Acta Societatis Botanicorum Poloniae
Journal homepage: pbsociety.org.pl/journals/index.php/asbp
ORIGINAL RESEARCH PAPER Received: 2010.10.07 Accepted: 2011.04.21 Published electronically: 2012.03.30 Acta Soc Bot Pol 81(1):17-21 DOI: 10.5586/asbp.2012.001

Evaluation of polysaccharides content in fruit bodies and their antimicrobial activity of four *Ganoderma lucidum* (W Curt.: Fr.) P. Karst. strains cultivated on different wood type substrates

Krystyna Skalicka-Woźniak[1]*, Janusz Szypowski[1], Renata Łoś[2], Marek Siwulski[3], Krzysztof Sobieralski[3], Kazimierz Głowniak[1], Anna Malm[2]

[1] Department of Pharmacognosy with Medicinal Plant Laboratory, Medicinal University of Lublin, 20-093 Lublin, Poland

[2] Department of Pharmaceutical Microbiology, Medicinal University of Lublin, 20-093 Lublin, Poland

[3] Department of Vegetable Crops, Poznań University of Life Sciences, 60-594 Poznań, Poland

Abstract

Quantitative determination of polysaccharides in *Ganoderma lucidum* fruit bodies from different sawdust cultivation substrates and their antibacterial activity was done. Thirty six samples were analyzed. Four strains of *Ganoderma lucidum* (GL01, GL02, GL03 and GL04) were cultivated on the growth substrates of three different sawdust types: birch (Bo), maple (Kl) or alder (Ol) amended with wheat bran in three different concentrations: 10, 20 and 30% (w/w). Even though the richest in polysaccharides was GL01 strain, the highest yields of the polysaccharides were determined in GL04Kl3 sample and was 112.82 mg/g of dry weight. The antibacterial activity of polysaccharides was determined in vitro using micro-dilution broth method. The panel of eight reference bacterial strains was used. All the polysaccharide samples tested showed the broad spectrum and the moderate antibacterial activity. *Micrococcus luteus* ATCC 10240 strain was the most sensitive with *MIC* (minimal inhibitory concentration) = 0.63 – 1.25 mg/mL.

Keywords: *Ganoderma lucidum*, polysaccharides, extraction, antibacterial activity

Introduction

Ganoderma lucidum (Curt.: Fr.) Karst. is a species of Basidiomycetes, wich belongs to Ganodermataceae (or Polyporaceae) of Aphylloporales [1]. This mushroom grows on a wide variety of dead or dying deciduous trees, especially *Quercus*, *Acer*, *Alnus*, *Betula*, *Carpinus*, *Castanea*, *Fagus*, *Fraxinus*, *Juglans*, *Malus*, *Populus*, *Pyrus*, and *Robinia* species. Species of the genus *Ganoderma* have been reported to occur throughout the world. Over 250 species of this mushroom are known [2]. Investigations of the representatives of the Ganodermataceae in Central European phytocenoses were carried out particularly intensively since the middle of the 20th century. Research in this area has been particularly abundant and there are numerous excellent domestic articles presenting and characterizing proportions of these representatives of cap mushrooms in individual communities of forest and non-forest national parks and nature reserves. The above-mentioned publications describe most frequently *Ganoderma applanatum*, *G. adspersum* (frequently in urbicenosis) as well as *G. lucidum* [3]. Artificial cultivation of *G. lucidum* began in early 1970s, and since 1980, its production has developed rapidly, particularly in China [4]. This mushroom have been cultivated in solid substrates such as lignocellulosic materials, especially sawdust, supplemented with wheat or rice bran [5].

G. lucidum as a medicinal mushroom has been widely used in China (named Ling Zhi) and Japan (named Reishi, Mannentake) for hundreds of years. It was used as a health tonic to promote longevity for more than two thousand years. Many bioactive components have been identified from its fruit bodies, mycelia, spores, and culture media. It was found that the major bioactive components were polysaccharides and triterpenes. Currently available data suggests that *G. lucidum* polysaccharides, mostly β-glucanes, activate host's immune responses exerting anti-cancer functions, whereas anti-tumor effect of triterpenes seems to be related to their cytotoxic activity against the tumor cells directly [6-8]. It is also believed that *Ganoderma lucidum* acts as an immunological agent. It has been used as a natural adjuvant for immunotherapy [9].

