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ORIGINAL ARTICLE

Lipid and fatty acid profile of the edible fungus *Laetiporus sulphurous*. Antifungal and antibacterial properties

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Abstract *Laetiporus sulphureus* is a saprophyte belonging to a specific group of wood-decomposing *Basidiomycetes* growing on deciduous trees. This fungus has been characterized as a herbal medicine and is also known for its antimicrobial properties. In the present study, high energy extraction techniques using different solvents were compared to obtain maximum yield of the edible fungus *Laetiporus sulphureus* total lipids. The lipid classes and fatty acid composition of the fruiting bodies' total lipids has been studied using GC-FID and Iatroscan TLC-FID analysis. Among the lipids, the neutral lipids predominated followed by phospholipids and glycolipids. Triglycerides were the most abundant in the neutral lipid fraction, whereas phosphatidylcholine in phospholipids. The existence of relatively high amount of sterols may be correlated to fungus pharmaceutical properties. Total lipids were found to contain high unsaturated degree fatty acids (UFA/SFA>3.4) and dominated of C18:2ω-6, C18:1ω-9 and C16:0 fatty acids. Antibacterial and antifungal properties of mushrooms' lipid extracts from two different solvents were also examined. Results indicated that hexane extracts possessed better antifungal and slightly better antibacterial activity compared to chloroform extracts though both were less active than the commercial antimicrobial agents.

Keywords *Laetiporus sulphureus* · Microwave Assisted Extraction (MAE) · Ultrasound-Assisted Extraction (UAE) · Lipid profile · Fatty acids · Antimicrobial activity

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Introduction

Laetiporus sulphureus is a species of bracket fungus found mainly in Europe and North America. Its common names are sulphur polypore, sulphur shelf, and chicken of the woods because of its taste which may also resemble the taste of crab or lobster. Its fruit bodies grow as golden-yellow shelf-like structures on tree trunks and branches. *Laetiporus sulphureus* is a saprophyte belonging to a specific group of wood-decomposing *Basidiomycetes* growing on deciduous trees. It belongs to Polyporaceae of Aphyllophorales and is the most readily recognized among macro fungi owing to its striking yellow or orange color. This specie is widely distributed in Europe, Asia, and North America (Vasaitis et al. 2009). Since the genus Laetiporus was first described by Murrill, at least 12 new species have been found (Kalač 2009; Borchers et al. 2004).

Laetiporus sulphureus produces large and strong fruiting bodies which are edible when young, and whose wet biomass sometimes reaches a few kilograms. Moreover, this fungus

has long been used in Asian herbal medicine and is also known as a source of antioxidant, antimicrobial, cytostatic and immunostimulative agents and a producer of HIV-1 reverse transcriptase inhibitors (Turkoglu et al. 2007; Mlinarič et al. 2005).

L. \sulphureus can also be cultivated on a larger scale in a laboratory both as fruiting bodies in a solid-state surface culture and as a hyphal mycelium in a fermented submerged culture (Davoli et al. 2005).

In general, mushrooms have been exploited in the last years as an alternative source of new antimicrobials. Wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional and/or medicinal properties (Kalač 2009; Borchers et al. 2004; Poucheret et al. 2006).

Main bioactive compounds in mushrooms include low (mostly secondary metabolites) and high (peptides and proteins) molecular weight compounds. Previous studies have indicated a higher antimicrobial activity of mushroom extracts especially against gram-positive bacteria. Among all the mushrooms, *Lentinus edodes* is the most studied species and seems to have a broad antimicrobial action against both gram-positive and gram-negative bacteria. Plectasin peptide, obtained from *Pseudoplectania nigrella*, is the isolated compound with the highest antimicrobial activity against gram-positive bacteria, while 2-aminoquinoline, isolated from *Leucopaxillus albissimus*, presents the highest antimicrobial activity against gram-negative bacteria (Alves et al. 2012).

Although *L. Sulphureus* has been used as a food for a long time, information especially about its lipid but also on the antibacterial activity about this mushroom are still rare. Most studies have been published focusing on the identification of compounds or categories of compounds of *L. Sulphureus* fruiting bodies and mycelia fractions with promising biological activities. These mainly include α-glucans and other mono and polysaccharides, carotenoid pigments, phenol contents and their antioxidant components (Alves et al. 2012). Hence, this study focuses on: (i) the characterization of the lipid and fatty acid profile under different extraction techniques and (ii) the determination of the antimicrobial activity of *L. Sulphureus* lipid extracts against bacterial and fungal strains.

