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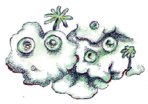
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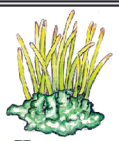
Hornwort

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Determining Antibacterial Activity of Some Mosses (*Cinclidotus riparius* (Host ex Brid.) Arn., *Calliergonella cuspidata* (Hedw.) Loeske, *Thamnobryum alopecurum* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll. Hal., *Cirriphyllum crassinervium* (Taylor) Loeske & M.Fleisch.)

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Abstract

In this study, the antibacterial activity of 5 different moss extracts (*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.Fleisch.) which were common in Zonguldak province and its environs were tested in vitro against the 13 different microorganisms. The extracts were prepared within ethanol, acetone, methanol, and ethyl acetate (96%). Disk diffusion method was applied for the determination of antibacterial activity of moss extracts. In addition, standard antibiotic disks and blank solvent disks were used respectively for comparison and control.

It is observed at the end of the study that *T. alopecurum* extracts in methanol and acetone and *L. juniperoideum* extract in methanol have the greatest antimicrobial activities against. Escherichia coli ATCC 11230 among all the other studied moss species. Additionally, the antibacterial activities of *T. alopecurum* extracts in methanol and acetone were the same with those of CTX30 (Cefotaxime), but higher than those of AK30 (Amikacin) which were among the studied standard antibiotic disks. Moreover, the antibacterial effect of *L. juniperoideum* extract in methanol was found higher only than AK30 (Amikacin).

Key words: Antibacterial activity, Bryophyta, Mosses.

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Bazı Karayosunu Türlerinin (*Cinclidotus riparius* (Host ex Brid.) Arn., *Calliergonella cuspidata* (Hedw.) Loeske, *Thamnobryum alopecurum* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll. Hal., *Cirriphyllum crassinervium* (Taylor) Loeske & M.Fleisch.) Antibakteriyel Aktivitesinin Belirlenmesi

Öz

Bu çalışmada, Zonguldak çevresinde bol bulunan 5 farklı karayosunu (*Cinclidotus riparius* (Host ex Brid.) Arn., *Calliergonella cuspidata* (Hedw.) Loeske, *Thamnobryum alopecurum* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll. Hal., *Cirriphyllum crassinervium* (Taylor) Loeske & M.Fleisch.) ekstraktlarının, 13 farklı bakteri kültürüne karşı antibakteriyel etkileri incelenmiştir. Ekstreler etanol, aseton, metanol ve etil asetat (%96) içinde hazırlanmıştır. Karayosunu ekstraktlarının antibakteriyel aktivitesinin belirlenmesi için Disk Difüzyon yöntemi uygulanmıştır. Buna ilaveten, sadece çözgenlerin emdirilmiş olduğu disklerle birlikte standart antibiyotik diskler de kıyas amaçlı kullanılmıştır.

Çalışma sonunda, *Escherichia coli* ATCC 11230'ye karşı *T. alopecurum*'un metanol ve aseton ekstreleri ile *L. juniperoideum*'un metanol ektresinin diğer çalışılan tüm karayosunu türlerinden daha fazla antibakteriyel aktivite gösterdikleri belirlenmiştir. Ayrıca *T. alopecurum*'un etanolik ve asetonik ekstrelerinin aktiviteleri standart olarak çalışılan antibiyotik disklerinden AK30 (Amikasin)'in etkisinden fazla CTX30 (Sefotaksim)'in etkisi ile aynıydı. Bununla birlikte *L. juniperoideum*'un metanol ekstresinin antibakteriyel etkisinin yalnızca AK30 (Amikasin)'den daha yüksek olduğu görülmüştür.

Anahtar Kelimeler: Antibakteriyel aktivite, Bryophyta, Karayosunu.

1. Introduction

Plants were used in the treatment of various diseases for centuries (Jones, 1996). Today, it is outstanding that, in many developed countries, 80% of the materials used in the treatment of diseases are plant origin (Baytop, 1999). To discover new active substances which can be used for the treatment of diseases, scientists research constantly medical uses of plants, such as antimicrobial and antitumor (Rajakaruna et al., 2002).

