INTRODUCTION

The genuine mosses are a large group of nonvascular higher plants consisting of about 14,000 species. Generally, bryophytes are not damaged by microorganisms, insects, snails, slugs, and small mammals. To date, over several hundred new compounds have been isolated from bryophytes and their structures elucidated (Asaka et al., 1995, 1999, 2001). In spite of a number of secondary metabolites identified from various mosses, chemical profiles of most species are insufficiently known or even unknown. The secondary metabolites from mosses identified so far are: terpenoids, flavonoids, and bibenzyls, and derivatives of fatty acids (Borel et al., 1993); acetophenols (Lorimers et al., 1993); aryl benzofurans (Von Reusz and König, 2004). Basile et al. (1999) showed that 7-O-flavonoids (apigenin, apigenin-7-O-triglycoside, luteolin-7-O-neohesperidoside, lucenin-2, saponarin, and vitexin) possess antimicrobial activity. Markham and Given (1987) demonstrated that species of the genus Bryum are rich in flavonoid glycosides (apigenin and luteolin glycosides and their 6’malonyl esters, 8-hydroxyapigenin-7-O-glucoside and 8-hydroxyluteolin-7-O-glucoside). Apart from monoflavonoids, mosses are characterized by biflavonoids, which are not present in liverworts. It has been shown that mosses rich in flavonoids possess strong antimicrobial activity.

A number of bryophytes, mosses in particular, have been widely used as medicinal plants. Some species are used in traditional medicine for treating skin infections and other diseases. Thus, Marchantia polymorpha has been used to cure liver and gall bladder diseases. About 40 moss species are used in Chinese traditional medicine because of their medicinal properties. Some species are still in use for treatment of hepatitis and inflammatory processes (Hu, 1987). Sphagnum spp. are used for treatment of eye diseases. Rhodobryum species have been used for treatment of cardiovascular disorders. In North America and the Himalayas, Indians used Bryum, Mnium, and Philonotis species to make various pre-

ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF FONTINALIS ANTIPYRETICA, HYPNUM CUPRESSIFORME, AND CTENIDIUM MOLLUSCUM

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Abstract — Antibacterial and antifungal activities of methanol extracts of the moss species Fontinalis antipyretica Hedw. var. antipyretica, Hypnum cupressiforme Hedw., and Ctenidium molluscum (Hedw.) Mitt. were analyzed. Antimicrobial activity was tested against Gram (+) (Bacillus subtilis, Micrococcus flavus, and Staphylococcus epidermidis) and Gram (-) (Escherichia coli and Salmonella enteritidis) bacteria. Antifungal activity of extracts was tested using the following microfungi: Trichoderma viride, Penicillium funiculosum, P. ochrochloron, Aspergillus fumigatus, A. flavus, and A. niger. The methanol extract of Fontinalis antipyretica showed the strongest activity against the tested bacteria and microfungi. The antibacterial effect of methanol extracts was higher against the G (-) (Escherichia coli and Salmonella enteritidis) than against the G (+) bacteria tested.

Key words: Mosses, methanol extracts, antibacterial activity, antifungal activity

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parations (for treatment of burns and wounds) and *Marchantia polymorpha* for treatment of furuncles and blisters (Flowers, 1957).

The active compounds isolated from these species are biflavones, flavone glycosides, and diglycosides (Markham and Given, 1988; Cambie, 1996). Clinical tests have shown that some mosses are effective in treatment of skin diseases. *Plagiochasma appendiculatum* possesses significant antibacterial and antifungal activities (Singh et al., 2006). Pharmacological investigations of mosses have intensified over the last two decades. *Hypnum cupressiforme* contains several biflavonoids (hypnogenol B1 and hipnumflavonoid A) which showed antibacterial activity (Sievers et al., 1993; Dulger et al., 2005). Investigations of Sabovljević et al. (2006) demonstrated the antimicrobial activity of ethanol extracts of *Bryum argenteum*. Our very recent investigations also showed that methanol extracts of selected genuine mosses (*Pleurozium schreberi*, *Palustriella commutata*, *Homalothecium philippeanum*, *Anomodon attenuatus*, *Rhytidium rugosum*, *Hylocomium splendens*, *Dicranum scoparium*, and *Leucobryum glaucum*) possess antimicrobial activity (Veljić et al., 2005).

The aim of this work was to test the activity of methanol extracts of *Fontinalis antipyretica* var. *antipyretica*, *Hypnum cupressiforme*, and *Ctenidium molluscum* collected in Serbia against selected bacteria and micromycetes.

**MATERIAL AND METHODS**

In this experiment, methanol extracts of the following species were used: *Fontinalis antipyretica* var. *antipyretica*, *Hypnum cupressiforme*, and *Ctenidium molluscum* collected in Serbia against selected bacteria and micromycetes.

**RESULTS AND DISCUSSION**

The results of testing the antibacterial activity of moss methanol extracts are presented in Tables 1 and 2. Those obtained by the disk diffusion method are presented in Table 1.

It is evident that all extracts showed bactericidal activity at a concentration of 20 mg/ml. *Escherichia coli* and *S. enteritidis* were more susceptible (reacting to a concentration of 10 mg/ml). A strong bactericidal effect was exerted by extract of the moss *Hypnum cupressiforme* (10 mg/ml) against *S. enteritidis*. The values of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) are given in Table 2.

