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Biological activities of selected basidiomycetes from Yemen

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In a previous paper we demonstrated the results of biological screening of Yemeni basidiomycetes. The present study was aimed to investigate the antimicrobial and the antioxidant activity of further basidiomycetes collected in Yemen. Dichloromethane, methanol and aqueous extracts of the fruiting bodies of 25 species were screened *in vitro* for their antibacterial activities against three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus flavus*) and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), against six human fungal pathogens (*Candida albicans*, *Candida krusei*, *Aspergillus fumigatus*, *Mucor sp.*, *Microsporium gypseum*, *Trichophyton mentagrophytes*) and against one non human pathogenic fungus (*Candida maltosa*). The results indicated that 75 extracts exhibited activity against one or more of the bacteria. The methanol extracts of *Agaricus cf. bernardii*, *Agrocybe pediades*, *Chlorophyllum molybdites*, *Coriolopsis polyzona*, *Ganoderma xylonoides*, *Pycnoporus sanguineus*, *Trametes lactinea* and *Trametes cingulata* showed activity against all tested bacteria. The highest antibacterial activity was exhibited by methanol extracts from *Chlorophyllum molybdites*, *Ganoderma xylonoides* and *Trametes cingulata* and *Agaricus cf. bernardii*, *Agrocybe pediades*, *Coriolopsis polyzona*, *Pycnoporus sanguineus* and *Trametes lactinea*. The methanol extracts of *Chlorophyllum molybdites*, *Ganoderma xylonoides* and *Pycnoporus sanguineus* showed considerable antifungal activities against the tested fungal strains. Strong antioxidative effects employing the DPPH assay were exhibited by methanol extracts from *Chlorophyllum molybdites*, *Ganoderma xylonoides*, *Hexagonia velutina*, *Pycnoporus sanguineus*, *Trametes lactinea* and *Trametes cingulata*. Our previous and presented studies about 48 basidiomycetes collected in Yemen provide evidence that basidiomycetes from the Arabic region so far should attract more attention as potential source for new biologically active agents.

1. Introduction

In continuation of our research for natural products from Yemen (Al-Fatimi et al. 2005, 2006, 2007), we have focused on the investigation of basidiomycetes (higher fungi, macrofungi, macrofungi, macrofungi, macrofungi), collected in this country (Table 1). Up to the beginning of our field excursions in 1998, the knowledge about the macrofungi of Yemen was very limited. Only 15 species had been collected and described by some European botanists in the period between 1880 and 1890 (Cooke 1881, 1882, 1888). Until now we could add more than 60 species, including 48 species of basidiomycetes, to the macrofungi flora of Yemen (Kreisel and Al-Fatimi 2004, 2008).

In some localities of Yemen, macrofungi are used in traditional medicine. For instance, *Podaxis pistillaris* is used for the treatment of skin diseases (Al-Fatimi et al. 2006). This together with the increasing importance of basidiomycetes from the colder regions as medicinal mushrooms and as source of bioactive compounds (Lindequist et al. 2005, 2010; Tidke and Rai 2006; Wasser and Weis 1999; Wasser 2010) stimulated us to screen the collected basidiomycetes for biological activities and bioactive compounds. The screening results of the first 23 species have been published in 2005 (Al-Fatimi et al. 2005). From *Podaxis pistillaris*, epidithioketopiperazines could be iso-

lated as responsible antibacterial and cytostatic constituents (Al-Fatimi et al. 2006). Here we report the screening results of further 25 macrofungi species collected at different localities in Yemen

2. Investigations and results

The mycological data of the collected basidiomycetes are summarized in Table 1. 16 of the 25 collected species belong to the order Agaricales, 7 to the order Poriales, one to Boletales and one to Phallales (Table 1). They are attributable to 14 different families, most of them to Agaricaceae (7 species) and Polyporaceae (6 species) (Table 1, Kreisel and Al-Fatimi 2004, 2008).

