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Antimicrobial and Antioxidant Activities of Some Mosses

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Abstract

This study investigated the in vitro antimicrobial and antioxidant activity of six mosses: *Plagiomnium medium* (Bruch & Schimp.) T.J.Kop, *Leptodon smithii* (Hedw.) F.Weber & D.Mohr, *Rhynchostegium alopecuroides* (Brid.) A.J.E.Sm., *Nogopterium gracile* (Hedw.) Crosby & W.R.Buck, *Pylasia polyantha* (Hedw.) and *Timmia bavarica* (Hessl.). The antimicrobial activity of prepared moss ethanol extracts was determined using the disk diffusion method against 20 strains. In addition, the antioxidant activities to determine for studied mosses 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity determination method was used As a result of the antimicrobial activity test, all moss samples, except *Rhynchostegium alopecuroides* (Brid.) A.J.E. Sm, were effective on multi-drug resistant and standard *Staphylococcus aureus* strains, which is an important nosocomial infection. As a result of the antioxidant activity test, an antioxidant effect was observed in all samples, and it was determined that the *P. medium* sample had the highest effect with an EC_{50} value of 6.0826 µg/mL.

Keywords: Antimicrobial activity, Antioxidant activity, Disk diffusion method, DPPH, Moss

Bazı Karayosunlarının Antimikrobiyal ve Antioksidan Aktiviteleri

Öz

Bu çalışmada, *Plagiomnium medium* (Bruch & Schimp.) T.J.Kop, *Leptodon smithii* (Hedw.) F.Weber & D.Mohr, *Rhynchostegium alopecuroides(Brid.) A.J.E.Sm., Nogopterium gracile (Hedw.) Crosby & W.R.Buck, Pylasia polyantha* (Hedw.) ve *Timmia bavarica* (Hessl.)'nın in vitro antimikrobiyal ve antioksidan aktiviteleri araştırılmıştır. Hazırlanan karayosunu etanol ekstraktlarının antimikrobiyal aktivitesi 20 suşa karşı disk difüzyon yöntemi kullanılarak belirlenmiştir. Ayrıca çalışılan karayosunları için antioksidan aktiviteleri belirlemek amacıyla 2,2-difenil-1-pikrilhidrazil (DPPH) antioksidan kapasite belirleme yöntemi kullanılmıştır. Antimikrobiyal aktivite testi sonucunda önemli bir hastane enfeksiyonu olan çoklu ilaca dirençli ve standart *Staphylococcus aureus* suşlarına *Rhynchostegium alopecuroides* (Brid.) A.J.E.Sm hariç tüm karayosunu örnekleri etki göstermiştir. Antioksidan aktivite testi sonucunda tüm örneklerde antioksidan etki gözlenmiş ve 6,0826 µg/mL EC_{50} değeri ile *P. medium* örneğinin en yüksek etkiye sahip olduğu belirlenmiştir.

Anahtar kelimeler: Antimikrobiyal aktivite, Antioksidan aktivite, Disk difüzyon yöntemi, DPPH, Karayosunu

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1. Introduction

Various methods and drugs have been used to combat diseases throughout history. The raw materials of the drugs used in this ongoing struggle are mostly natural products. Natural products used as pharmaceutical raw materials are provided from many different plant groups and one of these plant groups is bryophytes (Benek et al., 2021).

The plants that make up the group called bryophytes consist of mosses (Bryophyta), liverworts (Marchantiophyta), and hornworts (Anthocerotophyta). The bryophyte group is the second largest plant group in the world after angiosperms (Asakawa et al., 2013). Bryophytes generally have a simple morphological structure but show a great deal of chemical diversity in their simple structures. It is thought that secondary metabolites in their structures play an essential role in the interaction of bryophytes with their environment. The chemical richness due to their secondary metabolites is necessary because they do not have mechanical protection like vascular plants (Whitehead et al., 2018; Chen et al., 2018).

It is known that bryophytes have various biological activities due to the secondary metabolites they contain (Krzaczkowski et al., 2009). In recent years, there has been an increased interest in the study of the medicinal properties possessed by plants. For these reasons, bryophytes are important candidates for new active pharmaceutical compound research to be carried out (Onbasli and Yuvali, 2021).

