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Investigation of the Antimicrobial and Antifungal Activities of Some Moss Species (*Scleropodium touretii* (Brid.) L.F.Koch, *Hypnum cupressiforme* Hedw. var. *lacunosum* Brid., *Brachythecium glareosum* (Bruch ex Spruce) Schimp.)

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Abstract

This study evaluated the antibacterial and antifungal activities in ethanol, methanol, acetone, chloroform, hexane, ethyl acetate and isopropanol extracts of moss species; *Scleropodium touretii* (Brid.) L.F.Koch, *Hypnum cupressiforme* Hedw. var. *lacunosum* Brid. and *Brachythecium glareosum* (Bruch ex Spruce) Schimp., which was collected from the Zonguldak province of Turkey against 10 different microorganisms, including 4 gram-positive bacteria, 4 gram-negative bacteria and 2 yeast fungi species. Different fractions of the mosses were screened using the disc diffusion (qualitative), the agar-well diffusion method, and the minimum inhibitory concentration (MIC) methods. Inhibition of bacterial growth was compared with that of ciprofloxacin, eritromicin, penicillin and also, for fungal growth flukanozol-B as positive control and solvents and distilled water as negative control. The tests were performed in triplicate for each microorganism evaluated. The final results were presented as the arithmetic averages. The results showed that the most effective solvents were methanol and acetone, while hexane had the lowest antimicrobial activity. When evaluated in terms of microorganisms; *Enterococcus faecalis, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* showed the highest sensitivity to moss extracts. However, *Pseudomonas aeruginosa* and *Candida albicans* strains were found to be resistant to these extracts. In addition, among the moss species, *H. cupressiforme* var. *lacunosum* was determined to be the most effective species in terms of the number of affected microorganisms. These findings indicate the potential of mosses for the development of antimicrobial and antifungal agents. This study provides a basis for the investigation of alternative therapeutic agents that can be obtained from natural plant sources and paves the way for further studies in this field.

Keywords: Moss, Disk diffusion test, Agar well diffusion method, Minimum inhibitory concentration (MIC) test.

Bazı Karayosunu Türlerinin (*Scleropodium touretii* (Brid.) L.F.Koch, *Hypnum cupressiforme* Hedw. var. *lacunosum* Brid., *Brachythecium glareosum* (Bruch ex Spruce) Schimp.) Antimikrobiyal ve Antifungal Aktivitelerinin Araştırılması

Öz

Bu çalışmada, Türkiye'nin Zonguldak ilinden toplanan *Scleropodium touretii* (Brid.) L.F.Koch, *Hypnum cupressiforme* Hedw. var. *lacunosum* Brid. ve *Brachythecium glareosum* (Bruch ex Spruce) Schimp. karayosunu türlerinin etanol, metanol, aseton, kloroform, hekzan, etil asetat ve izopropanol özütlerindeki antibakteriyel ve antifungal aktiviteler, 4 gram-pozitif bakteri, 4 gram-negatif bakteri ve 2 maya mantarı türü olmak üzere 10 farklı mikroorganizmaya karşı değerlendirilmiştir. Karayosunlarının farklı fraksiyonları disk difüzyon (kalitatif), agar-kuyu difüzyon yöntemi ve minimum inhibitör konsantrasyon (MİK) yöntemleri kullanılarak tarandı. Bakteriyel büyümenin inhibisyonu siprofloksasin, eritromisin, penisilin antibiyotik diskleri ve ayrıca fungal büyüme için pozitif kontrol olarak flukanozol-B antifungal diskleri ve negatif kontrol olarak da çözücüler ve damıtılmış su ile karşılaştırıldı. Değerlendirilen her mikroorganizma için testler üçlü olarak gerçekleştirildi. Sonuçlar aritmetik ortalamalar alınarak

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sunuldu. Çalışmadan elde edilen sonuçlar en etkili çözücülerin metanol ve aseton olduğunu, en düşük antimikrobiyal aktiviteye sahip olanın ise hekzan olduğunu gösterdi. Mikroorganizmalar açısından ise; *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* ve *Bacillus subtilis*'in çalışılan karayosunu özütlerine en yüksek duyarlılığı olduğu görüldü. Ancak *Pseudomonas aeruginosa* ve *Candida albicans* suşlarının ise bu özütlere karşı dirençli olduğu bulundu. Ayrıca çalışılan karayosunu türleri arasında *H. cupressiforme* var. *lacunosum*'un etkilenen mikroorganizma sayısı açısından en etkili özüte sahip olduğu belirlendi. Bu bulgular bize karayosunların antimikrobiyal ve antifungal ajanların geliştirilmesinde potansiyelini göstermektedir. Bu çalışma, doğal bitki kaynaklarından elde edilebilecek alternatif terapötik ajanların araştırılması için bir temel sağlamakta ve bu alanda daha ileri çalışmalara zemin hazırlamaktadır.

