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Research Article

Antioxidant potentialities of some strains belonging to endophytic, entomopathogenic and saprophytic fungi

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ABSTRACT

Antioxidants have recently become the topic of interest as radical scavengers, which inhibit the free radical mediated processes. This study was carried out to investigate the antioxidant activity of 100 fungal strains (26 endophytes, 42 entomopathogens and 32 saprophytes). Three different assays (reducing power, total phenolic contents and flavonoid contents) were determined and used to evaluate the antioxidant potential of the fungal ethanolic extracts. The results revealed that all fungal strains under study showed antioxidant activity up to varying extent. A total of 21, 35 and 19 out of the tested endophytic, entomopathogenic and saprophytic strains, respectively, had a reducing power activity. High reducing power activities (≥ 0.6 mg/ml) were recorded by 9, 20 and 14 strains of the three tested groups, respectively. All tested strains have the ability to produce phenolic compounds with levels ranged from 0.92 to 63.44 mg/ml. The highest levels of total phenolic contents (≥ 40 mg/ml) observed in the extract of 12, 28 and 18 strains of endophytes, entomopathogens and saprophytes, respectively. Finally, all tested strains produced flavonoids with levels of 0.166 to 68.806 mg/ml. The highest flavonoid producers (formed ≥ 35 mg/ml) were only one strain of each of the endophytic and entomopathogenic fungi and three strains of saprophytic fungal group. The obtained results suggest that the tested strains, especially those of endophytes, had the potentiality as sources of strong natural and safe antioxidants for application in food and cosmetics industries.

Keywords: Antioxidant; Fungi; Flavonoids; Phenols.

1. INTRODUCTION

For thousands of years, fungi have been recognized as nutritious, highly palatable functional foods in many societies and are now accepted as a valuable source for the development of medicines and nutraceuticals [1, 2]. Fungi have proven to be a rich source of bioactive and novel organic compounds with interesting biological activities and a high level of biodiversity [3, 4]. Fungi produce a diverse array of secondary metabolites. Secondary metabolites have a tremendous impact on society and are exploited for their antibiotics and pharmaceuticals activities such as anticancer, antitumor, immuno-stimulatory, and antioxidants [5]. It is clear that, fungi represent a largely untapped source of potentially powerful new pharmaceutical products [2, 6].

Endophytes are microorganisms that colonize internal plant tissue and can live there for all or part

of their life cycle without causing any apparent damage or disease [7]. Entomopathogenic fungi are ecologically classified as fungi that grow either inside of insect bodies or on the surface of their exoskeleton, which eventually causes the death of the host insect [8]. Fungi play an important role in the research for antitumor compounds and might also represent an alternative source for the production of therapeutic agents that are not easily obtained by chemical synthesis. Generally these fungi are a store house of novel secondary metabolites including antibiotics, antioxidants, anticancer and immunosuppressant compounds [9-11].

Antioxidants are critical for the maintenance of normal cell function, health and well-being. They are compounds which prevent the initiation or propagation of oxidizing chain reactions which in turn inhibits or delays oxidative damage related to aging and disease. Although have developed natural mechanisms to protect cells from free radical damage by neutralizing them, the amount of antioxidant produced under normal conditions is not always sufficient. Fungi are a well-known source of antioxidants which can be used to prevent oxidative damage and as such, can limit their deleterious effects in humans and animals alike.

Antioxidants are the molecules, which prevent cellular damage by reducing the oxidative stress and therefore have a beneficial effect on human health [12]. Antioxidants may be characterized by their mode of action in preventing oxidative damage, being classified as preventative, scavenging, and repair or *de novo* antioxidants [13]. Antioxidants prevent the formation of reactive oxygen and nitrogen species ROS/RNS by reducing hydrogen peroxide and lipid hydro peroxidases, respectively, or by sequestering metal ions such as iron and copper [14].

Phenolic compounds are aromatic hydroxylase compounds possessing at least one aromatic ring with one or more hydroxyl groups [15]. By this means, a structure-function relationship exists between phenolic compounds; with their antioxidant activity depending on the number and position of the hydroxyl groups and the nature of substitutions on the aromatic rings [16]. These compounds are common in fungi and are important sources of bioactive substances [15]. Generally, antioxidants are obtained from fungal sources include phenolic compounds (tocopherol, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, amino acids, and amines) or carotenoids as well as ascorbic acid [17, 18].

