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Antimicrobial activities of some bryophytes collected from Trabzon, Türkiye and preparation of herbal soap and cream using *Pellia epiphylla* extract for the first time

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Abstract

Bryophytes are terrestrial plants and they contain many important medical aspects. In this study, six different bryophytes (*Polytrichastrum formosum, Plagiomnium cuspidatum, Hypnum jutlandicum, Sphagnum palustre, Rhizomnium punctatum* and *Pellia epiphylla*) were collected from Trabzon, Türkiye. The effect of the extract of these bryophytes on some human pathogenic microorganisms especially causing skin diseases (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Candida albicans*) was investigated. The highest antimicrobial activity was obtained by *P. formosum* extract against *S. aureus, B. cereus, E. coli,* and *S. epidermidis. P. epiphylla* was also effective towards *S. aureus, B. cereus,* and *S. epidermidis*. No activity was obtained for *C. albicans*. MIC values of *P. formosum, P. cuspidatum, R. punctatum,* and *P. epiphylla* plant extracts were determined by using *S. aureus, B. cereus,* and *S. epidermidis* microorganisms. Because the plant with the lowest MIC values was *P. epiphylla*, soap and cream formulations were prepared using the extract of this plant. Antimicrobial properties of suspended soap/cream samples were determined against *B. cereus, S. aureus,* and *S. epidermidis* microorganisms. It was observed that both soap and cream samples containing plant extract inhibited bacterial growth more than control samples of soap and cream.

Keywords: Bryophyte, herbal soap, herbal cream, Trabzon, Türkiye

Trabzon, Türkiye'den toplanan bazı biryofitlerin antimikrobiyal aktiviteleri ve *Pellia epiphylla* özü kullanılarak ilk kez bitkisel sabun ve krem hazırlanması

Öz

Briyofitler, karasal bitkiler olup tıbbi açıdan pek çok önemli özelliğe sahiptirler. Bu çalışmada, Trabzon'dan altı farklı briyofit türü (*Polytrichastrum formosum, Plagiomnium cuspidatum, Hypnum jutlandicum, Sphagnum palustre, Rhizomnium punktatum* ve *Pellia epiphylla*) toplandı. Bu biryofitlerden elde edilen ekstraktların, özellikle deri hastalıklarına neden olan bazı insan patojenik mikroorganizmaları (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* ve *Candida albicans*) üzerindeki etkisi araştırıldı. En yüksek antimikrobiyal aktivite, *Staphylococcus aureus, Bacillus cereus, Escherichia coli* ve *Staphylococcus epidermidis*'e karşı *P. formosum* özütü ile elde edildi. Ayrıca, *P. epiphylla* bitkisi de *S. aureus, B. cereus* ve *S. epidermidis*'e karşı etkiliydi. Ancak, *C. albicans* için herhangi bir aktivite elde edilmedi. *P. formosum, P. cuspidatum, R. punktatum* ve *P. epiphylla* bitki ekstraktlarının *S. aureus, B. cereus* ve *S. epidermidis* mikroorganizmalarına karşı MİK değerleri belirlendi. En düşük MİK değerine sahip bitki *P. epiphylla* olduğu için bu bitkinin ekstraktı kullanılarak sabun ve krem formülasyonları hazırlandı. Bu sabun/krem örneklerinin *B. cereus, S. aureus* ve *S. epidermidis* mikroorganizmalarına karşı antimikrobiyal özellikleri belirlendi. Bitki ekstraktı içeren sabun ve krem örneklerinin bakteri üremesini kontrol sabun ve krem örneklerine göre daha fazla engellediği gözlendi.

Anahtar kelimeler: Briyofit, bitkisel sabun, bitkisel krem, Trabzon, Türkiye

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1. Introduction

Bryophytes are non-vascular plants and are divided into three groups: mosses, liverworts, and hornworts. They are represented by 14000 to 15000 species around the world (Asakawa, 1995; Hallingbäck and Hodgetts, 2015).

Bryophytes have an important role in keeping ecosystems because they provide a remarkable buffer system for other plants. Because of their small size, many people do not have much knowledge about bryophytes. As a result, they are not neglected for extensive use. They are widely found in the world from desert to polar regions except for seas. Bryophytes were used for packing, plugging, and decoration from ancient times (Harris, 2008).

