Acta Pharm. 55 (2005) 69–79 Original research paper

Screening of selected wood-damaging fungi for the HIV-1 reverse transcriptase inhibitors

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Received February 25, 2004
Accepted January 20, 2005

Extracts obtained using methanol and dichloromethane from 57 species of wood damaging fungi were investigated for their ability to inhibit HIV-1 reverse transcriptase activity *in vitro* using a non-radioactive assay. Sixty-three samples were tested all together; some species were represented more than one isolate. Thirteen methanolic extracts exhibited more than 40% inhibition and two among them inhibited the enzyme by more than 80%. All extracts obtained with dichloromethane were inferior to methanolic extracts in their inhibitory activity. The most active fungal species discovered in the first screening were *Laetiporus sulphureus* and *Poria monticola*, followed by *Poria vaillanti* and *Chondrostereum purpureum*. In the second screening, *Laetiporus sulphureus* was selected for detailed examination and different isolates were tested. Preliminary findings confirmed the presence of an acidic compound with the amino group in the most active fraction.

Keywords: HIV-1 reverse transcriptase, screening, fungi, *Laetiporus sulphureus*

Searching for novel inhibitors of the HIV replication cycle is one of the main interests of numerous investigators and enormous efforts have been dedicated to finding promising lead compounds, both synthetic and natural (1). HIV-1 reverse transcriptase (HIV-1 RT) is one of the main targets for inhibiting the reproduction of HIV. This enzyme is responsible for transcription of viral RNA into a DNA, which is later integrated into the host cell and carries the information for the synthesis of new viral particles. Inhibition of HIV-1 RT, besides the later discovered HIV protease and integrase inhibition, was the first therapeutic approach successfully applied in prolonging the life of infected patients (2). Many inhibitors have been and still are intensively discovered in small and big scale screenings while only a small number is used in therapy. These compounds are nucleoside and non-nucleoside inhibitors (3).

HIV exhibits a high ability to develop resistance against therapeutic agents and therefore new promising substances have to be discovered. One of the commonly used

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methods in discovering new leading compounds is also screening of synthetic and natural substances. Hence, screening strategies and bioassays have been developed. The early developed assays to screen for HIV-1 RT inhibitory activity were relatively complicated, time consuming and included work with radioactive material (4). Recently, a simple, non-radioactive ELISA based assay became commercially available, and enabled rapid, reliable and safe screenings with a minimum amount of sample.

Numerous natural inhibitors of HIV-1 reverse transcriptase have been described, most of them obtained from plants (5–7), but not from fungi. The kingdom of fungal organisms represents a vast, promising and often overlooked source of novel therapeutic agents. Especially some lignicolous or woods destructing fungi are common ingredients of traditional medicines (*Lentinus edodes*, *Trametes* spp., *Ganoderma lucidum*, *Coriolus versicolor)* (8). The medicinal purpose for which this type of fungi has been most extensively investigated is their antitumour activity. Antiviral activity was also studied in some cases (9–12).

In order to find novel therapeutic agents, we decided to perform screenings of some Slovenian plants and fungi for their potential therapeutic activity (13). Fungal species currently examined belong to *Basidiomycotina* and *Ascomycotina*. All of them are connected with wood. Among them some species cause white-rot decay, some soft-rot decay, some species are connected with blue stain, and some species are symbiotic with wood insects (so-called Ambrosia bugs). To our knowledge, this preliminary study represents the first attempt to search for HIV-1 RT inhibitors in the wood-damaging fungi.

EXPERIMENTAL

Fungal material

Fungal species that we selected belong to *Basidiomycotina* (31 species) and *Ascomycotina* (25 species). Fungi specimens were obtained from the Collection of Wood Fungi of the Department for Wood Science and Technology, Biotechnical Faculty, University of Ljubljana (Slovenia) where voucher specimens are deposited. One sample of *Laetiporus sulphureus* was obtained from the field (Slovenska Bistrica, Slovenia) and used in the second screening.

