http://dergipark.org.tr/tr/pub/anatolianbryology

DOI: 10.26672/anatolianbryology.1810483

Anatolian Bryology
Anadolu Briyoloji
Dergisi
Research Article
e-ISSN:2458-8474
Online

Screening of Bioactive Potential of *Plasteurhynchium striatulum*: Antibiofilm Effects and Phytochemical Profile

Gizem GÜL[1,*], Elif GÖNÜL[2], Cenker YAMAN[1], Melike ERSİN[2], Selime Deniz BOZKURT[1], Gülcan GÖREN[2], Ezginur DUMAN[2], Atakan BENEK[3], Ayşe Dilek UNAN[4], Mustafa Eray BOZYEL[5], Kerem CANLI[2,3]

[1]*Dokuz Eylül University, Graduate School of Natural and Applied Science, Department of Biology, İzmir, TÜRKİYE*
[2]*Dokuz Eylül University, Faculty of Science, Department of Biology, Izmir, TÜRKİYE*
[3]*Dokuz Eylül University, Fauna and Flora Research and Application Center, İzmir, TÜRKİYE*
[4]*Zonguldak Bülent Ecevit University, Faculty of Science and Art, Department of Biology, Zonguldak, TÜRKİYE*
[5]*Department of Biology, Faculty of Arts and Science, Canakkale Onsekiz Mart University, Çanakkale, TÜRKİYE*

Received: 30 June 2025 **Revised: 30 September 2025** **Accepted: 18 October 2025**

Abstract

In the present study, the bryophyte *Plasteurhynchium striatulum* (Spruce) M. Fleisch was extracted using three different solvents: ethanol, methanol, and n-hexane. Subsequently, the antimicrobial activities of the extracts were evaluated against a range of microorganisms including foodborne isolates, clinical isolates, multidrug-resistant strains, and standard strains using disk diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibiofilm assays. In addition, antioxidant activity was assessed through the DPPH radical scavenging assay. The biochemical constituents of the extracts were identified via gas chromatography–mass spectrometry (GC-MS) analysis. According to the disk diffusion test results, activity was observed against eight bacterial strains, most notably *Escherichia coli* and *Providencia rustigianii*, both of which exhibit multidrug resistance. The highest MIC value was recorded as 0.8816 mg/mL against the *Salmonella infantis* strain. The strongest antioxidant performance was observed at a concentration of 1 mg/mL. GC-MS analysis revealed that the major components of the extract were Tris(2,4-di-tert-butylphenyl) phosphate (52.45%), A'-Neogammacer-22(29)-ene (25.00%), and Neophytadiene (12.72%). In conclusion, *P. striatulum* was found to possess notable antimicrobial and antioxidant activities, along with a rich biochemical composition. These findings suggest that *P. striatulum* holds promise as a potential source for the development of novel antimicrobial and antioxidant agents. Additionally, its biochemical characteristics could offer important information for upcoming studies.

Keywords: Bryophyte, Antimicrobial Activity, Antioxidant Activity, Gas Chromatography-Mass Spectrometry (GC-MS).

Plasteurhynchium striatulum'un Biyoaktif Potansiyelinin Taranması: Antibiyofilm Etkileri ve Fitokimyasal Profili

Öz

Gerçekleştirilen bu çalışmada bir bryofit olan *Plasteurhynchium striatulum* (Spruce) M. Fleisch'un etanol, metanol ve n-hekzan olmak üzere üç farklı solvent ile ekstraksiyonu gerçekleştirilmiştir. Ardından gıda izolatları, klinik izolatlar, çoklu ilaca dirençli ve standart suşlar dahil olmak üzere çeşitli mikroorganizmalara karşı antimikrobiyal aktiviteleri disk difüzyon, minimum inhibitör konsantrasyon (MİK), minimum bakterisidal konsantrasyonu (MBC) ve antibiyofilm testleri ile tespit edilmiştir. Bununla birlikte antioksidan aktivitelerinin tespiti DPPH testi ile gerçekleştirilmiştir. Biyokimyasal içerikleri Gaz kromatografisi-kütle spektrometrisi (GC-MS) yöntemi kullanılarak belirlenmiştir. Disk difüzyon testi bulgularında başta çoklu ilaç direncine sahip *Escherichia coli* ve *Providencia rustigianii* olmak üzere toplamda 8 bakteride etki gözlemlenmiştir. MİK testinde en yüksek sonuç *Salmonella infantis*

* Corresponding author: gizeemmgull@gmail.com

To cite this article: *Gül G. Gönül E. Yaman C. Ersin M. Bozkurt S.D. Gören G. Duman E., Benek A. Unan A.D. Bozyel M.E. Canlı K. 2025. Screening of Bioactive Potential of Plasteurhynchium striatulum: Moderate Antibiofilm Effects and Complex Phytochemical Profile. Anatolian Bryology. 11:2, 111-130.*

suşunda 0,8816 mg\mL olarak belirlenmiştir. Antioksidan aktivite testinde en yüksek temizleme performansı ise 1 mg/mL konsantrasyonunda gerçekleşmiştir. GC-MS analizi sonucunda majör madde olarak Tris(2,4-di-tert-butylphenyl) phosphate (%52,45), A'-Neogammacer-22(29)-ene (%25.00) ve Neophytadiene (%12,72) tespit edilmiştir. Sonuç olarak, *P. striatulum*'un antimikrobiyal ve antioksidan aktivitesinin yanı sıra oldukça zengin bir biyokimyasal içeriğe sahip olduğu belirlenmiştir. Bu durum *P. striatulum*'un gelecekte antimikrobiyal ve antioksidan etkinliğe sahip yeni ajanların oluşturulması hususunda umut verici bir nitelik taşırken biyokimyasal yapısının aydınlatılması gelecekteki çalışmalar için önemli bilgiler sunmaktadır.
Anahtar kelimeler: Bryofit, Antimikrobiyal Aktivite, Antioksidan Aktivite, Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS)

1. Introduction

Bryophytes are non-vascular land plants classified into three main groups: hornworts (Anthocerotopsida), liverworts (Hepaticopsida), and mosses (Bryopsida) (Chandra et al., 2017; Das et al., 2022). Globally, bryophytes represent a crucial component of biodiversity and are typically found in moist and high-altitude regions; however, they can also be encountered in deserts, polar areas, and tropical climates (Öztürk et al., 2018; Dziwak et al., 2022). These small plants, which play a critical role in ecosystem stability and plant conservation, have been used by humans for centuries in the treatment of various diseases, skin disorders, and respiratory ailments aimed at preserving human health (Asakawa and Ludwiczuk, 2017; Muhammad et al., 2018; Ursavaş and Keçeli 2019).

The high abundance of bioactive compounds in bryophytes has led to increasing interest in these plants (Klavina, 2015). These plants, lacking evolved structural adaptations to protect against environmental stressors, adapt to stress conditions through their chemical diversity (Cianciullo et al., 2021). Therefore, the metabolites they produce are highly diverse and abundant. These metabolites not only play a significant role in bryophytes' defense against environmental factors, but also function in their interactions with other organisms within the ecosystem (Peters et al., 2019). Bryophytes possess a rich content of bioactive compounds, including glycosides, terpenoids, phenolic compounds, and fatty acids (Akatin et al., 2024). However, bryophytes often go unnoticed due to their small size and inconspicuous nature in the natural environment. Their widespread distribution across diverse substrates such as soil, animals, dead wood, and leaves poses challenges for the collection of pure samples. Additionally, difficulties in identifying and analyzing existing species resulting from their small morphology have led to the neglect of research on these plants (Öztopçu et al., 2017; Ismaeel et al., 2022). However, with the increasing interest in these plants, studies focused on the isolation of their bioactive compounds have been steadily growing (Horn et al., 2021). These studies are crucial for the identification of their chemical constituents. Bryophytes still contain compounds that have not yet been discovered or fully characterized. Therefore, comprehensive analysis of bioactive compounds present in medicinal plants and accurate quantification of therapeutically relevant components are of great importance (Turu et al., 2024).

When the literature is examined, numerous studies are found on the antimicrobial effects of bioactive secondary metabolites derived from plant sources. However, there is a notable lack of sufficient data regarding simple terrestrial plants such as bryophytes. Due to their underdeveloped morphological barriers against environmental stressors, bryophytes rely on their potent chemical compositions as their most effective defense mechanisms (Rodrigues et al., 2020; Valeeva et al., 2022). The well-developed secondary metabolites of bryophytes indicate that they are among the best candidates for the discovery and characterization of novel pharmaceutical compounds (Önbaşlı and Yuvalı, 2021). Some bioactive compounds derived from bryophytes are known to exhibit antimicrobial activity due to the presence of biflavonoids in their composition (Joshi et al., 2023). Bacterial resistance mechanisms against antibiotics are increasing day by day (Alan et al., 2018). Antibiotic resistance, which has become a global issue, leads to an increase in mortality rates associated with infectious diseases. The increasing rate of bacterial resistance reduces the effectiveness of existing antibiotics and necessitates the discovery of compounds with novel mechanisms of action. In this context, the development of antimicrobial agents from plants and the use of plants in alternative therapeutic approaches remain of significant importance. Scientific research in this field contributes valuable knowledge to the literature and holds great importance (Woo et al., 2023; Kırmacı et al., 2024).

In addition to their antimicrobial effects, phytochemicals are also known to be effective against the formation of biofilms, which is one of the antimicrobial resistance mechanisms. The high concentration of various bioactive compounds in plants has been observed not only to enhance

antibiofilm activity but also to prevent the development of resistance. Moreover, pharmacological substances derived from plants offer greater advantages over existing drugs due to their high therapeutic efficacy and cost-effectiveness. However, the incomplete characterization of bioactive compound contents in plants poses a barrier to the efficient utilization of their antibiofilm effects (Rather et al., 2021; Ali and Neelakantan, 2022; Mahamud et al., 2024).

Living organisms produce reactive oxygen species (ROS) as by-products during the metabolic processing of oxygen. These reactive oxygen species cause the degradation of biological molecules and induce oxidative stress. As simple-structured plants, bryophytes possess effective defense mechanisms that confer antioxidant properties; these mechanisms help reduce the harmful effects of reactive oxygen species and minimize cellular damage. As simple-structured plants, bryophytes possess effective defense mechanisms that confer antioxidant properties; these mechanisms help reduce the harmful effects of reactive oxygen species and minimize cellular damage. (Provenzano et al., 2019; Chaves et al., 2020; Bozkurt et al., 2024;). Additionally, the volatile phenolic compounds present in bryophytes contribute to health promotion by exhibiting antioxidant properties that aid in the prevention of age-related diseases and aging (Mitra et al., 2019). Antioxidants obtained through diet or natural sources may be beneficial in combating oxidative stress, which adversely affects human health and contributes to aging. Due to their pharmaceutical effects, these antioxidants have potential applications as health supplements (Amorati and Valgimigli, 2018).