Bryophytes contain valuable chemicals such as norsesquiterpenoids, riccionidins sesquiterpenoids, anthocyanidins, etc. It has shown that these compounds have activities such as cytotoxic, antimicrobial, antifungal, insecticidal, molluscicidal, antitumor, cardiotonic, and plant growth regulatory (Asakawa, 1995; Marko et al., 2001). Bryophytes having these key properties are traditionally used in India to cure wounds, cuts, burns, and skin diseases (Pant and Tewari, 1990; Saxena, 2004; Singh et al., 2011).

It is known that natural products have been used in skincare for centuries. Today, they are becoming more prevalent in formulations because of synthetic ingredients or chemical substances are no longer preferred by people. The main benefit of plant extracts, used in skincare, is their antimicrobial activities (Ribeiro et al., 2015). Herbal skincare products containing natural ingredients having antibacterial and antifungal activities are prepared from different parts of plants such as stem, leaves, bark, fruit or root. They are administered topically and may be applied in the form of soap, cream, ointment, etc. (Gata-Goncalves et al., 2003; Melendez et al., 2006; Wagate et al., 2009). Gels, creams and soap formulations prepared by using plant extracts have been used to treat different skin disorders caused by microbial infections (Semkina, 2005; Nebedum et al., 2009).

In this study, six different bryophytes (*Polytrichastrum formosum, Plagiomnium cuspidatum, Hypnum jutlandicum, Sphagnum palustre, Rhizomnium punctatum,* and *Pellia epiphylla*) were collected from Trabzon, Türkiye. The effect of the extract of these bryophytes on some human pathogenic microorganisms especially causing skin diseases (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus epidermidis,* and *Candida albicans*) was investigated. Because the best antimicrobial activity was obtained with *P. epiphylla* extract, soap and cream formulations were prepared using the extract of this plant. After then, antimicrobial properties of suspended soap/cream samples were determined by using *B. cereus, S. aureus,* and *S. epidermidis* microorganisms.

When the literature is examined, it is seen that only one soap and only one cream formulation was prepared by using bryophytes and they were not examined in terms of antimicrobial activity. Therefore, this study is very important in this respect.

2. Materials and Methods

2.1. Plant material

Bryophyte samples were collected from Trabzon (Black Sea Region, Türkiye) during March-June 2018: *Polytrichum formosum* from Taflancik Village (Hayrat, Trabzon), *Pellia epiphylla* from Sinlice Village (Salpazarı, Trabzon), *Rhizomnium punctatum* from Erikbeli High Plateau (Tonya Trabzon), *Plagiomnium cuspidatum* from Erikbeli High Plateau (Tonya Trabzon), *Sphagnum palustre* from Camburnu (Surmene, Trabzon), *Hypnum jutlandicum* from Camburnu (Surmene, Trabzon). Information about the collection locations of bryophyte samples can be seen in Table 1. The plants were identified and deposited in the Herbarium at the Department of Biology, Faculty of Science, Karadeniz Technical University. They were carefully cleaned and washed by using distilled water to remove the contaminants. Samples were dried at room temperature under the shade.

2.2. Test microorganisms

Staphylococcus aureus ATCC 25923, *Bacillus cereus* RSKK 709, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans* ATCC 10231 microorganisms were grown on Muller Hinton Agar or Muller Hinton Broth and stored at 4 °C.

Table 1. Information about collection places of bryophyte samples

Bryophyte	Locality	Coordinate	Altitude (m)	Date
P. formosum	Trabzon (Hayrat): Taflancık Village	40º50'03.38"N/ 40º24'11.85"E	478	23.03.2018
R. punctatum ***P. cuspidatum***	Trabzon (Tonya): Erikbeli High Plateau	40º45'00.80"N/ 39º13'39.18"E	1440	05.06.2018
P. epiphylla	Trabzon (Salpazarı): Sinlice Village	40º48'09.20"N/ 39º13'37.70"E	1418	24.05.2018
S. palustre ***H. jutlandicum***	Trabzon (Surmene): Camburnu	40º55'14.74"N/ 40º13'04.53"E	185	24.03.2018

2.3. Preparation of plant extracts

Bryophyte extracts were prepared by using a modified method. For this purpose, 250 mL of 80% methanol solution was added to 25 g of the plant samples which were powdered with liquid nitrogen. Maceration was performed by shaking at 200 rpm for 2 days at 28 °C. After then, the extracts were filtered and the filtrates were left in an incubator at 30 °C to remove the solvent completely. The remaining solid was dissolved in a solution containing 1% methanol, 0.05% Tween 80, and 1% DMSO. The supernatants were collected by centrifugation at 4 °C, 7000 rpm for 10 minutes, and used for antimicrobial analysis (Esimone et al., 2008; Yeo et al., 2014; Klavina et al., 2015).

