergillus niger Van Tieghem	47.15	48.00	58.90	70.77
Penicillium lividum Westling	65.09	66.70	73.45	75.20
Aspergillus niger Van Tieghem	68.82	69.10	72.30	74.10
Penicillium brevicompactum Dierckx	14.44	15.20	17.03	20.01

Table 4: Biomass of different isolated fungal strains

Scientific name	Dry biomass (mg/ml)
Aspergillus candidus Link	2.4
Aspergillus terreus Thom	1.2
Aspergillus flavus Link	0.65
*Penicillium. Sp*Pitt	19.67
Aspergillus niger Van Tieghem	5.93
Penicillium lividum Westling	2.26
Aspergillus niger Van Tieghem	1.74
Penicillium brevicompactum Dierckx	2.08

The obtained data in Table (4), showed that, *Penicilliumsp* isolate No. 4NRC have the highest dry weight (19.67 mg/ml), although *Penicilliumsp* have moderate antioxidant activity in each of aqueous and ethyl acetate solution. Thus, there no correlation between the biomass which revealed to the fungal growth and the physiology of fungi and their biological activity.

DISCUSSION

Antioxidants are natural compounds known for their high scavenging activity toward free radical molecules that can damage the healthy cells of human body. Antioxidants can disturb the oxidation process by reacting with free radicals and acting as oxygen scavengers so they are very important for human health [22]. All tested isolates belonging to *Aspergillus* genus have the ability to scavenge the DPPH free radical well but this ability is varying degree. Production of effective antioxidant substances by members of *Aspergillus* genus was previously recorded such as *A. niger* [23,24], *A. Candidus* [25], *A. flavus* [26] and *A. terreus* [27]. However, the obtained results from this study showed that the antioxidant produced from *A. terreus* exhibited the highest antioxidant activity among other *Aspergillus* species. To our knowledge, this is the first study to produce high antioxidant activity of *A. terreus* reaches to 93.20% while *Penicillium* isolates have less ability to scavenge the DPPH free radical well in comparison with *Aspergillus*. However, they showed higher results in aqueous solution than *Aspergillus* species. Some members of *Penicillium* genus were previously recorded as antioxidant producers such as *Penicilliumsp*[28] and *Penicillium lividum*[29]. However, our knowledge indicates that this study is the first to produce natural effective antioxidant substances by *Penicillium brevicompactum.*

Aspergillus terreus is a saprotrophic fungus dominant in tropical and subtropical areas. It grows on decomposing vegetation and dust**.** *Aspergillus terreus* is highly used in industrial field to produce various organic acids, including itaconic acid, and enzymes as xylanase. In addition, it is used as source for many drugs. For example, it produces lovastatin used to lower serum cholesterol [30]. It is known for its ability to produce antioxidants however there are relatively few studies of antioxidant from *A. terreus* compared to other *Aspergillus* species such as *A. candidus*. In this study, *A. terreus* showed higher results in ethyl acetate solution in comparison to aqueous solution. It, also, presented the best scavenging ability after 120 mins reached 93.20 %. The high antioxidant activity of *Aspergillus terreus* was previously recorded by Dewi et al.[27] who demonstrated in his study that terreic acid and terremutin from ethyl acetate extract of *A. terreus* exhibited significant antioxidant activity. It is important to note that our study showed higher antioxidant activity compared to the previous study.

CONCLUSION

In present study, a number of species were used to measure the scavenging ability of each antioxidant produced in mycelial biomass using submerged liquid cultivation. It was determined that the various antioxidants produced were radical scavengers but their scavenging ability were varying degree, *Aspergillus* species showed better results than *Penicillium* species. In addition, the study demonstrated that depending on the process of extraction, different yields of antioxidants were recovered. Additionally, ethyl acetate extracts were typically more effective in antioxidant properties than aqueous extracts. Therefore, it was concluded that the overall antioxidant capacity of these filamentous fungi was attributed mainly to their phenolic content. These species of fungi show promising activity for further applications, especially *Aspergillus terreus* and *Aspergillus candidus.*