In this study, the biological activities of the phytochemicals present in the moss *Plasteurhynchium striatulum* (Spruce) M. Fleisch were thoroughly investigated. Its antimicrobial potential was assessed using the disk diffusion method, followed by the determination of the Minimum Inhibitory Concentration (MIC) value. The antibiofilm activity of the plant was determined by analyzing bacterial biofilm formation. Additionally, the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed to evaluate its antioxidant capacity. The richness of *P. striatulum*'s phytochemical content was demonstrated by GC-MS analysis, and the obtained active secondary metabolites were assessed to discuss their biological efficacy.

2. Materials and Methods

P. striatulum moss was collected and identified by Dr. Ayşe Dilek Unan from Merkez/Zonguldak (41°27'14.3"N 31°45'49.2"E). The plant material was deposited at the Dokuz Eylül University Fauna and Flora Research and Application Center, Buca, İzmir, Turkey, and is preserved under the herbarium code FFDEU-MEB4.

2.1. Preparation of extracts from Plasteurhynchium striatulum

After drying, the collected samples were ground into small pieces using a grinder (IKA, MF10 basic). Thirty grams of the powdered plant material was placed in an Erlenmeyer flask, to which 200 mL of ethanol was added. The mixture was then shaken at 140 rpm for 2 days. Following the completion of the shaking step, the mixture was passed through Whatman No. 1 filter paper into flasks. Subsequently, the solvent was evaporated using a rotary evaporator, and the remaining residue in the flask was measured and noted. The extract was prepared for further use (Canlı et al., 2019). Extraction was also conducted separately with three distinct solvents ethanol, n-hexane, and methanol by applying the same protocol. The amounts of extract obtained were calculated and recorded as 0.529 g in 25 mL for ethanol, 1.149 g for methanol, and 0.147 g for n-hexane.

2.2. Microorganisms

This research assessed antimicrobial effects on 48 microbial strains in total, comprising 17 standard strains, 7 isolates from food sources, 13 clinical isolates, and 11 multidrug-resistant (MDR) strains. The strains used in this study were sourced from the Biology Department at the Faculty of Science, Dokuz Eylül University (İzmir, Turkey).

2.3. Inoculum preparation

A sterile 0.9% sodium chloride (NaCl) solution was prepared for the microbial strains utilized in the study and microbial suspensions were adjusted to a density of 1.5×10^7 CFU/mL^{-1} for yeasts and 1.5×10^8 CFU/mL^{-1} for bacteria in accordance with the 0.5 McFarland standard (Benek et al., 2024).

2.4. Disc diffusion test

In order to compare the antimicrobial effects of ethanol, methanol, and n-hexane extracts of *P. striatulum* moss on microorganisms, the disk diffusion method was employed. A volume of 25 mL was used for each solvent, and the extract yields were calculated as 0.529 g for ethanol, 1.149 g for methanol, and 0.147 g for n-hexane. Next, to assess antimicrobial activity, sterile petri

dishes were filled with Mueller-Hinton agar that had been prepared beforehand. Antimicrobial test disks were impregnated with 110 µL of ethanol extract and 150 µL each of methanol and n-hexane extracts. Prepared microbial inocula were spread onto agar plates, and the extract-impregnated disks were placed upside down to facilitate more effective diffusion. Following the placement of the disks, petri plates with bacterial strains were incubated at 37°C for 24 hours, whereas those containing yeast strains were incubated at 28°C for 48 hours. Following incubation, the inhibition zones surrounding the disks were measured and documented in tables. Gentamicin served as the positive control. (Şimşek et al., 2023).

2.5. Determination of minimum inhibitor concentrations (MIC)

MIC of *P. striatulum* plant extract against microorganisms that showed antimicrobial sensitivity in the disk diffusion test was determined using the broth microdilution method. For the preparation of the DMSO-water extract required for the MIC test, the ethanol-based extract was first concentrated by rotary evaporation at 40 °C. The concentrated material was then dissolved in dimethyl sulfoxide (DMSO) (Iron Chemistry, Turkey) and processed. Subsequently, dilution was performed with ultrapure water obtained from a distillation system (Thermo Fisher Scientific, USA) to prepare a solution containing 1% DMSO (Gül et al., 2025). Following this procedure, the extract amounts were calculated and recorded as 0.16 g for ethanol-DMSO, 0.377 g for methanol-DMSO, and 0.057 g for n-hexane-DMSO in 15 mL of extract. Mueller-Hinton broth was used as the suitable culture medium for the microorganisms, and the microbial density was adjusted according to the 0.5 McFarland standard. The plant extracts were transferred into sterile 96-well microplates at a volume of 100 µL per well. Subsequently, 50 µL of microbial suspension was added to all wells. Antimicrobial activity was evaluated by visual inspection. For the negative control, wells contained only the culture medium and microbial inoculum, while for the positive control, only the culture medium was added to monitor any contamination. The plates were incubated for 24 hours to determine the necessary concentration to inhibit bacterial growth. The test was conducted in triplicate (Canlı et al., 2023a, 2023b).

2.6. Determination of minimum bactericidal concentration (MBC)

MBC is defined as the minimum concentration of an antimicrobial substance required to eliminate 99.9% of the initial bacterial population. After establishing the MIC value of *P. striatulum* plant extract against the tested microorganisms, 10 µL samples were collected from wells showing inhibition and streaked onto agar plates, which were then incubated at 37°C for 24 hours. The MBC value was determined by examining the agar plates for the presence or absence of microbial growth (Benek et al., 2024).

2.7. Biofilm activity test

The biofilm activity assay was adapted from the study conducted by Tunca-Pınarlı and Canlı (2021). The assessment of biofilm activity was carried out through a two-step process: first, determining the conditions necessary for biofilm formation, and second, evaluating the antibiofilm activity of the moss extracts.

2.7.1. Assessment of conditions for biofilm formation

To determine the antibiofilm activity of *P. striatulum,* a total of five bacterial strains were used: *Escherichia coli* 1209212 (CI), *Listeria innocua* (FI), *E. coli ATCC* 25922, *Listeria monocytogenes* ATCC 7644, and *Bacillus subtilis DSMZ* 1971. The bacterial strains used were standardized to a density of 1.5×10^8 CFU/mL^{-1} in sterile physiological saline solution, in accordance with the 0.5 McFarland standard. Subsequently, Luria–Bertani (LB) broth media containing six different glucose monohydrate concentrations (0.0%, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) were loaded into 96-well microplates. The bacterial suspensions were then added, and the plates were incubated at 37°C for 24-8 hours. Following the completion of incubation, the microplates were washed and allowed to dry. After drying, each well was stained with 200 µL of crystal violet solution and allowed to sit for 15 minutes. Following staining, the plates were rinsed with distilled water and dried once more. Finally, 200 µL of a solution composed of 30% acetone and 70% ethanol was added to every well and incubated for 15 minutes before transferring the contents to clean microplates. The absorbance of the wells was measured at 550 nm using a microplate reader (Biotek). As a result, the optimal conditions for high biofilm production were determined for each microorganism.

2.7.2. Antibiofilm activity detection

To evaluate the antibiofilm activity of the moss extracts, concentrations corresponding to ½ of the MIC value were selected. The bacterial strains *Escherichia coli* 1209212 (CI), *E. coli* ATCC 25922, *Bacillus subtilis* DSMZ 1971. *Listeria monocytogenes* ATCC 7644 and *Listeria innocua* (FI) were standardized to a 0.5 McFarland turbidity standard. Subsequently, 100 µL of LB

broth medium, along with 50 µL each of the liquid extract and bacterial suspension, were added to each well of the microplates. After loading, the microplates were incubated under the optimal incubation conditions previously determined for each microorganism. Upon completion of incubation, the plates were washed and allowed to dry. Once dried, 200 µL of a solution composed of 30% acetone and 70% ethanol was added to each well and incubated for 15 minutes. The contents were then transferred to clean microplates. Halamid at a concentration of 3 mg/ml was used as a reference substance for the positive control. The absorbance of each well was measured and recorded at 550 nm using a microplate reader (Yaman et al., 2025).

2.8. Assessment of antioxidant properties

The antioxidant activity of *P. striatulum* extracts was evaluated based on their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. The method used relies on measuring the efficiency of antioxidants present in the plant extract to neutralize the DPPH radical. A quantity of 0.0039 g DPPH was measured and dissolved in 50 mL of ethanol using a glass Erlenmeyer flask to prepare the DPPH solution. The flask was then covered with aluminum foil to shield the solution from light. Extracts and DPPH solution were added to 96-well microplates at concentrations ranging from 7.8125 to 1000 µg/mL. The plates were then kept in the dark at room temperature for 30 minutes. After this incubation period, absorbance was read at 515 nm using a microplate reader and the results were documented. Ascorbic acid served as the positive control, and all tests were performed in triplicate. (Turu et al., 2024; Bozkurt et al., 2024).

2.9. Gas chromatography-mass spectrometry method (GC-MS)

The biochemical content analysis of the moss sample was performed using the methods described by Benek et al. (2024). GC-MS analysis was carried out using the Agilent GC 8890-Agilent GC/MSD 5977B system, with helium gas used as the carrier. Compound identification was performed by matching the acquired spectra against the reference data from the NIST and Wiley databases. Chemical constituents present at concentrations higher than 0.5% were recorded as major components.

2.10. Statistical analysis

The statistical evaluation was carried out using the parametric one-way ANOVA technique. The Pearson correlation coefficient was also determined. All tests were performed in triplicate. P-values of 0.05 or lower ($p \leq 0.05$) were regarded as statistically significant. All statistical analyses were performed using R Studio.

3. Result

3.1. Disk diffusion test

Using the disk diffusion technique, the antimicrobial effects of *P. striatulum* extracts prepared with ethanol, methanol, and n-hexane were assessed against a diverse range of 48 microbial strains.