2.4. Determination of antimicrobial activities of the plant extracts

For the determination of the antimicrobial activity of the plant extracts, agar well diffusion tests and minimum inhibitory concentration (MIC) tests were performed.

2.4.1. Agar well diffusion tests

25 mL of Sterile Muller Hinton Agar was poured into Petri dishes with a thickness of 20-25 mm. After cooling, the microorganisms prepared according to McFarland 0.5 scale were spread to the agar surface with a sterile swab. Then 6-7 mm diameter wells were drilled on the agar surface and 50-100 µL of plant extracts were placed in each well. A negative control solution containing 1% methanol, 0.05% Tween 80 and 1% DMSO was used. The Petri dishes were incubated for 24 hours at 25 °C for *C. albicans* and at 37 °C for other microorganisms. Zone diameters around the wells were measured at the end of the period (Bukvicki et al., 2012; Adeniyi et al., 2017).

2.4.2. Determination of minimum inhibition concentration (MIC) values

The minimum inhibition concentration (MIC) values of the samples having antibacterial effects were determined by the liquid microdilution method. In this method, 96-well microplates were used. 100 µL of sterile Muller Hinton Broth was added to each well in the microplate. Then, 100 µL of plant extract was added to the first column on the microplate. 100 µL of the mixture in this column was transferred to the second column and 2-folds dilutions were made. After then 5 µL of the microorganism culture were added to the wells. After the microplates were incubated at 37 °C for 12-18 hours with shaking, bacterial density was determined using an ELISA reader. The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of extract that prevents the visible growth of bacteria (Oliveira et al., 2012).

2.5. Preparation of herbal soap formulation

12 g of coconut oil and 54 g of palm oil were mixed for 15 minutes at 250 rpm at room temperature. Then 51 mL of 20% NaOH solution was slowly added to this mixture and stirred for about 30 minutes until soap formation was observed. After the plant extract having concentration at MIC value was added, the solid soap formed was poured into the molds and left to stand for 12 hours. The soaps removed from the molds were allowed to dry outdoors for 7 days. Control soap was prepared in the same manner but without the addition of the plant extract (Wongthongdee and Inprakhon, 2013).

2.6. Preparation of herbal cream formulation

While the oil phase of cream contained 4% stearic acid, 4% stearyl alcohol, 2% cetyl alcohol, 5% lanolin and 8% isopropyl myristate, the water phase contained 5% propylene glycol, 5% glycerin, 0.75% triethanolamine, 0.18% methylparaben, 0.02% propylparaben, 0.05% EDTA and sufficient water. Each prepared phase was heated to 65 °C, separately. The aqueous phase was then added in portions to the oil phase with continuous stirring and stirred continued until it cooled down. The plant extract was then added to the mixture at a concentration of MIC value. Control cream was prepared by the same procedure without the addition of plant extract (Lachman et al., 1987; Gupta et al., 2015).

2.7. Antimicrobial analysis of herbal soap and cream formulations

10 mL of sterile distilled water was added over 2 g of soap/cream and suspended with stirring.

Vigorous agitation was performed to homogenize the suspension, disperse the foam and dissolve the soap. 200-300 μL of this suspension was spread over the Muller Hinton Agar in a Petri dish and completely dried. 6 μL of the microorganisms prepared according to McFarland 0.5 scale was dropped in specific regions on the Petri dish. After the incubation at 37 °C for 24 hours, the zone diameters of the growing bacteria were measured (Esimone et al., 2008).

3. Results

3.1. Agar well diffusion tests

The antimicrobial activity of 6 different bryophyte extracts was examined (Table 2). The highest activity was obtained with *Polytrichastrum formosum* extract against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Staphylococcus epidermidis* microorganisms. *Rhizominium punctatum* and *Pellia epiphylla* extracts also had the highest activity towards *S. aureus*, *B. cereus* and *S. epidermidis*. But, no activity was obtained against *Candida albicans*. Therefore, it can be said that plant extracts are more effective against Gram (+) bacteria.

As shown in Table 2, the best antimicrobial activities were obtained with *P. formosum*, *P. cuspidatum*, *R. punctatum* and *P. epiphylla* plants. For this reason, determination of minimum inhibition concentration (MIC) values were carried out with these plant extracts.