The main cause of mortality and morbidity in the world is atherosclerosis, the accumulation of oxysterol, cholesterol, and peroxide lipids in arteries, generated by free radicals which lead to heart attack. Hence, there has been an increased interest in the application of antioxidants [19].

Natural compounds such as ascorbic acid, vitamin E, carotenoids, flavones and phenolic acids which are common to fungi possess the ability to scavenge free radicals in the human body. They play a key role in health maintenance and prevention of chronic and degenerative diseases such as atherosclerosis, carcinogenesis, neurodegenerative diseases, DNA damage and aging [20]. Antioxidants serve as the defensive factor against free radicals in the body. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tert-butylhydroquinone (TBHQ) are usually used as food additives by the food industry to prevent lipid peroxidation. 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 [21]. A number of plants and mushrooms are commonly known to produce antioxidants but there are few reports on lower fungi [22]. These include *Penicillium roquefortii*, *Aspergillus candidus*, *Emericella falconensis*, *Acremonium* sp., *Colletotrichum gloeosporioides* [22], *Chaetomium* sp., *Cladosporium* sp., *Phoma* sp. etc. [23]. A lot more fungi still needs to study. Keeping above in mind the present study was aimed to determine the total phenolic, flavonoid content and reducing power of ethanolic extracts of a total 100 strain of endophytic (26 strains), saprophytic (32) and entomopathogenic (42) fungi collected from different sources.

2. MATERIALS AND METHODS

2.1. Collection of fungal strains

A total of 100 fungal strains (26 endophytic, 32 saprophytic and 42 entomopathogenic fungal

strains) isolated from different sources were kindly provided by the Assiut University Mycological Centre (AUMC), Assiut University, Assiut, Egypt.

2.2. Preparation of fungal inoculum

Inocula of tested fungi were prepared by suspended 1cm from seven day-old culture of each organism on potato dextrose agar (PDA) in 5 ml of sterile distilled water supplemented with 0.01% of Tween 80 and suspending the spores with a sterile loop [24]. This spore suspension was used as inoculum for cultivation of each organism.

2.3. Preparation of crude fungal extracts

Fungal strains were grown on potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks. Cultures were incubated for 10 days at 28 ± 2 ^{0}C. The mycelia and the fermentation broth of each fungal strain were blended with 150 ml ethanol in electric blender; the extracts were filtered using filter paper to remove the mycelia. Mislabel extracts were individually transferred into rotatory evaporator under reduced pressure at 35 ^{0}C till semisolid residue was obtained.

2.4. Antioxidant assays

Three different assays including reducing power, phenolic content and flavonoids were used to evaluate the antioxidant potential of fungal extracts. Each experiment was done in triplicate and mean values were taken.

2.4.1. Determination of antioxidant activity by reducing power measurement

The reducing power of the extract was determined according to Chang et al. [25] with slight modification as follows: an aliquot of 0.5 ml extract was added to 0.1 ml of 1% (w/v) potassium ferricyanide. After incubating the mixture at 50 ^{0}C for 30 min, during which the ferricyanide was reduced to ferrocyanide, it was supplemented with 0.1 ml of 1% (w/v) trichloroacetic acid and 0.1% $FeCl_3$ and left for 20 min. Absorbance was read at 700 nm to determine the amount of ferric ferro-cyanide (Prussian blue) formed. Higher absorbance of the reaction mixture indicates higher reducing power of the sample. Ascorbic acid concentrated of 10 to 200 µg/ml was used as standard.

2.4.2. Determination of total phenolic content

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu (F-C) colorimetric method [26]. Briefly, 50 µl of sample and 50 µl of F-C reagent were pipetted into an eppendorf tube. The contents were vortexed for 10 second and then left to stand at room temperature for 2 min before the reaction was stopped by adding 500 µl of 5% (w/v) sodium carbonate solution and 400 µl of distilled water, and the volume was adjusted to 1 ml. The mixture was then vortexed and incubated at 45°C for 30 min before cooling rapidly with ice. The absorbance of the solution was measured at 760 nm. Gallic acid concentrations ranging from 10 to 300 µg/ml were prepared and a calibration curve was obtained using a linear fit.