Anahtar kelimeler: Karayosunları, Disk difüzyon testi, Agar kuyucuk difüzyon metodu, Minimum inhibitör konsantrasyon (MİK) testi.

1. Introduction

The fight against rapidly increasing epidemics worldwide is becoming more difficult as time goes by. Fungi, bacteria and other pathogenic microorganisms cause many problems for both humans and other living things used in animal and agricultural production. Therefore, in recent years, the use of plants, particularly medicinal and aromatic species, has increased in the search for bioactive substances that can be used to control diseases caused by microorganisms. Although many synthetic drugs have been produced with the developing technology in this fight process, after a certain point, these are not enough and bacteria become resistant to antibiotics over time, leading scientists to search for new antimicrobial and antifungal substances. In this context, bryophytes, which have been used as medicinal plants in traditional medicine for centuries due to the chemical components they contain throughout history, are remarkable in the search for new antimicrobial and antifungal substances. In fact, in many scientific studies conducted since the 1960s, it has been determined that bryophytes contain hundreds of new compounds, including many aromatics and terpenoids, and that these compounds exhibit various activities such as antimicrobial, antifungal, cytotoxic, antitumor and insecticidal properties that can be used in agricultural and medical processes (Asakawa, 2007; Üçüncü et al., 2010; Pant 1998; Saxena and Harinder, 2004). Therefore, in this study, we wanted to investigate the antimicrobial and antifungal activities of the extractions obtained from some species of mosses, on which no active substance was studied. The presence of such a variety of secondary metabolites in this plant group has resulted in their evolutionary success in surviving against fluctuating climatic conditions. Today, bryophytes are the oldest known small non-vascular terrestrial plants that constitute the largest part of the plant kingdom after angiosperms (Shaw and Renzaglia, 2004). They are also classified as seedless plants because they reproduce by spores instead of seeds, or as cryptogams because their gamete-producing structures are hidden. The small size of these plants means they produce less biomass, which makes them less known to most people (Harris, 2008). Evolutionarily, they are between algae and vascular plants (Smith, 2004). It is estimated that there are approximately 15,000-20,000 bryophyte species on Earth, with more than 1,000 genera, and they are the most primitive living plants (Smith, 2004). Systematically, bryophytes are studied under the sub-kingdom Bryobiotina in the plant kingdom, divided into 3 sections. These are; liverworts (Marchantiophyta), hornworts (Anthocerotophyta) and mosses (Bryophyta) (Kürschner and Frey, 2011). Bryophytes, which have an important place in terms of biodiversity, can be found on various substrates such as soil, rocks and trees, usually in moist and shady places.

2. Materials and Methods

2.1 Collection and identification of moss species

Moss samples were collected in April 2024 from six different stations within the Kozlu, Devrek, and Çatalağzı districts of the Zonguldak province. The samples were identified by Prof. Dr. Güray UYAR. The moss species selected for this study were *Brachythecium glareosum* (Bruch ex Spruce) Schimp. and *Scleropodium touretii* (Brid.) LF Koch. and *Hypnum cupressiforme* var. *lacunosum* Brid. The location details of these samples are presented in Table 1.

2.2. Used microorganisms

A total of 10 different microorganisms were selected for evaluating the antimicrobial and antifungal activities of the extracts obtained in the experimental study. This selection comprised 4 gram-negative bacterial species, 4 gram-positive bacterial species, and 2 fungal species, as detailed in Table 2.

Table 1. Location information of collected moss species

Station No.	Altitude	Coordinates	Vegetation and Location
1.	47 m	41º29'35.2"N 031º52'49.2"E	Çatalağzı district in Zonguldak province, mixed leafy forest (*Fagus orientalis* Lipsky. and *Carpinus betulus* L.) roadside on rocks
2.	30 m	41º30'00.2"N 31º53'56.8"E	Çatalağzı district in Zonguldak province, mixed forest edge, open area next to dam reservoir.
3.	88 m	41º30'31.3"N 31º57'19.2"E	Muslu district in Zonguldak province, roadside slopes.
4.	342 m	41º23'38.7"N 031º42'44.5"E	Değirmenağzı region of Kozlu district in Zonguldak province, close to roadside on the way to hospital, mixed leafy forest (*Fagus orientalis* Lipsky. and *Carpinus betulus* L.)
5.	504 m	41º08'02.7"N 032º01'37.6"E	Devrek district of Zonguldak province, Beldibi mixed leafy forest (*Fagus orientalis* Lipsky. and *Carpinus betulus* L.)
6.	450 m	41º23'28.2"N 031º50'44.7"E	Roadside slopes in urban forest of Zonguldak province

