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ORIGINAL RESEARCH PAPER

Chemical compounds of extracts from *Sarcodon imbricatus* at optimized growth conditions

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Abstract
The effect of carbon and nitrogen sources and initial pH and temperature of the medium on the mycelial growth of *Sarcodon imbricatus* (L.) P. Karst. in axenic liquid culture was investigated. The optimal composition of the medium was found to be: 5% fructose, 1% hydrolysate of casein, 1% yeast extract, and 0.3% KH_2PO_4 at pH = 6 and incubation temperature of 20°C. In this condition the maximum biomass growth was observed, yielding 10.2 g L^{-1} of dry weight after 3-week of growth. The medium regarded as optimal for growth of *S. imbricatus* mycelium was used for the production of the biomass and further chemical analysis. The quantitative and qualitative composition of phenolic acids, fatty acids, and sterols were determined using chromatographic methods. The total content of phenolic acids was 1.86 mg × 100 g^{-1} DW, with the largest amount of protocatechuic acid (1.27 mg × 100 g^{-1} DW). Nineteen fatty acids were estimated, including five unsaturated fatty acids, e.g., oleic and α-linolenic acid. The analysis of sterols composition revealed the presence of ergosterol and ergosterol peroxide (197.7 and 200.47 mg × 100 g^{-1} DW, respectively). These compounds were isolated and confirmed by ^{1}H-NMR. Presented study constitutes the first report on the accumulation of substances (phenolic acids, fatty acids, and sterols) with multidirectional biological activity in the mycelial axenic culture of *Sarcodon imbricatus*.

Keywords
Sarcodon imbricatus; in vitro cultures; secondary metabolites

Introduction

An alternative method to obtain secondary metabolites with therapeutic properties from fungal fruiting bodies is to use the biosynthetic capability of in vitro mycelial cultures. The main advantage of in vitro cultures is their independence from the environmental conditions and the ability to produce continuously high-quality material. The intensity of metabolites' synthesis and their further accumulation can be controlled by the nutrient composition of the medium and the environmental parameters during culture. For this reason, it is important to optimize the conditions for in vitro culture of fungi [1,2].

The genus *Sarcodon* includes some species with therapeutic potentials, e.g., *Sarcodon aspratus* was found effective in antitumor activity and *Sarcodon scabrosus* in anti-inflammatory actions [3–5]. Interesting biological properties of this genus

are associated with the occurrence of ergosterol peroxide (3β-hydroxy-5α,8α-epidioxyergosta-6,22-dien), which exhibits a series of activities such as antileukemic and antitumor [6] and inflammatory activity [7]. Recent studies have demonstrated that *Sarcodon imbricatus* is also important as an agent that stimulates neuronal growth and promotes brain health [8]. Our previous studies of *S. imbricatus* indicated antimicrobial activity against five strains of Gram-positive and Gram-negative bacteria, antiviral activity against HPV-1 virus, and cytotoxic activity toward B16 murine melanoma tumor cell lines, sarcoma XC, and human breast cancer cell line in vitro of selected polysaccharide fractions isolated from mycelial cultures of this fungal species [9,10].

This mushroom is ectomycorrhizal species associated with *Picea*, common in the south-west of Europe, but becoming rare in Poland [11].

Fruiting bodies are considered edible, though it can have a slightly bitter taste and therefore the culinary value of *S. imbricatus* is considered moderate [12]. When eaten raw it can be even poisonous.

The aim of the present study was to initiate in vitro culture of *S. imbricatus* to determine the optimal conditions for mycelial growth and accumulation level of compounds with biological activity in extracts from the obtained biomass. Presented results are the first report on the quantitative and qualitative analysis of a group of compounds with multidirectional therapeutic effects such as phenolic acids, fatty acids, and sterols obtained from the biomass of mycelial cultures of *S. imbricatus*. These compounds show various biological activities such as antitumor, immunostimulating, antioxidant, and protection of cardiovascular system [13].

