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Original article

Isolation, identification and anti-candidal activity of filamentous fungi from Saudi Arabia soil

Nouf M. Al-Enazi [a], Amani S. Awaad [b,*], Monerah R. Al-Othman [c], Nour K. Al-Anazi [d], Saleh I. Alqasoumi [e]

[a] Biology Department, College of Science and Humanity Studies, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia
[b] Pharmacognosy Department, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia
[c] Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia
[d] Pharmaceutics Department, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia
[e] Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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ABSTRACT

Ten fungal strains; namely, *Penicillium melinii*, *Petriella setifera*, *Aspergillus pseudo-niger*, *Alternaria chlamydospora*, *Pythium nayoroense*, *Phoma glomerata*, *Mucor ramosissimus*, *Mucor racemosus*, *Fusarium chlamydosporum* and *Rhizopus azygosporus* were isolated from soil. The extra- and intra-cellular extracts of the fungal strains grown on malt extract and yeast-extract sucrose media were screened for their anticandidal activity against different clinically-isolated *Candida* species. Most of the fungal extracts showed activity against different *Candida* species. However, the fungal strains grew on malt extract showed greater activities than those grew on yeast extract sucrose media. The activity of the intracellular was higher than the extracellular metabolites. All fungal extracts (extra and intra) were similar in chemical constituent; they contained carbohydrates and/or glycosides, unsaturated sterols and/or triterpens, tannins and traces of coumarins. Alkaloids, flavonoids, saponins, anthraquinones and cardenolides were no detected. The intra-cellular extracts contained more compounds than the extra-cellular extracts.

1. Introduction

Soils are very complex, having numerous constituents performing different functions mainly due to the activity of soil organisms (Ullah et al., 2017; Raja et al., 2017; Kostadinova et al., 2009). The microorganisms plays significant role in soil ecosystem. The soil quality is determining by microbial composition and functioning changes during decomposition of organic matter, recycling of nutrients and biological control (Stefanis et al., 2013). Fungi are very vital for the soil ecosystem since they play a key role in different essential processes including elemental release by mineralization and organic matter decomposition (Christensen, 1989). Moreover, the fungi are responsible for the decomposition of organic compounds and their activity contributes in the bio-deterioration and biodegradation of toxic substances in the soil (Rangaswami and Bagyaraj, 1998).

Fungi; eukaryotic microorganisms, can occur as unicellular (yeasts), filamentous (molds) form. Fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases. Yeasts are microscopic fungi consisting of single cells that reproduce by budding while molds, in contrast, occur as long filaments known as hyphae, which grow by apical extension (Aggarwal, 2010; Baron, 1996). Generally, soil is an oligotrophic habitat for fungi because the fungal growths are limited and readily present for short periods in a restricted zone. Accordingly, fungi are either dormant, or metabolize and grow very slowly utilizing a range of organic molecules (Ratna Kumar et al., 2015). Fungi are playing a significant role in the daily life of human beings in addition to their participation in industry, agriculture, medicine, food industry, bioremediation, natural cycling, bio-fertilizers and other ways leading to human welfare (Karthikeyan et al., 2014; Dick, 2009; Kirk, 2004).

Fungi produce many antibiotics, having antibacterial and antifungal activity, which are widely used as drugs over the world especially the penicillin, cephalosporin and fusidic acid (Dobashi et al., 1998). The recent decades are characterized by the novel discoveries of microorganisms capable of producing compounds,

* Corresponding author at: P.O. Box 173, Riyadh 11942, Saudi Arabia.
E-mail address: amaniawaad@hotmail.com (A.S. Awaad).
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as a potential source of new antibiotics (Ullah et al., 2017). Knowing this information in mind, the present study aimed at determining the diversity of fungi in the soil of Al-Qassim governorate, Saudia Arabia and making an assessment of their anticandidal activity.

2. Material and methods

2.1. Fungal isolation and identification

2.1.1. Fungal isolation

2.1.1.1. Samples collection. Soil samples from different places in Al-Qassim region, KSA, were collected after 10–15 cm deep pits dug. The samples were collected in sterile zipper polythene bags and stored at 4 °C until used.