Based on the outcomes of the disk diffusion assay, *P. striatulum* extracts demonstrated antimicrobial activity against a total of eight different strains. The microbial strains showing inhibition zones with the ethanol extract were identified as *Escherichia coli* (7 mm), *Pseudomonas aeruginosa* (7 mm), *Staphylococcus aureus* (7 mm), *Salmonella infantis* (7 mm), and *Streptococcus mutans* (7 mm). The methanol extract exhibited antimicrobial activity against *Listeria monocytogenes* (7 mm), *Staphylococcus aureus* (7 mm), *Salmonella infantis* (7 mm), and *Streptococcus mutans* (7 mm). In the disk diffusion test performed using the n-hexane extract, activity was recorded only against the *Providencia rustigianii* strain (7 mm). Statistical evaluation indicated that there was no significant variation among the results obtained from the different solvents ($p > 0.05$). Furthermore, when ethanol, methanol, and n-hexane extracts were evaluated separately, no significant difference was observed in the levels of antimicrobial activity against different bacterial species ($p = 0.5 > 0.05$). This finding indicates that a substantial portion of the variance is attributed not to the bacterial species but to other experimental variables. According to the results obtained, the inhibitory effects of the applied biological agents on bacterial species occurred at similar levels, and the differences in sensitivity among species were not statistically significant. This suggests that the tested agents may exhibit a homogeneous effect profile on the target microorganisms or that the bacterial species included in the study possess similar resistance levels. The zones of inhibition surrounding the disks containing *P. striatulum* extracts are shown in Tables 1–4, corresponding to standard isolates (Table 1), food isolates (Table 2), clinical isolates (Table 3), and multidrug-resistant (MDR) strains (Table 4).

Table 1. Disk diffusion test results of *P. striatulum* moss on standard isolated strains

Microorganisms	**Ethanol (110µl)**	**Methanol (150µl)**	**n-hexane (150µl)**	**Gen (10 µg)**
Bacillus subtilis DSMZ l97l	-	-	-	30±0.00
Candida albicans DSMZ l386	-	-	-	12±0.00
Enterobacter aerogenes ATCC l 3048	-	-	-	24±0.00
Enterococcus faecalis ATCC 292l2	-	-	-	12±0.00
Escherichia coli ATCC25922	7±0.00	-	-	22±0.00
Listeria monocytogenes ATCC 7644	-	7±0.00	-	28±0.00
Pseudomonas aeruginosa DSMZ 50071	7±0.00	-	-	15±0.00
Pseudoıuonas fluorescens P 1	-	-	-	13±0.00
Salmonella enteritidis ATCC 13076	-	-	-	21±0.00
Salmonella typhimuriuın SL1344	-	-	-	24±0.00
Staphylococcus aureus ATCC 25923	7±0.00	7±0.00	-	21±0.00
Staphylococcus epiderınidis DSMZ 20044	-	-	-	22±0.00
Staphylococcus hominis ATCC 27844	-	-	-	18±0.00
Staphylococcus warneri ATCC 27836	-	-	-	23±0.00
Bacillus cereus RSKK 863	-	-	-	24±0.00
Shigella flexneri RSKK l84	-	-	-	18±0.00
Acinetobacter baumannii CECT 9111	-	-	-	13±0.00
Gen: Gentamicin, (-): No effect was observed.				

Table 2. Disk diffusion test results of *P. striatulum* moss on food isolate strains

Microorganisms	**Ethanol (110µl)**	**Methanol (150µl)**	**n-hexane (150µl)**	**Gen (10 µg)**
Enterococcus durans	-	-	-	11±0.00
Enterococcus faecium	-	-	-	28±0.00
Klebsiella pneumoniae	-	-	-	19±0.00
Listeria innocua	-	-	-	13±0.00
Salmonella infantis	7±0.00	7±0.00	-	17±0.00
Salmonella kentucky	-	-	-	12±0.00
Escherichia coli	-	-	-	0±0.00
Gen: Gentamicin, (-): No effect was observed.				

Table 3. Disk diffusion test results of *P. striatulum* moss on clinical isolate strains

Microorganisms	Ethanol (110µl)	Methanol (150µl)	n-hexane (150µl)	Gen (10 µg)
Staphylococcus aureus	-	-	-	22±0.00
Streptococcus mutans	7±0.00	7±0.00	-	22±0.00
Staphylococcus hominis	-	-	-	9±0.00
Staphylococcus haemolyticus	-	-	-	10±0.00
Staphylococcus lugdunensis	-	-	-	17±0.00
Shigella boydi	-	-	-	20±0.00
Actinobacter baumannii	-	-	-	18±0.00
Shigella flexneri	-	-	-	16±0.00
Staphylococcus aureus	-	-	-	22±0.00
Enterococcus faecalis	-	-	-	12±0.00
Klebsiella pneumoniae	-	-	-	18±0.00
Candida tropicalis	-	-	-	0±0.00
Candida glabrata	-	-	-	7±0.00
Gen: Gentamicin, (-): No effect was observed.				

Table 4. Disk diffusion test results of *P. striatulum* moss on multidrug-resistant (MDR) strains

Microorganisms	Ethanol (110µl)	Methanol (150µl)	n-hexane (150µl)	Gen (10 µg)
Escherichia coli	7±0.00	-	-	8±0.00
Klebsiella pneumoniae	-	-	-	15±0.00
Acinetobacter baumannii	-	-	-	0±0.00
Enterobacter aerogenes	-	-	-	16±0.00
Serratia odorifera	-	-	-	7±0.00
Proteus vulgaris	-	-	-	11±0.00
Streptococcus pneumonia	-	-	-	10±0.00
Staphylococcus aureus MRSA	-	-	-	0±0.00
Staphylococcus aureus MRSA+ MDR	-	-	-	22±0.00
Providencia rustigianii	-	-	7±0.00	16±0.00
Achromobacter sp.	-	-	-	9±0.00
Gen: Gentamicin, (-): No effect was observed.				

3.2. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC/MFC)

The MIC test results of the ethanol extract of *P. striatulum* are presented in Table 5, the methanol extract results in Table 6, and the n-hexane extract results in Table 7. Among the 11 different strains tested, the MIC value against S. infantis was determined to be 0.8816 mg/mL. Furthermore, the MBC test performed on the strain that showed antimicrobial activity in the MIC test revealed that the extract exhibited a bacteriostatic effect. The MBC test was not performed on microorganisms that showed no effect in the MIC test. Test readings were based on visual inspection. All tests were repeated three times.

Table 5. MIC and MBC/MFC Results of the Ethanol Extract of *P. striatulum*

Microorganisms	MIC (mg/ml)	MBC/MFC (mg/ml)
Escherichia coli ATCC 25922	>7,0533	-
Pseudomonas aeruginosa DSMZ 50071	>7,0533	-
Staphylococcus aureus ATCC 25923	>7,0533	-
Salmonella infantis (FI)	0,8816	>0,8816
Escherichia coli (MDR)	>7,0533	-
Streptococcus mutans (CI)	>7,0533	-
(-): MBC test was not performed for strains where no MIC was determined.		

Table 6. MIC and MBC/MFC Results of the Methanol Extract of *P. striatulum*

Microorganisms	MIC (mg/ml)	MBC/MFC(mg/ml)
Listeria monocytogenes ATCC 7644	>15,32	-
Staphylococcus aureus ATCC 25923	>15,32	-
Salmonella infantis (FI)	>15,32	-
Streptococcus mutans (CI)	>15,32	-
(-): MBC test was not performed for strains where no MIC was determined.		

Table 7. MIC and MBC/MFC Results of the n-Hexane Extract of *P. striatulum*

Microorganisms	MIC (mg/ml)	MBC/MFC(mg/ml)
Providencia rustigianii (MDR)	>1,96	-
(-): MBC test was not performed for strains where no MIC was determined.		

3.3. Findings of the tests determining biofilm formation conditions

As a result of the tests conducted to determine the optimal conditions for biofilm formation, it was found that the *B. subtilis* DSMZ 1971 strain produced the highest level of biofilm after 48 hours of incubation in a medium containing 0% glucose. Similarly, the *E. coli* ATCC 25922 strain formed the highest amount of biofilm after 24 hours of incubation in a 0% glucose medium. The *L. monocytogenes* ATCC 7644 and *L. innocua* strains exhibited maximum biofilm production after 48 hours of incubation in a 0% glucose medium. Lastly, the *E. coli* 1209212 (CI) strain showed the highest level of biofilm formation after 24 hours of incubation in a medium with 0% glucose.

3.4. Antibiofilm activity findings

The antibiofilm effects of *P. striatulum* extracts prepared with ethanol, methanol, n-hexane, and water were examined in this study. The concentrations of the active constituents in the 15 mL extracts were calculated and recorded as follows: 10.67 mg/mL for the ethanol-DMSO extract, 25.13 mg/mL for the methanol-DMSO extract, 3.80 mg/mL for the n-hexane-DMSO extract, and 32.67 mg/mL for the aqueous extract.

The antibiofilm activity results of ethanol, methanol, n-hexane, and aqueous extracts of the bryophyte *P. striatulum* against the standard isolate *B. subtilis* DSMZ 1971 are presented in Figure1.

As a result of the study, all *P. striatulum* (PS) extracts prepared with ethanol, methanol, n-hexane, and water were found to significantly inhibit the biofilm formation of the standard isolate *Bacillus subtilis* DSMZ 1971 at MIC/2 concentrations. The antibiofilm activity of the extracts was found to be much higher than that of the positive control, Halamid (1.347%). The highest antibiofilm effect was observed in the ethanol extract (52.29%), followed by the n-hexane (47.27%) and methanol (44.38%) extracts, respectively. The lowest activity was detected in the aqueous extract (18.79%); however, this extract also exhibited stronger inhibition than the positive control. The standard isolate *E. coli* ATCC 25922 are presented in Figure 2.