Despite the widespread usage of plants as a source of antimicrobial agents, bryophytes are not commonly used for that purposes. However, it is known that for centuries that mosses have been used to reduce the risks of infection in wounds and wound healing.

Bryophytes belong to the group of the oldest known land plants, which includes liverworts, hornworts and mosses. There are nearly 22,000 members of the mosses (Bryophyte) in the world (Zinsmeister and Mues, 1987). Today, mosses are interesting for biotechnological use in medicine, agriculture and pharmacology (Frahm, 2001; Decker et al., 2003; Asakawa, 2008). The very special features that helped bryophytes to maintain existence in today's flora is their biologically active compounds they contain (Asakawa, 1990; Bodade et al., 2008). They possess several medicinal properties and also show anticancer and antimicrobial

Uyar G. et al., 2016. Determining Antibacterial Activity of Some Mosses (*Cinclidotus riparius* (Host ex Brid) Arn., *Calliergonella cuspidate* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll. Hal., *Cirriphyllum crassinervium* (Taylor) Loeske & M.Fleisch.) . *Anatolian Bryology*. 1-2(2): 1-8.

activity due to their unique chemical constituents (Banerjee and Sen, 1979; Asakawa, 2008).

In presented paper, the antibacterial activity of 5 common bryophyte species (Ören et al., 2015) (*Cinclidotus riparius* (Host ex Brid.) Arn., *Calliergonella cuspidata* (Hedw.) Loeske, *Thamnobryum alopecurum* (Hedw.) Gangulee, *Leucobryum juniperoideum* (Brid.) Müll.Hal., *Cirriphyllum crassinervium* (Taylor) Loeske & M.Fleisch. collected from Zonguldak province were evaluated.

2. Materials and Methods

2.1 Plant material

Samples of all tested plants were collected from their native habitats in Zonguldak province and the specimens were identified. The plant material was carefully cleaned under tap water to remove attached litter and finally washed with sterile distilled water.

2.2 Preparation of the extracts

Dried samples grinded mechanically in aseptic condition and 15 g grinded powder were mixed with 150 mL ethanol, acetone, methanol, and ethyl acetate (96%) solvents, then all extracts were obtained after 12 hours and stored + 4 °C.

2.3 Preparation of cultures of microorganisms and extracts containing disk

Antibacterial activity of the bryophytes fractions were determined by agar diffusion method according to the National Committee for Clinical Laboratory Standards (Gould and Bowie, 1952; Anna and Brown, 2001; NCCLS, 2002). Petri plates containing sterilized Mueller-Hinton Agar (OXOID) were used as base layer.

According to this method, paper disks of 6 mm in diameter were soaked in 50 µL extracts. To refresh the bacterial cultures Brain Heart Infusion Broth (OXOID), was used and bacteria strains taken from cultured stock were suspended in 4-5 mL broth separately and incubated for 2-5 hours in incubator. After this, the bacterial suspension was adjusted with sterile saline to McFarland standard tube, cultivation was carried out.

After 24 hours incubation in broth, it was dealed out to petri dishes and waited for 15 min for solidification. After the solidification, the disks with different extracts were placed on petri dishes. The bacterial dishes were incubated at 35°C for 24 hours. The antibacterial activity was measured in terms of the zone of inhibition (mm). Solvents soaked disks were used as negative control and AK30 (Amikacin) and CTX30 (Cefotaxime) were used for positive control. The tests were performed in triplicates.

2.4 Test microorganisms

The microorganism cultures were supplied from the Microbiology Research Laboratory in Canakkale Onsekiz Mart University, Biology Department. In present study, *Staphylococcus aureus* ATCC 6538P, *Salmonella typhimurium* CCM 5445, *Escherichia coli* ATCC 11230, *Escherichia coli, Listeria inocule, Micrococcus luteus* CCM 169, *Staphylococcus epidermis, Streptococcus faecalis, Proteus mirabilis* ATCC 14153, *Proteus vulgaris* ATCC 6337, *Bacillus cereus* ATCC 7064, *Citrobacter freundii* ATCC 8090 and *Serratia marcescens* bacterial cultures were used.

