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Evaluation of the activity of crude extracts from semi-arid soil fungi against clinical yeasts and molds

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Abstract

In recent years, the incidence and severity of fungal diseases has increased, particularly in populations with a broad list of immunocompromised conditions. Moreover, the emergence of azole resistance is arising. For these reasons and the small number of available antifungal agents, searching for new and effective compounds with antifungal activity is mandatory. The aim of this study was to evaluate the antifungal activity of crude extracts from semi-arid soil fungi against strains of clinical molds and yeasts. For screening purposes crude extracts of fifteen isolates, corresponding to ten fungal species, were preliminarily explored against *Candida albicans* ATCC6414 by diffusion methodology. Special focus was placed on *Aspergillus tatenoi*, *Leiothecium ellipsoideum*, *Subplenodomus violicola* and *Trichoderma saturnisporum* extracts because of their preceding antifungal performance. Crude extracts of these species were once more examined and assayed against 54 yeast and 24 molds including the genera *Aspergillus*, *Candida* and *Cryptococcus*. Antifungal susceptibility testing by microdilution methodology was performed. Activity, as the percentage of clinical strains inhibited by different extract concentrations, ranged from 60.5% to 100% for *A. tatenoi*, *L. ellipsoideum* and *T. saturnisporum* extracts. On the other hand, *S. violicola* extract was active against all the strains tested with MIC values ≤ 0.25 µg/mL. This study dealt with active crude extracts; particularly, the extract from *S. violicola* has shown a potent and promising antifungal activity. The composition of the active fractions and the mechanisms of action involved remain to be studied and warrant further investigations.

Key words – antifungal activity – *Aspergillus* – *Candida* – crude extracts – minimal inhibitory concentration

Introduction

All living organisms synthesize chemical compounds that can be classified into primary and secondary metabolites. Sugars, lipids, proteins, and nucleic acids are considered as primary

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metabolites; they are abundant and essential for the development of living organisms. Secondary metabolites, also called natural products, correspond to low molecular weight structures and are generally found in low quantity. Fungi, plants, and bacteria are the major kingdoms of life with well-developed secondary metabolism (Bills & Gloer 2007).

About 500,000 natural products have been described to date, and 15,600 are of fungal origin (Bills & Gloer 2007. It is considered that there are approximately 5 million species of fungi in nature (Blackwell 2011, Hawksworth & Lücking 2017). This estimation makes fungal natural products a vast unexplored source of unique chemical structures that have been optimized by evolution in response to constant communication and adaptation in their habitats (Gloer 2007, Gamboa Angulo & de la Rosa García 2008).

Numerous compounds with therapeutic utility were isolated from ascomycetes: penicillins from *Penicillium chrysogenum*, cephalosporins from *Acremonium strictum* (currently *Sarocladium strictum*), cyclosporine from *Tolypocladium inflatum*, lovastatin from *Aspergillus terreus* among others (Bennett 1998). On the other hand, fungal endophytes that live within the organs of all vascular plants, without causing any harm to their host, produce secondary metabolites that can increase resistance and improve adaptation to the habitat of the plants (Hardoim et al. 2015). In the last years, other bioactive compounds have been reported from fungi with several properties and continue being investigated (Xu et al. 2006, Garrigues et al. 2018, Cai et al. 2020).

Soil, in particular, is an ecological niche widely studied as a reservoir for microorganisms that make biologically active natural products (Kumar et al. 2010). Most antibiotics and antifungals were isolated from common soil dwellers (Lihan et al. 2004). A high proportion of antimicrobial-producing fungal strains are isolated from environments with extreme conditions, being their survival related with suitable metabolism and strongly influenced by natural selection (Gloer 2007). For these reasons, microorganisms isolated from previously unexplored areas and/or extreme environments constitute an interesting choice for searching potential new bioactive metabolites (Phoebe et al. 2001).

In recent years, the incidence and severity of fungal diseases has increased, particularly in populations with a broad list of immunocompromised conditions, such as cancer, AIDS, solid-organ and hematopoietic stem cell transplantation (Shao et al. 2007, Castón-Osorio et al. 2008). Although the most common agents found are *Candida* or *Aspergillus*, other fungi difficult to treat, such as *Scedosporium*, *Fusarium*, *Lomentospora* among others, are increasingly isolated. Moreover, an emergence of azole resistance is arising. Besides, panresistant strains such as *C. auris* appeared, or some non-*Candida albicans* less susceptible to echinocandins such as *C. glabrata* have recently come forth as a therapeutic challenge (Shao et al. 2007, Tobudic et al. 2012, Dudiuk et al. 2014, Spivak & Hanson 2018). Treatments are based on using systemic conventional drugs like polyenes (nystatin, amphotericin B); azoles (fluconazole, itraconazole, isavuconazole, voriconazole, posaconazole); allylamines (terbinafine) or echinocandins (caspofungin, micafungin, anidulafungin) (Andriole 1999, Odds et al. 2003).