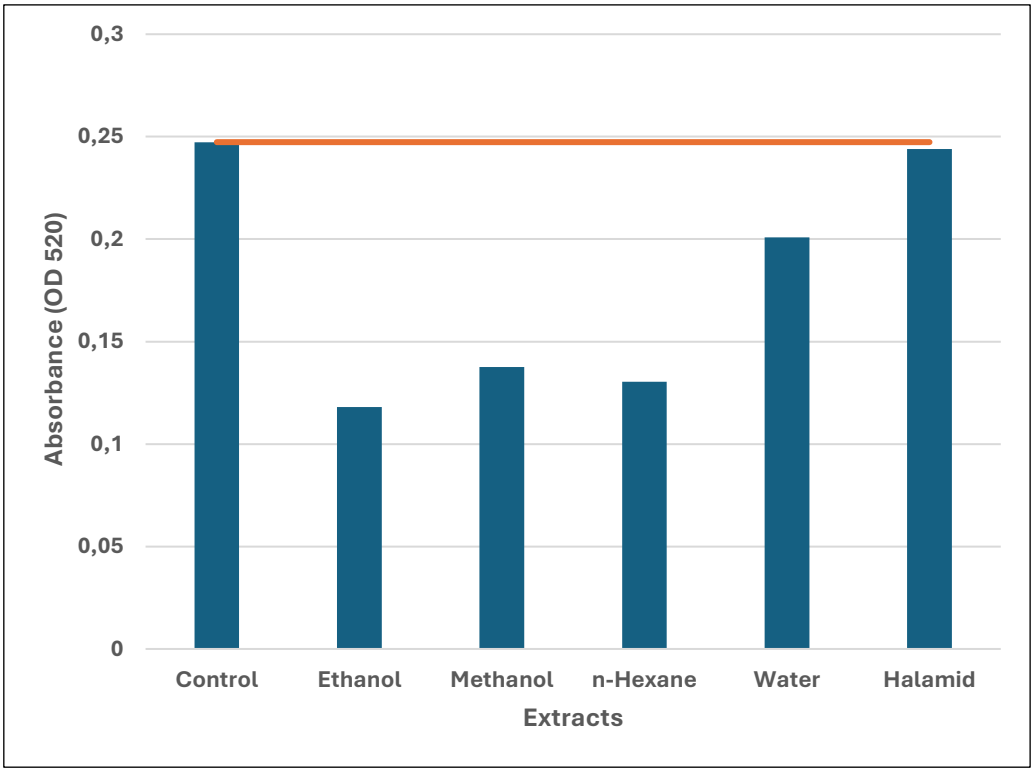

Figure 1. Effect of ethanol, methanol, n-hexane, and aqueous *P. striatulum* extracts on *B. subtilis* DSMZ 1971 biofilm formation (OD values)

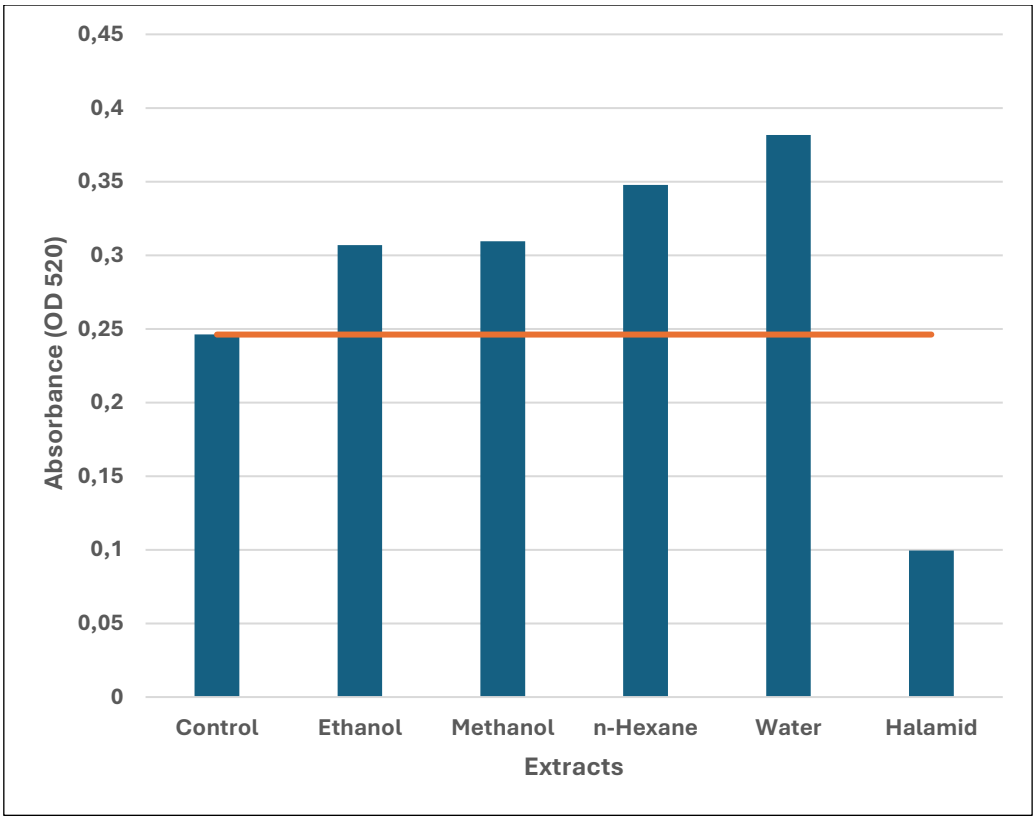

Figure 2. Effect of ethanol, methanol, n-hexane, and aqueous *P. striatulum* extracts on *E. coli* ATCC 25922 biofilm formation (OD values)

The study revealed that all *P. striatulum* (PS) extracts prepared with ethanol, methanol, n-hexane, and water increased rather than inhibited biofilm formation of the standard *E. coli* ATCC 25922 isolate at MIC/2 concentrations. The aqueous extract showed the highest induction of biofilm formation (–55.01%), followed by the n-hexane (–41.25%), methanol (–25.70%), and ethanol (–24.71%) extracts, respectively. This suggests that the extract may contain signaling components capable of stimulating biofilm development or that this concentration may provide a favorable environment for bacterial growth and biofilm formation. *Striatulum* against the standard isolate *L. monocytogenes* ATCC 7644 are presented in Figure 3.

As a result of the study, the *P. striatulum* (PS) n-hexane extract (17.14%) significantly inhibited biofilm formation of the standard isolate *L. monocytogenes* ATCC 7644 at MIC/2 concentrations. In addition, the water extract (3.49%) also exhibited a noticeable antibiofilm activity. Although the methanol extract (0.22%) showed the lowest antibiofilm effect, the positive control, Halamid (–3.574%), demonstrated a stimulatory effect on biofilm formation. Similarly, the ethanol extract (–15.10%) was also found to promote biofilm development. The antibiofilm activity results of ethanol, methanol, n-hexane, and water extracts of *P. striatulum* moss agains the food isolate *L. innocua* strain are presented in Figure 4.

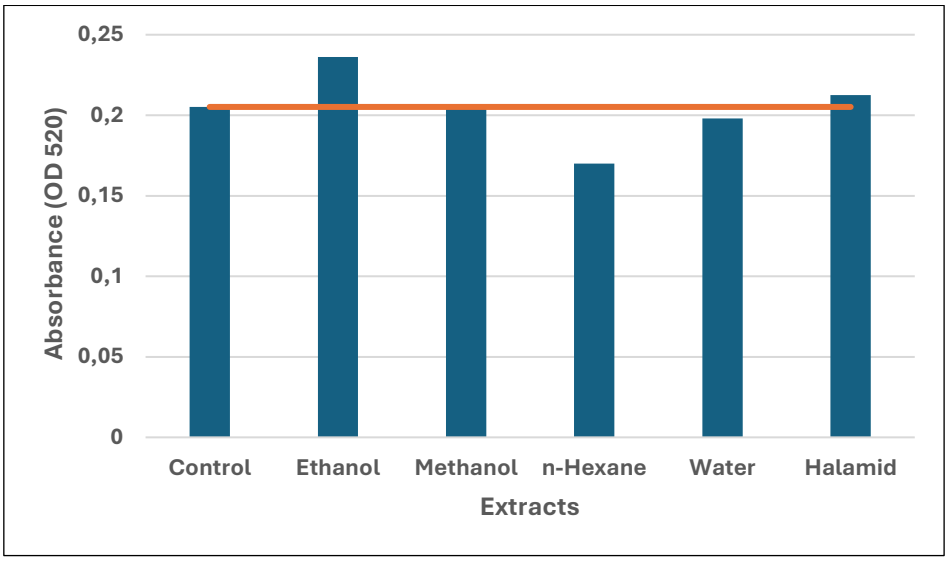

Figure 3. Effect of ethanol, methanol, n-hexane, and aqueous *P. striatulum* extracts on *L. monocytogenes* ATCC 7644 biofilm formation (OD values)

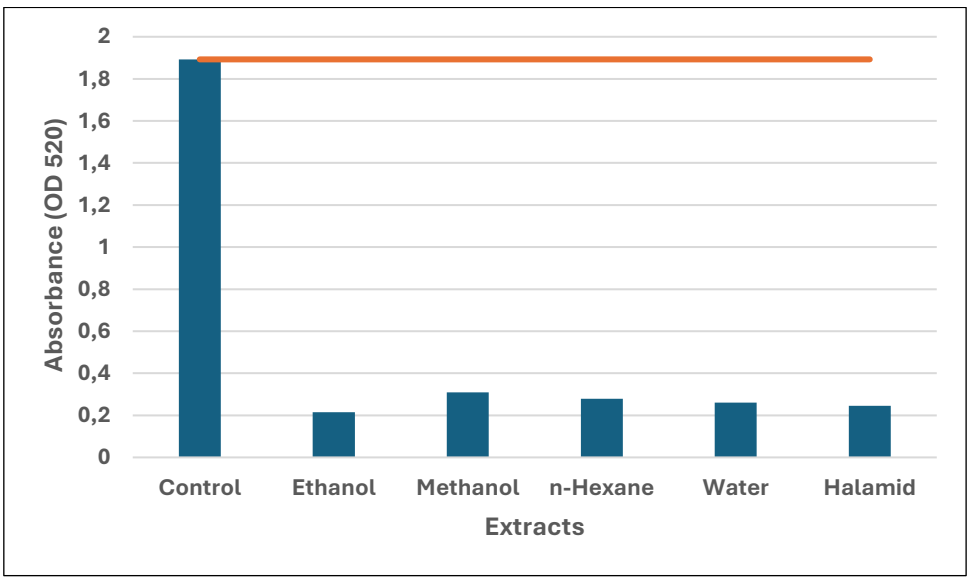

Figure 4. Effect of ethanol, methanol, n-hexane, and aqueous *P. striatulum* moss extracts on biofilm formation of *L. innocua* food isolate (OD values)

As a result of the study, all *P. striatulum* moss extracts prepared with ethanol, methanol, n-hexane, and water at MIC/2 concentrations were found to effectively inhibit biofilm formation of the food isolate *L. innocua*. The highest antibiofilm activity was observed in the ethanol extract (88.65%), followed by the aqueous (86.21%), n-hexane (85.28%), and methanol (83.63%) extracts, respectively. Notably, the ethanol extract exhibited a higher inhibition capacity than the positive control halamid (87.02%), indicating that *P. striatulum* extracts may be effective in preventing biofilm formation and could be considered as potential alternative antibiofilm agents against foodborne bacteria. The antibiofilm activity results of ethanol, methanol, n-hexane, and water extracts of *P. striatulum* moss against the clinically isolated *Escherichia coli* 1209212 strain are presented in Figure 5.

