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A potential antimicrobial, extracellular enzymes, and antioxidants resource: Endophytic fungi associated with medicinal plants

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Abstract

This study aimed for the production of antimicrobial, extracellular enzymes and antioxidants by endophytic teleomorphic Ascomycota associated with medicinal plants. A total of eleven teleomorphic species were isolated from four medicinal plant species. *Chaetomium grande* and *Sordaria fimicola* were the most frequently isolated species and represented by 12 (Chg1-Chg12) and 7 (Sf1-Sf7) isolates respectively. The minimum inhibitory concentration (MIC) of all the isolates was determined against nine reference strains of bacteria and fungi. Effectiveness of 100-300µg/ ml DEMSO then in H2O of the ethyl acetate fractions of the most effective two isolates Chg 5 and Sf 3 on the tested reference strains revealed a different inhibitory effecst. Saturated disc of Streptomycin and Rifampin (0.165mg/ml) were used for bacteria and amphotericin B and fluconazole were used for yeasts and fungi as a positive control. Enzymatically, Chg 5 isolate considered as a resource of amylase, cellulase, protease, lipase, and chitinase. however, Sf3 isolates considered as a resource of amylase, laccase, and chitinase out of six screened enzymes. Total phenolics (TP), total flavonoids (TF) and antioxidant activity of the Sf3 and Chg 5 extracs were measured. The TP values were expressed as milligram gallic acid equivalents per gram of dry extract of Sf3 and Chg 5, which equal to 53.9±0.35 and 97.9±0.48 respectively. TF present in both Sf3 and Chg5 isolates extracts with values equal to 2.44±0.01 and 7±0.05 respectively expressed as routine equivalents. *in vitro*, the antioxidant activity of the extracts was investigated using DPPH radical-scavenging assay, and equal to 0.06% and 0.39% respectively in the extract of both taxa.

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Introduction

Fungi from extreme environments are considered as good potential candidates for the isolation of new bioactive compounds (Chávez *et al* ., 2015) and this interest has been reflected in the gradual increase of published articles reporting new compounds from these fungi in Egypt (Abdel-Azeem *et al*., 2012; Abdel-Azeem and Salem, 2012; Salem and Abdel-Azeem, 2014; Selim *et al*., 2014; Selim *et al*., 2016; Abdel-Azeem *et al*., 2016; Abdel-Azeem *et al*., 2018a). Out of about 1 million natural products around 25% are biologically active, of these; about 60% come from plants. (Newman and Cragg, 2007; Demain, 2014). Endophytic fungi are symbiotically associated biota of living plant tissues that induce symptomless disease to their hosts (Petrini, 1991, Cohen, 2006) .In the beginning of this century pharmacological and pharmaceutical studies have been directed to endophytic fungi (Strobel and Daisy, 2003) e.g. antimicrobial, antitumor, anti-inflammatory, liver curative and antiviral activities (Ali *et al*., 2008; Liu *et al*., 2008).

In Egypt, about 55 species and one variety of the genus Chaetomium have been recorded till now. (Blanchette *et al*., 2017; Abdel-Azeem *et al*., 2018b). *Chaetomium* has attracted the attention of researchers as an important genus in Ascomycota because of the variety of biological and biotechnological applications of this species in different areas (Abdel-Azeem *et al*., 2016). To the best of our knowledge, more than 200 compounds, associated with unique and diverse structural types have been isolated and chemically identified from the genus *Chaetomium*(Fujimoto *et al*., 2004; Jiao *et al*., 2004; Bashyal *et al*., 2005; Kobayashi *et al*., 2005; Ding *et al*., 2006; Isham *et al*., 2007).

Genus *Sordaria* Ces. & De Not. includes approximately 10 species, except for *S. humana* and *S. fimicola* that are cosmopolitan species and are frequent in soil, while the remaining species have a coprophilic habitat (Guarro *et al*., 2012) . Different secondary metabolites e.g. sordarin, sordarin B, sordariol, neosordarin, tricyclic uronic acid, immunosuppressive constituents were studied from different species of *Sordaria* by several investigators. (Hauser and Sigg, 1971; Bouillant *et al*., 1989; Schneider *et al*.,1995; Okada *et al*., 1995; Fujimoto *et al*.,1999; Davoli *et al*., 2002; Weber *et al*., 2005). Only 3 species of *Sordaria* were recorded in Egypt (Moustafa and Abdel-Azeem., 2011).

Some important medicinal plant species were widely distributed in arid Sinai, Egypt. It has been reported to possess many medicinal properties. (Hanafi and Abdel-Wahab., 2000; Hameed *et al*., 2015). Some endophytic fungi can produce the same bioactive compounds as their host plants (Stierle *et al*., 1993; Zhao *et al*., 2010). Due to the previous demonstration, further researches on medicinal plants and the isolated endophytes must be done to fill the gap in this area , to conserve the medicinal plant as possiple, and to employ the isolated endophytes as a resources of prospective valuable components, Thus, this study was accompanied to isolate different species of endophytes from arid Sinai, Egypt., and to to evaluate antioxidant , antimicrobial activities and valuable enzymes.
of the endophytic fungi as a prospective sources.