For the reasons described above and the small number of available antifungal agents, searching for new and effective compounds with antifungal activity is mandatory (Pfaller 2012, Kathiravan et al. 2012). The aim of this study was to evaluate the antifungal activity of crude extracts from semi-arid soil fungi against strains of clinical molds and yeasts.

Materials & Methods

Fungal strains

Fifteen fungal strains from semi-arid soils of Argentina were used; twelve of these isolates were heat resistant. Five g of soil of each sample were transferred to 100 mL of melted (45–50ºC) Malt Extract Agar (MEA, Oxoid CM0059) prepared with the addition of 50 ppm of chloramphenicol, and heated at 75ºC for 30 min. The mixture was plated into 150 mm glass Petri dishes and incubated at 30ºC for up to 30 d (Samson et al. 2000). Two strains were isolated by treatment of the soil with ethanol and transferred to Potato Carrot Agar (PCA) according to

Bills et al. (2004). One additional xerophilic strain was included. It was isolated spreading soil on the surface of Dichloran Glycerol Agar 18% (DG18), according to the methodology for isolation of xerophilic fungi (Pitt & Hocking 2009).

The isolates were identified at species level according to its macroscopic and micromorphological characteristics in Malt Extract Agar (MEA) and Oatmeal Agar (OA) following von Arx et al. (1988), Domsch et al. (2007), Guarro et al. (2012), Samuels et al. (1998), Samson et al. (2000), Boerema et al. (2004), Pitt & Hocking (2009), and Peterson et al. (2010). Table 1 details the species, the strain numbers, the isolation techniques used and geographical location of the soil-source samples. The strain selection for testing in this work was made considering publications where bioactive compounds were mentioned (Brian & Hemming 1947, Dennis & Webster 1971, Ghisalberti & Sivasithamparam 1991, Liang 2008, Reino et al. 2008) as well as species that are rarely isolated, for example the case of those that have been found in our country.

Table 1 Selected isolates for biological activity assays.

Species	Strain number	Isolation technique	Geographical location
Achaetomium luteum	192	EP	28°40′30″S, 66°30′2″W
Aspergillus laciniosus	022	HT	29°33′35″S, 64°52′56″W
Aspergillus tatenoi	222	HT	29°33′35″S, 64°52′56″W
Gilmaniella humicola	3821	HT	28°15′31″S, 66°08′47″W
Hamigera paravellanea	0525	HT	28°13′5″S, 66°22′41″W
Hamigera paravellanea	0416	HT	28°55′15″S, 66°08′46″W
Hamigera paravellanea	5721	HT	27°00′18″S, 66°21′35″W
Leiothecium ellipsoideum	0311	HT	29°30′41″S, 65°37′57″W
Leiothecium ellipsoideum	5311	HT	27°26′50″S, 66°24′26″W
Sordaria fimicola	104	EP	28°13′17″S 66°08′37″W
Subplenodomus violicola	0327	X	29°30′41″S, 65°37′57″W
Trichocladium pyriforme	021	HT	28°42′3″S, 65°46′83″W
Trichoderma saturnisporum	0352	HT	29°30′41″S, 65°37′57″W
Trichoderma saturnisporum	1019	HT	28°13′17″S 66°08′37″W
Trichoderma saturnisporum	0312	HT	29°30′41″S, 65°37′57″W

EP: ethanol pasteurization, HT: heat-resistant, X: xerophilic

Preparation of crude extracts

Isolates were grown in sterile rice (30 g of rice, 50 ml of water) for 15 days at 25°C. The cultures were extracted with 50 mL of ethyl acetate for 18 h with 50 ml of ethyl acetate, then filtered and dried on a rotary evaporator (35ºC). The dry extracts were resuspended in chloroform and quantitatively transferred to previously tared vials. They were dried again under a stream of nitrogen and the mass of each was determined using an analytical balance (OHAUS, ± 0.0001 g). The extracts were kept dry at -30ºC until use.

