Investigation of Antimicrobial Activity of Different *Trametes versicolor* Extracts on Some Clinical Isolates

Farklı *Trametes versicolor* Özütlerinin Bazı Klinik İzolatlar Üzerindeki Antimikrobiyal Aktivitesinin İncelenmesi

Research Article

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ABSTRACT

Intensive drug uses against species which have clinical significance and cause severe diseases have triggered the development of resistance in these species. It is necessary to discover new potential drugs against clinical isolates because many antimicrobial drugs become ineffective in the coming years. In this research, we used different extracts from *Trametes versicolor* fruiting bodies for investigating antibacterial and antifungal activities on some clinical isolates. For this purpose, five bacterial species (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) and a fungal species (*Candida albicans*) were selected as clinically important isolates and four different crude extracts (chloroform, water, ethyl acetate and ethyl alcohol) of *Trametes versicolor* were tried on these species in terms of antimicrobial sensitivity with disc diffusion method. Obtained data were analyzed using SPSS 14 software. At the result, some of extracts were found to have antimicrobial activity on clinical isolates although all extracts generally show antimicrobial effect. Chloroform extract was found to have the highest antimicrobial activity on *S. aureus* and *E. faecalis* while ethanol extract was observed as more effective on *P. aeruginosa*, *E. coli*, *B. subtilis* and *C. albicans* isolates. It can therefore be suggested that, some of *Trametes versicolor* extracts are promising antimicrobial agents.

Key Words
Trametes versicolor, Antimicrobial activity, Antifungal activity, Clinical isolates.

ÖZET

Klinik önemi olan ve ciddi hastalıklara sebep olan türlere karşı yoğun ilaç kullanımı bu türlerde direnç gelişimini tetiklemektedir. İlerleyen yıllarda birçok antimikrobiyal ilaç etkisiz hale geleceğinden bu türlere karşı yeni potansiyel ilaçların keşfedilmesi gereklidir. Bu araştırmada, *Trametes versicolor* üreme yapılarından elde edilen farklı özütler, bazı klinik izolatlar üzerinde antibakteriyel ve antifungal aktiviteleri araştırmak için kullanılmıştır. Bu amaç doğrultusunda, klinik önemi olan izolatlar olarak 5 bakteri türü *(Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa)* ile bir fungus türü (*Candida albicans*) seçildi ve *Trametes versicolor*'un 4 farklı ham özütünün (kloroform, su, etil asetat, etil alkol), bu türler üzerinde antimikrobiyal duyarlılık açısından disk difüzyon yöntemi kullanılarak denendi. Elde edilen veriler SPSS 14 programı kullanılarak analiz edildi. Çalışmanın sonunda, tüm özütler genel olarak antimikrobiyal etki gösterse de özütlerden bazılarının klinik izolatlar üzerinde antimikrobiyal aktiviteye sahip olduğu bulunmuştur. Kloroform özütünün *S. aureus* ve *E. faecalis* üzerinde en yüksek antimikrobiyal aktiviteye sahip olduğu bulunurken etanol özütünün *P. aeruginosa*, *E. coli*, *B. subtilis* ve *C. albicans* izolatları üzerinde daha etkili olduğu gözlenmiştir. Bu nedenle, bazı *Trametes versicolor* özütlerinin umut verici antimikrobiyal ajanlar olabileceği söylenebilir.

Anahtar Kelimeler
Trametes versicolor, Antimikrobiyal aktivite, Antifungal aktivite, Klinik izolatlar.

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INTRODUCTION

Fungi have been a very good source of many medical compounds for thousands of years. Fungal substances have been as important source of lead structures for new drug compounds. Due to the discovery of penicillin that led to later discoveries of potent antibiotics isolated from microbial habitats [1,2]. In the last decades, problem with antibiotic resistant bacteria has emerged because of the wrong and intensive drug uses. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and nowadays, the need to discover new and more potent of these agents as alternative to antibiotic therapy is stronger [3-5].

There are many different studies about antimicrobial activity of different types of fungal extracts from Slovakia, Nigeria, India and China [3,6-9]. In these studies, fungal extracts have been shown to be a potential drug against bacterial and fungal disease agents. Studies with macromycetes, especially *Ganoderma lucidum* and *Trametes versicolor*, revealed that these fungi have effective microbial inhibitory properties.

