

EXPERIMENTAL PAPER

Antibacterial activity of ethanolic extracts of some moss species

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Summary

Introduction: For centuries, mosses have been used in traditional medicine due to their antibacterial, anti-fungal, and antiviral activities.

Objective: The present study was designed to evaluate the antibacterial activity of ethanolic extracts obtained from 12 moss species: *Brachythecium albicans*, *Bryum argenteum*, *Ceratodon purpureus*, *Dicranum scoparium*, *Dryptodon pulvinatus*, *Orthotrichum anomalum*, *Oxyrrhynchium hians*, *Plagiomnium undulatum*, *Polytrichum juniperinum*, *P. piliferum*, *Schistidium crassipilum*, and *Syntrichia ruralis*.

Methods: The antimicrobial activity of extracts was investigated against three Gram(+) bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) and two Gram(-) bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), using the agar disc-diffusion method.

Results: The high activity against all investigated bacteria was determined for extracts of *D. pulvinatus*, *P. undulatum*, *B. argenteum*, *S. crassipilum*, *O. anomalum* (mean inhibition zone: 11.3–13.1 mm) and to a lesser extent in the case of *D. scoparium* (8.3 mm). Extracts from *P. juniperinum* and *P. piliferum* showed activity only against Gram-positive bacteria, with an inhibition zone from 7.3 to 9.7 mm. Four species: *B. albicans*, *C. purpureus*, *O. hians*, and *S. ruralis* had not antibacterial properties.

Conclusions: The obtained results indicate that mosses could be a significant source of antibacterial agents. For the first time, we presented antibacterial activity of ethanolic extracts from *S. crassipilum* and *O. anomalum*.

Key words: *antibacterial activity, mosses, agar disc-diffusion method, Dryptodon pulvinatus, Schistidium crassipilum, Orthotrichum anomalum*

INTRODUCTION

Bryophytes are the large group of plants with about 20 000 species [1]. In Poland, they consist of about 950 species, mainly mosses (around 700 taxa) [2], also liverworts (about 250 species) and only 4 taxa of hornworts [3]. Bryophytes are of a great ecological importance, often colonizing uninhabited places like rocks, walls, tree trunks, soils. They grow from tropical zone to arctic and alpine regions, possessing a high bioindication value [4-6].

In many countries, bryophytes are widely used, first of all in horticulture as a substrate for growing plants (peat), but also as a material for decoration, packing, bedding, building, heating, etc. They play an important role in the oriental gardens and in the traditional medicine of China, Japan and India [7]. Nowadays, the increase of the antibiotic resistance and clinical problems in infection treatment are observed. Therefore, it returns to alternative treatment methods like bacteriophages, cationic antimicrobial peptides and active substances extracted from plants [8, 9].

Bryophytes were used as medicines for centuries in Asia and North America. Over 400 years ago, the Chinese used some *Polytrichum* and *Fissidens* species as diuretics and hair growth stimulators. Traditional cultures in India and North America utilized *Bryum*, *Mnium*, *Philonotis* spp. and *Polytrichum juniperinum* for healing burns, bruises and wounds. *Marchantia polymorpha* was used in the treatment of liver disorders. During World War I, dried peat (*Sphagnum*) was used instead of bandages [10, 11]. In Poland, a mud is used – a substance based on the remains of bryophytes, exhibiting biological activity and medicinal properties, utilized in rheumatology, dermatology, gynaecology, ophthalmology, orthopedics, and dentistry [12]. Literature data show antibacterial [13-15], antifungal [16, 17] and antiviral [13, 18] activities of some liverwort and moss species. In many liverworts and moss of *Polytrichum juniperinum*, anticancer properties were also documented [19, 20]. In bryophytes, a lot of bioactive compounds, such as flavonoids, terpenoids, alcohols, fatty acids, and essential oils were determined [19-21].

The aim of this study was the *in vitro* investigation of antibacterial activity of ethanolic crude extracts obtained from 12 moss species (*Brachythecium albicans*, *Bryum argenteum*, *Ceratodon purpureus*, *Dicranum scoparium*, *Dryptodon pulvinatus*,

Orthotrichum anomalum, *Oxyrrhynchium hians*, *Plagiomnium undulatum*, *Polytrichum juniperinum*, *P. piliferum*, *Schistidium crassipilum*, and *Syntrichia ruralis*) against 5 bacterial strains (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*).