Materials and methods

Chemicals, standards and solvents

The lipid standards used were: cholesteryloleate, cholesterol, tristearoyl-glycerol, lauric acid, oleic acid, linoleic acid, 1,2-distearoyl-glycerol, 1-monostearoyl-rac-glycerol, phosphatidylcholine, phosphatidylethanolamine, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyeline standards of the Sigma Chemical Co (Sigma-Aldrich Company. Dorset. Great Britain and St. Louis, MO). Fatty acid methyl esters used as GC-FID standard mixtures were: Supelco TM 37 Component FAME Mix C4-C24, 100 mg Neat. And Supelco PUFA No.1, Marine Source, 100 mg Neat. All solvents used for sample preparation were of analytical grade and the solvents used for GC and Iatroscan TLC-FID analyses were of HPLC grade from Merck (Darmstadt. Germany). Double distilled water was used throughout this work. All reagents used were of analytical grade and they were purchased from Mallinckrodt Chemical Works (St. Louis, MO) and from Sigma Chemical Co (Sigma-Aldrich Company, UK).

Preparation of mushroom extracts; Mushroom Samples

Specimens of *Laetiporus sulphureus* were collected in *Salix alba* in May of 2011, from Jojkićev branch of Danube River, near Pančevo, Northern Serbia, and authenticated by Dr. Jasmina Glamočlija (Institute for Biological Research). A voucher specimen has been deposited at the Fungal Collection Unit of the Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research "Siniša Stanković", Belgrade, Serbia, under the number Ls-610-2011. Fresh mushrooms were randomly divided and oven-dried at 40 °C, before analysis. The specimens were lyophilised (LH Leybold, Lyovac GT2, Frenkendorf, Switzerland, respectively), reduced to a fine dried powder (20 mesh), mixed to obtain homogenous samples and stored in a desiccator, protected from light at -20ºC until further analysis.

Extraction methodologies

Different types of extractions including high energy techniques (ultrasound and microwave assisted extractions) were employed to identify the optimum conditions for higher yield of lipid isolation.

Microwave assisted extraction (MAE) procedure: 2 g of lyophilized mushroom sample and 20 mL solvent (n-hexane or chloroform) were placed in a 100 mL vessel equipped with a condenser and irradiated with CEM Discover LabMate apparatus for 10 min at 25 W, maximum temperature 50 °C. After this the extracts were concentrated in rotary evaporator and the dry residues were re-dissolved in 30 % solvent.

Ultrasound-Assisted Extraction (UAE) procedure: 2 g of lyophilized mushroom sample and 50 mL solvent (n-hexane or chloroform) were placed in a 250 mL three-neck vessel in ice-bath, maximum temperature 40 °C and sonicated using Sonics & Material INC., Vibra-Cell VCX750 (20 kHz, 750 W) ultrasonics processor, equiped with piezoelectric converter and 13 mm diameter probe fabricated from Titanium alloy Ti-6Al-4 V. The amplitude was 80 % and the pulse sonication sequence was 10 s ON and 5 s OFF. After this

the extracts were concentrated in rotary evaporator and the dry residues were re-dissolved in 30 % solvent.

Iatroscan analysis of neutral and polar lipids

Lipid classes were separated on silicic acid-coated quartz rods, chromarods (Type SIII, 5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan) and they were quantified using a thin layer chromatography–flame ionization detection system. Iatroscan TLC–FID analysis was performed by an Iatroscan thin-layer chromatograph (Model MK-6 TLC/FID – FPD Analyser Iatron Laboratories, Tokyo, Japan) equipped with a flame ionization detector. Individual lipid classes were quantified as described by Sinanoglou et al. (2013). The neutral solvent system consisted of n-hexane-diethyl ether-formic acid (n-H:DE:FA) 60:15:1.5 (by vol) and the polar system of chloroform:methanol:water (C:M:W) 50:20:2 (by vol).

