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Some Biological Activities of the Moss *Brachythecium populeum* (Hedw.) Bruch, Schimp. & W.Gumbel (Bryophyta)

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Highlights

- The paper focuses on antibacterial, antifungal and anticancer activities of extracts of *B. populeum*.
- Acetone and C extracts of *B. populeum* showed significant antibacterial activities.
- Higher doses of extract A of *B. populeum* decreased the survival of rat glioma cells in 48 h.

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Abstract

The present study assessed the antimicrobial and anticancer activities of *Brachythecium populeum (Hedw.) Bruch, Schimp. & W.Gümbel (Brachytheciaceae)* extracts using two different extracting methods. The antibacterial and antifungal activity of seven solvent extracts of *Brachythecium populeum* was examined against six species of bacteria and seven species of fungi by using well diffusion method. The minimal inhibitory concentration (MIC) of the extracts of acetone and C was determined. Extract C has the highest effect against *E. faecalis* (13 mm), *B. subtilis* and *S. aureus* (12 mm). The inhibition zone was showed by acetone extract against *E. faecalis* (13 mm), *P. aeruginosa* (10 mm) and *B. subtilis* (9 mm). Furthermore, all extracts exhibited different activities on *P. aeruginosa*. The MIC ranges acetone and C extracts against bacterial strains were from 93.8-375 µg/mL. Cytotoxic activities of extracts of acetone, A and C of *B. populeum* (0.17, 1.7, 17, 85 and 170 µg/mL) were tested against rat glioma cells by using MTT assay, after 24 and 48 h. Extracts of acetone, A and C showed a moderate toxicity, however high concentrations of extract A significantly decreased the survival of glioma cells in 48 h. The study confirms the antimicrobial and cytotoxic activities of *B. populeum*, extracted using various solvents. It is suggested that the active substances to be obtained by using different solvents from *B. populeum* may be the active substances of various drugs in the future. Further studies are needed.

1. INTRODUCTION

Bryophytes are non-vascular lands plants. It spread in many areas on the Earth. They consist of three divisions: mosses, liverworts and hornworts. The bryophytes consist of about 25.000 plant species. Turkish mosses consist of 726 species, subspecies and varieties representing 164 genera and 42 families [1,2].

Bryophytes, which have the ability to live in various habitats both inside and outside the ecosystem, are one of the important rings of the chain that make up the ecosystem. These plants usually continue their lives by forming colonies in different areas such as rocks, walls, tree trunks and soils. Bryophytes have been used in the Far East for therapeutic purposes such as in burns, cuts, wounds and so on since ancient times. Bryophytes include different bioactive compounds, such as essential oils, flavonoids, terpenoids, fatty acids and alcohols. Some bryophytes exhibit characteristic fragrant scents and an intense hot and bitter or saccharine-like taste. Mosses are not damaged by microorganisms, fungi, insects and slugs, but most of them have biochemical components and pharmacological properties that are under investigation [3-5]. Bryophytes indicated interesting biological activities such as antibacterial, antifungal, cytotoxic, antitumor, antiviral, antioxidant and so on. There has been a rapid increase in studies carried out since 2000's [6–9].

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There are no reports of the effects of extracts from *Brachythecium populeum* (Brachytheciaceae) on cytotoxic activities. The present study investigated antimicrobial and cytotoxic activities of different *B. populeum* extracts.

2. MATERIAL METHOD

2.1. Extraction Procedure

B. populeum was botanized on the Sundiken Mountains (Yukari Danisment, Eskisehir), at a height of 1370 m, on rock in June 2018. The specimen was identified by Savaroglu (Savaroglu 335) and was deposited at the Herbarium of the Department of Biology in Eskisehir Osmangazi University. Only green shoots were used for experimental work. *B. populeum* was treated in 0.8% Tween 80 aqueous solution and was then dried at room temperature. They were used by grinding. Extractions were done according to Savaroglu et al. (2018) and Jones and Kinghorn (2012) [9,10].