Dichloromethane (A), methanol (B) and water (C) extracts of the fruiting bodies of each species were tested *in vitro* for their antibacterial effects against five bacterial strains (three Gram-positive and two Gram-negative bacteria strains) (Table 2). The methanolic extracts were tested additionally against seven fungal strains (Table 3) and for their antioxidative activities using spectrophotometric 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method (DPPH assay) (Table 4).

Table 1: Mycological data of studied basidiomycetes from Yemen

Order/Family	Basidiomycetes names Species	Herbarium specimen No.	Site of collection from Yemen
Agaricales/ Agaricaceae	<i>Agaricus aff. campestris</i>	MAF 221	Taiz
Agaricales/ Agaricaceae	<i>Agaricus sp</i> Type V	MAF 84	Ibb
Agaricales/ Agaricaceae	<i>Agaricus sp</i> Type VI	MAF 197	Ibb
Agaricales/ Agaricaceae	<i>Agaricus sp</i> Type VII	MAF 287	Taiz, Sharaab
Agaricales/ Agaricaceae	<i>Agaricus cf. bernardii</i> QUÉL	MAF 220	Taiz, Sharaab
Agaricales/ Agaricaceae	<i>Coprinus disseminatus</i> (PERS.: FR.) GRAY	MAF281	Aden
Agaricales/ Agaricaceae	<i>Coprinus sterquilinus</i> (FR.) FR.	MAF 277	Lahaj, Subeiha
Agaricales/ Bolbitiaceae	<i>Agrocybe pediades</i> (FR.) FAYOD	MAF 297	Taiz
Agaricales/ Broomeiaceae	<i>Broomeia congregata</i> BERK.	MAF 177	Aldhalee, Qadaba
Agaricales/ Cortinariaceae	<i>Gymnopilus junonius</i> (FR.) P. D. ORTON	MAF 298	Taiz
Agaricales/ Lepiotaceae	<i>Chlorophyllum molybdites</i> (G. MEY.: FR. 1821) MASSEE	MAF185	Aldhalee, Nischam
Agaricales/ Lycoperdaceae	<i>Calvatia fragilis</i> (VITTAD.) MORG.	MAF 288	Taiz, Gabal Saber
Agaricales/ Pluteaceae	<i>Volvariella gloiocephala</i> (DC.: FR.) BOEKHOUT & ENDERLE	MAF 266	Lahaj
Agaricales/ Schizophyllaceae	<i>Schizophyllum commune</i> FR.: FR.	MAF 146	Soqotra, Qalansiyah
Agaricales/ Tulostomataceae	<i>Schizostoma mundkurii</i> (S. AHMAD) LONG & STOUFFER	MAF 248	Aden
Agaricales/ Tricholomataceae	<i>Pleurotus nebrodensis</i> (INZENG) QUÉL. var. <i>nebrodensis</i>	MAF 309	Haga
Boletales/ Gasterosporiaceae	<i>Gastrosporium sp.</i>	MAF 300	Aden
Phallales/ Phallaceae	<i>Phallus roseus</i> DELILE	MAF 263	Hadramout, Daoaan
Porales/ Ganodermataceae	<i>Ganoderma xylonoides</i> STEYAERT	MAF 155	Taiz, Alahkoom
Porales/ Polyporaceae	<i>Cerrena meyenii</i> (KLOTZSCH) L. HANSEN	MAF 260	Aldhalee, Quadaba
Porales/ Polporaceae	<i>Coriopsis polyzona</i> (PERS.) RYVARDEN	MAF144	Modyah, Khama'a
Porales/ Polyporaceae	<i>Hexagonia velutina</i> PAT. & HAR.	MAF 140	Soqotra, Heibaq
Porales/ Polyporaceae	<i>Pycnoporus sanguineus</i> (L.: FR.) MURRILL	MAF 292	Taiz
Porales/ Polyporaceae	<i>Trametes cingulata</i> BERK.	MAF 293	Taiz
Porales/ Polyporaceae	<i>Trametes lactinea</i> (BERK.) PAT.	MAF 306	Albedha, Alzاهر

2.1. Antibacterial activities

The results of the antibacterial tests are presented in Table 2. The highest antibacterial activity was shown by methanol extracts from *Chlorophyllum molybdites*, *Ganoderma xylonoides*, and *Trametes cingulata*, followed by the methanol extracts of *Agaricus cf. bernardii*, *Agrocybe pediades*, *Cerrena meyenii*, *Coriopsis polyzona*, *Pycnoporus sanguineus* and *Trametes lactinea* (Table 2).