Table 2. Inhibition zone diameters (mm) obtained by agar well diffusion method

	S. aureus	*S. epidermidis*	*C. albicans*	*E. coli*	*B. cereus*	*P.aeruginosa*
P. formosum	12.45±0.70	13.26±1.12	ND	12.71±4.14	7.19±0.53	ND
P. cuspidatum	8.64±0.28	8.39±0.93	ND	ND	7.25±0.80	ND
H. jutlandicum	8.81±0.07	ND	ND	ND	ND	ND
S. palustre	10.48±0.01	ND	ND	ND	ND	ND
R. punctatum	7.09±0.71	7.94±0.85	ND	ND	6.18±0.32	ND
P. epiphylla	13.45±0.58	15.18±0.43	ND	ND	8.68±0.42	ND

Inhibition zone diameters (mm)±standard deviation ND: Not determined

3.2. Determination of minimum inhibition concentration (MIC) values

The minimum inhibition concentration (MIC) values of the plant samples having good antibacterial effect were determined by using liquid microdilution method. Table 3 shows the MIC values obtained for each plant against *S. aureus*, *B. cereus* and *S. epidermidis* microorganisms. It was determined that *P. epiphylla* is the plant having the lowest MIC values. Therefore, the extract of this plant was used in the preparation of herbal soap and cream formulations.

Table 3. Minimum inhibition concentration (MIC) values of the plant samples

	MIC (mg/mL)		
	S. epidermidis	***S. aureus***	***B. cereus***
P. formosum	33.3	33.3	66.6
P. cuspidatum	129.4	64.7	129.4
R. punctatum	217.5	217.5	217.5
P. epiphylla	14.6	7.3	1.8

3.3. Preparation of herbal soap formulation

Herbal soap production was carried out using *P. epiphylla* plant extract. The plant extract was added to the soap at a final concentration of 14.6 mg/mL. Control soap was prepared without addition of plant extract (Figure 1).

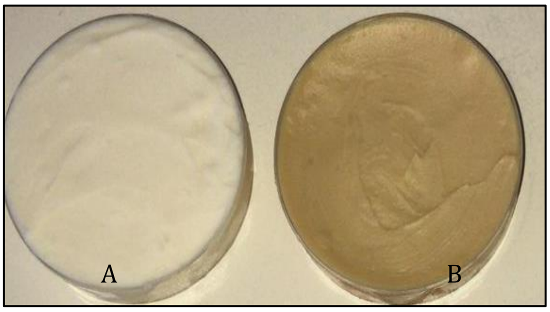

Figure 1. Soaps produced: A) Control soap B) Soap prepared using *P. epiph*ylla plant extract

3.4. Preparation of herbal cream formulation

Herbal cream was prepared such that the concentration of *P. epiphylla* plant extract was 14.6 mg/mL. The control cream was prepared without adding the plant extract (Figure 2).

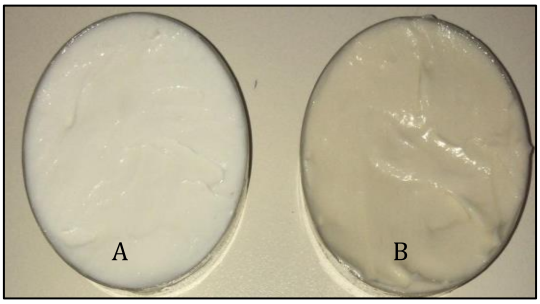

Figure 2. Cream produced: A) Control cream B) Cream prepared using *P. epiphylla* plant extract

3.5. Antimicrobial analysis of herbal soap and cream formulations

Antimicrobial properties of suspended soap/cream samples were determined by using *B. cereus, S. aureus* and *S. epidermidis* microorganisms. The zone diameters of the growing bacteria were measured. As can be seen from Table 4, *P. epiphylla* soap inhibited the growth of *S. epidermidis* and *S. aureus* bacteria more than control soap. But the soap did not inhibit the growth of *B. cereus*. In the case of *P. epiphylla* cream, it is seen that the growth of all three bacteria used was more inhibited than the control cream depending on the presence of plant extract.