2.1.1.2. Isolation of fungi. Different media including potato dextrose, czapek's dox, malt extract, and yeast extract sucrose and yeast malt extract agar media were used. Sprinkle plates were used as isolation techniques. Sprinkle plates were prepared by uniformly distributing the soil directly on the surface of the medium. The plates were incubated for 5–7 days at 25 °C. Fungi growing on the agar plates were purified by single spore and hyphal-tip technique and transferred to malt extract slants and then maintained as a stock culture.

2.1.2. Fungal identification

The isolated fungi were identified to the genus and the species level on the basis of their morphological characters and microscopic analysis by using suitable media, slide cultures (obtained by inoculating microfungi directly on a small square of agar

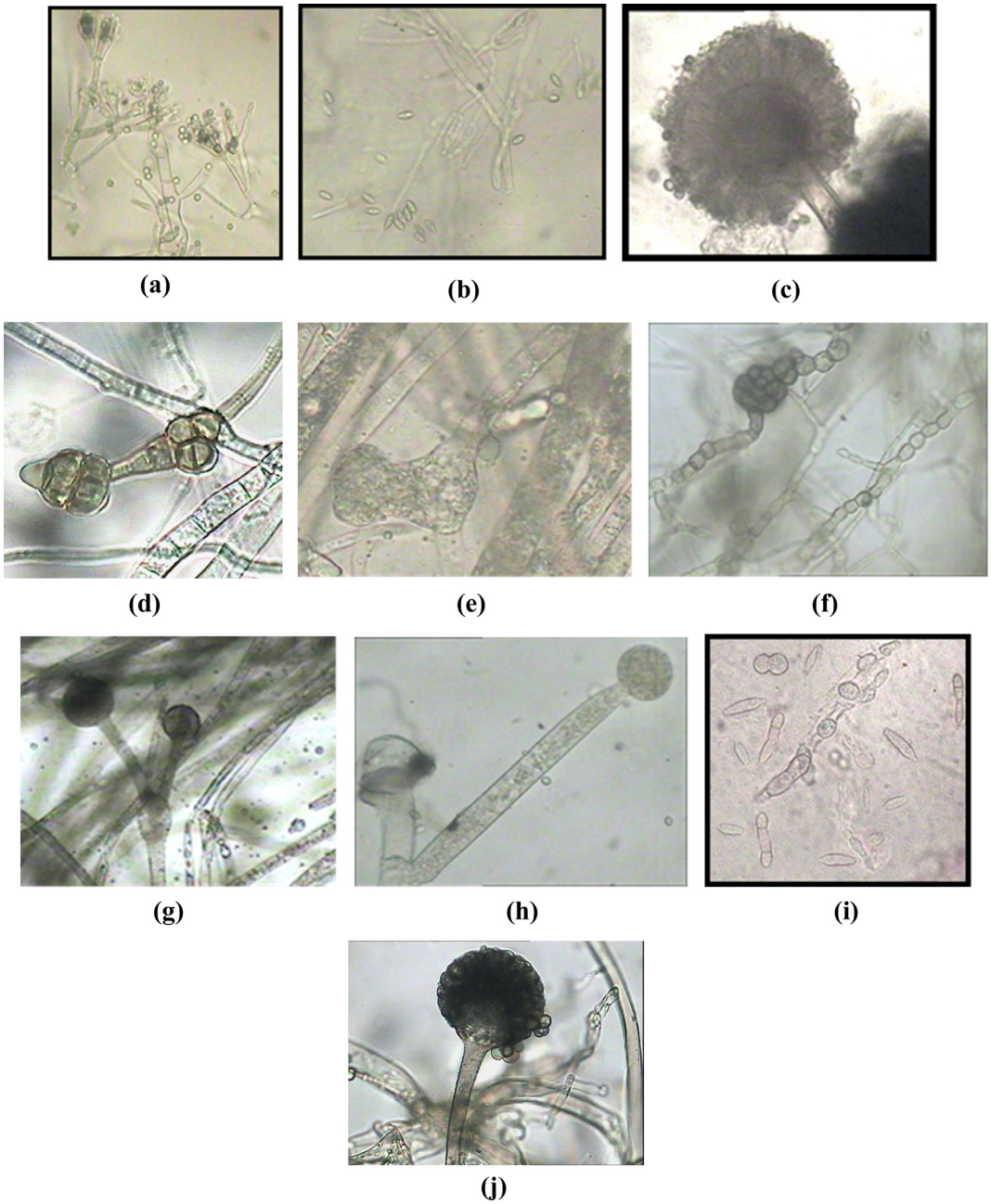

Fig. 1. The vegetative and reproductive structures of fungal isolates; *Penicillium melinii* (a), *Petriella setifera* (b), *Aspergillus pseudo-niger* (c), *Alternaria chlamydospora* (d), *Pythium nayoroense* (e), *Phoma glomerata* (f), *Mucor ramosissimus* (g), *Mucor racemosus* (h), *Fusarium chlamydosporum* (i) and *Rhizopus azygosporus* (j).

medium) and the most taxonomic guides and standard procedures (Juan et al., 2014; Casero et al., 2017)) .

2.2. Extraction of fungal materials

2.2.1. Mycelial mat and media

For obtaining the mycelial mat, 9 mm diameter discs of agar media (using 9 mm diameter sterile cork borer) containing the fungal materials were picked up from the margin of actively growing colonies and each disc was transferred into 100 ml liquid medium; in 250 ml conical flasks. The flasks were then incubated at 30 °C for 14 days. Mycelia were harvested by filtration using Buchner funnel. Then the mycelia were washed thoroughly with distilled water and the excess water was removed by plotting with filter papers. Both mycelia and the growth media obtained were kept for extraction.