Material and methods

Mushroom material and obtaining in vitro culture

The fruiting bodies of *S. imbricatus* were collected under spruce trees (*Picea* sp.) in the Forest District of Krynica (southern Poland) in August 2008. Four fruiting bodies were found and after taxonomic identification [14,15], one of them was used to obtain mycelial cultures. Representative voucher specimens were deposited at the Department of Pharmaceutical Botany, Pharmaceutical Faculty, Jagiellonian University Medical College, Kraków. The in vitro cultures were obtained from the hymenial part of the fruiting body. Pieces of fruiting body were sterilized in 0.1% NaOCl for 10 min and inoculated on a Petri dishes containing an agar-solidified standard medium (SM) of a composition described by Turło [16] (glucose 5%, yeast extract 1%, casein hydrolysate 1%, KH_2PO_4 0.3%, agar 10%). This initial cultures were grown at 22°C ±2°C and were sub-cultured every three weeks. The obtained strain UJCMBF-51MC (PCM 2719) was deposited in the Polish Collection of Microorganisms (PCM), Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

After growing on the solid medium the pieces of mycelium were inoculated in the Erlenmeyer flask (500 mL) containing 250 mL of the standard medium as described above and were maintained as agitated liquid cultures.

Optimization of culture conditions

Two types of liquid cultures, agitated and stationary ones were used for optimization of the culture conditions. An effect of carbon and nitrogen source and pH of the medium was evaluated in the agitated culture and an effect of temperatures was tested in stationary cultures

To find the most beneficial carbon source for the growth of *S. imbricatus* mycelium, various carbon sources such as monosaccharides (fructose and glucose), disaccharides (maltose and sucrose), and polysaccharide (starch) were compared as a carbon source in a standard medium.

To analyze the effect of nitrogen sources on the mycelial growth, *S. imbricatus* mycelium was cultivated on a standard medium with casein hydrolysate, urea, ammonium sulfate, and ammonium phosphate as a nitrogen source.

To test the effect of pH on growth of *S. imbricatus* mycelium, the pH value of the standard medium was adjusted from 3.0 to 7.5 at 0.5 increments using 0.1 M HCl or 0.1 M NaOH. The pH values were checked using a digital pH-meter (CP-505 – Elmetron, Poland).

The liquid cultures were maintained in an Erlenmeyer flask (500 mL) containing 250 mL of a liquid medium. The culture was shaken at a rate of 140 rpm (shaker ALTEL, Poland), under 16-h light (900 lx / 8 dark) at 22°C ±2°C.

The effect of temperature on the liquid stationary cultures was determined in an incubator (ST500/B/40, POL-EKO-APARATURA, Poland) at various temperatures (15°C, 20°C, 25°C, 30°C, and 35°C). Cultures were maintained in an Erlenmeyer flask (500 mL) containing 250 mL of a liquid standard medium.

After 3-weeks of growth, the mycelium was separated from the liquid medium using a filter paper on a Büchner funnel, rinsed with redistilled water, frozen and dried by lyophilization (lyophilizer Freezone 4.5, Labconco, USA; temperature: –40°C) and the dry biomass was determined.

Chemical analyzes

The final composition of the culture medium selected during the optimization process was used for the cultivation of biomass for further chemical analysis.

Determination of phenolic acids. The extraction process was carried out as follows: 2 g of powdered lyophilized biomass was twice extracted with 100 mL of boiling methanol (at 67.4°C) for 2 h under a reflux condenser. The combined extracts (200 mL) were concentrated to dryness using a rotary vacuum evaporator at 40°C. Then the residues were dissolved in 10 mL of methanol.

The HPLC method was followed according to the procedure developed by Ellnain-Wojtaszek and Zgórka with some modifications [17]. The qualitative and quantitative HPLC analyzes were conducted using HPLC VWR Hitachi apparatus: autosampler L-2200, pump L-2130, RP18 column (250 × 4 mm, 5 μm) thermostated at 25°C, column oven L-2350, and diode array detector (DAD) L-2455 at UV range 200–400 nm. The mobile phase consisted of solvent A: methanol / 0.5% acetic acid 1:4 (v/v) and solvent B: methanol. The gradient was as follows: (A:B) 100:0 for 0–25 min; 70:30 for 35 min; 50:50 for 45 min; 0:100 for 50–55 min; 100:0 for 57–67 min. The comparison of the UV spectra at $\lambda = 254$ and retention times with standard compounds enabled the identification of phenolic acids present in the analyzed samples. The quantitative analysis was performed using a calibration curve with the assumption of linear size of the area under the peak and the concentration of the reference standard.