The strongest effect was manifested by extract of *Fontinalis antipyretica*. Extract of this species was active against *M. flavus* at a concentration of 0.5 mg/disk. The species *C. molluscum* and *H. cupressiforme* were also active. The extract of *C. molluscum* did not show activity against *M. flavus*. *Hypnum cupressiforme* showed the strongest effect against *M. flavus* at a concentration of 2 mg/disk.
The most resistant bacterial species, as in the microdilution method, was *Staphylococcus epidermidis*. Extracts at a concentration of 0.5 mg/disk were not active against *Micrococcus flavus*, while higher concentrations showed wide zones of inhibition. The most susceptible bacteria were *Escherichia coli* and *Bacillus subtilis*. When this method was used, Amoxycilin showed activity significantly stronger than that of moss extracts.

Antifungal activity of moss extracts was analyzed by the microdilution method against six micromycetes: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium funiculosum*, *Penicillium ochrochloron*, and *Trichoderma viride*. The minimal fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) are presented in Table 3.

All tested fungi were very susceptible at extract concentrations of 10 and 5 mg/ml, and MFC values were 5 mg/ml in the majority. The most active was methanol extract of *Fontinalis antipyretica*, since it inhibited growth of most micromycetes at a concentration of 2.5 mg/ml. Bifonazol showed an effect significantly stronger than those of the analyzed extracts (MIC 0.1-0.5 mg/ml; MFC 0.1-1 mg/ml).

Antimicrobial activity of methanol extract of *Hypnum cupressiforme* was also analyzed recently by Dulger et al. (2005). According to those results, the extract inhibited growth of bacteria and fungi at a concentration of 30 mg/ml. The isolated substances were polycyclic hydrocarbons, biflavonoids, and dihydroflavonols. In the present work, methanol extract of *H. cupressiforme* was active against the tested bacteria at concentrations of 10 and 20 mg/ml and against micromycetes at a concentration of 5 mg/ml.

In our study, methanol extract of *Fontinalis antipyretica* possessed moderate antimicrobial activity, while *Ctenidium molluscum* showed low activity against the bacteria and micromycetes tested.
The antifungal activity of the analyzed moss species was higher than their antibacterial activity. The antibacterial effect of methanol extracts was higher against G (-) (Escherichia coli and Salmonella enteritidis) than against G (+) bacteria.

Together with previously published data, our results indicate that mosses and liverworts could be useful as sources of new antibacterial and especially antifungal agents. Fractionation, isolation, and characterization of secondary metabolites might lead to introduction of new active compounds for possible application in pharmacy after further pharmacological tests.

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REFERENCES


Table 2. Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of methanol extracts of investigated mosses.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Minimal inhibitory concentration (MIC) (mg/ml)</th>
<th>Minimal bactericidal concentration (MBC) (mg/ml)</th>
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<tr>
<td></td>
<td>F. antipyretica</td>
<td>C. molluscum</td>
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<tr>
<td>E. coli</td>
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</tr>
<tr>
<td>S. enteritidis</td>
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</tr>
<tr>
<td>S. epidermidis</td>
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<td>20.0</td>
</tr>
<tr>
<td>B. subtilis</td>
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<td>20.0</td>
</tr>
<tr>
<td>M. flavus</td>
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<td>20.0</td>
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</table>

Table 3. Antifungal activity of methanol extracts of selected mosses. Abbreviations as in Table 2.

<table>
<thead>
<tr>
<th>Mosses</th>
<th>Minimal inhibitory concentration (MIC) (mg/ml)</th>
<th>Minimal bactericidal concentration (MBC) (mg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F. antipyretica</td>
<td>C. molluscum</td>
</tr>
<tr>
<td>A. flavus</td>
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<td>5.0</td>
</tr>
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<td>A. fumigatus</td>
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</tr>
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<td>A. niger</td>
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</tr>
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<td>P. funiculosum</td>
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<td>P. ochrochloron</td>
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<td>5.0</td>
</tr>
<tr>
<td>T. viride</td>
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ANIMICROBNA AKTIVNOST METANOLNIH EKSTRAKATA
FONITALIS ANTIPYRETICA, HYPNUM CUPRESSIFORME I CTENIDIIUM MOLLUSCUM

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Аналитирана је антимикробна и антифунгалина активност метанолних екстраката врста Fontinalis antipyretica Hedw. вар. antipyretica, Hypnum cupressiforme Hedw. и Ctenidium molluscum (Hedw.) Mitt. Антимикробна активност је тестирана на грам (+) (Bacillus subtilis, Micrococcus flauvs и Staphylococcus epidermidis) и грам (-) бактерије (Escherichia coli и Salmonella enteritidis). Као подлога за антифунгалну активност коришћене су гљиве: Trichoderma viride, Penicillum funiculosum, P. ochrochloron, Aspergillus fumigatus, A. flavus и A. niger. Метанолни екстракт Fontinalis antipyretica показао је најјаче дејство на тестиране бактерије и микромицете. Антибактеријски ефекат метанолних екстраката је био знатно јачи на грам (-) (Escherichia coli и Salmonella enteritidis) него на грам (+) бактерије.