2.2.1.1. Fungal mycelial mat extraction. The mycelial mat (100 g) for each fungi separately was harvested, washed with distilled water, extracted by refluxing in boiled methanol (2 L) for 2 h, and then filtered off. The mycelial residue was re-extracted again for three times. The combined filtrates were concentrated under reduced pressure at temperature not exceeding 35 °C. The obtained residues were kept for investigation.

2.2.1.2. Fungal growth medium extraction. The growth medium (1 L) was extracted by n-butanol (2 L). This step was repeated until complete extraction for each fungus. The butanolic extract was filtered on anhydrous sodium sulphate. The combined filtrates were subjected to concentration using reduced pressure at temperature not exceeding 35 °C. The obtained residues were kept for investigation.

2.2.2. Phytochemical screening and chromatographic investigation

2.2.2.1. Phytochemical screening. Each fungal extract (extra and intra) were separately subjected to phytochemical screening according to the standard published methods (Tiwari et al. 2011).

2.2.2.2. Chromatographic investigation. Each fungal extracts (extra and intra) was chromatographically investigated using three systems a, b and c, system (a) ethyl acetate: methanol: water 90: 5:4 v/v/v, system (b) chloroform: methanol 95: 5 v/v and system (c) benzene: ethyl acetate 86: 14 v/v. Visualization of the spots were carried out under UV before and after sprays with antimony trichloride ($SbCl_3$).

2.3. Anticandidal activity

2.3.1. Test organisms

Ten clinically isolated Candida species; Candida albicans, C. dubliniensis, C. famata, C. glabrata, C. inconspicua, C. kefyr, C. krusei, C. norvegensis, C. parapsilosis and C. tropicalis were used as test organisms.

2.3.2. Anticandidal screening

The anticandidal activity of total alcoholic extracts of the isolated fungi (both mycelia mat & growth medium) were determined by using two methods Disc-diffusion method (Akinyeye et al., 2014) and well-diffusion method (Zain et al., 2012). Petri dishes containing 20 ml of malt extract agar medium were seeded with 48-h cultures of candidal inoculums. Wells (6 mm in diameter) were cut off from the agar, and 50 μL of fungal extracts were added and incubated at 37 °C for 24–48 h. The anticandidal activity was determined by measurement of the diameter of the inhibition zone formed around the well.

