



## Antiviral activity of total polysaccharide fraction of water and ethanol extracts of *Pleurotus pulmonarius* against the influenza A virus

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Vlasenko VA, Ilyicheva TN, Teplyakova TV, Svyatchenko SV, Asbaganov SV, Zmitrovich IV, Vlasenko AV 2020 – Antiviral activity of total polysaccharide fraction of water and ethanol extracts of *Pleurotus pulmonarius* against the influenza A virus. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 10(1), 224–235, Doi 10.5943/cream/10/1/22

### Abstract

New data of antiviral properties of species of the genus *Pleurotus* are given in the article. Seven samples of one species of pleurotoid fungi, *Pleurotus pulmonarius*, collected in various habitats and substrates of the Novosibirsk Region, was studied for antiviral activity against the current highly pathogenic strain of influenza virus A/California/07/09(H1N1pdm). As a result, it was found that ethanol extracts from fungi exhibit a more pronounced antiviral effect than aqueous extracts. In this connection, ethanol extraction is a more promising method for extracting the total polysaccharide fraction of fungi, in order to create promising preparations with antiviral properties. Fruiting bodies of *P. pulmonarius* are potential sources of drugs, with antiviral action against socially significant viral infections caused by the influenza virus. Fruit bodies and cultivated mycelium of *P. pulmonarius* are a promising source of extracts with fungal polysaccharides.

**Key words** – antiviral properties – pleurotoid fungi – fungal polysaccharides – new sequences – influenza A virus – Novosibirsk Region

### Introduction

Mushrooms are becoming more and more important in our diet for their nutritional characteristics. The high protein, essential nutrients, and low energy contents make them excellent foods. Besides their use as nutritious food, mushrooms have been used as health-promoting food supplements or nutraceuticals (Barros et al. 2007, 2008, Ogbe et al. 2008).

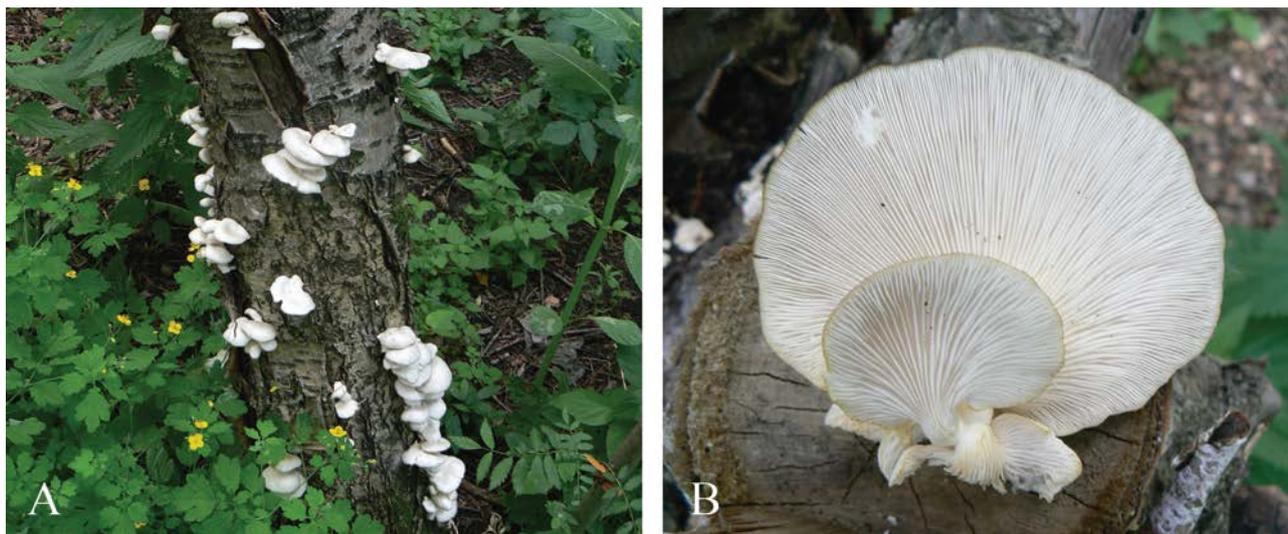
Basidiomycetes contain a wide range of various drugs such as polysaccharides, organic acids, lipids, steroids, tetracyclic triterpenes, which are of interest for medical applications (Lindequist et al. 2005, Wasser 2002, 2010, Teplyakova et al. 2012).