3. Result and Discussion

The disc diffusion method was used to determine the inhibition zones of mosses extracts. The zones around the discs were measured with three different angles. The tables showed the zone of inhibition of moss extracts with different solvents and antibiotic discs for control against 13 different microorganisms. Table 1 shows the diameter of the zone of inhibition caused by discs soaked with *C. riparius* extract applied to bacterial cultures.

Table 1. Antibacterial activity of *C. riparius.*

	Zone of inhibition (mm)					
	Extracts				**Standard antibiotics**	
Type of Microorganisms	**Ethanol**	**Methanol**	**Ethyl acetate**	**Acetone**	**AK30**	**CTX30**
Staphylococcus aureus ATCC 6538P	13.0	10.5	8.0	10.0	24.2	18.0
Salmonella typhimurium CCM 5445	11.0	10.0	9.0	8.8	19.2	21.0
Escherichia coli ATCC 11230	16.5	15.0	9.0	9.5	17.2	18.0
Escherichia coli	8.5	8.0	9.0	6.5	17.0	17.0
Listeria inocule	10.0	8.5	6.5	10.0	20.6	16.7
Micrococcus luteus CCM169	9.5	8.0	8.5	9.0	24.4	18.0
Staphylococcus epidermis	13.0	11.5	14.0	11.0	23.0	22.0
Streptococcus faecalis	10.0	10.5	7.3	7.0	20.0	21.0
Proteus mirabilis ATCC 14153	13.0	12.0	17.5	10.0	20.0	18.0
Proteus vulgaris ATCC 6337	10.0	11.0	10.5	11.0	18.0	18.0
Bacillus cereus ATCC 7064	14.0	9.5	10.5	11.0	16.0	14.0
Citrobacter freundii ATCC 8090	10.0	8.5	6.0	7.0	20.0	20.0
Serratia marcescens	8.5	8.0	10.0	7.5	20.0	20.0

As shown on the table, the zone diameters formed against microorganism are 6-16.5 mm. Also the *C. riparius* extracts with ethanol and methanol showed effective results against *E. coli* ATCC 11230 as standard antibiotic discs.

In general, it can be said that the extracts with ethanol shows best results. In addition to this, ethyl acetate extracts are more effective against *P. mirabilis* ATCC 14153 than others.

According to the results of the studies with *C. cuspidata* extracts, the zone of inhibition was evaluated on Table 2.

It was found out that, the diameter of zones are 7.0-16.0 mm. The methanol and acetone extraction of *C. cuspidata* showed high antimicrobial activity against *E. coli* ATCC 11230. And also the ethyl acetate extract of this species was effective to *P. vulgaris* ATCC 6337.

Table 2. Antibacterial activity of *C. cuspidata.*

	Zone of inhibition (mm)					
	Extracts				**Standard antibiotics**	
Type of Microorganisms	**Ethanol**	**Methanol**	**Ethyl acetate**	**Acetone**	**AK30**	**CTX30**
Staphylococcus aureus ATCC 6538P	7.0	9.0	7.5	12.0	24.2	18.0
Salmonella typhimurium CCM 5445	9.0	7.0	8.0	11.0	19.2	21.0
Escherichia coli ATCC 11230	8.0	14.0	9.0	16.0	17.2	18.0
Escherichia coli	8.0	7.5	6.0	10.0	17.0	17.0
Listeria inocule	7.0	6.0	9.0	9.0	20.6	16.7
Micrococcus luteus CCM169	9.5	6.0	8.0	12.0	24.4	18.0
Staphylococcus epidermis	11.0	8.0	11.0	10.5	23.0	22.0
Streptococcus faecalis	9.5	10.0	8.0	11.0	20.0	21.0
Proteus mirabilis ATCC 14153	13.0	11.0	11.0.	11.5	20.0	18.0
Proteus vulgaris ATCC 6337	10.0	8.0	14.0	10.0	18.0	18.0
Bacillus cereus ATCC 7064	11.0	9.5	7.0	12.5	16.0	14.0
Citrobacter freundii ATCC 8090	11.0	7.5	12.0	11.5	20.0	20.0
Serratia marcescens	10.0	7.0	8.0	8.0	20.0	20.0

