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Antimicrobial, cytotoxic and antioxidant activity of selected basidiomycetes from Yemen

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Dedicated to Doz. Dr. Wolf-Dieter Jülich on the occasion of his 60th birthday

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Dichloromethane, methanol and aqueous extracts of 23 selected Basidiomycetes species fruiting bodies collected in Yemen were screened *in vitro* for their antibacterial activities against three Gram-positive bacteria (*Staphyloccocus aureus*, *Bacillus subtilis, Micrococcus flavus*), two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and against one yeast fungus (*Candida maltosa*), as well as for their cytotoxic and antioxidant activity. The highest antibacterial activity was shown by extracts from *Agaricus* sp. (Type 1), *Coriolopsis caperata*, *Ganoderma colossus*, *Ganoderma resinaceum*, *Phellorinia herculea* and *Tulostoma obesum*. Strong antioxidative effects employing the DPPH assay were exhibited by methanol extracts from *Ganoderma resinaceum, Inonotus ochroporus, Phellinus rimosus* and *Phellorinia herculea*. The results provide evidence that some of the studied fungi might be potential sources for new biologically active agents.

1. Introduction

The first scientific collection of basidiomycetes in Yemen was carried out between 1880 and 1889. The European botanists Deflers from France, Balfour from Great Britain and Schweinfurth from Germany collected basidiomycetes from the Yemeni regions Aden and Hodidah and from the island Socotra during this period (Cooke 1881, 1882, 1888). After this date there has been no further scientific collection of basidiomycetes in Yemen.
We collected more than thirty species between 1999 and 2004 and classified them taxonomically (Al-Fatimi 2001; Kreisel and Al-Fatimi 2004). In total, 39 taxa of larger fungi (macromycetes: 36 basidiomycetes and three ascomycetes), including former records from the literature, were enumerated and annotated from the territory of Yemen. In recent times 22 taxa have been collected; from which 16 were new records for Yemen. Currently, eight taxa are known from the highlands, 16 taxa from the coastal lowland, and 15 taxa (only very old records) from the island Socotra. Members of the macromycete flora of Yemen are species with south-temperate to mediterranean distribution (mainly represented in the highlands), species distributed along the subtropical to tropical (mainly represented in the lowlands) and even cosmopolitical species (Kreisel and Al-Fatimi 2004).
Basidiomycetes of the colder regions were found to be interesting sources for biological active compounds and numerous substances with antibacterial and antifungal effects have been isolated (Lindequist et al. 1990; Wasser and Weis 1999). There have been no reports about biological, pharmacological and phytochemical investigations of mushrooms from Yemen apart from our studies about *Podaxis pistillaris* (Al-Fatimi et al. 2001).
This report presents the results of biological screening investigations carried out with several extracts from recently collected fungi from Yemen. Besides the ecological function of antimicrobial secondary metabolites for fungi these substances could be of medicinal importance against infectious diseases. Therefore the screening investigations were focused on tests for antibacterial and antifungal activity. Furthermore tests for cytotoxic and antioxidant activities were accomplished.

2. Investigations and results

Dichloromethane (A), methanol (B) and water (C) extracts of 23 basidiomycete species, collected in Yemen, were tested for their antimicrobial effects against six microorganism strains. The mycological data were summarized in Table 1. The results of the antimicrobial tests were presented in Table 2.
The most susceptible bacterial species was *Staphylococcus aureus* (24 extracts with good activity). Fourteen extracts showed good activity against *Pseudomonas aeruginosa*, 12 against *Micrococcus flavus* and against *Bacillus subtilis* and only 4 against *Escherichia coli*. Four methanol extracts (*Ganoderma colossus, Ganoderma resinaceum, Tulostoma obesum* and *Agaricus* sp.) demonstrated weak activity against *Candida maltosa*. The most active extracts were the methanol extracts (20 extracts with good activity against one or more microbial strains). Ten water and seven di-