Fungi were grown on the potato-dextrose-agar (PDA) medium (Fluka, Switzerland) consisting of potato extract (4 g L^{-1}), dextrose (20 g L^{-1}) and agar (15 g L^{-1}) in 9-cm diameter Petri dishes. Medium was prepared by dissolving 39 g of PDA mixture in 1 L of distilled water and autoclaved (121 °C, 202.6 kPA, 20 min). Samples were incubated at 25 °C and 100% relative moisture until the medium was overgrown with mycelium. Subsequently, the medium and mycelium were freeze-dried.

For the second screening, the most active fungi were grown on the PDA medium, in liquid malt medium (1%, *m/V* in water) (malt extract Biolife, Italy) and in the following liquid medium (g per litre aq-solution): KH_2PO_4, 0.2, NH_4Cl, 0.2, $Ca(NO_3)_2$, 0.05, $MgSO_4 \times 7H_2O$, 0.15; $FeCl_3$, 0.12, malt extract, 0.5, glucose, 5). Liquid media were autoclaved after preparation as stated above.

Preparation of extracts

Freeze-dried PDA medium with mycelium was pulverised. The powder (500 mg of each) was extracted with methanol or dichloromethane (10 mL). Extraction procedure was performed as follows: maceration at room temperature for 30 min, extraction in the ultrasonic bath for 30 min, maceration at room temperature for 12 h and repetition of the procedure in the ultrasonic bath. Extracts were filtered and the solvent was evaporated to dryness under reduced pressure and temperature not exceeding 35 °C. Dry extracts were dissolved in sterile DMSO (Fluka, 10%, *V/V*, aqueous solution), diluted to a final concentration of 1 mg mL^{-1} and tested immediately. DMSO was used as a solvent instead of methanol because of its lower inhibitory activity towards HIV-1 RT.

In the case of liquid medium, a mixture of mycelium and medium was separated by filtration and both fractions were freeze-dried, extracted with 50% (*V/V*) and pure methanol and prepared for testing as described above.

Extracts of freeze-dried fresh media, prepared as described, were used as negative controls.

HIV-1 RT assay

Reverse Transcriptase Assay, non-radioactive (No. 1 468 120), was purchased from Roche Diagnostics (Switzerland). The assay was performed according to the manufacturer's instructions. HIV-1 RT (recombinant, 2.5 ng dissolved in 20 μL of Tris buffer: 50 mmol L^{-1} Tris, 80 mmol L^{-1} KCl, 2.5 mmol L^{-1} DTT, 0.75 mmol L^{-1} EDTA dissolved in 0.5% Triton® X-100, pH 7.8) and the sample dissolved in DMSO (20 μL) were added to a microtiter plate well. The reaction was started with addition of 20 mL of the reaction mixture containing RNA template/primer hybrid, DIG-dUTP, biotin-dUTP and dTT nucleotides. The mixture was incubated for 1 h at 37 °C. The microtiter plate was washed five times with 200 μL of washing buffer, and subsequently 20 μL of anti DIG-POD conjugated antibodies dissolved in sodium phosphate buffer pH 7.4 (Roche) were added. The mixture was incubated again for 1 h at 37 °C. After the incubation period, the wells were washed with 200 μL of washing solution (Roche, composition not disclosed). Later on, 250 μL of ABTS substrate was added (ABTS dissolved in phosphate buffer containing sodium perborate and citric acid, Roche). Absorbance was measured after 15 minutes with a Biolise Rainbow (Tecan, Austria) microtiter plate reader at 405 nm. The inhibition rates were calculated by comparison with the sample-free control and corrected by comparison with the enzyme-free control.

Preparative HPLC

Separation of the most active extract was performed by preparative HPLC (preparative column: Eurospher 100 C_{18} 5 mm, length 120 mm, diameter 16 mm, injector Midas Spark, pumps: Knauer-HPLC pump K-501, mixing chamber: Knauer, fraction collector: Bio-Rad, model 2128). Gradient elution was performed starting with 12% methanol in water (15 min), continuing with gradient (12–50% methanol) for 10 min and ending with 12% methanol for 5 min. Injection volume (sample) was 250 μL, flow rate was 4 mL min^{-1}. Fractions were collected at 1 minute intervals and fractions were evaporated to dryness *in vacuo*.