Determination of fatty acids. One gram of powdered lyophilized biomass was extracted with chloroform/methanol solution, 2:1 (v/v). The fatty acid methyl esters (FAME) were synthesized using 20% BF3 in methanol at 100°C. The FAME analyzes were done with gas chromatography – Agilent 6890 N with capillary column J&W DB – 23 (60 m, ID 0.25 mm, 0.25 μm) and FID detector. The chromatograph parameters: FID 260°C, injector 250°C, split ratio 50:1, oven 140°C for 5 min, ranged from 140 to 190°C at 4°C/min, 190°C for 15 min, and ranged from 190 to 240°C at 2.75°C/min, 240°C for 4 min, carrier gas – helium. For the identification of the fatty acids (FA), retention times of standards FAME from Supelco (47801) were used. The peak areas were measured with an integrator (ChemStation). The results for FA composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in the samples were expressed as relative percentage of the total fatty acids.

Determination of sterols. Five grams of powdered lyophilized biomass was extracted with a mixture of methanol/dichloromethane 7.5:2.5 (v/v). The mixture was sonicated at 40 kHz for 10 min. After 2 hours, the extract was centrifuged at 12 000 rpm for

10 min and decantated. The extraction procedure was repeated twice and the obtained extracts were mixed together and evaporated under reduced pressure.

The HPLC analysis was conducted on a Hitachi (Merck, Germany) liquid chromatograph equipped with a detector UV-Vis L-7400. Sterols were separated and analyzed using an RP18 column (4.6 × 250 mm, 5 µm) at 30°C. The mobile phase consisted of solvent A: methanol/water, 80:20 (v/v) and solvent B: methanol/dichloromethane 75:25 (v/v). A gradient procedure was used as follows: starting with the sample injection, 60% of solvent B for 5 min, a linear gradient from 60 to 100% of solvent B for 10 min, and 100% for 10 min. The flow rate was 1.0 mL min^{-1} [18]. The quantitative analysis was performed using a calibration curve with the assumption of linear size of the area under the peak and the concentration of the reference standard.

The isolation and purification of ergosterol and ergosterol peroxide were performed with chromatographic methods using HP-TLC plates (Merck Millipore, Germany). The ^{1}H-NMR spectra were recorded using the Mercury 300-BRÜKER ^{1}H 300.08 MHz apparatus in NMR Spectroscopy Laboratory of the Department of Organic Chemistry at Jagiellonian University Medical College in Kraków.

Statistical analysis

All received data were presented as mean ± standard deviation (*SD*) and subjected to statistical analysis (STATISTICA version 12 PL software package, StatSoft). Statistical significance of differences between studied groups was estimated with non-parametric Mann–Whitney *U* test.

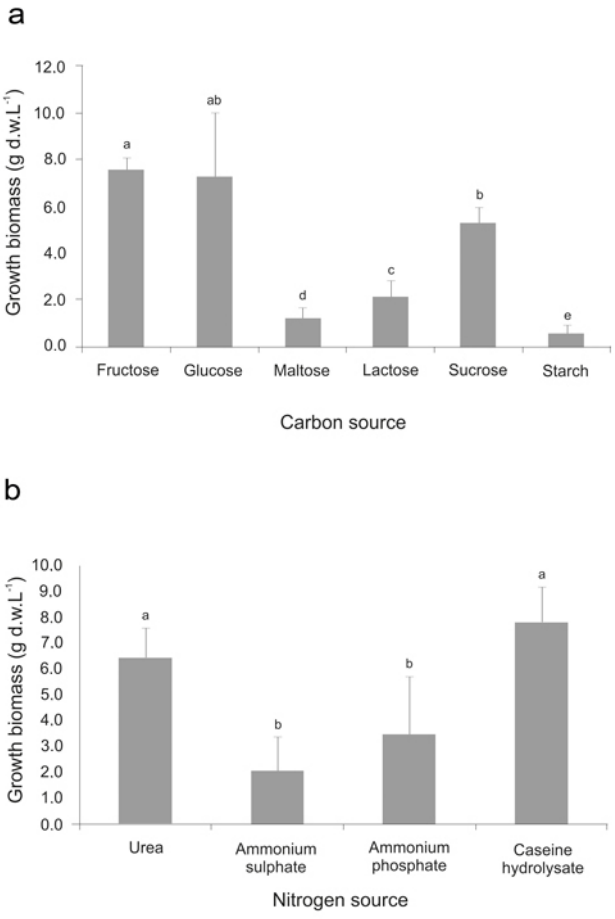

Fig. 1 Effect of carbon (**a**) and nitrogen (**b**) sources on the mycelium biomass growth of *Sarcodon imbricatus*. Different letters indicate significant differences with $p < 0.05$.