2.4.3. Determination of flavonoid content

The total flavonoid content was determined according to the aluminum chloride method [27]. Briefly, 0.5 ml of sample and 300 µl of $NaNO_2$ (1:20 w/v) were pipetted into a test tube and the contents were vortexed for 10 second and left to stand at room temperature for 5 min. After standing, 300 µl of $AlCl_3$ (1:10 w/v), 2 ml of NaOH (1 M) and 1.9 ml of distilled water were added to the reaction mixture, which was then vortexes for 10 s, and the absorbance was measured at 510 nm. Narnginine concentrations ranging from 10 to 800 µg/ml were prepared and a standard calibration curve was obtained using a linear fit.

3. RESULTS AND DISCUSSION

Epidemiological studies show that human body is damaged by reactive oxygen and nitrogen species. Thus, it is considered important to increase the intake of antioxidants in the human diet. Some synthetic antioxidants may exhibit toxicity with carcinogenic potential, show lower efficiency than natural antioxidants, and require high manufacturing costs. Thus, there is a need to identify natural and possibly more economic and effective antioxidants

[21, 28, 29]. So, in present study, ethanolic extracts of a total 100 fungal strains belonging to endophytic (26), saprophytic (32) and entomopathogenic (42) strains were investigated for antioxidant potential by using three different methods, all extracts showed antioxidant activity up to varying extent.

3.1. Reducing power

The reducing power evaluation of the fungal extracts is an important parameter related to assessing the antioxidant activity. Reducing power measure the ability of a sample to act as an electron donor and therefore, reacts with free radicals converting them to more stable products and thereby terminate radical chain reaction. In order to examine the reducing power of fungal extracts, the reaction of Fe^{3+} was observed. The reducing power of the extracts was determined according to Change et al. [25]. Increase absorbance of the reaction mixture indicated increased reducing power of the sample. Absorbance was read to determine the amount of ferric Ferro cyanide (Prussian blue) formed. Ascorbic acid was taken as the standard. The results revealed that a total of 21, 35 and 19 out of 26, 42 and 32 strains of endophytes, entomopathogens and saprophytes, respectively, had a reducing power activity and formed ferric ferro cyanide with activities ranged between 0.01 and 1.116 mg/ml fungal extract (Tables 1-3). These producer strains can be classified according to their reducing power activities into three groups. The highest reducing power activities (which formed ≥ 0.6 mg/ml) were represented by 9, 20 and 14 strains of the three tested groups of fungi, respectively (Tables 1-3). The highest one of endophytic fungi was *Emericella nidulans* AUMC 8854 (recorded 0.972 ± 0.04 mg/ml) followed by *Aspergillus versicolor* AUMC 6872 (formed 0.942 ± 0.001 mg/ml) (Table 1). *Beauveria bassiana* AUMC 3873 was the greatest strain of entomopathogenic group and recorded 1.042 ± 0.01 mg/ml followed by *Aspergillus niger* AUMC 9890 which yield 1 ± 0.005 mg/ml (Table 2). On the other side, the best strain of saprophytic fungi was *Phoma herbarum* AUMC 3509 (formed 1.116 ± 0.01 mg/ml) followed by *Aspergillus terreus* AUMC 3101 and *Botryotrichum piluliferum* AUMC 6467, they yield ferric ferro cyanide with activities equal to 1.022 ± 0.003 and 1.020 ± 0.003 mg/ml, respectively (Table 3).

Moderate activities of reducing power (from 0.4 to > 0.6 mg/ml) were observed in the extract of 4, 3 and 2 strains of endophytes, entomopathogens and saprophytes, respectively. On the other hand, 8, 12 and 3 strains of endophytic, entomopathogenic and saprophytic fungi under study, respectively, were recorded as lower producers for reducing power compounds with activities less than 0.4 mg/ml (Tables 1-3). Only 5, 7 and 13 out of the tested strains of endophytic, entomopathogenic and saprophytic fungi, respectively, could not to produce any detectable amounts of reducing power compounds (Tables 1-3).

These results are in agreement with those obtained by Chandra and Arora [29]. They determined the reducing power of 51 strains of fungi isolated from different area of Indian soil and recorded that only 32 fungal strains showed reducing power ranged from 0.115 to 1.6 mg/ml. Recently, Kumaresan et al. [30] reported that the reducing power of the extracts of four endophytic fungi *Chaetomium* sp., *Curvularia* sp., *Colletotrichum* sp. and *Trichoderma* sp. were ranged between 0.935 and 1.241 mg/ml extract. They found that *Chaetomium* sp. exhibited maximum reducing power (1.241 mg/ml) and *Colletotrichum* sp. showed the lowest reducing power (0.935 mg/ml).