Gas chromatography analysis of fatty acid methyl esters

Fatty acid methyl esters (FAME) of total lipids (TL) were prepared according to the procedure described by Sinanoglou and Miniadis-Meimaroglou (1998). Both quantitative and qualitative analysis were performed on an Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector, as described by Sinanoglou et al. (2013). DB-23 capillary column (60 m× 0.25 mm i.d. 0.15 μm film) (50 %-cyanopropyl-methylpolysiloxane) (Agilent Technologies) was used. The temperature program selected was a trade-off between the resolution of fatty acid structural isomers or positional isomers or geometrical isomers or homologues and the maximum elution time. In order to validate the method in terms of linearity, standard solutions containing different concentrations of 37 Component FAME Mix were prepared and subjected to GC analysis. The standard curves for individual FAME were obtained by plotting concentration ratio against area ratio and the correlation coefficient (r2) was calculated with the linear equations used for quantitation. The detection limit (DL) and quantitation limit (QL) were tested. Three different concentrations of 37 Component FAME Mix were used to assess precision. Each sample was run three times within a single day to evaluate repeatability. Reproducibility was also tested.The instrumental parameters on the integrator were appropriate to the peak widths at various times during analysis. The above separation of FAME by GC-FID offers several advantages, such as high repeatability and reproducibility of retention times and high precision in quantitation based on peak area measurements. The results showed that the selection of a capillary column with appropriate length resulted in the best separation of FAME isomers (Sinanoglou et al. (2013)).

The individual FAME were identified by comparing their retention times with those of the authentic standard mixtures. The relative content of fatty acids in the sample was determined according to Sinanoglou et al. (2013).

Evaluation of Antimicrobial Activity

Antibacterial Activity

The following Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas* aeruginosa (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311) and the following Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538) were used. Bacteria were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research 'Siniša Stanković', University of Belgrade, Serbia. The antibacterial assay used was carried out by micro-dilution method (Clinical and Laboratory Standards 2009; Tsukatani et al. 2012). Bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The inocula were prepared daily and stored at 4 C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. Different mushroom extracts were dissolved in the 5% of DMSO and added (at a concentration ranging from 0.15-4.0 mg/mL) in Triptic Soy broth (TSB) medium (100 μL) containing bacterial inoculum (1.0×10^4 CFU per well), so as to achieve the appropriate concentrations. Microplates were incubated at a rotary shaker (160*rpm*) for 24 h at 37 °C. The following day, 30 μL of 0.2 mg/mL solution of INT (*p*-iodonitrotetrazolium violet) was added to the plates, followed by ½h incubation so as to ensure adequate color reaction. Inhibition of bacterial growth was indicated by a clear solution or a definite decrease in color reaction. The lowest concentrations without visible growth (at the binocular microscope) were defined as the concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 μL into microtitre plates containing 100 μL of broth per well, followed by an additional 24 h incubation period. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5 % killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by microplate manager 4.0 (Bio-Rad Laboratories) and compared with that of the blank and positive control. The antibiotics streptomycin

and ampicillin were used as positive controls (1 mg/mL in sterile physiological saline) for all tested bacteria.

Antifungal Activity

The antifungal activity of various *L. sulphureus* extracts was tested against the following micromycetes: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Trichoderma viride* (IAM 5061), *Penicillium ochrochloron* (ATCC 9112) and *Penicillium verrucosum* var. *cyclopium*. These fungal species were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research "Siniša Stanković", and University of Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures were stored at 4 °C and sub-cultured once per month. The antifungal assay was carried out by a modified micro-dilution technique (Espinel-Ingroff 2001). The fungal spores were washed from the surface of the agar plates with sterile 0.85% saline containing 0.1 % Tween 80 (v/v). The spore suspension was adjusted, with sterile saline, to a concentration of approximately 1.0×10^5, in a final volume of 100 μL per well. The inocula were stored at 4 °C for further use. Dilutions of the inoculum were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. MIC determinations were performed by a serial dilution technique using 96-well microtiter plates. The examined fractions were diluted in appropriate solvents (1.5-17 mg/mL) and added in broth malt medium (MA) containing inoculum. Microplates were incubated at a rotary shaker (160 rpm) for 72h at 28 C. The lowest concentrations at which no visible fungal growth was observed (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial sub-cultivation of 2 μL and inoculated for 72 h, into microtiter plates containing 100 μL of broth per well, followed by an additional 72 h incubation at 28 ° C. The lowest concentration with no visible growth was defined as MFC indicating 99.5 % killing of the original inoculum. The commercial antifungal agents bifonazole and ketoconazole were used as positive controls (at a concentration ranging from 1-3500 μg/mL).