Trametes versicolor (L.) which commonly known as turkey tail is a common polypore mushroom found throughout the world. The top surface of the cap shows typical concentric zones of different colours. The fruiting body is 1–3 mm thick and has leathery texture. *T. versicolor* contains biologically active compounds with an enormous variety of chemical structures including the protein-bound PSP, B-1,3-B-1,4 glucans, tetracyclic triterpenoids of lanostane, fungisterol and B-sitosterol [10-12]. Furthermore, Polysaccharide-K (PSK) which can be obtained from mushrooms displays anticancer activity in laboratory studies and preliminary human researches [13,14]. Therefore, *Trametes* spp. could be useful in the search of new potent antimicrobial agents.

The aim of this study is to investigate antibacterial and antifungal activities of different extracts from *Trametes versicolor* fruiting bodies against some clinical isolates including Gram-positive, Gram-negative bacteria and *Candida albicans*.

MATERIALS and METHODS

Preparation of Different *Trametes versicolor* Extracts

Trametes versicolor fruiting bodies were collected almost 500 gram from different forest areas in Ankara and Zonguldak provinces, Turkey during the spring season 2015 and macroscopic identification of harvested fungi were done. Fruiting bodies of *T. versicolor* were dried at 70°C and pulverized with laboratory blender. The obtained powder was weighed and divided into four groups. Each group was treated with 500 mL of four different solvent (chloroform, water, ethyl acetate and ethyl alcohol) to find out which solvent requires to obtain active compounds from *T. versicolor* fruiting bodies. Powdered substances in each extract were dissolved with the aid of sonicator. After this process, each mixture was filtered to remove solid residues and solvents evaporated with rotary evaporator. The crude residue of each extract was frozen at -80°C and lyophilized [3]. These fungi extracts were stored at + 4°C in refrigerator until use.

Test Microorganisms

Six clinically important strains of microorganisms were tested in this research. Three Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) were selected for antibacterial activity test. *Candida albicans* ATCC 10231, a clinically important yeast strain, was also selected for antifungal activity test. *Staphylococcus aureus* and *Enterococcus faecalis* were cultured in the blood agar (Sigma-Aldrich, USA) at 37°C, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were cultured in the nutrient agar (Merck, Germany) at 37°C and *Candida albicans* was cultured in the sabouraud dextrose agar (Merck, Germany) at 30°C.

Antimicrobial Assay

Antibacterial and antifungal activity tests were performed with disc diffusion method to find antimicrobial sensitivities of selected clinical isolates. Different Fungal extracts dissolved in

DMSO were prepared to a final concentration of 200 μg/mL. These solutions were regarded as 100% concentration and were diluted 1:1 ratio with DMSO to obtain 50% concentrations. Suspensions of all test microorganisms were prepared in eppendorf tubes with using sterile distilled water. Each tube was adjusted with microbial suspension at the final density of 0.5 McF°. Antibiotics known to be inhibition effect on isolates were used as a positive control and DMSO was used as a negative control during disc diffusion method. Vancomycin was used for *S. aureus* and *E. faecalis*, Erythromycin for *B. subtilis*, Norfloxacin for *E. coli*, Piperacillin-tazobactam for *P. aeruginosa* and Polymyxin B for *C. albicans* as an antibiotic. To obtain meaningful results, microbial inoculations were repeated three times for each parameter. 100 μl of microbial suspension was inoculated into respective medium for each petri dish by spread plate technique at disc diffusion method. Discs were placed inoculated petri dishes and 100% and 50% concentrations of crude extracts were added 15 μl on blank discs (5 mm). After incubation at 37°C for bacteria and 30°C for yeast for 36 hours, the inhibition of microbial growth was evaluated by measuring zone diameters.

Statistical Analysis

After disc diffusion method, SPSS 14 software was used for statistical evaluation of zone diameters (in millimeters) measurement data. The comparison of different extracts in each isolate and between isolates was performed with one-way ANOVA. These results were obtained in 95% confidence intervals. The most effective extract was also evaluated with Duncan test for each clinical isolate.