Materials and methods

Study area, sampling, and isolation of endophytic fungi

One hundred samples from the four plant species under investigation were collected from Wadi Itlah (28°58'72.3" N, 33°92'01.7" E, 1385m above sea level), Wadi Tala (28°34'02.3" N, 33°55'55.8" E, 1450-1670m above sea level, Wadi El-Arbaein (28°54'54" N, 33°55'36" E, 1385-1859m above sea level) and Gebel Ahmar (28°52'83" N, 33°61'83" E, 1892m above sea level) respectively. Aerial parts (25 sample/ plant) from *Artemisia herba-alba* Asso; *Chiliadenus montanus* (Vahl) Brullo; *Origanum*

syriacum L.; and *Verbascum sinaiticum* Benth. were sampled according to (Salem and Abdel-Azeem., 2014). For isolation of endophytic microfungi, small parts of the shoot system of each plant were surface-sterilized according to (Abdel-Azeem and Salem., 2012) Sterilized pieces were plated out on 400 plates of different isolation media (Potato Dextrose Agar, Czapek's Yeast Extract Agar, Malt Extract Agar, Czapek's Yeast Extract Agar) supplemented with bacteriostatic Rosebengal and bacteriocidal chloramphenicol and incubated at 28°C.

Phenotypic identification

Phenotypic identification of recovered microfungi was primarily based on the relevant identification keys for *Penicillium* (Pitt, 1979); *Aspergillus* (Klich, 2002). Dematiaceous hyphomycetes (Ellis 1971, 1976). *Fusarium (*Leslie and Summerell, 2006) different taxa (Domsch *et al*., 2007) ascomycetes of soil. (Guarro *et al*., 2017) *Chaetomium* and *Alternaria* (Simmons, 2007) Taxonomic position, assignments and name corrections of all recovered taxa were checked against the Index Fungorum website database (Kirk, 2018).

Molecular identification

As potent isolates, Chg5 and Sf3 were molecularly identified. DNA of single spore culture was extracted by adapted chloroform procedure (Arenz and Blanchette, 2011). The internal transcribed spacer (ITS) region of ribosomal DNA was targeted for PCR amplification with the primers ITS1 and ITS4 for large subunit amplification. PCR amplifications were done using Amplitaq Gold PCR Master-mix (Applied Biosystems, Foster City, CA) and 1 ml of template DNA using the following parameters: 94°C for 5 min, 35 cycles of 94°C for 1 min, 50 °C for 1 min, 72°C for 1 min, and a final extension step of 5 min at 72°C. PCR amplicons were visualized on a 1% agarose gel using SYBR green 1 (Life Technologies, Grand Island, NY) prestain and a Dark Reader DR45 transilluminator (Clare Chemical Research, Denver, CO). Primers used for PCR were used for sequencing reactions on automated DNA sequencer (Model 3100; PerkinElmerInc/Applied Biosystems-Bioneer, South Korea), according to the manufacturer's protocol. Consensus sequences were assembled using Geneious 9.0 (Kearse *et al*., 2012) and compared to those in Gen Bank using BLASTn for identification.

Extraction of active metabolites

Isolates of *Ch. grande* and *S. fimicola* under investigation were grown on Oat Meal Agar at 28°C for 15 days. Each taxon was prepared by inoculation in 2L Erlenmeyer flasks containing 1L autoclaved potato dextrose broth and shaking at 180 rpm at 28°C for 21 days. The fermentation broth of each species was filtered and fresh mycelia were washed three times with distilled water and stored in a freezer. Ethyl acetate was used for the extraction of active metabolites. The filtrate was extracted three times with equal volumes of ethyl acetate and collected separately (aqueous and organic phases). The frozen mycelia were ground and extracted three times in the organic solvent, and combined with organic extracts of the filtrate and evaporated till dryness under reduced pressure according to the procedures outlined by (Salem and Abdel-Azeem 2014) After evaporation, the dried extract was stored in darkness in a refrigerator until further use. For antimicrobial studies, a freshly prepared solution of solid metabolites was applied through re-suspension in the DMSO solution and water.

Antimicrobial screening

The following test microorganisms were used for screening of antimicrobial activities: *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 6629, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Shigella flexneri* ATCC 12022, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC10231, *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus niger* ATCC 16404.

The bacterial and fungal pathogens were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The agar well diffusion method was used to test the antimicrobial effects of the tested fungal isolates extract against the tested pathogenic microbes, via measuring the diameter of the inhibition zone. Concisely, the hole-plate diffusion method consisted of performing a uniform spread of the tested isolates suspension (about 100 µL, corresponding to Mc Farland standard 2) on Potato dextrose agar plate followed by the making of wells of 6 mm diameter on labeled positions using cork borers and filling particular wells with 100 µL (corresponding to 100, 200 and 300 µg/ ml of DEMSO then repeated by water as a solvent per well of Chg 5 and Sf3 prepared extract). Saturated disc of Streptomycin and Rifampin (0.165mg/ml) were used for bacteria and also amphotericin B and fluconazole were used for yeasts and fungi as positive control while Water and DMSO served as the negative control (Valgas *et al*., 2007). The plates were incubated at 37°C and inhibition zones were observed after 24h for bacteria and *C. albicans* and 72h for fungi. Triplicates of each sample were used. Both antibacterial and antifungal activities were determined by measuring the diameter of the inhibition zone mean value ± standard error.