3.2. Total phenolic content (TPC)

The total phenolic content was determined according to Folin-Ciocalteu (F-C) colorimetric method as described by Cicco et al. [26]. The TPC of ethanolic fungal extracts have been expressed as gallic acid equivalent (GAE). TPCs are known to be responsible for antioxidant activity and the high TPC is positively correlated with the antioxidant potential of an organism. All tested strains could be producing TPCs with levels ranged from 0.92 to 63.44 mg/ml fungal extract. Total phenolic compounds produced by endophytic fungi were ranged from 18.3 to 63.44 mg/ml while those produced by saprophytic and entomopathogenic fungi fluctuated between 0.92 to 60.54 and 6.6 to 61.72 mg/ml, respectively (Tables 1-3).

Most of research achieved on detected the total phenolic compounds produced by fungi using endophytic fungi [11, 31]. In this study out of the

total 26 tested endophytic strains, 12 strains (46.15%) were recorded as highly producer strains which secreted total phenolic compounds with activities equal to or more than 40 mg/ml. The superior endophytic fungal strain was *Emericella nidulans* AUMC 8854 which formed TPCs with activity reached to 63.44 ± 0.001 mg/ml, followed by *Aspergillus oryzae* AUMC 8863 which formed activity reached to 52.72 ± 0.008 mg/ml (Table 1). On the other side, 28 and 18 fungal strains represented 66.67% and 56.25% out of the tested strains of entomopathogenic and saprophytic fungi were recorded as highly producers for TPC and secreted total phenolic compounds with activities equal to or more than 40 mg/ml in (Tables 2, 3). The greatest strain of entomopathogenic fungi was *Epicoccum nigrum* AUMC 3148 which yield 61.72 ± 0.06 mg/ml extract in (Table 2). While the greatest two strains of saprophytic fungi were *Paecilomyces lilacinus* AUMC 6499 and *Penicillium roquefortii* AUMC 6398 which recorded 61.6 ± 0.03 mg/ml and 60.54 ± 0.01 mg/ml, respectively (Table 3).

Table 1. Total phenolic compounds, flavonoids and reducing power as antioxidant activities of some endophytic fungi recorded as mg/ml fungal extracts.

