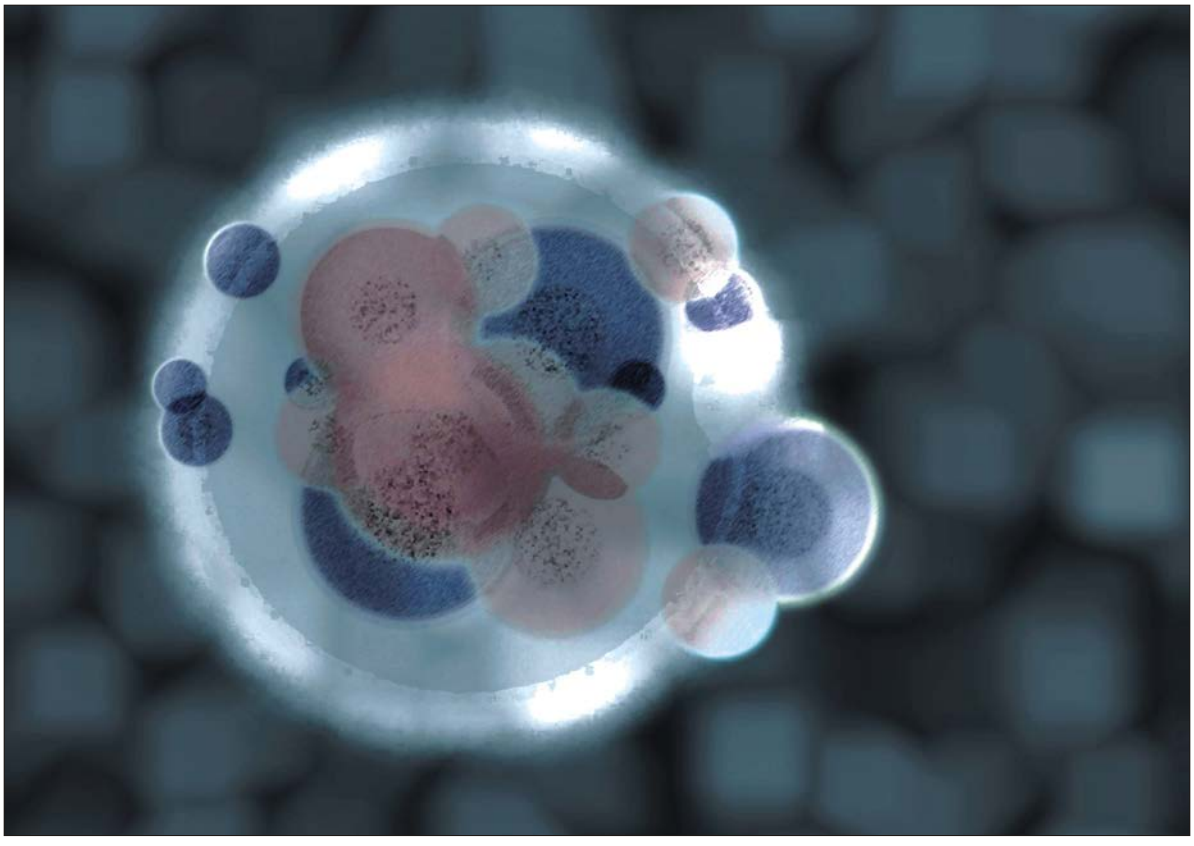

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Evaluation of Antimicrobial Activity of the Macrofungus *Phellinus torulosus*

Basaran Dulger, Tulay Bican Suerdem, Deniz Yesilyurt, Nurcihan Hacioglu and Aytac Camdeviren
Department of Biology, Faculty of Science and Arts, Canakkale Onsekiz Mart University, Canakkale, Turkey