Extracellular enzymes

Both taxa were screened for production of six extracellular enzymes by plate assay method and were assessed by placing 5 mm mycelial plugs on solid media with substrates specific to the respective enzyme, starch for amylase, carboxymethyl cellulose for cellulase, guiacol for laccase, olive oil for lipase, colloidal chitin for chitinase and gelatin for protease respectively (Kouker and Jaeger 1987; Maria *et al*., 2005; Sharaf *et al*., 2012; Pavithra *et al*., 2012). After incubation at room temperature for 5 days, the plates were examined for the presence of a clear zone in the agar around the colony, indicating extracellular enzyme activity. Amylase and protease activities were detected by staining the plates with Lugol's solution and Coomassie brilliant blue solution, respectively. Relative enzyme activity (RA) was calculated according to (Krishnan *et al*., 2014).

Total phenolic, flavonoid and antioxidant activity

Total phenolic content of organic extracts of both taxa was estimated using Folin-Ciocalteau reagent based assay using Gallic acid as standard (Oliveira *et al*., 2008). The total flavonoid content was determined by the aluminum chloride colorimetric method (Chang *et al*., 2002). The percentage of antioxidant activity of both isolates was assessed by 1, 1 diphenyl-2-picryl hydrazyl-(DPPH) free radical assay according to (Shiban *et al*., 2012).

Statistical analysis

Statistical analyses were performed using the IBM-SPSS statistical software version 23. A one-way analysis of variance was used to determine whether a significant difference existed between the treated groups and the positive control. Data were expressed as mean values of three replicates and differences were considered statistically significant if $P < 0.05$.

Ethics Statement

All participants gave their verbal, written and informed consent to participate in the study after they were verbally read all the elements of written consent. Samples were collected under the permission of the Saint Katherine Protectorate (permit no. 15/2017) for scientific purposes and no endangered species were involved in the study. All relevant data are within the paper.

Results and discussion

Study area, sampling, and isolation of endophytic fungi

During the present study of endophytic fungi hosted four plant species in Saint Katherine protectorate we recovered 387 CFU from all plant segments.

Our observations showed that 46 species belonging to 20 genera were isolated and assigned to 2 phyla, 3 classes, 7 orders, and 10 families. Order Eurotiales accommodates the greatest range of species (16 species) followed by, Hypocreales and Pleosporales (9 species each) and Sordariales (8 species). Other orders were represented by one or two species. *Chiliadenus montanus* and *Origanum syriacum* are by far the richest plant by showing a spectrum of 25 species, followed by *Artemisia herba-alba* (24 species). *Verbascum sinaiticum* considered the poorest plant by recorded 16 species. Regarding sociability of endophytic fungal species, i.e., association with specific host plant, the results showed that while some taxa are likely able to exist on more than one plant species such as *Alternaria alternata, Chaetomium globosum,* and *Nigrospora oryzae,* others showed clear tendency for a restricted occurrence, like *Sordaria fimicola* which restricted to *Origanum syriacum* and *Chaetomium grande* to *Verbascum sinaiticum.* The ordination showed that some species are not specific in their occurrences such as *Chaetomium globosum*, *Aspergillus niger, Alternaria alternate, Trichoderma harzianum* and *Penicillium chrysogenum* which are common to a wide range of plants (Table 1).

The sequences obtained from *C. grande* and *S. fimicola* isolates were 542 and 553 bp in length respectively. *C. grande* and *S. fimicola* sequences were checked against the NCBI database using the BLAST homology search. Native *C. grande* and *S. fimicola* were deposited in the GenBank database under accession number MF787599 and MF787600 respectively.

Table 1. Total count of fungal taxa recovered from plants under investigation on isolation media at 28°C.

Species	*Artemisia herba-alba*	*Chiliadenus montanus*	*Origanum syriacum*	*Verbascum sinaiticum*	Total
Teleomorphic taxa					
Chaetomium bostrychodes Zopf.	0	2	3	0	5
Ch. brasiliense Bat. & Pontual	1	0	0	0	1
Ch. globosum Knuze	2	3	1	1	7
Ch. grande Asgari & Zare	0	0	0	12	12
Ch. nigricolor L.M. Ames	0	1	2	0	3
Ch. piluliferum J. Daniels	3	0	0	0	3
Ch. senegalense L.M. Ames	0	1	2	0	3
Emericella nidulans (Eidam) Vuill.	1	0	3	0	4
Eurotium chevalieri L. Mangin	0	1	0	0	1
Microascus trigonosporus C.W. Emmons & B.O. Dodge	1	0	1	0	2
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	0	0	9	0	9
Anamorphic taxa					
Acremonium alternatum Link	1	0	0	1	2
A. murorum (Corda) W. Gams	0	0	2	1	3
A. rutilum W. Gams	1	0	0	2	3
Alternaria alternata (Fr.) Keissl.	5	5	5	3	18
A. atra (Preuss) Woudenb. & Crous	5	2	4	4	15
A. botrytis (Preuss) Woudenb. & Crous	0	1	0	0	1
Alternaria tenuissima (Kunze) Wiltshire	0	2	1	0	2
Aspergillus alliaceus Thom & Church	2	0	0	1	3
A. candidus Link	0	0	2	0	2
A. flavus Link	2	10	14	0	26
A. fumigatus Fresen.	1	0	0	0	1
A. japonicus Saito	0	0	1	0	1
A. niger Tiegh.	8	28	31	11	87
A. terreus Thom	1	11	0	0	12
A. versicolor (Vuill.) Tirab.	0	0	2	0	2
Cladosporium cladosporioides (Fresen.) G.A. de Vries	2	3	2	0	7
Curvularia lunata (Wakker) Boedijn	0	0	5	0	5
Drechslera australiensis Bugnic. ex M.B. Ellis	0	0	0	2	2
D. hawaiiensis Bugnic. Ex Subram. & B.L. Jain	0	1	2	1	4
D. spicifer (Bainier) Arx	0	1	0	0	1
Embellisia phragmospora (Emden) E.G. Simmons	0	0	0	1	1
Fusarium oxysporum Schltdl.	8	0	4	2	14
F. solani (Mart.) Sacc.	0	5	0	0	5