RESULTS

The amounts of crude residues which are lyophilisates of four different extracts made from *Trametes versicolor* fruiting bodies are shown in Table 1. Even though the products were obtained with different amounts of each extract, standardization is made using the residue in the same proportions during disc diffusion method.

The antimicrobial activity (expressed as mm) of four different fungi extracts from *Trametes versicolor* against various strains of bacteria and yeast are summarized in Table 2. Although all extracts generally show antimicrobial effect, some of them were found to have antimicrobial activity on clinical isolates.

The comparison of zone diameters showed that there is a significant difference between the zones when extracts of 100% and 50% concentrations compared with positive and negative controls ($p < 0.05$). Antibiotics used as positive control were found to be most effective on all isolates. However, Different *Trametes versicolor* extracts were found to have microbial inhibition due to the negative control by Duncan test (Table 3). Although it was not seen a significant difference between the concentrations of the extracts, it was found to be more effective in 100% concentration of extracts ($p > 0.05$).

There was no significant difference between the extracts when the antimicrobial activity of the extracts on all isolates compared with one way ANOVA ($p > 0.05$). However, there were significant differences in the comparisons for each isolate ($p < 0.05$). Chloroform extract was found to have the highest antimicrobial activity on *S. aureus* and *E. faecalis* (Figure 1) while ethanol extract was observed to be more effective on *P. aeruginosa*, *E. coli*, *B. subtilis* and *C. albicans* isolates (Figure 2). Furthermore, we did not determine significant antimicrobial activity of tested water and ethyl acetate extracts against selected clinical isolates.

DISCUSSION

Many researchers who tested different macromycetes extracts against bacteria and yeast observed their antimicrobial activities. Macromycetes extracts contain lots of active chemical compounds which show potential antimicrobial activities. For example, terpenes, organic acids, benzoic acid derivates and quinolone were isolated from some species such as *Ganoderma spp., Lentinus edodes* and *Leucopaxillus albissimus* showed activity against many different species of bacteria and yeast [1,10,15-18].

Many different species of macrofungi extracts were tested against different bacteria

Table 1. The amounts of crude residues obtained from four different *Trametes versicolor* extracts.

Extracts	Weight (mg)
Ethyl Alcohol	98
Ethyl Acetate	83
Chloroform	124
Water	149

Table 2. Antimicrobial activity of different *Trametes versicolor* extracts against clinical isolates tested by disc diffusion method.

	Ethanol		Ethyl acetate		Chloroform		Water	
	100%	50%	100%	50%	100%	50%	100%	50%
P.aeruginosa	10.33	9.33	6.00	6.67	7.33	7.33	7.00	7.33
S.aureus	5.33	5.00	7.33	5.67	10.00	12.33	5.00	5.00
E.faecalis	6.33	6.33	5.67	6.33	6.33	7.33	5.33	5.33
B.subtilis	9.33	8.67	6.00	5.00	6.33	8.33	8.67	7.00
E.coli	9.67	9.33	6.33	6.33	7.00	8.67	9.00	8.67
C.albicans	9.00	6.67	7.00	5.67	6.33	6.33	7.33	5.00

Values are mean inhibition zone of three replicates (mm).

Table 3. The comparison of 100% (A) and 50% (B) concentration of extracts with positive and negative controls.

CONS	N	Subset for alpha = 0.05		
		1	2	3
Duncan[a] N	72	5.01		
50%	72		7.07	
100%	72		7.21	
P	72			19.83
Sig.		1.000	0.79	1.000

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 72.000.

by Suay et al., [12]. These researchers determined and confirmed antimicrobial activity of crude macrofungi extracts.

From the obtained results, it could be observed that ethanol was the best solvent for extracting antimicrobial compounds from *Trametes versicolor*. This suggestion was based on the number of organisms inhibited and the diameter of inhibitory zones produced. Furthermore, chloroform extract of *Trametes versicolor* has effect against *S. aureus* and *E. faecalis* known as infectious disease agents. These results demonstrated that various active compounds can be isolated from different fungal extracts. Thus, fungal extraction with different solvents should be performed in order to obtain active compounds to inhibit each clinical isolate. Comparison of the concentrations (100% and 50%) of extracts revealed that there is no significant difference between the concentrations (Table 3). According to this result, inhibition of clinical isolates can be performed with the lesser amount of active compounds.

