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Antimicrobial Activity of the Macrofungus *Cantharellus cibarius*

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Abstract: In this study, ethyl acetate, acetone, chloroform and ethanol extracts of *Cantharellus cibarius* Fr. (*Cantharellaceae*) 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, *Corynebacterium xerosis* CCM 2824, *Corynebacterium glutamicum* ATCC 13022, *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, *Sarcina lutea*, *Micrococcus luteus* LA 2971, *Staphylococcus aureus* ATCC 6538P, *Stapylococcus epidermidis* NRRL B-4877, *Alcaligenes faecalis* CCM 3763, *Alcaligenes eutrophus, Salmonella parathyphi* B, *Salmonella typhi* ATCC 19430, *Salmonella tyhimurium* 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* NRRL 2415, *Candida albicans* ATCC 10231, *Candida utilis* LA 991, *Hansenula* sp., *Rhodotorula rubra*, *Debaryomyces* sp., *Saccharomyces cerevisiae* ATCC 9763, *Schizosaccharomyces* sp., *Torulopsis* sp., *Torula* sp., *Aspergillus oryzae, Aspergillus flavus, Botrytis cineriae, Fusarium oxysporium, Streptomyces murinus* ISP 5091 and *Nocardia cornea* IFO 14403. From the present study it was found that *Cantharellus cibarius* Fr. revealed antimicrobial activity against some Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes also used in this study.

Key words: *Cantharellus cibarius* Fr., antimicrobial activity

INTRODUCTION

The medicinal value of fungi has gained great international significance since 1929, when antibiotics had been found from *Penicillium*. The dominant category of fungi engendering antibiotics pertains chiefly to Fungi Imperfecti and Basidiomycetes with Ascomycetes coming next. The antimicrobial effect produced by macrofungi covers a wide area. It tends to inhibit the growth of bacteria, fungi, protozoa and cancerous cells. The researches undertaken for the activity of macrofungus antibiotics, therefore have laid emphasis on their inhibitory effect upon the activity of bacteria, fungi, protozoa, viruses and tumours[1-9]. Many species of Basidiomycetes have been found to contain polysaccharide which markedly inhibit the growth of sarcoma in white mice. There are more than sixty species of edible Basidiomycetes which have been found to possess the same effect[10].

Many species of edible fungi have the effect of lowering blood cholesterol. Among these the effect of Donko, a strain of *Lentinus edodes*, is particularly marked, next come *Agaricus bisporus* and *Auricularia polytricha*. Among the inedible species of fungi, *Polyporus fomentarius* has also been found to have the effect of lowering chelosterol. A small dose of certain poisonous mushroom like *Amanita muscaria* produces a soporific effect. *Inocybe fastigiata* tends to cure eczemia. Moreover, *Tyromyces sulphureus*, *Poria cocos, Lentinus lepideus, Fomitopsis officinalis*, etc., may produce eburicoic acid, which can be used for synthesising steroid medicines[2,6-8,10].

The sporophore of *Cantharellus cibarius* which is edible contains Vit A. Mycelia from submerged culture are source of proteins and amino acids essential to human body. It contains protein 21.5%, fat 5%, total carbohydrate 64.9%, fibre 11.2%, ash 8.6% and 353 kcal.[1]. Eating this fungus frequently prevents one from abnormal

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sight ophthalmia, night blindness, dryness of skin and mucous membrane from losing its power of secretion. It resist certain infectious diseases of the respiratory tract. In addition, the decoction is used for the treatment of abscesses and wounds. Ethanol extract of sporophore of this fungus has an inhibitory effect on sarcoma 180 in white mice[10].

This study was aimed to determine the antimicrobial activity of the extracts of *Cantharellus cibarius* Fr. against various microorganisms.