Species	*Artemisia herba-alba*	*Chiliadenus montanus*	*Origanum syriacum*	*Verbascum sinaiticum*	Total
Mucor hiemalis Wehmer	8	0	0	0	8
Nigrospora oryzae (Berk. & Broome) Petch	5	21	10	10	46
Paecilomyces variotii Bainier	0	2	0	0	2
Penicillium brevi-compactum Dierckx	0	4	4	0	8
P. chrysogenum Thom	6	6	4	3	19
P. citrinum Thom	5	0	0	0	5
P. notatum Westling	0	2	0	0	2
P. rubrum Stoll	2	0	0	0	2
Sarocladium strictum (W. Gams) Summerb.	0	1	0	0	1
Stachybotrys chartarum (Ehrenb.) S. Hughes	1	2	4	0	7
Trichoderma harzianum Rifai	1	2	0	0	3
T. viride Pers.	3	4	3	7	17

Antimicrobial activity

It was declared that the crude ethyl acetate extracts (100 µL) of taxa Sf3 and Chg 5 (corresponding to 100, 200 and 300) µg/ ml of DEMSO per well revealed an efficiently in suppressing the growth of eight tested isolates with variable strength. As indicated in Table (2).

The mean inhibition zone according to the Sf3 effect ranged from 1.37 to 2.50 on *S. aureus,* 2.10-2.40 on *E. coli,* 2.50-2.67 on *B. cereus.* 8.50-9.00 on *S. flexneri,* 3.33-3.60 on *S. Typhimurium,* 2.27-3.27 on *C. albicans,* 0.00-2.33 on *A. brasiliensis* and 0.00-2.47 on *A. niger* indicating a remarkable antimicrobial effect when compared with that of the positive control of bacteria and fungi, According to Chg 5 isolate effect the mean inhibition zone ranged from1.27 to 1.47 on *S. aureus ,* 1.47-1.77 on *E. coli,* 2.07-2.60 on *B. cereus.* 8.50-9.03 on *S. flexneri,* 3.47-3.73 on *S. Typhimurium,* 2.30-2.57 on *C. albicans,*0.00-2.60 on *A. brasiliensis,* and 0.00-2.37 on *A. niger,* revealed a remarkable antimicrobial effect when compared with that of the positive control of bacteria and fungi, however, the both isolate crude solvent extract had no effect on *K. pneumoniae*

Also in the present study, It was demonstrated that the crude aqueous extracts (100 µL) of both taxa Sf3 and Chg 5 (corresponding to 100, 200 and 300 µg/ ml of H2O per well revealed an efficiently in suppressing the growth of seven and eight isolates out of the tested nine isolates by Sf3 and Chg 5 respectively with variable strength. As indicated in Table 2.

Regarding the Sf3 isolate effect, the mean inhibition zone ranged from 2.13-2.33 on *E. coli,* 2.17-2.30 on *B. cereus.* 2.50-2.80 on *S. flexneri,* 3.37-3.60 on *S. Typhimurium,* 0.00-2.67 on *C. albicans,* 0.00-2.63 on *A. brasiliensis,* and 0.00-2.27 on *A. niger* indicating a notable antimicrobial effect when compared with that of the positive control of bacteria and fungi, however, no effect had shown on *S. aureus* and *K. pneumoniae* . Regarding the Chg 5 isolate the mean inhibition zone ranged from from 1.27 to 1.47 on *S.aureus* 2.50-2.63 on *E. coli,* 2.17-2.63 on *B. cereus.* 2.40-2.47 on *S. flexneri,* 3.07-3.23 on *S. Typhimurium,* 0.00-2.37 on *C. albicans,* 0.00-1.87 on *A. brasiliensis,* and 0.00-2.43 on *A. niger* indicating a notable antimicrobial effect when compared with that of the positive control of bacteria and fungi, however, no effect had shown on *K. pneumoniae..*

Regarding the *in vitro* study, the results manifested that both the solvent and the aqueous extract of Sf3 and Chg 5 isolates gained a strong antimicrobial activity against the tested pathogenic bacteria and fungi in a dose-dependent manner. In comparing the tested extract with the standard positive control antibiotic Rifampin and Streptomycin and the Antifungal positive control Fluconazole and Amphotericin it revealed their significant antimicrobial activities.

The increasing prevalence of fungi and bacteria that resistant to some drug, lead to search for new natural components that have a potent antimicrobial effects and low sides effects on humans.

The variety of the biologically active components (novel bioactive secondary metabolites) with potential employment in the medical and agricultural fields extracted from endophytes led to extensive focus and research on these organisms (Bilal *et al*., 2018).