Staphylococcus aureus was the most susceptible bacterial species. 29 extracts had good activity against this bacterial strain, 7 against *Micrococcus flavus* and 12 against *Bacillus subtilis*. Seven extracts showed good activity against *Pseudomonas aeruginosa*, and only three against *Escherichia coli* (Table 2).

The methanolic extracts showed better antibacterial activities than the dichloromethane extracts. The aqueous extracts were only very weak or not active. In general Gram-positive bacteria were more sensitive than the Gram-negative ones (Table 2).

For the methanolic extracts minimal inhibition concentrations (MIC) have been determined. MIC values lower than 500 µg against *Staphylococcus aureus* could be found for *Agaricus cf. bernardii*, *Agrocybe pediades*, *Chlorophyllum molybdites*, *Coriopsis polyzona*, *Ganoderma xylonoides*, *Pycnoporus sanguineus*, *Trametes cingulata* and *Trametes lactinea*.

These results are comparable with the activity of the reference antibiotics penicillin G and gentamicin (Table 2).

2.2. Antifungal activities

The results of the antifungal tests are presented in Table 3. The most active fungal species, presenting inhibiting activity against at least 4 human fungi strains were *Cerrena meyenii*, *Chlorophyllum molybdites*, *Coriopsis polyzona*, *Ganoderma*

xylonoides, *Pycnoporus sanguineus*, *Schizophyllum commune*, *Trametes cingulata* and *Trametes lactinea*. *Chlorophyllum molybdites* and *Hexagonia velutina* showed considerable antifungal activity against *Trichophyton mentagrophytes* stronger than the activity of the reference antibiotic nystatin (Table 3). *Trichophyton mentagrophytes* was the most susceptible fungal species. Seven of the tested basidiomycetes did not possess antifungal activities. On other hand, no extract showed antifungal activities against all human pathogenic fungi. The extracts from *Chlorophyllum molybdites*, *Ganoderma xylonoides*, *Pycnoporus sanguineus* and *Trametes lactinea* were active against fungi as well as against bacteria (Table 3).

2.3. Antioxidant activities

The results of tests for antioxidant activities are presented in Table 4. Extracts with inhibition values greater than 90% (concentration of 500 mg) were those from *Chlorophyllum molybdites*, *Ganoderma xylonoides*, *Hexagonia velutina*, *Pycnoporus sanguineus*, *Trametes cingulata* and *Trametes lactinea*. The strongest antioxidative activities with more than 90 % at 500 µg/ml showed *Ganoderma xylonoides*, *Hexagonia velutina* and *Pycnoporus sanguineus*. *Chlorophyllum molybdites* showed at 1000 µg/ml an antioxidant activity with 97.1% similar to the reference compound ascorbic acid, followed by *Pycnoporus sanguineus* with 96.2% (Table 4).

3. Discussion

The present study is a part of our ongoing studies about bioactive plants and fungi in Yemen, their use in Yemeni ethnomedicine and their potential as source for new drugs (Al-Fatimi et al. 2005, 2006, 2007). Because of very limited knowledge about basid-