The prevention and treatment of viral diseases remain an urgent problem in modern medicine. Virus infection is one of the challenging global issues with regular widespread epidemics or pandemics resulting in high mortality worldwide (Kamps et al. 2006). A number of mushroom species have been identified as potential sources of drugs, with antiviral action against socially significant viral infections caused by the influenza virus (Mothana et al. 2003, Ohta et al. 2007),

HIV-1 (Wang & Ng 2001a), HSV-1 (Doğan et al. 2018), HSV-2 (Amoros et al. 1997, Oh et al. 2000).

The genus *Pleurotus* comprises some most popular edible mushrooms due to their favorable organoleptic and medicinal properties, vigorous growth and undemanding cultivation conditions (Yildiz et al. 1998, Bonatti et al. 2004, Gregori et al. 2007, Teplyakova & Kosogova 2015). It can be cultivated on log and a wide variety of agroforestry products, weeds and wastes for the production of food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification (Croan 2000, Isikhuemhen et al. 2000, Stamets 2000, Zhang et al. 2003, Kalmis & Sargin 2004, Mandeel et al. 2005, Pavlik 2005, Salmones et al. 2005, Das & Mukherjee 2007).

In nature, these species are widespread, they grows on many species of woody plants, most often deciduous, but can also grow on coniferous trees (Fig. 1).



**Fig. 1** – Substrates and appearance of *Pleurotus pulmonarius* in the Novosibirsk Region. A Fruiting body of *P. pulmonarius* on a live trunk of *Sorbus sibirica*. B Fruiting body of *P. pulmonarius* on dead wood. Photos by V. Vlasenko.

*Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties, and other beneficial effects. Oyster mushrooms are a good source of dietary fiber and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities (Çaglarırmak 2007).

Oyster mushrooms have medicinal properties, the polysaccharides contained in them exhibit a high antitumor, antioxidant, immunomodulatory, antiviral and other effects (Vlasenko & Vlasenko 2018). Mushrooms contain substances that exert direct or indirect antiviral effects as a result of the immunostimulatory activity (Brandt & Piraino 2000).

The antiviral properties of pleurotoid fungi began to be studied in 1969. Was studied the antitumor activity of a polysaccharide extracted from the fruiting bodies of *P. ostreatus* (Watanabe 1969).

Polysaccharides of oyster mushrooms were first isolated from *P. ostreatus*. It was an insoluble polysaccharide Pleuran (Karácsonyi & Kuniak 1994), commercial name Imunoglukan. It possesses immunomodulating properties that make the body more resistant to infections and cancer (Luzio 1985) and has other properties.

Water extracts with polysaccharides *P. ostreatus* and *P. pulmonarius* were active against the herpes simplex virus of the second type. The antiviral activity of water extracts of fungi is associated with the presence of polysaccharides and increases with increasing concentration of polysaccharides (Chihara et al. 1970). Two water-insoluble  $\beta$ -glucans isolated from *P. tuber-regium* sclerotia, their corresponding water-soluble sulfated derivatives exert antiviral activities against herpes simplex virus type 1 and type 2. The effect is presumably elicited by the binding of

sulfated  $\beta$ -glucans to viral particles, thus preventing them from infecting the host cells (Zhang et al. 2003, 2004).

The antiviral properties of aqueous and methanol extracts of *P. ostreatus* were evaluated against herpes simplex virus type 1 (HSV-1). Antiviral activity correlates with the beta-glucans present in the polysaccharide fraction, which showed higher antiviral activity than aqueous extracts (Santoyo et al. 2012).

Extracts with polysaccharides obtained from water extracts from the genus *Pleurotus* completely suppress the infectious activity of at least 1000 TCID<sub>50</sub> of the West Nile virus (Razumov et al. 2010).

EI Fakharany et al 2010 reported that a laccase has been purified from *P. ostreatus* mushroom, which is capable to inhibit the hepatitis C virus entry into peripheral blood cells and hepatoma HepG2 cells and its replication (Ei-Fakharany et al. 2010).

It was found that the purified lectins of *P. ostreatus* as an adjuvant (1 mg/ml) also enhance the immunogenicity of the hepatitis B DNA vaccine (Gao et al. 2013).