In conclusion, this study has shown that different extracts (ethyl alcohol, ethyl acetate, chloroform and water) have been used in-vitro

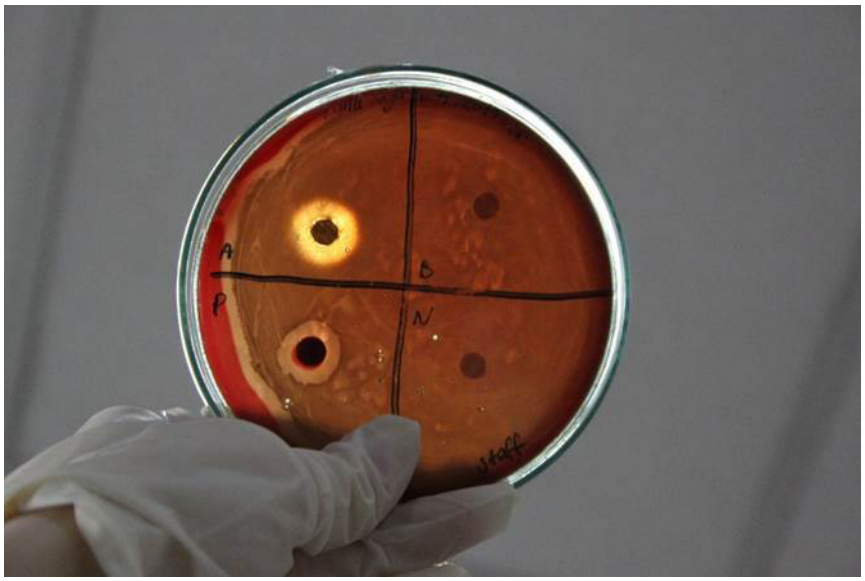

Figure 1. Evaluation of antimicrobial effect of chloroform extracts of *Trametes versicolor* on *Staphylococcus aureus* by disc diffusion method.

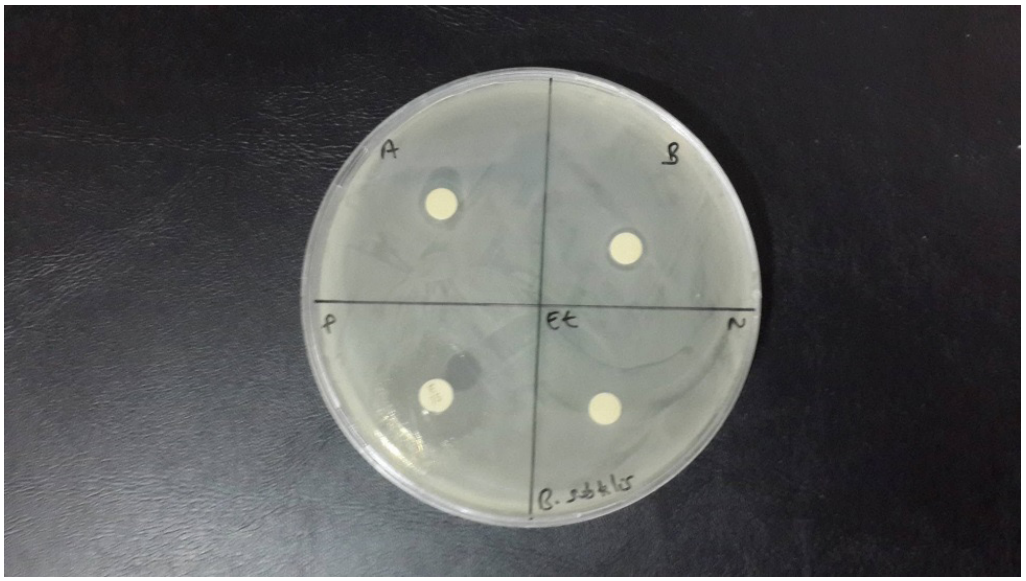

Figure 2. Evaluation of antimicrobial effect of ethanol extracts of *Trametes versicolor* on *Bacillus subtilis* by disc diffusion method.

to inhibit the growth of some disease causing bacteria and yeast. It can therefore be suggested that, some of extracts are promising antimicrobial agents.

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