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Antimicrobial Activity of Two Wild Mushrooms *Clitocybe alexandri* (Gill.) Konr. and *Rhizopogon roseolus* (Corda) T.M. Fries collected from Turkey

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Two edible wild mushrooms, namely *Clitocybe alexandri* (Gill.) Konr. (*Tricholomataceae*) and *Rhizopogon roseolus* (Corda) T.M. Fries (*Rhizopogonaceae*), collected from the southwest of Turkey, were tested for their antimicrobial activity by using the disc diffusion method. The ethanol, methanol, diethyl ether, water, ethylacetate and *n*-hexane extracts from the fruit bodies of mushrooms were assayed against 13 microorganisms. In comparison with the test antibiotics penicillin, novobiocin, nalidixic acid and ampicillin, the methanol extract obtained from the two mushrooms presented significant activity against *E. coli*, *Bacillus subtilis* and *Enterobacter aerogenes*. On the other hand, the ethylacetate extract from *C. alexandri* was found to be active against *Candida albicans* and *Saccharomyces cerevisiae*, whereas the ethanol extract of *Rhizopogon roseolus* was active against *Saccharomyces cerevisiae*. This research has shown that various extracts obtained from two macrofungi could be used *in vitro* to inhibit the growth of some important bacteria and fungi.

Keywords: antimicrobial activity; *Clitocybe alexandri*; *Rhizopogon roseolus*.

INTRODUCTION

Macrofungi have long been used as a valuable food source and as traditional medicines around the world, especially in Japan and China. A number of medicinal mushrooms, such as *Ganoderma lucidum*, *Tremella fuciformis* and *Lentinula edodes*, are deemed to belong to the highest class of medicines (Wasser and Weis, 1999). Furthermore, screening programmes aimed at the discovery of new bioactive metabolites from macrofungi have been performed (Rosa *et al.*, 2003; Dulger *et al.*, 2002, 2004).

In research, extracts of more than 75% of the polypore mushroom species surveyed showed antimicrobial activity and 45% of 204 mushroom species inhibited the growth of a wide variety of microorganisms (Suay *et al.*, 2000).

This experimental study is part of a programme focusing on screening of wild edible mushrooms collected from the west region of Turkey. The antimicrobial activities of ethanol, methanol, diethyl ether, water, ethylacetate and *n*-hexane extracts of two wild edible mushrooms are reported here for the first time.

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MATERIALS AND METHODS

Fungal organisms. *Clitocybe alexandri* (Gill.) Konr. (*Tricholomataceae*) and *Rhizopogon roseolus* (Corda) T.M. Fries (*Rhizopogonaceae*) were collected in nature during field trips between 2004 and 2005, from the southwest of Turkey. The morphological and ecological characteristics 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 (Watling, 1973; Moser, 1983).

Preparation of macrofungi extracts. The dried and powdered fruit bodies of macrofungi were reduced to coarse powder. 20 g of each sample was extracted with 100 mL of ethanol, methanol, ethylacetate, diethyl ether and *n*-hexane at room temperature, with stirring for 2 days. The water extracts were prepared by 2% infusion. 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 13 strains was used: *Bacillus cereus* CM 99, *B. subtilis* ATCC 6683, *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 6997, *Klebsiella pneumoniae* CCM 2318, *Saccharomyces cerevisiae* ATCC 9763, *Pseudomonas fluorescens*, *Micrococcus luteus* ATTC 9341, *Enterobacter aerogenes*

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ATCC 13048, *Salmonella typhimidium* CCM 5445, *Serratia marcescens* CCM 583, *Staphylococcus aureus* ATCC 6538P and *Candida albicans* ATTC 10239.

Assay for antimicrobial activity. Antimicrobial activity was assayed by measuring the inhibition zones against used strains in agar plates. The sterilized medium at 45–50 °C was poured into petri dishes. The agar depth was 4 mm. 25 mL medium was used for plates with 90 mm diameter. The paper disks of 7 mm diameter impregnated with 20 μL of the macrofungi extracts (5 mg/mL) were dried at 35 °C and placed into the bacteria and yeast petri dishes. Discs injected with 20 μL of pure ethanol, methanol, ethylacetate, diethyl ether and *n*-hexane served as negative controls. The treated petri dishes were incubated overnight at 37 °C. The antimicrobial effect was identified and measured as the zone of inhibition. Each experiment was replicated three times and the results were expressed as average values. The standard antibacterial agents, novobiocin (5 μg/mL), penicillin G (20 μg/mL), nalidixic acid (30 μg/mL) and ampicillin (10 μg/mL) were used as positive controls for bacteria and the standard antifungal agent, nystatin (10 μg/mL) as a positive control for fungi.