Preliminary screening for antifungal activity

Preliminary tests of the antifungal activity of the crude extracts against *Candida albicans* ATCC 6414 were performed by diffusion methodology according to the M44-A2 document (CLSI 2009). The weighted crude extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain stock solutions (s.s.). Dilutions were made in sterile distilled water to obtain final concentrations in a range of 5120-640 µg/ mL.

In vitro susceptibility testing of crude extracts against yeasts and molds

Crude extracts that showed activity by the preliminary screening were selected to perform the broth microdilution methodology. These were tested against 54 yeasts and 24 molds of clinical

origin (Table 2). The strains were isolated and maintained at the Ramos Mejía Hospital (Parasitology Unit, Mycology Section) in Buenos Aires, Argentina. Susceptibility testing for yeasts and molds were based on the Clinical and Laboratory Standards Institute (CLSI), M27A3 and M38-A2, respectively (CLSI 2008a, b). Briefly, RPMI 1640 medium with glutamine and without sodium bicarbonate (Gibco BRL, Life Technologies) buffered to pH 7.0 with 0.165 M morpholinopropanesulphonic acid (MOPS) (Sigma Chemical Co, St. Louis, MO, USA) were used. Isolates were cultured onto Sabouraud for 48 h at 37ºC (for yeasts) and Potato Dextrose Agar (PDA) slants at 35ºC for up to 7 d (for molds). Inocula were prepared to obtain a starting inoculum of 0.5–5 × 10^6 CFU/mL and dilutions were made in the media and after inoculation in the plates, to a final inoculum of 0.5–5 × 10^3 and 0.5–5 × 10^4 CFU/mL for yeast and molds respectively. Stock solutions of the extracts were prepared and both, the inoculum and the extracts were diluted to half their original concentration, being the final concentration of the extracts 256-0.25 µg/mL. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were quality control strains. MICs were read visually. Endpoints were defined as the lowest concentration of the extracts that showed 100-50% of inhibition compared with the growth control. Minimal fungicidal concentration (MFC) was established following the incubation time for the MIC determination. Thirty µL from each well with complete growth inhibition was inoculated onto SGA plates and incubated at 30ºC for up to 72 h. The MFC was defined as the lowest concentration of the drug at which there was either no growth or a growth up to seven colonies, which corresponds to a 99.9% kill (Pfaller et al. 2004). The methods should be adequately detailed or referenced to other work.

Table 2 Species, number, and origin of the studied strains

Species	Strain number	Origin
Aspergillus niger	75	ND
Aspergillus flavus	916	S
Aspergillus flavus	596	PF
Aspergillus flavus	593	NB
Aspergillus flavus	1115	NB
Aspergillus flavus	591	NB
Aspergillus flavus	1321	PNS
Aspergillus flavus	838	ND
Aspergillus flavus	ATCC 204304	SP
Aspergillus flavus	40	ND
Aspergillus flavus	1271	SP
Aspergillus flavus	592	PF
Aspergillus flavus	1273	SP
Aspergillus fumigatus	1519	ND
Aspergillus fumigatus	76	ND
Aspergillus fumigatus	ATCC 204305	SP
Aspergillus fumigatus	653	SP
Aspergillus fumigatus	1005	SP
Aspergillus fumigatus	812	TN
Aspergillus fumigatus	1100	SP
Aspergillus terreus	105	ND
Aspergillus terreus	108	ND
Aspergillus terreus	109	ND
Aspergillus terreus	110	ND
Candida albicans	6746	SP
Candida albicans	6846/1585	MS
Candida albicans	6708/1571	SP
Candida albicans	1517	MS
Candida albicans	1516	MS
Candida albicans	982879	ND
Candida albicans	982891	ND

Table 2 Continued.