MATERIALS AND METHODS

Macrofungal Material: *Cantharellus cibarius* Fr. collected from Uludag mountain, Bursa-Turkey in 1997. The macrofungus was identified by Prof. Dr. Fahrettin Gucin. A voucher specimen (BD--MF12) has been deposited at the herbarium of Department of Biology, Canakkale Onsekiz Mart University, Canakkale-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, *Corynebacterium xerosis* CCM 2824, *Corynebacterium glutamicum* ATCC 13022, *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, *Sarcina lutea*, *Micrococcus luteus* LA 2971, *Staphylococcus aureus* ATCC 6538P, *Stapylococcus epidermidis* NRRL B-4877, *Alcaligenes faecalis* CCM 3763, *Alcaligenes eutrophus, Salmonella parathyphi* B, *Salmonella typhi* ATCC 19430, *Salmonella tyhimurium* 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* NRRL 2415, *Candida albicans* ATCC 10231, *Candida utilis* LA 991, *Hansenula* sp., *Rhodotorula rubra*, *Debaryomyces* sp., *Saccharomyces cerevisiae* ATCC 9763, *Schizosaccharomyces* sp., *Torulopsis* sp., *Torula* sp., *Aspergillus oryzae, Aspergillus flavus, Botrytis cineriae, Fusarium oxysporium, Streptomyces murinus* ISP 5091 and *Nocardia cornea* IFO 14403. Test microorganisms 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 g 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[13-16].

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) was 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 Standard 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° 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[17,18].

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[19-21]. 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[17].

RESULTS AND DISCUSSION

The zone diameters of the plates after incubation are given in Table 1. According to the present finding; all of the extracts *Cantharellus cibarius* have been found to be ineffective against *Corynebacterium xerosis* CCM 2824, *Corynebacterium glutamicum* ATCC 13022, *Klebsiella pneumoniae* UC57, *Salmonella typhimurium* CCM 5445, *Salmonella typhi* ATCC 19430, *Salmonella parathyphi* B, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427, *Sarcina lutea, Micrococcus luteus* LA 2971, *Micrococcus flavus* ATCC 14452, *Micrococcus roseus*, *Citrobacter freundii* ATCC 8090, *Bordatella bronchiseptica* ATCC 4617, *Erwinia amylovora, Pseudomonas fluorescens* and *Pseudomonas extorquens*. The various extracts of *Cantharellus cibarius* Fr. have been determined to be less effective than that of AK30 used as comparison antibiotic against *Aeromonas hydrophila* ATCC 7966, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* NRRL B-4877, *Serratia marcescens* NRRL 3284, *Xanthomonas campestris, Pseudomonas putida* and *Pseudomonas aeruginosa* ATCC 27853. All the extracts of the macrofungus have been found more active against *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Alcaligenes feacalis* CCM 3763, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Bacillus brevis* ATCC

Table 1: Antimicrobial activity of the extracts of *Cantharellus cibarius* Fr. on some bacteria and yeasts.

	Zones of Inhibition (mm)			Comparison antibiotic	
Tested microorganisms	Acetone	Chloroform	Ethyl Acetate	Ethanol	AK30
Aeromonas hydrophila ATCC 7966	9.0	10.0	9.0	13.0	21.0
Listeria monocytogenes ATCC 19117	25.0	24.0	20.0	26.0	20.0
Escherichia coli ATCC 11230	20.0	24.0	22.0	26.0	17.0
Enterobacter aerogenes ATCC 13048	24.0	22.0	19.0	30.0	18.0
Corynebacterium xerosis CCM 2824	-	-	-	-	20.0
Proteus vulgaris ATCC 8427	-	-	-	-	18.0
Serratia marcescens NRRL 3284	11.0	-	-	18.0	20.0
Bacillus cereus ATCC 7064	22.0	20.0	26.0	28.0	16.0
Bacillus subtilis ATCC 6633	18.0	18.0	18.0	22.0	16.0
Bacillus sphaericus	22.0	21.0	21.0	28.0	20.0
Bacillus brevis ATCC 9999	24.0	31.0	30.0	32.0	18.0
Bacillus megaterium	30.0	30.0	32.0	34.0	20.0
Mycobacterium smegmatis CCM 2067	-	-	-	-	18.0
Sarcina lutea	-	-	-	-	26.0
Micrococcus luteus LA 2971	-	-	-	-	24.0
Staphylococcus aureus ATCC 6538P	10.0	8.0	12.0	14.0	24.0
Staphylococcus epidermidis NRRL B-4877	-	-	-	10.0	23.0
Alcaligenes faecalis CCM 3763	22.0	26.0	24.0	31.0	19.0
Salmonella parathyphi B	-	-	-	-	20.0
Salmonella thyphi ATCC 19430	-	-	-	-	20.0
Salmonella thyphimurium CCM 5445	-	-	-	-	19.0
Klebsiella pneumoniae UC57	-	-	-	-	20.0
Micrococcus roseus	-	-	-	-	24.0
Micrococcus flavus ATCC 14452	-	-	-	-	20.0
Citrobacter freundii ATCC 8090	-	-	-	-	20.0
Bordatella bronchiseptica ATCC 4617	-	-	-	-	16.0
Erwinia amylovora	-	-	-	-	19.0
Corynebacterium glutamicum	-	-	-	-	20.0
Pseudomonas putida	-	-	-	8.0	20.0
Pseudomonas extorquens	-	-	-	-	18.0
Pseudomonas fluorescens	-	-	-	-	20.0
Pseudomonas aeruginosa ATCC 27853	8.0	-	-	11.0	19.0
Xanthomonas campestris	-	10.0	-	12.0	20.0
Alcaligenes eutrophus	24.0	24.0	20.0	28.0	20.0
Candida utilis LA 991	-	-	-	-	NT
Candida albicans ATCC 10231	-	-	-	-	NT
Kluyveromyces fragilis NRRL 2415	18.0	20.0	20.0	24.0	NT
Hansenula sp.	23.0	22.0	24.0	26.0	NT
Debaryomyces sp.	13.0	8.0	10.0	18.0	NT
Saccharomyces cerevisiae ATCC 9763	15.0	12.0	10.0	16.0	NT
Rhodotorula rubra	19.0	26.0	22.0	20.0	NT
Schizosaccharomyces sp.	10.0	8.0	8.0	12.0	NT
Torulopsis sp.	-	-	-	-	NT
Torula sp.	-	-	-	-	NT