Results and discussion

Optimization of culture conditions

From among the tested carbon sources, the highest mean dry biomass 8.34 g L^{-1} DW was observed for the medium containing fructose. The biomass growth on the medium supplemented with glucose was also high in compare to other carbon sources (7.24 g L^{-1} DW) (Fig. 1a).

Glucose and fructose are commonly used as carbon sources for in vitro cultures of higher fungi. Of the six carbon sources tested by Joo [19], including glucose, fructose, maltose, and sucrose, the largest mycelium growth in *S. aspratus* was noted on the medium contained glucose and fructose [19]. When considering the analyzed nitrogen sources, the highest mean dry biomass (7.76 g L^{-1} DW) was observed on the medium containing casein hydrolysate; also the growth was substantial on the medium supplemented with urea (6.43 g L^{-1} DW) (Fig. 1b). The low biomass of *S. imbricatus* mycelium was noted on the medium with the addition of inorganic nitrogen sources.

The studies by Joo proved that peptone was the best nitrogen source for biomass growth in *S. asptatus* [19]. The studies conducted in other research centers have indicated the preference for using organic nitrogen sources for biomass growth in mycelial cultures over inorganic sources [20]. This was also demonstrated by Newcomb and Jennison [21], who tested the nitrogen requirement when it was delivered from inorganic sources (ammonium salts) vs. organic sources (amino acids, casein, and peptone) in 42 species of

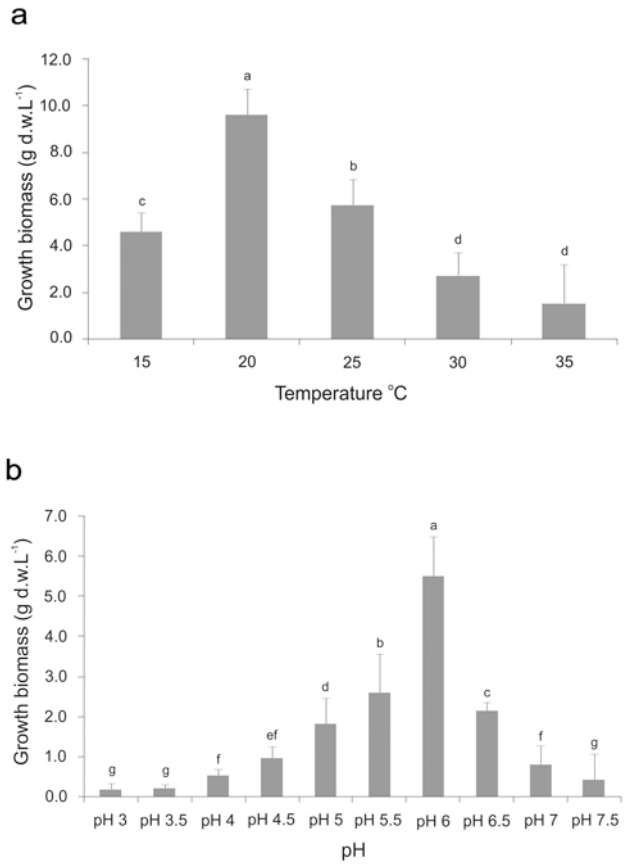

Fig. 2 Effect of temperature (**a**) and initial pH (**b**) of the medium on the mycelial biomass growth of *Sarcodon imbicatus*. Different letters indicate significant differences with $p < 0.05$.

fungi, including three polyporales species, e.g., genera *Peniophora*, *Polyporus*, and *Trametes*. These authors reported that only a single species, *Trametes serialis*, grew in the presence of ammonium chloride as an exclusive source of nitrogen [21]. Thus our results confirmed the previous data about the suitability of organic nitrogen sources in mycelial cultures of Basidiomycetes.