Statistical Analysis

All determinations were carried out at least three times (three separate samplings) and in triplicate. Values were averaged and reported along with the standard deviation (S.D.) or standard error (S.E.). All data were analyzed with One-Way ANOVA Post Hoc Tests and pairwise multiple comparisons were conducted with the Tukey's honestly significant difference test. Possibilities less than 0.05 were considered as statistically significant ($P< 0.05$). All statistical calculations were per-formed with the SPSS package (IBM SPSS Statistics, version 19.0, Chicago, IL, USA) statistical software for Windows.

Results and Discussion

Extract profile of *L. sulphureus*

The effect of high energy extraction procedures (ultrasound and microwave assisted extractions) by using two different solvents, on the lipid yield and fatty acid composition of total lipid of *Laetiporus sulphureus* was investigated. Chloroform and n-hexane were selected and compared. Several studies have been presented using UAE and MAE as fast extraction techniques, however most of them focus on the class of polysaccharides and polyphenols (Tiana et al. 2012) from other types of mushrooms. For this reason, similar to literature (Araujo et al. 2013) extraction protocols were used with certain modifications after testing different extraction parameters (power, time). Chloroform and hexane were used for our study as the most common solvents to recover total lipids. Both high energy techniques resulted in greater extraction yield using Chloroform. Ultrasound assisted extraction with n-hexane did not improve the lipid yield. Therefore, the total fat content was found 2.12±0.38 g/100 g dry weight, depending on the extraction procedure. Our results were quite similar to those reported by: a Kavishree et al. (2008) for the Indian mushrooms, *Cantharellus cibarius*, *Russula brevepis*, *Termitomyces microcarpus* and *Termitomyces mummiformis*, b) Barros et al. (2008a) for the wild mushrooms Agaricus *silvaticus* and *Agaricus silvicola*, c) Beluhan and Ranogajec (2011) for the edible mushroom species *Macroleptiota procera* and *Pleurotus ostreatus* and d) Hung and Nhi (2012) for five Vietnamese edible mushrooms (*Pleurotus ostreatus*, *Volvariella volvacea*, *Lentinula edodes*, *Auricularia polytricha* and *Ganoderma lucidum*). Hung and Nhi (2012) also reported that mushroom fat content may present high variation depending on the nutritive of substrate for mushroom growth, genus of species and environment conditions.

The lipid composition of *Laetiporus sulphureus* extracts was investigated. Individual neutral and polar lipids were separated by TLC-FID and their composition in respect to the method of extraction is presented in Table 1. Neutral lipids (NL) predominated in all extracts, mainly consisted of triglycerides (TG) followed by sterols. Concerning the mushroom polar lipids (PL), phosphatidylcholine (PC) seemed to have the highest content followed by phosphatidylethanolamine (PE).

The individual neutral and polar lipid percentages were significantly dependent on the solvent and the method of extraction used (Table 1). Results showed that extraction with chloroform was more efficient in extracting glyco- and phospholipids rather than n-hexane. On the contrary, extraction

Table 1 *Total lipids* (TL) and Lipid classes (% of TL) of *Laetiporus sulphurous* extracts

Samples	Microwave assisted extraction with hexane	Microwave assisted extraction with chloroform	Ultrasound assisted extraction with hexane	Ultrasound assisted extraction with chloroform
Total lipid (% dw)	2.05±0.05a	2.51±0.03b	1.57±0.04c	2.50±0.07b
Wax–wax esters	11.25±0.26a	6.49±0.21b	10.77±0.52a	5.03±0.17c
TG	63.95±0.51a	48.71±0.83b	56.31±0.69c	45.97±0.73d
Sterols	12.02±0.23a	11.02±0.28b	10.52±0.25b	6.84±0.29c
GL	2.81±0.09a	7.06±0.31b	2.94±0.07a	6.30±0.24c
PhL	9.97±0.18a	26.72±0.49b	19.46±0.16c	35.86±0.45d
PE	1.46±0.05a	3.88±0.12b	2.78±0.09c	5.37±0.16d
PC	8.51±0.17a	22.84±0.28b	16.88±0.25c	30.49±0.38d