REFERENCES

[1] M Precival. *Clin. Nutri. Insights*,**1996**, 3(5), 1-4.
[2] P Held. An Introduction to Reactive Oxygen Species: Measurement of ROS in Cells. BioTek Instrument, Inc., USA,**2015**; 1-21.
[3] F Lledias,P Rangel,W Hansberg.*J. Biolog. Chemist*., **1998**, 273, 10630-10637.
[4] V Teixeira,H Valente,S Casal,L Pereira,F Marques,P Moreira. *NRC Res. Press*,**2009**, 34(4), 716-724.
[5] T Rahman. *Appl.Microbiol.Biotechnol*., **2007**, 73(6), 121-130.
[6] N Ito,S Fukushima,H Tsuda. *Crit. Rev.Toxicol*.,**1985**, 15(2), 109-150.
[7] LL Zaika,JL Smith. *J. Sci. Food Agric*., **1975**,26, 1357-1369.
[8] Y Nakakita,K Yomosa,A Hirota,H Sakai. *Agric. Biol. OMm.*,**1984**, 48, 239-240.
[9] Y Ishikawa,K Morimoto,T Hamasaki. *J. Jap. Oil OMm. Soc*.,**1986**, 35, 903-906.
[10] K Morimoto,T Yoshiwa,Y Ishikawa,T Hamasaki. *J. Jap. Oil OMm. Soc.*,**1987**, 36, 10-15.
[11] OH El-Sayed, MMS Asker, SMShash, SR Hamed. *Int. J.ChemTech Res*.,**2015**, 8(1), 58-66.
[12] MA Hossain,M Furuichi, YYone. *NipponSuisanGakkaishi*,**1987**, 53, 1629-1632.
[13] M Matsuo. *Nippon NogeikagakuKaishi*,**1989**, 63,1765-1770.
[14] GC Ainsworth. Ainsworth and Bisby's Dictionary of the fungi. Commonwealth Mycological Institute, Kew, Surrey, England, **1971**.
[15] MA Klich,JI Pitt. A laboratory guide to the common *Aspergillus* species and their teleomorphs. Commonwealth scientific and industrial research organization, division of food processing, North Ryde, Australia, **1992**.
[16] C Ramirez. Manual and Atlas of Penicillia. Elsevier Biomedical Press, Amsterdam, Netherlands, **1982**.
[17] JI Pitt. The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. Academic Press, INC, LTD, London, **1979**.
[18] JI Pitt. A laboratory guide to common *Penicillium* species. Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, N.S.W. Australia, **1985**.
[19] W Masoud,C Kaltoft. *Inter. J. Food Microbiol*., **2006**,106, 229-234.
[20] N Serizawa,K Nakagawa,K Kamano,Y Tsujita,A Terahara,H Kuwano. *J. Antibiotics*, **1983**, 36, 604-607.
[21] W Brand-Williams,ME Cuvelier,C Berset. *LebensmittelWissenschaftTechnologie*, **1995**, 28, 25-30.
[22] W Droge. *Physiol.Rev*.,**2002**, 82, 47-95.
[23] Y Kawai,M Otaka,M Kakio,Y Oeda,N Inoue,H Shinano. *Bull. Fac. Fish. Hokkaido Univ.*,**1994**, 45(1), 26-31.
[24] K Aldaco,E Martinez,R Herrera,C Aguilar. *Inter J. Engineering Technol*.,**2009**,1(4), 288-290.
[25] M Elaasser,M Marwa,A Rasha.*J. Microbial. Biotech. Res*., **2011**, 1(4), 5-17.
[26] A Soniyamby,S Lalitha, B Praveesh. *Asian J. Pharmaceut.Clin.Res*., **2011**, 4, 174-177.
[27] RT Dewi, S Tachibana, K Itoh, M Ilyas. *J. Microbial Biochem. Technol*., **2012**, 4, 010- 014.
[28] D Arora,P Chandra, GJ Kaur. *Curr.Biotechnol*.,**2012**, 1, 2-10.
[29] RH Shimaa, SS Mohamed, MS Selim, RS Al-Wasify, OH El Sayed. *J. Chem. Pharm. Res.*, **2015**, 7(9), 173-179.
[30] G Szakacs,G Morovjan,R Tengerdy. *Biotechnol. Lett*.,**1998**,20(4), 411–415.