To investigate the effect of temperature has on mycelial growth we incubated stationary liquid cultures on the standard medium at temperatures ranging from 20 to 35°C. The most suitable incubation temperature for mycelial cultures was found to be 20°C. The final biomass after a 3-week incubation at 20°C was 10.96 g L^{-1} DW and was eight times higher than the biomass noted at 35°C (2.72 g L^{-1} DW) (Fig. 2a). Production of mycelium occurs over the wide range of temperature. Numerous studies have found an optimum temperature for growths of Basidiomycota between 20 and 30°C [22,23]. Obtained result is comparable with those of many kinds of mushrooms that exhibit relatively low-temperature optima, ranging from 20 to 25°C in their submerged cultures [24].

The optimum initial pH value was estimated to be 6.0. When this value was achieved, a biomass growth of 6.0 g L^{-1} DW was noted after the 3-weeks incubation period, which was 20 times higher than that observed on the medium with lowest pH value (3.0) amounting only to 0.28 g L^{-1} DW (Fig. 2b).

For the mycelium growth of different species of higher fungi, the optimal pH value ranges from 5.0 to 6.0, except for the arboreal species in which the optimal pH can be lowered down to 4.0 [23,25]. In our research, the highest amount of mycelium biomass of *S. imbricatus* was obtained at the initial medium pH = 6. Moreover, further lowering (to pH = 3) or increasing (up to pH = 7.5) of the pH of culture medium results in many times smaller mycelial growth. Our results confirm that the optimal pH value for axenic culture of *S. imbricatus* amounts around 6.0, and increase up to 7.5 results in a significant decrease in the biomass growth.

Many researchers consider that the acidity of the medium is critical for the biomass formation and metabolites accumulation. They suggest that a suitable pH value of the medium is crucial for normal functioning of the cellular membranes, maintenance of cell structure, and morphology, and also for the intake of nutrients and synthesis of active metabolites [26]. Based on the obtained results expressed as a dry biomass increments, the medium variant containing fructose at 50 g L^{-1} as a carbon source and casein hydrolysate at 10 g L^{-1} as nitrogen source was chosen for further studies. The optimum incubation temperature was determined as 20°C and the initial pH value was set at 6.0. The best increase in biomass was observed during 3-week growth cycles in shaking liquid cultures and averaged at 10.2 g L^{-1} DW.

This increase in biomass and the dynamics of mycelium growth differ from the results that we had obtained for *Xerocomus badius* and *Tricholoma equestre* cultures studied earlier [27]. Obtained during the optimization of biomass *S. imbricatus* increases were higher than in previously conducted mycelial cultures of *X. badius* and *T. equestre*. The increments of biomass amounted to 5.2 and 7.2 g L^{-1} DW for *X. badius* and *T. equestre*, respectively. In all of the studied cultures were obtained homogeneous biomass in the form of spherical aggregates [27]. Mycelial cultures of these species were maintained on the basic variant of a medium, without the modification of the culture condition. The obtained results indicate the need to carry out the optimization of mycelial cultures.

Tab. 1 Amounts (mg × 100 g^{-1} DW) of phenolic acids and sterols in biomass extracts from in vitro cultures of *Sarcodon imbricatus* cultivated on the optimized medium described by Turło [16], during 3-weeks growth cycles.

Name of compound	Amount
Phenolic acids	
p-Hydroxybenzoic acid	0.31 ±0.002
Protocatechuic acid	1.27 ±0.020
Syringic acid	0.29 ±0.054
Total content	1.86 ±0.040
Sterols	
Ergosterol	197.70 ±1.99
Ergosterol peroxide	200.47 ±1.08
Total content	398.17 ±0.99

All experimental data are mean ± standard deviation (*SD*) of triple determination.

Determination of phenolic acids

Among the 14 phenolic acids studied, only three of them were detected in the tested extracts (Tab. 1). The total amount of free phenolic acids was 1.86 mg × 100 g^{-1} DW. From the results of analyzes, it was found that mycelium from in vitro cultures of *S. imbricatus* contained derivatives of benzoic acid: *p*-hydroxybenzoic, protocatechuic, and syringic acids. In quantitative terms, the main metabolite was protocatechuic acid (1.27 mg × 100 g^{-1} DW). The available literature contains data on the occurrence and amounts of phenolic acids in the fruiting bodies. However, up to now phenolic acids, has not been identified in the biomass from in vitro cultures of Basidiomycota.