As a result of the study, all *P. striatulum* moss extracts prepared with ethanol, methanol, n-hexane, and water at MIC/2 concentrations were found to inhibit biofilm formation of a clinically isolated *E. coli* strain. The highest activity was observed in the methanol extract (56.24%), followed by the ethanol (54.28%) and aqueous (49.45%) extracts. The lowest activity was recorded in the n-hexane extract (41.46%). The positive control, halamid, achieved 87.20% inhibition.

Biofilm inhibition percentages were calculated using the following formula:

$$\%\text{Inhibition} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Statistical analysis revealed that the antibiofilm effects of *P. striatulum* extracts varied significantly among different bacterial strains ($p = 0.00000414 < 0.05$). However, no statistically significant differences were observed in the inhibition results among extracts prepared with different solvents ($p = 0.39 > 0.05$).

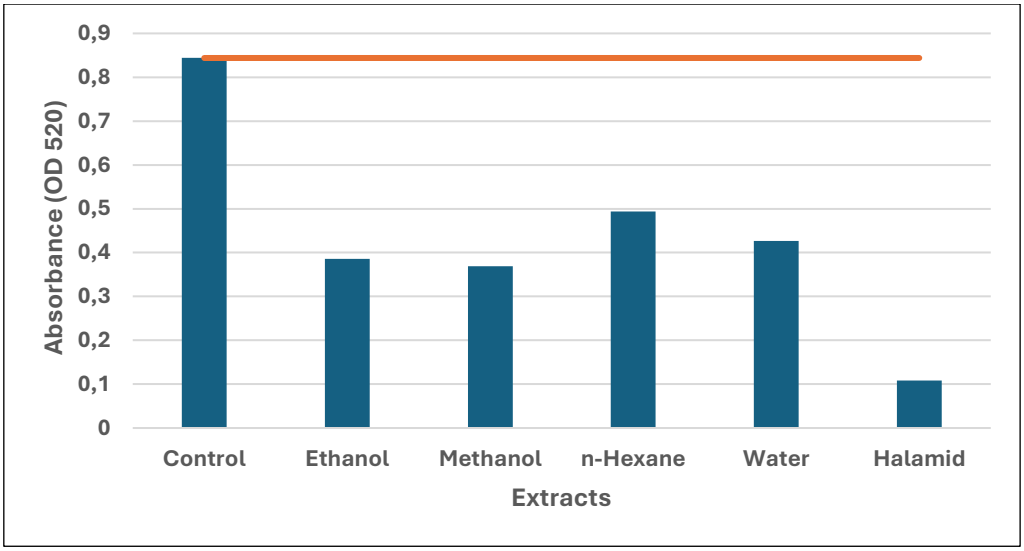

Figure 5. Effect of ethanol, methanol, n-hexane, and aqueous *P. striatulum* moss extracts on biofilm formation of clinically isolated *E. coli* 1209212 (OD values)

3.5. Comprehensive analysis of the biochemical constituents of P. striatulum

The biochemical contents of ethanol, methanol, and n-hexane extracts obtained from *P. striatulum* moss are presented in Table 8.

The biochemical contents of ethanol, methanol, and n-hexane extracts obtained from *P. striatulum* moss were determined by GC-MS analysis. In the ethanol extract, 23 different compounds were identified, with only three remaining unidentified. In the methanol extract, 24 different compounds were detected, four of which could not be identified. Finally, in the n-hexane extract, 24 different compounds were detected, seven of which could not be identified. In the ethanol extract, the compound A'-Neogammacer-22(29)-ene was found to be the most abundant, with a relative percentage of 25.00%. In the methanol extract, Neophytadiene (%12.72) was identified as the major component. In the n-hexane extract, Tris(2,4-di-tert-butylphenyl) phosphate was the most predominant compound, with a relative abundance of 52.45%. The compounds commonly found in the extracts prepared with the three different solvents were identified as n-Hexadecanoic acid, Octadecanoic acid, Vitamin E, and A'-Neogammacer-22(29)-ene.

Table 8. GC-MS analysis results of ethanol, methanol, and n-hexane extracts of *P. striatulum* moss.

RT	Compound name	Formula	MW (g/mol)	PS Ethanol extract	PS Methanol extract	PS n-Hexane extract
35.130	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276,4	-	-	0.86
36.641	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256,4	3.73	7.43	1.89
41.814	Linoelaidic acid	$C_{18}H_{32}O_2$	280,4	4.56	-	1.59
41.955	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264. 4	-	-	1.15
41.873	Octadecanoic acid	$C_{18}H_{36}O_2$	284,5	0.89	1.09	0.65
45.211	Tricosane	$C_{23}H_{48}$	324,6	-	-	4.04
47.905	Tetracosane	$C_{24}H_{50}$	338,7	-	-	1.25
50.696	Heptacosane	$C_{27}H_{56}$	380,7	-	-	5.08
53.605	Hexacosane	$C_{26}H_{54}$	366,7	-	-	1.87
59.685	Octacosane	$C_{28}H_{58}$	394,8	-	-	3.13
63.907	2,6,10,14,18-Pentamethyl-2,6,10,14 ,18-eicosapentaene	$C_{25}H_{42}$	342,6	-	-	1.59
65.748	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	415,2	-	-	1.37
67.108	E,Z-2,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308,5	-	-	0.79
68.807	Celidoniol, deoxy-	$C_{29}H_{60}$	408,8	-	-	2.15
68.703	Vitamin E	$C_{29}H_{50}O_2$	430,7	5.47	2.02	4.10
73.218	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662,9	-	-	52.45
73.781	A'-Neogammacer-22(29)-ene	$C_{30}H_{50}$	410,7	25.00	8.59	3.96
6.653	2-Furancarboxaldehyde	$C_5H_4O_2$	96.08	-	1.52	-
20.222	2-Furaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126.11	-	9.04	-
32.270	Neophytadiene	$C_{20}H_{38}$	278,5	22.02	12.72	-
34.931	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270,5	4.18	1.59	-
39.582	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294,5	-	0.82	-
39.787	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292,5	-	0.89	-
40.524	Methyl stearate	$C_{19}H_{38}O_2$	298,5	-	0.51	-
41.439	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278,4	2.15	7.70	-
44.098	5,8,11,14-eicosatetraenoic acid, methyl ester(all-Z)-	$C_{21}H_{34}O_2$	318,5	-	0.97	-
46.424	5,8,11,14,17-eicosapentaenoic acid	$C_{20}H_{30}O_2$	302,5	-	2.01	-
46.554	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	$C_{22}H_{36}O_2$	332,5	-	1.12	-
52.530	Beta sitosterol	$C_{29}H_{50}O$	414.7	0.75	0.59	-
59.362	Squalene	$C_{30}H_{50}$	410,7	1.66	1.06	-
63.768	trans-Geranylgeraniol	$C_{20}H_{34}O$	290,5	-	4.32	-
66.454	Stigmastan-3,5-diene	$C_{29}H_{48}$	396,7	0.56	0.76	-
73.931	Tricyclo[4.3.0.0(7,9)]Nonane, 2,2,5,5,8,8-Hexamethyl-, (1.Alpha.,6.BEta.,7.Alpha.,9.Alpha.)-	$C_{15}H_{26}$	206,37	-	0.59	-
32.332	(2E)-3,7,11,15-Tetramethyl-2-Hexadecene	$C_{20}H_{40}$	280.5	0.51	-	-
36.345	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	4.18	-	-
39.664	Phytol	$C_{20}H_{40}O$	296.5	0.62	-	-
40.882	Ethyl (9Z,12Z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	308.5	1.39	-	-
44.553	Dodecane, 1,1'-oxybis-	$C_{24}H_{50}O$	354.7	0.70	-	-
45.762	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	4.83	-	-

62.905	Bisabolol	$C_{15}H_{26}O$	222.3	5.74	-	-
72.912	Hop-21-ene	$C_{30}H_{50}$	410,7	0.86	-	-
74.440	A-Norcholestan-3-one, 5-ethenyl-,(5.beta.)-	$C_{28}H_{46}O$	398,7	0.80	-	-
14.527	Unknown	-	-	-	1.90	-
16.497	Unknown	-	-	-	2.10	-
33.665	Unknown	-	-	-	5.28	-
51.838	Unknown	-	-	-	1.26	-
18.618	Unknown	-	-	0.56	-	-
33.324	Unknown	-	-	6.06	-	-
50.821	Unknown	-	-	1.05	-	-
51.668	Unknown	-	-	-	-	0.54
55.792	Unknown	-	-	-	-	0.87
60.010	Unknown	-	-	-	-	1.25
64.144	Unknown	-	-	-	-	0.78
67.827	Unknown	-	-	-	-	0.57
72.064	Unknown	-	-	-	-	1.82
75.827	Unknown	-	-	-	-	1.73

3.6. Determination of antioxidant activity

The findings of the DPPH antioxidant assay for the ethanol extract of *P. striatulum* moss are presented in Table 9.

The DPPH radical scavenging test was conducted exclusively on the ethanol extract of *P. striatulum*. The findings are presented in Table 9. At a concentration of 1000 µg/mL, the ethanol extract of *P. striatulum* moss demonstrated the greatest DPPH radical scavenging activity, achieving an inhibition rate of 23.39%. At the same concentration, the positive control, ascorbic acid, showed a scavenging activity of 94.66%, indicating that *P. striatulum* moss exhibited lower antioxidant activity compared to the positive control at an identical concentration. One-way analysis of variance (ANOVA) was employed to perform the statistical analysis a parametric method, and the p-value was determined as 0.0658 ($p > 0.05$). This indicates that the increase in extract concentration did not result in a statistically significant difference in DPPH radical scavenging activity.

Table 9. DPPH antioxidant activity results of the ethanol extract of *P. striatulum* moss.

Concentration (µg/ml)	DPPH (%)	Ascorbic acid (%)
1000	23,39973	94,665
500	5,818852	93,391
250	2,47028	92,077
125	1,117735	90,086
62,5	2,991073	69,943
31,25	1,82574	35,794
15,625	1,640431	17,698
7,8125	1,041601	8,739

4. Discussion and Conclusions

Plants synthesize a wide variety of phytochemicals throughout their lifespan. These compounds are generally classified into two main groups: primary metabolites and secondary metabolites. Primary metabolites are responsible for the growth and developmental processes of the plant and include components such as nucleic acids, proteins, and various carbohydrates. Secondary metabolites, on the other hand, enhance the plant's survival capacity and facilitate its adaptation to environmental conditions. Among the most well-known phytochemical compounds are phenolics, terpenoids, tannins, coumarins, and alkaloids. These compounds are particularly notable for their diverse biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Demir and Akpınar, 2020; Bitwell et al., 2023). The World Health Organization has also emphasized that medicinal and aromatic plants may serve as potential sources for the development of novel drugs (Vaou et al., 2021).