Table 2: *In vitro* antibacterial activities of the fruiting bodies extracts

Species	Extract and % yield ^a	Inhibition zones (mm) against				
		Gram + ve			Gram -ve	
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>M. flavus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<i>Agaricus aff. campestris</i>	A (2.5)	10	0	0	10	10
	B (8.3)	15	0	10	10	10
	C (10.2)	8	0	0	0	0
<i>Agaricus sp.</i> Type V	A (43)	10	8	0	0	0
	B (14.5)	15	10	0	10	10
	C (19.0)	8	0	0	0	0
<i>Agaricus sp.</i> Type VI	A (2.2)	10	0	0	0	0
	B (5.5)	15	10	8	0	8
	C (13.5)	8	0	0	0	0
<i>Agaricus sp.</i> Type VII	A (3.2)	10	0	0	0	0
	B (10.1)	15	8	0	0	10
	C (13.2)	8	0	0	0	0
<i>Agaricus cf. bernardii</i>	A (3.3)	15	8	0	10	0
	B (13.6)	25	15	10	15	
	C (15.9)	10	0	0	0	0
<i>Agrocybe pediades</i>	A (2.4)	10	8	0	0	10
	B (5.9)	25	15	15	8	10
	C (10.2)	10	0	10	0	
<i>Broomeia congregata</i>	A (4.0)	10	0	0	0	0
	B (7.9)	10	10	10	0	0
	C (9.2)	8	0	0	0	0
<i>Calvatia fragilis</i>	A (3.1)	15	10	10	0	0
	B (7.3)	15	10	10	0	
	C (8.9)	10	0	0	0	0
<i>Cerrena meyenii</i>	A (2.9)	15	10	10	0	0
	B (9.2)	20	15	10	10	0
	C (12.2)	10	0	0	0	0
<i>Chlorophyllum molybdites</i>	A (5.2)	20	10	10	0	0
	B (8.5)	30	20	15	10	15
	C (10.8)	10	0	0	0	10
<i>Coprinus disseminatus</i>	A (2.8)	15	10	10	0	0
	B (9.7)	15	10	10	0	0
	C (5.3)	10	0	0	0	0
<i>Coprinus sterquilinus</i>	A (3.0)	15	10	10	0	0
	B (8.7)	15	10	10	0	0
	C (6.2)	10	0	0	0	0
<i>Coriolopsis polyzona</i>	A (5.2)	10	12	10	10	10
	B (17.8)	25	15	20	10	15
	C (20.2)	10	10	10	0	10
<i>Ganoderma xylonoides</i>	A (6.2)	20	10	18	0	10
	B (15.9)	30	15	20	15	15
	C (17.8)	10	0	0	0	10
<i>Gastrosporium sp.</i>	A (3.4)	10	0	10	0	0
	B (7.2)	10	0	10	0	0
	C (9.8)	8	0	0	0	0
<i>Gymnopilus junonius</i>	A (3.3)	10	8	0	0	0
	B (7.6)	15	10	0	8	8
	C (8.2)	8	0	0	0	0
<i>Hexagonia velutina</i>	A (3.2)	15	18	10	0	15
	B (10.9)	15	15	15	0	15
	C (12.3)	10	10	0	0	0
<i>Phallus roseus</i>	A (3.2)	10	0	0	0	10
	B (7.4)	15	10	0	0	0
	C (8.2)	8	0	0	0	0
<i>Pleurotus nebrodensis</i>	A (3.4)	10	10	0	10	10
	B (13.2)	15	10	0	0	10
	C (10.3)	8	0	0	0	0
<i>Pycnoporus sanguineus</i>	A (2.8)	10	0	0	10	0
	B (11.4)	25	15	10	20	15
	C (15.1)	10	10	0	0	10

Table 2: Continued

Species	Extract and % yield ^a	Inhibition zones (mm) against				
		Gram + ve			Gram -ve	
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>M. flavus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<i>Schizophyllum commune</i>	A (4.0)	8	0	0	0	0
	B (8.9)	15	10	0	0	10
	C (7.8)	8	0	0	0	0
<i>Schizostoma mundkurii</i>	A (2.1)	10	0	0	0	0
	B (8.4)	10	8	0	8	8
	C (13.7)	10	0	0	0	0
<i>Trametes cingulata</i>	A (6.3)	15	8	8	0	0
	B (13.9)	30	15	10	10	15
	C (15.4)	10	0	0	0	0
<i>Trametes lactinea</i>	A (5.9)	20	8	18	0	10
	B (10.8)	25	15	10	10	10
	C (14.2)	15	10	8	0	0
<i>Volvariella gloiocephala</i>	A (3.2)	10	0	0	0	0
	B (7.4)	10	8	0	0	0
	C (9.8)	8	0	0	0	0
Penicillin G (10 µg)		40	35	30	nd	nd
Gentamicin (10 µg)		nd	25	nd	20	20