Fungal strains	Reducing power	Level	Total phenolic	Level	Flavonoids	Level
Alternaria						
A. alternata AUMC 6836	0.71 ± 0.02	H	19.82 ± 0.01	L	0.586 ± 0.001	L
A. alternata AUMC 8840	0.712 ± 0.05	H	39.2 ± 0.001	M	7.726 ± 0.02	L
A. alternata AUMC 8841	0.388 ± 0.001	L	40.32 ± 0.1	H	8.046 ± 0.04	L
Aspergillus						
A. awamori AUMC 8855	0.762 ± 0.01	H	44.4 ± 0.05	H	9.626 ± 0.001	L
A. fumigatus AUMC 8872	0.74 ± 0.5	H	42.3 ± 0.2	H	12.686 ± 0.2	L
A. niger AUMC 8852	L.D.	N	40.82 ± 0.06	H	9.486 ± 0.03	L
A. niger AUMC 8856	0.348 ± 0.001	L	48.64 ± 0.002	H	19.086 ± 0.03	M
A. oryzae AUMC 8863	0.224 ± 0.007	L	52.72 ± 0.008	H	19.346 ± 0.01	M
A. versicolor AUMC 6872	0.942 ± 0.001	H	37.34 ± 0.009	M	0.406 ± 0.003	L
Circinella muscae AUMC 8861	0.078 ± 0.001	L	38.2 ± 0.1	M	6.946 ± 0.05	L
Chaetomium globosum AUMC 8862	L.D.	N	33.94 ± 0.09	M	7.846 ± 0.004	L
Fusarium						
F. lateritium AUMC 6833	0.592 ± 0.001	M	40.4 ± 0.02	H	9.986 ± 0.002	L
F. oxysporum AUMC 6827	0.552 ± 0.02	M	48.28± 0.001	H	9.926 ± 0.03	L
F. semitectum AUMC 6816	L.D.	N	19.06 ± 0.01	L	1.106 ± 0.02	L
F. scirpi AUMC 8858	0.744 ± 0.05	H	49.72 ± 0.005	H	3.746 ± 0.001	L
F. subglutinans AUMC 8839	L.D.	N	33.54 ± 0.008	M	3.506 ± 0.001	L
Gliocladium solani AUMC 6802	0.102 ± 0.002	L	18.3 ± 0.005	L	4.086 ± 0.001	L
Emericella						
E. nidulans AUMC 8854	0.972 ± 0.04	H	63.44 ± 0.001	H	10.286 ± 0.01	L
E. rugulosa AUMC 8867	L.D.	N	41.54 ± 0.2	H	12.106 ± 0.004	L
Exophiala costellanii AUMC 8865	0.264 ± 0.2	L	37 ± 0.003	M	7.366 ± 0.05	L
Papulaspora irregularis AUMC 8843	0.58 ± 0.002	M	23.08 ± 0.03	M	0.466 ± 0.05	L
Penicillium						
P. aurantiogriseum AUMC 8847	0.35 ± 0.03	L	38.86 ± 0.001	M	4.826 ± 0.002	L
P. funiculosum AUMC 8850	0.762 ± 0.01	H	44.4 ± 0.05	H	9.626 ± 0.001	L
P. raistrickii AUMC 7265	0.45 ± 0.01	M	36.72 ± 0.01	M	13.346 ± 0.05	L
Penicillium sp. AUMC 8859	0.392 ± 0.03	L	22.7 ± 0.03	M	5.866 ± 0.003	L
Pleospora tarda AUMC 8871	0.862 ± 0.3	H	24.9 ± 0.03	M	61.826 ± 0.003	H

Table 2. Total phenolic compounds, flavonoids and reducing power as antioxidant activities of some entomopathogenic fungi recorded as mg/ml fungal extracts.