3. Results and discussion

3.1. Isolation and identification of fungal strains

A total of 21 isolates were obtained from the analyses of different soil samples taken from the area of Al-Qassim governorate, KSA through soil sprinkle-plate techniques. Ten strains were isolated from agar plates incubated at 28 °C for 7 days. All fungal isolates were obtained in pure cultures by single conidial transfer onto beer agar plates. The fungal isolates were identified as *Penicillium melinii*, *Petriella setifera*, *Aspergillus pseudo-niger*, *Alternaria chlamydospora*, *Pythium nayoroense*, *Phoma glomerata*, *Mucor ramosissimus*, *Mucor racemosus*, *Fusarium chlamydosporum* and *Rhizopus azygosporus* (Fig. 1). It is known that the soil serves as a reservoir for many microorganisms which plays major role in soil ecosystem (Stefanis et al., 2013).

3.2. Phytochemical screening and chromatographic investigation

The phytochemical screening of all fungal extracts (extra and intra) showed that all extra-cellular extracts were similar in chemical constituent; they contained carbohydrates and/or glycosides, unsaturated sterols and/or triterpens, tannins and traces of coumarins. There were no saponins, flavonoids, anthraquinones, alkaloids and cardenolides (Table 1). On the other hand, the thin layer

Table 1
Phytochemical screening of the extra- and intra- cellular extracts of the isolated fungi.

Test	Fungi																			
	Pen. mul.		*Pet. seti.*		*Asp. pseu.*		*Alt. chl.*		*Pyth. nay.*		*Ph. glom.*		*Muc. ram.*		*Muc. race.*		*Fus. chl.*		*Rhiz. azyg.*	
	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in
Alkaloids	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbohydrates and /or glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saponins	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tannins	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Unsaturated sterols and/or Triterpens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Proteins and/or amino acids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins and lactones	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Cardenolides	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

+, present; –, absent; ±, traces; Pen. mul., *Penicillium melinii*; Pet. seti., *Petriella setifera*; Asp. pseu., *Aspergillus pseudo-niger*, Alt. chl., *Alternaria chlamydospora*; Pyth. nay., *Pythium nayoroense*; Ph. glom., *Phoma glomerata*; Muc. ram., *Mucor ramosissimus*; Muc. race., *Mucor racemosus*; Fus. chl., *Fusarium chlamydosporum*; Rhiz. azyg., *Rhizopus azygosporus*.

Table 2
Thin layer chromatography extra- and intra- cellular extracts of the isolated fungi.

Spot			Fungi																			
R_f	Col-our	Sys-tem	*Pen. mul.*		*Pet. seti.*		*Asp. pseu.*		*Alt. chl.*		*Pyth. nay.*		*Ph. glom.*		*Muc. ram.*		*Muc. race.*		*Fus. chl.*		*Rhiz. azyg.*	
			ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in
0.01	Brown	c	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
0.02	Brown	c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+
0.03	Blue	c	+	–	+	–	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	–
0.03	Brown	b	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–
0.04	Brown	b	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+
0.11	Blue	b	+	–	–	–	+	+	+	–	–	–	+	+	–	–	+	+	+	+	+	+
0.14	Blue	b	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	+	–	+	–	+
0.18	Blue	c	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
0.20	Blue	a	–	–	–	–	–	+	–	+	+	–	+	–	+	–	+	+	–	+	–	–
0.21	Blue	b	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	+	–	+	–	+
0.28	Blue	b	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	+	+	+
0.32	Green	c	–	+	–	+	–	+	–	+	–	+	–	–	–	+	–	+	–	+	–	+
0.41	Brown	c	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+	–	+	–	–
0.42	Blue	a	+	–	+	–	+	+	–	–	–	–	–	–	–	–	+	+	+	–	–	+
0.45	Blue green	c	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	+	+
0.55	Blue green	c	+	+	+	–	+	–	+	–	–	–	–	–	+	–	–	+	+	–	+	+
0.55	Blue	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
0.60	Blue green	b	–	–	–	+	–	+	–	+	–	–	–	+	–	–	–	+	–	–	–	–
0.62	Blue	a	–	–	–	+	+	+	–	–	–	+	–	–	–	–	+	+	+	+	–	–
0.67	Blue	b	–	–	–	+	–	+	+	–	–	–	+	+	+	+	–	–	+	+	–	–
0.70	Blue	b	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	+	–	+	–	+
072	Yellow	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
0.73	Yellow	a	+	–	+	–	–	–	+	–	+	–	+	+	+	–	+	+	+	+	+	+
0.73	Brown	b	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	+	–	–	–	–
0.74	Blue	b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
0.83	bLue	a	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–
0.94	Blue	c	–	+	–	+	–	–	–	+	–	–	–	–	–	+	–	+	–	–	–	–
0.97	Brown	c	–	+	–	–	+	–	–	–	+	–	+	+	–	–	+	+	–	–	–	+
0.99	Blue	c	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–