As a result of the studies carried out, it was determined that there are approximately 1059 (±204 liverwort, ±851 moss, ± 4 hornwort) bryophyte taxa in our country (Kürschner and Frey, 2020; Özen-Öztürk et al., 2023). Our aim in this study, *P. medium*, *L. smithii*, *R. alopecuroides*, *N. gracile*, *P. polyantha*, and *T. bavarica* moss samples' antimicrobial activity was investigated against 20 strains. The moss samples' antioxidant activities were determined using of DPPH method.

2. Material and Method

2.1 Moss Samples

The moss samples used in this study were collected and identified by Assoc. Prof. Dr. Kerem CANLI from Akdağ Mountain, Amasya. Voucher specimens were deposited for further reference in Dokuz Eylül University, Research and Application Center for Fauna Flora (FAMER), İzmir, Turkey. Moss samples location information are given in Table 1.

Table 1. Moss samples locality information

Sample	Coordinate	Altitude (m)	Habitat
Plagiomnium medium (Bruch & Schimp.) T.J.Kop	N 40° 41.006′ E 036° 3,902′	872	Soil
Leptodon smithii (Hedw.) F.Weber & D.Mohr	N 40° 46.786′ E 035°55,621′	2040	Trees
Rhynchostegium alopecuroides(Brid.) A.J.E.Sm.	N 40° 48.080′ E 036° 7,876′	1230	Rocks
Nogopterium gracile (Hedw.) Crosby & W.R.Buck	N 40° 45.227′ E 035°36,509′	483	Rocks and Trees
Pylasia polyantha (Hedw.)	N 40° 46.296′ E 035°59,988′	1330	Trees
Timmia bavarica (Hessl.)	N 40° 48.249′ E 036° 9,532′	1060	Rocks

2.2 Microorganisms

In this study, 9 standard strains, 5 multidrug resistance (MDR) strains, 3 clinical isolated strains and 3 food isolated strains were used to determine the antimicrobial activities of mosses. Standart strains; *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Salmonella enteritidis* ATCC 13076, *Acinetobacter baumannii* CECT 9111 and *Candida albicans* DSMZ 1386. Multidrug resistance (MDR) strains; *Staphylococcus aureus* MRSA, *Klebsiella pneumoniae* MDR, *Escherichia coli*, *Streptococcus pneumoniae* and *Proteus vulgaris.* Clinical isolated strains; *Klebsiella pneumoniae*, *Streptococcus mutans* and *Candida tropicalis.* Food isolated strains; *Listeria innocua*, *Enterococcus durans*, and *Salmonelle kentucky.*

2.3 Microorganism Inoculum Preparation

All strains used in the study were standardized at 0.5 McFarland value in sterile 0.9% NaCl solution. Bacteria were incubated at 37 °C for 24 hours, and the only yeast strain *Candida albicans* (C.P. Robin) used was incubated at 27 °C for 48 hours solution (Canli et al., 2015).

2.4 Extraction Method

After all the moss samples were collected, they were dried at room temperature, and the samples were ground with a grinder until they turned into

powder. The active ingredients were extracted by shaking the powder samples in 200 mL of pure ethyl alcohol (Sigma-Aldrich) at 130 rpm at room temperature for 3 days. Afterwards the extraction was complete, samples were filtered through Whatman No. 1 filter paper into glass balloons. The ethyl alcohol in balloons was evaporated with a rotary evaporator (Buchi R3) at 35°C. After the evaporation process was completed, the amount of moss remaining in the glass balloon was determined with a precision balance. After the weighing process, moss extracts were prepared by adding 99% pure ethyl alcohol to dissolve the dry matter adhering to the surfaces in the balloon. After the extract in the balloon was transferred to the tubes, the glass balloon was weighed again and the amount of extract contained in the moss extracts was determined (Altuner et al., 2014).