The phenolic acids are the main group of phenolic compounds produced by mushrooms. Their strong antioxidant activity and ability to protect important cellular structures such as cellular membranes, structural proteins, enzymes, cell membrane lipids, as well as nucleic acids against oxidative stress are the reason for their wide biological action [28]. Most common phenolic acids in fungi are gallic, ferulic, gentisic, *p*-hydroxybenzoic, *p*-coumaric, caffeic, protocatechuic, syringic, and vanillic acids [29]. The analyzes carried out on 23 species of mushrooms growing in their natural state (India) showed that among the phenolic acids present in mushrooms, gallic, gentisic, and protocatechuic acids occurs in large quantities. The highest amount of gallic acid is observed in *Morchella conica* (12.85 mg g^{-1}), and protocatechuic and gentisic acids in *Helvella crispa* (18.48 mg g^{-1} and 4.89 mg g^{-1}, respectively). Other phenolic acids, i.e., ferulic, caffeic, coumaric, syringic, and vanillic are present in small or trace amounts in fungi [30]. Compared to edible mushrooms, medicinal mushrooms have a higher total content of phenolic compounds [31].

In the studies conducted by Barros, levels of phenolic acids had been determined in more than a dozen species of fungi from the genera *Agaricus*, *Lactarius*, and *Lycoperdon* commonly found in Central Europe [32]. The study also included the fruiting bodies of the *S. imbricatus* in which *p*-hydroxybenzoic acid was determined at 3.32 mg × 100 g^{-1}. In other fruiting bodies of Basidiomycota analyzed by Barros, protocatechuic acid was found in considerable amounts, e.g., 34.2 mg × 100 g^{-1} in the species *Ramaria botrytis* [32].

Determinations of fatty acids

The present study is the first of its kind which determined the composition of free fatty acids (FFA) in the biomass from mycelia cultures of *S. imbricatus* and showed that the in vitro cultures were a good source of therapeutically important FFA. The percentage of fatty acids in the biomass from mycelial cultures is shown in Tab. 2. One of quantitatively predominant compounds in our studies was α-linolenic acids (*cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid) (23.32%). This compound possesses multidirectional therapeutic action: is indispensable for normal lipid transport, participating in prostaglandin synthesis, lowering blood pressure, and changing blood coagulation ability [33]. Free fatty acids are the common components of the species belonging to Basidiomycota and their presence in spores is an important determinant for taxonomy [34].

FFA contents range from 20% to 30% and the substantial amount of unsaturated fatty acids, even up to 70% of the total fatty acids, is conspicuous [35].

The previous studies showed the presence of palmitic, oleic, and linoleic acid in fruiting bodies of Basidiomycota. The unsaturated fatty acids mainly belonged to the ω6 and 9 family indicating the carboxyl-directed desaturation as a major metabolic pathway [36]. Low fatty acid contents are characteristic of the fruiting bodies of the species *Agaricus bisporus* and *Pleurotus ostreatus* (0.3 and 0.486 mg × 100 g^{-1} DW,

Tab. 2 Contents (%) of fatty acids in biomass extract from in vitro cultures of *Sarcodon imbricatus* cultivated on the optimized medium described by Turło [16], during 3-week growth cycles.

Systematic name	Lipid numbers	%	*SD*
SFA			
Octanoic acid	C8:0	3.55	0.45
Decanoic acid	C10:0	0.96	0.07
Dodecanoic acid	C12:0	1.82	0.11
Tridecanoic acid	C13:0	0.94	0.05
Tetradecanoic acid	C14:0	0.91	0.01
Pentadecanoic acid	C15:0	0.73	0.04
Hexadecanoic acid	C16:0	18.64	0.50
Heptadecanoic acid	C17:0	0.32	0.02
Octadecanoic acid	C18:0	0.54	0.01
Eicosanoic acid	C20:0	31.09	0.39
Docosanoic acid	C22:0	0.52	0.04
MUFA			
9-Tetradecenoic acid	C14:1*cis*9	0.20	0.05
7-Hexadecenoic acid	C16:1ω7	0.66	0.51
trans-9-Octadecenoic	C18:1ω9 *trans*	0.83	0.02
cis-1-Eicosanoic	C20:1	0.34	0.01
9-*cis*-Octadecenoic acid	C18:1ω9	12.77	0.41
cis-13-Docosenoic	C22:1ω9	0.67	0.03
PUFA			
cis,*cis*,*cis*-9,12,15-Octadecatrienoic acid	C18:3ω3	23.32	0.37
5,8,11,14-all-*cis*-Eicosatetraenoic acid	C20:4w6	1.19	0.08