Pen. mul., *Penicillium melinii*; Pet. seti., *Petriella setifera*; Asp. pseu., Aspergillus *pseudo-niger*, Alt. chl., *Alternaria chlamydospora*; Pyth. nay., *Pythium nayoroense*; Ph. glom., *Phoma glomerata*; Muc. ram., *Mucor ramosissimus*; Muc. race., *Mucor racemosus*; Fus. chl., *Fusarium chlamydosporum*; Rhiz. azyg., *Rhizopus azygosporus*.

Table 3
Anticandidal activity of extra- and intra-cellular extracts of the isolated fungi grown on malt extract medium.

Candida species	Fungi Diameter of inhibition zone (mm)																			
	Pen. mul.		*Pet. seti.*		*Asp. pseu.*		*Alt. chl.*		*Pyth. nay.*		*Ph. glom.*		*Muc. ram.*		*Muc. race.*		*Fus. chl.*		*Rhiz. azyg.*	
	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in
Candida albicans	07	08	19	24	11	12	07	09	00	00	00	00	00	00	00	07	07	10	00	00
C. dubliniensis	07	07	15	18	07	10	00	09	00	07	00	00	07	08	00	07	07	09	00	00
C. famata	07	07	16	19	07	10	00	07	00	00	00	00	00	00	08	08	07	08	00	00
C. glabrata	07	09	15	19	08	10	07	07	00	00	00	00	00	00	07	07	00	08	08	08
C. inconspicua	07	07	15	17	00	09	07	07	00	00	00	00	00	00	00	07	07	09	00	00
C. kefyr	00	08	15	15	07	10	07	08	00	00	00	00	00	00	00	07	07	07	00	00
C. krusei	08	10	16	18	08	11	07	09	00	00	00	00	07	08	07	07	00	07	00	00
C. norvegensis	00	08	11	16	07	10	00	00	00	07	00	00	00	00	07	07	07	07	00	08
C. parapsilosis	07	09	13	19	10	15	07	07	00	00	00	00	00	07	00	08	07	07	00	07
C. tropicalis	00	07	15	20	00	09	07	07	00	00	00	00	00	07	00	00	00	07	00	07

Pen. mul., *Penicillium melinii*; Pet. seti., *Petriella setifera*; Asp. pseu., Aspergillus *pseudo-niger*, Alt. chl., *Alternaria chlamydospora*; Pyth. nay., *Pythium nayoroense*; Ph. glom., *Phoma glomerata*; Muc. ram., *Mucor ramosissimus*; Muc. race., *Mucor racemosus*; Fus. chl., *Fusarium chlamydosporum*; Rhiz. azyg., *Rhizopus azygosporus*.

chromatography (TLC) investigation revealed that the intra- cellular extracts contained more compounds than extra-cellular extracts (Table 2).