^a percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight x 100. A: dichloromethane extract; B: methanole extract; C: water extract; 2 mg dried extract/disc nd = none detected. Inhibition zone: 15 mm or greater- good antibacterial activity; 12–14 – moderate antibacterial activity; 8–10 – weak antibacterial activity.

iomycetes in Yemen, our special interest is directed on this group of organisms (Kreisel and Al-Fatimi 2004, 2008). After the first report about the biological screening of 23 species (Al-Fatimi et al. 2005) we present here the results obtained with 25 further species. As most interesting species with remarkable activities *Cerrena meyenii*, *Chlorophyllum molybdites*, *Coriolopsis polyzona*, *Ganoderma xylonoides*, *Hexagonia velutina*, *Pleurotus*

nebrodensis, *Pycnoporus sanguineus*, *Trametes cingulata* and *Trametes lactinea* could be identified.

From these species *Cerrena meyenii*, *Coriolopsis polyzona*, *Ganoderma xylonoides*, *Hexagonia velutina*, *Trametes cingulata* and *Trametes lactinea* have not been investigated before. But there are some results for related species from the same genera. *Coriolopsis caperata* has shown moderate antimicrobial

Table 3: In vitro antifungal activity of the methanol fruiting bodies extracts

Botanical name	Inhibition zones (mm) against ^a						
	Cm	Ca	Ck	Af	Ms	Mg	Tm
<i>Agaricus aff. campestris</i>	–	–	–	–	–	–	10
<i>Agaricus sp</i> Type V	10	–	10	–	–	–	–
<i>Agaricus sp</i> Type VI	10	–	–	–	–	10	10
<i>Agaricus sp</i> Type VII	–	–	10	–	–	–	10
<i>Agaricus cf. bernardii</i>	15	–	–	–	–	–	14
<i>Agrocybe pediades</i>	15	–	–	–	–	10	10
<i>Broomeia congregata</i>	–	–	–	–	–	–	–
<i>Calvatia fragilis</i>	10	10	–	–	–	–	–
<i>Cerrena meyenii</i>	10	10	–	10	10	17	20
<i>Chlorophyllum molybdites</i>	15	20	21	10	–	16	30
<i>Coprinus disseminatus</i>	–	–	–	–	–	–	–
<i>Coprinus sterquilinus</i>	–	–	–	–	–	–	–
<i>Coriolopsis polyzona</i>	10	–	–	10	10	10	18
<i>Ganoderma xylonoides</i>	15	10	15	–	10	12	14
<i>Gastrosporium sp.</i>	–	–	–	–	–	–	–
<i>Gymnopilus junonius</i>	–	–	–	–	–	–	–
<i>Hexagonia velutina</i>	10	–	–	–	–	–	30
<i>Phallus roseus</i>	–	–	–	–	–	–	–
<i>Pleurotus nebrodensis</i>	10	–	–	–	–	–	–
<i>Pycnoporus sanguineus</i>	10	10	16	–	–	15	20
<i>Schizophyllum commune</i>	10	10	–	10	–	10	10
<i>Schizostoma mundkurii</i>	–	–	–	–	–	–	15
<i>Trametes cingulata</i>	10	–	10	–	10	10	10
<i>Trametes lactinea</i>	10	10	10	9	10	–	10
<i>Volvariella gloiocephala</i>	–	–	–	–	–	–	–
Nystatin	25	25	26	22	24	22	25

^a Fungi: Cm, *Candida maltosa*; Ca, *Candida albicans*; Ck, *Candida krusei*; Af, *Aspergillus fumigatus*; Ms, *Mucor sp.*; Ms, *Microsporium gypseum*; Tm, *Trichophyton mentagrophytes*; Nystatin (100 µg/disc).

