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Determination of the Antimicrobial Activity of Some Moss Samples Collected From the Dokuz Eylül University Tinaztepe Campus

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Abstract: *When examining studies conducted worldwide, it becomes evident that plant species are utilized for both disease treatment and wound healing. This study investigates the antimicrobial activities of ethyl alcohol extracts from various moss species, namely Grimmia pulvinata (Hedw.), Pleurochaete squarrosa (Brid.), Bryum capillare (Hedw.), Grimmia orbicularis (Bruch), Oxyrrhynchium hians (Hedw), Homalothecium lutescens (Hedw.), Hypnum cupressiforme (Hedw.), and Palustriella falcate (Hedw.), against 15 strains comprising 5 gram-positive bacteria, 7 gram-negative bacteria, and 3 yeast. Following the tests, it was determined that Oxyrrhynchium hians (Hedw.), Homalothecium lutescens (Hedw.), Hypnum cupressiforme (Hedw.), and Palustriella falcate (Hedw.) moss species exhibited antimicrobial activity. Notably, mosses displayed the highest antimicrobial activity against the Staphylococcus aureus strain among the strains used in this study.*

Keywords: Moss, Disk diffusion, antimicrobial activity

1. INTRODUCTION

The ability of numerous plant species worldwide to treat diseases has been evident throughout human history. Various plant species were crushed and applied directly to open wounds or boiled to extract their medicinal properties. In some cases, plants were consumed as food without any specific treatment [1].

Infections account for approximately half of all deaths, particularly in tropical countries. In Africa, around 300,000 children die annually due to infectious diseases caused by *E. coli*, *Shigella* spp., and *Salmonella* spp. This situation is not surprising considering the socioeconomic status of these countries. Conversely, developed nations are experiencing an increase in infectious diseases and related fatalities [2].

Given the global research and emerging trends, there is an urgent need to prevent infectious diseases and explore new treatment methods. The effectiveness of drugs is decreasing over time, emphasizing the growing importance for pharmacologists and microbiologists to identify and develop antimicrobial agents. Utilizing plants and investigating their active ingredients has become a natural option in the pursuit of new antimicrobial agents [3].

Antimicrobial susceptibility testing (AST) is conducted to assess the in vitro activity of antimicrobial agents against specific bacterial species. In clinical practice, the selection of antimicrobial therapy heavily relies on the results obtained from susceptibility testing. Due to the rising antibiotic resistance resulting from excessive usage, susceptibility testing is crucial before initiating treatment to prevent unfavorable outcomes and uncontrolled increases in resistance rates [4].

Moss and liverworts have been traditionally used for healing diseases and wounds, making it important to conduct antimicrobial activity tests, identify effective substances in these plants, and evaluate their potential as future drugs. Numerous studies and applications have been conducted in our country to investigate the antimicrobial activities of moss and liverwort species [5].

It is known that plants possess antimicrobial effects due to the presence of phytochemicals in their structures. Studies have indicated that mosses primarily exhibit antimicrobial activity through their primary metabolites. These metabolites are essential for all organisms and originate from primary metabolic reactions [6].

In this study, the antimicrobial activities of ethanol extracts from *Grimmia pulvinata* (Hedw.), *Pleurochaete squarrosa* (Brid.), *Bryum capillare* (Hedw.), *Grimmia orbicularis* (Bruch), *Oxyrrhynchium hians* (Hedw), *Homalothecium lutescens* (Hedw.), *Hypnum cupressiforme* (Hedw.), and *Palustriella falcate* (Hedw.) species were determined against 15 strains using the disk diffusion test.

2. MATERIAL AND METHOD

2.1 Moss Samples

As a result of the fieldwork carried out in the Dokuz Eylul University Tınaztepe campus, 8 different species were collected and the specimens were identified by Associate Professor Dr. Kerem CANLI. The places where the specimens were collected and the species names are given in the table. Witness samples are kept in Dokuz Eylul University Biology Department.

FAMILY	SPECIES
Grimmiaceae	*Grimmia pulvinata (Hedw.)*
Pottiaceae	*Pleurochaete squarrosa (Brid.)*
Bryaceae	*Bryum capillare (Hedw.)*
Grimmiaceae	*Grimmia orbicularis (Bruch)*
Brachytheciaceae	*Oxyrrhynchium hians (Hedw)*
Brachytheciaceae	*Homalothecium lutescens (Hedw.)*
Hipnaceae	*Hypnum cupressiforme (Hedw.)*
Amblystegiaceae	*Palustriella falcate (Hedw.)*

2.2 Extraction Method

The collected moss samples were dried under appropriate conditions. Subsequently, the samples were ground into a powder using a grinder. For each ground sample, 200 ml of ethanol was added and allowed to shake for 48 hours. After the shaking process, the mixture was filtered using Whatman No. 1 filter paper into a flask. The alcohol was then removed from the filtered samples using a rotary evaporator. The samples were weighed sequentially, and the extraction process was repeated by adding ethanol once again [7].

2.3 Microorganisms

In order to determine the antimicrobial activity of moss extracts, 15 different strains were used. 5 gram-positive, 7 gram-negative bacteria and 3 yeast. *Candida albicans*, *Candida tropicalis*, *Candida glabrata* were selected as yeast.

2.4 Inoculum Preparation

It was prepared according to the 0.5 Mcfarland turbidity standard in saline in order to standardize the microorganisms to be used in determining the antimicrobial activity [8].