Abstract: In this study, ethyl acetate, acetone, chloroform and ethanol extracts of *Phellinus torulosus* (Pers.) Bourd. and Galz. (*Polyporaceae*) were tested for antimicrobial activity by Disc Diffusion method on the following test microorganisms: *Aeromonas hydrophila* ATCC 7966, *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* ATCC 8427, *Serratia marcescens* NRRL 3284, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Bacillus brevis* ATCC 9999, *Bacillus sphaericus*, *Bacillus megaterium*, *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* LA 2971, *Staphylococcus aureus* ATCC 6538P, *Stapylococcus epidermidis* NRRL B-4877, *Alcaligenes faecalis* CCM 3763, *Alcaligenes eutrophus, Salmonella typhi* ATCC 19430, *Salmonella typhimurium* CCM 5445, *Klebsiella pneumoniae* UC57, *Micrococcus roseus*, *Micrococcus flavus* ATCC 14452, *Citrobacter freundii* ATCC 8090, *Bordatella bronchiseptica* ATCC 4617, *Erwinia amylovora*, *Xanthomonas campestris*, *Pseudomonas extorquens*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas putida*, *Kluyveromyces fragilis* ATCC 8608, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 10403, *Aspergillus oryzae, Aspergillus flavus, Botrytis cineriae, Fusarium oxysporium, Streptomyces murinus* ISP 5091 and *Nocardia cornea* IFO 14403. As a result of study, we have found that *Phellinus torulosus* (Pers.) Bourd. and Galz. revealed antimicrobial activity against some Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes used in this study.

Key words: *Phellinus torulosus* (Pers.) Bourd. and Galz., antimicrobial activity

INTRODUCTION

Many antibiotics in clinical use developed from fungal and actinomycetes metabolites. Large-scale screening programs of the 1940's for the detection of antibiotic activity included a variety of fleshy basidiomycetes[1-3]. A number of more recent reports recorded additional general observation of microbial antagonism with basidiomycetes[4]. Unfortunately, the identities of the basidiomycete metabolites responsible for the antimicrobial effects are still unknown in most instances.

The polyacetylenes 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 the phenolic metabolites[1,4].

Phellinus torulosus is the causal agent of white rot that infects especially the roots and the collar of old trees and shrubs of many species. Although there are many investigations on *Phellinus torulosus* in different subjects[5-8], antimicrobial activity of this macrofungus has not been previously investigated. Therefore, in the present study aim was to determine the antimicrobial effects of the extracts of *Phellinus torulosus* (Pers.) Bourd. and Galz. against various microorganisms.

MATERIALS AND METHODS

Macrofungal material: *Phellinus torulosus* (Pers.) Bourd. and Galz. collected from Uludag mountain, Bursa-Turkey in 1997. The macrofungus was identified by Prof. Dr. Fahrettin Gucin, Fatih University, Faculty of Science and Arts, Department of Biology, Istanbul. A voucher specimen (BD-MF18) has been deposited at the herbarium of Department of Biology, Uludag University, Bursa-Turkey.

Microorganisms: In this study, the following microorganisms were used: *Aeromonas hydrophila* ATCC 7966, *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* ATCC 8427, *Serratia marcescens* NRRL 3284, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC

Corresponding Author: Dr. Basaran Dulger, Department of Biology, Faculty of Science and Arts, 17100, Canakkale Onsekiz Mart University, Canakkale, Turkey E-mail: dbasaran@comu.edu.tr

6633, *Bacillus brevis* ATCC 9999, *Bacillus sphaericus*, *Bacillus megaterium*, *Mycobacterium smegmatis* CCM 2067, *Micrococcus luteus* LA 2971, *Staphylococcus aureus* ATCC 6538P, *Stapylococcus epidermidis* NRRL B-4877, *Alcaligenes faecalis* CCM 3763, *Alcaligenes eutrophus, Salmonella typhi* ATCC 19430, *Salmonella typhimurium* CCM 5445, *Klebsiella pneumoniae* UC57, *Micrococcus roseus*, *Micrococcus flavus* ATCC 14452, *Citrobacter freundii* ATCC 8090, *Bordatella bronchiseptica* ATCC 4617, *Erwinia amylovora*, Xanthomonas campestris, *Pseudomonas extorquens*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas putida*, *Kluyveromyces fragilis* ATCC 8608, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 10403, *Aspergillus oryzae, Aspergillus flavus, Botrytis cineriae, Fusarium oxysporium, Streptomyces murinus* ISP 5091 and *Nocardia cornea* IFO 14403. Test microorgamisms were obtained from culture collection of Aegean University, Faculty of Science, Basic and Industrial Microbiology Department.

Preparation and antimicrobial activity of extracts: The material was ground to a fine powder. Fifteen gram of this material was subjected to Soxhlet extraction for 12 h each using 150 mL of the following solvents ethyl acetate, acetone, chloroform and ethanol. The extracts were kept at + 4°C[9,10].