The zone of inhibition of the discs with *T. alopecurum* is presented on the Table 3. The diameter of zones is 6.0-18.0 mm. Remarkable results have revealed that the methanol and acetone extraction of *T. alopecurum* showed higher antimicrobial activity against *E. coli* ATCC 11230 than others and also showed the same or higher activity than standards antibiotics as controls.

Table 3. Antibacterial activity of *T. alopecurum.*

	Zone of inhibition (mm)					
	Extracts				**Standard antibiotics**	
Type of Microorganisms	**Ethanol**	**Methanol**	**Ethyl acetate**	**Acetone**	**AK30**	**CTX30**
Staphylococcus aureus ATCC 6538P	8.0	10,5	11.0	11.0	24.2	18.0
Salmonella typhimurium CCM 5445	8.0	11.0	11.0	10.0	19.2	21.0
Escherichia coli ATCC 11230	6.0	**18.0**	10.0	**18.0**	17.2	18.0
Escherichia coli	9.0	12.0	9.0	10.0	17.0	17.0
Listeria inocule	9.0	7.0	8.0	7.0	20.6	16.7
Micrococcus luteus CCM 169	8,5	10,5	9.0	8,5	24.4	18.0
Staphylococcus epidermis	8.0	12.0	11,5	9.0	23.0	22.0
Streptococcus faecalis	9.0	9.0	10.0	8.0	20.0	21.0
Proteus mirabilis ATCC 14153	10.0	11.0	12.0	10.0	20.0	18.0
Proteus vulgaris ATCC 6337	11.0	10,5	10,5	9,5	18.0	18.0
Bacillus cereus ATCC 7064	10.0	10,5	12.0	8.0	16.0	14.0
Citrobacter freundii ATCC 8090	6.0	9.0	10,5	11.0	20.0	20.0
Serratia marcescens	9.0	11.0	9.0	7,5	20.0	20.0

However, the least antimicrobial activity was found in ethanol extracts. To evaluate the antimicrobial activity of *L. juniperoideum* (Table 4), the inhibition zones of different *L. juniperoideum* extracts were performed and found out 6.0-17.5 mm. Higher antagonistic activities were found against *E. coli* ATCC 11230 and *S. faecalis* with methanol and ethyl acetate extracts.

Table 4. Antibacterial Activity of *L. juniperoideum.*

	Zone of inhibition (mm)					
	Extracts				**Standard antibiotics**	
Type of Microorganisms	**Ethanol**	**Methanol**	**Ethyl acetate**	**Acetone**	**AK30**	**CTX30**
Staphylococcus aureus ATCC 6538P	10.0	12.0	11.0	7.0	24.2	18.0
Salmonella typhimurium CCM 5445	6.0	10.5	14.0.	7.0	19.2	21.0
Escherichia coli ATCC 11230	9.0	**17.5**	16.0	12.0	17.2	18.0
Escherichia coli	6.0	12.0	10.5	7.0	17.0	17.0
Listeria inocule	7.0	8.0	8.0	7.0	20.6	16.7
Micrococcus luteus	7.0	7.5	8.0	8.0	24.4	18.0
Staphylococcus epidermis	7.0	7.5	11.0	11.0	23.0	22.0
Streptococcus faecalis	7.0	16.0	17.5	7.6	20.0	21.0
Proteus mirabilis ATCC 14153	11.0	7.5	9.0	11.0	20.0	18.0
Proteus vulgaris ATCC 6337	7.0	8.0	10.0	7.5	18.0	18.0
Bacillus cereus ATCC 7064	10.0	11.0	10.0	7.0	16.0	14.0
Citrobacter freundii ATCC 8090	11.0	14.0	12.0	7.0	20.0	20.0
Serratia marcescens	7.0	9.0	13.0	7.0	20.0	20.0