Values are mean±SD calculated from three different experiments performed in triplicate. Means with different letters in the same column are significantly different ($p<0.05$)

with n-hexane resulted in significant ($P<0.05$) higher wax – wax ester and triglyceride proportions than chloroform, with MAE giving higher rates compared to UAE. Furthermore, MAE with n-hexane has given the highest sterol proportion. Ultrasound assisted extraction with chloroform gave the highest ($P<0.05$) glycolipid and phospholipid and the lowest neutral lipid classes' proportions. Both high energy techniques may reduce the extraction time and can be used with any other solvent for extracting a wide variety of natural compounds. Furthermore, UAE didn't result in any temperature increase.

Among the examined lipid extracts, ultrasound assisted lipid extract with chloroform contained the highest amount of PC and PE, providing 0.76 and 0.13 g of PC and PE per 100 g dry matter, respectively (data calculated from Table 1). Choline is an important dietary nutrient and an intake of 425 and 550 mg of choline per day for women and men, respectively, is adequate (Food and Nutrition Board, Institute of Medicine 2000). Since choline is present in foods as lecithin (PC), *L. sulphureus* fat appeared to be a good source of choline. Microwave assisted lipid extract with chloroform contained the highest amount of sterols, providing 0.28 g per 100 g dry matter (data calculated from Table 1). As phytosterols can reduce cholesterol absorption, lowering plasma and LDL cholesterol, *L. sulphureus* could be possibly selected as a supplement to the human diet.

Fatty acid profile of *L. sulphureus*

The fatty acid profiles *of L.sulphureus* total lipids are shown in Table 2. Thirty-one saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were identified. The main saturated fatty acid of mushroom lipids was found to be palmitic acid (C16:0), the main monounsaturated was oleic acid (C18:1ω-9) and the main polyunsaturated fatty acid was linoleic acid (C18:2ω-6). Percentages of palmitic (C16:0), oleic (C18:1ω-9) and limoleic (C18:2ω-6) acid varied greatly depending on the mushroom type (Kavishree et al. 2008; Barros et al. 2007a). Thus, our results were comparable to those reported by Longvah and Deosthale (1998) for the Indian mushrooms, *Schizophyllum commune* and *Lentinus edodes* (C16:0: ~20%, C18:1ω-9: ~10% and C18:2ω-6: ~65% of TFA), by Barros et al. (2008a) for *Cantharellus cibarius* and *Calocybe gambosa* (C16:0: 13.08 and 18.10%, C18:1ω-9: 10.78 and 15.16% and C18:2ω-6: 53.59 and 57.7% of TFA, respectively), by Barros et al. (2007a) for *Agaricus arvensis* (C16:0: 14.55 %, C18:1ω-9: 15.46% and C18:2ω-6: 56.11% of TFA) and by Kavishree et al. (2008) for *Pleurotus sajor-caju* and *Termitomyces mummiformis* (C16:0: 13.9 and 16.0%, C18:1ω-9: 16.4 and 18.2% and C18:2ω-6: 53.8 and 58.0%of TFA respectively).

Fatty acid profile in total lipid varied depending on the extraction method or type of solvent used. Thus, iso-C15:0, C18:4ω-3 and C22:5 ω-3 were identified in detectable levels in microwave assisted extracts with hexane, whereas were absent in the other extracts. Besides, C12:0, C14:1, cyclo-C17:0, C17:0, C18:0, C18:1ω-9, C18:3ω-6, C22:5 ω-3 and C24:1ω-9 percentages varied greatly depending on the solvent and the method of extraction used (Table 2). Additionally, fatty acids were not present in the same proportions in the studied extracts, which may be due to the selectivity in neutral and polar lipid molecular species extracted. Furthermore, linoleic acid (C18:2ω-6) and ω-6 fatty acid proportions as well as UFA/SFA ratio presented no significant variations regardless the extraction method or type of solvent used. MUFA and EPA (C20:5ω-3) proportions resulted statistically the same values for the same solvent. Finally, oleic acid (C18:1ω-9) proportion was not significantly different for the same extraction method performed.