TLC and identification

Thin-layer chromatography was performed on Kieselgel 60 and Kieselgel 60 F_{254} (Merck, Germany) plates using mobile phase containing dichloromethane and methanol (9:1) and different spraying reagents such as $FeCl_3$ (32), vaniline solution in H_2SO_4 (32), anysaldehide (32), rodamin G6 (32), nynhidrine and bromcresol green (32).

RESULTS AND DISCUSSION

In the first screening total of 63 samples were tested because some species were represented by a larger number of isolates.

The experimental results, presented in Table I, indicate that the methanolic extracts of 13 samples and dichloromethane extracts of only 1 sample exhibited more than 40% *in vitro* inhibition of HIV-1 reverse transcriptase. Dichloromethane extracts were inferior to methanolic extracts in their inhibitory activity. This may be due to the presence of active polar inhibitory compounds in investigated methanolic extracts. In Table II, inhibitory activity of the most active extracts is presented.

The most active *Ascomycotina* species were *Cladosporium herbarum*, *Leptographium lundbergii* and *Pichia anomala* with inhibitory activities of their methanolic extracts of 50.7%, 54.7% and 48.7%, respectively. No HIV and general antiviral activity was previously reported for these three moulds. The only available reference of chemical composition of *Leptographium lundbergii* notes the presence of africanols, sesquiterpene derivatives (29) with no biological activity described. The sponge derived *Cladosporium herbarum* contains macrolide substances pandangolides and cladospolides, which have phytotoxic activity. The presence of antimicrobial furane carboxylic acids was also reported (14) but these data may not correspond with our data obtained for the strain, which was isolated from wood. *Pichia anomala* is the producer of a killer toxin (PaKT) with known antimicrobial properties (15) and may also be responsible for the anti HIV-RT activity discovered in our study.

The *Basidiomycotina* member with outstanding activity was *Laetiporus sulphureus*, whose inhibitory activity was 90.1%. There is no published data concerning this fungi and its anti-HIV activity. The most significant pharmacological activity of the extract obtained from *L. sulphureus*, which was observed by Okamura (16), is the inhibition of thrombin. The substance responsible for this activity has not been reported to date. Chemical composition of this fungi was partly reported by Rapior (17) who searched for volatile compounds and discovered twenty-six components. The major constituents were 3-methylcinnamaldehyde, 2-phenylethanol, benzaldehyde and *N*-phenylethylformamide. He also identified some sulphur compounds, which may be responsible for the odour of *L. sulphureus*. A cyclodepsipeptide beauvericin with antimicrobial activity was also isolated from *L. sulphureus* (30). This fungus was also reported to cause hallucinations and atoxia in children upon ingestion. Two triterpenoids, 15α-hydroxytrametenolic acid and sulfurenic acid, showed dopamine D_2 receptor agonistic activity in monkeys (30). The most recent discovery was laetiporic acid, a new polyene pigment. No pharmacological activity was reported yet (31). In one report (18), the isolation of a new benzofurane glycoside, masu-

Table I. In vitro HIV-1 RT inhibitory activity of extracts obtained from wood-damaging fungi