Species	Strain number	Origin
Candida albicans	6878/1595	MS
Candida albicans	1532	SP
Candida albicans	1513	SP
Candida albicans	6527/1537	MS
Candida albicans	522	MS
Candida albicans	509	MS
Candida albicans	514	MS
Candida guilliermondii	6636/1566	S
Candida guilliermondii	02150	ND
Candida guilliermondii	21150	ND
Candida krusei	671	MS
Candida krusei	842	MS
Candida krusei	521	FN
Candida krusei	ATCC 6815	ND
Candida krusei	688	BAL
Candida krusei	827	SP
Candida parapsilosis	ATCC 22019	ND
Candida parapsilosis	6634/1565	TN
Candida parapsilosis	1543	MB
Candida parapsilosis	1545	FN
Candida parapsilosis	1552	MBAL
Candida parapsilosis	525	FN
Candida parapsilosis	544	FN
Candida parapsilosis	507	BAL
Candida parapsilosis	ATCC 90018	BC
Candida parapsilosis	547	U
Candida tropicalis	6784/1580	FN
Candida tropicalis	6800/1583	MS
Candida tropicalis	1515	S
Candida tropicalis	6846	ND
Candida tropicalis	1531	BC
Candida tropicalis	1542	TN
Cryptococcus neoformans	1424	CSF
Cryptococcus neoformans	1437	ND
Cryptococcus neoformans	1438	CSF
Cryptococcus neoformans	1421	BC
Cryptococcus neoformans	6641/1540	CSF
Cryptococcus neoformans	3145	ND
Cryptococcus neoformans	1534	CSF
Cryptococcus neoformans	28/1069	BC
Cryptococcus neoformans	25/1058	CSF
Cryptococcus neoformans	13/869	CSF
Cryptococcus neoformans	43/1312	CSF
Cryptococcus neoformans	44/1313	BC
Cryptococcus neoformans	6/554	CSF
Cryptococcus neoformans	39/1285	CSF
Cryptococcus neoformans	16/944	CSF

BAL: bronchoalveolar lavage, BC: blood culture, CSF: cerebrospinal fluid, FN: fingernail, MB: mucosa biopsy, MBAL: mini BAL, NB: nasal biopsy, MS: mouth swab, PF: pleural fluid, PNS: paranasal sinuses, S: skin, SP: sputum, TN: toenail, U: urine, ND = no determined

Results

Antifungal activity by diffusion screening against *Candida albicans* ATCC 6414 was

observed in the crude extracts from *A. tatenoi* (222), *S. violicola* (0327), *L. ellipsoideum* (0311) and *T. saturnisporum* (0312) (Table 3). For this reason, these extracts were selected to perform susceptibility testing.

Table 3 Diameters of inhibition halos produced by extracts of different concentration (µg/mL)

Extract source species	Strain number	Halo diameter (mm)			
		5120*	2560	1280	640
Achaetomium luteum	192	-	-	-	-
Aspergillus laciniosus	022	-	-	-	-
Aspergillus tatenoi	222	20	17	17	14
Gilmaniella humicola	3821	-	-	-	-
Hamigera paravellanea	0416	-	-	-	-
Hamigera paravellanea	0525	-	-	-	-
Hamigera paravellanea	5721	-	-	-	-
Leiothecium ellipsoideum	0311	20	12	10	8
Leiothecium ellipsoideum	5311	-	-	-	-
Sordaria fimicola	104	-	-	-	-
Subplenodomus violicola	0327	20	14	12	9
Trichocladium pyriforme	021	-	-	-	-
Trichoderma saturnisporum	0352	-	-	-	-
Trichoderma saturnisporum	1019	-	-	-	-
Trichoderma saturnisporum	0312	32	29	25	17

*: extracts concentration in µg/mL

The minimal inhibitory concentration (MIC) was performed following the CLSI guidelines (CLSI 2008a, b). The endpoint is defined as the lowest concentration of the drug tested that caused significant growth diminution, compared to the growth control. The determination of the endpoint depends on the antifungal and is fixed in relation to multicenter studies correlated with the clinical response. In the present work, crude extracts (extractive mixtures of unknown composition) were evaluated. For this reason, the MIC values that produce 50 and 100% inhibition of fungal growth were analyzed. Table 4 summarizes the in vitro susceptibilities of the 78 isolates tested to the four extracts as determined by the broth microdilution procedures. The data are presented as MIC ranges and geometric mean (Gm). In general, lower MICs values were observed for 50% of inhibition, being the highest activity for extracts obtained from of *S. violicola*. The MIC value for all the strains was <0.25 µg/mL for both 50 and 100% inhibition. Concerning with 50% of inhibition, *T. saturnisporum*, *A. tatenoi* and *L. ellipsoideum* were active against all yeasts and molds tested, being less active for *C. tropicalis*. *T. saturnisporum* extract showed the highest MIC values for this species, with a Gm = 294.07 µg/mL. Moreover, the three extracts mentioned were more active against *Aspergillus* spp. (Gm: <0.25-0.19 µg/mL) compared with *Candida* and *Cryptococcus* species. *C. neoformans* was more susceptible than *Candida* spp. (Gm 0.24-0.27 µg/mL).

