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# *In Vitro* Antimicrobial Activity of *Brachythecium campestre* and *Eurhynchium pulchellum* Extracts

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The purpose of this research was to examine the *in vitro* antimicrobial activity of ethanol extracts of *Brachythecium campestre* ($E_1$) and *Eurhynchium pulchellum* ($E_2$). The antimicrobial activity of the $E_1$ and $E_2$ was evaluated according to the disc diffusion method against 10 test bacteria, including 4 Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 122228, *Streptococcus faecalis* NRRLB-14617, *Bacillus cereus* ATCC 11778), 6 Gram-negative bacteria (*Salmonella typhi* CCM 5445, *Proteus vulgaris* ATTC 68, *Enterobacter aerogenes* ATCC 13043, *Pseudomonas aureginosa* ATTC 27853, *Escherichia coli* ATCC 25922, *Neisseria canis*) and 9 test yeasts (*Saccharomyces cerevisiae* ATCC 9763, *Saccharomyces colombia*, *Saccharomyces uvarum*, *Schizosaccharomyces pombe*, *Kluyveromyces marxianus*, *Debarymyces hansenii*, *Candida lypolitica*, *Geotricum candidum* and *Rhodotorula rubra* DSM 70403). Present results showed that the extracts possessed antibacterial and antiyeast effect against the majority of the bacteria and all of the yeasts tested.

**Key Words: Antimicrobial activity, *Brachythecium campestre*, *Eurhynchium pulchellum*.**

## INTRODUCTION

Herbarium specimens usually need special treatment against insects and microorganisms, but bryophytes are almost free from attack by microorganisms. The Chinese, Europeans and North Americans have used bryophytes as medicine for hundreds of years. More than 400 years ago Chinese used some *Fissidens* sp. and *Polytrichum* sp. species as diuretics and hair growth stimulation tonics. North American Indians used *Bryum, Mnium, Philonotis* sp. and *Polytrichum juniperinum* to heal burns, bruises and wounds[1]. Several liverworts (*Bazzania, Frullania, Marchantia, Plagiochila, Porella* and *Radula* sp.) extracts have been used in antimicrobial, antifungal and antiviral activity[2]. *Conocephalum conicum, Mnium*

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*undulatum* and *Leptodictyum riparium* showed maximum antibacterial activity to pathogenic bacterial species[3]. *Rhynchostegium riparioides* extract has good antibacterial activity against Gram-negative bacteria in particular, *Echerichia coli, Proteus-mirabilis, Enterobacter cloaceae* and *Pseudomonas aeruginosa*[4]. *Pleurocheata squarrosa* extract showed antibacterial activity to some Gram-negative bacteria[5]. *Sphagnum junghuhnianum, Barbula javanica, Barbula arcuata, Brachythecium populeum, B. rutabulum, Mnium marginatum* and *Entodon cf rubicundus* were found to be most active against 5 Gram-positive, 6 Gram-negative bacteria and 8 fungi[6]. Sabovljevic *et al.*[7] showed that *Bryum argenteum* exhibited lower antimicrobial activities compared to the standard antibiotic. Dülger *et al.*[8] found that moss species such as *Grimmia pulvinata, Tortula subulata, Weisia contreversa, Leucodon sciuroides, Hypnum cupressiforme, Homalothecium sericeum, Neckera complanata* and *Mnium undulatum* had moderate activity against Gram-positive and Gram-negative bacteria.

The aim of this research was to investigate the possible activity of *Brachythecium campestre* and *Eurhynchium pulchellum* extracts against 10 bacteria and 9 yeasts.

## EXPERIMENTAL

*Brachythecium campestre* and *Eurhynchium pulchellum* were collected from Gokyar Mount, Hatay-Turkey in 2002. Voucher specimens were identified by Dr. Ozlem Tonguc Yayintas and deposited Canakkale Onsekiz Mart University Department of Biology Herbarium.