In vitro antimicrobial studies were carried out by the Agar-Disc Diffusion method against test microorganisms. As a consequence of Mueller Hinton Agar (OXOID) was used as the most suitable medium for antimicrobial activity studies. The sterilized medium at 45-50°C was poured into petri dishes. Agar depth was 4 mm. For 90 mm diameter plates 25 mL medium was used. According to this method, ethanol, ethyl acetate, acetone and chloroform extracts were impregnated as four discs in ranging concentrations from 50 μL. Then all discs were dried in 50°C and placed into the bacteria and yeasts petri dishes. Each disc was 6 mm diameter. For each experiment a fifth disc which contained only solvent was used as control disc. As reference, antibiotic AK30 (=Amikasin) for bacteria and NY100 (=Nystatin) for the yeast cultures were used. Experiments were repeated three times and the results were expressed as average values.

Bacteria and yeast cultures were suspended in 4-5 mL Brain Heart Infusion Broth (OXOID). Bacteria were incubated in 37°C for 2-5 h. Yeast cultures were incubated in 30°C for 5-7 h. When a visible turbidity was obtained at the end of this time, the turbidity of bacterial suspension was adjusted against Macfarland Standart Tube [0.5] with physiologic serum and inoculation was performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. Streak the entire Mueller Hinton Agar surface in 3 different directions by rotating the plate 60°C angles after each streaking. Yeast cultures were inoculated into Mueller-Hinton Agar (10^2 cfu mL^{-1}). All petri dishes after inoculation were allowed to dry for 15-20 min in room temperature. For bacteria (35°C) and yeasts (30°C), inhibition zone diameters were measured after 24-48 h using Agar-Disc Diffusion method[11, 12].

Spore suspension of filamentous fungi and actinomycetes were cultured on Sabouraud's Dextrose agar (10^5 cfu mL^{-1}) by plate dilution techniques using Thoma and Howard slides[3,13,14]. It was observed that Agar-Disc diffusion method was generally not suitable for filamentous fungi and actinomycetes. Therefore this method was used after modification. In this experiment, the solutions (from 10 to 200 μg mL^{-1}) were added into the medium after autoclaving. Erythromycine (15 μg mL^{-1}) was used as a comparison antibiotic against filamentous fungi. The antibiotic was added into the medium. The evaluation of filamentous fungi and actinomycetes was carried out by means of reproduction on the medium and reduction of the colony numbers at the end of the seven days[11].

RESULTS AND DISCUSSION

According to the present findings all of the extracts *Phellinus torulosus* have been found to be ineffective against *Salmonella typhimurium*, *Salmonella typhi*, the acid-fast bacterium *Mycobacterium smegmatis*, *Proteus vulgaris* ATCC 8427 and *Erwinia amylovora*. The various extracts of *Oudemansiella melanotricha* have been determined to be less effective than that of AK30 used as comparison antibiotic against *Aeromonas hydrophila*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Xanthomonas campestris*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas exterquens*, *Pseudomonas flourescens*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Micrococcus flavus* and *Citrobacter freundii*. Notably, all the extracts of the macrofungus have been found more active against especially *Bacillus* and *Alcaligenes* strains than those of the standard comparison antibiotic AK30.

The extracts of *Phellinus torulosus* have shown antiyeast activity against the yeasts. The extracts were found to be effective against *Kluyveromyces fragilis*, *Rhodotorula rubra* and *Candida albicans*, as compared to standard antibiotics Nystatin.

Table 1: Antimicrobial activity of the extracts of *Phellinus torulosus* (Pers.) Bourd. and Galz. on some bacteria and yeasts