The highest attention is paid to polysaccharides from *G. lucidum*, which activate macrophages, lymphocytes, NK cells, proinflmmatory cytokines such as TNF or interleukins, essential for host survival from infection, and are also required for the repair of tissue injury [10,11]. Polysaccharides extracted from the fruit bodies, mycelium, and spores of *G. lucidum* can promote the function of macrophages as well as B cells [12].

* Corresponding author. Email: kskalicka@pharmacognosy.org

Additionally, it has been shown that the anti-oxidant property of *Ganoderma* polysaccharide peptide decreased the oxidation of low density lipoprotein and exhibited anti-oxidant effect by scavenging reactive oxygen species in mice [13]. Other studies demonstrated that *G. lucidum* contained antibacterial constituents [14-16]. The aqueous extract from the carpophores of *G. lucidum* inhibited 15 types of bacteria [17].

It is known that the content of polysaccharides is different depending on the growth substrate. Nowadays almost all of the important medicinal mushrooms have been subjected to large-scale artificial cultivation by solid substrate or low moisture fermentation. Various substrates for *Ganoderma lucidum* cultivation have been investigated as a supplement for substrate mixture. Erkel [18], Siwulski and Sobieralski [19] stated that yielding of *G. lucidum* depended on the type of sawdust, meaning the tree species it originated from. Kim et al. [20] reported that different media in submerged culture of *G. lucidum* influenced the mycelial growth and exobiopolymer production. The aim of our study was quantitative determination of polysaccharides in *G. lucidum* fruit bodies from different sawdust cultivation substrates and their antibacterial activity.

Material and methods

Plant material

Four strains of *Ganoderma lucidum* (Fr.) Karst. fructifications were used in experiment, i.e. GL01, GL02, GL03 and GL04. All strains originated from the Culture Collection of the Department of Vegetable Crops of Poznań University of Life Sciences. As a growth substrate birch (Bo), maple (Kl) or alder (Ol) sawdust were used and they were amended with 10, 20 and 30% (w/w) of wheat bran, correspondingly assigned as (1), (2) and (3). After mixing the components, the substrates were watered up to the moisture content of 65%. In experiment the substrates were packed into PP foil bags with filter (0.02 μm). Each bag contained 1.5 kg of substrate. Bags with the substrates were sterilized at 121°C for 2 h. After cooling to the room temperature (ca. 21°C) the substrates were inoculated with the mycelium of tested *Ganoderma lucidum* strains in the amount equal to 5% of the substrate dry weight. Next, substrates were incubated at 25°C and 80-85% RH until all the substrate volume was overgrown by the fungus. After incubation bags were opened by cutting in the upper part and substrates were transferred into cultivation room with temperature of 17-18°C and RH 85-90%. Cultures were illuminated with fluorescent light (Day-Light) of 500 lx intensity for 12 h a day. Fruiting bodies were set on the upper surface of the substrate. They were harvested once, after the end of maturation, by cutting them back at the substrate surface. The experiment was set in a randomised design with 3 replicates during the spring of 2009.

Reagents and instruments

Ethanol used for preparation of the extract from fungal material was of analytical grade and purchased together with phenol and sulphuric acid from the Polish Reagents (POCH, Gliwice, Poland). Water was purified using a Millipore laboratory ultra-pure water system (SimplicityTM system, Millipore, Molsheim, France). A Specol-11 spectrophotometer (Carl Zeiss, Germany) was employed for reading the absorbance.

Tab. 1 The content of determined polysaccharides in examined samples of *Ganoderma lucidum*.