The *iso*-methyl branched fatty acids, *iso*-C15:0, *iso*-C16:0 and iso-C17:0, together with cyclo-C17:0 and the odd-chain MUFA, C15:1ω-5 and C17:1ω-7, were also identified in detectable levels. The above findings were in agreement with the results reported for other basidiomycetes (Yilmaz et al. 2006; Barros et al. 2007b; Ribeiro et al. 2009; Papaspyridi et al. 2013).

Table 2 Fatty acid composition (% w/w) of total fatty acids of *Laetiporus sulphureus* total lipids

Fatty acids	Microwave assisted extraction with hexane	Microwave assisted extraction with chloroform	Ultrasound assisted extraction with hexane	Ultrasound assisted extraction with chloroform
C10:0	0.64±0.00a	0.85±0.00b	0.69±0.01c	0.95±0.01d
C12:0	0.35±0.00a	1.29±0.01b	1.92±0.01c	1.04±0.01d
C14:0	0.35±0.00a	0.46±0.01b	0.37±0.00c	0.41±0.01d
C14:1	0.08±0.00a	0.42±0.01b	0.17±0.00c	0.26±0.00d
iso–C15:0	0.13±0.00	–	–	–
C15:0	1.72±0.00a	1.65±0.01b	1.68±0.01c	1.63±0.01b
C15:1ω–5	0.12±0.00a	0.25±0.00b	0.10±0.00c	0.17±0.00d
C16:0	10.80±0.03a	11.38±0.05b	11.07±0.04c	10.74±0.03a
iso–C16:0	0.45±0.00a	0.46±0.00a	0.44±0.01b	0.43±0.00b
C16:1ω–9	0.65±0.00a	0.56±0.00b	0.67±0.01a	0.62±0.01c
iso–C17:0	0.18±0.00a	0.27±0.00b	0.14±0.00c	0.16±0.00d
cyclo–C17:0	1.32±0.01a	1.27±0.01b	0.86±0.01c	0.95±0.01d
C17:0	0.88±0.01a	0.54±0.01b	0.82±0.01c	0.69±0.01d
C17:1ω-7	0.43±0.00a	0.48±0.00b	0.45±0.00c	0.47±0.00d
C18:0	1.63±0.02a	1.95±0.02b	1.89±0.02c	2.04±0.03d
C18:1ω-9	13.91±0.14a	13.92±0.13a	14.22±0.11b	14.38±0.16b
C18:1ω-7	0.54±0.00a	0.48±0.00b	0.52±0.02a	0.55±0.01a
C18:2ω-6	57.00±0.56a	56.12±0.75a	56.10±0.71a	56.45±0.59a
C18:2*cis*–9*trans*-11	0.58±0.00a	0.57±0.00b	0.54±0.01c	0.56±0.01cb
C18:3ω-6	0.56±0.00a	0.33±0.00b	0.48±0.00c	0.42±0.00d
C18:3ω-3	0.09±0.00a	0.13±0.00b	0.08±0.00c	0.10±0.00d
C18:4ω-3	0.09±0.00	-	-	-
C20:0	0.16±0.00a	0.19±0.00b	0.15±0.00c	0.18±0.00d
C20:1ω-9	0.09±0.00a	0.12±0.00b	0.10±0.00c	0.11±0.00d
C20:2ω–6	0.30±0.00a	0.36±0.00b	0.35±0.01bc	0.34±0.01c
C20:5ω–3	0.09±0.00a	0.17±0.00b	0.09±0.00a	0.17±0.00b
C22:5ω–6	1.10±0.01a	1.04±0.01b	1.24±0.01c	1.13±0.01d
C22:5ω-3	0.91±0.00	–	–	–
C24:0	2.03±0.04a	1.86±0.02b	2.23±0.02c	2.15±0.02d
C22:6ω–3	1.62±0.03a	1.27±0.01b	1.51±0.02c	1.34±0.02d
C24:1ω–9	1.20±0.03a	1.62±0.01b	1.12±0.03c	1.56±0.01d
Σω:0 (SFA)	20.64±0.12a	22.18±0.12b	22.26±0.14b	21.37±0.15c
Σω:1 (MUFA)	17.02±0.17a	17.85±0.15b	17.35±0.13a	18.12±0.14b
Σω:n (PUFA)	62.34±0.61a	59.98±0.57b	60.39±0.46b	60.51±0.52b
Σω:3	2.80±0.04a	1.57±0.02b	1.68±0.04c	1.61±0.03bc
Σω:6	59.53±0.57a	58.41±0.56a	58.71±0.49a	58.90±0.52a
ω-6/ω-3	21.24±0.08a	37.21±0.19b	34.95±0.15c	36.58±0.17d
PUFA/SFA	3.02±0.01a	2.70±0.01b	2.71±0.01b	2.83±0.01c
MUFA/SFA	0.82±0.00a	0.80±0.00b	0.78±0.01b	0.85±0.01c
UFA/SFA	3.84±0.35a	3.47±0.31a	3.49±0.28a	3.68±0.36a
C18:2ω-6/C18:1ω-9	4.14±0.00a	4.34±0.00b	3.95±0.02c	3.93±0.02c