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUF – polyunsaturated fatty acids. All experimental data are mean ± standard deviation (*SD*) of triple determination.

respectively) [37]. However, *Cantharellus cibarius* and *Boletus edulis* contain up to 8–10 times greater quantities of fatty acids. Lauric acid (dodecanoic acid), myristic acid (tetradecanoic acid), and palmitic acids (hexadecanoic acid) dominate among the saturated fatty acids, whereas oleic (9-*cis*-octadecenoic acid) and linoleic (*cis*,*cis*-9,12-octadecadienoic acids) prevail among unsaturated ones. More recent data describe that the coriolinic acid (1,3-hydroxy-9-*cis*,11-*trans*-octadecadienoic acid) and linoleic acid isolated from *Pleurotus pulmonarius* showed anthelminthic properties [38].

In the study of Barros et al. [39], the major fatty acids found in *S. imbricatus* were linoleic and oleic acids followed by palmitic acid. This observation is in agreement with the results of our study, but we also detected a high amount of linolenic acid in the mycelial cultures which may suggest the conversion of linoleic acid into linolenic one. The sum of unsaturated fatty acids was similar to the content of these acids detected by Barros [39]. Our studies suggest an influence of the medium components on the synthesis of fatty acids.

Determination of sterols

HPLC analysis of the extracts from the biomass cultured in vitro revealed peaks with retention times corresponding to the retention time of ergosterol (ergosta-5,7,22-trien-3β-ol) and ergosterol peroxide (5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3β-ol) standards. Its contents were determined from a standard curve and showed that content from in vitro cultures amounted 197.70 and 200.47 86 mg × 100 g^{-1} DW, respectively (Tab. 1).

There are no data on the content of ergosterol in in vitro cultures of *S. imbricatus* and the present study is the first one that documented the identity of sterols in these cultures with spectral methods. The identity of ergosterol and ergosterol peroxide were confirmed by comparing the obtained spectral data with literature data [40,41].

The sterols are widespread chemical compounds of fungi. Ergosterol and its peroxide occur in a majority of representatives of Basidiomycota. For instance, the mean ergosterol content in the genus *Lactarius* is 269.01–300.86 mg × 100 g^{-1} DW and in *Cantharellus* 304.12–377.86 mg × 100 g^{-1} DW. It was proven that these compounds are essential for the normal development of hyphae of higher fungi while ergosterol is the main part of fungal cell membranes. Although it occurs in a majority of Basidiomycota species, the highest content of this compound was noted in saprophytic fungi [42].

The ergosterol irradiated by UV light is transformed into vitamin D_2. It is also a precursor of cortisol, an adrenal cortex hormone producing anti-inflammatory actions [43]. Many studies have demonstrated that ergosterol and its peroxidation products may give potential health benefits and significant pharmacological activities, including reducing pain related to inflammation, reducing the incidence of cardiovascular disease, and inhibiting cyclooxygenase enzyme (COX), antioxidant, antimicrobial, anticomplementary, and antitumor activities [44,45]. Moreover, ergosterol peroxide isolated from the acetone extract of fruiting bodies of *Sarcodon aspratus* Berk. S. Ito inhibits proliferation and induce apoptosis of acute lymphoblastic leukemia (HL60) cells [41].

Apart from ergosterol and its peroxide, among representatives of mushrooms, attention should be paid to: brassicasterol, ergosta-5,24(28)-dienol, stigmasta-7,24(28)-dienol, stigmast-7-enol, 5-dihydroergosterol, or 22-dihydroergosterol, and cholesterol and its derivatives: 24-methylene-cholesterol, 24,25-methylene-cholesterol, 24-ethyl–cholesterol, lanosterol, desmosterol, and episterol [46].

Conclusions

Optimization of axenic culture of *S. imbricatus* resulted in defining the medium with the greatest efficiency of biomass growth. The chemical analyzes of obtained homogeneous mycelium demonstrated the ability to the endogenous accumulation of free phenolic acids, fatty acids, and sterols. These metabolites are characterized by a wide spectrum of biological activity. Further studies are planned to optimization of mycelial cultures aimed at stimulation of metabolites production by the addition of compounds precursors.

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