**Preparation of the extracts:** Samples of *Brachythecium campestre* and *Eurhynchium pulchellum* were treated with 0.8 % Tween 80 aqueous solution to remove epiphytic hosts normally found on the surface. They were washed in tap and distilled water and dried on filter paper. The samples were then extracted with 80 % ethanol in water under refluxed for 2 h at 40 ºC. The extracts were filtered through a 0.45 µm cellulose acetate membrane. The filtrates were oven-dried at 45 ºC. Then 80 mg of the dry residue was dissolved in 1 mL of dimethylsulfoxide[9].

**Test microorganisms:** A total of 19 microorganisms including 10 bacteria and 9 yeasts which were used in this study are presented successively as in the following: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 122228, *Streptococcus faecalis* NRRLB-14617, *Bacillus cereus* ATCC 11778, *Salmonella typhi* CCM 5445, *Proteus vulgaris* ATTC 6889, *Enterobacter aerogenes* ATCC 13043, *Pseudomonas aureginosa* ATTC 27853, *Escherichia coli* ATCC 25922, *Neisseria canis, Saccharomyces cerevisiae* ATCC 9763, *Saccharomyces colombia, Saccharomyces uvarum, Schizosaccharomyces pombe, Kluyveromyces marxianus, Debarymyces hansenii, Candida lypolitica, Geotricum candidum* and *Rhodotorula rubra* DSM 70403. All of the test microorganisms were obtained from culture collection of Ege University, Faculty of Science, Biology Department, Basic and Industrial Microbiology Section.

**Determination of antimicrobial activity:** The disc diffusion method was used to screen *in vitro* antimicrobial activity of moss extracts. Bacteria and yeasts cultures

were grown on Mueller-Hinton Agar (MHA) and Malt Extract Agar (MEA) plates, respectively. Bacteria were incubated at 37 ºC for 24 h by inoculation into Mueller-Hinton Broth (MHB), while yeasts were incubated at 30 ºC for 48 h by inoculation into Malt Extract Broth (MEB). Bacterial and yeast suspensions contained $10^8$ and $10^7$ cells per milliliter, successively. Mueller-Hinton Agar and Malt Extract Agar were sterilized in a flask and cooled to 45-50 ºC. Then, they were distributed in sterilized petri dishes with a diameter of 9 cm. The sterile paper discs (6 mm in diameter) were individually impregnated with 30 µL of the extract solutions. Then all discs were dried in 50 ºC and placed onto the agar plates which had previously been inoculated with the test microorganisms. The petri dishes were kept at 4 ºC for 2 h. The plates were then incubated at 37 ºC for 24 h for the bacteria and at 30 ºC for 48 h for the yeasts. The diameters of the inhibition zone were measured in millimeters. The extracts were tested in triplicate and the experiment was performed four times. Norfloxacin was used in parallel experiments in order to control the sensitivity of the test organisms. The results were expressed as mean values of all experiments[10,11].

## RESULTS AND DISCUSSION

Inhibition zones obtained from *Brachythecium campestre* ($E_1$), *Eurhynchium pulchellum* ($E_2$) and control against 10 test bacteria and 9 yeasts are presented in Table-1. It was found that inhibition zones obtained from $E_1$ and $E_2$ varied from 7 mm to 11 mm for bacteria, 7 mm to 18 mm for yeasts. The results indicated that $E_1$ and $E_2$ had different antimicrobial activity against tested bacteria. $E_1$ exhibited antibacterial activity to all the bacteria tested except Gram-positive *Bacillus cereus* and Gram-negative *Pseudomonas aureginosa*. Gram-positive *Staphylococcus epidermidis* was the most sensitive bacterium among all bacteria tested with the $E_1$, with the strongest inhibition zone of 11 mm.

This extract also displayed high antibacterial activity against some of Gram-positive bacteria such as *Staphylococcus aureus* (10 mm), *Streptococcus fecalis* (9 mm) and the Gram-negative bacteria *Salmonella typhi* (10 mm), *Enterobacter aerogenes* (10 mm), *Neisseria canis* (10 mm) and *Escherichia coli* (9 mm). $E_2$ showed antimicrobial activity against all Gram-positive and Gram-negative bacteria except *Bacillus cereus*. *Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli*, with the inhibition zone of 10 mm were more sensitive than the other three bacteria, *Salmonella typhi, Proteus vulgaris* and *Neisseria canis*, having the inhibition zone of 7 mm to the extract.