Table 1: Mycological data of basidiomycetes studied

Order/species	Herbarium specimen No.	Site of collection in Abyan Region
Agaricales/*Agaricus* sp. Type I	MAF 9	Lawdar, Imsha'a
Agaricales/*Agaricus devoniensis* P. D. Orton	MAF 8	Lawdar, Imsha'a
Agaricales/*Agaricus* sp. Type III	MAF 14	Lawdar, Imsha'a
Agaricales/*Agaricus* sp. Type IV	MAF 2	Lawdar, Imsha'a
Agaricales/*Amanita nana* Singer	MAF 113	Lawdar, Imsha'a
Agaricales/*Gyrophragmium dunalii* (Fr.) Zeller	MAF 96	Lawdar, Imsha'a
Agaricales/*Montagnea haussknechtii* Rabenh.	MAF 5	Modya, Imkhudeerah
Agaricales/*Montagnea radiosa* (Pallas) Šebek	MAF 4	Modya, Imkhudeerah
Agaricales/*Podaxis pistillaris* (L.: Pers.) Fr. emend. Morse	MAF 24	Lawdar, Imsha'a
Agaricales/*Lepiota* sp.	MAF 87	Modya, Khamah
Agaricales/*Pluteus* sp.	MAF 120	Lawdar, Imsha'a
Hymenochaetales/*Inonotus ochroporus* (Van der Byl) Pegler	MAF 81	Lawdar, Imsha'a
Hymenochaetales/*Phellinus rimosus* (Berk.) Pilát	MAF 86	Lawdar, Imsha'a
Polyporales/*Coriolopsis caperata* (Berk.) Murrill	MAF 98	Lawdar, Imsha'a
Polyporales/*Ganoderma colossus* (Fr.) Baker	MAF 97	Lawdar, Imsha'a
Polyporales/*Ganoderma resinaceum* Boud.	MAF 99	Khanfar, Zingibar
Polyporales/*Laetiporus baudonii* (Pat.) Ryvarden	MAF 93	Khanfar, Zingibar
Polyporales/*Lentinus strigosus* (Schwein.: Fr.) Fr.	MAF 126	Lahej, Al-Musemier
Tulostomatales/*Battarrea stevenii* (Liboschitz) Fr.	MAF 6	Lawdar, Imsha'a
Tulostomatales/*Phellorinia herculea* (Pallas: Pers.) Kreisel	MAF 11	Lawdar, Modya
Tulostomatales/*Queletia* sp.	MAF 3	Lawdar, Imsha'a
Tulostomatales/*Schizostoma laceratum* Ehrenb.	MAF 108	Lawdar, Imsha'a
Tulostomatales/*Tulostoma obesum* Cooke & Ellis	MAF 7	Modya, Dathina

Table 2: *In vitro* antimicrobial studies of selected basidiomycetes from Yemen

Species	Extract and % yield[a]	Inhibition zones (mm) against						IC_{50} against FL-cells (μg/ml)
		S. aureus	*B. subtilis*	*M. flavus*	*E. coli*	*P. aeruginosa*	*C. maltosa*	
Agaricus sp. Type I	A(4.5)	8	8	15	20	20	r	
	B(10.2)	15	15	10	10	15	r	560
	C(16.2)	10	10	15	r	15	r	
Agaricus devoniensis	A(5.3)	10	8	18	8	8	r	
	B(11.5)	20	10	8	15	10	8	630
	C(18.3)	18	15	r	r	10	r	
Agaricus sp. Type III	A(3.2)	r	r	r	r	r	r	
	B(9.8)	10	8	8	r	8	r	670
	C(17.5)	r	r	r	r	r	r	
Agaricus sp. Type IV	A(2.5)	r	r	r	r	r	r	
	B(12.4)	15	8	r	r	r	r	590
	C(19.6)	r	r	r	r	r	r	
Amanita nana	A(2.3)	r	8	r	r	r	r	
	B(11.6)	15	8	10	r	8	r	nd
	C(17.3)	15	15	10	r	10	r	
Battarrea stevenii	A(2.0)	8	8	r	r	r	r	
	B(7.9)	15	10	10	r	r	r	390
	C(8.2)	10	r	10	10	r	r	
Coriolopsis caperata	A(4.9)	10	12	20	r	10	r	
	B(18.2)	15	15	r	8	15	r	980
	C(19.4)	15	15	r	r	r	r	
Ganoderma colossus	A(5.3)	10	10	20	8	10	r	
	B(17.9)	15	15	18	10	15	8	550
	C(16.5)	25	15	15	8	20	r	
Ganoderma resinaceum	A(5.8)	10	10	10	8	15	r	
	B(19.2)	10	15	10	15	10	r	290
	C(17.3)	20	15	10	8	20	r	
Gryophragmium dunalii	A(2.5)	10	8	r	r	r	r	
	B(6.3)	15	10	r	8	8	r	nd
	C(7.4)	r	r	r	r	r	r	
Inonotus ochroporus	A(4.6)	8	r	r	8	10	r	
	B(13.8)	15	10	8	r	15	r	380
	C(20.2)	15	10	r	r	20	r	