Although several studies in the literature have investigated the biological activities of secondary metabolites synthesized by bryophytes (Bozkurt et al., 2025), research on *P. striatulum* remains rather limited. Therefore, in the present study, *P. striatulum* was extracted using three different solvents (ethanol, methanol, and n-hexane). The

aim was to evaluate the antimicrobial, biofilm-inhibitory, and antioxidant activities of the obtained extracts, as well as to perform a biochemical content analysis.

In another study reported in the literature, Benek et al. (2023) evaluated the antimicrobial activities of ethanol extracts from various moss species using the disk diffusion method. These species included *Nogopterium gracile*, *Timmia bavarica*, *Rhynchostegium alopecuroides*, *Pylasia polyantha*, *Plagiomnium medium*, and *Leptodon smithii*. As a result of the disk diffusion test conducted on a total of 20 microorganisms, including nine standard strains, five multidrug-resistant (MDR) strains, three clinical isolates, and three food isolates, *P. medium* exhibited antimicrobial activity against *E. aerogenes* ATCC 13048 and *S. aureus* MRSA strains. *L. smithii* was effective against *S. aureus* MRSA, *E. coli* ATCC 25922, and *P. vulgaris* strains, while *R. alopecuroides* demonstrated activity against *E. aerogenes* ATCC 13048, *L. monocytogenes*, and *K. pneumoniae*. *N. gracile* showed inhibitory effects against *S. aureus* ATCC 25923, *S. kentucky*, and *S. aureus* MRSA strains. Finally, *P. polyantha* exhibited antimicrobial activity against *E. aerogenes* ATCC 13048, *S. aureus* ATCC 25923, and *S. aureus* MRSA, whereas *T. bavarica* was active against *E. faecalis*, *S. aureus* ATCC 25923, and *S. aureus* MRSA strains.

The strains used in the studies and the disk diffusion method applied were the same. The results showed particular similarities against *E. coli*, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. mutans*, and *P. aeruginosa* DSMZ 50071. In addition, the ethanol extract of *P. striatulum* demonstrated activity against the *S. infantis* strain in the disk diffusion test. The compound neophytadiene, identified in both the ethanol and methanol extracts during the biochemical profiling, is known for its antimicrobial, antioxidant, and anti-inflammatory properties (Al-Rajhi et al., 2022), which supports the observed antimicrobial effect. However, no antimicrobial activity was detected against the other strains. The limited antimicrobial activity observed in the study is primarily attributed to the insufficient concentration of the extracts. The administered dose may not have reached the minimum inhibitory concentration of the active compounds, resulting in the detection of small inhibition zones only in a limited number of strains. Furthermore, the inadequate diffusion of the complex extract composition within the agar medium may also have contributed to the apparently low activity. To overcome these limitations and to further support the findings, MIC and MBC values were additionally determined in the study.

Following the disk diffusion assay, minimum inhibitory concentration (MIC) testing was performed on the microorganisms that exhibited antimicrobial activity. The MIC test of the ethanol extract was conducted against *P. aeruginosa* DSMZ 50071, *S. mutans* (CI), *E. coli* ATCC 25922, *S. infantis* (FI), *E. coli* (MDR), and *S. aureus* ATCC 25923 strains. The methanol extract was tested against *S. aureus* ATCC 25923, *S. mutans* (CI), *S. infantis* (FI), and *L. monocytogenes* ATCC 7644, whereas the n-hexane extract was tested against the *P. rustigianii* (MDR) strain. As a result, the ethanol extract exhibited antimicrobial activity against the foodborne *S. infantis* strain at a concentration of 0.881 mg/mL. No MIC value was determined for the methanol and n-hexane extracts. *S. infantis* is one of the five serovar strains responsible for salmonellosis in humans (Zeng et al., 2021), a disease characterized by symptoms such as nausea, diarrhea, and fever, which can be life-threatening particularly in children and the elderly (Montoro-Dasi et al., 2023). During the MBC assay, bacterial growth observed in this strain indicated that the effect was bacteriostatic rather than bactericidal.

Öztürk et al. (2022) lyophilized four bryophyte species (*Palamocladium euchloron*, *Cratoneuron filicinum*, *Plasteurhynchium striatum*, and *Campyliadelphus chrysophylus*) and extracted them with methanol, ethanol, chloroform, acetone, and water, aiming to determine their MIC values. The tested strains included *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, MRSA ATCC 43300, and *E. coli* ATCC 25922. None of the extracts exhibited antimicrobial activity. Similarly, in the present study, no inhibition was observed against the reference strain *E. coli* ATCC 25922. However, the determination of an MIC value against the foodborne *S. infantis* strain provides a noteworthy contribution to literature.

A review of the literature reveals that no studies to date have investigated the antibiofilm activity of *P. striatulum*. Thus, the present study represents the first systematic attempt to elucidate the antibiofilm potential of this moss. Biofilms are complex structures formed by bacteria adhering to biotic or abiotic surfaces, providing microorganisms with protection against environmental stresses and antimicrobial agents (Liu et al., 2023). Produced by both Gram-positive and Gram-negative bacteria, biofilms play a critical role in the chronicity of infectious diseases and the

development of treatment resistance (Shree et al., 2023). Furthermore, bacterial cells within established biofilms exhibit low metabolic activity, rendering them insensitive to antibiotics, which are effective primarily against metabolically active cells (Razdan et al., 2022). Therefore, biofilm inhibition is a comparatively rare phenomenon, standing out as a biologically significant property beyond ordinary antimicrobial effects.

In the study conducted by Uyar et al. (2023) on the moss *Dichodontium pellucidum*, the antibiofilm activities of ethanol, methanol, ethyl acetate, and acetone extracts were evaluated against various bacterial and fungal strains. Among these, the ethyl acetate extract at the MIC concentration (10.0 µg/mL) exhibited the highest inhibition, achieving 90.03% against *B. subtilis* ATCC 6633. At 1/2 MIC (5.0 µg/mL), the highest results were observed for all extracts ethanol, methanol, ethyl acetate, and acetone against *P. aeruginosa* ATCC 27853, with inhibition rates of 75.33%, 65.05%, 68.07%, and 67.04%, respectively.

In this study, the ethanol, methanol, n-hexane, and aqueous extracts were evaluated for their antibiofilm activities against *L. monocytogenes* ATCC 7644, *E. coli* ATCC 25922, *E. coli* (CI), *L. innocua* (FI), and *B. subtilis* DSMZ 1971 strains. Initially, the optimal conditions for biofilm formation were determined, and subsequently, the effects of the extracts were tested. The results revealed that, at 1/2 MIC concentrations, some extracts stimulated biofilm activity, whereas others significantly inhibited biofilm formation.

Specifically, while the positive control of the test, halamid, exhibited 1.34% inhibition against the Gram-positive *B. subtilis* DSMZ 1971 strain, which shows a high adaptive capacity to environmental conditions, the ethanol extract demonstrated 52.29% inhibition (Arnaouteli et al., 2021; Iqbal et al., 2023). In addition, the n-hexane extract inhibited *L. monocytogenes* ATCC 7644 by 17.14%, whereas halamid was observed to stimulate biofilm formation (-3.57%). The ethanol extract exhibited 88.65% inhibition against *L. innocua*, while the positive control halamid showed 87.02% inhibition. The fact that some of the tested extracts exhibited higher inhibition than positive control indicates their potential for use in the development of new antibiofilm agents.The extracts of *P. striatulum* showed no antibiofilm activity against *E. coli* ATCC 25922; on the contrary, a stimulatory effect on biofilm formation was detected. The methanol extract exhibited 56.24% inhibition against the clinical isolate of *E. coli*. The variability observed in the obtained results can be attributed to differences in materials, methods, solvents, and microbial strain diversity. GC-MS analyses revealed the presence of octadecanoic acid in all solvent-based extracts. Previous studies have reported that this compound exhibits antifungal and antimicrobial properties (AlRaddadi et al., 2024).

The findings suggest that *P. striatulum* can be considered a potential antibiofilm agent. Furthermore, the extract's antibiofilm activity against both Gram-negative and Gram-positive bacteria indicates that it may serve as an effective therapeutic candidate for the treatment of chronic infections. In addition, the presence of yet unidentified compounds in the biochemical profiles of the extracts suggests that these compounds may contribute to the antibiofilm activity of *P. striatulum*. Future studies focusing on the isolation and characterization of these compounds will provide a more comprehensive understanding of the biological potential of this moss. This study not only reveals the existing antibiofilm effects but also provides valuable insights for future research aimed at developing new potential agents against treatment-resistant and biofilm-forming bacteria.

Under conditions such as extreme heat and nutrient deficiency, the generation of reactive oxygen species (ROS) in plants leads to oxidative stress. Certain bioactive compounds present in the biochemical composition of plants exhibit strong antioxidant properties. In particular, phenolic compounds, which belong to the class of secondary metabolites, play a crucial role in mitigating oxidative stress due to their antioxidant activities. Previous studies have shown that the consumption of plants with high phenolic content exerts beneficial effects on human health (Chaves et al., 2020). In recent years, interest in antioxidants has increased due to the adverse effects of free radicals on human metabolism (Gülçin & Alwasel, 2023).

In this study, the antioxidant potential of the ethanol extract of *P. striatulum* was evaluated using the DPPH assay, based on absorbance measurements at 517 nm. The results indicated that the antioxidant capacity of the extract was lower than that of ascorbic acid, which was used as a positive control. While ascorbic acid exhibited strong antioxidant activity at much lower doses, the *P. striatulum* extract required higher concentrations to achieve a comparable effect. The DPPH scavenging rates ranged from 1.04% to 23.39%, with an EC_{50} value of 1.3849. GC-MS analysis revealed the presence of n-hexadecanoic

acid (3.73%) and vitamin E (5.47%) in the biochemical composition of the extract. Although these compounds are reported in the literature to possess strong antioxidant properties, their low absolute concentrations limited the radical scavenging capacity in the DPPH assay (Ganesan et al., 2024; Packer, 2023).

In the literature, a study by Öztürk et al. (2022) evaluated the antioxidant activities of methanol extracts of *C. chrysophyllus*, *P. euchloron*, *C. filicinum*, and *P. striatum* using the DPPH assay. The highest activity was reported for *C. filicinum* (65%), while the *P. striatum* extract exhibited a radical scavenging capacity of 55.22%. The lower antioxidant activity observed in the present study may be attributed to factors such as the different polarity profile of the solvent used, variations in extraction conditions, and the complex matrix structure of the natural extract.