MATERIAL AND METHODS

Plant material

Moss samples were collected in the area of Poznań (West Poland) on February–May 2017. Voucher specimens were identified by authors, checked by Dr. Anna Rusińska (Natural History Collections, Adam Mickiewicz University, Poznań, Poland) and deposited in the Institute of Natural Fibres and Medicinal Plants (Poznań, Poland). In this work, 12 common moss species from 9 families were investigated (behind the name of the species, a habitat is given):

Brachytheciaceae

- *Brachythecium albicans* (Hedw.) Schimp, sand soil among the grassland
- *Oxyrrhynchium hians* (Hedw.) Loeske (syn. *Eurhynchium hians* /Hedw./ Sande Lac.), soil in the park

Bryaceae

- *Bryum argenteum* Hedw., slits between paving tiles

Dicranaceae

- *Dicranum scoparium* Hedw., ground in thickets
- *Ditrichaceae*
- *Ceratodon purpureus* (Hedw.) Brid., soil

Grimmiaceae

- *Dryptodon pulvinatus* (Hedw.) Brid. (syn. *Grimmia pulvinata* /Hedw./ Sm.), concrete wall
- *Schistidium crassipilum* H.H. Blom, concrete wall

Mniaceae

- *Plagiomnium undulatum* (Hedw.) T.J. Kop., wet ground in thickets

Orthotrichaceae

- *Orthotrichum anomalum* Hedw., concrete wall

Polytrichaceae

- *Polytrichum juniperinum* Hedw., psammophilous grassland
- *Polytrichum piliferum* Hedw., psammophilous grassland

Pottiaceae

- *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr, soil-gravel base

Extract preparation

Plant material was treated with 0.8% Tween 80 aqueous solution to remove organic and inorganic pollutants. Then, samples were washed in tap and distilled water, and dried at room temperature for 1–2 weeks [15]. Plant extracts were prepared according to the methods described by Elibol *et al.* [22] and More *et al.* [23], with slight modifications. Air-dried plant material (50 g) was extracted with 96% ethanol for 4 days at a room temperature. A 5:1 ratio (solvent:plant material) was used. The extracts were filtered through a 22 µm membrane filter, and then the solutions were evaporated at 30°C until dryness. The prepared crude mixtures were dissolved in 10% water solution of dimethyl sulfoxide – DMSO (Sigma) to a final concentration of 100 mg/ml, and stored at -20°C until use.

Antimicrobial activity

In this study, clinical strains of bacteria were used. Antimicrobial activity of crude moss extracts were investigated against three Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*), and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The species of bacteria were grown at 35°C for 24 h, in Mueller-Hinton Agar and Mueller-Hinton Broth (Oxoid).

The microbial growth inhibitory potential of the extracts was determined by using the agar disc-diffusion method according to recommendations of CLSI [24]. Inocula were prepared by mixing a few microbial colonies with sterile 0.9% NaCl solution to receive the concentration of 0.5 McFarland [25] which is equivalent to $(1-2) \times 10^8$ CFU/ml. 100 µl of all bacterial suspensions were inoculated on Mueller-Hinton Agar (Oxoid). The extracts dissolved in 10% water solution of DMSO (Sigma) in final concentration of 100 mg/ml were used. 20 µl (2 mg/disc) of the plant extracts were transferred onto sterile filter papers (6 mm diameter). Additionally, sterile filter papers were soaked 20 µl of 2% chlorhexidine digluconate (positive control) and 20 µl of 10% DMSO (negative control). The plates were incubated at 35°C for 18 h. The experiments were three times repeated.

Statistical analysis

Values shown in the table are means of three parallel measurements. Data were processed using Statistica for Windows. Statistical analysis of the results was based on Mann-Whitney U-test. Differences of $p < 0.05$ were considered significant.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

The values of growth inhibition zones (ZOI) of tested bacteria obtained in the agar disc-diffusion assay are shown in table 1. Among 12 investigated moss species, 8 taxa exhibited antibacterial activity. *Dryptodon pulvinatus* (ZOI = 11.7–15.0 mm) (fig. 1-2) and *Plagiomnium undulatum* (ZOI = 11.0–14.3 mm) showed the highest antibacterial properties. Significant antibacterial activity had also ethanolic extracts of *Bryum argenteum*, *Schistidium crassipilum*, and *Orthotrichum anomalum*. Extract from *Dicranum scoparium* had weaker antibacterial activity than above-mentioned ones. On the other hand, two extracts (from *Polytrichum juniperinum* and from *P. piliferum*) showed weak activity against Gram-positive bacteria and lack of activity against Gram-negative bacteria. In this study, the tendency of bacterial sensitivity to active moss extracts was observed as follows: *Streptococcus pyogenes* > *Staphylococcus aureus* > *Enterococcus faecalis* > *Escherichia coli* > *Klebsiella pneumoniae*. However, there were no significant differences ($p > 0.05$) between ZOI values of individual bacteria species. In the case of four ethanolic extracts of mosses (*Brachythecium albicans*, *Ceratodon purpureus*, *Oxyrrhynchium hians*, and *Syntrichia ruralis*), antibacterial activity was not observed.