As seen clearly in Table-1, the inhibition zones obtained from $E_1$ and $E_2$ varied for yeasts. *Debarymyces hansenii, Saccharomyces uvarum* and *Kluyveromyces marxianus* were mostly sensitive to $E_1$ and $E_2$. Extracts also exhibited high antiyeast activity against *Rhodotorula rubra*. In addition, while *Candida lypolitica* was resistant to standard antibiotic, this yeast was sensitive to both $E_1$ and $E_2$.

TABLE-1
ANTIMICROBIAL ACTIVITY OF $E_1$ AND $E_2$ AND CONTROL (10 µL)

| Microorganisms | Zone of inhibition (mm)* | | |
|---|---|---|---|
| | $E_1$ | $E_2$ | Control** |
| | (30 µL) | | (10 µL) |
| **Gram-negative bacteria** | | | |
| *Salmonella typhi* | 10 | 7 | 27 |
| *Proteus vulgaris* | 7 | 7 | 12 |
| *Enterobacter aerogenes* | 10 | 10 | 23 |
| *Pseudomonas aureginosa* | - | 9 | 25 |
| *Escherichia coli* | 9 | 10 | 30 |
| *Neisseria canis* | 10 | 7 | 25 |
| **Gram-positive bacteria** | | | |
| *Staphylococcus aureus* | 10 | 9 | 20 |
| *Staphylococcus epidermidis* | 11 | 10 | 23 |
| *Streptococcus fecalis* | 9 | 8 | 21 |
| *Bacillus cereus* | - | - | 25 |
| **Yeasts** | | | |
| *Saccharomyces cerevisiae* | 7 | 8 | 25 |
| *Saccharomyces colombia* | 7 | 10 | 30 |
| *Saccharomyces uvarum* | 15 | 15 | 27 |
| *Kluyveromyces marxianus* | 15 | 12 | 26 |
| *Candida lypolitica* | 9 | 7 | - |
| *Rhodotorula rubra* | 10 | 10 | 25 |
| *Debarymyces hansenii* | 16 | 18 | 28 |
| *Schizosaccharomyces pombe* | 11 | 9 | 27 |
| *Geotricum candidum* | 8 | 8 | 23 |

*Values, including diameter of the filter paper disc (6.0 mm), are means of three replicate
**Norfloxacin was used as control for bacteria and yeasts
– Absence of inhibition.

The antibacterial activity of mosses against microorganisms has been shown earlier in different investigations; for instance, *Rhyncostegium riparioides* and *Pleurochaete squarrosa* extracts were more active against Gram-negative bacteria than Gram-positive bacteria[4,5]. In another study, Ilhan *et al.*[12] found that acetone extract of *Palustriella commutata* was effective against all Gram-negative bacteria tested and some Gram-positive bacteria such as *Bacillus mycoides*, *B. cereus*, *B. subtilis* and *Micrococcus luteus*. However, acetonic and methanolic extracts of *P. ommutata* were inactive against yeast and mould strains. Moreover, Dulger *et al.*[8] showed that while 8 different species of mosses were active against both Gram-positive and Gram-negative bacteria, these mosses displayed weak antiyeast activity. All these studies suggest that mosses have potential antimicrobial activity. The determination of the antimicrobial activities of mosses and their utilization in the preparation of the new antimicrobials are crucial.

In present study, it was found that $E_1$ and $E_2$ showed antimicrobial activity against most of the bacteria and all of the yeasts tested. The results of this study indicate that *Brachythecium campestre* and *Eurhynchium pulchellum* may be used for protection against both of the bacteria and yeasts in some applications. Further investigations are also needed to obtain more information on chemical composition of the moss species and their mechanism of action on microbial cells.

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