*P. djamour*, *P. sajor-caju* and *P. citrinopileatus* extracts had stronger antiviral activities against both pox virus and infectious bursa disease virus (Kidukuli et al. 2010). The name "*Pleurotus sajor-caju*" used in modern mycology has two meanings: a strict taxonomical meaning refers to its nomenclatural synonym *Lentinus sajor-caju*, whereas more widely distributed biotechnological meaning refers to a strain determined as "*Pleurotus sajor-caju*", but belongs to true *Pleurotus*, namely a tropical ecotype of *P. pulmonarius* (*P. pulmonarius* var. *stchangii*) named in honor of Prof. S.T. Chang, who introduced this taxon into mushroom industry (Zmitrovich & Wasser 2016).

Ubiquitin-like glycoprotein was isolated from *P. ostreatus*, which inhibited the development of the human immunodeficiency virus (Wang & Ng 2000).

A laccase from *P. eryngii* was examined by Wang and Ng for inhibitory activity against HIV (Wang & Ng 2001b). The result demonstrated that the laccase was active against HIV-1 growth with an IC<sub>50</sub> of 2.2  $\mu$ M by inhibiting HIV-1 reverse transcriptase. A protease designated pleureryn, isolated from fresh fruiting bodies of the edible mushroom, caused (23.1  $\pm$  0.6)% and (91.4  $\pm$  3.2)% inhibition of HIV-1 reverse transcriptase at 3 and 30 mM, respectively. These results indicated that there was a dose-dependent relationship for protease showing anti-HIV protease activity.

Inhibitory activity against human immunodeficiency virus HIV-1 reverse transcriptase has recently been demonstrated for *P. sajor-caju* and *P. pulmonarius* hot water extracts (Wang et al. 2007).

The *P. citrinopileatus* lectin was a homodimeric 32.4 kDa protein able to inhibit the HIV-1 reverse transcriptase with the concentration of the substance required to reduce the plaque number in Vero cells by 50% (IC<sub>50</sub>) being 0.3  $\mu$ mol l<sup>-1</sup> (Li et al. 2008).

Antiviral effect of aqueous and methanol extracts of *P. ostreatus* and *P. pulmonarius* was shown in relation to the influenza viruses. Aquatic extracts of *P. pulmonarius* fruiting bodies showed high activity against influenza viruses (6.06  $\pm$  0.18 lg for H5N1 and 5.73  $\pm$  0.14 lg for H3N2) (Kabanov et al. 2011).

The antiviral activity of the in vitro mycelium of higher fungi against influenza A virus (serotype H1N1) and herpes simplex virus type 2 (HSV-2), strain BH was investigated. *P. ostreatus* inhibited the propagation of the influenza A/FM/1/47 (H1N1) virus strain in MDCK cells that reduce the infectious titer by 2.0-6.0 lg ID<sub>50</sub>. It was determined that *P. ostreatus* is effective against HSH-2 strain BH in RK-13 cells with similar levels of inhibition as for influenza (Krupodorova et al. 2014).

Thus, based on the above studies, it can be concluded that polysaccharides (glucans) are the main active component of mushroom extracts.

Polysaccharides, are a kind of macromolecule sugar polymer, found in fungi, for example, *Pleurotus ostreatus* (Palacios et al. 2012). They have various biological properties, including, antitumor, antiviral activity, immunomodulation, antilipidemic impact and oxidation protection (Jing et al. 2016).

A water-soluble polysaccharide, FI, and water-insoluble polysaccharides, FII and FIII were extracted from *Pleurotus citrinopileatus* mushrooms (Zhang et al. 1994).

In study *Pleurotus ostreatus* mycelia were grown in submerged culture and the bioactive polysaccharides were extracted from mycelia & culture broth by repeated precipitation with ethanol. The structure of the polysaccharide is mainly glucan. The C13 NMR signals indicating that the polysaccharides is highly branched mainly 1-3 and 1-6 linkage. The results showed that the polysaccharides possess anticancer activity against Ehrlich ascites carcinoma & oesophageal cancer cell line. Mushroom polysaccharides showed direct & indirect antitumor activity, immune potentiating activity by stimulation of T cell mediated immune response (Daba 2006).