High MIC values for the three extracts were observed for all the strains tested for 100% of inhibition, with the exception of *C. albicans* (Gm = 1.64-3.45), and *A. terreus* for *L. ellipsoideum* extract (Gm <0.25 µg/ml). For this crude extract, less activity was observed for *C. parapsilosis*, compared with the other *Candida* spp. (MIC range: 16-128 µg/ml). However, it was the most active extract against *C. albicans*.

The MIC distributions showed that most of the strains had MIC ≤0.25 µg/ml, considering 50% inhibition. The values were as follows: for *S. violicola* 100% of the strains; for *T. saturnisporum* 60.5% for *Candida* species, 86.7% for *C. neoformans*, 91.6% for *Aspergillus* species; for *L. ellipsoideum* 76.3% for *Candida* species, 86.7% for *C. neoformans*, 95.8% for *Aspergillus* species; for *A. tatenoi* 81.6% for *Candida* species, 86.7% for *C. neoformans*, 100% for *Aspergillus* species (Table 5).

Table 4 MIC for 50 and 100% inhibition (µg/mL) of different crude extracts in relation to yeasts and molds of clinical origin

Species	Extracts source															
	T. saturnisporum				*A. tatenoi*				*L. ellipsoideum*				*S. violicola*			
	50%		100%		50%		100%		50%		100%		50%		100%	
	R	Gm	R	Gm	R	Gm	R	Gm	R	Gm	R	Gm	R	Gm	R	Gm
C. albicans (n = 14)	<0.25-4	0.43	<0.25->256	3.45	<0.25->256	0.48	<0.25->256	2.44	<0.25-4	0.32	<0.25-64	1.64	<0.25	<0.25	<0.25	<0.25
C. guilliermondii (n = 3)	<0.25	n.d.	<0.25->256	n.d.	<0.25	n.d.	<0.25-128	n.d.	<0.25-128	n.d.	<0.25-25	n.d.	<0.25	n.d.	<0.25	n.d.
C. krusei (n = 6)	<0.25-256	0.50	8->256	57.20	<0.25-16	0.28	8->256	25.4	<0.25-8	0.28	4-64	22.63	<0.25	<0.25	<0.25	<0.25
C. parapsilosis (n = 10)	<0.25-128	0.47	2->256	181.02	<0.25-128	0.41	0.5->256	128	<0.25-32	0.35	32-128	48.50	<0.25	<0.25	<0.25	<0.25
C. tropicalis (n = 5)	64->256	294.07	128->256	388.02	125-512	13.93	128->256	388.02	<0.25-8	0.76	<0.25-64	12.13	<0.25	<0.25	<0.25	<0.25
C. neoformans (n = 15)	<0.25->256	0.27	64->256	445.72	<0.25-512	0.27	64->256	337.79	<0.25-32	0.24	<0.25->256	30.55	<0.25	<0.25	<0.25	<0.25
A. niger (n = 1)	128	n.d.	>256	n.d.	<0.25	n.d.	>256	n.d.	0.25	n.d.	>256	n.d.	<0.25	<0.25	<0.25	<0.25
A. flavus (n = 12)	<0.25-16	0.19	>256	512	<0.25	<0.25	64->256	430.54	<0.25-64	0.21	32->256	322.54	<0.25	<0.25	<0.25	<0.25
A. fumigatus (n = 7)	<0.25	0.13	8->256	282.65	<0.25	<0.25	256->256	463.73	<0.25	<0.25	32->256	231.87	<0.25	<0.25	<0.25	<0.25
A. terreus (n = 4)	<0.25	0.13	>256	512	<0.25	<0.25	16->256	215.27	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25

R: ranges
Gm: geometric mean

Given the observed values for *S. violicola* extract, the minimal fungicidal concentration (MFC) was performed. The range and Gm in µg/mL, respectively were: (<0.25->256) (4.6) for *C. albicans*; (0.5->256) (90.51) for *C. parapsilosis*; (0.5->256) (Gm = 128) for *C. krusei* and (<0.25) (<0.25) for *C. tropicalis*; (<0.25->256) (32.10) for *C. neoformans*; (<0.25->256) (271.22) for *A. flavus*; (<0.25->256) (156.91) for *A. fumigatus* and (1->256) (54) for *A. terreus*. From this data it is observed that fungistatic activity is exhibited.

A brief description of *S. violicola* is presented below because this extract was the most active and constitutes the first report of this species for Argentina.