Figure 1.

Dryptodon pulvinatus

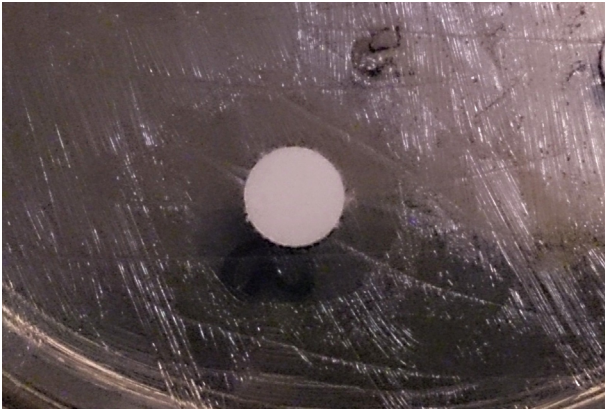


Figure 2.

Inhibition zone near disk with ethanolic extract of *Dryptodon pulvinatus* in the culture of *Streptococcus pyogenes* on Mueller-Hinton agar plate

DISCUSSION

Survey of the literature data shows that the studies of the antibacterial activity of ethanolic extracts from *B. albicans*, *C. purpureus*, *O. anomalum*, *O. hians*, and *S. crassipilum* were made for the first time. Among them, two species: *S. crassipilum* (ZOI range of 9.7–14.3 mm) and *O. anomalum* (9.7–13.7 mm) showed high antibacterial activities, especially in the case of Gram-positive bacteria: *E. faecalis*, *S. pyogenes*,

and *S. aureus*. Additionally, high antibacterial activity against selected Gram-positive and Gram-negative bacteria was determined for *B. argenteum*, *D. scoparium*, *D. pulvinatus*, and *P. undulatum* (tab. 1).

Antibacterial activity of *B. argenteum* was presented by Sabovljevic *et al.* [26]. They determined antimicrobial activities of different ethanolic extracts from *B. argenteum* against all the species tested, including *S. aureus* and *E. coli*. However, they used other method for determining antibacterial activity, namely the microdilution, in which minimal inhibition concentration (MIC) is tested. Also Krishnan *et al.* [27] presented good antibiotic activity of *B. argenteum* in microdilution method, using benzene, chloroform, petroleum ether, methanol and water extracts. Whereas, Bodade *et al.* [28] described relatively low activity (ZOI range of 9–11 mm) of *B. argenteum* ethanol and chloroform extracts against *S. aureus*, *E. coli*, and *K. pneumoniae*.

In our work, ethanolic extract of *D. pulvinatus* had the best antibacterial activity. Dulger *et al.* [29] presented, that the methanol extracts of mosses (including *Grimmia pulvinata* = *D. pulvinatus* and *Mnium undulatum* = *P. undulatum*) possess a moderate activity against Gram-positive and Gram-negative bacteria, including *S. aureus*, *E. coli* and *K. pneumoniae*. For extracts from above two mosses in their studies, ZOI were lower than in our results. Moreover, they showed lack of activity of *D. pulvinatus*

Table 1.

Antibacterial activity of ethanolic moss extracts determined by the agar disc-diffusion method

Moss species	Bacteria and diameter of inhibition zone (ZOI) [mm]					Mean of ZOI
	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	
<i>Dryptodon pulvinatus</i>	15.0	13.3	11.7	13.0	12.3	13.1
<i>Plagiomnium undulatum</i>	14.3	11.0	12.0	13.3	11.7	12.5
<i>Bryum argenteum</i>	14.7	12.3	11.0	10.3	11.0	11.9
<i>Schistidium crassipilum</i>	14.3	13.0	11.7	10.3	9.7	11.8
<i>Orthotrichum anomalum</i>	13.7	11.7	11.3	9.7	10.3	11.3
<i>Dicranum scoparium</i>	10.7	7.7	8.0	7.7	7.3	8.3
<i>Polytrichum juniperinum</i>	9.7	8.3	8.3	6.0	6.0	7.7
<i>Polytrichum piliferum</i>	8.7	8.0	7.3	6.0	6.0	7.2
<i>Brachythecium albicans</i>	6.0	6.0	6.0	6.0	6.0	6.0
<i>Ceratodon purpureus</i>	6.0	6.0	6.0	6.0	6.0	6.0
<i>Oxyrrhynchium hians</i>	6.0	6.0	6.0	6.0	6.0	6.0
<i>Syntrichia ruralis</i>	6.0	6.0	6.0	6.0	6.0	6.0
Mean of ZOI	10.4	9.1	8.8	8.4	8.2	
CHX	20.3	18.7	18.0	18.0	18.0	18.6
DMSO	6.0	6.0	6.0	6.0	6.0	6.0

CHX – chlorhexidine digluconate, DMSO – dimethyl sulfoxide

extract against *K. pneumoniae*. These differences may result from the use of other solvents: we applied ethanol, whereas Dulger *et al.* used methanol [29]. In our work, ethanolic extract of *P. undulatum* had the second best antibacterial activity and it worked on all bacterial strains. However, our results differ from work of Nikolajeva *et al.* [14] in which antibacterial activity against *S. aureus* was not established for *P. undulatum* ethanolic and water extracts.