2.2. Microorganisms and Antimicrobial Activity

B. subtilis (NRRL B-209), *E. coli (*ATCC 25922), *E. faecalis (*ATCC 29212), *P. aeruginosa* (ATCC 27853), *S. typhimurium* (ATCC 14028), *S. aureus* (ATCC 25923), *A. flavus* (ATCC 9807), *A. fumigatus* (NRRL 163), *A. niger* (ATCC 10949), *A. parasiticus* (NRRL 465), *F. graminearum*, *F. solani* and *G. candidum* were used for antimicrobial evaluation. The antimicrobial activities were reported utilizing the method described by the The Clinical & Laboratory Standards Institute (CLSI). Bacteria were incubated in Mueller-Hinton Broth (MHB) at 35 ºC for 24 h. Fungi were incubated in Potato Dextrose Agar (PDA) at 27 ºC for 7 days. Bacteria and fungi suspensions were cultivated with a sterile swab on MHA and Sabouraud 4% Glucose Medium plates. Six wells, each 6 mm, were cut from the agar, and 20 µL of extract was placed into the well. Petri dishes with bacteria were incubated at 37°C for 24 h, and fungal strains were incubated at 27°C for 48 h. At the end of the incubation they were kept at 4°C for 2 h and their inhibition diameters were measured with a caliper in mm. Paper discs with only DMSO were used as negative controls. Studies were conducted in duplicate [8,9,11,12].

2.3. Minimum Inhibitory Concentration (MIC)

The microdilution method was used to determine MIC values according to CLSI [8].

2.4. Cytotoxic Activity

The rat glioma (C6) cells were purchased from American Type Culture Collection (ATCC) and were stored by freezing, and they were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich), 1% 100 U/mL penicilin and 100 µg/mL streptomycin solution (Biochrom) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The C6 cells were trypsinized and live cell quantity was measured by trypan blue (Sigma-Aldrich) assay. Then, the cells seeded into $2x10^4$ cells/well in 96 well plates for 24 h. Extracts were solved in DMSO, then concentrations were adjusted in DMEM (1:10). Extracts were wrapped in aluminum foil to protect them from light. Five different concentrations were added at least in eight wells and all experimental stages were repeated three times. After 24 or 48 h, extracts' toxicity was evaluated by MTT (Applichem) assay [13]. Formazan dye's absorbance was read by a microplate reader at 550 nm, and percentages of cell viability were determined by formula [8]. All data were analyzed statistically using one-way analysis of variance (ANOVA) and following Tukey's multiple comparison tests. $p<0.05$ was found to be significant.

3. RESULTS

3.1. Antimicrobial Activity

Antimicrobial activity results of all extracts from *B. populeum* are presented in Table 1. Acetone extract and Extract C showed significant antibacterial activities. Extract C has the high effect against *E. faecalis* (13 mm), *B. subtilis* (12 mm) and *S. aureus* (12 mm). The inhibition zone was produced by acetone extract against *E. faecalis* (13 mm), *P. aeruginosa* (10 mm) and *B. subtilis* (9 mm). Furthermore, all extracts had a certain effect on *P. aeruginosa*. Applied concentration of all extracts of *B. populeum* did not demonstrate any activity against fungal strains except extract C. The MIC of the extracts of acetone and C ranged from 93.8-375 µg/mL (Table 2). DMSO, which is used as a negative control, has no effect on microorganisms.

Table 1. *The in vitro antimicrobial activity of B. populeum extracts as zone of inhibition (mm)*

BACTERIA	**Me**	**K**	**As**	**A**	**B**	**C**	**D**	**C1***	**C2****
B. subtilis 209	—	9	9	10	—	12	—	13±0,2	24±0,1
E. coli 25922	—	—	—	—	—	—	—	30±0,2	25±0,1
E. faecalis 29212	—	—	13	7	—	13	—	27±0,2	15±0,1
P. aeruginosa 27853	11	11	10	11	9	10	8	30±0,2	20±0,1
S. typhimurium 14028	—	—	—	—	—	—	—	21±0,2	18±0,1
S. aureus 25923	—	—	—	10	—	12	—	35±0,2	27±0,1
FUNGI								**C3*****	
A. flavus 9807	—	—	—	—	—	—	—	7±0,1	
A. fumigatus 163	—	—	—	—	—	8	—	15±0,1	
A. niger 10949	—	—	—	—	—	—	—	13±0,1	
A. parasiticus 465	—	—	—	—	—	—	—	14±0,1	
F. graminearum	—	—	—	—	—	—	—	16±0,1	
F. solani	—	—	—	—	—	—	—	13±0,1	
G. candidum	—	—	—	—	—	—	—	11±0,1	

*Me, methanol; K, chloroform; As, acetone; A, B, C and D are the extracts from the second extraction method. Shown are mean values of duplicate tests; "—" indicates no significant inhibitory effect (<6 mm). *Penicillin (10 µg, Bioanalyses), **tetracycline (30 µg, Bioanalyses) and ***amphotericin B (10 µg, Sigma) were used as reference discs for control*

Table 2. *The MIC of the extracts of acetone and C ranged from 93.8-375 µg/mL*

Bacteria	As	C	Penicilin (µg/ml)	Tetracycline (µg/ml)
B. subtilis		187,50	*<1,5*	*<1,5*
E. faecalis	375,00	375,00		
S. aureus		93,80	*<1,5*	*<1,5*