From the given data it was demonstrated that both taxa Sf3 and Chg5 exhibited different strengths of antimicrobial activities which may be attributed to the extraction procedure culture condition and the test strain used for the antimicrobial analysis (Hoffman *et al*., 2008). In many studies Chaetomium is genus of ascomycete reported to produce a lot of bioactive compounds as reported by Zhangb *et al*., 2012; Wang *et al*., 2017. Studying the secretion of antimicrobial components by promising endophytic fungi gained a lot of attention due to the increasing resistance rate of pathogenic bacteria and fungi to the most antibiotics and antifungal. and also as a result of the serious side effects of both the synthetic antibacterial and the antifungal (Hoffman *et al*., 2008). The current study will be concerned with the effect of some endophytic fungal extract on some pathogenic bacteria and fungi, which give inhibitory effects against them with different degrees. This confirmed by Ananda *et al*., 2012 who reported that the endophytes become novel sources of antimicrobial components. and the beneficial role of these endophytes may be due to increasing the resistance of plant host immunity to the different pathogenic fungi and bacteria . Noteworthy there are many studies reported with the antimicrobial compounds produced by endophytes in cultures that were active against plant and human pathogenic microorganisms (Pandey *at al.,* 2004; Ogundare *et al*., 2006 Chareprasert *et al*., 2006).

With the finding of our study , it was previously reported by Ibrahim *et al.* (2018) that the naturally extracted fungal metabolites possessed remarkable antibacterial, antifungal, antioxidant, radical scavenging.

Table 2. Antimicrobial effects of *Chaetomium grande* and *Sordaria fimicola* fungi against different bacterial and fungal species.

Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	Fungal strains	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%

Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%	97.9 ±0.48	7.0±0.05	*Sordaria fimicola*	0.39%
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	*Fungal strains*	Reducing power
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%	53.9±0.35	2.44±0.01	*Chaetomium grande*	0.06%

Extracellular enzymatic potential

Both endophytes *Chaetomium grande* and *Sordaria fimicola* display an extracellular enzymatic potential with a variable degree as demonstrated in Table. 3. where *Chaetomium grande* had the activity of Amylase RA= 1.6, Cellulose RA= 1.5, Protease RA= 1.4, Lipase RA= 0.9 and Chitinase RA= 1.3 secretion . *Sordaria fimicola* had the potential of Amaylase RA= 1.9 Laccase RA= 1.2 and Chitinase RA= 1.9 secretion.

From the given data it was declared that both taxa Sf3 and Chg 5 exhibited different strengths of enzymatic activity and so it considered as rich sources of enzymes that are used in the large scale in medicine and industry. knowledge of the types, amounts, and characteristics of enzymes produced by the endophytic fungi would help for industrial requirements.

Studied taxa must gain attention because they considered a rich important origin of enzymes that are more stable at different temperature ranges and diverse pH than enzymes derived from plants and animals (Mari *et al*., 2005) and it is helpful in agriculture, industries (food processing, production of beverages, textiles and leather industry) and human health.

Recently, enzymes producer endophytic fungi have become very fascinating resources of some valuable enzymes that used in a lot of applications in life sciences. Amylase has an industrial and agricultural use, and active in a wide range of temperatures and pH, Proteases are used in medical application especially in diabetic patient's treatment(Ananda *et al*., 2012). The extracellular cellulase used in industrial applications of the paper industry and lipase used fats as energy sources (Amirita *et al*., 2012). Endophytic fungi differ in their ability to secrete enzymes, and their activity is controlled by many factors as climatic states and geographical places.

Table 3. Relative enzyme activities (RA) of *Chaetomium grande* and *Sordaria fimicola.*

Fungal strains	Reducing power					
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	Fungal strains	Reducing power	Total phenolic mg/mL
Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml	Fungal strains	Reducing power	Total phenolic mg/mL
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01	Chaetomium grande	0.06%	53.9±0.35

Total phenolic, flavonoid and antioxidant activity

Total phenolics (TP), total flavonoids (TF) and the antioxidant activity of the Sf3 and Chg5 isolates extracts were represented in Table 4. The TP values were expressed as milligram gallic acid equivalents per gram of dry extract of Sf3 and Chg5 isolates which equal to 53.9±0.35 and 97.9±0.48 respectively.

TF present in both Sf3 and Chg5 isolates extract with a value equal to 2.44±0.01 and 7±0.05 respectively. expressed as routine equivalents. In the present study, *in vitro* antioxidant activity of the extracts was investigated using DPPH radical-scavenging assay, and equal to 0.06% and 0.39% in both taxa respectively.

Antioxidants have to pay attention to their effects in preventing disease due to their oxidative stress, moreover, the epidemiological studies display that reactive oxygen and nitrogen species could damage the human body. Therefore it is important to increase the intake of antioxidants in the human diet. But it was demonstrated that the synthetic antioxidants might exhibit toxicity with carcinogenic potential and also has a low efficiency than natural antioxidants (Lob *et al*., 2010). So it is critical to obtain natural antioxidant with low cost.

Table 4. Total phenolic compounds, flavonoids and reducing power as antioxidant activities of *Chaetomium grande* and *Sordaria fimicola.*

Fungal strains	Reducing power	Total phenolic mg/mL	Flavonoids mg/ml
Chaetomium grande	0.06%	53.9±0.35	2.44±0.01
Sordaria fimicola	0.39%	97.9 ±0.48	7.0±0.05

In the present study it was detected a phenolic and flavonoids compounds in the Sf3 and Chg5 isolates extracts,. which are recognized to achieve several functions in plants, and may display many pharmacological beneits as effective agents in prevention and treatment of various diseases (Shehab *et al*., 2015). Also, the scavenging capability of DPPH free radical is commonly used to explore the antioxidant property of the tested extracts (Moukette *et al*., 2015).