Table 4: Antioxidative activity of the methanol basidiomycetes fruiting bodies extracts

Extracts	Radical scavenging activity %				
	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
<i>Agaricus aff. campestris</i>	5.7	7.4	10.8	21.4	40.5
<i>Agaricus sp.</i> Type V	4.5	7.2	7.6	18.4	36.2
<i>Agaricus p.s</i> Type VI	7.7	15.4	17.5	39.8	47.2
<i>Agaricus sp.</i> Type VII	6.3	13.9	21.2	43.3	52.1
<i>Agaricus cf. bernardii</i>	8.9	11.2	17.8	35.4	70.8
<i>Agrocybe pediatas</i>	3.3	4.1	7.9	33.8	65.5
<i>Broomeia congregata</i>	2.8	5.5	6.6	17.5	30.3
<i>Calvatia fragilis</i>	10.2	18.4	32.6	37.7	69.8
<i>Cerrena meyenii</i>	13.4	28.4	30.8	45.5	75.6
<i>Chlorophyllum molybdites</i>	9.9	32.8	59.2	90.8	91.7
<i>Coprinus disseminatus</i>	9.4	12.8	16.4	38.8	42.8
<i>Coprinus sterquilinus</i>	8.5	19.7	28.3	45.2	52.7
<i>Coriolopsis polyzona</i>	17.4	26.8	47.9	72.1	75.2
<i>Ganoderma xylonoides</i>	23.9	34.6	55.8	94.5	97.1
<i>Gastrosporium sp.</i>	12.4	16.5	37.9	57.8	65.9
<i>Gymnopilus junonius</i>	5.7	6.4	8.2	18.2	37
<i>Hexagonia velutina</i>	30.4	47.6	72.8	90.7	91.1
<i>Phallus roseus</i>	8.7	9.4	13.2	36.2	45
<i>Pleurotus nebrodensis</i>	10.9	13.6	54.8	81.5	95.1
<i>Pycnoporus sanguineus</i>	39.8	70.0	93.1	94.2	96.2
<i>Schizophyllum commune</i>	1.7	2.4	3.2	13.2	34
<i>Schizostoma mundkurii</i>	2.3	2.8	2.4	16.8	35
<i>Trametes cingulata</i>	17.5	39.2	58.3	90.5	92.9
<i>Trametes lactinea</i>	16.5	40.2	62.3	90.9	91.7
<i>Volvariella gloiocephala</i>	7.5	10.4	32.5	34.2	46.5
Ascorbic acid	48.8	97.0	97.1	97.2	97.2

and antioxidant activities in previous studies (Al-Fatimi et al. 2005). The genus *Ganoderma* includes the most famous medicinal mushroom species, *Ganoderma lucidum* (Lindequist et al. 2010). Besides, *G. applanatum* (Lee et al. 2006), *G. colossus* (El Din et al. 2008, Ofodile et al. 2005), *G. pfeifferi* (Mothana et al. 2000), *G. resinaceum* (Ding et al. 2010, Niu et al. 2007) and some other have been investigated. In our previous study, *Ganoderma colossus* and *Ganoderma resinaceum*, both collected in Yemen, showed good antimicrobial and antioxidant activities (Al-Fatimi et al. 2005). In the present study, the third Yemeni *Ganoderma* species, *Ganoderma xylonoides*, was studied for its biological activities for the first time. Antimicrobial activities have also been reported for the related species *Hexagonia hydnoidea* (Rosa et al. 2003). *Pleurotus nebrodensis* is a very well known edible mushroom. For some *Pleurotus* species strong antioxidative properties have been described (Badalyan et al. 2003a; Jose et al. 2002, Kim et al. 2008). The antimicrobial activity of *Pycnoporus sanguineus* has already been reported (Rosa et al. 2003). Cinnabarine, an orange pigment is known as antimicrobial, antiviral and cytotoxic compound from *Pycnoporus sanguineus* (Smânia et al. 1995, Smânia et al. 1999, 2003). *Chlorophyllum molybdites*, Lepiotaceae, is evaluated as a poisonous mushroom (Kreisel and Al-Fatimi 2008; Hashimoto et al. 2000, Whitaker and Box 1985; Yokoyama and Gonmori 2009). From this species the pyrrolidine alkaloids lepiotin A and B (Ohta et al. 1998) and two cytotoxic steroids (Yoshikawa et al. 2001) were isolated. For *Trametes cingulata* and *T. marianna* antibacterial activities have been described (Ofodile et al. 2008). *Trametes versicolor* shows antioxidant activities (Badalyan 2003b) and is known for its immunomodulating effects (Lindequist et al. 2005). *Ganoderma tsugae* (Mau et al. 2002), *Agrocybe aegerita* (Zhang et al. 2003) and *Agrocybe cylindracea* have been studied for their antioxidant activities (Kim et al. 1997).