Glucan with different active unit linkage such as (1→3), (1→6)-β-glucan and (1→3)- α -glucans constitute mushroom polysaccharides which perform immunomodulator activity as they are biological response modifiers (BRMs). Beta-glucan obtained from the *Pleurotus ostreatus* is known in the scientific literature as pleuran. It is structural cell wall compound. It stimulates the body's defense system against infections (bacterial, viral, yeast, and parasitic) and modulates the blood producing activity of bone marrow (El Enshasy et al. 2012).

Among the several molecules with bioactivity produced by fungi, polysaccharides seems to be the main responsible by the antitumor effect (Ren et al. 2012).

Several polysaccharidic extracts from the fruiting bodies with antitumor activity have been reported (Patel et al. 2012, Assis et al. 2013, Liu et al. 2015, Wisbeck et al. 2017).

## Materials & Methods

### Fungal isolation

Fruiting bodies of fungi were collected in the field studies in various habitats of the Novosibirsk Region. Dried specimens were preserved in the herbarium, they were used for molecular genetic studies and for the preparation of extracts. An overview of all taxa used for antiviral studies, shows the species names, date of collections, habitats and substrates given in Table 1.

**Table 1** Pleurotoid fungi used for antiviral studies.

Species	Extract №	Herbarium voucher	Habitat	Substrate
<i>Pleurotus pulmonarius</i>	1	NSK 1014216	birch forest	<i>Betula pendula</i>
<i>Pleurotus pulmonarius</i>	2	NSK 1014217	riverine forest	<i>Populus</i> sp.
<i>Pleurotus pulmonarius</i>	3	NSK 1014218	floodplain forest	<i>Salix alba</i>
<i>Pleurotus pulmonarius</i>	4	NSK 1014214	birch forest	<i>Sorbus sibirica</i>
<i>Pleurotus pulmonarius</i>	5	NSK 1014279	birch forest	<i>Betula pendula</i>
<i>Pleurotus pulmonarius</i>	6	NSK 1014280	pine-birch-aspen forest	<i>Populus tremula</i>
<i>Pleurotus pulmonarius</i>	7	NSK 1014215	birch forest	<i>Betula pendula</i>

### Morphological examination

The initial morphological examination was performed using a Carl Zeiss Stemi DV4 stereomicroscope, a Carl Zeiss Axiolab E re-light microscope and a Carl Zeiss Axioskop-40 light microscope. The examination of microstructures under the light microscope was made after boiling the preparation in cotton blue. Voucher specimens of the species are stored in the MG Popov Herbarium (NSK), Novosibirsk, Russia.

## DNA extraction and sequencing

Specimens of *Pleurotus* genus was used for molecular analyses. A fragment of fungal fruiting body (50 µg) was homogenized in 300 µl lysis buffer and extract the DNA with NucleoSpin Plant II kit was used. The ITS1-5.8S-ITS2 region of the rDNA was amplified by PCR with the primers ITS1F and ITS4B. For PCR, HS Taq DNA Polymerase (Evrogen, Moscow) was used. PCR reactions were performed in a C1000 Thermal Cycler (Bio-Rad, USA). PCR results were checked at Gel Doc XR+ Imager (Bio-Rad, USA). DAN amplicons sequencing performed in SB RAS Genomics Core Facilities (Novosibirsk, Russia).

## Phylogenetic analyses

Additional 4 ITS sequences of other *Pleurotus* species based on BLAST results and 2 ITS sequences of other species were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>). We first generated a 7 new sequence for ITS1-5.8S-ITS2 region. The final dataset consisted of 13 ITS sequences. An overview of all taxa and on sequences used for tree reconstruction, shows the species names, herbarium vouchers/strain and GenBank accession numbers given in Table 2.