The investigation of the biochemical components of bryophytes is important for understanding their metabolism and identifying the secondary metabolites responsible for their biological activities (Klavina et al., 2015). In the literature, Adebiyi and Tedela (2023) examined the biochemical composition of *Barbula lambaranensis* and *Philonotis hastata* using GC-MS analysis of their n-hexane extracts. A total of 23 different compounds were identified in the n-hexane extract of *P. hastata*, with the major compound being 9-octadecenoic acid, methyl ester (24.10%). In the n-hexane extract of *B. lambaranensis*, 31 compounds were detected, with hexadecanoic acid, methyl ester (11.76%) identified as the predominant compound.

Although there are studies in the literature focusing on the biochemical composition of certain moss species, no research has been conducted on the biochemical profile of *P. striatulum*. This study is the first to characterize the biochemical components of *P. striatulum*. The biochemical composition of its ethanol, methanol, and n-hexane extracts was analyzed using the GC-MS method.

In conclusion, a total of 23 different compounds were identified in the ethanol extract, with A'-Neogammacer-22(29)-ene (25.00%) being the predominant compound. In the literature, Garuba et al. (2023) reported the antiviral activity of this compound. In the methanol and n-hexane extracts, 24 compounds were detected. The major compound in the methanol extract was identified as Neophytadiene (12.72%), which was also observed in the ethanol extract (22.02%). Studies have indicated that neophytadiene possesses analgesic, anti-inflammatory, antimicrobial, and antioxidant properties (Willie et al., 2021). The primary compound in the n-hexane extract was Tris(2,4-di-tert-butylphenyl) phosphate (52.45%), with Heptacosane (5.08%) also detected in the biochemical profile. While Tris(2,4-di-tert-butylphenyl) phosphate has been reported in the literature for its anticancer activity, Heptacosane has been documented as a compound exhibiting antioxidant activity (Zahara et al., 2022; Akpuaka et al., 2013).

In our study, tris(2,4-di-tert-butylphenyl) phosphate was detected at a high concentration (~52%) in the GC-MS analysis of *P. striatulum*. Vinuchakkaravarthy et al. (2010) successfully isolated this compound from the leaves of *Vitex negundo* and reported its antioxidant activity. Based on the information provided in the literature, it can be concluded that this compound is naturally produced by plants. Another study listed this compound as a phytochemical with antioxidant properties (Jain et al., 2024). Similarly, Afrin et al. (2019) identified tris(2,4-di-tert-butylphenyl) phosphate in the biochemical profile of *Cuscuta reflexa* (Roxb.) and reported its antioxidant activity. However, some studies in the literature have also mentioned that this compound may exhibit toxic effects (Wang et al., 2025). According to the results obtained in our study, the high concentration of this compound could contribute to the observed antioxidant activity, while also raising the possibility of contamination. This aspect should be considered in subsequent studies following this preliminary investigation.

Moreover, n-Hexadecanoic acid, commonly found in plant tissues and known for its antimicrobial and antioxidant activities, was detected in all three extracts (Ganesan et al., 2024; Ravi & Krishnan, 2017). Additionally, Octadecanoic acid, Vitamin E, and A'-Neogammacer-22(29)-ene were also common to all three extracts. Octadecanoic acid has been attributed with anticancer, antitumor, antioxidant, and anti-inflammatory effects (Parvathi et al., 2022).

The observed antimicrobial, antibiofilm, and antioxidant activities of *P. striatulum* are consistent with the properties of the compounds identified by GC-MS and their reported biological activities in the literature. However, the potential contribution of synergistic interactions among the compounds present in the biochemical composition of the extracts should not be overlooked. Furthermore, it is suggested that compounds not identified by the GC-MS library

may also contribute to these effects and should be taken into consideration.

Declaration

Author contributions

Idea/Concept: GG, EG, KC; Conceptualization and design: GG, EG, DT; Auditing consulting: AB, KC; References: KC; Materials: ADU; Data collection and/or processing: GG, EG, CY, SDB; Analysis and/or interpretation: GG, GG, ED, EG; Literature search: GG, EG, DT; Writing phase: GG, EG; Critical review: MEB, AB, KC

Conflict of interest

It is declared by the authors that no conflict of interest exists concerning the findings and overall content of the study.

Funding

This study was supported by the he Scientific and Technological Research Council of Turkey (TÜBİTAK) under the BİDEB 2218 program, project number 122C197. The authors are grateful to TÜBİTAK for its financial support.

Ethical approval

This research was conducted under in vitro conditions and therefore does not require ethical approval.

References

Adebiyi A.O. Tedela P.O. 2023. Phytochemical profiling and GC-MS analysis of extracts of two tropical moss species. Scholars Academic Journal of Biosciences. 5:181-190.

Afrin N.S. Hossain M.A. Saha K. 2019. Phytochemical screening of plant extracts and GC-MS analysis of n-hexane soluble part of crude chloroform extract of Cuscuta reflexa (Roxb.). Journal of Pharmacognosy and Phytochemistry. 8:2, 560-564.

Akatin Y.M. Ayaz F.A. Boyraci G.M. Er Kemal M. Batan N. Colak N. 2024. An evaluation of the antioxidant potential and in vitro enzyme inhibition profile of selected bryophytes from Northeast Anatolia (Türkiye). Journal of Biomolecular Structure and Dynamics. 1-13.

Akpuaka A. Ekwenchi M.M. Dashak D.A. Dildar A. 2013. Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. Nature and Science. 11:5, 141-147.

Alan Ş. Koçer F. Uyar G. 2018. Antimicrobial activities of some bryophytes in Turkey. Communications Faculty of Sciences University of Ankara Series C Biology. 27:2, 17-22.

Ali I.A. Neelakantan P. 2022. Antibiofilm activity of phytochemicals against *Enterococcus faecalis*: A literature review. Phytotherapy Research. 36:7, 2824-2838.

Al-Rajhi A.M.H. Qanash H. Almuhayawi M.S. Al Jaouni S.K. Bakri M.M. Ganash M. Salama H.M Selim S. Abdelghany T.M. 2022. Molecular interaction studies and phytochemical characterization of *Mentha pulegium* L. constituents with multiple biological utilities as antioxidant, antimicrobial, anticancer and anti-hemolytic agents. Molecules. 27:15, 4824.

Amorati R. Valgimigli L. 2018. Methods to measure the antioxidant activity of phytochemicals and plant extracts. Journal of Agricultural and Food Chemistry. 66:13, 3324-3329.

Arnaouteli S. Bamford N.C. Stanley-Wall N.R. Kovács Á. T. 2021. *Bacillus subtilis* biofilm formation and social interactions. Nature Reviews Microbiology. 19:9, 600-614.

Asakawa Y. Ludwiczuk A. 2017. Chemical constituents of bryophytes: structures and biological activity. Journal of Natural Products. 81:3, 641-660.

Benek A. Canlı K. Altuner E. M. 2023. Antimicrobial and antioxidant activities of some mosses. Anatolian Bryology. 9:1, 42-49.

Benek A. Turu D. Canlı K. 2024. Determination of biological activity and biochemical content of ethanol extract from fruiting body of *Tricholoma bufonium* (Pers.) Gillet. Journal of Fungi. 10:11, 761.

Bitwell C. Indra S. S. Luke C. Kakoma M. K. 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African. 19: e01585.

Bozkurt S.D. Turu D. Gül G. Yaman C. Benek A. Canlı K. 2024. Determination of antioxidant activity and biochemical content of *Homalothecium philippeanum* (Spruce) Schimp. Anatolian Bryology. 10:2, 169-178.

Canlı K. Bozyel M.E. Turu D. Benek A. Simsek O. Altuner E.M. 2023. Biochemical, antioxidant properties and antimicrobial activity of steno-endemic *Origanum onites*. Microorganisms. 11:8, 1987.

Canlı K. Turu D. Benek A. Bozyel M. E. Simsek Ö. Altuner E. M. 2023. Biochemical and antioxidant properties as well as antimicrobial and antibiofilm activities of *Allium scorodoprasum* subsp. *jajlae*

(Vved.) Stearn. Current Issues in Molecular Biology. 45:6, 4970-4984.

Canlı K. Yetgin A. Benek A. Bozyel M. E. urat Altuner E. 2019. In vitro antimicrobial activity screening of ethanol extract of *Lavandula stoechas* and investigation of its biochemical composition. Advances in Pharmacological and Pharmaceutical Sciences. 2019:1, 3201458.

Chandra S. Chandra D. Barh A. Pandey R.K. Sharma I. P. 2017. Bryophytes: hoard of remedies, an ethno-medicinal review. Journal of Traditional and Complementary Medicine. 7:1, 94-98.

Chaves N. Santiago A. Alías J.C. 2020. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used. Antioxidants. 9:1, 76.

Cianciullo P. Maresca V. Sorbo S. Basile A. 2021. Antioxidant and antibacterial properties of extracts and bioactive compounds in bryophytes. Applied Sciences. 12:1, 160.

Das K. Kityania S. Nath R. Das S. Nath D. Talukdar A. D. 2022. Bioactive compounds from bryophytes. In: Talukdar A. D. (Ed.), Bioactive compounds in bryophytes and pteridophytes (pp. 1–15). Cham: Springer International Publishing.

Demir T. Akpınar Ö. 2020. Bitkilerde bulunan fitokimyasalların biyolojik aktiviteleri (*Biological Activities of Phytochemicals in Plants*). Turkish Journal of Agriculture-Food Science and Technology. 8:8, 1734-1746.

Dziwak M. Wróblewska K. Szumny A. Galek R. 2022. Modern use of bryophytes as a source of secondary metabolites. Agronomy. 12:6, 1456.

Ganesan T. Subban M. Christopher Leslee D.B. Kuppannan S.B. Seedevi P. 2024. Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. Biomass Conversion and Biorefinery. 14:13, 14547-14558.

Ganesan T. Subban M. Christopher Leslee D.B. Kuppannan S.B. Seedevi P. 2024. Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. Biomass Conversion and Biorefinery. 14:13, 14547-14558.

Garuba T. Govender R. Isah H.A. Sabiu S. 2022. Metabolites profiling and molecular docking identification of putative leads from endophytic *Phyllosticta capitalensis* as modulators of key druggable structural targets of rotavirus A. Transactions of the Royal Society of South Africa. 77:3, 207-217.