Codes of samples	C (mg/g)	*SD*	*RSD*
GL01Kl1	46.12	0.0006	0.001
GL01Kl2	59.46	0.0006	0.001
GL01Kl3	43.47	0.0000	0.000
GL01Bo1	45.13	0.0006	0.001
GL01Bo2	61.20	0.0021	0.003
GL01Bo3	88.45	0.0012	0.001
GL01Ol1	58.58	0.0006	0.001
GL01Ol2	82.15	0.0006	0.001
GL01Ol3	71.72	0.0010	0.001
GL02Kl1	76.51	0.0006	0.001
GL02Kl2	65.02	0.0000	0.000
GL02Kl3	36.84	0.0010	0.003
GL02Bo1	50.71	0.0000	0.000
GL02Bo2	37.19	0.0006	0.002
GL02Bo3	39.08	0.0000	0.000
GL02Ol1	35.38	0.0000	0.000
GL02Ol2	37.39	0.0010	0.003
GL02Ol3	37.08	0.0000	0.000
GL03Kl1	28.05	0.0000	0.000
GL03Kl2	41.39	0.0006	0.001
GL03Kl3	33.86	0.0000	0.000
GL03Bo1	33.85	0.0012	0.003
GL03Bo2	35.86	0.0006	0.002
GL03Bo3	49.04	0.0012	0.002
GL03Ol1	45.89	0.0000	0.000
GL03Ol2	44.73	0.0010	0.002
GL03Ol3	41.12	0.0006	0.001
GL04Kl1	54.66	0.0000	0.000
GL04Kl2	63.39	0.0000	0.000
GL04Kl3	112.82	0.0000	0.000
GL04Bo1	24.10	0.0006	0.002
GL04Bo2	28.39	0.0000	0.000
GL04Bo3	24.06	0.0006	0.002
GL04Ol1	24.71	0.0000	0.000
GL04Ol2	18.45	0.0000	0.000
GL04Ol3	25.30	0.0000	0.000

Each value is the mean mg/g of dry sample $n = 3$. Birch, maple, alder sawdust amended with 10, 20 and 30% (w/w) of wheat bran – (1), (2) and (3) respectively. Bo – birch; C – concentration; Kl – maple; Ol – alder; *RSD* – relative standard deviation; *SD* – standard deviation.

Extraction

Polysaccharides were isolated by the method proposed by Pillai [21] with some modifications. Ten grams of fungal material was extracted three times with 200 mL of hot water (85°C) for 5 h. Water extracts were filtered, combined and concentrated to 100 mL. Then 300 mL of chilled ethanol was added and it was left in a cold place (4°C) for 24 h. The precipitate was collected after centrifugation (5000 rpm, 10 min), washed with ethanol and dried. Crude polysaccharides were obtained.

Determination of total sugar in crude polysaccharide

The total sugar in a sample of crude polysaccharide of *G. lucidum* was determined using a phenol-sulphuric acid method [22]. One milligram of each sample was dissolved in 10 mL

Tab. 2 Minimal inhibitory concentrations and minimal bactericidal concentrations of tested polysaccharides isolated from *Ganoderma lucidum* against bacterial reference strains.

	MIC (*MBC*) in mg/mL							
	S. epidermidis	***S. aureus***	***B. subtilis***	***M. luteus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***P. mirabilis***
Codes of samples	**ATCC 12228**	**ATCC 25923**	**ATCC 6633**	**ATCC 10240**	**ATCC 25922**	**ATCC 13883**	**ATCC 9027**	**ATCC 12453**
GLO1Kl1	2.5 (2.5)	2.5 (2.5)	2.5 (5.0)	1.25 (2.5)	2.5 (2.5)	1.25 (5.0)	2.5 (2.5)	2.5 (5.0)
GLO1Kl2	2.5 (2.5)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	1.25 (5.0)	1.25 (2.5)	2.5 (5.0)
GLO1Kl3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	1.25 (5.0)	1.25 (2.5)	2.5 (5.0)
GLO1Bo1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	0.63 (5.0)	2.5 (2.5)	1.25 (2.5)	2.5 (2.5)	2.5 (2.5)
GLO1Bo2	2.5 (5.0)	2.5 (2.5)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	1.25 (2.5)	1.25 (2.5)	2.5 (5.0)
GLO1Bo3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (2.5)	2.5 (5.0)
GLO1Ol1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	0.63 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO1Ol2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	0.63 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO1Ol3	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	0.63 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (2.5)	2.5 (5.0)
GLO2Kl1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Kl2	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Kl3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Bo1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Bo2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Bo3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Ol1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (2.5)	2.5 (5.0)	2.5 (2.5)	2.5 (5.0)
GLO2Ol2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO2Ol3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	1.25 (5.0)	1.25 (5.0)	2.5 (5.0)
GLO3Kl1	2.5 (5.0)	5.0 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Kl2	2.5 (5.0)	5.0 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Kl3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Bo1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Bo2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Bo3	2.5 (5.0)	5.0 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Ol1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Ol2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO3Ol3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (2.5)	2.5 (5.0)
GLO4Kl1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Kl2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Kl3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Bo1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	1.25 (5.0)	1.25 (2.5)	2.5 (5.0)
GLO4Bo2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)
GLO4Bo3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Ol1	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Ol2	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)
GLO4Ol3	2.5 (5.0)	2.5 (5.0)	2.5 (5.0)	1.25 (2.5)	2.5 (5.0)	2.5 (5.0)	2.5 (2.5)	2.5 (5.0)