Table 2: continued

Species	Extract and % yield[a]	Inhibition zones (mm) against						IC_{50} against FL-cells (μg/ml)
		S. aureus	*B. subtilis*	*M. flavus*	*E. coli*	*P. aeruginosa*	*C. maltosa*	
Laetiporus baudonii	A(2.7)	10	8	r	r	8	r	
	B(9.8)	15	10	8	r	r	r	nd
	C(16.4)	10	8	r	r	r	r	
Lentinus strigosus	A(3.2)	8	8	10	r	r	r	
	B(6.4)	10	8	15	r	r	r	nd
	C(9.5)	r	r	r	r	r	r	
Lepiota sp.	A(2.9)	8	8	10	r	10	r	
	B(5.8)	15	10	15	r	10	r	nd
	C(10.3)	r	r	r	r	r	r	
Montagnea haussknechtii	A(1.9)	10	r	r	10	10	r	
	B(10.4)	15	r	r	10	10	r	450
	C(17.3)	r	12	r	r	r	r	
Montagnea radiosa	A(1.6)	8	8	8	r	r	r	
	B(9.8)	10	10	10	r	15	r	430
	C(16.8)	r	r	15	r	r	r	
Phellinus rimosus	A(4.2)	8	8	8	r	r	r	
	B(8.4)	10	15	8	r	8	r	880
	C(13.4)	10	r	r	r	10	r	
Phellorinia herculea	A(2.4)	20	18	r	20	25	r	
	B(12.3)	15	10	r	10	r	r	390
	C(18.9)	10	15	r	r	15	r	
Pluteus sp.	A(1.5)	8	r	8	r	10	r	
	B(7.5)	15	10	10	r	10	r	nd
	C(13.4)	r	r	r	r	r	r	
Podaxis pistillaris	A(1.7)	r	r	r	r	r	r	
	B(15.7)	8	8	r	r	8	r	450
	C(20.3)	r	r	r	r	r	r	
Queletia sp.	A(3.1)	10	r	r	r	r	r	
	B(8.4)	10	8	r	r	r	r	nd
	C(15.5)	8	8	r	r	r	r	
Schizostoma laceratum	A(3.4)	r	r	r	r	r	r	
	B(7.8)	15	10	r	r	10	8	750
	C(15.6)	r	r	r	r	r	r	
Tulostoma obesum	A(2.2)	10	8	18	8	10	r	
	B(5.7)	15	8	20	8	10	8	330
	C(14.7)	15	10	8	r	15	r	
Penicillin G (10 μg)		40	35	30				
Gentamicin (10 μg)			25		20	20		

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

Table 3: Antioxidative activity of the methanolic basidiomycetes extracts

Extracts	Radical scavenging activity %				
	10 μg/ml	50 μg/ml	100 μg/ml	500 μg/ml	1000 μg/ml
Agaricus sp. Type I	4.8	6.2	6.7	19.4	30.3
Agaricus devoniensis	3.3	4.1	7.9	33.8	65.5
Agaricus sp. Type IV	5.5	9.6	11.6	27.6	45.5
Amanita nana	9.9	12.8	19.2	79.8	91.7
Coriolopsis caperata	9.4	12.8	16.4	38.8	62.8
Ganoderma resinaceum	8.5	19.7	38.0	93.2	96.7
Inonotus ochroporus	21.2	50.1	60.5	90.7	92.1
Phellinus rimosus	14.5	30.2	52.3	93.0	93.8
Phellorinia herculea	10.9	13.6	24.8	81.5	95.1
Podaxis pistillaris	1.4	1.8	1.8	14.7	32
Ascorbic acid	48.8	97.0	97.1	97.2	97.2

chloromethane extracts displayed good activities against one or more microbial strains. The most active fungal species, presenting good activity against at least five bacterial strains, were an unidentified *Agaricus* sp. (Type 1), *Coriolopsis caperata*, *Ganoderma colossus*, *Ganoderma resinaceum*, *Phellorinia herculea* and *Tulostoma obesum*. Obviously the basidomycetes growing on plants show stronger antibacterial activity than the basidiomycetes growing on soil (Tables 1 and 2, Kreisel and Al-Fatimi 2004).