3.3. Anticandidal activity

The obtained fungal strains were screened for their anticandidal activity against different clinically-isolated *Candida* species. Each fungal strain was grown on Malt Extract (ME) and Yeast Extract Sucrose (YES) at 28 ± 2 °C for 14 days. Both the intra- and extra-cellular metabolites of the fungal isolates were screened for their anticandidal activity. The anticandidal activities of extracts of both mycelial mat (intracellular metabolites) and growth media (extracellular metabolites) of all the obtained fungal strains were determined (Tables 3–6). The obtained results revealed that the extract of majority of the fungal strains showed activity against different *Candida* species. The fungal strains grew on malt extract medium showed greater activities than those grew on yeast extract sucrose media. Moreover, the activity of the intracellular metabolites was higher than the extracellular ones (Tables 3–6).

The intracellular activity of metabolites produced by fungal isolates grown on malt extract showed that the highest activity was obtained by *Petriella setifera* against *Candida albicans* (24 mm), *C. tropicalis* (20 mm),and *C. famata*, *C. glabrata*, *C. parapsilosis* (19

Table 4
Anticandidal activity of extra- and intra-cellular extracts of the isolated fungi grown on yeast sucrose extract medium.

Candida species	Fungi Diameter of inhibition zone (mm)																			
	Pen. mul.		*Pet. seti.*		*Asp. pseu.*		*Alt. chl.*		*Pyth. nay.*		*Ph. glom.*		*Muc. ram.*		*Muc. race.*		*Fus. chl.*		*Rhiz. azyg.*	
	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in	ex	in
Candida albicans	00	00	08	11	08	08	00	07	00	00	00	00	00	00	00	00	00	00	00	00
C. dubliniensis	00	07	00	07	07	00	00	07	00	00	00	00	08	00	00	00	00	00	00	00
C. famata	00	07	00	00	07	00	00	07	00	00	00	00	00	00	00	00	00	07	00	07
C. glabrata	08	00	00	13	00	07	00	00	00	07	07	00	00	00	00	07	07	00	00	00
C. inconspicua	00	00	07	11	00	07	00	07	00	00	00	00	00	00	00	00	00	07	00	00
C. kefyr	00	00	07	12	07	00	00	00	00	00	00	00	00	00	00	00	00	07	00	07
C. krusei	00	00	07	12	07	07	00	00	00	00	00	00	00	00	00	00	07	07	00	00
C. norvegensis	00	00	07	09	00	08	00	00	00	07	09	00	00	00	00	07	07	07	00	00
C. parapsilosis	00	07	08	10	00	11	00	07	00	00	00	00	00	00	00	00	00	07	00	08
C. tropicalis	00	00	07	00	00	00	00	07	00	00	00	00	00	00	00	00	08	00	00	07

Pen. mul., *Penicillium melinii*; Pet. seti., *Petriella setifera*; Asp. pseu., *Aspergillus pseudo-niger*, Alt. chl., *Alternaria chlamydospora*; Pyth. nay., *Pythium nayoroense*; Ph. glom., *Phoma glomerata*; Muc. ram., *Mucor ramosissimus*; Muc. race., *Mucor racemosus*; Fus. chl., *Fusarium chlamydosporum*; Rhiz. azyg., *Rhizopus azygosporus*.

mm), All of the previously mentioned areas in mm, corresponds to the diameter of inhibition zone (Table 1). The extract of Aspergillus *pseudo-niger* exhibited a good anticandidal activity against *C. parapsilosis* (15 mm), *C. albicans* (12 mm), and *C. krusei* (11 mm).

The extracts of *Phoma glomerata* showed no anticandidal activity against all the investigated *Candida* species (Table 3). On the other hand, the extracts of most fungal isolates showed moderate anticandidal activities against the most of the *Candida* species. The current results revealed an anticandidal activity of the fungi isolated from soil, similarly, a huge number of antibiotics produced by filamentous fungi have been reported (Awaad et al., 2017; Ullah et al., 2017; Zain et al., 2014).

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References

Aggarwal, A., 2010. Medical Microbiology. International Scientific Publishing Academy. OUP 25, 384.

Akinyeye, A., Solanke, E., Adebiyi, I., 2014. Phytochemical and antimicrobial valuation of leaf and seed of Moringa olifera extracts. Int. J. Res. Med. Health Sci. 4 (6), 307–2083.