Our finding was confirmed with other studies that Endophytic fungi are previously reported as resources of ample bioactive compounds and secondary metabolites. These bioactive compounds and secondary metabolites have applications in biological control. Thus, there is an urgent to extract and identify natural components that have more economical and effective antioxidants characters (Mathew and Abraham, 2006, Kumar and Chattopadhaya, 2007 Chandra and Arora, 2009) Oduntan *et al*., 2017.

Conclusion

The present study declared that the endophytic teleomorphic Ascomycota *Chaetomium grande* and *Sordaria fimicola* hosted medicinal plants in Saint Katherine Protectorate, Egypt extract acts as an antimicrobial agent., the principal mechanisms of action may rely on its antioxidant potential. Thus, the extracted natural components are recommended as antimicrobial agents against some pathogenic fungi and bacteria. The extracted natural components considered as another source of safe antioxidants for integration into some foodstuffs and supplements and also in preventing many free radical-mediated diseases and used in healthy cosmetics. Beside that they acquire an enzymatic activity, which recommended to be used in biotechnology. The present study recommended further and continuous research on endophytic fungi to detect ,purify and identify more of the bioactive compounds.

Conflicts of interest

The authors have no conflicts of interest to disclose.

References

Abdel-Azeem AM, Blanchette RA, Held BW. 2018b. New record of *Chaetomium grande* Asgari & Zare (Chaetomiaceae) for the Egyptian and African mycobiota. Phytotaxa **343(3),** 283-288.

Abdel-Azeem AM, Omran MA, Mohamed RA. 2018a. Evaluation of the curative probability of bioactive metabolites from endophytic fungi isolated from some medicinal plants against paracetamol-induced liver injury in mice. LAP LAMBERT Academic Publishing. ISBN: 978-613-9-89820-6.

Abdel-Azeem AM, Salem FM. 2012. Biodiversity of laccase producing fungi in Egypt. Mycosphere **3(6),** 900-920.

Abdel-Azeem AM, Salem FM, Mohamed HM, Rashad HM, Mohamed RM, Khalil WF. 2012. Bioprospecting of Egyptian fungi for ecological and medical applications. TWAS-ARO 8th meeting, Bibilotheca Alexandrina, December 30-31.

Abdel-Azeem AM, Zaki SM, Khalil WF, Makhlouf NA, Farghaly LM. 2016. Anti-rheumatoid activity of secondary metabolites produced by endophytic Chaetomium globosum. Frontiers in Microbiology **7(1477),** 1-11.

Aly AH, Edrada-Ebel R,Wray V, Muller WEG, Kozytska S, Hentschel U. 2008. Bioactive metabolites from the endophytic fungus *Ampelomyces* sp. Isolated from the medicinal plant *Urospermum picroides*. Phytochemistry **69,** 1716-1725. DOI: 10.1016/j.phytochem.02.013

Amirita A, Sindhu P, Swetha J, Vasanthi NS, Kannan KP. 2012.Enumeration of endophytic fungi, from medicinal plants and screening of extracellular enzymes. Wold J Sci Technol **2,**13-9.

Ananda K, Pavithra LN, Sathish L. 2012. Antimicrobial and enzyme activity of Endophytic fungi isolated from Tulsi. J Pharm Biomed Sci **16,** 1-6.

Arenz B, Blanchette R. 2011. Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys. Soil Biol. Biochem **43,** 308-315. https://doi.org/10.1016/j.soilbio.2010.10.016

Bashyal BP, Wijeratne EM, Faeth SH, Gunatilaka AA. 2005. Globosumones A-C, cytotoxic orsellinic acid esters from the Sonoran desert endophytic fungus *Chaetomium globosum*. J. Nat. Prod **68,** 724-728.

Blanchette RA, Held BW, Abdel-Azeem AM. 2017.New record of *Chaetomium iranianum* MF787598 (Chaetomiaceae) for the Egyptian and African mycobiota. Microbial Biosystems Journal **2(2),** 6-9.

Bouillant ML, Bernillon J, Favrebonvin J, Salin N. 1989. New hexaketides related to sordariol in *Sordaria-macrospora*. Z Naturforsch C **44,** 719-723.

Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robison R, Condron MAM, Teplow DB, Stevens D, Yaver D. 2002. Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562 endophytic on *Kennedia nigriscans.* Microbiology **148(9),** 2675-2685.

Chandra P, Arora DS. 2009. Antioxidant activity of fungi isolated from soil of different areas of Punjab, India. J Appl Nat Sci **1,** 123-128.

Chang C, Yang M. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal **10,** 178-182.

Chareprasert S, Piapukiew J, Thienhirun S, Whalley A, Sihanonth P. 2006. Endophytic fungi of teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr. World J. Microbiol. Biotechnol **22,** 481-486.

Chávez R, Fierro F, García-Rico RO, Vaca I. 2015. Filamentous fungi from extreme environments as a promising source of novel bioactive secondary metabolites. Front Microbiol **6,** 903. Published Sep 9. DOI: 10.3389/fmicb. 00903

Cohen SD. 2006. Hosts electivity and genetic variation of *Discula umbrinella* isolates from two oak species: Analyses of *Intergenic equences* of ribosomal DNA. Microb. Ecol. **52,** 463-469. DOI: 10.1007/s00248-006-9073-5.