**Table 2** Sequences used in the phylogenetic analyses.

Species	Herbarium voucher/Strain	Genbank accession numbers
<i>Pleurotus pulmonarius</i>	NSK 1014216	MN179415
<i>Pleurotus pulmonarius</i>	NSK 1014217	MN179416
<i>Pleurotus pulmonarius</i>	NSK 1014218	MN179417
<i>Pleurotus pulmonarius</i>	NSK 1014214	MN179418
<i>Pleurotus pulmonarius</i>	NSK 1014279	MN179419
<i>Pleurotus pulmonarius</i>	NSK 1014280	MN179420
<i>Pleurotus pulmonarius</i>	NSK 1014215	MN179421
<i>Pleurotus pulmonarius</i>	FPPMK-L	JX429930
<i>Pleurotus pulmonarius</i>	ATCC 62887	JX535494
<i>Pleurotus pulmonarius</i>	UNIP30	KT273376
<i>Pleurotus pulmonarius</i>	DMRP-10	MG819729
<i>Pleurotus cf. eryngii</i>	C1	FJ514549
<i>Pleurotus ostreatus</i>	6689	AY450345

Sequences were align using ClustalW methods (Higgins et al. 1994) in MEGA 7 (Kumar et al. 2016). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). On dendrograms, next to the branches, values in the bootstrap test (1000 repetitions) (Felsenstein 1985) are more than 75%. The optimal tree with the sum of branch length = 0.0144 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura & Kumar 2002). The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 597 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

## Preparation of water and ethanol extracts

Used 5 grams of dry matter of fruiting bodies of fungi. Samples were ground in a mill, placed in glass jars, 20 ml of distilled water was added, and placed in a water bath. Extraction was carried out at a temperature of 80 degrees Celsius. Water extraction was carried out for 1.5 hours, ethanol (40%) – 0.5 hours. The extracts were cooled, filtered, centrifuged for 10 minutes at 3500 rpm. After centrifugation, the supernatant was taken, placed in sterile containers. 1 ml of extracts were placed in Eppendorf tubes to study their antiviral activity.

## Research methods antiviral activity of extracts

Evaluation of the activity of anti-influenza drugs in the neutral red adsorption test. The method is based on the ability of living cells to absorb and accumulate neutral red supravital dye in lysosomes due to electrostatic attraction. Damage to the lysosomal membranes leads to a decrease in the accumulation of the dye, so the intensity of staining is proportional to the number of living cells.

Materials: 96-well flat-bottom plates (OrangeScientific, Belgium); Cellulose acetate membrane filter with a pore diameter of 0.22  $\mu\text{m}$  (Corning, cat. No. 430320); Single and multichannel pipettes from 1 to 1000  $\mu\text{l}$  (Lenpipet, Russia); CO<sub>2</sub> incubator (Heraeus, USA); ELISA reader (Bio-Rad, USA); Neutral red (Sigma, USA, cat. No. N4638); Nutrient medium for cell culture (Gibco).

The stock solution of the dye 3.33 g of neutral red was dissolved in 1 l of distilled water, the solution was passed through filter paper. The dye extraction solution was mixed in equal volumes of ethanol and 0.1 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> (pH = 3.5).

The first and second days of the experiment: MDCK cells were seeded on the first day of the experiment. Did the dilution of the drug in a supportive environment, removed the growth medium from the tablet with the cell monolayer. The virus was injected at a dose of 100 TCID<sub>50</sub> / well to the wells to determine the effective dose. Then in all the wells made Wednesday with dilutions of the drug. Cell control – wells with a cell monolayer in growth medium (without virus and without drug). Virus control – wells with a cell monolayer infected with a virus in growth medium (without preparation).

The third day of the experiment: Preparing the required volume of the solution of neutral red in the growth medium in order to obtain a 1:3 dilution (1 part of the stock solution of the dye to 2 parts of the growth medium). The concentration of the neutral red working solution was thus 1.11 mg/ml. Without discharging the growth medium, 50  $\mu\text{l}$  of a neutral red working solution was added to each well of the plate using a multi-channel dispenser (final dye concentration –0.22 mg/ml). The prepared plates were placed in a CO<sub>2</sub> incubator for 1.5 hours. The medium from the plates was removed, the inverted plates were dried with filter paper. 300  $\mu\text{l}$  of phosphate-saline buffer (PBS) was added to each well of the plate. The FSB was shaken out of the plate, and the remnants were removed by tapping on filter paper. The tablet was washed twice. 100  $\mu\text{l}$  of the dye extraction solution was applied from the cells. The tablets were left for 30–90 minutes at room temperature. Took readings on an ELISA reader at a wavelength of 490 nm. Calculated 50% inhibitory and toxic concentrations of drugs using graphs. Charts were plotted using average OD values for each group of three infected or uninfected wells with different concentrations of the drug.