Table 2. Information on the strains of the used microorganisms

Family	Species of used microorganisms	Laboratory from which it was supplied
Pseudomonadaceae	*Pseudomonas aeruginosa* ATCC 27853	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Enterobacteriaceae	*Enterobacter aerogenes* ATCC 13048 *Klebsiella pneumoniae* MTCC 109 *Escherichia coli* ATCC 35218	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Enterococcaceae	*Enterococcus faecalis* ATCC 29212	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Staphylococcaceae	*Staphylococcus aureus* ATCC 25923	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Bacillaceae	*Bacillus subtilis* ATCC 6633	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Streptococcaceae	*Streptococcus pyogenes* ATCC 12344	Gazi University, Faculty of Dentistry, Department of Medical Microbiology
Saccharomycetaceae	*Candida albicans* ATCC 10231 *Saccharomyces cerevisiae* ATCC 9763	Gazi University, Faculty of Dentistry, Department of Medical Microbiology

2.3 Extraction Process

Following the removal of foreign substances, the mosses collected from various stations were subjected to a series of treatments. Initially, they were washed with distilled water and dried on blotting paper. The mosses were then ground into a powder using a laboratory blender. The resulting powder was transferred to 5-g zip-lock bags and placed in volumetric flasks. Subsequently, 250 ml of different solvents (methyl alcohol, ethyl alcohol, acetone, chloroform, ethyl acetate, hexane, and isopropanol) were added to the flasks. The mouths of the flasks were sealed with Parafilm, and the samples were left at room temperature for one day. The temperature was adjusted to a value close to the boiling point of the solvent in each solution, and a magnetic stirrer was added. The extraction process was then carried out in a Soxhlet system by stirring the samples for 6 hours. The resulting extracts were collected using a filter paper and a funnel, and the solvents were separated from the extracts. The extracts were then placed in a rotary evaporator device for concentration. In this process, the solvents were evaporated under vacuum, low temperature, and pressure conditions until 2-4 ml of the extract remained. Once the desired volume was reached, the extracts were transferred to 2.0 ml capacity Eppendorf tubes using sterile pipettes (Şimşek, 2012).

2.4. Determination of the antimicrobial and antifungal activities of the mosses

The study employed disk diffusion, agar well diffusion, and minimum inhibitory concentration (MIC) tests to assess the antimicrobial and antifungal properties of three distinct moss samples against a range of microorganisms. These methods facilitated the measurement and comparison of the efficacy of the moss extracts.

2.5. Disk diffusion and agar well method

The antimicrobial susceptibility of eight bacterial species, including *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae*, and *Escherichia coli,* was evaluated using Mueller-Hilton Agar (MHA) medium for disk diffusion and agar well diffusion tests. Mueller-Hinton Broth (MHB) was employed as

the liquid medium in the minimum inhibitory concentration (MIC) tests for these bacteria. In contrast, Sabouraud Dextrose Agar (SDA) was utilized for the disk diffusion and agar well diffusion tests of Candida albicans and Saccharomyces cerevisiae yeasts, while Sabouraud Dextrose Broth (SDB) liquid medium was used to determine the MIC values. To prepare the MHA medium, 38 grams of the medium was dissolved in 1 liter of distilled water. The medium was subsequently sterilized by autoclaving at 121 degrees Celsius for 15 minutes. The prepared medium was then poured into Petri dishes and allowed to harden at room temperature before being used for microbiological testing (Balouiri et al., 2016).

2.7. Minimum inhibitory concentration (MIC) method

Standardization of the microorganisms and inoculation was performed in accordance with the 0.5 McFarland standard. A suspension of the microorganisms was prepared by taking a small amount of the sample into a test tube using a sterile loop and mixing it. The turbidity of the suspension was measured and compared to the 0.5 McFarland standard turbidity, as described by Şenol et al. (2007) and Andrews (2001). A sample of the microorganism solution that met the 0.5 McFarland standard was then taken using a sterile swab stick and spread evenly across the medium in petri dishes in three different directions. Following inoculation, 6 mm sterile empty disks were placed at equal intervals in the medium using tweezers and impregnated with 20 µL of extract for the disk diffusion test. Four disks were used in each 90-mm sterile plastic petri dish. The disks were left to dry for 24 hours to allow the extracts to evaporate and dry, as recommended by Altuner (2008). For the agar well diffusion method, 5-6 mm diameter wells were opened in the solidified medium using sterile pipette tips at equal intervals. Duplicate samples of each extract were added to the wells in a volume of 60 µL. The prepared petri dishes were then incubated in an oven at 37°C for 18-24 hours to provide suitable conditions for the growth of microorganisms. After incubation, the petri dishes were removed from the oven and the diameter of the growth was measured using a ruler from one end to the other, as described by Altuner (2008).

2.6. Standardization of the microorganisms and inoculation

The positive control of the study consisted of antibiotic disks containing Penicillin (P10), Gentamicin (CN10), Tobramycin (TOB10), Erythromycin (E15), Tetracycline (TE30) and Ciprofloxacin (CIP5) for bacteria and fluconazole disks for fungi. These disks served as an effective positive control because of their sensitivity to antibiotics. Conversely, the negative control of the experiment involved the use of ethyl alcohol, methyl alcohol, hexane, chloroform, ethyl acetate, acetone and isopropanol solvents.