2.5 Antimicrobial Activity Test

The antimicrobial activity of moss ethanol extracts was determined using the disk diffusion method as described by Andrews (Andrews, 2007). In the first stage, all moss extracts were loaded to 6 mm radius Oxoid Antimicrobial Susceptibility Test Disks with 50-100-200 µL (containing *P. medium* 0.17 mg, 0.34 mg, 0.69 mg, *L. smithii* 0.11 mg, 0.23 mg, 0.46 mg, *R. alopecuroides* 0.13 mg, 0.26 mg, 0.53 mg, *N. gracile* 0.10 mg 0.21mg, 0.43 mg, *P. polyantha* 0.09 mg, 0.19 mg, 0.38 mg, *T. bavarica* 0.23 mg, 0.46 mg, 0.92 mg). The disks were left to dry overnight at 30°C under sterile conditions to evaporate residual ethyl alcohol, which could alter the test results. Mueller Hinton Agar (BD Difco, USA) was then poured into a sterile 90 mm petri dish at a thickness of 4.0 mm ± 0.5 mm. Microorganisms standardized at 0.5 MacFarland value in sterile NaCl solution were inoculated into Petri dishes. After inoculation, extract-loaded disks were placed on the microorganism culture medium and incubated. After the incubation period was completed, the diameters of the inhibition zones around the disks were measured in mm and recorded. In this study, sterile blank disks used to load the extract were used as negative controls. *Gentamicin* and *tobramycin* antibiotics were used as positive controls to compare the results obtained (Bozyel et al. 2019).

The experiments were carried out in triplicate, and the R Studio v 3.3.2 program was used to statistically evaluate the difference between the repetitions. The distribution of the data between the groups was evaluated with the ANOVA test. The difference between groups was considered significant when the p-value was <0.05. On the other hand, the Pearson correlation coefficient was calculated to reveal whether the increased amount of extract increased the effect (Core R Team, 2016).

2.6 Antioxidant activity test

The DPPH (2,2-diphenyl-1-picrylhydrazil) method was used to determine the antioxidant activity of moss extracts. This method is based on evaluating the DPPH radical scavenging properties of the antioxidant compounds of moss. To prepare the DPPH solution to be used in the study, 3.9432 mg DPPH was added to 50 mL of ethanol (Mensor et al., 2001). The prepared DPPH mixture and plant extracts were mixed in a 96-well plate and incubated in the dark for 30 minutes at room temperature. At the end of the incubation period, a spectrophotometer (Biotek Microplate Spectrophotometer, USA) was used to measure the absorbance of the sample on the plate at 515 nm. Ascorbic acid was used as a positive control in this study (Turu et al., 2020).

3. Results

As a result of the antimicrobial activity research of *P. medium, L. smithii, R. alopecuroides, N. gracile, P. polyantha,* and *T. bavarica* ethanol extracts against 20 strains, an effect on 9 strains was determined as seen in graphics. *P. medium* ethanol extract was effective against *Enterobacter aerogenes* ATCC 13048 (7 mm) and *Staphylococcus aureus* MRSA (11 mm) strains. *L. smithii* ethanol extract created a zone of inhibition against *Escherichia coli* ATCC 25922 (7 mm), *Proteus vulgaris* (7 mm), and *Staphylococcus aureus* MRSA (7 mm) strains. *R. alopecuroides* ethanol extract was effective against *Enterobacter aerogenes* ATCC 13048 (7 mm), *Listeria monocytogenes* (7 mm), and *Klebsiella pneumoniae* (7 mm) strains. *N. gracile* ethanol extract was effective against *Staphylococcus aureus* ATCC 25923 (11 mm), *Salmonella kentucky* (7 mm), and *Staphylococcus aureus* MRSA (14 mm) strains. *P. polyantha* ethanol extract was effective against *Enterobacter aerogenes* ATCC 13048 (7 mm), *Staphylococcus aureus* ATCC 25923 (11 mm), and *Staphylococcus aureus* MRSA (11 mm) strains. *T. bavarica* ethanol extract created a zone of inhibition against *Enterococcus faecalis* (7 mm), *Staphylococcus aureus* ATCC 25923 (9 mm), and *Staphylococcus aureus* MRSA (13 mm) strains.

The DPPH scavenging percentage results of extracts and ascorbic acid in terms of their EC_{50} and EC_{90} values are given in Table 2.