2.5 Antimicrobial Activity Test

The antimicrobial activity test was conducted using the disk diffusion method, as described by Andrews [9]. Petri dishes were filled with Müller-Hinton medium. The process of spreading microorganisms, which was standardized to a 0.5 McFarland value, was performed on the medium in the prepared petri dishes. Discs loaded with moss extract in amounts of 50 µl, 100 µl, and 200 µl were placed in the respective petri dishes. Bacterial strains were incubated at 37 degrees Celsius for 24 hours, while yeast strains were incubated at 27 degrees Celsius for 48 hours [10].

3. RESULTS

As a result of the antimicrobial activity study conducted on 8 moss samples collected from their natural habitats, using the disc diffusion method and testing against 15 strains, the findings presented in the table were obtained. The antimicrobial activity test revealed the following results:

Oxyrrhynchium hians exhibited antimicrobial activity against B. subtilis (8mm), E. coli (8mm), and S. aureus (8mm).

Homalothecium lutescens showed antimicrobial activity against C. albicans (7mm), P. fluorescens (9mm), and S. aureus (9mm).

Hypnum cupressiforme demonstrated antimicrobial activity against L. monocytogenes (7mm), P. fluorescens (7mm), and S. aureus (8mm).

Palustriella falcate displayed antimicrobial activity against B. subtilis (7mm) and S. aureus (8mm).

On the other hand, *Grimmia pulvinata*, *Pleurochaete squarrosa*, *Bryum capillare*, and *Grimmia orbicularis* species did not exhibit any antimicrobial activity against the 12 bacteria and 3 yeast strains included in the study.

Microorganisms/ Moss extracts	*Oxyrrhynchium hians*			*Homalothecium lutescens*			*Hypnum cupressiforme*			*Palustriella falcate*			*Grimmia pulvinata*			*Pleurochaete squarrosa*			*Bryum capillare*			*Grimmia orbicularis*		
	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl
B.subtilis	**7.00**	**7.00**	**8.00**	-	-	-	-	-	-	**7.00**	**7.00**	**7.00**	-	-	-	-	-	-	-	-	-	-	-	-
C.albicans	-	-	-	**7.00**	**7.00**	**7.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.aeogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.faecalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	**7.00**	**7.00**	**8.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L.monocytogenes	-	-	-	-	-	-	**7.00**	**7.00**	**7.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.fluorescens	-	-	-	**7.00**	**8.00**	**9.00**	**7.00**	**7.00**	**7.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.enteritidis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.aureus	**7.00**	**8.00**	**8.00**	**7.00**	**8.00**	**9.00**	**7.00**	**8.00**	**8.00**	**7.00**	**8.00**	**8.00**	-	-	-	-	-	-	-	-	-	-	-	-
S.epidermidis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.baumannii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.tropicalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.glabrata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4. DİSCUSSİON

Antimicrobial activity studies are gaining increasing importance as the world faces a growing number of diseases caused by pathogens. In this study, significant results were obtained from 4 out of 8 moss species collected from the Tınaztepe campus.

The completed antimicrobial activity test revealed that the moss species *Oxyrrhynchium hians* exhibited effectiveness against *B. subtilis*, *E. coli*, and *S. aureus* species. *Homalothecium lutescens* showed an effect against *C. albicans*, *P. fluorescens*, and *S. aureus* species. Similarly, *Hypnum cupressiforme* demonstrated an effect against *L. monocytogenes*, *P. fluorescens*, and *S. aureus* species. Antimicrobial activity was observed from *Palustriella falcate* against *S. aureus* species. However, no results were obtained from the other 4 moss species studied.

The study revealed that *Staphylococcus aureus*, which showed the most remarkable results, poses a serious danger and causes various diseases. Four different moss extracts exhibited efficacy against this strain at different doses.

In a study by Benek et al. [11], moss extracts prepared using ethyl alcohol did not show any zone formation against *P. fluorescens* bacteria. However, in this study, the ethyl alcohol extracts exhibited zone sizes of 8mm and 9mm against the same bacteria at different doses.

E. coli, which is responsible for urinary system disorders and intestinal infections, is a pathogen that commonly resides in the human intestines. Colak et al. [12] analyzed the *H. cupressiforme* strain using different solvents in an antimicrobial activity study. The *H. cupressiforme* extract formed zones of 6mm and 9mm against *E. coli* bacteria. However, in this study, no zone formation was observed against *E. coli* bacteria.

P. aeruginosa is a dangerous pathogen found in soil and water, causing respiratory distress in individuals with compromised immune systems and opportunistic infections in open wounds. Young colonies of this bacterium exhibit a blue-green pigment, which is an important characteristic for recognition. In Tekerlek's antimicrobial activity thesis study [13], no zone formation was observed against *P. aeruginosa* bacteria. Similarly, no zone formation was observed against this bacterial species in the current study.

C. albicans is a fungus that assists in nutrient absorption and digestion but can cause problems if it overgrows without control. In the antimicrobial activity study conducted by Altuner et al. [14], the ethyl alcohol extract did not exhibit any zone formation against *C. albicans* yeast. However, in this study, one of our moss extracts yielded results against *C. albicans* yeast.

The study was conducted using doses of 50 µl, 100 µl, and 200 µl. It is believed that increasing the applied doses will lead to larger zone formations in a positive manner.

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