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

Table 2: Antimicrobial activity of the extracts of *Cantharellus cibarius* Fr. on some filamentous fungi and actinomycetes

		The colony numbers after incubation*				
Tested organisms	Concentrations (μg mL^{-1})	Ethyl Acetate	Acetone	Chloroform	Ethanol	Erythromycin(15 μg mL^{-1})
Aspergillus oryzae	10	52	42	41	21	
	50	37	21	24	7	
	100	-	-	-	-	15
	200	-	-	-	-	
Aspergillus flavus	10	45	43	27	16	
	50	36	21	16	5	
	100	-	-	-	-	18
	200	-	-	-	-	
Botrytis cineriae	10	64	61	49	41	
	50	48	51	37	27	
	100	-	-	-	-	15
	200	-	-	-	-	
Fusarium oxysporium	10	40	60	37	29	
	50	25	38	22	17	
	100	-	-	-	-	16
	200	-	-	-	-	
*Streptomyces murinus*ISP 5091	10	54	50	42	41	
	50	25	36	28	20	
	100	-	-	-	-	NT
	200	-	-	-	-	
Nocarida cornea IFO 14403	10	36	21	25	20	
	50	27	9	11	7	
	100	-	-	-	-	NT
	200	-	-	-	-	

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

9999, *Bacillus sphaericus*, *Bacillus megaterium* and *Alcaligenes eutrophus* than that of AK30.

The extracts of *Cantharellus cibarius* Fr. have shown antiyeast activity against the yeasts. All the extracts were found to be effective against *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra, Saccharomyces cerevisiae* ATCC 9763, *Schizosaccharomyces* sp., *Debaryomyces* sp. and *Hansenula* sp. except for *Candida albicans* ATCC 10231, *Candida utilis* La 991, *Torulopsis* sp. and *Torula* sp.

The 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[22,23].

According to literature data, the extracts of *Cantharellus cibarius* Fr. Cooke showed antimicrobial activity against Gram (+) and Gram (-) bacteria. Its inhibition rates against Sarcoma 180 and *Ehrlich carcinoma* are 90 and 100%, respectively[10]. Present findings were partly parallel to the ones in the above study. It can be said that the effect of *Cantharellus cibarius* extracts on the microorganisms used is expected to vary according to the antimicrobial properties of the materials contained in these extracts.

According to literature, ethanol extract was the most effective extracts in disc diffusion method on macrofungi[8]. In Table 1, Antimicrobial activities of ethanol extract were higher than others (Table 1). So, it can be said that solvent of the fungal compounds shown antimicrobial activity is ethanol.

As the result, *Cantharellus cibarius* Fr. 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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