	Zones of inhibition (mm)				Comparison antibiotic
Tested microorganisms	Acetone	Chloroform	Ethyl acetate	Ethanol	AK30/NY100
Aeromonas hydrophila ATCC 7966	11.0	11.4	10.2	11.8	21.2
Listeria monocytogenes ATCC 19117	10.2	10.2	10.6	12.2	20.6
Escherichia coli ATCC 11230	-	11.2	-	10.6	17.2
Enterobacter aerogenes ATCC 13048	10.4	10.8	-	11.2	18.6
Proteus vulgaris ATCC 8427	-	-	-	-	18.0
Serratia marcesceus NRRL 3284	11.0	-	-	18.0	20.0
Bacillus cereus ATCC 7064	17.2	18.8	16.8	20.6	16.2
Bacillus subtilis ATCC 6633	18.0	17.6	18.2	22.2	16.4
Bacillus sphaericus	18.8	18.0	19.6	24.2	20.0
Bacillus brevis ATCC 9999	17.2	17.4	16.8	22.0	18.6
Bacillus megaterium	20.4	22.8	21.0	23.2	20.4
Mycobacterium smegmatis CCM 2067	-	-	-	-	18.2
Micrococcus luteus LA 2971	10.8	-	-	11.4	24.4
Micrococcus flavus ATCC 14452	-	-	-	12.2	20.0
Staphylococcus aureus ATCC 6538P	10.0	12.6	12.2	13.8	24.2
Staphylococcus epidermidis NRRL B-4877	-	-	10.6	10.8	23.2
Alcaligenes faecalis CCM 3763	16.8	18.2	20.0	22.2	19.8
Alcaligenes eutrophus	20.8	17.8	21.8	24.0	20.2
Salmonella typhi ATCC 19430	-	-	-	-	20.6
Salmonella typhimurium CCM 5445	-	-	-	-	19.2
Klebsiella pneumoniae UC57	-	-	-	10.4	20.0
Citrobacter freundii ATCC 8090	-	9.6	-	9.0	20.0
Erwinia amylovora	-	-	-	-	19.4
Pseudomonas pntida	-	-	-	9.0	20.2
Pseudomonas extorqneus	-	-	9.0	10.2	18.6
Pseudomonas fluoresceus	-	-	-	9.6	20.4
Pseudomonas aeruginosa ATCC 27853	9.0	-	-	11.0	19.6
Xanthomonas campestris	-	10.0	-	12.0	20.2
Candida albicans ATCC 10231	18.2	18.8	20.6	22.2	19.8
Kluyveromyces fragilis ATCC 8608	18.6	19.0	20.2	20.0	17.8
Rhodotorula rubra DSM 70403	19.4	26.4	18.0	20.0	18.2

(-): No Inhibition Zones, (NT): Not Tested

Table 2: Antimicrobial activity of the extracts of *Phellinus torulosus* (Pers.) Bourd. and Galz. on some filamentous fungi and actinomycetes

			The colony numbers after incubation*			
Tested organisms	Concentration (μg mL^{-1})	Ethyl acetate	Acetone	Chloroform	Ethanol	Erythromycin (15 μg mL^{-1})
Aspergillus oryzae	10	62	54	49	28	
	50	26	31	28	12	
	100	-	-	-	-	15
	200	-	-	-	-	
Aspergillus flavus	10	42	58	45	22	
	50	32	27	29	9	
	100	-	-	-	-	18
	200	-	-	-	-	
Botrytis cineriae	10	54	64	63	41	
	50	32	29	27	20	
	100	-	-	-	-	15
	200	-	-	-	-	
Fusarium oxysporium	10	63	66	34	28	
	50	27	35	18	12	
	100	-	-	-	-	16
	200	-	-	-	-	
Streptomyces murinus ISP 5091	10	52	55	48	36	
	50	21	30	20	15	
	100	-	-	-	-	NT
	200	-	-	-	-	
Nocarida cornea IFO 14403	10	46	35	35	24	
	50	37	17	22	11	
	100	-	-	-	-	NT

*: Datas are the average of n=3 experiments -: No growth NT: Not Tested

Table 2 shows that the colony numbers of filamentous fungi and actinomycetes were reduced between 99.95 and 99.98% for the concentrations of 10 and 50 μg mL^{-1} of the related compounds after the incubation whilst the concentrations 100 and 200 μg mL^{-1} of these compounds inhibited filamentous fungi and actinomycetes growth completely. All results showed that colony numbers were reduced because of the activity of the compounds contained in the extracts.

Inhibition zone diameters around control disc were measured between 0-1 mm.

The macrofungus differ significantly in their activity against tested microorganisms. These differences may be attributed to fact that the cell wall in gram-positive bacteria of a single layer, whereas the gram-negative cell wall is multi-layered structure and the yeast cell wall is quite complex[15]. In addition, microorganisms variable sensitivity to chemical substances relates to different resistance levels between the strains[16].

According to literature, ethanol extract was the most effective extracts in disc diffusion method on macrofungi[1,17]. In Table 1, antimicrobial activities of ethanol extract were higher than others. So, it can be said that solvent of the fungal compounds shown antimicrobial activity is ethanol.

As the result, *Phellinus torulosus* has antimicrobial activity against some Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes. All the extracts showed more antifungal activities than antibacterial activities.

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