Subdivision	Species	Inhibition	
		Methanolic extract	Dichloro-methane extract
Ascomycotina	*Alternaria alternata* (Fr.: Fr.) von Keissler	–	–
Ascomycotina	*Aspergillus terreus* Thom	–	–
Ascomycotina	*Calcarisporium arbuscula* Preuss	–	–
Ascomycotina	*Ceratocystis coerulescens* (Münch) Bakshi	+	+
Ascomycotina	*Chaetomium globosum* (Kunze: Fr.)	–	+
Ascomycotina	*Cladosporium herbarum* (Pers.) Link: Fr.	++	+
Ascomycotina	*Epicoccum nigrum* Link	–	–
Ascomycotina	*Gilmaniella humicola* Barron	–	+
Ascomycotina	*Hormonema dematioides* Lagerberg & Melin	–	–
Ascomycotina	*Humicola grisea* Traaen	–	–
Ascomycotina	*Hymenula cerealis* Ellis & Everh.	+	–
Ascomycotina	*Leptographium lundbergii* Lagerberg & Melin	++	+
Ascomycotina	*Nectria vilior* Starb.	–	–
Ascomycotina	*Ophiostoma araucarie*	–	–
Ascomycotina	*Ophiostoma piliferum (*Fr.: Fr) H. & P. Sydow	–	–
Ascomycotina	*Phialophora aurantiaca*	–	–
Ascomycotina	*Phialophora mutabilis* (van Beyma) Schol-Schwartz	+	–
Ascomycotina	*Pichia anomala* (Hansen) Kurtzman	++	+
Ascomycotina	Raffaelea ambrosia	–	–
Ascomycotina	*Sclerophoma pythiophila* (Corda) Höhn.	–	–
Ascomycotina	*Scytalidium album* (isolate 2[a)]	–	–
Ascomycotina	*Sporotrichum pulverulentum* Novobranova	–	–
Ascomycotina	*Trichoderma harzianum* Rifai	–	+
Ascomycotina	*Tricholus spiralus*	+	–
Ascomycotina	Verticillium lecanii (Zimm.) Viegas	+	–
Basidiomycotina	*Agrocybe aegerita* (Brig.) Fayod	–	–
Basidiomycotina	*Antrodia vaillanti* (Fr.) Ryv. (isolate 4)	++	++
Basidiomycotina	*Aureobasidium pullulans* (de Bary) Arnaud (isolate 2)	–	–
Basidiomycotina	*Bjerkandera adjusta* (Willd.: Fr.) Karst	+	–
Basidiomycotina	*Chondrostereum purpureum* (Pers.: Fr.) Pouz. (isolate 1)	++	–
Basidiomycotina	*Chondrostereum purpureum* (isolate 2)	+	–
Basidiomycotina	*Chondrostereum purpureum* (isolate 3)	–	–
Basidiomycotina	*Chondrostereum purpureum* (isolate 4)	++	–
Basidiomycotina	*Coniophora puteana* (Schum.: Fr.) Karst.	+	+
Basidiomycotina	*Creolophus cirrhatus* (Pers.: Fr.) Karst.	–	–
Basidiomycotina	*Daedalea quercina* (L.: Fr.) Pers.	+	+

Basidiomycotina	*Daedaleopsis confragosa* (Bolt.: Fr.) Schroet.	–	–
Basidiomycotina	*Flammulina velutipes* (Curt.: Fr.) Karst.	–	–
Basidiomycotina	*Fomitopsis pinicola* (Sow.: Fr.) Karst.	+	–
Basidiomycotina	*Ganoderma applanatum* (Pers.) Pat.	–	–
Basidiomycotina	*Ganoderma lucidum* (Leyss.: Fr.) Karst.	–	–
Basidiomycotina	*Gloeophyllum trabeum* (Pers.) Murr. (isolate 2)	+	–
Basidiomycotina	*Grifola frondosa* (Dicks.: Fr.) Gray	–	–
Basidiomycotina	*Heterobasidium annosum* (Fr.: Fr.) Bref.	–	–
Basidiomycotina	*Laetiporus sulphureus* (Bull.: Fr.) Murrill	+++	+
Basidiomycotina	*Lentinula edodes* (Berk.) Pegl.	–	–
Basidiomycotina	Marasmius stiptitarius	–	–
Basidiomycotina	*Peniophora gigantea* (Fr.) Massee	–	–
Basidiomycotina	*Pholiota adiposa* (Fr.) Kumm.	+	–
Basidiomycotina	*Pleurotus ostreatus* (Jacq.: Fr.) Kumm. (isolate 2)	–	–
Basidiomycotina	Pleurotus ostreatus (isolate 3)	–	–
Basidiomycotina	*Poria monticola* (isolate 2)	+++	+
Basidiomycotina	*Poria vaillanti* (DC. & Lamarck) Fries (isolate 1)	++	+
Basidiomycotina	*Poria vaillanti* (isolate 2)	++	+
Basidiomycotina	*Schizophyllum commune* (Fr.: Fr.) (isolate 2)	–	+
Basidiomycotina	*Serpula lacrymans* (Wulf.: Fr.) Schroet. (isolate 2)	+	+
Basidiomycotina	*Serpula lacrymans* (isolate 4)	+	–
Basidiomycotina	*Sistostrema brinkmannii* (Bres.) Erikss.	++	–
Basidiomycotina	*Stereum hirsutum* (Wild.: Fr.) Fr.	+	+
Basidiomycotina	*Stereum rugosum* (Pers. ex Fr.) Fr.	++	+
Basidiomycotina	*Stereum subtomentosum* (Pouz.)	++	+
Basidiomycotina	*Trametes gibbosa* (Pers.: Fr.) Fr.	+	+
Basidiomycotina	*Trametes versicolor* (L.: Fr.) Lloyd (isolate 2)	–	–