Table 5 MIC distributions (50%) for the four extracts tested against *Candida* spp., *Cryptococcus neoformans* and *Aspergillus* spp. (µg/mL)

Extract	Strain	MIC (µg/ml)										
		≥256	128	64	32	16	8	4	2	1	0.5	≤0.25
T. saturnisporum	*C. albicans*							2	2	1	2	7
	C. guilliermondii											3
	C. krusei	1										5
	C. parapsilosis		1	1								8
	C. tropicalis	4		1								
	C. neoformans	1						1				13
	A. niger		1									
	A. flavus					1						11
	A. fumigatus											7
	A. terreus											4
A. tatenoi	*C. albicans*	1		1						2		10
	C. guilliermondii											3
	C. krusei					1						5
	C. parapsilosis		1			1						8
	C. tropicalis	2	1									2
	C. neoformans	1						1				13
	A. niger											1
	A. flavus											12
	A. fumigatus											7
	A. terreus											4
L. ellipsoideum	*C. albicans*						1	2			1	10
	C. guilliermondii											3
	C. krusei						1					5
	C. parapsilosis				1	1						8
	C. tropicalis						2					3
	C. neoformans				1		1					13
	A. niger											1
	A. flavus			1								11
	A. fumigatus											7
	A. terreus											4
S. violicola	*C. albicans*											14
	C. guilliermondii											3
	C. krusei											6
	C. parapsilosis											10
	C. tropicalis											5
	C. neoformans											15
	A. niger											1
	A. flavus											12
	A. fumigatus											7
	A. terreus											4

Subplenodomus violicola (P. Syd.) Gruyter, Aveskamp & Verkley, Stud. Mycol. 75: 23, 2012

Fig. 1

Colonies on Oatmeal Agar (OA), 25ºC, 7 days, in darkness, 53-54 mm diam., olive green to dark green, light brown aerial mycelium, reverse with the same color as the anverse; after additional 7 days, 25°C, under light cycles, (40 cm below cool white tubes, 8 h light, 16 h darkness), covering the whole culture plate. On MEA, 25ºC, 7 days, 44-50 mm diam., white or dark grey to greenish in color, light greyish edges, floccose; reverse brown black with reddish edges; after additional 7 days, 25°C, under light cycles, covering the whole culture plate (Fig. 1a-c).

Conidiomata pycnidial subglobose, 80-290 µm diam., mostly uni-ostiolate, papillate or with a cylindrical neck, mostly aggregated but sometimes solitary (Fig. 1d-f). Presence of micropycnidia in the aerial mycelium. Conidiogenous cells hyaline, ampulliform to doliiform, 6-7 µm long.

Conidia cylindrical, smooth, hyaline, 10-13 × 2-3 μm diam., usually biguttulate, but in some cases with 3 guttules, terminal to subterminal (Fig. 1i). Chlamydospores in irregular botryoid-alternarioid clumps, unicellular chlamydospores and pseudosclerotioid masses also present (Fig. 1g-h).