In the present study, we observed that extract from *D. scoparium* had weak antibacterial activity. In the paper of Veljić *et al.* [30], antibacterial activity of methanol extracts of this species in disc-diffusion method only against *Staphylococcus epidermidis* was determined. In relation to other bacteria, including *S. aureus* and *E. coli*, they stated lack of activity of this extract. Nikolajeva *et al.* [14] indicated that the ethanolic extract of *D. scoparium* had highest antimicrobial activity against *S. aureus* among studied moss extracts.

Two extracts – from *P. juniperinum* and *P. pili-ferum* showed weak activity against Gram-positive bacteria and lack of activity against Gram-negative bacteria (tab. 1). In work of Savaroglu *et al.* [31], methanol, chloroform and acetone extracts of *P. juniperinum* indicated the best inhibitory effect against *S. aureus* and *Pseudomonas aeruginosa*. At the same time, they have shown that only chloroform extract of this moss had activity against *E. coli*.

In our studies, four ethanolic extracts from mosses (*B. albicans*, *C. purpureus*, *O. hians*, and *S. ruralis*) had not antibacterial activity. Elibol *et al.* [22] indicated that ethanolic and methanolic extracts of *S. ruralis* had an inhibition effect against *E. coli*, *Salmonella* and yeast *Saccharomyces cerevisiae*, while it did not work against *S. aureus*, *Pseudomonas* and *Bacillus*. Moreover, acetone and chloroform extracts were inactive against all tested microorganisms.

Giving above into consideration, our results indicate that mosses could be a significant source of antibacterial agents. Additionally, for the first time we presented antibacterial activity of ethanolic extracts from *S. crassipilum* and *O. anomalum*.

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Conflict of interest: Authors declare no conflict of interest.

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Aktywność przeciwbakteryjna wyciągów etanolowych wybranych gatunków mchów

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Streszczenie

Wstęp: Od wieków mchy były stosowane w tradycyjnej medycynie, ze względu na ich działanie przeciwbakteryjne, przeciwgrzybicze i przeciwwirusowe.

Cel: Celem niniejszej pracy było określenie aktywności przeciwbakteryjnej wyciągów etanolowych otrzymanych z 12 gatunków mchów: *Brachythecium albicans*, *Bryum argenteum*, *Ceratodon purpureus*, *Dicranum scoparium*, *Dryptodon pulvinatus*, *Orthotrichum anomalum*, *Oxyrrhynchium hians*, *Plagiomnium undulatum*, *Polytrichum juniperinum*, *P. piliferum*, *Schistidium crassipilum* i *Syntrichia ruralis*.

Metody: Działanie przeciwbakteryjne wyciągów badano wobec trzech bakterii Gram-dodatnich (*Enterococcus faecalis*, *Staphylococcus aureus* i *Streptococcus pyogenes*) oraz dwóch bakterii Gram-ujemnych (*Escherichia coli* i *Klebsiella pneumoniae*), stosując metodę dyfuzyjno-krążkową.

Wyniki: Wysoką aktywność przeciwko wszystkim badanym bakteriom stwierdzono w przypadku wyciągów uzyskanych z *D. pulvinatus*, *P. undulatum*, *B. argenteum*, *S. crassipilum* i *O. anomalum* (średnia strefa zahamowania wzrostu: 11.3–13.1 mm), a niższą dla *D. scoparium* (8.3 mm). Wyciągi z *P. juniperinum* i *P. piliferum* wykazały aktywność jedynie wobec bakterii Gram-dodatnich. Ekstrakty z *B. albicans*, *C. purpureus*, *O. hians* i *S. ruralis* nie posiadały właściwości przeciwbakteryjnych.

Wnioski: Uzyskane wyniki wskazują, że mchy mogą mieć znaczenie jako źródło związków działających przeciwbakteryjnie. Były to pierwsze badania wykazujące przeciwbakteryjną aktywność wyciągów etanolowych uzyskanych z *S. crassipilum* i *O. anomalum*.

Słowa kluczowe: *aktywność przeciwbakteryjna, mchy, metoda dyfuzyjno-krążkowa, Dryptodon pulvinatus, Schistidium crassipilum, Orthotrichum anomalum*