+++ > 80% inhibition, ++ 40–80% inhibition, + < 40% inhibition, – no inhibition
[a] Certain species are represented by a larger number of isolates in the Wood fungi collection of the Department for Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia).

Table II. In vitro *HIV-1 RT inhibitory activity of methanolic extracts (first screening)*

Species	Inhibition (%)[a]
Chondrostereum purpureum (isolate 1)	64.3 ± 4.8
Chondrostereum purpureum (isolate 4)	62.3 ± 5.0
Cladosporium herbarum	50.7 ± 4.3
Laetiporus sulphureus	90.1 ± 3.6
Leptographium lundbergii	54.7 ± 7.0
Pichia anomale	48.7 ± 3.9
Poria monticola (isolate 2)	86.1 ± 8.4
Poria vaillanti (isolate 2)	53.3 ± 2.1
Poria vaillanti (isolate 1)	68.7 ± 5.2

[a] Mean ± SD, $n = 3$.

takeside I and a new acetylenic acid, masutakic acid, from the fruiting bodies of *L. sulphureus* var. *miniatus* that grows in Japan was reported. No biological activity of these substances was reported. Zjawiony (30) reported in his recent review that compounds such as egonol, demethoxyegonol and egonol glucoside, all isolated from *L. sulphureus* var. *miniatus*, exhibited low cytotoxic activity.

The second most active species was *Poria monticola* (isolate 2), whose inhibitory activity of HIV-1 RT was 86.1%. The related species *Poria vaillanti* and *P. vaillanti* (isolate 2) showed 68.7% and 53.3% inhibition, respectively. A sample of the same species, stored under the synonym *Antrodia vaillanti (*isolate 4) showed similar inhibition as *P. vaillanti.* The sample of *Poria monticola* (isolate 2) was further diluted and tested in dilutions of 0.1 and 0.01 mg mL^{-1}, which showed inhibitions of 42.4% and 16.4%, respectively. This species is relatively abundant in Europe and is known under numerous synonyms (*Poria placenta, Oligoporus placenta, Tyromyces placenta).* It causes brown rot decay, mostly on softwood constructions in buildings. *Poria* species degrade cellulose, leaving a brown residue of lignin. Until this research, there were no reports of therapeutic uses of *Poria monticola.* Reports of its chemical constitution are scarce. The presence of volatile metabolites such as acroleine, butanal, octanal, acetone and some terpenes was reported in one study but these compounds may also be connected with the specific source from which this fungus was isolated (19). *P. monticola* also produces oxalic acid and hydrogen peroxide, which are believed to be involved in the degradation of wood carbohydrates (20).