Table 2. Antioxidant test results

	EC_{50} (µg/mL)	EC_{90} (µg/mL)
P. medium	6.0826	10.2626
L. smithii	6.0869	9.2269
R. alopecuroides	7.5317	11.5917
N. gracile	10.5152	20.3592
P. polyantha	50.239	67.263
T. bavarica	40.202	58.222
Ascorbic acid	0.359	0.359

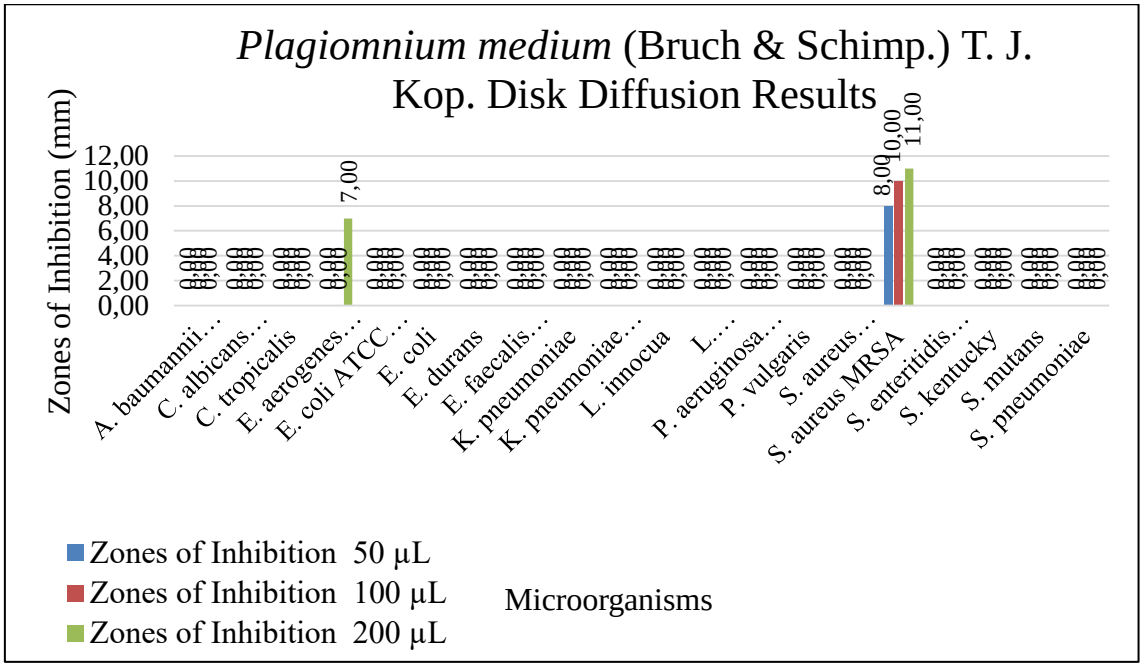