RESULTS AND DISCUSSION

Tables 1 and 2 show the antimicrobial activities of the extracts obtained from *Clitocybe alexandri* and *Rhizopogon roseolus*, respectively. As clearly seen from Table 1, with an inhibition zone of 25 mm, the methanol extract of the fruit bodies of *C. alexandri* presented significant activity against *Bacillus subtilis.* Of all the extracts only the ethylacetate extract had antiyeast activity against *Candida albicans* and *Saccharomyces cerevisiae*. In comparison with ethanol and ethylacetate extracts, *n*-hexane and water extracts with the same inhibition zone were more active against *Bacillus cereus*. Compared with the ethanol extract, the methanol extract was twice as active against *Micrococcus luteus*. Activity against *Enterobacter aerogenes* was found from the water, diethyl ether and methanol extracts with inhibition zones of 13, 15 and 18 mm, respectively. On the other hand, the methanol extract was found to be the most active extract against *Escherichia coli*. As listed in Table 1, none of the extracts were active against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella*

Table 1. Antimicrobial activity of *Clitocybe alexandri* (zones of inhibition, mm)

Test organism	1	2	3	4	5	6	7	8	9	10	11
Bacillus cereus	13	18	–	–	14	18	–	20	20	22	–
Bacillus subtilis	–	–	–	25	–	–	15	–	24	22	–
Enterobacter aerogenes	–	13	15	18	–	–	20	25	22	20	–
Escherichia coli	10	–	9	15	9	–	20	–	26	25	–
Klebsiella pneumoniae	–	–	–	–	–	–	–	–	–	–	–
Micrococcus luteus	10	–	–	20	–	–	–	–	–	–	–
Proteus vulgaris	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas fluorescens	–	–	–	–	–	–	–	–	–	–	–
Salmonella typhimidium	–	–	–	–	–	–	–	–	–	–	–
Serratia marcescens	–	–	–	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–	–	–	–	–
Candida albicans	–	–	–	–	14	–	–	–	–	–	22
Saccharomyces cerevisiae	–	–	–	–	12	–	–	–	–	–	20

1, ethanol extract; **2**, water extract; **3**, diethyl ether extract; **4**, methanol extract; **5**, ethylacetate extract; **6**, *n*-hexane extract; **7**, novobiocin; **8**, penicillin G; **9**, nalidixic acid; **10**, ampicillin; **11**, nystatin.

Table 2. Antimicrobial activity of *Rhizopogon roseolus* (zones of inhibition, mm)

Test organism	1	2	3	4	5	6	7	8	9	10	11
Bacillus cereus	11	–	–	–	13	–	–	20	20	22	–
Bacillus subtilis	–	–	–	18	–	–	15	–	24	22	–
Enterobacter aerogenes	12	–	–	16	–	–	20	25	22	20	–
Escherichia coli	–	–	–	17	–	–	20	–	26	25	–
Klebsiella pneumoniae	–	–	–	–	–	–	–	–	–	–	–
Micrococcus luteus	–	–	–	–	10	–	–	–	–	–	–
Proteus vulgaris	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas fluorescens	–	–	–	–	–	–	–	–	–	–	–
Salmonella typhimidium	–	–	–	–	–	–	–	–	–	–	–
Serratia marcescens	–	–	–	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–	–	–	–	–
Candida albicans	–	–	–	–	–	–	–	–	–	–	22
Saccharomyces cerevisiae	11	–	–	–	–	–	–	–	–	–	20

1, ethanol extract; **2**, water extract; **3**, diethyl ether extract; **4**, methanol extract; **5**, ethylacetate extract; **6**, *n*-hexane extract; **7**, novobiocin; **8**, penicillin G; **9**, nalidixic acid; **10**, ampicillin; **11**, nystatin.

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typhimidium, *Serratia marcescens* and *Staphylococcus aureus*.

Table 2 presents the results of the antimicrobial activity of *Rhizopogon roseolus* extracts. Water, diethyl ether and *n*-hexane extracts prepared from the fruit bodies of *Rhizopogon roseolus* showed no inhibitory effects against the selected microorganisms. The only extract that had antifungal activity against *Saccharomyces cerevisiae* was the ethanol extract with an inhibition zone of 11 mm. The methanol extract had activity against *Bacillus subtilis*, *Enterobacter aerogenes* and *Escherichia coli* while the ethyl acetate extract was active against *Bacillus cereus* and *Micrococcus luteus*. The extracts had no inhibitory properties on *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella typhimidium*, *Serratia marcescens*, *Staphylococcus aureus* and *Candida albicans*.

In a previous study ethanol was observed as the best solvent for extracting antimicrobial substances from *Lycoperdon pusilum* and *L. giganteum* (Jonathan and Fasidi, 2003). In contrast, our results showed that water and methanol were good solvents for extracting *C. alexandri* and *R. roseololus* fruit bodies. Suay *et al.* (2000) reported that the methanol extract of *C. nebularis* (Tricholomataceae) was active against *Staphylococcus aureus* (<15 mm), *Bacillus subtilis* (>15 mm) and *Aspergillus fumigatus* (weak inhibition zone). In the same study, simple hot-water extracts of *Lepista nuda* (Tricholomataceae) were found to be active against *C. albicans*. However, this study observed activity against *C. albicans* only from the ethylacetate extract of *C. alexandri*.

These results confirm that bioactive components of any macrofungi may differ in their solubility depending on the extractive solvents. In a comparison of the two wild edible mushrooms, *C. alexandri* which is easily found and collected from nature exerted more inhibitory effects than *R. roseolus* which has no stem and cap.

In the light of our data it is concluded that *C. alexandri* is a prospective edible wild mushroom for the isolation of new antibiotics or chemical substances. Although further investigations are clearly necessary to clarify and identify the bioactive constituents we believe that our results presented herein may be a contribution for other researchers, who would carry out further studies on the antimicrobial activity of macrofungi.

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