Davoli P, Engel G, Werle A, Sterner O, Anke T. 2002. Neosordarin and hydroxysordarin, two new antifungal agents from *Sordaria araneosa*. Journal of spacer regions Antibiotics **55,** 377-382.

Demain AL. 2014. Importance of microbial natural products and the need to revitalize their discovery. J Int. Microbiol Biotechnol **41,** 185-201.

Ding G, Song YC, Chen JR, Xu C, Ge H M, Wang X T. 2006. Chaetoglobosin U, acytochalasan alkaloid from endophytic *Chaetomium globosum* IFB-E019. J. Nat. Prod. **69,** 302-304.

Domsch KH, Gams W, Anderson TH. 2007. Compendium of Soil Fungi (2. ed., ta). Eching: IHW-Verl.

Ellis MB. 1976. More dematiaceous hyphomycetes. Kew, Eng: Commonwealth Mycological Institute.

Ferreira MC, Vieira MLA, Zani CZ, Alves TMA, Sales Junior PA, Murta SMF, Romanha AJ, Gil LHVG, Carvalho AGO, Zilli JE, Vital MJS, Rosa CA, Rosa LH. 2015. Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). Biochemical Systematics and Ecology **59,** 36-4.

Fujimoto H, Fujimaki T, Okuyama E, Yamazaki M. 1999.Immunomodulatory constituents from an ascomycete, *Microascus tardifaciens*. Chem. Pharm. Bull. (Tokyo) **47,** 1426-1432.

Fujimoto H, Sumino M, Okuyama E, Ishibashi M. 2004. Immunomodulatory constituents from an ascomycete, *Chaetomium seminudum.* J. Nat. Prod **67,** 98-102.

Guarro J, Gene J, Stchigel AM, Figueras MJ. 2012. Atlas of soil ascomycetes. Issue 10 of CBS biodiversity series, Holland.

Hameed I H, Hussein H J, Kareem M A, Hamad N S. 2015. Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography-mass spectrometry (GC-MS). J. Pharm. Phytoth **7,** 107-125.

Hanafi Y, Abdel-Wahab M. 2000. Wild medicinal plants in Sinai. Arabian Gulf of Est. Egypt. (In Arabic) 337pp.

Hauser D, Sigg HP. 1971. Isolierung und A babu von Sordarin. Helv. Chim. A cta **54,**1187-1190.

Hoffman M, Mayer SG, Strobel GA, Hess WM, Sovocool GW, Grange AH, Kelley-Swift EG. 2008. Purification, identification and activity of phomodione, a furandione from endophytic Phoma species. Phytochem **69,** 1049-56.

Ibrahim SRM, Mohame GA, Al Haidari RA, El-Kholy AA, Zayed MF, Khayat MT. 2018. Biologically active fungal depsidones: Chemistry, biosynthesis, structural characterization, and bioactivities. Fitoterapia **129,** 317-365. [CrossRef].

Isham CR, Tibodeau JD, Jin W, Xu R, Timm MM, Bible KC. 2007. Chaetocin: A promising new antimyeloma agent within vitro and in vivo activity mediated via imposition of oxidative stress. Blood **109,** 2579-2588.
DOI: 10.1182/ blood-07-027326.

Jiao W, Feng Y, Blunt JW, Cole AL, Munro MH. 2004. Chaetoglobosins Q, R, and T, three further new metabolites from Chaetomium globosum. J. Nat.Prod **6,** 1722-1725.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P. Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28,** 1647-1649.

Klich MA. 2002. Identification of common *Aspergillus* species. Centralbureau voor Schimmelcultures, Utrecht, Netherlands, 116 pp.

Kobayashi M, Kanasaki R, Sato I, Abe F, Nitta K, Ezaki M. 2005. FR207944, an antifungal antibiotic from *Chaetomium* sp. 217I. Taxonomy, fermentation, and biological properties. Biosci. Biotechnol. Biochem **69,** 515-521.
DOI: 10. 1271/bbb.69.515

Kouker G, Jaeger KE. 1987. Specific and sensitive plate assay for bacterial lipases. Appl Environ Microbial **53,** 211-3.

Krishnan A, Alias SA, Wong CMVL. 2011. Extracellular hydrolase enzyme production by soil fungi from King George Island, Antarctica. Polar Biology **39,** 65-76.

Kumar A, Chattopadhaya S. 2007.DNA damage protecting activity and antioxidant potential of Pudina extracts. Food Chem **100,** 1377-1384.

Leslie JF , Summerell BA. 2006. The *Fusarium* laboratory manual. 1st ed. Ames, Iowa: Blackwell Publishing.

Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou J. 2008.Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methyl coumarin. Appl. Microbiol. Biotechnol **78,** 241-247. DOI: 10.1007/s00253-007-1305-1

Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health Pharmacogn Rev. Jul-Dec **4(8),** 118-126. DOI: 10.4103/0973-7847.70902

Maria GL, Sridhar KR, Raviraja NS. 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. Journal of Agricultural Technology **1,** 67-80.

Mathew S, Abraham TE. 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum Verum*) bark extracts, through various in vitro models. Food Chem **94(4),** 520-528.

Moustafa AF, Abdel-Azeem, AM. 2011. An annotated check-list of Ascomycota reported from soil and other terricolous substrates in Egypt. Journal of Basic and Applied Mycology **2,** 1-27.

