ANTIMICROBIAL ACTIVITY OF SOME MACROFUNGI EXTRACTS

Ergin Murat ALTUNER[1], Ilgaz AKATA[2]

[1] Kastamonu University, Faculty of Science and Arts, Department of Biology, TR-37100, Kuzeykent, Kastamonu, TURKEY
[2] Ankara University, Faculty of Science, Department of Biology, TR-06100, Tandoğan, Ankara, TURKEY
Email: ergin.murat.altuner@gmail.com

Abstract

Natural products have potential of containing agents for various diseases. When extensive drug resistance is taken into account, the need for the investigation of new antimicrobial substances is essential. In this study the antimicrobial activity of *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius deliciosus* and *Phellinus hartigii i*s investigated against *Candida albicans* ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhimirium SL 1344 and *Shigella flexneri* (clinical isolate). As a result it is observed that all extracts had an antibacterial activity against *S. flexneri* and all extracts except *P. hartigii* had an antibacterial activity against *P. aeruginosa*.

Keywords: Macrofungi, antimicrobial activity, disc diffusion test

BAZI MAKROMANTAR EKSTRAKTLARININ ANTİMİKROBİYAL AKTİVİTESİ

Özet

Doğadan elde edilen maddeler, birçok hastalığın tedavisinde kullanılabilecek ajanları içerme potansiyeline sahiptirler. İlâca karşı geliştirilen yüksek direnç dikkate alındığında, yeni antimikrobiyal ürünlerin araştırılmasındaki ihtiyaç kaçınılmazdır. Bu çalışmada, *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius deliciosus* ve *Phellinus hartigii* türlerinin *Candida albicans* ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhimirium SL 1344 ve *Shigella flexneri* (klinik izole) üzerine antimikrobiyal etkileri incelenmiştir. Sonuç olarak *S. flexneri* üzerine bütün ekstraktların, *Phellinus hartigii* hariç diğer bütün ekstraktların *P. aeruginosa* üzerine antibakteriyel etki gösterdiği gözlenmiştir.

Keywords: Makromantar, antimikrobiyal aktivite, disk difüzyon testi

I. INTRODUCTION

Although there is tremendous progress in human medicine; bacterial, fungal and viral diseases are still threaten the public health especially in the developing countries [1]. Relative unavailability of medicines in these countries and in addition to this the extensive drug resistance has a large impact on human health [2]. Therefore, further researches about investigation of new antimicrobial substances should be conducted.

Natural products have potential of containing therapeutic agents for various conditions, including infectious diseases [3]. It is reported that natural products have been used for hundreds of years to treat several diseases such as caused by bacteria, fungi, viruses and parasites [4]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads but only a minute portion of the available diversity among fungi, marine fauna and flora, bacteria and plants has yet been explored for such purposes [1].

Mushrooms have a potential of using both as nutritive and medicinal food stuff [5, 6, 7, 8]. Mushrooms are not only sources of nutrients but also could be used to prevent diseases such as hypertension, hypercholesterolemia and cancer [9, 10].

Researchers isolated and identified some compounds, originating from mushrooms; show other medicinal properties, such as immunemodulatory, liver protective, antifibrotic, antiinflammatory, antidiabetic, antiviral and antimicrobial activities [11, 12, 13, 14, 15].

In this study the antimicrobial activity of *Infundibulicybe geotropa* (Bull.) Harmaja, *Lactarius controversus* (Pers.) Pers., *Lactarius deliciosus* (L.) Gray and *Phellinus hartigii* (Allesch. & Schnabl) Pat. is investigated against *Candida albicans* ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhimirium SL 1344 and *Shigella flexneri*.

Materials and Methods

1.1. Macrofungi samples

Infundibulicybe geotropa (Bull.) Harmaja, *Lactarius controversus* (Pers.) Pers., *Lactarius deliciosus* (L.) Gray and *Phellinus hartigii* (Allesch. & Schnabl) Pat. samples were used in this study. All macrofungi samples were collected and identified by Ilgaz Akata. Voucher specimens were deposited for further reference in ANK Herbarium.

1.2. Extraction Procedure

All macrofungi samples were dried out after collection and the samples were grounded by mortar and pestle. In order to extract as much as active substance grounded samples were extracted subsequently with a solvent cocktail, dH_2O:ethyl alcohol:methyl alchol:acetone: CH_2Cl_2 (1:2.5:2.5:2:2) for 3 days. After 3 days the extracts were filtered through Whatman No. 1 filter paper and the filtrate was evaporated by rotary evaporator at 30°C. The residue was used to prepare 300mg.mL^{-1} extracts.

1.3. Microorganisms

Candida albicans ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa* (clinical isolate), *Salmonella enterica* serotype Typhimirium SL 1344 and *Shigella flexneri* (clinical isolate) were used in the study (Kastamonu University, Department of Biology, Botanical Research Laboratory Culture Collection).