MIC – minimal inhibitory concentration; *MBC* – minimal bactericidal concentration.

of distilled water. One milliliter of this solution was mixed with 1 mL of 5% phenol solution and 5 mL of concentrated sulphuric acid. The mixture was shaken for 30 min and then the absorbance was measured at 490 nm. The total sugar was calculated based on the standard curve of glucose. All experiments were repeated three times and the *SD* (standard deviation) and *RSD* value (the percentage of relative standard deviation) were calculated.

Calibration procedure

The calibration curve was analyzed three times with five different concentrations as follow: 0.055, 0.114, 0.155, 0.239, 0.475 mg/mL. The quantification was performed by measuring the absorbance. The calibration curves were characterized by their regression coefficient, slope of the line (b) and intercept of the straight line with *y* axis (a).

Antibacterial assay in vitro

Minimal inhibitory concentration (*MIC*) of the polysaccharide fractions obtained from *G. lucidum* fruit bodies from different sawdust cultivation substrates for eight reference strains, including four Gram-positive bacteria (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240) and four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453), was performed by the micro-dilution broth method according to Skalicka-Woźniak et al. [23]. Briefly, the stock solutions of polysaccharides fractions were prepared in dimethyl sulphoxide (DMSO).

The series of two-fold dilution of this stock solutions, ranging from 0.075 to 5 mg/mL, were prepared in Mueller-Hinton broth (Biocorp, Poland) in 96-well microtiter plates. Bacterial inoculum was added to each well to obtain final optical density corresponding to 5 × 105 CFU/mL. After incubation at 35°C for 24 h, the *MIC* was assessed visually as the lowest concentration of polysaccharides showing total inhibition of bacterial growth. Appropriate DMSO, growth and sterile controls were carried out. Minimal bactericidal concentration (*MBC*) of polysaccharide fractions towards all bacterial strains tested was defined by subculturing 0.1 mL from each well that showed bacterial growth inhibition onto Mueller-Hinton agar (Biocorp, Poland) plates. The plates were incubated at 35°C for 24 h and the *MBC* was defined as the lowest concentration of essential oil at which there was no bacterial growth. Each experiment was repeated in triplicate. Representative data are presented.

Results and discussion

The calibration curve was linear (R^2 = 0.9993) in a concentration range 0.055-0.475 mg/mL and was $y = 1.7465x + 0.0106$. The *RSD* value as a measure of repeatability was lower than 0.003. The amounts of polysaccharides in the analyzed samples are shown in Tab. 1.

Thirty six samples were analyzed. Four strains of *Ganoderma lucidum* (GL01, GL02, GL03 and GL04) were cultivated on the growth substrates of three different sawdust types: birch, maple or alder amended with wheat bran in three different concentrations: 10, 20 and 30% (w/w). Even though the highest production of polysaccharides was noticed in GL01 strain, the highest amount of polysaccharides were calculated in GL04Kl3 sample. The lowest level of production of polysaccharides was in the GL03 strain, low level was noticed also for GL04 strain. However, it was the GL04Kl3 sample that appeared to be the richest in interesting compounds. Alder sawdust was found as the best growth substrate for GL01 and GL03 strains, while for strains GL02 and GL04 it was a maple sawdust. Sugars, as the carbon sources, can influence mycelium growth and polysaccharide production [24]. It is known that lactose exerts more favourable impact on cell growth and production of intercellular polysaccharide than glucose. The effect of different carbon sources on the biomass production in *G. lucidum* was reported by Erkel [25], Avtonomova et al. [26] and Yang et al. [27]. Baabitskaia et al. [28] maintain that polysaccharide production by *G. lucidum* depends on such conditions as the initial pH of the substrate, C:N ratio and incubation temperature. The influence of substrate pH on polysaccharide production by *G. lucidum* was also reported by Kim et al. [29] and Lee et al. [30]. According to Prosiński [31], wood of various tree species differs with regard to the content of cellulose, lignin, hemicellulose, sugars, protein as well as other substances and shows differences in pH. It is possible that these properties led to differences in the polysaccharide content of the fruiting bodies of *G. lucidum* obtained from the substrates used in our own experiments.