Moukette BM, Pieme CA, Njimou JR, Biapa CPN, Marco B, Ngogang JY. 2015. In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a nontimber forest product used as spice: *Monodora myristica.* Biol Res **48(1),** 15.

Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat.Prod **70,** 461-477. DOI: 10.1021/np068054v

Oduntan AO, Akinfasoye JA, Fasoyiro SB. 2017. Ascorbic Acid, Total Phenolic, Flavonoid and Antioxidant Activity of Two Cultivars of *Basella alba*. Food Science and Technology **5(4),** 92-96.

Ogundare AO, Adetuyi FC, Akinyosoye FA. 2006. Antimicrobial activities of *Vernonia tenoreana*. Afr. J. Biotechnol **5(18),** 1663-1668.

Okada H, Kamiya S, Shina Y, Suwa H, Nagashima M, Nakajima S, Shimokawa H, Sugiyama E, Kondo H, Kojiri K, Suda H. 1998. BE-31405, a new antifungal antibiotic produced by *Penicillium minioluteum*. I. Description of producing organism, fermentation isolation, physico-chemical and biological properties. Journal of Antibiotics **51,** 1081-1086.

Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L, Pereira JA. 2008. Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food Chem Toxicol. Jul 46(7), 2326-31.
DOI: 10.1016/ j.fct.2008.03.017.Epu Mar 2.

Pandey B, Ghimire PA, grawal VP. 2004. Studies on the antibacterial activity of the actinomycetes isolated from the Khumbu region of Nepal. J. Biol. Sci. **23,** 44-53.

Pavithra N, Sathish L, Kulal A. 2012. Antimicrobial and enzyme activity of endophytic fungi isolated from tulsi. J. Pharm Biomed Sci **16(16),**1-6.

Petrini O. 1991. Fungal endophytes of tree leave. In: Andrews JH, Hirano SS, editors. Microbial ecology of leaves. New York: Springer. 179-197.

Pitt JI.1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London; New York: Academic Press.

Salem FM, Abdel-Azeem AM. 2014. Screening of anticancer metabolites produced by endophytic fungi. LAP LAMBERT Academic Publishing. ISBN 98-3-659-53697-7.

Schneider G, Anke H, Sterner O. 1995. Xylarin, an antifungal *Xylaria* metabolite with an unusual tricyclic uronic acid moiety. Natural Products Letters **7,** 309-316.

Selim KA, El-Beih AA, Abdel-RahmanTM, El-Diwany AI. 2014. Biological evaluation of endophytic fungus, Chaetomium globosum JN711454, as potential candidate for pimroving drug discovery. Cell Biochem.Biophys **68,** 67-82.

Selim KA, El-Beih AA, Abdel-Rahman TM, El-Diwany AI. 2016. High expression level of antioxidants and pharmaceutical bioactivities of endophytic fungus Chaetomium globosum JN711454. Preparative biochemistry & biotechnology **46,** 131-140.

Sharaf EF, El-Sarrany AEQ, El-Deeb M. 2012. Biorecycling of shrimp shell by *Trichoderma viride* for production of antifungal chitinase. Af. J. Microbiol. Res **6(21),** 4538-4545.

Shehab NG, Abu-Gharbieh E, Bayoumi FA. 2015. Impact of phenolic composition on hepatoprotective and antioxidant effects of four desert medicinal plants. BMC Complement Altern Med **15(1),** 401.

Shiban MS, Al-Otaibi MM. 2012. Antioxidant Activity of Pomegranate (*Punica granatum* L.) Fruit Peels. Food Nutr Sci **3,** 991-996.

Simmons EG. 2007. *Alternaria*: an Identification manual. CBS biodiversity series 6. Utrecht: Centraalbureau voor Schimmelcultures.

Souza ADL, Rodrigues-Filho E,Souza AQL,Pereira JO ,Calgarotto AK,Maso V. 2008. Koninginins, phospholipase A2 inhibitors from endophytic fungus *Trichoderma Koningii.* Toxicon **51,** 240-250. DOI: 10.1016/j.toxicon.09.009

Stierle A, Strobel G, Stierle D.1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science **260,** 214-216.

Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews **67(4),** 491-502.

Valgas C, de Souza SM, Smânia EFA, Smânia AJR. 2007. methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology **38(2),** 369-380.

Wang XY, Yan X, Fang MJ, Wu Z, Wang D, Qiu YK. 2017. Two new cytochalasan derivatives from *Chaetomium globosum* SNSHI-5, a fungus derived from extreme environment. Nat. Prod. Res **31,** 1669-1675. [CrossRef] [PubMed].

Weber RW, Meffert A, Anke H, Sterner O. 2005. Production of sordarin and related metabolites by the coprophilous fungus *Podospora pleiospora* in submerged culture and in its natural substrate. Mycol Res **109(5),** 619-26.

Zhao J, Zhou L, Wang J, Shan T, Zhong L, Liu X. 2010. Endophytic fungi for producing bioactive compounds originally from their host plants, in Current Research, Technology and Education Topics in Applied Microbiology Microbial Biotechnology, ed. M.A. Vilas (Badajoz: Formatex Research Center, 567-576.

Zhangb Q, Li HQ, Zong SC, Gao JM, Zhang AL. 2012. Chemical and bioactive diversities of the genus Chaetomium secondary metabolites. Mini-Rev. Med. Chem **12,** 127-148. [CrossRef].