Gulcin İ. Alwasel S.H. 2023. DPPH radical scavenging assay. Processes. 11:8, 2248.

Gül G. Yaman C. Turu D. Benek A. Pala R. Sayım M.Ü. Bozyel M.E. Canlı K. 2025. Determination of the antimicrobial and biochemical content of black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.). Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi. 25:2, 253-260.

Horn A. Pascal A. Lončarević I. Volpatto Marques R. Lu Y. Miguel S. Bourgaud F. Thorsteinsdóttir M. Cronberg N. Becker J. vd. 2021. Natural products from bryophytes: from basic biology to biotechnological applications. Critical Reviews in Plant Sciences. 40:3, 191–217.

Iqbal S. Begum F. Rabaan A.A. Aljeldah M. Al Shammari B.R. Alawfi A. Alshengeti A. Sulaiman T. Khan A. 2023. Classification and Multifaceted Potential of Secondary Metabolites Produced by Bacillus subtilis Group: A Comprehensive Review. Molecules. 28:3, 927.

Ismaeel M. Ismaeel M. Mokrani L.M.L. Ibrahim A.I.A. 2022. Characterization of fatty acids profile in some moss species in Syria. Jordan Journal of Chemistry (JJC). 17:1, 27-33.

Jain P. Kumar V. Teotia D. Goyal H. Agrawal A. Malik V. 2024. Phytochemical composition of Sida rhombifolia ssp. retusa (L.) Bross.: a comprehensive GC/MS analysis. Journal of Xi'an Shiyou University, Natural Sciences Edition. 67:1.

Joshi S. Singh, S. Sharma R. Vats S. Alam A. 2023. Gas chromatography-mass spectrometry (GC–MS) profiling of aqueous methanol fraction of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Sphagnum fimbriatum* Wilson for probable antiviral potential. Vegetos. 36:1, 87-92.

Kırmacı M. Önder A. Çınar Koç A. S. Rızvanoğlu S. S. Eryılmaz M. Acıkara Ö. B. Aslan G. Carbonell Barrachina A. A. 2024. First screening of volatile constituents and antibacterial, antibiofilm, and anti-quorum sensing activities of *Cinclidotus* species. İstanbul Journal of Pharmacy. 54:3, 424–434.

Klavina L. 2015. A study on bryophyte chemical composition–search for new applications. Agronomy Research. 13:4, 969-978.

Klavina L. Springe G. Nikolajeva V. Martsinkevich I. Nakurte I. Dzabijeva D.

Steinberga I. 2015. Chemical composition analysis, antimicrobial activity and cytotoxicity screening of moss extracts (moss phytochemistry). Molecules. 20:9, 17221-17243.

Liu X. Yao H. Zhao X. Ge C. 2023. Biofilm formation and control of foodborne pathogenic bacteria. Molecules. 28:6, 2432.

Mahamud U.A.S. Nahar S. Ashrafudoulla M. Park S.H. Ha S.D. 2024. Insights into antibiofilm mechanisms of phytochemicals: Prospects in the food industry. Critical Reviews in Food Science and Nutrition. 64:6, 1736-1763.

Mitra S. Manna A. Rai R. 2019. Phytochemical screening and in-vitro antioxidant potential of two ethnomedicinally important mosses of Dicranaceae from Darjeeling hills. Journal of Pharmacognosy and Phytochemistry. 8:1, 649-654.

Montoro-Dasi L. Lorenzo-Rebenaque L. Marco-Fuertes A. Vega S. Marin C. 2023. Holistic strategies to control *Salmonella Infantis*: an emerging challenge in the European broiler sector. Microorganisms. 11:7, 1765.

Muhammad N. Ali N. Uddin N. Wadood S. F. Khan M. K. Alam N. & Haq I. 2018. Evaluation of informants consensus factor of medicinal uses of bryophytes in Swegalai Valley KPK, Pakistan. Journal of Biodiversity and Environmental Sciences (JBES). 12:5, 57-63.

Onbasli D. Yuvali G. 2021. In vitro medicinal potentials of *Bryum capillare*, a moss sample, from Turkey. Saudi Journal of Biological Sciences. 28:1, 478-483.

Ozturk M. Gökler İ. Altay V. 2018. Medicinal bryophytes distributed in Turkey. Plant and Human Health, Volume 1: Ethnobotany and Physiology. 323-348.

Öztopçu-Vatan P. Savaroğlu F.İ. İşcen C. F. Kabadere S. Öztürk N. İlhan S. 2017. Screening of antimicrobial, cytotoxic effects and phenolic compounds of the moss *Aulacomnium androgynum* (Hedw.) Schwagr (Bryophyta). Journal of Animal and Plant Sciences. 27:6, 1909–1917.

Öztürk Ş. Hazer Y. Kaşkatepe B. Ören M. 2022. Determination of total phenol contents, antibacterial and antioxidant activity of some mosses species. Karaelmas Fen ve Mühendislik Dergisi. 12:1, 86-92.

Packer L. 2023. Vitamin E: biological activity and health benefits: overview. Vitamin E in Health and Disease, 977-982.

Parvathi K. Kandeepan C. Sabitha M. Senthilkumar N. Ramya S. Boopathi N.M. Jayakumararaj R. Ramanathan L. 2022. In-silico absorption, distribution, metabolism, elimination and toxicity profile of 9,12,15-octadecatrienoic acid (ODA) from *Moringa oleifera*. Journal of Drug Delivery and Therapeutics. 12:2-S, 142-150.

Peters K. Treutler H. Döll S. Kindt A.S. Hankemeier T. Neumann S. 2019. Chemical diversity and classification of secondary metabolites in nine bryophyte species. Metabolites. 9:10, 222.

Provenzano F. Sánchez J. L. Rao E. Santonocito R. Ditta L. A. Borrás Linares I. Passantino R. Campisi P. Dia M. G. Costa M. A. vd. 2019. Water extract of *Cryphaea heteromalla* (Hedw.) D. Mohr bryophyte as a natural powerful source of biologically active compounds. International Journal of Molecular Sciences. 20:22, 5560.

Rather M.A. Gupta K. Mandal M. 2021. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. Brazilian Journal of Microbiology. 52:1701–1718.

Ravi L. Krishnan K. 2017. Cytotoxic potential of N-hexadecanoic acid extracted from *Kigelia pinnata* leaves. Asian Journal of Cell Biology. 12, 20-27.

Razdan K. Garcia-Lara J. Sinha V.R. Singh K.K. 2022. Pharmaceutical strategies for the treatment of bacterial biofilms in chronic wounds. Drug Discovery Today. 27:8, 2137-2150.

Rodrigues C.K. Júnior E.L. Ethur E.M. Scheibel T. de Moura N.F. 2020. Evaluation of the antimicrobial activity of ethanol extracts from *Orthostichella rigida* (Mull. Hal.) BH Allen Magill (Bryophyta) on pathogenic microorganisms. Journal of Medicinal Plants Research. 14:3, 98-104.

Shree P. Singh C.K. Sodhi K.K. Surya J.N. Singh D. K. 2023. Biofilms: Understanding the structure and contribution towards bacterial resistance in antibiotics. Medicine in Microecology. 16, 100084.

Simsek O. Canli K. Benek A. Turu D. Altuner E. M. 2023. Biochemical, Antioxidant Properties and Antimicrobial Activity of Epiphytic Leafy Liverwort *Frullania dilatata* (L.) Dumort. Plants. 12:9, 1877.

Tunca Pınarlı, Y. Canlı K. 2021. Determination of Antibiofilm Activity of *Sideritis trojana*. International Journal of Academic and Applied Research (IJAAR), 5:11, 135-138.

Turu D. Bozkurt S.D. Yaman C. Gül G. Benek A. Canlı K. 2024. Determination of Biochemical Content and Antioxidant Activity of *Calliergonella cuspidata* (Hedw.) Loeske. Anatolian Bryology. 10:1, 25-33.

Ursavaş S. Keçeli T. 2019. *Weissia multicapsularis*, a rare moss species new to Turkey and Asia. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology, 153:5, 669–672.

Uyar G. Demir N. Hacıoğlu N. 2023. *Dichodontium pellucidum* (Hedw.) Schimp. Karayosununun antimikrobiyal, antibiyofilm ve antioksidan aktivitelerinin araştırılması. Türk Tarım ve Doğa Bilimleri Dergisi. 10:3, 548-555.

Valeeva L.R. Dague A.L. Hall M.H. Tikhonova A.E. Sharipova M.R. Valentovic M.A. vd. 2022. Antimicrobial activities of secondary metabolites from model mosses. Antibiotics. 11:8, 1004.

Vaou N. Stavropoulou E. Voidarou C. Tsigalou C. Bezirtzoglou E. 2021. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 9:10, 2041.7

Vinuchakkaravarthy T. Sangeetha C.K. Velmurugan D. 2010. Tris (2,4-di-tert-butylphenyl) phosphate. Structure Reports. 66:9, o2207-o2208.

Wang H. Wang D. Zheng Q. He Y. Yang Q. Du J. Wang J. Zhan X. 2025. Tris (2,4-di-tert-butylphenyl) phosphate is the key toxicant in aged polyvinyl chloride microplastics to wheat (*Triticum aestivum* L.) roots. ACS Agricultural Science & Technology. 5:2, 166-175.

Willie P. Uyoh E.A. Aikpokpodion P.O. 2021. Gas chromatography-mass spectrometry (GC-MS) assay of bio-active compounds and phytochemical analyses in three species of Apocynaceae. Pharmacognosy Journal. 13:2, 383-392.

Woo S. Marquez L. Crandall W.J. Risener C.J. Quave C.L. 2023. Recent advances in the discovery of plant-derived antimicrobial natural products to combat antimicrobial resistant pathogens: insights from 2018–2022. Natural Product Reports. 40:7, 1271-1290.

Yaman C. Gül G. Benek A. Turu D. Bozkurt S.D. Akata I. Canlı K. 2025. Chemical composition and biological activity of *Pholiota aurivella* (Batsch) P. Kumm. Journal of Faculty of Pharmacy of Ankara University. 49:2, 10-10.

Zahara K. Bibi Y. Masood S. Nisa S. Sher A. Ali N. Aljahani A. H. 2022. Isolation and identification of bioactive compounds from *Bidens* spp. using HPLC-DAD and GC-MS analysis and their biological activity as anticancer molecules. Molecules. 27:6, 1927.

Zeng H. De Reu K. Gabriël S. Mattheus W. De Zutter L. Rasschaert G. 2021. Salmonella prevalence and persistence in industrialized poultry slaughterhouses. Poultry Science. 100:4, 100991.