Popular medicinal fungi, such as *Ganoderma applanatum*, *Ganoderma lucidum, Grifola frondosa, Lentinula edodes* and *Trametes versicolor,* did not exhibit HIV-1 RT inhibitory activity in our *in vitro* test, although many reports of their antiviral activity exist in the literature. The authors reported inhibition of the binding of HIV with lymphocites, caused by the polysaccharides isolated from *T. versicolor,* as one of the mechanisms of antiviral activity (30). According to Chihara (21), a lentinan compound, obtained from *Lentinus edodes*, enhanced host resistance to infections by bacteria as well as by fungi, parasites and viruses, including the agents of AIDS. Lentinan reduced the toxicity of azidothymidine (HIV-1 RT inhibitor used in therapy). Prevention of the onset of AIDS symptoms through potentiation of host defence was also described (21, 22). Antiviral activity of water and methanol soluble substances from *Ganoderma lucidum* was reported (9). Isolated substances were estimated to be polysaccharides and proteins and were inhibitory to *Herpes simplex* virus (HSV-1 and HSV-2) (9). Triterpenes, especially ganoderic acid b, isolated from fruiting bodies of *Ganoderma lucidum*, exhibited HIV protease inhibitory activity (23, 30). The same species also produced compounds the ganoderiol F and ganodermanotriol, which have anti-HIV-1 activity (30). Although a report on HIV reverse transcriptase inhibiton caused by cerebrosides isolated from *G. lucidum* was published (24), we did not observe such activity. This fact may also be connected with solvents used for extraction of fungi in our study.

It appears that genus *Stereum* would be also interesting for further investigation because both methanolic and dichloromethane extracts of the three tested species were active, although not remarkably, and may contain common inhibitory compound(s). We have not found any previous reports of the anti HIV activity of the tested species *S. hirsutum, S. rugosum* and *S. subtomentosum*. There are many reports on the chemical composition of *S. hirsutum*, which is known for its antimicrobial, antitumour, antioxidant and phytotoxic activities (25–27).

Another interesting species is *Sistostrema brinkmanii*, with inhibitory activity of methanolic extract reaching 48.2%. There are no scientific data about this fungus, obtainable from the available sources. Interesting aspects were the differences in inhibitory activity between different isolates of the same species, *Chondrostereum purpureum*. Methanolic extracts of isolate 1 and of isolate 4 exhibited inhibitions of 64.3% and 62.3%, while inhibition of isolate 2 was 12.7% whereas isolate 3 did not show any inhibitory activity. This species contains sterpurene sesquiterpenes (28) with phytotoxic activity.

In an effort to confirm anti-HIV-1 RT activity and clarify the nature of the most active extracts, further investigations were performed in our laboratory. In the second screening, the most active species were grown on PDA and liquid media. Results of the second screening are shown in Table III. Liquid media were separated from the mycelium, separately extracted and tested. In the latter case, we were able to quantify and compare more precisely the amount of mycelia used for extraction. In the second screening, three isolates of *Laetiporus sulphureus*, among them one obtained from the field, and one isolate of *Poria monticola* (in concentrations of 1.0, 0.01 and 0.001 mg mL^{-1}) were tested. According to this screening, *Laetiporus sulphureus* was chosen for further research. It was evident that aqueous-methanolic extracts were more potent than the methanolic ones. The most potent extract obtained from *L. sulphureus* (obtained from the field) was analysed by preparative HPLC; 5 fractions were collected and tested for inhibitory activity (results shown in Table IV). The most active fraction, namely fraction 1, was analysed by TLC and preliminarily tested for the presence of different compounds. Tests with $FeCl_3$, vaniline and rodamin G6 were negative and we concluded that no compounds with a phenolic hydroxyl group, terpenoid and phenylpropanoid structure and no lipids were present. The test with anysaldehyde was positive, but due to the unspecific nature of this reagent, no detailed conclusions could be made. Spraying with bromcresol green revealed the presence of acidic compounds and the test with ninhydrin confirmed the presence of compounds with an amino group in the most active fraction.

Table III. In vitro *HIV-1 RT inhibitory activity (2) of the most active extracts (second screening)*

	Extract concentrations (mg mL^{-1})[a]			
Species	Solvent	1.0	0.01	0.001
Laetiporus sulphureus (from the field)	MeOH	90.84 ± 4.68	8.75 ± 3.05	3.10 ± 5.76
Laetiporus sulphureus[b] (from the field)	MeOH (50%)	96.19 ± 6.23	30.08 ± 6.55	10.03 ± 4.32
Laetiporus sulphureus (isolate 2)	MeOH (50%)	79.54 ± 3.78	24.55 ± 4.20	11.39 ± 4.03
Laetiporus sulphureus (isolate 3)	MeOH (50%)	60.32 ± 6.32	36.10 ± 5.89	24.14 ± 3.30
Poria monticola (isolate 2)	MeOH	70.30 ± 7.20	13.84 ± 4.08	8.90 ± 5.03
Poria monticola (isolate 2)	MeOH (50%)	27.70 ± 4.88	12.35 ± 5.56	3.21 ± 5.34

[a] Mean ± SD, $n = 3$.
[b] Used for fractionation by HPLC.