## Criteria for evaluating the antiviral effect of chemotherapy drugs in cell culture

The main criterion for studying the specific antiviral effect of compounds is the value of the chemotherapeutic index (CTI), or the selectivity index (IS), which is calculated by the formula  $IS = CD_{50}/ED_{50}$  where  $CD_{50}$  is the concentration of the drug, which causes a cytotoxic effect in 50% of the cultured cells, and  $ED_{50}$  is the minimum concentration of the drug that effectively inhibits virus-induced cytopathic changes by 50%. Compounds with IS equal to 10 and more are expressed in the high activity. Such compounds can be considered promising for further research in animal studies. Suppose, according to the experimental data. On the abscissa axis are the dilutions of the drug, on the ordinate axis – the percentage of living cells. For 100%, cell control is always taken – KK – green line. The minimum number of living cells is observed in the control of the virus – KV (purple line). Toxicity of the drug is measured when cells are incubated in medium with the drug without being infected with a virus (blue curve). It can be seen from the graph that only 20% of the cells survive by diluting the drug – 2 times the drug is toxic, 50% of the cells surviving 4 times, this is the 50% toxic dose of the drug,  $CD_{50}$ . The effectiveness of the drug is estimated by the number of living cells after infection with the virus and the cultivation of cells in an environment with different concentrations of the drug. On the graph is a red curve. When the drug is diluted 2 and 4 times a few cells survive because of the toxicity of the drug. Then, at dilutions of 8–128, more than

50% of the cells survive, in these concentrations the drug protects the cells from the action of the virus, i.e. the drug is effective. 50% of the cells survive the dilution of the drug about 200 times – 50% effective dose, ED<sub>50</sub>.

The chemotherapeutic index, or selectivity index, IS, is calculated by dividing the 50% toxic dose by the 50% effective dose, CD<sub>50</sub>/ED<sub>50</sub>. In this case, it is 200/4 = 50.

### **Analysis antiviral activity**

To analyze the antiviral activity of experimental drugs, wells of a 96-well plate were seeded with MDCK cell culture, with a seed dose of  $2 \times 10^4$  cells per well. After forming 90% of the monolayer (20 hours of incubation at 37°C in an atmosphere of 5% CO<sub>2</sub>), the influenza virus A / California/07/09 (H1N1pdm09) was introduced at a dose of 100 TCID<sub>50</sub> per well. This dose is equivalent to a multiplicity of infection of 0.001 infectious particles per cell. 30 minutes after infection, the test sample was introduced into the wells and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 72 hours. Then neutral red (final concentration 0.34%) was added to each well of the plate, the cells were washed out after 1.5 hours, the dye extraction solution was added (0.1 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and 96% ethanol in equal volumes) and the optical density of the released dye at a wavelength of 490 nm. The antiviral activity of the compound was calculated as the dose (concentration) of the experimental drug, which inhibits viral reproduction by 50%, or IC<sub>50</sub>.

To analyze the toxicity of the compounds, wells of a 96-well plate were seeded with MDCK cell culture, with a seed dose of  $2 \times 10^4$  cells per well. After 20 hours of incubation at 37°C in an atmosphere of 5% CO<sub>2</sub>, compounds dissolved in MEM (Gibco) containing 5% fetal bovine serum were added. After 3 days of incubation, the percentage of cell proliferation inhibition was assessed using NK as described above. The toxicity of the compounds was calculated as the dose (concentration) of the experimental drug, in which 50% of the cells die, or CD<sub>50</sub>.

The chemotherapeutic index, or selectivity index, IS, was calculated by dividing the 50% toxic dose by the 50% effective dose, CD<sub>50</sub>/ED<sub>50</sub>.

### **Results**

The molecular phylogenetic analyses placed the specimens of *Pleurotus* genus from Novosibirsk Region close to *P. pulmonarius* (Fig. 2).

The influenza virus A/California/07/09(H1N1pdm09) was obtained from the WHO Collaborating Center (Atlanta, USA), and has been developed in MDCK cell culture. 2 virus pools were obtained, titration was performed.

In the work on the determination of the antiviral activity of drugs, a dose of 100 TCID<sub>50</sub> was used.