3. Findings

3.1. Zone diameter tables

The zone diameters obtained from the disk diffusion, agar well, and minimum inhibition methods are presented in the tables below.

The zone diameters of the *B. glareosum* extract obtained via the disk diffusion method on the studied microorganisms are presented in millimetres. As shown in Table 3, the plant extracts prepared in the chloroform solution exhibited a greater effect on the E. coli (11 mm), *E. faecalis* (12 mm), *B. subtilis* (10 mm), and *E. aerogenes* (10 mm) strains than the solvents.

Table 3 Disk diffusion results of *B. glareosum* extracts prepared using different filters for various microorganisms, highlighting the antimicrobial efficacy of the extracts against a range of pathogens.

B.glareosum	S.pyogenes	S.aureus	E.faecalis	B.subtilis	K.pneumonia	E.aerogenes	P.aeroginosa	E.coli	C.albicans	S.cerevisiae
1-1.methanol	9	6	6	7	6	6	6	6	6	12
methanol	6	8	10	8	7	9	8	9	10	12
1-2.ethanol	10	10	10	10	9	9	10	9	10	10
ethanol	10	9	10	8	8	15	18	10	20	15
1-3.chloroform	6	10	12	10	9	10	12	11	6	6
chloroform	7	10	10	8	10	7	13	10	12	12
1-4.aseton	6	9	9	8	6	6	6	7	6	4
aseton	10	7	10	8	9	11	9	10	15	13
1-5.isopropanol	10	11	11	10	11	12	8	11	12	9
isopropanol	12	11	14	18	14	14	14	18	14	8
1-6.etil asetat	7	8	8	8	12	7	7	8	7	8
etil asetat	6	8	8	7	12	11	11	8	7	7
1-7.hexane	14	6	6	6	6	6	6	6	6	6
hexane	6	6	6	6	6	6	6	6	6	6

Table 4. Agar Well Diffusion results of *B. glareosum* extracts prepared with different wells for various microorganisms

B.glareosum	*S.pyogenes*	*S.aureus*	*E.faecalis*	*B.subtilis*	*K.pneumonia*	*E.aerogenes*	*P.aeroginosa*	*E.coli*	*C.albicans*	*S.cerevisiae*
1-1 (methanol)	14	6	6	7	6	6	6	6	6	12
methanol	10	9	10	8	7	9	8	12	12	12
1-2(ethanol)	10	8	10	11	12	9	10	9	10	10
ethanol	12	8	9	7	12	15	18	10	20	15
1-3(chloroform)	6	10	15	10	9	8	12	12	6	6
chloroform	9	9	7	6	10	6	13	7	12	12
1-4(aseton)	6	8	9	8	6	6	6	7	6	4
aseton	10	10	10	8	9	11	9	10	15	13
1-5isopropanol)	10	11	11	10	11	12	8	16	12	9
isopropanol	12	10	14	10	14	14	14	10	14	8
1-6(etil asetat)	7	10	8	6	12	7	7	10	7	8
etil asetat	12	7	11	6	12	11	11	10	12	7
1-7(hexane)	6	6	6	6	6	6	6	6	6	6
hexane	10	6	6	6	6	6	6	6	6	6

Table 5. MIC values of *B. glareosum* extracts prepared with different reagents for various microorganisms

B.glareosum	*S.pyogenes*	*S.aureus*	*E.faecalis*	*B.subtilis*	*K.pneumonia*	*E.aerogenes*	*P.aeroginosa*	*E.coli*	*C.albicans*	*S.cerevisiae*
1-1.methanol	1,25									
methanol	2,5	1,25						2,5	2,5	
1-2.ethanol		1,25			2,5					
ethanol	2,5	1,25	1,25		2,5			0,625		
1-3.chloroform								1,25		
chloroform	2,5	2,5	2,5	2,5	2,5			1,25		
1-4.aseton										
aseton		2,5								
1-										
isopropanol		1,25						0,625	1,25	
1-6.etil asetat				1,25				1,25		
etil asetat		2,5	2,5					1,25	2,5	
1-7.hexane	1,25									
hexane										

The zone diameters obtained using the agar well diffusion method for the *B. glareosum* extract on the studied microorganisms are presented in millimetres. As indicated in Table 4, the plant extract dissolved in the isopropanol solution exhibited greater activity than the solvent against E. coli (16 mm), *S. aureus* (11 mm), and *S. cerevisiae* (9 mm) strains.

The ethanolic extract of *B. glareosum* species exhibited antibacterial activity against *S. aureus* and *K. pneumoniae* strains at concentrations of 1.25 µl/ml and 2.5 µl/ml, respectively. Notably, the solvent ethanol also demonstrated comparable inhibition values against these microorganisms.

Table 6 presents the zone diameters in millimeters obtained via the disk diffusion method of S. touretii extract on the investigated microorganisms. Notably, the plant extract prepared in methanol solution yielded a significantly wider inhibition zone diameter on the *E. aerogenes* (11 mm) strain compared to the solvent.