1.4. Preparation of Inocula

All bacterial strains were incubated in atmospheric air at 37 ˚C for 24 hours and *C. albicans* at 27 ˚C for 48 hours. Inocula were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard having approximately 10^8 cfu/ml for bacteria and 10^7cfu/ml for *C. albicans* [16]. Mueller - Hinton Agar (Merck) medium is used for bacteria, where *C. albicans* strain was plated on Sabouraud dextrose agar (Merck).

1.5. Disc Diffusion Method

Disc diffusion test was performed as described previously in BSAC [17]. The culture medium was poured into 120 mm sterile Petri dish to give a mean depth of 4.0 mm ± 0.5 mm [18]. 10 µl, 20 µl and 30 µl aliquots of each extract was applied on sterile paper discs of 6 mm diameter end up with 3 mg.µl^{-1}, 6 mg.µl^{-1} and 9 mg.µl^{-1} sample on each disc [19]. To get rid of any residual solvent which might interfere with the results, discs were left to dry overnight at 30°C in sterile conditions [20]. The surface of the plates was inoculated using previously prepared inocula containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5 minutes at room temperature before applying the discs. Discs were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimetres.

1.6. Controls

All extraction solvents and empty sterile discs were used as negative controls.

1.7. Statistics

All extracts were tested in triplicate and MACANOVA (version 5.05) was used for statistical analysis of the data. *P* values of <0.05 were considered statistically significant.

Results

The diameter of the inhibition zones recorded as the diameter of the zones in millimetres for the samples are given in Table 1.

No activity was observed for the negative controls; solvents and empty sterile discs.

Table 1 clearly puts forward that all extracts were presented antimicrobial activity against *S. flexneri*. According to the results it could be concluded that *Infundibulicybe geotropa* and *Lactarius controversus* had very close activity against *S. flexneri* which were the lowest where *Phellinus hartigii* had the highest activity among others.

Table 1. Disc diffusion test results (Inhibition zones in mm)

	I. geotropa			***L. controversus***			***L. deliciosus***			***P. hartigii***		
	a	b	c	a	b	c	a	b	c	a	b	c
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	9	10	12	-	-	9	7	8	12	-	-	-
S. enterica	-	-	-	-	-	-	-	-	-	-	-	-
S. flexneri	7	8	9	7	8	10	9	11	12	20	22	23

a.10µL b.20µL c.30µL
"-": no activity observed.

Table 1 also presents that all the extracts, except *Phellinus hartigii* had antimicrobial activity against *P. aeruginosa*. If the results are compared it could be observed that *Lactarius controversus* had the lowest activity but *Infundibulicybe geotropa* and *Lactarius deliciosus* had antimicrobial activity higher than *Lactarius controversus*.

II.DISCUSSION

Previous studies showed that *Infundibulicybe geotropa* has antimicrobial activity against *Staphylococcus aureus* ATCC 6538P, *Escherichia coli*, *Sarcina lutea* ATCC 9341NA, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* CCM 5445, *Proteus vulgaris* ATCC 6897, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13047D and *Candida albicans* ATCC 10231 [21]. In addition to these results the antimicrobial activity against *Saccharomyces cerevisiae* NRRL-Y-2034, *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Shigella sonnei*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Clostridium perfringens* and *Listeria monocytogenes* in several studies has also been reported [22, 23]. But the antimicrobial activity of these fungi against *S. flexneri* and *P. aeruginosa* is observed in this study.

There are several studies about the insecticidal [24], antitumor properties [25] and the chemical composition [26] of *Lactarius controversus*. But not much information could be found about the antimicrobial activity of *Lactarius controversus* in the literature.

The antimicrobial activity of some *Lactarius* species, namely *Lactarius deterrimus*, *Lactarius sanguifluus*, *Lactarius semisanguifluus*, *Lactarius piperatus*, *Lactarius deliciousus* and *Lactarius salmonicolor* were previously studied [27]. These *Lactarius* species showed no antimicrobial activity against *C. albicans*. But in this study we observed an antimicrobial activity against *C. albicans*.

The water and methanol extract of *Phellinus hartigii* is reported to inhibit sarcoma 180 (67.9%-100%) and Ehrlich carcinoma (90%) in white mice [28, 29]. But no record could be found related with the antimicrobial activity of *Phellinus hartigii*.

As a result, *Infundibulicybe geotropa*, *Lactarius controversus* and *Phellinus hartigii* may be suggested as a new potential source of natural antimicrobial agents against *S. flexneri* and *P. aeruginosa*, but further researches are needed to be conducted in order to analyse the active substances in details.

III.REFERENCES

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