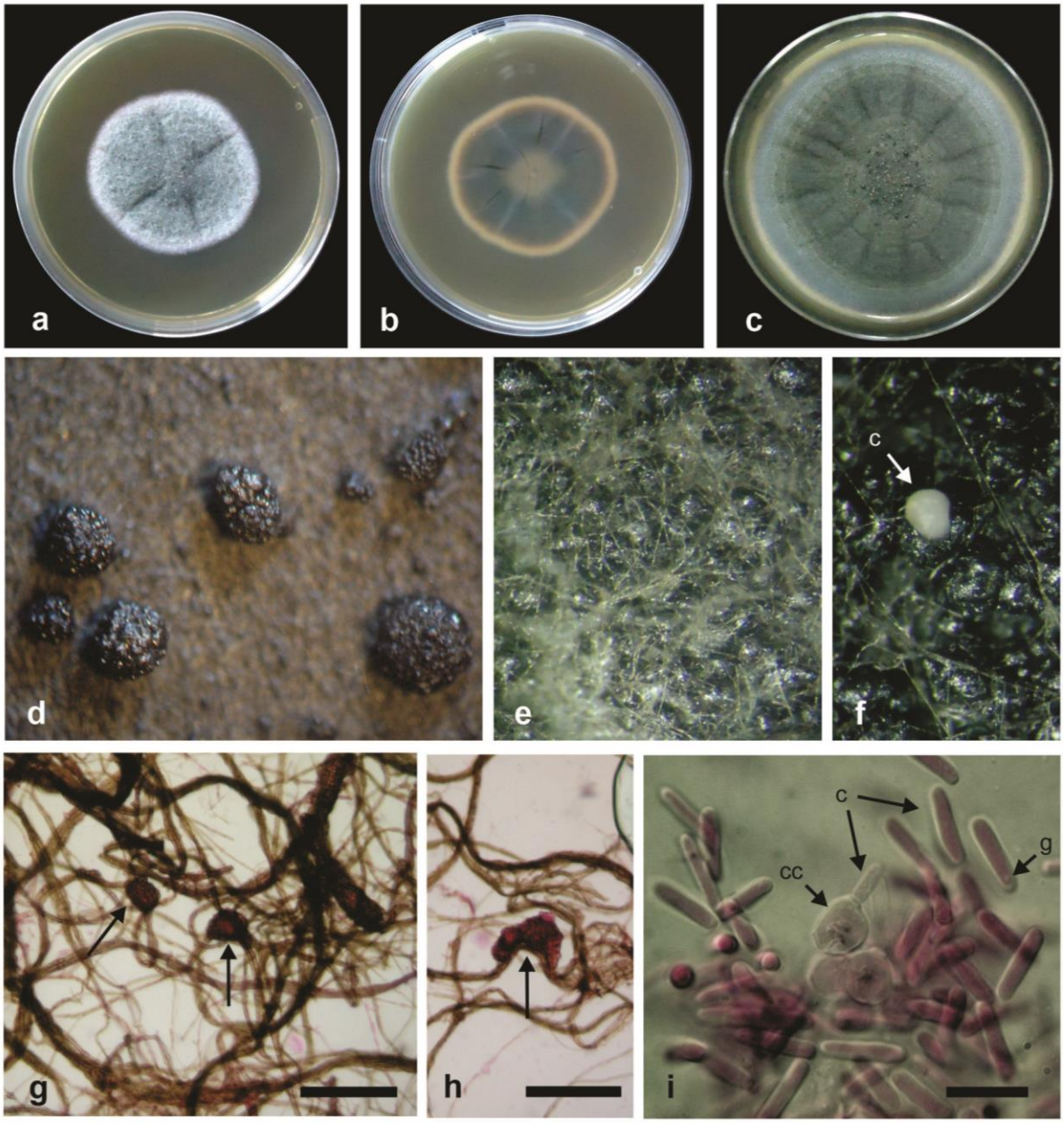

Fig. 1 – *Subplenodomus violicola*. a-c Colonies at 25ºC. a Malt Extract Agar (MEA), 7 days. b Reverse on MEA, 7 days. c MEA, 14 days. d-f Conidiomata. c = cirrus. g-h. Chlamydospores and pseudosclerotioid masses (arrows). i. Conidiogenous cell and conidia. cc = conidiogenous cell. c = conidia. g = guttules. Scale bars: g, h = 50 μm, i = 10 μm.

Discussion

In this study crude extracts from 15 fungi isolated from semi-arid soil were analyzed. Screening was performed by diffusion methodology against *Candida albicans* ATCC 6414 since inhibition is better visualized in yeast than in filamentous fungi when using an agar diffusion method. Activity was observed in four extracts. None of the fungal strains studied was inhibited by the *Sordaria fimicola* extract. Another species of the genus, *S. araneosa*, produces sordarin, which inhibited *C. albicans* growth (Liang 2008).

Yim et al. (Yim et al. 2014) isolated from *A. tatenoi* a new meroterpenoid, named tatenoic acid, together with five known compounds such as aszonapyrones A. This compound exhibited antimalarial activity against *Plasmodium falciparum* and present cytotoxic effect against two cancer cell lines.

Some species of the genus *Trichoderma* were intensively studied as potential sources of biocontrol agents, enzymes, and bioactive secondary metabolites producers (Ghisalberti & Sivasithamparam 1991, Reino et al. 2008). *T. saturnisporum* was reported to have antibacterial activity by peptaiboles production against *S. aureus* (Rebuffat et al. 1993) and *Bacillus megaterium* (Ritieni et al. 1993). Interesting to observe is that in contrast to our findings in which *T. saturnisporum* was active against *Aspergillus*, *Cryptococcus* and some species of *Candida*, antifungal activity was not observed by other authors. In one report by Sharma & Shanmugam (Sharma & Shanmugam 2012), antagonism was found against *Fusarium oxysporum*, a genus that was not tested in our work.

