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Antimicrobial Activity of Three *Macrolepiota* Species from Turkey

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Three *Macrolepiota* species (*M. heimii, M. rachodes* and *M. puellaris*) were tested for their antimicrobial activity by using the disc diffusion method. The ethanol extract from the fruit bodies of mushrooms were assayed against 16 microorganisms. In comparison with the test antibiotics amikacin, chloramphenicol and nystatin, the ethanol extract obtained from *M. heimii* presented significant activity against *Micrococcus* species and *Citrobacter freundii*. The extracts have weak anti-yeast activity against the yeast cultures.

Key Words: Antimicrobial activity, *Macrolepiota*, Turkey.

INTRODUCTION

Many antibiotics in clinical use were isolated from fungi. Although production of important antibiotics such as penicillin, cephalosporin and griseofulvin by fungi is well-known, the occurrence of antibiotics in mushroom is less well documented for discovery of new antibiotics with different structural types. It has been known since Greek and Roman antiquity that macrofungi are used as food and medicine and thus these may be a source of new and useful bioactive compounds[1].

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans. As a matter of fact, produce a large number of metabolites that show antibacterial, antifungal, antiviral, antitumor, hypoglycemic, antiallergic, immunomodulating, antiinflammatory, hypolipidemic and hepatoprotective activity[1-4].

In this study, antimicrobial activities of three *Macrolepiota* species (*M. rachodes* (Vitt.) Sing., *M. heimii* Locq.:Bon and *M. puellaris* (Fr.) Moser) collected from different localities of Turkey have been investigated by disk diffusion methods.

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EXPERIMENTAL

Fungal organism: Three Macrolepiota species (*M. rachodes* (Vitt.) Sing., *M. heimii* Locq.:Bon and *M. puellaris* (Fr.) Moser) were collected in nature during field trips in August-September, 2003, from the different localities in Turkey. The morphological and ecological characterization of the collected macrofungi were recorded and photographed in their natural habitats. Dried specimens were numbered and placed in locked bags. The specimens were identified according to macroscopic and microscopic features and the related literature[5].

Preparation of macrofungi extracts: The dried and powdered fruit bodies of macrofungi were reduced to coarse powder. 50 g of each sample was extracted with 150 mL of ethanol at room temperature with stirring for 2 d. The extraction solvent was evaporated to dryness. Sample solutions were prepared by dissolving the extracts in extraction solvents (5 mg/mL).

Microbial test organisms: A total of 16 strains was used: *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Bacillus brevis* ATCC 9999, *Micrococcus luteus* La 2971, *Micrococcus flavus* ATCC 14452, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Alcaligenes faecalis* ATCC 8090, *Candida albicans* ATCC 10231, *Kluyveromyces fragilis* ATCC 8608 and *Rhodotorula rubra* DSM 70403.

Assay for antimicrobial activity: The dried extracts were dissolved in 10 % aqueous dimethyl sulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 µm membrane filter. Empty sterilized antibiotic disks having a diameter of 6 mm (Schleicher & Schull no. 2668, Dassel, Germany) were each impregnated with 50 µL of extract (10 mg/disk) at a concentration of 200 mg/mL. All the bacteria mentioned above were incubated at 35 ± 0.1 ºC for 24 h by inoculation into nutrient broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in malt extract broth (Difco Laboratories) at 25 ± 0.1 ºC for 48 h an inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The disks injected with the yeast cultures were incubated at 25 ± 0.1 ºC and bacteria were incubated at 35 ± 0.1 ºC for 24 h[6]. At the end of the period, inhibition zones formed on the medium were evaluated in millimeters. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disc was applied, depending on the test microorganism for comparison.

RESULTS AND DISCUSSION

Table-1 shows antimicrobial activity of the extracts obtained from three *Macrolepiota* species.