Figure 1. *Plagiomnium medium* (Bruch & Schimp.) T.J.Kop Disk Diffusion Results

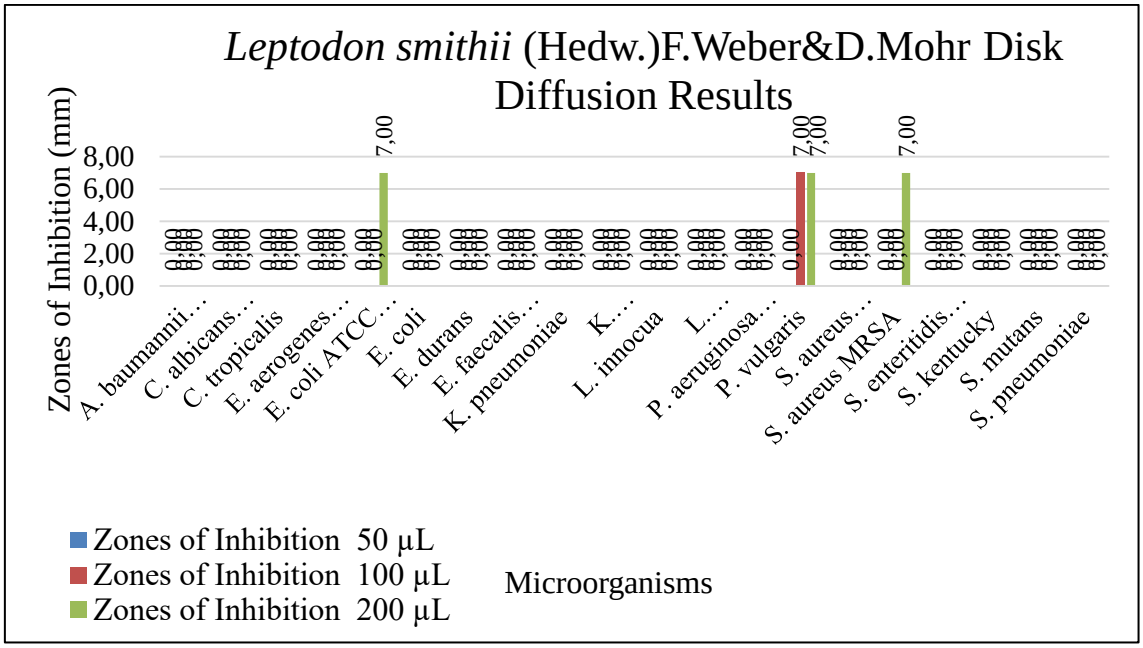

Figure 2. *Leptodon smithii* (Hedw.) F.Weber & D.Mohr Disk Diffusion Results

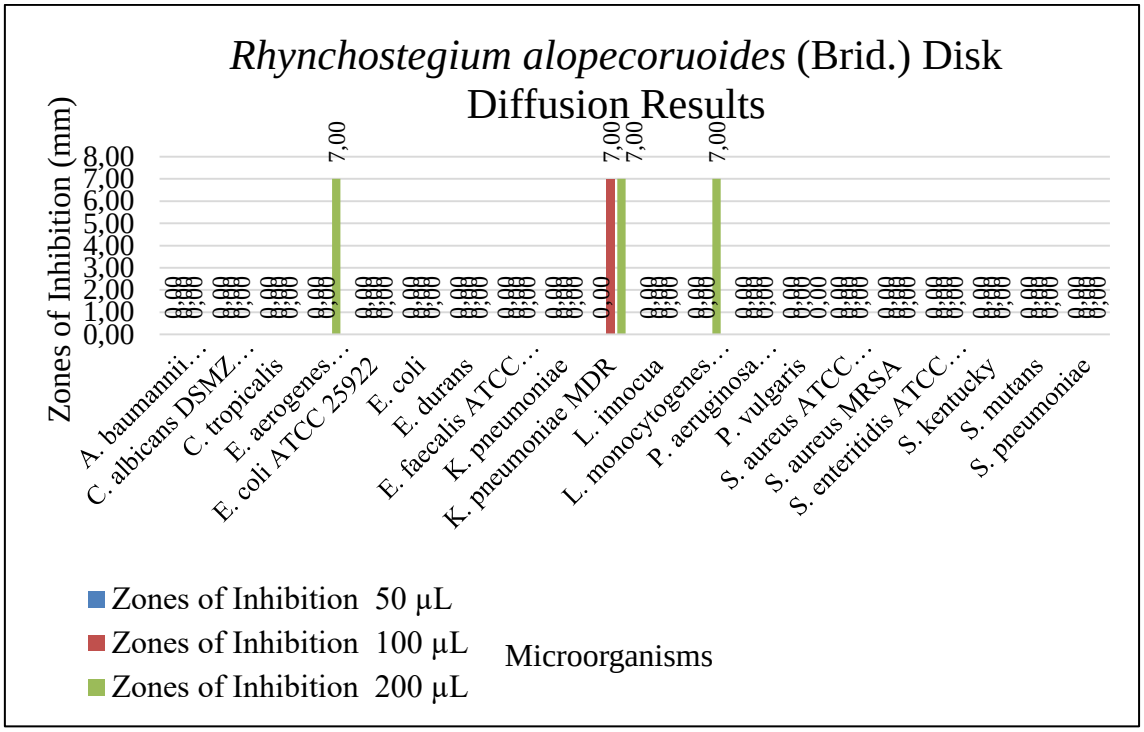

Figure 3. *Rhynchostegium alopecuroides* (Brid.) A.J.E.Sm. Disk Diffusion Results