Table 7 presents the zone diameters obtained via the agar well diffusion method for the studied microorganisms treated with the *S. touretii* extract. Notably, the plant extract dissolved in ethanol exhibited greater efficacy than the solvent alone against S. aureus (9 mm), *E. faecalis* (10 mm), and *B. subtilis* (8 mm) strains.

Examination of Table 8 revealed that the ethanolic and chloroform extracts exhibited inhibitory effects on bacterial growth at a concentration of 1.25 µl/ml in the *K. pneumoniae* strain. Conversely, the effectiveness of the ethanol and chloroform solvents was observed at a concentration of 2.5 µl/ml, indicating that the plant extracts are capable of inhibiting bacterial growth at lower concentrations than the respective solvents.

Table 6 The disk diffusion results of *S. touretii* extracts prepared using different filters for various microorganisms.

S.touretii	S.pyogenes	S.aureus	E.faecalis	B.subtilis	K.pneumonia	E.aerogenes	P.aeroginosa	E.coli	C.albicans	S.cerevisiae
2-1 (methanol)	8	6	6	6	6	11	6	8	6	10
methanol	9	8	10	8	7	9	8	9	10	12
2-2 (ethanol)	9	9	10	8	10	10	11	9	10	12
ethanol	10	9	10	8	8	15	18	10	20	15
2-3 (chloroform)	9	8	10	8	12	6	14	9	9	7
chloroform	10	10	10	8	10	7	13	10	12	12
2-4 (aseton)	7	6	7	6	6	6	6	7	7	6
aseton	10	7	10	8	9	11	9	10	15	13
2-5 (isopropanol)	8	8	9	9	9	10	9	8	12	10
isopropanol	18	11	14	18	14	14	14	18	14	8
2-6 (etil asetat)	8	8	10	7	10	6	8	8	7	12
etil asetat	8	8	8	7	12	12	11	8	7	7
2-7 (hexane)	6	6	6	7	6	6	6	6	6	6
hexane	6	6	6	6	6	6	6	6	6	6

Table 7. Agar Well Diffusion results of *S. touretii* extracts prepared with different reagents for various microorganisms

S.touretii	S.pyogenes	S.aureus	E.faecalis	B.subtilis	K.pneumonia	E.aerogenes	P.aeroginosa	E.coli	C.albicans	S.cerevisiae
2-1(methanol)	8	6	6	6	6	7	6	8	6	10
methanol	10	9	10	8	7	9	8	12	12	12
2-2(ethanol)	9	9	10	8	10	10	11	9	10	12
ethanol	12	8	9	7	12	15	18	10	20	15
2-3(chloroform)	8	8	10	8	10	6	14	9	9	7
chloroform	9	9	7	6	10	6	13	7	12	12
2-4(aseton)	7	6	7	6	6	6	6	7	7	6
aseton	10	10	10	8	9	11	9	10	15	13
2-5(isopropanol)	8	8	9	9	9	10	9	8	12	10
isopropanol	18	10	14	10	14	14	14	10	14	8
2-6(etil asetat)	8	8	17	7	10	6	8	10	7	12
etil asetat	12	7	11	6	12	12	11	10	12	7
2-7(hexane)	6	6	6	7	6	6	6	6	6	6
hexane	10	6	6	6	6	6	6	6	6	6

Table 8. The minimum inhibitory concentration (MIC) values of *S. touretii* extracts prepared with different reagents against various microorganisms.

S.touretii	S.pyogenes	S.aureus	E.faecalis	B.subtilis	K.pneumonia	E.aerogenes	P.aeroginosa	E.coli	C.albicans	S.cerevisiae
2-1 (methanol)										
methanol	2,5	1,25						2,50	2,5	
2-2 (ethanol)					1,25					
ethanol	2,5	1,25	1,25		2,5			0,63		
2-3 (chloroform)	2,5				1,25					
chloroform	2,5	2,5	2,5	2,5	2,5			1,25		
2-4 (aseton)										
aseton		2,5								
2-5 (isopropanol)										
isopropanol		1,25						0,63	1,25	
2-6 (etil asetat)										
etil asetat		1,25	2,5	2,5				1,25	2,5	
2-7 (hexane)										
hexane										

Table 9. Disk Diffusion results of *H. cupressiforme* var. *lacunosum* extracts prepared with different filters for various microorganisms