Table 5. Antibacterial activity of *C. crassinervium.*

	Zone of inhibition (mm)					
	Extracts				**Standard antibiotics**	
Type of Microorganisms	**Ethanol**	**Methanol**	**Ethyl acetate**	**Acetone**	**AK30**	**CTX30**
Staphylococcus aureus ATCC 6538P	13.0	13.0	14.0	12.0	24.2	18.0
Salmonella typhimurium CCM 5445	8.0	10.0	12.0	13.0	19.2	21.0
Escherichia coli ATCC 11230	9.0	14.0	16.0	12.0	17.2	18.0
Escherichia coli	7.0	11.0	11.0	10.0	17.0	17.0
Listeria inocule	8.0	9.0	9.0	10.0	20.6	16.7
Micrococcus luteus	7.0	8.0	7.0	10.0	24.4	18.0
Staphylococcus epidermis	7.0	7.0	10.0	10.0	23.0	22.0
Streptococcus faecalis	7.0	13.0	12.0	10.0	20.0	21.0
Proteus mirabilis ATCC 14153	10.0	7.0	8.0	12.0	20.0	18.0
Proteus vulgaris ATCC 6337	10.0	8.0	14.0	12.0	18.0	18.0
Bacillus cereus ATCC 7064	10.0	13.0	14.0	9.0	16.0	14.0
Citrobacter freundii ATCC 8090	9.0	11.0	12.0	12.0	20.0	20.0
Serratia marcescens	7.0	12.0	10.0	10.0	20.0	20.0

According to the results of the studies with *C. crassinervium* extracts, the zone of inhibition was evaluated on Table 5. It was found out that, the diameter of zones are between 7,0-16,0 mm The ethyl acetate and acetone extraction of *C. crassinervium* showed nearly the same antimicrobial activity with standard antibiotics.

On the other hand it was found out that, the discs only soaked with solvent showed the diameter of zones between 0-1 mm.

In this study, the extracts of the mosses showed close antimicrobial activity with standard antibiotics. Yet, methanol and acetone extracts of *T. alopecurum* against *E. coli* ATCC 11230 showed the same activity with CTX30 and higher activity than AK30. Also, methanol extracts of *L. juniperoideum* antimicrobial activity was higher than AK30.

In our study, the antimicrobial activity of 5 different moss extracts with different solvents (methanol, ethanol, ethyl acetate, acetone) were tested in vitro against the 13 different microorganisms.

Of Bryophyte extracts, the simplest land plants, isoflavonoids, flavonoids and bioflavonoids have been reported to be possible chemical barriers against microorganims (Basile et al., 1999; Saxena and Harinder, 2004). Terpenoids, phenolic and volatile constituents have also investigated in some Bryophyte species (Saritas et al., 2001). It was found that a methanolic extract of *H. aduncus* inhibited the growth of pathogenic fungi *Botrytis cineria*, *Rhizoctonia soloni* and *Pythium debaryanum*, whereas petroleum-ether extracts of *Barbula* and *Timmiella* species were found to be active against both gram-positive and gram-negative bacteria. In previos study, the ethanol extracts of *Plasteurhynchium meridionale* and *Anomodon viticulosis* have shown antimicrobial activity against some Gram-positive, Gram-negative bacteria and some yeast cultures especiallay *S. aureus*, *P. mirabilis* and *Candida albicans* (Dulger et al., 2009). Altuner et al. (2014) have found that *C. cuspidata* has no any antagonistic activity on some bacteria and yeast cultures. But the results in this study have shown that *C. cuspidata* has an important antagonistic activity against *E.coli*, *P. mirabilis* and *P. vulgaris*. There is no data in literature on other studied mosses.This antibiotic activity might be attributed due to the presence of non-ionized organic acids and poloyphenolic compounds (Saxena and Harinder, 2004). The light of the obtained results, using these kind of plants to evaluate the antimicrobial activity is very important.

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