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# Antimicrobial Activity of Some Lactarius Species

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### Abstract

The extracts obtained from six *Lactarius* species [Lactarius deterrimus Grager, Lactarius sanguifluus (Paul.: Fr.) Fr., Lactarius semisanguifluus Heim et Leclair, Lactarius piperatus Scop. ex Fr., Lactarius deliciosus (L. ex Fr.) S.F. Gray and Lactarius salmonicolor Heim et Leclair] have been investigated for their antimicrobial activity. Growth inhibition using agar disk diffusion assays was determined against: Escherichia coli ATCC 11230, Micrococcus luteus ATCC 2971, Stapylococcus aureus ATCC 6538P, Salmonella thyphi ATCC 19430, Klebsiella pneumoniae UC57, Pseudomonas aeruginosa ATCC 27853, Corynebacterium xerosis CCM 2824, Bacillus cereus ATCC 7064, Bacillus megaterium DSM 32, Mycobacterium smegmatis CCM 2067, Candida albicans ATCC10231 and Saccharomyces cerevisiae ATCC 9763. As a result of this study, we have found that *Lactarius* species revealed antimicrobial activity against some Gram (+) and Gram (-) bacteria, but showed no antagonistic effect against yeasts used in this study.

Keywords: Lactarius, antimicrobial activity.

# Introduction

Large-scale screening programs from the 1940s for the detection of antibiotic activity include a variety of fleshy basidiomycetes (Benedict & Brady, 1972; Espanshade & Griffith, 1966; Broadbent, 1966). A number of more recent reports recorded additional general observations of microbial antagonism with basidiomycetes (Conchran, 1978). Unfortunately, the identities of the basidiomycete metabolites responsible for the antimicrobial effects are still unknown in most instances.

The polyacetlylenes are the most extensively characterized group of antagonistic mushroom constituents. More than 50 of these unsaturated antibiotic substances are known from one or more species of *Aleurodiscus*, *Clitocybe*, *Coprinus*, *Cortinellus*, *Marasmius*, *Merulies*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella* and *Tricholoma*. Other known antagonist compounds from basidiomycetes include phenolic metabolites (Benedict & Brady, 1972; Conchran, 1978).

In this study, we aimed to determine the antimicrobial activity of the extracts of some *Lactarius* species. Extracts were tested for antimicrobial activity against representative Gram-positive and Gram-negative bacteria as well as yeasts.

# Material and methods

#### Materials

Six species of *Lactarius [Lactarius deterrimus* Grager, *Lactarius sanguifluus* (Paul.: Fr.) Fr., *Lactarius semisanguifluus* Heim et Leclair, *Lactarius piperatus* Scop. ex Fr., *Lactarius deliciosus* (L. ex Fr.) S.F.Gray and *Lactarius salmonicolor* Heim et Leclair] were collected at Bursa-Uludag and Balikesir-Savastepe in Turkey.

#### Extraction

Macrofungus material was extracted twice with an appropriate amount of 80% aq. methanol. The extracts were evaporated to dryness, and stored at -20 °C until further analysis.

#### Bioassays

*In vitro* antimicrobial studies were carried out by the agardisk diffusion method against test micro-organisms (Collins & Lyne, 1987; NCCLS, 1993; Board & Lovelock, 1975; Favel et al., 1994). Five hundred micrograms of crude

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Microorganisms	1	2	3	4	5	6
Escherichia coli ATCC 11230	++	+++	++	++	+++	+++
Micrococcus luteus La 2971	_	_	_	_	_	_
Proteus vulgaris ATCC 8427	++	++	++	++	+++	+++
Staphylococcus aureus ATCC 6538P	+	+	+	+	+	+
Klebsiella pneumoniae UC57	_	(+)	_	_	(+)	_
Pseudomonas aeruginosa ATCC 27853	_	_	_	_	(+)	_
Corynebacterium xerosis CCM 7064	_	_	_	_	_	(+)
Bacillus cereus ATCC 7064	+	+	+	+	+	+
Bacillus megaterium DSM 32	+	+	+	+	+	+
Mycobacterium smegmatis CCM 2067	++	++	++	+++	+++	+++
Candida albicans ATCC 10231	_	_	_	_	_	-
Saccharomyces cerevisiae ATCC 9730	-	_	-	-	-	-
Collector/Collection number	FG4147	FG4136	FG4137	FG4085	Y78	Y41

Table 1. Survey of antimicrobial activity in Lactarius species.