H. cupressiforme	*S.pyogenes*	*S.aureus*	*E.faecalis*	*B.subtilis*	*K.pneumonia*	*E.aerogenes*	*P.aeroginosa*	*E.coli*	*C.albicans*	*S.cerevisiae*
3-1 methanol	7	6	6	6	6	6	6	6	6	6
methanol	6	8	10	8	7	9	8	9	10	12
3-2 ethanol	11	11	10	9	8	10	10	10	12	12
ethanol	10	9	10	8	8	5	18	10	20	15
3-3 chloroform	7	10	11	8	8	14	8	9	8	8
chloroform	7	10	10	8	10	7	13	10	12	12
3-4 aseton	7	10	10	9	7	9	7	9	7	10
aseton	10	7	10	8	9	11	9	10	15	13
3-5 isopropanol	12	10	10	10	8	11	14	10	12	6
isopropanol	12	11	14	18	14	14	14	18	14	8
3-6 etil asetat	6	10	11	11	10	8	7	11	10	6
etil asetat	12	7	11	6	12	12	11	10	12	7
3-7(hexane)	6	6	14	12	6	6	6	6	6	6
hexane	10	6	6	6	6	6	6	6	6	6

Table 10. Agar Well Diffusion results of *H. cupressiforme* var. *lacunosum* extracts prepared with different reagents for various microorganisms

H. cupressiforme	*S.pyogenes*	*S.aureus*	*E.faecalis*	*B.subtilis*	*K.pneumonia*	*E.aerogenes*	*P.aeroginosa*	*E.coli*	*C.albicans*	*S.cerevisiae*
3-1(methanol)	7	6	6	6	6	6	6	6	6	6
methanol	10	9	10	8	7	9	8	12	12	12
3-2(ethanol)	11	9	10	9	8	10	10	10	12	12
ethanol	12	8	9	7	8	5	18	10	20	15
3-3(chloroform)	7	10	11	8	8	14	8	9	8	8
chloroform	9	9	7	6	10	6	13	7	12	12
3-4(aseton)	7	9	10	9	7	9	7	9	7	10
aseton	10	10	10	8	9	11	9	10	15	13
3-5(isopropanol)	12	10	10	10	8	11	14	10	12	6
isopropanol	12	10	14	10	14	14	14	10	14	8
3-6(etil asetat)	6	8	12	6	10	8	7	14	7	6
etil asetat	12	7	11	6	12	12	11	10	12	7
3-7(hexane)	6	6	14	12	6	6	6	6	6	6
hexane	10	6	6	6	6	6	6	6	6	6

Table 9 presents the zone diameters obtained via the disk diffusion method for the extract of H. cupressiforme var. lacunosum on the investigated microorganisms. The efficacy of the plant extract prepared in ethanolic solution was found to be greater than that of the solvent in S. aureus (11 mm), B. subtilis (9 mm), E. aerogenes (10 mm) and S. pyogenes (11 mm) strains.

Table 10 presents the zone diameters of the *H. cupressiforme* var. *lacunosum* extract obtained via the agar well diffusion method on the investigated microorganisms. The results indicate that the antimicrobial activity of the plant extract prepared in an ethanolic solution was more pronounced against S. aureus (9 mm), E. faecalis (10 mm), B. subtilis (9 mm) and *E. aerogenes* (10 mm) strains compared to the solvents used.

Data from Table 11 indicate that bacterial growth was observed at a concentration of 1.25 µl/ml in both the plant extract and ethanol solvent in the *S. aureus* strain, suggesting that the antimicrobial activity of ethanol is comparable to that of the plant extract.

Table 11. MIC values of *H. cupressiforme* var. *lacunosum* extracts prepared with different reagents for various microorganisms

H. cupressiforme	*S.pyogenes*	*S.aureus*	*E.faecalis*	*B.subtilis*	*K.pneumonia*	*E.aerogenes*	*P.aeroginosa*	*E.coli*	*C.albicans*	*S.cerevisiae*
3-1(methanol)	7	6	6	6	6	6	6	6	6	6
methanol	10	9	10	8	7	9	8	12	12	12
3-2(ethanol)	11	9	10	9	8	10	10	10	12	12
ethanol	12	8	9	7	8	5	18	10	20	15
3-3(chloroform)	7	10	11	8	8	14	8	9	8	8
chloroform	9	9	7	6	10	6	13	7	12	12
3-4(aseton)	7	9	10	9	7	9	7	9	7	10
aseton	10	10	10	8	9	11	9	10	15	13
3-5(isopropanol)	12	10	10	10	8	11	14	10	12	6
isopropanol	12	10	14	10	14	14	14	10	14	8
3-6(etil asetat)	6	8	12	6	10	8	7	14	7	6
etil asetat	12	7	11	6	12	12	11	10	12	7
3-7(hexane)	6	6	14	12	6	6	6	6	6	6
hexane	10	6	6	6	6	6	6	6	6	6

3.2. Statistical calculations of zone diameters

Statistical calculations of the zone diameters were conducted with three replications. The obtained data were subjected to statistical analysis, which included the Kruskal-Wallis Variance test, Mann–Whitney U test, and Pearson Chi-square analysis.

The difference between the median values of the data belonging to three parallel groups was analysed with the Kruskal-Wallis test. As a result of the analysis, no significant difference was observed between the mean ranks (p>0.081). This result shows that the repetitions between the parallel groups are close to each other and the test results are compatible with each other (Table 12).