According to our results presented in Tab. 2 the analyzed samples of polysaccharides exhibited inhibitory effect against all bacterial strains tested with *MIC* values ranging from 0.62 to 5.0 mg/mL, however, *M. luteus* was the most sensitive strain (*MIC* = 0.62 – 1.25 mg/mL). The *MBC* values for all analyzed samples were comparable (2.5 or 5.0 mg/mL). Only slight differences of *MICs* and *MBCs* for polysaccharide samples obtained from the particular *G. lucidum* strains and the cultivate conditions were observed. The low values of *MBC*/*MIC* ratio (1-8) suggest that polysaccharides acted as bactericidal agents.

The screening of antibacterial activity indicates that there were no significant differences in the spectrum or the power of activity between the polysaccharides obtained from four strains of *G. lucidum* fruit bodies from different sawdust cultivation substrates. All the polysaccharide samples tested in our study show the broad spectrum of antibacterial activity, covering both Gram-positive and Gram-negative strains, however, the power of this activity is moderate. Our results are in agreement with other studies reporting the moderate activity and the broad antimicrobial spectrum of the different extracts from *G. lucidum* [17,32,33]. Its polysaccharides are confirmed to be the bioactive components, which show the significant antibacterial activity. Similarly to data obtained by Yoon et al. [17], the polysaccharides tested in our study exerted the strongest inhibitory effect towards *M. luteus* (*MIC* 0.62 or 1.25 mg/mL).

Acknowledgements

The authors wish to acknowledge the financial support of a part of the studies by project grant No. NN310083636 from the Polish Ministry of Science and Higher Education.

References

1. Moncalvo JM, Ryvarden L. A nomenclatural study of the Ganodermataceae Donk. Oslo: Fungiflora; 1997. (Synopsis fungorum; vol 11).
2. Gottlieb AM, Saidman BO, Wright JE. Isoenzymes of *Ganoderma* species from southern South America. Mycol Res. 1998;102(04):415-426. http://dx.doi.org/10.1017/S0953756297005352
3. Sokół S. Ganodermataceae Polski: taksonomia, ekologia i rozmieszczenie. Katowice: Silesian University Press; 2000.
4. Wasser PS. Reishi or Ling Zhi (*Ganoderma lucidum*). In: Coates PM, Blackman M, Cragg G, Levine M, Moss J, White JD, editors. Encyclopedia of dietary supplements. New York: Marcel Dekker; 2005. p. 603-622.
5. Stamets P. Growing gourmet and medicinal mushrooms. Berkeley: Ten Speed Press; 2000.
6. Wasser SP, Weis AL. Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. Crit Rev Immunol. 1999;19(1):65-96.
7. Lin ZB, Zhang HN. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol Sin. 2004;25(11):1387-1395.
8. Kuo MC, Weng CY, Ha CL, Wu MJ. *Ganoderma lucidum* mycelia enhance innate immunity by activating NF-kappaB. J Ethnopharmacol. 2006;103(2):217-222. http://dx.doi.org/10.1016/j.jep.2005.08.010
9. Chan WK, Lam DTW, Law HKW, Wong WT, Koo MWL, Lau ASY, et al. *Ganoderma lucidum* mycelium and spore extracts as natural adjuvants for immunotherapy. J Altern Complement Med. 2005;11(6):1047-1057. http://dx.doi.org/10.1089/acm.2005.11.1047
10. Gao Y, Gao H, Chan E, Tang W, Xu A, Yang H, et al. Antitumor activity and underlying mechanisms of ganopoly, the refined polysaccharides extracted from *Ganoderma*