Among the extracts tested for cytotoxicity none showed considerable cytotoxic activity against FL-cells. All determined IC 50 values were greater than about 300 µg/ml (Table 2).

Ten out of 23 methanol extracts tested demonstrated remarkable activity in the DPPH assay.

Extracts with inhibition values greater than 90% (concentration of 500 µg) were those from *Ganoderma resinaceum, Inonotus ochroporus, Phellinus rimosus* and *Phellorinia herculea*. This is comparable with the activity of the reference compound ascorbic acid (Table 3).

3. Discussion

Recent investigations of plants used in the Yemeni ethnomedicine (Awadh et al. 2001) revealed a great potential of organisms of this geographic region as source for pharmacologically active agents. As opposed to plants, mushrooms are not used in the traditional medicine in Yemen. The inhabitants think that mushrooms are bad products of some animals like fox and call them "fox" (Al-Fatimi 2001). The use of mushrooms as food is also unusual. Hence, scientific investigations of fungi in Yemen are very rare. On that account this opens the possibility to detect new drugs.

The results of our biologic screening investigations show definitively that some basidiomycete species collected during the last years in Yemen (Kreisel and Al-Fatimi 2004) possess remarkable antimicrobial and antioxidant activity. Most interesting species are *Coriolopsis caperata*, *Ganoderma colossus*, *G. resinaceum*, *Inonotus ochroporus, Phellinus rimosus, Phellorinia herculea* and *Tulostoma obesum*. Other species of the genera *Ganoderma, Inonotus* and *Phellinus* have been well investigated. *Ganoderma lucidum,* known as "Reishi" or "Ling zhi", is the best investigated medicinal mushroom worldwide. It contains more than 120 triterpenes, bioactive polysaccharides etc. and is used as a drug or as a food supplement for many indications (Lindequist 1998; Wasser and Weis 1999). The antibacterial activity of the European species *G. pfeifferi* is caused by sesquiterpenoid hydroquinones named ganomycins (Mothana et al. 2000). In Eastern Europe the sclerotium of *Inonotus obliquus,* known as "Tschaga", has been used as a folk medicine for cancer and stomach diseases since the 16th or 17th century. Several triterpenes contribute to the activity (Molitoris 1994). The related species *I. hispidus* produces e.g. the antiviral metabolites hispolon and hispidin (Awadh et al. 2003). Polysaccharides from *Phellinus linteus* act as biological response modifier (Han et al. 1999). The phytochemical and pharmacological knowledge about the *Ganoderma*, *Inonotus* and *Phellinus* species reported here is very limited. The antioxidant activity of *Phellinus rimosus* was recently described (Ajith 2002) and is in accordance with our results. To our knowledge chemical compositions and biological activities of *Coriolopsis caperata*, *Phellorinia herculea* and *Tulostoma obesum* have not been investigated until now.

4. Experimental

4.1. Basidiomycetes material

The fruiting bodies of the basidiomycetes were collected from different localities of Abyan province, Yemen, in the time from January 1999 to August 2004 (Table 1). They were identified by Prof. Dr. H. Kreisel at the Department of Microbiology, Ernst-Moritz-Arndt-University, Greifswald, Germany. Authenic (voucher) specimen (MAF1-MAF139) are deposited in Kreisel's herbarium at the Department of Microbiology, Ernst-Moritz-Arndt-University, Greifswald, Germany.

4.2. Extraction

The fruiting bodies were allowed to dry in the air and were afterwards pulverized in a grinder. Twenty g 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 an exsiccator until use.

4.3. Determination of antimicrobial activities

The following microbial strains were used: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6059), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus flavus* (SBUG 16) and *Candida maltosa* (SBUG17).

The antimicrobial activities of the extracts (2 mg dried extract per disc) were determined applying the agar diffusion assay according to Awadh et al. (2001). 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.

4.4. Cytotoxicity assay

The cytotoxicity was measured by the neutral red uptake assay (Lindl and Bauer 1989) using FL-cells according to Awadh et al. (2001).

4.5. Determination of antioxidant activity

Estimation of a 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 scavening activity of sample was determined by comparison with an ethanol treated control group. Ascorbic acid was used as positive control.

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