TABLE-1
ANTIMICROBIAL ACTIVITY OF *Macrolepiota* SPECIES

Microorganisms		Zone of inhibition (mm)*					
		Ethanol extracts (50 mg/mL)			Standards**		
		1	2	3	AK	CHL	NYS
Bacteria							
Staphylococcus aureus ATCC6538P	(Gr+)	11.2	12.4	12.2	24.2	18.2	NT
Bacillus cereus ATCC 7064	(Gr+)	6.0	12.8	10.8	16.2	16.4	NT
Bacillus subtilis ATCC 6633	(Gr+)	6.0	12.6	11.0	16.4	16.2	NT
Bacillus brevis ATCC 9999	(Gr+)	6.0	13.4	12.4	16.0	16.6	NT
Micrococcus luteus La 2971	(Gr+)	16.8	18.2	17.0	24.4	16.4	NT
Micrococcus flavus ATCC 14452	(Gr+)	19.0	19.6	19.0	20.0	17.2	NT
Pseudomonas aeruginosa ATCC 27853	(Gr-)	12.0	14.2	13.8	20.2	24.0	NT
Proteus vulgaris ATCC 8427	(Gr-)	6.0	13.8	11.2	18.0	18.0	NT
Escherichia coli ATCC 11230	(Gr-)	12.6	14.6	12.2	17.2	18.4	NT
Enterobacter aerogenes ATCC 13048	(Gr-)	6.0	11.4	6.0	18.6	18.2	NT
Alcaligenes faecalis CCM 3763	(Gr-)	10.2	14.8	12.4	19.8	18.6	NT
Salmonella typhimurium CCM 5445	(Gr-)	11.4	14.2	13.2	19.2	16.0	NT
Citrobacter freundii ATCC 8090	(Gr-)	18.2	19.4	17.6	20.0	16.6	NT
Fungi							
Candida albicans ATCC 10231		6.0	12.2	6.0	NT	NT	18.4
Kluyveromyces fragilis ATCC 8608		6.0	14.0	12.2	NT	NT	17.8
Rhodotorula rubra DSM 70403		11.0	12.0	11.4	NT	NT	18.2

1 = *Macrolepiota rachodes*; 2 = *Macrolepiota heimii*; 3 = *Macrolepiota puellaris*
*Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates; NT = not tested; **AK = Amikacin (30 µg/disc); CHL = Chloramphenicol (10 µg/disc); NYS = Nystatin (25 µg/disc).

As can seen from Table-1, the ethanol extracts of the mushrooms were significantly active against the bacteria Gram(+) and Gram(-) and the yeast cultures studied. The extracts of *M. heimii* were inactive *Bacillus species, Proteus vulgaris, Enterobacter aerogenes* and the yeast culture except for *Rhodotorula rubra*. Similarly *Enterobacter aerogenes* and *Candida albicans* were resistant to the extracts of *M. puellaris*. Notably, the extracts of *M. heimii* showed antimicrobial activity against all tested microorganisms. When the results obtained are compared to those of standard antibiotics, it is determined that all extracts have more effective than those of chloramphenicol against *Micrococcus* species and *Citrobacter freundii*. Some of the results

reported in this study are consisted with those from earlier studies[7-9]. In previous study[7], the extracts of *Macrolepiota procera* have shown antimicrobial activity against some Gram (+) and Gram (-) bacteria especially *Proteus vulgaris, Enterobacter aerogenes* and against some yeast cultures especially *Candida utilis* and *Hansenula* sp. It was found that extracts from mycelial cultures of *Lepista nuda, Polyporus arcularius* and several *Ganoderma* species have antibacterial activity[8]. It is determined that high antimicrobial activity from several *Basidiomycetes* species against bacteria and yeasts[9]. In another study, the extracts obtained from six *Lactarius* species (*L. deterrimus, L. sanguifluus, L. semisanguifluus, L. piperatus, L. deliciosus* and *L. salmonicolor*) have been investigated for their antimicrobial activity. It is found that *Lactarius* species revealed high antimicrobial activity against some Gram (+) and Gram (-) bacteria, but showed no antimicrobial effect against yeasts[10]. The intra-specific genetic differences have already been observed[8]. The production secondary metabolites by co-specific isolates in fungi were reported in the literature[11]. Thus, it is important to keep and screen for antimicrobial activity of differences/isolates of the same species of the *Basidiomycetes* in the collections.

In previous study, ethanol was observed as the best solvent for extracting antimicrobial substances from *Lycoperdon pusilum* and *L. giganteum*[12]. The results in this study with ethanol are similar to those reported in the mentioned study.

Although more pharmacological investigations are necessary to classify and identify the bioactive constituents the results presented here in may be a contribution for other researchers.

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