lucidum, in mice. Immunol Invest. 2005;34(2):171-198. http://dx.doi.org/10.1081/IMM-200055813
11. Ahmadi K, Riazipour M. Effect of *Ganoderma lucidum* on cytokine release by peritoneal macrophages. Iran J Immunol. 2007;4(4):220-226.
12. Shao BM, Dai H, Xu W, Lin ZB, Gao XM. Immune receptors for polysaccharides from *Ganoderma lucidum*. Biochem Biophys Res Commun. 2004;323(1):133-141. http://dx.doi.org/10.1016/j.bbrc.2004.08.069
13. Paterson RR. *Ganoderma* – a therapeutic fungal biofactory. Phytochemistry. 2006;67(18):1985-2001. http://dx.doi.org/10.1016/j.phytochem.2006.07.004
14. Kim DH, Bae EA, Jang IS, Han MJ. Anti-*Helicobacter pylori* activity of mushrooms. Arch Pharm Res. 1996;19(6):447-449. http://dx.doi.org/10.1007/BF02986009
15. Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Díez MT, et al. Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek. 2000;78(2):129-139.
16. Gao Y, Zhou S, Huang M, Xu A. Antibacterial and antiviral value of the genus *Ganoderma* P. Karst. species (Aphyllophoromycetideae): a review. Int J Med Mushrooms. 2003;5(3):235-246. http://dx.doi.org/10.1615/InterJMedicMush.v5.i3.20
17. Yoon SY, Eo SK, Kim YS, Lee CK, Han SS. Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. Arch Pharm Res. 1994;17(6):438-442.
18. Erkel EI. The effect of different substrate mediums on yield of *Ganoderma lucidum* (Fr.) Karst. J Food Agric Environ. 2009;7:841-844.
19. Siwulski M, Sobieralski K. Mycelium growth and yielding of two strains of *Ganoderma lucidum* (Fries) Karst. cultivated on sawdust substrate. Veg Crops Res Bull. 2001;54:101-104.
20. Kim SW, Hwang HJ, Park JP, Cho YJ, Song CH, Yun JW. Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. Lett Appl Microbiol. 2002;34(1):56-61. http://dx.doi.org/10.1046/j.1472-765x.2002.01041.x
21. Pillai TG, Nair CKK, Janardhanan KK. Polysaccharides isolated from *Ganoderma lucidum* occurring in southern parts of India, protects radiation induced damages both in vitro and in vivo. Environ Toxicol Pharmacol. 2008;26(1):80-85. http://dx.doi.org/10.1016/j.etap.2008.02.004
22. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28(3):350-356. http://dx.doi.org/10.1021/ac60111a017
23. Skalicka-Woźniak K, Los R, Głowniak K, Malm A. Antimicrobial activity of fatty acids from fruits of *Peucedanum cervaria* and *P. alsaticum*. Chem Biodivers. 2010;7(11):2748-2754. http://dx.doi.org/10.1002/cbdv.201000008
24. Hsieh C, Tseng MH, Liu CJ. Production of polysaccharides from *Ganoderma lucidum* (CCRC 36041) under limitations of nutrients. Enzyme Microb Technol. 2006;38(1-2):109-117. http://dx.doi.org/10.1016/j.enzmictec.2005.05.004
25. Erkel EI. Yield performance of *Ganoderma lucidum* (Fr.) Karst cultivation on substrates containing different protein and carbohydrate sources. Afr J Agric Res. 2009;4(11):1331-1333.
26. Avtonomova AV, Krasnopol'skaya LM, Maksimov VN. Optimization of nutrient medium for submerged cultivation of *Ganoderma lucidum* (Curt.: Fr.) P. Karst. Microbiology. 2006;75(2):148-153. http://dx.doi.org/10.1134/S002626170602007X
27. Yang FC, Ke YF, Kuo SS. Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. Enzyme Microb Technol. 2000;27(3–5):295-301. http://dx.doi.org/10.1016/S0141-0229(00)00213-1
28. Babitskaya VG, Shcherba VV, Puchkova TA, Smirnov DA. Polysaccharides of *Ganoderma lucidum*: Factors affecting their production. Applied Biochemistry and Microbiology. 2005;41(2):169-173. http://dx.doi.org/10.1007/s10438-005-0029-1
29. Kim HM, Park MK, Yun JW. Culture pH affects exopolysaccharide production in submerged mycelial culture of *Ganoderma lucidum*. Appl Biochem Biotechnol. 2006;134(3):249-262. http://dx.doi.org/10.1385/ABAB:134:3:249
30. Lee KM, Lee SY, Lee HY. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor. J Biosci Bioeng. 1999;88(6):646-650. http://dx.doi.org/10.1016/S1389-1723(00)87094-2
31. Prosiński S. Wood chemistry. Warszawa: PWRiL; 1984.
32. Klaus A, Niksic M. Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Zbornik Matice srpske za prirodne nauke. 2007;(113):219-226. http://dx.doi.org/10.2298/ZMSPN0713219K
33. Keypour S, Riahi H, Moradali MF, Rafati H. Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae), from Iran. Int J Med Mushrooms. 2008;10(4):345-349. http://dx.doi.org/10.1615/IntJMedMushr.v10.i4.70