Fungal strains	Reducing power	Level	Total phenolic	Level	Flavonoids	Level
Aspergillus						
A. flavus AUMC 9881	L.D.	N	49.98 ± 0.006	H	29.406 ± 0.04	M
A. flavus AUMC 9885	L.D.	N	58.74 ± 0.03	H	6.086 ± 0.001	L
A. flavus AUMC 9903	0.834 ± 01	H	39.72 ± 0.4	M	6.226 ± 0.001	L
A. flavus AUMC 9904	0.682 ± 0.006	H	48.6 ± 0.07	H	14.986 ± 0.09	L
A. niger AUMC 9882	0.292 ± 0.1	L	42.64 ± 0.01	H	24.806 ± 0.05	M
A. niger AUMC 9890	1 ± 0.005	H	44.26 ± 0.08	H	18.106 ± 0.03	M
A. sydowii AUMC 9888	0.814 ± 0.09	H	41.2 ± 0.01	H	12.406 ± 0.07	L
A. tamarii AUMC 9902	0.27 ± 0.05	L	47 ± 0.08	H	25.086 ± 0.3	M
Beauveria						
B. bassiana AUMC 3847	0.76 ± 0.1	H	18.46 ± 0.00	L	5.906 ± 0.01	L
B. bassiana AUMC 3848	0.712 ± 0.001	H	44.2 ± 0.1	H	5.743 ± 0.03	L
B. bassiana AUMC 3849	0.646 ± 0.01	H	47.34 ± 0.09	H	7.746 ± 0.006	L
B. bassiana AUMC 3850	0.858 ± 0.03	H	55.4 ± 0.04	H	5.266 ± 0.05	L
B. bassiana AUMC 3852	0.486 ± 0.04	M	35.94 ± 0.1	M	2.706 ± 0.002	L
B. bassiana AUMC 3853	0.81 ± 0.1	H	41.6 ± 0.006	H	5.906 ± 0.1	L
B. bassiana AUMC 3854	0.058 ± 0.01	L	19.42 ± 0.001	L	1.986 ± 0.001	L
B. bassiana AUMC 3855	0.306 ± 0.002	L	44.26 ± 0.03	H	5.926 ± 0.05	L
B. bassiana AUMC 3856	0.75 ± 0.1	H	39.54 ± 0.4	M	7.726 ± 0.06	L
B. bassiana AUMC 3858	0.74 ± 0.001	H	16.22 ± 0.008	L	0.606 ± 0.003	L
B. bassiana AUMC 3859	0.374 ± 0.02	L	11.86 ± 0.07	L	16.686 ± 0.01	M
B. bassiana AUMC 3860	0.516 ± 0.06	M	55.28 ± 0.2	H	11.906 ± 0.04	L
B. bassiana AUMC 3862	0.598 ± 0.04	M	53.16 ± 0.09	H	8.626 ± 0.03	L
B. bassiana AUMC 3864	0.72 ± 0.01	H	48.22 ± 0.001	H	5.786 ± 0.07	L
B. bassiana AUMC 3866	0.764 ± 0.001	H	41.24 ± 0.004	H	15.526 ± 0.06	M
B. bassiana AUMC 3867	0.39± 0.07	L	60.83 ± 0.3	H	12.666 ± 0.03	L
B. bassiana AUMC 3869	0.182 ± 0.01	L	11.58 ± 0.3	L	0.806 ± 0.04	L
B. bassiana AUMC 3870	0.726 ± 0.002	H	40.9 ± 0.03	H	7.586 ± 0.001	L
B. bassiana AUMC 3873	1.042 ± 0.01	H	45 ± 0.005	H	10.626 ± 0.03	L
B. bassiana AUMC 9894	0.33 ± 0.06	L	41 ± 0.02	H	9.446 ± 0.1	L
B. bassiana AUMC 9895	0.09 ± 0.001	L	42.96 ± 0.2	H	19.126 ± 0.05	M
B. bassiana AUMC 9896	0.266 ± 0.06	L	37.74 ± 0.003	M	10.246 ± 0.4	L
B. bassiana AUMC 9908	0.91 ± 0.08	H	6.6 ± 0.02	L	6.413 ± 0.003	L
Epicoccum						
E. nigrum AUMC 3148	0.804 ± 0.01	H	61.72 ± 0.06	H	36.626 ± 0.07	H
E. nigrum AUMC 3149	0.348 ± 0.003	L	47.2 ± 0.11	H	12.206 ± 0.03	L
Fusarium						
F. nygamai AUMC 9891	L.D.	N	41 ± 0.3	H	18.506 ± 0.1	M
F. nygamai AUMC 9892	L.D.	N	54 ± 0.007	H	10.566 ± 0.02	L
F. pseudocircinatum AUMC 9899	L.D.	N	30 ± 0.09	M	4.166 ± 0.03	L
F. solani AUMC 9893	0.254 ± 0.3	L	33.4 ± 0.001	M	6.086 ± 0.006	L
F. verticillioides AUMC 9889	L.D.	N	45.58 ± 0.005	H	11.126 ± 0.01	L
Metarhizium anisoplea AUMC 5130	L.D.	N	32.6 ± 0.02	M	8.446 ± 0.05	L
Penicillium						
P. corylophilum AUMC 9900	0.838 ± 0.1	H	33 ± 0.008	M	7.666 ± 0.004	L
P. oxalicum AUMC 9898	0.79 ± 0.003	H	52.92 ± 0.1	H	16.086 ± 0.05	M
Penicillium sp. AUMC 9901	0.834 ± 0.07	H	42.6 ± 0.005	H	7.226 ± 0.04	L

Table 3. Total phenolic compounds, flavonoids and reducing power as antioxidant activities of some saprophytic fungi recorded as mg/ml fungal extracts.

