Arch. Biol. Sci., Belgrade, 61 (2), 225-229, 2009 DOI:10.2298/ABS0901225V

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

M. VELJIĆ[1], ALEKSANDRA ĐURIĆ[1], MARINA SOKOVIĆ[2], ANA ĆIRIĆ[2], JASMINA GLAMOČLIJA[2,] and P. D. MARIN[1]

[1]*Institute of Botany and Jevremovac Botanical Garden, Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia
[2]*Siniša Stanković Institute for Biological Research,* 11060 Belgrade, Serbia

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 micromycetes: *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 micromycetes. 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

UDC 582.32:581.2:579.6

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 (Asakawa, 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); arylbenzofurans (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 blader 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-

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., 2008).

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* Hedw. var. *antipyretica*, collected 23.11.1994, locality: Sušica (Voucher No. 16166); *Hypnum cupressiforme* Hedw., collected 03.06.1997, locality: Djavolja Varoš (Voucher No. 16170); and *Ctenidium molluscum* (Hedw.) Mitt., collected 14.06.2006, locality: Rača-Ladjevac (Voucher No. 16169). All species were identified by the senior author.

The extracts were tested against the following bacteria: *Staphylococcus epidermidis* (Winslow & Winslow) Evans (ATCC 12228), *Micrococcus flavus* Trevisan (ATCC 10240), *Bacillus subtilis* Cohn (ATCC 10707), *Escherichia coli* (Migula) Castellani & Chalmers (ATCC 25922), *Salmonella enteritidis* (Geartner) Castellani & Chalmers (ATCC 13076), and the synthetic antibiotic Amoxycilin. Antibacterial assays were carried out by a modified disk-diffusion method (Verpoorte et al., 1983) and the microdilution method (Hanel and Raether, 1988; Daouk et al., 1995).

Antifungal activity was tested using the following species: *Aspergillus flavus* Link ex Fr. (ATCC 9170), *Aspergillus fumigatus* Fresenius (human isolate), *Aspergillus niger* Linx ex Fr. (ATCC 6275), *Penicillium funiculosum* Thom (ATCC 10509), *Penicillium ochrochloron* Biourge (ATCC 9112), *Trichoderma viride* Pers. ex Fr. (ATCC IAM 5061), *Candida albicans* (Robin) Berkhout (isolated directly from patients at the Center for Preventive Medicine, MMA, Belgrade, Serbia), and the synthetic fungicide Bifonazol. The disk diffusion method was used to test antifungal activity of extracts.

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.

Table 1. Antibacterial activity of methanol extracts of *Fontinalis antipyretica*, *Hypnum cupressiforme*, and *Ctenidium molluscum* as determined by the disk diffusion method.

Bacteria	Zone of inhibition (mm)	Concentration of moss extract: 2 mg/disk Concentration of Amoxycilin: 0.04 mg/disk		
	F. antipyretica	*C. molluscum*	*H. cupressiforme*	Amoxycilin
E. coli	14.00	6.67	9.00	13.00
S. epidermidis	9.33	5.00	5.00	14.30
B. subtilis	14.67	8.67	8.00	24.00
M. flavus	12.00	-	16.00	42.00
		Concentration of moss extract: 1 g/disk Concentration of Amoxycilin: 0.02 mg/disk		
E. coli	9.00	7.00	8.00	12.00
S. epidermidis	5.00	5.00	5.00	14.00
B. subtilis	9.67	8.67	5.00	20.00
M. flavus	9.00	-	7.50	40.00
		Concentration of moss extract: 0.5 g/disk Concentration of Amoxycilin: 0.01 mg/disk		
E. coli	7.33	6.33	7.67	10.00
S. epidermidis	5.00	5.00	5.00	-
B. subtilis	9.00	8.00	5.00	-
M. flavus	-	-	-	36.00

The most resistent 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.

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

Bacteria	Minimal inhibitory concentration (MIC) (mg/ml) Minimal bactericidal concentration (MBC) (mg/ml)			
	F. antipyretica	*C. molluscum*	*H. cupressiforme*	Amoxycilin
E. coli	10.0 20.0	10.0 20.0	10.0 20.0	0.1 0.2
S. enteritidis	10.0 20.0	20.0 20.0	10.0 10.0	0.05 0.1
S. epidermidis	20.0 20.0	20.0 20.0	20.0 20.0	0.2 0.2
B. subtilis	20.0 20.0	20.0 20.0	20.0 20.0	0.1 0.1
M. flavus	20.0 20.0	20.0 20.0	20.0 20.0	0.01 0.01

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

Mosses	*F. antipyretica*		*C. molluscum*		*H. cupressiforme*		Bifonazol	
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. flavus	2.5	5.0	5.0	5.0	5.0	5.0	0.1	0.1
A. fumigatus	5.0	5.0	5.0	5.0	5.0	5.0	0.1	0.2
A. niger	5.0	5.0	5.0	5.0	5.0	5.0	0.1	0.1
P. funiculosum	2.5	5.0	5.0	5.0	2.5	5.0	0.5	1.0
P. ochrochloron	2.5	5.0	5.0	5.0	5.0	5.0	0.1	0.2
T. viride	2.5	5.0	5.0	5.0	5.0	5.0	1.0	1.0

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.

Acknowledgment — The authors are grateful to the Ministry of Science and Technological Development of Serbia for financial support (Grants 143049 and 143041).