3.2. Cytotoxic Activity of Extracts

The cytotoxic activity of all extracts at different concentrations on C6 cells were estimated in 24 and 48 h. While 170 µg/mL of acetone extract in 24 h decreased cell viability by 17 % ($p<0.001$), all concentrations of acetone extract decreased C6 cell viability by 13, 14, 14, 25 and 43 % ($p<0.001$), in 48 h, respectively (Figures 1, 2). Extract A at 85 and 170 µg/mL reduced C6 viability by 18 and 25 % in 24 h, respectively ($p<0.00$, Figure 1). All doses of extract A in 48 h diminished C6 number ($p<0.001$), the most effective concentration was found to be 85 and 170 µg/mL and reduced the survival by 55 and 67 %, respectively (Figure 2). Although 0.17 ($p<0.01$) and 1.7 µg/mL ($p<0.05$) of extract C concentrations stimulated C6 growth by 16 and 15 %, the highest concentration of extract C showed moderate reducing effect (15 %, $p<0.001$) on C6 viability in 24 h (Figure 1). In 48 h, all doses of extract C reduced C6 viability ($p<0.001$, Figure 2). The dead cell rate was determined by 43 % at 170 µg/mL.

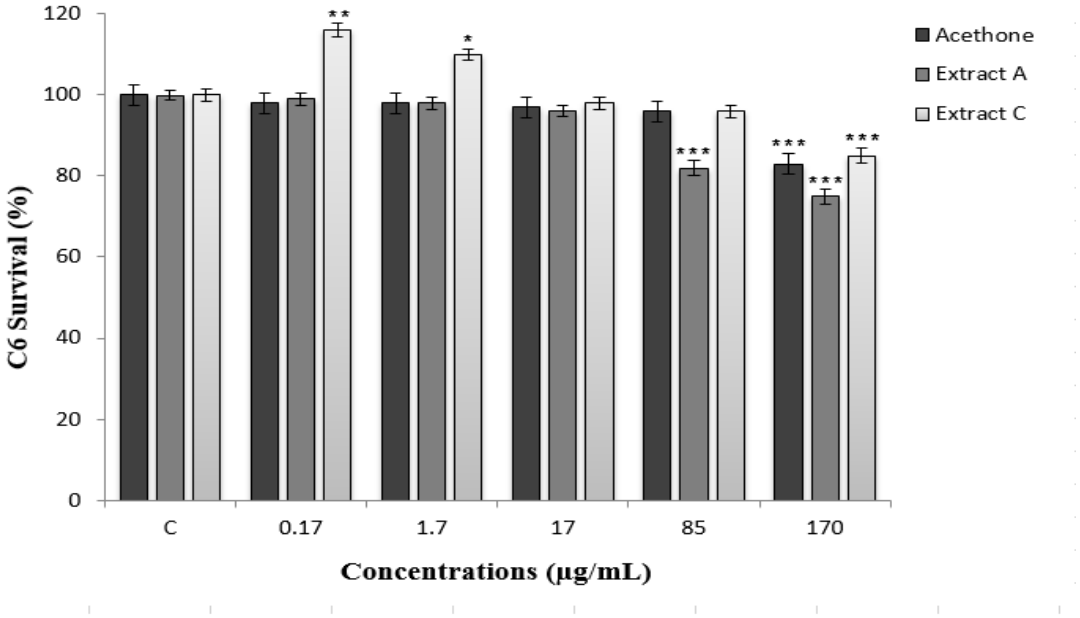

Figure 1. *The action of extracts of B. populeum on survival of rat glioma (C6) cells for 24 h (C: control, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)*