The broth microdilution methodology to determine the MIC was performed for crude extracts of *A. tatenoi*, *L. ellipsoideum*, *S. violicola* and T. *saturnisporum* against clinical isolates of yeasts and filamentous fungi. *S. violicola* extract was the most active one to all the strains tested. The MICs were < 0.25 µg/mL, being as active as azoles against *Candida* and *Aspergillus* species (St-Germain 2001). Fifty seven percent of the isolated *C. albicans* came from the oral mucosa, mainly from HIV patients with oropharyngeal candidiasis. This is a relatively common medical illness due to candidal infection. The widely treatment used is fluconazole, but other antifungals may also be indicated, depending on the isolated species and the patient's condition. The MIC values observed in the extracts analyzed are comparable to the values reported for *Candida* and azoles, especially for fluconazole where it is interesting to mention that, as observed in this study, *C. tropicalis*, presented higher MIC values (Cuenca-Estrella et al. 2002). *Subplenodomus* was erected by Gruyter et al. (2013). *Subplenodomus violicola* is a new combination for *Phoma violicola*. No biological activity was found from this species in the literature. This is the first report of *S. violicola* for Argentina. In a recent study, it was found activity against *Candida tropicalis*, *C. glabrata*, *Cryptococcus neoformans*, and *A. fumigatus* among others, with a MIC range of 4-8 µg/mL with campafungin A, a compound purified from fermentations of *Plenodomus enteroleucus* (Perlatti et al. 2020), that belongs together with *Subplenodomus* to the family Leptosphaeriaceae. Shibazaki et al. (2004) described a new antifungal compound from *Phoma* sp. and the MIC values for *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* observed were 2-16 µg/mL. Other studies reported antifungal activity from *Phoma*, however the identification of the biological activity at species level was not performed (Nagai et al. 2002, Herath et al. 2009, Qin et al. 2010, Wang et al. 2012). *Phoma lingam*, currently *Leptosphaeria maculans*, was cited as the producer of antifungal compounds such as fomenoic acid and lafomenolactone (Topgi et al. 1987, Devys et al. 1984, 1986). *Phoma etheridgei*, currently *Leptosphaeria etheridgei*, produced a compound that inhibited *Phellinus tremulae* (Ayer & Jimenez 1994).

The extracts of *L. ellipsoideum*, *T. saturnisporum* and *A. tatenoi* showed higher MIC values compared with azoles against the reference strains of *C. parapsilosis* (ATCC 22019), *A. fumigatus* (ATCC 204305), and *A. flavus* (ATCC 204304) (CLSI 2008a, b). It is interesting to note that although *S. violicola* was the most active compound, *L. ellipsoideum*, *T. saturnisporum* and *A. tatenoi* were also very active, especially against all the *Aspergillus* species tested. In general, low MIC values are reported for isavuconazole and voriconazole which are the preferred agents for first-line treatment of pulmonary invasive aspergillosis. For isavuconazole, MIC values of 0.25 µg/mL were reported for *A. terreus*, which is intrinsically resistant to amphotericin (a widely antifungal used) and for *A. nidulans* complex and *A. lentulus*, which are generally less sensitive to antifungal drugs (Pfaller et al. 2018, Ullmann et al. 2018). The activity of the conventional drugs reported are in agreement with the activity of the extracts for *Aspergillus* obtained in this study.

The diverse activity in the extracts may be due to the amount of the existing active fractions, thus, the bioactive compounds might be present in low proportion than other metabolites. It could also be hypothesized that some extracts have only one active fraction and others possess more than

one. Therefore, regarding the crude extracts activity, it is expected that synergistic or antagonistic effects have taken place in some extent.

In recent years, it has been increasingly reported the emergence of resistance in strains of the genus *Candida* to different antifungals, being worth of mentioning *C. auris* as a multidrug-resistant species, a health care-associated fungal pathogen (Spivak & Hanson 2018). Acquired resistance to azoles was mainly found in *Aspergillus fumigatus* and was first reported in the Netherlands and UK against itraconazole (Verweij et al. 2016). For these considerations, searching new compounds with antifungal activity is of utmost importance.

In conclusion, this study has demonstrated that *S. violicola*, *L. ellipsoideum*, *T. saturnisporum* and *A. tatenoi* extracts showed potential and promising activity against clinical important species of yeasts and molds. The composition of the active fractions and the mechanisms of action involved remain to be studied and warrants further investigations.

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