The Kruskal-Wallis test, as presented in Table 13, was conducted to determine whether variations in extract diameters were influenced by plant species. The analysis revealed a statistically significant difference between the plants ($p < 0.05$). Examination of the rank averages indicated that *H. cupressiforme* var. *lacunosum* exhibited the highest activity, whereas *S. touretii* displayed the lowest activity. These results demonstrate that the activities of the tested plants differed significantly and were dependent on the plant species.

Table 12. Repeat results of experiments

	Parallel	N	Average Rank	sd	Chi-square	p
Extract Diameter	1. Parallel	420	623,37	2	5,019	0,081
	2. Parallel	420	615,51			
	3. Parallel	420	622,61			

p<0,05

Table 13. Results of extract diameters according to mosses species

	Mosses	N	Rank Avg.	sd	Chi-square	p
Extract Diameter	*B. glareosum*	420	637	2	11,758	0,003
	S. touretii	420	585,03			
	H. cupressiforme var. *lacunosum*	420	669,48			

p<0,05

Table 14. Results of extract diameters according to solvent types

	Solvent Type	N	Rank Avg.	sd	Chi-square	p
Extract diameter	Methanol	180	372,44	6	524,388	0,00
	Ethanol	180	918,82			
	Chloroform	180	761,63			
	Acetone	180	465,86			
	Isopropanol	180	912,66			
	Etil Aseton	180	671,55			
	Hekzan	180	310,55			

p<0,05

Analysis of data from seven different solvents using the Kruskal-Wallis test revealed a statistically significant difference between the solvents at a p-value of less than 0.05. Examination of Table 14 indicates that the mean ranks of the solvents show ethanol (918.82) and isopropanol (912.66) to exhibit the highest activity, whereas hexane (310.55) demonstrated the lowest activity. These results highlight the significance of the solvent type on mosses and demonstrate the variability in activity among different solvents.

A comparison of the susceptibilities of gram-positive bacteria, gram-negative bacteria, and fungi was conducted using the Kruskal-Wallis test. The results indicated no statistically significant difference between the groups. Examination of the ranked means revealed that gram-positive bacteria exhibited slightly higher values than the other groups, although this difference was not statistically significant.

The sensitivity of 10 distinct microorganisms was assessed using the Kruskal-Wallis test, which revealed a statistically significant difference among the microorganisms. Examination of the rank averages presented in Table 16 indicates that *E. faecalis* exhibits the highest activity, whereas *S. pyogenes, E. aerogenes,* and *P. aeruginosa* display low activity.

The sensitivity difference between gram-positive and gram-negative bacteria was investigated using the Mann–Whitney U test. The mean rank for gram-positive bacteria was determined to be 521.62, while that for gram-negative bacteria was 487.38, as per the results presented in Table 17. Although the obtained p-value of 0.059 exceeded the 0.05 significance threshold, gram-positive bacteria exhibited a greater tendency toward sensitivity.

The antimicrobial activity status of the plant species in Table 18 was classified as "effective" and "not effective" and comparedwith theo observed and expected numbers. A Pearson chi-square value of 6.230 was obtained using the chi-square test, and the corresponding significance level was calculated as p=0.044. This outcome indicates a statistically significant difference between the groups at a significance level of p<0.05. Notably, *B. glareosum* and *H. cupressiforme* var. *lacunosum* exhibited higher "effective" status values than expected, whereas *S. touretii* showed a lower "effective" status than anticipated.

Table 15. Results of extract diameters according to the type of strain prepared

	Suş	N	Sıra Ort.	sd	Ki-kare	p
Ekstrakt çap	G Pozitif	504	656,03	2	4,229	0,121
	G Negatif	504	614,11			
	Mantar	252	612,2			

p<0,05

Table 16. Results of extract diameters according to microorganism type

	Microorganism Type	N	Rank Avg.	sd	Chi-square	p
Extract diameter	*S. pyogenes*	126	591,31	9	26,821	0,001
	S. aureus	126	634,65			
	E. faecalis	126	774,15			
	B. subtilis	126	624,03			
	K. pneumonia	126	609			
	A. erogenes	126	591,91			
	P. aeroginosa	126	591,89			
	E. coli	126	663,66			
	C. albicons	126	603,4			
	S. cerevisiae	126	621,01			

p<0,05

Table 17. Results of extract diameters according to gram positive and gram negative species.

Groups	N	Rank Avg.	Mann Whitney	Z Value	p
Gram Positive	504	521.62	118.382	-1,886	0,059
Gram Negative	504	487.38			

p<0,05

Table 18. Impact status results according to moss type.