1: Lactarius deterrimus Grager

2: Lactarius sanguifluus (Paul.: Fr.) Fr.

3: Lactarius semisanguifluus Heim et Leclair

4: Lactarius piperatus Scop. ex Fr.

5: Lactarius deliciosus (L. ex Fr.) S.F.Gray

6: Lactarius salmonicolor Heim et Leclair

FG: Fahrettin Gucin; Y: Fadime Yilmaz

(+) : Inhibition zone less than 1 mm surrounding the 6 mm paper disk.

+ : Inhibition less than

++ : Inhibition comparable to

+++ : Inhibition more than 10 µg penicillin or sulconazole/disk; Inhibition zones of references: 12–16 mm diam.

macrofungus extract dissolved in 20µl of 80% aq. methanol were applied to a 6mm diameter paper disk for every test. Penicillin for the bacteria, sulconazole for the yeasts (10µg/ disk; both obtained form Sigma), and 80% aq. methanol were used as controls. Mueller Hinton Agar plates (Oxoid) were plated with 200 µl of microbial cultures in the exponential growing phase (approximately 5 CPU). Escherichia coli ATCC 11230, Micrococcus luteus ATCC La 2971, Proteus vulgaris ATCC 8427, Staphylococcus aureus ATCC 6538P, Salmonella thyphi ATCC 19430, Klebsiella pneumoniae UC57, Pseudomonas aeruginosa ATCC 19430, Corynebacterium xerosis CCM 2824, Bacillus cereus ATCC ATCC 7064, Bacillus megaterium DSM 32, Mycobacterium smegmatis CCM 2067, Candida albicans ATCC 10231 and Saccharomyces cerevisiae ATCC 9763 were used to test antimicrobial activity. The degree of growth inhibition was qualitatively evaluated after 16h by comparison to growth inhibition resulting from the positive control.

#### **Results and discussion**

Table 1 gives a summary of the investigated *Lactarius* species and the results of the antimicrobial screening. No significant activity was found against yeasts. Antimicrobial

activity was most consistently detected in all *Lactarius* genera against *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 8427 and *Mycobacterium smegmatis* CCM 2067. Notably, the extract of *Lepista* species exhibited weak activity against *Staphylococcus aureus* ATCC 8427, *Bacillus cereus* ATCC 7064, *Bacillus megaterium* DSM 32 and *Salmonella thyphi* ATCC 19430, and no antimicrobial activity against *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Corynebacterium xerosis* CCM 2824 and *Micrococcus luteus* La 2971. In addition, *Lactarius deliciosus* (L. ex Fr.) S.F.Gray was more effective than other *Lactarius* species against tested micro-organisms used in this study.

According to literature, a hot water extract of *Lactarius piperatus* (L. ex Fr.) Gray inhibits Lewis pulmonary adenoma in white mice. Its inhibition rate against sarcoma 180 in white mice is 80%, that against Ehrlich carcinoma is 70% (Jayko et al., 1974). The extract of *Lactarius deliciosus* was found to be particularly effective against the acid-fast *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* (Ying et al., 1987). Our findings were parallel to those reported in the above study.

As a result of this study, we have found that *Lactarius* species revealed antimicrobial activity against some Gram (+) and Gram (-) bacteria but showed no antagonistic effect

against yeasts used in this study. In addition, *Mycobacterium smegmatis* CCM 2067, *Escherichia coli* ATCC 11230 and *Proteus vulgaris* ATCC 8427 were found to be the most sensitive bacteria against the extract of *Lactarius* species.

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