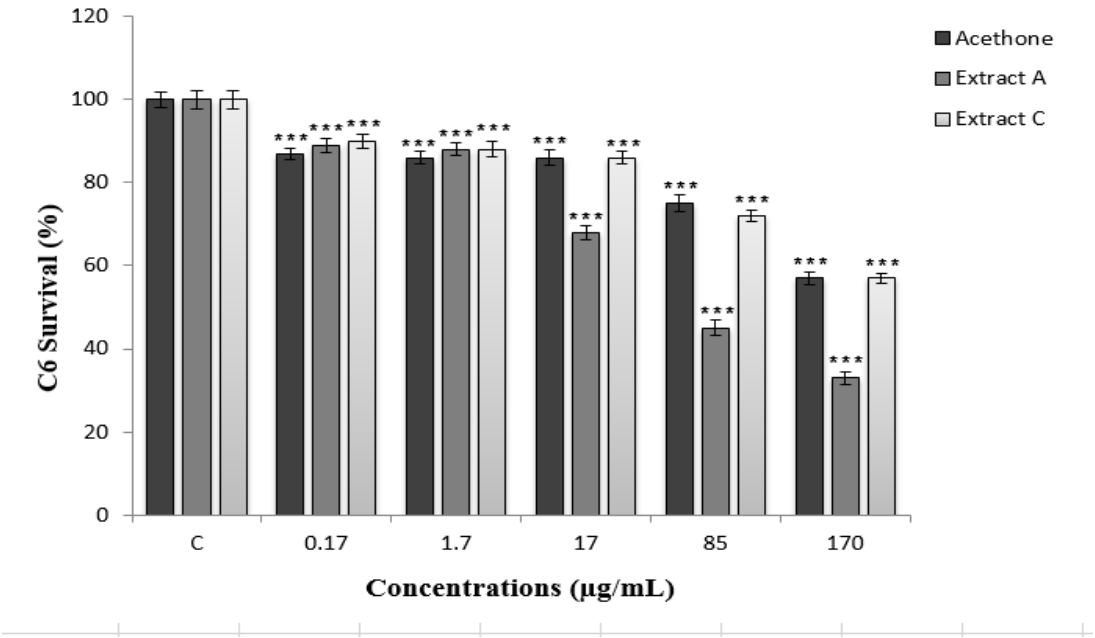

Figure 2. *The action of extracts of B. populeum on survival of rat glioma (C6) cells for 48 h (C: control, ***: p<0.001)*

4. DISCUSSION

We assayed the antimicrobial and growth inhibitory effects of some extracts of *B. populeum* against six bacterial, seven fungal species and a rat glioma cell line.

B. populeum extracts had activity against *E. faecalis*, *B. subtilis*, *S. aureus* and *P. aeruginosa*. The *B. populeum* extracts showed inhibitory effect against the gram positive and gram negative bacteria. Vollár et al. (2018) studied about antimicrobial activities of *B. rutabulum*, and reported that ethanol extract was the highest effect against gram positive, negative bacteria and fungi [14].

Acetone extract and extract C demonstrated most effective antimicrobial activities with 13 mm and 13 mm inhibition zone against *E. faecalis*. Yayıntas and Yapıcı (2009) reported that the ethanolic extract of *B. campestre* was the antimicrobial activity with 9 mm/30 µL inhibition zone against *S. faecalis* [15].

MIC values of the extracts were between 93.8-375 µg/mL. Extract C showed MIC of 93.8 µg/mL against *S. aureus*. Similarly, Singh et al. (2007) demonstrated that the ethanolic extract of *B. populeum* possessed the highest with a MIC of 1.56 µg/mL against *S. aureus* [16].

Extract A at the doses of 85 and 175 µg/mL has the strongest effect on C6 growth for 48 h. Only extract C at the 0.17 and 1.7 µg/mL stimulated proliferation of cells for 24 h, besides, it has not been determined after 48 h. Although cytotoxicity was prominent on C6 growth, depending upon dose and time for 48 h., 170 µg/mL of extract C suppresses proliferation for 24 h. Acetone extract, in the two highest concentrations, reduced cell viability by 25 and 43 % in 48 h. In support of our data, different studies showed that some bryophytes extracts have toxicity on cancer cells [17,18]. Ether extract of Frullania species decreased cell viability of two different cancer cells. The EC_{50} values were calculated 1.6 and 11.2 µg/mL (KB cells) and 6.7 and 1.6 µg/mL (HL-60 cells), respectively [19]. In addition, in our former articles, we determined that *Fontinalis antipyretica* (extract C) reduces the number of glioma cells in the highest two doses [20]. Similarly, it was observed that among three different extracts obtained from *Homalothecium sericeum* and *Aulocomnium androgynum*, extract C again has strong cytotoxic activity in glioma cells [8,21]. In another study with *Dicranum scoparium*, methanol, B and C extracts were tested on glioma cell viability, and it was determined that extract C had high cytotoxic activity [9]. Also, Vollár et al. (2018) obtained two different *Brachythecium rutabulum* extracts and examined the antiproliferative effects of extracts A and B

on cervix epithelial adenocarcinoma (HeLa), ovarian carcinoma (A2780), and invasive ductal breast carcinoma (T47D) cells for 72 h [14]. They found that both extracts have cell inhibitory action on all cell lines treated. Furthermore, extracts were most effective on HeLa cells.

Our study indicates that *B. populeum* extracts might possess different types of antimicrobial and cytotoxic molecule(s). Therefore, the compounds contained in different extracts could be investigated in prospective studies.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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