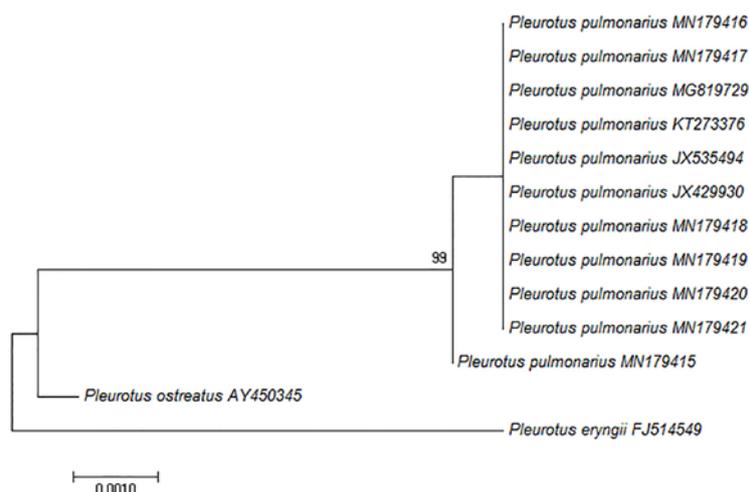
A study of antiviral activity and toxicity of fungal extracts was performed on 14 November and 21 November 2018.

The results of the experiment by definition toxic and effective dose and selectivity index of water and ethanol extracts from fungi against virus A / California / 07/09 (H1N1pdm09) are presented in Tables 3, 4.

Weak antiviral activity against influenza A/California/07/09 strain (H1N1pdm09) was shown by aqueous extracts 5, 6, 7.

Weak antiviral activity against the strain of influenza virus A/California/07/09(H1N1pdm09) showed ethanol extracts 2, 5. The ethanol extract 7 showed moderately expressed antiviral activity.

Ethanol extract No. 7 dated 21/11/2018 showed weak toxicity – CD<sub>50</sub> at a dilution of 1:4, and moderate antiviral activity – ED<sub>50</sub> at a dilution of 1:30. The therapeutic index of the extract is 7.5. As a result, it was found that ethanol extracts from fungi exhibit a more pronounced antiviral effect than aqueous extracts. In this connection, ethanol extraction is a more promising method for extracting the polysaccharide fraction of fungi, in order to create promising preparations with antiviral properties.



**Fig. 2** – Neighbor Joining (NJ) tree showing phylogenetic relationships between the *P. pulmonarius* species from Novosibirsk Region and other related species of *Pleurotus* genus based on the ITS rDNA sequences. *Pleurotus eryngii* (FJ514549) was used as the outgroup taxon. Values on the branches represent the percentage of 1000 bootstrap replicates and bootstrap values over 75% are shown in the tree. Genetic distance 0.005 with bootstrap to support *P. pulmonarius* branch 99%.

**Table 3** Toxic and effective dose and selectivity index of water extracts from fungi against virus A/California/07/09 (H1N1pdm09).

Extract number	Total fraction of polysaccharides, mg/ml	Toxic dose CC <sub>50</sub> mg/ml	Effective dose EC <sub>50</sub> mg/ml	Selectivity index IS
1	22	>5.5	does not reach	
2	25	>6.25	does not reach	
3	23	>5.75	does not reach	
4	20	>5.0	does not reach	
5	24	>6.0	3	>2
6	17	>4.25	2.1	>2
7	29	>7.25	1.8	>4

**Table 4** Toxic and effective dose and selectivity index of ethanol extracts from fungi against virus A/California/07/09 (H1N1pdm09).

Extract number	Total fraction of polysaccharides, mg/ml	Toxic dose CC <sub>50</sub> mg/ml	Effective dose EC <sub>50</sub> mg/ml	Selectivity index IS
1	17	1.7	0.85	2
2	28	7	1.75	4
3	27	>6.75	3.75	<2
4	27	>6.75	3.75	<2
5	32	>8	2	<4
6	19	4.75	1.9	2,5
7	23	5.75	0.77	7,5

### Acknowledgements

The reported study of the antiviral activity of extracts of fungi of the genus *Pleurotus* was funded by RFBR and Government of the Novosibirsk Region to the research project № 18-44-543018 r\_mol\_a. Herbarium specimens from MG Popov Herbarium (NSK), were used.

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