Table 4. Antimicrobial effects of soaps and creams

	S. epidermidis	*S. aureus*	*B. cereus*
Control soap	7.28±1.62	7.79±0.38	ND
***P. epiphylla* soap**	7.09±0.45	6.17±0.30	ND
Control cream	7.59±2.13	8.97±2.28	15.72±3.74
***P. epiphylla* cream**	6.65±1.41	7.46±1.33	12.08±4.01

Growth zone diameters (mm)±standard deviation ND: Not detected

4. Discussion

As indicated in the findings section, when the antimicrobial activity of six different bryophyte extracts was examined, the highest activity was examined in the case of *Polytrichastrum formosum* extract against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Staphylococcus epidermidis* microorganisms. In addition, *Plagiomnium cuspidatum, Rhizominium punctatum*, and *Pellia epiphylla* extracts were highly active against *Staphylococcus aureus, Bacillus cereus* and *Staphylococcus epidermidis.*

When the literature data is examined, it is seen that the number of detailed antimicrobial studies using bryophytes is not very high. In a study, it was found that crude ether and methanol extract obtained from *Mastigophora diclados* showed a good activity (MIC value 16 µg/mL) against *B. subtilis* and *S. aureus* (Komala et al., 2010).

The antimicrobial potential of extracts from *Scapania nemorea* against yeast and bacteria causing food degradation was determined *in vitro* by microdilution method. MIC values were determined between 0.5-3 mg/mL for bacteria and 0.2-1.0 mg/mL for yeasts (Bukvicki et al., 2012).

Antibacterial and antifungal activity of methanol extract of *Palustriella commutata, Pleurozium schreberi, Dicranum scoparium, Homalothecium philippeanum, Rhytidium rugosum, Anomodon attenuatus, Leucobryum glaucum*, and *Hylocomium splendens* were investigated against 6 bacteria and 7 fungal species. *A. attenuatus* extract showed the highest antimicrobial activity (MIC 1.25-5.0 mg/mL), while *L. glaucum* extract showed the lowest activity (MIC 20.0-25.0 mg / mL). The extracts were found to be more effective against Gram (+) bacteria and showed strong antifungal activity (Veljic et al., 2009).

The activity of ethanol, methanol, acetone, chloroform and water extracts of *Lunularia cruciate* was investigated against the pathogenic bacteria (*Agrobacterium tumefaciens, Escherichia coli, Xanthomonas phoseoli, Erwinia chrysanthemi*, and *Bacillus subtilis*) and fungus (*Alternaria alternata, Pyricularia oryzae* and *Sclerotinia sclerotiorum*). All organic solvent extracts exhibited remarkable antibacterial activity but no antifungal activity. The aqueous plant extract showed no activity. The maximum inhibition of ethanol extract in the agar well method was shown in *A. tumefaciens, X. phoseoli, E. coli* and *B. subtilis* (zone diameter 10-20 mm). This is followed by extracts of methanol, acetone and chloroform (Dhondiyal et al., 2013).

In this study, MIC values of *P. formosum, P. cuspidatum, R. punctatum* and *P. epiphylla* plants for *S. epidermidis, S. aureus* and *B. cereus* were determined using liquid micro dilution method. *P. epiphylla* was the plant that killed all three bacteria with the lowest concentration of plant extract.

Extracts from *Homaliodendron montagneanum, Papillaria fuscescens*, and *Campylopus latinervice* bryophytes were prepared using acetone, ethyl acetate and water. The MIC value of *H. montagneanum* acetone extract with the best antimicrobial activity was determined as 20 mg/mL against *S. aureus* (Williams et al., 2016).

Studies have shown that many bryophyte species have different antimicrobial activities. However, although these plant species are so rich in antimicrobial aspects, they are almost never used in soap production. The only example of this is the sphagnol soap produced by Peat Products in the UK. This soap was produced using sphagnol extract from *Sphagnum* species and was used by the British Red Cross to treat wounds during both world wars (Fall, 2004). The only example of ointment is *Conocephalum conicum* Dumort, and *Marchantia polymorpha* L. liverwort mixed with vegetable oils to treat burns, eczema, cuts and insect bites (Ding, 1982).

Within the scope of this study, both soap and cream were produced by using *P. epiphylla* plant extract. *P. epiphylla* soap was found to inhibit the growth of *S. epidermidis* and *S. aureus* bacteria more than control soap. In the case of cream, the growth of all three bacteria used was more inhibited depending on the presence of plant extract. Therefore, based on the results obtained from the study, it can be said that soap and cream containing *P. epiphylla* extract had more high antimicrobial activity than control soap and cream.

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