Awaad, A.S., AL-Mudhayyif, H.A., Al-Othman, M.R., Zain, M.E., El-Meligy, R.M., 2017. Amhezole, a novel fungal secondary metabolite from *Aspergillus terreus* for treatment of microbial mouth infection. Phytother. Res. https://doi.org/10.1002/ptr.5760.

Baron, S., 1996. Medical Microbiology. University of Texas Medical Branch at Galveston, Galveston (TX).

Casero, M.C., Ascaso, C., DiRuggiero, J., Meslier, V., Artieda, O., Quesada, A., Wierzchos, J., 2017. Biogeography at microscopic scale: diversity of endolithic microbial communities in icrohabitats of gypcrete from the Atacama Desert. In: Book of Abstracts VII International Conference on nvironmental Industrial and Applied Microbiology – BMW2017 Madrid (Spain), pp. 31–31.

Christensen, M., 1989. A view of fungal ecology. Mycologia 81, 1–19.

Dick, R., 2009. Lecture on Soil Fungus in Soil Microbiology, Personal Collection of R. Dick, The Ohio State University School of Environment and Natural Resources, Columbus, OH.

Dobashi, K., Matsuda, N., Hamada, M., Naganawa, H., Takita, T., Takeuchi, T., 1998. Novel antifungal antibiotics A and B: taxonomy, fermentation and isolation, physicochemical properties and biological activities. J. Antibiotics 41, 1525–1532.

Juan, Li., Hyde, K.D., Zhang, Ke-Qin., 2014. Methodology for Studying Nematophagous Fungi. Nematode-Trapping Fungi, pp. 13–40.

Karthikeyan, P., Kanimozhi, K., Senthilkumar, G., Panneerselvam, A., Ashok, G., 2014. Optimization of enzyme production in *Trichoderma viride* using carbon and nitrogen source. Int. J. Curr. Microbiol. App. Sci. 3 (1), 88–95.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.M., Lee, H., Trevor, J.T., 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169–188.

Kostadinova, N., Krumova, E., Tosi, S., Pashova, Angelova, M., 2009. Isolation and identification of filamentous fungi from island Livingston, Antarctica. Biotechnol. Biotechnolog. Equip. 23 (1), 267–270.

Raja, M., Praveena, G., William, S., 2017. Isolation and identification of fungi from soil in Loyola college campus, Chennai, India. Int. J. Curr. Microbiol. App. Sci. 6 (2), 1789–1795.

Rangaswami, G., Bagyaraj, D.J., 1998. Agricultural Microbiology. Prentice Hall of India Pvt Ltd, N Delhi.

Ratna Kumar, P.K., Hemanth, G.P., Shiny, N., Samuel, K.K., 2015. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal Srikakulam District. Int. J. Adv. Pharmacol. l4 (2), 484–490.

Stefanis, C., Alexopoulos, A., Voidarou, C., Vavias, S., Bezirtzoglou, E., 2013. Principal methods for isolation and identification of soil microbial communities. Folia Microbiol. (Praha) 58 (1), 61–68. https://doi.org/10.1007/s12223-012-0179-5.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, G., 2011. Phytochemical screening and extraction: a review. Int. Pharm. Sci., 98–106

Ullah, I., Khan, N.A., Jadoon, M.A., Hameed, U.R., Khan, H., Mujaddad, U.R., Hayat, A., Ali, S., Rehman, M., Khan, M.A., Maqsood, A., Anwar, S., 2017. Isolation and identification of different rhizospheres fungi of Mansehra region, Pakistan. J. Entomol. Zool. Stud. 5 (2), 437–442.

Zain, M.E., Awaad, A.S., Al-Othman, M.R., Alafeefy, A.M., El-Meligy, R.M., 2014. Biological activity of fungal secondary metabolites. Int. J. Chem. Appl. Biol. Sci. 1 (1), 14–22.

Zain, M.E., Awaad, A.S., Al-Outhman, M.R., El-Meligy, R.M., 2012. Antimicrobial activities of Saudi Arabia desert plants. Phytopharmacology 2, 106–113.