Taking together, our previous and present studies about till now 48 basidiomycetes collected in Yemen provide evidence that basidiomycetes from the Arabic region should receive more attention as potential source for new biologically active agents.

4. Experimental

4.1. Basidiomycetes material

The fruiting bodies of the basidiomycetes were collected from different localities of Yemen, in the time from January 2004 to August 2007 (Table 1). They were identified by Prof. Dr. H. Kreisel at the Department of Microbiology, Ernst-Moritz-Arndt-University, Greifswald, Germany. Authentic (voucher) specimen are deposited in Kreisel's herbarium at the Department of Microbiology, Ernst-Moritz-Arndt-University, Greifswald, Germany and in Al-Fatimi's Herbarium at Department of Pharmacognosy, Pharmacy Faculty, Aden University, Aden, Yemen,

4.2. Extraction

The fruiting bodies were allowed to air dry and afterwards pulverized in grinder. Twenty gram of the pulverized materials were successively extracted with 300 ml of dichloromethane, 300 ml of methanol and 300 ml of water at room temperature for 8 h each. The extracts were then concentrated under reduced pressure at 40 °C, freeze-dried and stored in exsiccator until use.

4.3. Determination of antimicrobial activities

The following microbial strains were used:

Bacterial strains:

Staphylococcus aureus (ATCC 29213), *Bacillus subtilis* (ATCC 6059), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Micrococcus flavus* (SBUG 16).

Fungal strains:

Candida maltosa SBUG17, *Candida albicans* ATCC 90028, *Candida krusei* ATCC 90878, *Aspergillus fumigatus* 13550/99, *Trichophyton mentagrophytes* 05/2004, *Microsporium gypseum* and *Mucor sp.*

The antimicrobial activities of the extracts (2 mg dried extract per disc) were determined applying the agar diffusion assay according to Al-Fatimi et al. (2007). penicillin G and gentamicin were used as positive, the solvents

dichlormethane and methanol as negative controls. Inhibition zone diameters include diameter of the disc (6 mm). An average zone of inhibition was calculated from three replicates. An inhibition zone of 15 mm or greater was considered as good antibacterial activity. Minimal inhibitory concentrations (MICs) were determined by the agar diffusion technique as described by Rajbhandari and Schöpke (1999). The highest concentration of extract tested during the experiment was 1 mg/ml. The MIC corresponds to the lowest concentration of the test extract able to inhibit any visible microbial growth. Yeasts and hyphomycetes were inoculated into sterile Mueller-Hinton agar (Becton Dickinson, Heidelberg) according to E DIN 58940-3 for the agar disc-diffusion assay. Nystatin was used as positive control and the solvents as negative control. Plates were incubated with yeasts for 48 h at 36 °C and with hyphomycetes for 72 h at 30 °C.

4.4. Determination of antioxidant activity

Estimation of radical scavenging effect was carried out by the DPPH assay according to the method of Brand et al. (1995). The reaction mixture contained 500 µl of test extract and 125 µl of DPPH in ethanol. Different concentrations of test samples were prepared while the concentration of DPPH was 1 mM in the reaction mixture. After incubation of reaction mixture at 37 °C for 30 min the absorbance was measured at 517 nm. Percentage radical scavenging activity of sample was determined by comparison with an ethanol treated control group. Ascorbic acid was used as positive control.

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