Effect Status	Mosses	N	Chi-Square	sd	p
No Effect	*B. glareosum*	313	6,23	2	0,044
	S. touretii	342			
	H. cupressiforme	320			
Yes Effect	*B. glareosum*	107			
	S. touretii	78			
	H. cupressiforme var. *lacunosum*	100			

p<0,05

Table 19. Effect status results according to solvent type

Solvent Type	No Effect	Yes Effect	N	Chi-Square	sd	p
Methanol	167	13	180	110,667	6	0,00
Ethanol	125	55				
Chloroform	110	70				
Acetone	166	14				
Isopropanol	160	20				
Etil Aseton	121	59				
Hekzan	26	154				

p<0,05

The efficacy of the seven distinct solvents was evaluated using the Pearson Chi-Square test. As a result of this analysis, a statistically significant relationship was established between the solvent types and their antimicrobial effects. Notably, hexane emerged as the most effective solvent within the "effect" group, implying that it may exhibit enhanced antimicrobial properties compared to other solvents against specific microorganisms.

The study involved the evaluation of three distinct moss species (*B. glareosum, S. touretii, H. cupressiforme var. lacunosum*) and seven different solvent extracts (methanol, ethanol, acetone, chloroform, hexane, ethyl acetate, and isopropanol) against a panel of 10 microorganisms. This panel consisted of 4 gram-positive bacteria, 4 gram-negative bacteria, and 2 fungi, specifically: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, Streptococcus pyogenes ATCC 12344, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* MTCC 109, Escherichia coli ATCC 35218, *Candida albicans* ATCC 10231, and Saccharomyces cerevisiae ATCC 9337. The antimicrobial and antifungal effects of these extracts were assessed.

This study conducted a comprehensive analysis of the activity levels against microorganisms and the impact of the solvents employed. The primary objective was to explore the potential therapeutic applications of bryophytes with the aim of addressing the growing issue of antimicrobial and antifungal drug resistance. The research findings revealed substantial variations between the solvents used and the types of microorganisms present.

Moss extracts typically demonstrated enhanced antimicrobial activity against gram-positive bacteria. Specifically, *B. glareosum* extracts displayed notable efficacy against gram-positive bacteria. Among the solvents employed, hexane extract yielded the highest level of inhibition, whereas methanol, acetone, and isopropanol were found to be ineffective. Furthermore, no antimicrobial or antifungal activity was observed against Pseudomonas aeruginosa and Candida albicans strains.

S. touretii extracts showed strong antimicrobial and antifungal activity, especially against Enterococcus faecalis, Bacillus subtilis and Saccharomyces cerevisiae. However, they were ineffective against other microorganisms. The highest activity in this species was observed in the ethyl acetate extract, while methanol, ethanol, acetone and chloroform did not show any effect.

Extracts of *H. cupressiforme* var. *lacunosum* exhibited antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis and Escherichia coli strains. Conversely, no antimicrobial activity was observed against *Streptococcus pyogenes*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans* and *Saccharomyces cerevisiae*. Notably, hexane and chloroform solvents yielded the highest antimicrobial activity, whereas methanol and isopropanol exhibited lower activity.

The results showed that ethanol and isopropanol were the most effective solvents, while methanol, acetone and hexane had the lowest antimicrobial activity. When evaluated in terms of microorganisms, *Enterococcus faecalis*, Escherichia coli, Staphylococcus aureus and Bacillus subtilis showed the highest sensitivity to the moss extracts. *Pseudomonas aeruginosa* and *Candida albicans* strains were found to be resistant to the extracts.

At the microorganism level, *Pseudomonas aeruginosa* and *Candida albicans* strains exhibited resistance to moss extracts, whereas Staphylococcus aureus, *Bacillus subtilis, Enterococcus faecalis,* and *Escherichia coli* demonstrated the highest sensitivity to these extracts.

The solvent diameters were ranked in the following order: ethanol > isopropanol > chloroform > ethyl acetate > acetone > methanol > hexane, as indicated by the statistical analysis results. The plant species exhibiting the highest activity were *H. cupressiforme* var. *lacunosum,*

Brachythecium glareosum, and *S. touretii*, in descending order of activity. In terms of microorganisms, the highest activity was observed in *E. faecalis*, followed by *E. coli, B. subtilis, S. cerevisiae, K. pneumoniae, C. albicans, E. aerogenes, P. aeruginosa*, and *S. pyogenes.*

A comparative analysis of microorganism species based on their diameters revealed that fungi exhibited greater resistance than gram-negative bacteria, which in turn demonstrated greater resistance than gram-positive bacteria. The antimicrobial efficacy of the plant extract was found to be lower in instances where the solvent diameters exceeded the diameter of the plant extract.

This investigation demonstrated that bryophytes exhibit substantial antimicrobial and antifungal properties against certain microorganisms, suggesting their potential as sources in drug development processes. The findings indicate that these plants can serve as a natural alternative in pharmaceutical and medical applications and may also inform further research in this area.

Observations have been made that extracts derived from mosses possess potential as natural antimicrobial and antifungal agents in pharmaceutical development. Consequently, it is considered essential to establish suitable methods for large-scale industrial applications and to investigate the use of these extracts within the pharmaceutical, agricultural, and food industries.

Declaration

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