REFERENCES

Asakawa, Y. (1995). Chemical constituents of the bryophytes, In: *Progress in the Chemistry of Organic Natural Products, 65* (Eds. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, and C. Tamm), 1-562. Springer, Vienna.

Asakawa, Y. (1999). Phytochemistry of bryophytes: biologically active terpenoids and aromatic compounds from liverworts, In: *Phytochemicals in Human Health Protection, Nutrition,* and *Plant Defense* (Ed. J. Romeo), 319-342. Kluwer Academic/Plenum Publishers, New York.

Asakawa, Y. (2001). Recent advances in phytochemistry of bryophytes – acetogenins, terpenoids, and bis(bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean, and European liverworts. *Phytochemistry* **56,** 297-312.

Basile, A., Giordano, S., López-Sáez, J. A., and *R. Castaldo-Cobianchini* (1999). Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry* **52,** 1479-1482.

*Borel, C., Welthy, H., Fern*and*ez, I.,* and *M. Colmenares* (1993). Dicranin, an antimicrobial and 15-lipoxygenase inhibitor from the moss *Dicranum scoparium*. *J. Nat. Prod.* **56,** 1071-1077.

Cambie, R. C. (1996). A phytochemical register of New Zeland plant species. *J. Roy. Soc. New Zeal*and **26 (4),** 483-527.

Daouk, D., Dagher, S., and *E. Sattout* (1995). Antifungal activity of the essential oil of *Origanum syriacum* L. *J. Food Protect.* **58,** 1147-**1149.**

Dulger, B., Yayintas, T., and *A. Gonuz* (2005). Antimicrobial activity of some mosses from Turkey. *Fitoterapia* **76,** 730-732.

Flowers, P. (1957). Ethnobryology of the Gosuite Indians of Utah. *Bryologist* **60**, 11-14.

Hanel, H., and *W. Raether* (1988). A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazoleans as an example. *Mycoses* **31,** 148-154.

Hu, R. L. (1987). *Bryology*. 465 pp. Higher Education Press, Beijing, China.

Lorimers, S. D., Perry, N. B., and *R. S. Tangney* (1993). Antifungal bibenzyls from the new Zealand liverwort *Plagiochila stephensoniana*. *J. Nat. Prod.* **56,** 1444-1450.

Markham, K. R., and *R. Given* (1987). The major flavonoids of an Antarctic *Bryum*. *Phytochemistry* **27 (2),** 2843-2845.

Saboljević, A., Soković, M., Saboljević, M., and *D. Grubišić* (2006). Antimicrobial activity of *Bryum argenteum*. *Fitoterapia* **77,** 144-145.

Saxena, K., and *S. Harinder* (2004). Uses of Bryophytes. *Resonance* **9 (6),** 56-65.

Sievers, H., Burkhardt, G., Becker, H., and *H. D. Zinsmeister* (1993). Further biflavonoids and 3'-phenylflavonoids from *Hypnum cupressiforme*. *Phytochemistry* **35 (3),** 795-798.

Singh, M., Rawat, A. K. S., and *R. Govindarajan* (2007). Antimicrobial activity of some Indian mosses. *Fitoterapia* **78 (2),** 156-158.

*Veljić, M., Tarbuk, M., Marin, P. D., **Ćirić, A., Soković, M.,*** and *M. Marin* (2008). Antimicrobial activity of methanol extracts of some genuine mosses from Serbia. *Pharmaceut. Biol.* (In press).

*Verpoorte, R., Van Beek, T. A., Thomassen, P. H. A. M., A*andeweil, *J.,* and *A. Baerheim-Svendsen* (1983). Screening of antimicrobal activity of some plants belonging to the Apocynanceae and Loganinaceae. *J. Ethnopharmacol.* **8,** 287-302.

Von Reusz, S. H., and *W. A. König* (2004). Corsifurans A-C, 2-arylbenzofurans of presumed stilbenoid origin from *Corsinia cori*and*rina* (Hepaticae). *Phytochemistry* **65 (23),** 3113-3118.

Wang, X., Yu, W., and *H. Lou* (2005). Antifungal constituents from the Chinese moss *Homalia trichomanoides*. *Chem. Biodivers.* **2 (1),** 139-145.

АНТИМИКРОБНА АКТИВНОСТ МЕТАНОЛНИХ ЕКСТРАКАТА *FONTINALIS ANTIPYRETICA, HYPNUM CUPRESSIFORME И CTENIDIUM MOLLUSCUM*

М. ВЕЉИЋ[1], АЛЕКСАНДРА ЂУРИЋ[1], МАРИНА СОКОВИЋ[2], АНА ЋИРИЋ[2], ЈАСМИНА ГЛАМОЧЛИЈА[2] и П. Д. МАРИН1

[1]*Институт за ботанику и Ботаничка башта "Јевремовац", Биолошки факултет, Универзитет у Београду,* 11000 Београд, Србија

[2]*Институт за биолошка истраживања "Синиша Станковић",* 11060 Београд, Србија

Анализирана је антимикробна и антифунгална активност метанолног екстракта врста *Fontinalis antipyretica* Hedw. вар. *antipyretica*, *Hypnum cupressiforme* Hedw. и *Ctenidium molluscum* (Hedw.) Mitt. Антимикробна активност је тестирана на грам (+) (*Bacillus subtilis*, *Micrococcus flavus* и *Staphylococcus epidermidis*) и грам (-) бактерије (*Escherichia coli* и *Salmonella enteritidis*). Као подлога за антифунгалну активност коришћене су гљиве: *Trichoderma viride*, *Penicillium 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.