Fungal strains	Reducing power	Level	Total phenolic	Level	Flavonoids	Level
Alternaria						
A. alternata AUMC 3128	0.926 ± 0.05	H	24.98 ± 0.1	M	9.726 ± 0.007	L
A. alternata AUMC 3131	0.026 ± 0.001	L	40.98 ± 0.006	H	8.126 ± 0.08	L
Aspergillus						
A. flavus AUMC 3200	L.D.	N	42.98 ± 0.1	H	9.886 ± 0.002	L
A. fumigatus AUMC 48	0.978 ± 0.09	H	13.04 ± 0.01	L	1.066 ± 0.001	L
A. terreus AUMC 3101	1.022 ± 0.003	H	16 ± 0.2	L	2.326 ± 0.004	L
A. terreus AUMC 3102	L.D.	N	59.8 ± 0.08	H	45.606 ± 0.06	H
Botryotrichum piluliferum AUMC 6467	1.02 ± 0.003	H	49.84 ± 0.2	H	14.086 ± 0.01	L
Chaetomium						
C. globosum AUMC 113	0.636 ± 0.01	H	41.6 ± 0.03	H	16.006 ± 0.02	M
C. globosum AUMC 114	0.652 ± 0.04	H	48.72 ± 0.01	H	11.886 ± 0.001	L
Cladosporium						
C. cladosporioides AUMC 132	0.432 ± 0.08	M	39.56 ± 0.02	M	16.746 ± 0.003	M
C. cladosporioides AUMC 133	0.708 ± 0.001	H	41.24 ± 0.01	H	11.906 ± 0.01	L
C. cladosporioides AUMC 3111	L.D.	N	41.6 ± 0.3	H	7.006 ± 0.007	L
C. cladosporioides AUMC 6091	0.946 ± 0.01	H	13.12 ± 0.09	L	8.386 ± 0.003	L
Fusarium						
F. oxysporum AUMC 3224	0.846 ± 0.03	H	5.28 ± 0.005	L	0.166 ± 0.07	L
F. proliferatum AUMC 3190	L.D.	N	40.46 ± 0.07	H	8.626 ± 0.1	L
F. solani AUMC 222	L.D.	N	21.62 ± 0.002	M	3.546 ± 0.006	L
F. solani AUMC 223	0.988 ± 0.02	H	15.36 ± 0.03	L	3.986 ± 0.05	L
Gliocladium						
G. catenulatum AUMC 6103	0.908 ± 0.05	H	11.06 ± 0.001	L	2.826 ± 0.3	L
G. roseum AUMC 3763	0.952 ± 0.003	H	51.04 ± 0.02	H	25.426 ± 0.01	M
Lecanicillium antillanum AUMC 9905	0.01 ± 0.006	L	45.8 ± 0.001	H	45.686 ± 0.2	H
Paecilomyces						
P. lilacinus AUMC 6275	L.D.	N	45.8 ± 0.01	H	14.126 ± 0.01	L
P. lilacinus AUMC 6499	L.D.	N	61.6 ± 0.03	H	10.606 ± 0.007	L
P. variotii AUMC 3112	0.514 ± 0.03	M	39.38 ± 0.05	M	8.546 ± 0.01	L
Papulaspora irregularis AUMC 3107	L.D.	N	49.4 ± 0.4	H	11.566 ± 0.03	L
Penicillium						
P. roquefortii AUMC 6397	L.D.	N	36.18 ± 0.06	M	11.906 ± 0.03	L
P. roquefortii AUMC 6398	L.D.	N	60.54 ± 0.01	H	64.806 ± 0.08	H
Periconia digitata AUMC 6235	0.956 ± 0.1	H	0.92 ± 0.03	L	0.746 ± 0.1	L
Phoma herbarum AUMC 3509	1.116 ± 0.001	H	54.36 ± 0.04	H	13.326 ± 0.01	L
Trichoderma						
T. longibranchiatum AUMC 3113	L.D.	N	35.16 ± 0.04	M	6.846 ± 0.09	L
T. pseudokoningii AUMC 6430	L.D.	N	50.1 ± 0.02	H	11.466 ± 0.003	L
Rhizopus stolonifer AUMC 9906	0.238 ± 0.01	L	30.56 ± 0.05	M	4.726 ± 0.05	L
Stachybotrys chartarum AUMC 1661	L.D.	N	44.4 ± 0.006	H	5.306 ± 0.008	L

Several previous studies have proved that the phenolic substances are considerably more potent antioxidants than vitamin C and vitamin E. These compounds have also been found to exhibit many other health related properties because of their antioxidant activities [32]. The interest in the phenolic compounds has increased tremendously due to their prominent free radical scavenging activity [33], attributed to their redox properties, which allow them to act as reducing agents or hydrogen atom donor [34].