Table IV. In vitro HIV-1 *RT inhibitory activity (2) of the most active fractions from Laetiporus sulphureus*[a] *after HPLC fractionation*

Fraction	Extract concentrations (mg mL^{-1})[b]		
	1.0	0.1	0.01
1	96.21 ± 4.67	62.01 ± 5.22	28.16 ± 7.02
2	71.34 ± 2.44	–	–
3	85.05 ± 3.56	17.13 ± 4.35	–
4	24.62 ± 5.18	0.93 ± 4.39	–
5	63.40 ± 6.45	12.06 ± 5.20	–

[a] From the field
[b] Mean ± SD, n = 3.

CONCLUSIONS

It would be premature to speculate about the possible mechanism of the reverse transcriptase inhibition at this stage of research. Both competitive and non-competitive specific inhibitors may be present, or non-specific inhibitors that would change the secondary or tertiary structure of the enzyme.

Direct extracts (without any subsequent purification and enrichment) of *Laetiporus sulphureus* and *Poria monticola* with 90% inhibition of HIV-1 RT, and also of *Poria vaillanti* and *Chondrostereum purpureum* showed remarkable activity. More detailed preliminary examination of *Laetiporus sulphureus* confirmed the possible presence of an acidic compound with amino group in the most active fraction of the methanolic extract.

Further detailed investigations will be performed in the future to reveal the structure of inhibitory compound(s).

Abbreviations. – ABTS – 2,2′-azino-bis (3-ethylbenzthialzoline-6-sulfonic acid), anti DIG-POD – anti digoxigenine-peroxidase, DIG-dUTP – digoxigenine-deoxyuridine triphosphate, dTT – deoxythymidine triphosphate, ELISA – Enzyme-Linked Immunosorbent Assay, HIV – Human Immunodeficiency Virus, HSV – Herpes Simplex Virus, PDA – potato-dextrose-agar, RT – reverse transcriptase.

Acknowledgement. – The authors are very grateful to Mr. Matej Pegam, M. Pharm. for his technical assistance.

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S A Ž E T A K

Iskanje inhibitorjev HIV-1 reverzne transkriptaze v lesnih glivah

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Iz 57 vrst lesnih gliv sta bili pripravljeni dve seriji izvlečkov. V prvi so bili izvlečki, pripravljeni z metanolom, v drugi pa z diklorometanom. Preizkušenih je bilo 63 vzorcev, saj so bile nekatere vrste zastopane z večjim številom izolatov. Izvlečkom smo *in vitro* preizkusili inhibitorno aktivnost na HIV-1 reverzno transkriptazo s pomočjo neradioaktivne metode. 13 metanolnih izvlečkov je inhibiralo encim več kot 40-odstotno, med njimi sta dva inhibirala encim več kot 80-odstotno. Najbolj učinkovita sta bila izvlečka gliv *Laetiporus sulphureus* in *Poria monticola*, sledita jim izvlečka vrst *Poria vaillanti* in *Chondrostereum purpureum*. V nadaljevanju raziskave smo ugotavljali inhibitorno aktivnost različnih izolatov glive *Laetiporus sulphureus*. Najbolj aktivne izvlečke smo frakcionirali s pomočjo preparativne tekočinske kromatografije. Domnevamo, da bi bila lahko v najbolj aktivni frakciji prisotna spojina ali spojine, ki so kisle narave in imajo v strukturi amino skupino.

Ključne besede: HIV-1 reverzna transkriptaza, rešetanje, glive, *Laetiporus sulphureus*

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