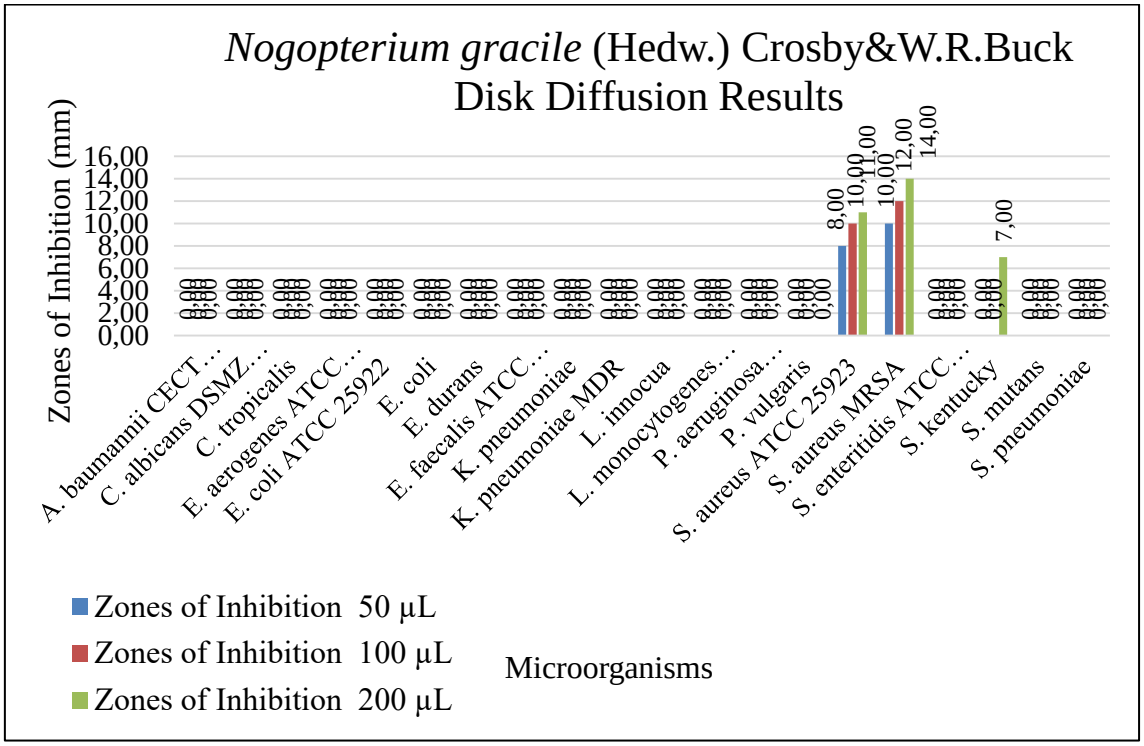

Figure 4. *Nogopterium gracile* (Hedw.) Crosby & W.R.Buck Disk Diffusion Results

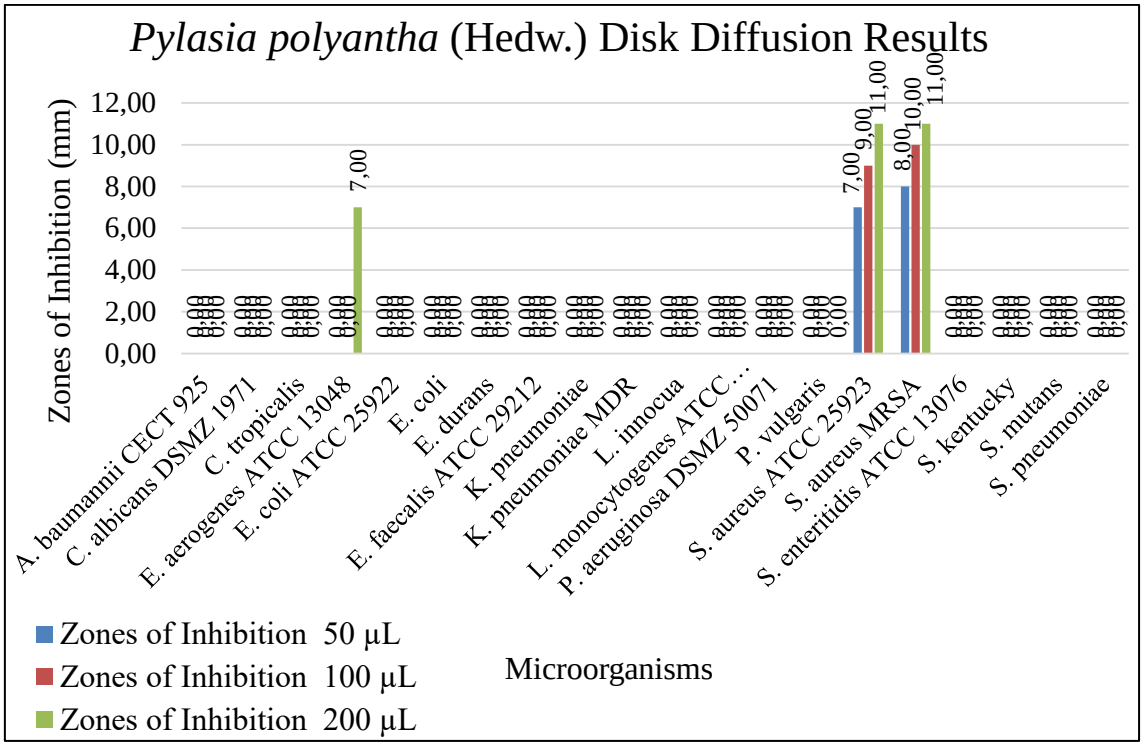

Figure 5. *Pylasia polyantha* (Hedw.) Disk Diffusion Results

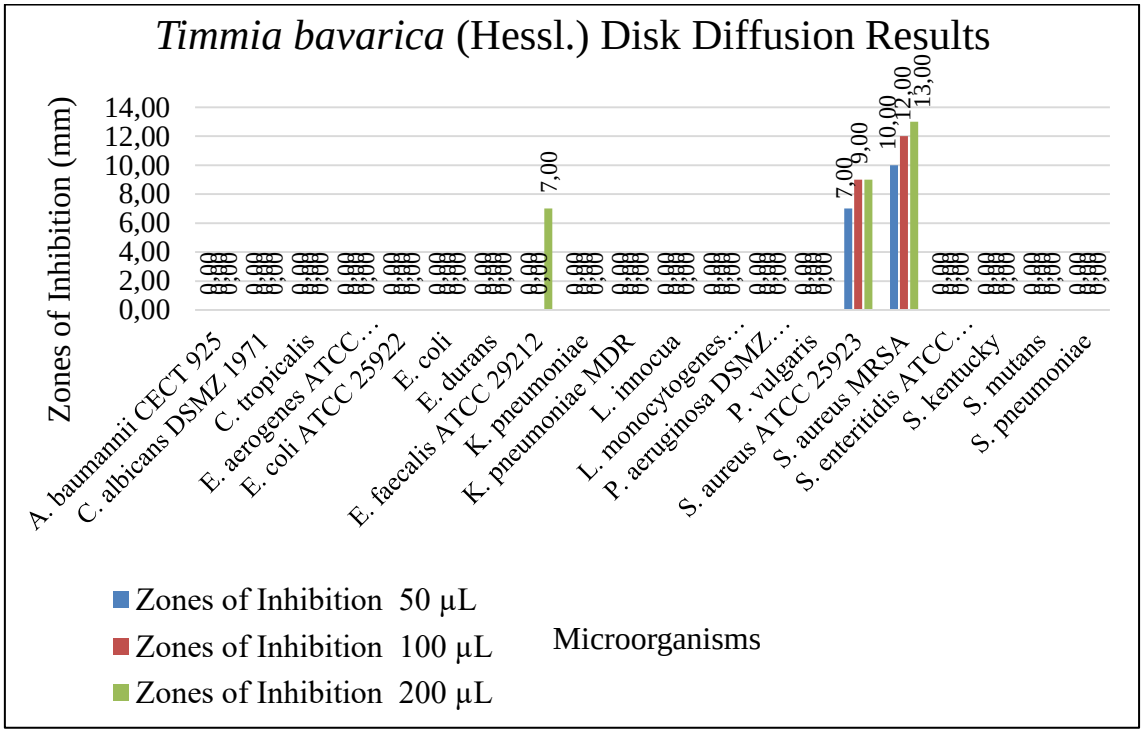

Figure 6. *Timmia bavarica* (Hessl.) Disk Diffusion Results

4. Discussion

According to the results of the disk diffusion test performed, the most sensitive microorganisms to the moss samples used are *S. aureus* ATCC 25923 affected by three different moss extracts at different concentrations, and *S. aureus* MRSA affected by four extracts. It was determined that all of the investigated moss samples had antimicrobial effects against at least two and at most three strains. The *P. medium* sample showed activity against two strains and was the least effective moss

species. All of the other moss samples investigated showed activity against all three strains.