The results in this study link with some previous finding specially those of endophytic fungi and their antioxidant activity. *Chaetomium* sp. isolated from *Nerium oleander* possessed the highest antioxidant capacity with phenolic content reached to 13.95 ± 0.11 mg/ml [35]. Chandra and Arora [29] examined 51 strains of soil fungi for TPCs production and found that all strains had the ability to produce TPC with levels ranged from 1.01 and 20.59 mg/ml extract. Also, the same authors in another paperArora and Chandra [36] examined four fungal isolates: *Aspergillus wentii* 1, *A. wentii* 2, *Penicillium citrinum* and *P. granulatum* for their antioxidant potential and detected 20.6, 12.1, 12.03 and 7.2 mg/ml extract of the four fungal isolates, respectively. Ruma et al. [31] reported that the TPC in different selected six endophytic fungal extracts ranged from 4 to 144 mg/ml extract. *Aspergillus terreus* isolated from *Ocimum sanctum* exhibited antioxidant activity with 14.96 ± 0.07 mg/g dry weight [37].

Yadav et al. [11] isolated 21 endophytic fungal isolates from *Eugenia jambolana* Lam in India and screened their ability to produce TPC. They found that their TPC varied from 4.20 to 60.13 mg/g of dry weight. Also, they observed the highest level of TPC was in the extract of *Chaetomium* sp. (60.13 mg) followed by *Aspergillus niger* (58.46 mg). Recently, Kumaresan et al. [30] studies the antioxidant potential of four endophytic fungi and reported that the TPCs in the extract of *Chaetomium* sp., *Curvularia* sp. *Colletotrichum* sp. and *Trichoderma* sp. were 28.5, 9.82, 10.63, and 7.51 mg/g dry weight, respectively. Sugiharto et al. [38] reported that the TPCs of *Acremonium charticola* and *Rhizopus oryzae* isolated from the Indonesian fermented dry cassava were 26.25 ± 0.39 and 16.08 ± 0.16 mg/100 g, respectively.

3.3. Flavonoids

The amount of total flavonoids compounds was determined as the naringenin equivalent using an equation obtained from a standard naringenin graph. The results in this study appeared that the tested fungal strains have the ability to produce flavonoids with levels of 0.166 to 68.806 mg/ml (Tables 1-3).

The highest flavonoid producers (formed ≥ 35 mg/ml) were only five of tested fungal strains of endophytes (1), entomopathogens (1) and saprophytes (3) shows in (Tables 1-3). Out of the 26 tested strains of endophytic fungi, only *Pleospora tarda* AUMC 8871 was recorded as highly producer strain and formed 61.826 ± 0.003 mg/ml followed by *Aspergillus oryzae* AUMC 8863 formed 19.346 ± 0.01 mg/ml as in (Table 1). The highest entomopathogenic strain was *Epicoccum nigrum* AUMC 3148 (recorded 36.626 ± 0.07 mg/ml) flowed by *Aspergillus flavus* AUMC 9881 (formed 29.406 ± 0.04 mg/ml) in (Table 2), while the highest three strains of saprophytic fungi were *Penicillium roquefortii* AUMC 6398, *Lecanicillium antillanum* AUMC 9905 and *Aspergillus terrus* AUMC 3102 which recorded 64.806 ± 0.08 mg/ml 45.686 ± 0.2 mg/ml and 45.606 ± 0.06 mg/ml, respectively (Table 3).

Similar results were obtained by several researchers. Kumaresan et al. [30] reported that all tested endophytic fungi had the ability to produce flavonoids in varied quantity ranged from 3.08 and 11.83 mg/g dry weight. Smith et al. [39] examined 10 species of filamentous fungi for their antioxidant capacity and reported that the total flavonoid compounds of the ten species were in the range of 0.02-3.90 mg/g. Sugiharto et al. [38] found that the total flavonoids of *Acremonium charticola* and *Rhizopus oryzae* isolated from the Indonesian fermented dried cassava were 2.92 ± 0.15 and 10.87 ± 0.37 mg/100 g dry weight, respectively.

4. CONCLUSION

The present study demonstrates the ability of fungi belonging to endophytes, entomopathogenes as well as saprophytes to produce compounds having significant antioxidant activities. High levels of total phenolic and flavonoid compounds were

recorded in the extracts of most tested fungal strains specially those belonging to endophytes. Production of these compounds by fungi will be helpful in the biotechnological mass production of safe alternative sources of antioxidants for incorporation into some food products and supplements preventing many free radical mediated diseases and healthy cosmetics.

AUTHORS' CONTRIBUTION

All authors contributed in design and execution the research plan point to point. Also, they contributed in writing, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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