In the study conducted by Vollár et al. (2018) antimicrobial activities of *Plagiomnium* spp. against the *S. aureus* ATCC 25923 strain were determined. According to this study, the disk diffusion results of *P. affine*, *P. cuspidatum*, and *P. undulatum* were 0.00 mm, 10.7 mm, and 8.00 mm respectively. The *P. medium* used in our study did not show any antimicrobial effect against *S. aureus* ATCC 25923. But it showed activity between 8.00 and 11.00 mm in 50 µL, 100 µL, and 200 µL applications against *S. aureus* MRSA strain. The antimicrobial activity knowledge of the species belonging to the genus *Plagiomnium* has been expanded with the present study carried out.

Uyar et al (2016) conducted a study in which samples of *Cinclidotus riparius* (Host ex Brid.) Arn., *Calliergonella cuspidata* (Hedw.) Loeske, *Thamnobryum alopecurum* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll. Hal., and *Cirriphyllum crassinervium* (Taylor) Loeske & M. fleish were extracted with four different solvents, and their antimicrobial activities against 13 different strains were determined. According to the results of the study, ethanol extracts of the moss samples produced zones of inhibition ranging from 6 to 9 mm against the *E. coli* strain used in both studies. However, in this study used moss samples no antimicrobial activity was observed against the *E. coli* strain.

The antimicrobial activity of *Palustriella commutata* (Hedw.) Ochyra moss against eleven bacteria, one yeast, and eight molds was determined by İlhan et al. (2006) using acetone and methanol extracts prepared through the disk diffusion method. In both studies, no results were observed against the commonly used strain *S. aureus* ATCC 25923, whereas the methanol extract exhibited a 7 mm zone and the acetone extract showed an 11 mm zone against the clinical isolate *K. pneumoniae*. In this study, the ethanol extract of *N. gracile* formed zones of 8-10-11 mm, *P. polyantha* exhibited zones of 7-9-11 mm, and *T. bavarica* showed zones of 7-9-10 mm against *S. aureus* ATCC 25923. Both studies demonstrate antimicrobial activity against *S. aureus* ATCC 25923, an important hospital infection, through different moss species and solvent effects.

The EC_{50} value of 0.359072 µg/mL of ascorbic acid used as a standard in this study was determined. Compared to the standard, the moss sample with the lowest antioxidant scavenging activity was *P. polyantha* with an EC_{50} value of 50,239 µg/mL. The moss sample with the highest antioxidant scavenging activity is the *P. medium* sample with an EC_{50} value of 6.0826 µg/mL.

Ertürk et al. (2015) conducted a study to determine the antioxidant capacity of some mosses, such as, *Hypnum cupressiforme* (Hedw.) EC_{50} 0.79±0.05 µg/mL, *Homalothecium sericeum* (Hedw.) EC_{50} 0.52±0.06 µg/mL, *Thuidium delicatulum* (Hedw.) EC_{50} 0.87±0.06 µg/mL, *Homalothecium lutescens* (Hedw.) EC_{50} 2.83±0.08 µg/mL, *Homalothecium nitens* (Hedw.) EC_{50} 4.40±0 .09 µg/mL, *Leucodon sciuroides* (Hedw.) EC_{50} 0.49±0.04 µg/mL, *Ctenidium molluscum* (Hedw.) EC_{50} 1.96±0.07 µg/mL, *Eurhynchium striatulum* (Spruce) EC_{50} 0,22±0.01 µg/mL. The lowest value obtained from the samples used in this study was taken from *P. medium* with an EC_{50} value of 6.0826 µg/mL and the highest value from *P. polyantha* with an EC_{50} value of 50,239 µg/mL. All remaining results are higher than the results of the study done by Ertürk et al. (2015) which means lower antioxidant activity.

Carranza et al. (2019) investigated the antioxidant activity of seven different moss species in the Philippines. In the study, the lowest result was found in *Gymnostomum recurvirostrum* (Hedw.) species EC_{50} 0.236 mg/mL, and the highest result in *Hypnum plumiforme* (Wilson) species EC_{50} 2.213 mg/mL. The *P. medium* sample used in this study with the lowest effect had an EC_{50} value of 6.0826 µg/mL, it is close but higher than the results obtained in the study by Carranza et al.

The antioxidant activity of the *R alopecuroides* sample used in this study was previously determined by Yayıntaş et al. (2019) using the CERAC and CUPRAC methods. In this study, the antimicrobial and antioxidant activities of the remaining samples were demonstrated for the first time. Further studies are required to determine from which substances the antimicrobial and antioxidant effects of mosses originate.

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