Values are mean±SD calculated from three different experiments performed in triplicate. Means with different letters in the same column are significantly different ($p<0.05$

L. sulphureus total lipids contained measurable quantities of conjugated linoleic acid (CLA) (C18:2ω-6cis-9trans-11) although the occurrence of trans fatty acids in mushrooms has not been previously reported (Kalač 2009).

Regarding the ω-3 fatty acids, C22:6ω-3 represented significantly higher ($P<0.05$) proportion than C20:5ω-3, whereas C18:3ω-3, C18:4ω-3 and C22:5ω-3 were in traces or absent depending the extraction method (Table 2). Minor

Table 3 Antibacterial activity of n-hexane extract of *Laetiporus sulfureus* (MIC/MBC in mg/mL)

Extract		*M. flavus*	*S. aureus*	*L. monocytogenes*	*E. coli*	*P. aeruginosa*	*S. typhimurium*
n–hexane	MIC	1.0±0.08^{c}	0.25±0.02^{b}	0.9±0.03^{c}	2.0±0.07^{c}	1.5±0.00^{c}	0.15±0.00^{a}
	MBC	2.0±0.1^{c}	0.5±0.00^{c}	3.6±0.03^{d}	4.0±0.00^{d}	2.0±0.09^{c}	0.3±0.02^{a}
chloroform	MIC	0.78±0.02^{b}	0.4±0.00^{d}	1.56±0.05^{d}	1.56±0.02^{b}	1.56±0.02^{c}	0.78±0.03^{c}
	MBC	1.56±0.03^{b}	0.78±0.02^{d}	3.125±0.00^{c}	3.125±0.03^{c}	3.125±0.04^{d}	3.125±0.04^{c}
Ampicillin	MIC	0.3±0.00^{a}	0.3±0.02^{c}	0.4±0.00^{b}	0.3±0.02^{a}	0.8±0.03^{b}	0.3±0.02^{b}
	MBC	0.4±0.02^{a}	0.4±0.02^{b}	0.5±0.00^{b}	0.5±0.02^{b}	1.25±0.09^{b}	0.5±0.00^{b}
Streptomycin	MIC	0.2±0.007^{a}	0.05±0.003^{a}	0.2±0.03^{a}	0.2±0.00^{a}	0.2±0.02^{a}	0.2±0.02^{a}
	MBC	0.3±0.00^{a}	0.1±0.00^{a}	0.3±0.02^{a}	0.3±0.03^{a}	0.3±0.00^{a}	0.3±0.02^{a}

Values are mean±SE calculated from three different experiments performed in triplicate. Means with different letters in the same column are significantly different ($p<0.05$)

contents of EPA and DHA were already reported by other authors for other mushroom species (Barros et al. 2008b; Ribeiro et al. 2009; Papaspyridi et al. 2013).

Concerning the sums of the fatty acids ΣPUFA occurred in the highest percentage followed by ΣSFA and ΣMUFA. *L. sulphureus* total lipids characterized by high unsaturated/saturated (UFA/SFA), ω-6/ω-3 and linoleic:oleic acids ratios (Table 2). Thus, total lipid UFA accounted from 77.74 to 79.36 % of total FA, depending on the solvent and the method of extraction used, corresponding to ratio of UFA/SFA accounted from 3.47 to 3.84. This result was in agreement with the observations reported for many species of basidiomycetes belonging to the families Agaricaceae, Amanitaceae, Boletaceae, Coprinaceae, Ganodermataceae and Tricholomataceae, stating that UFA predominated over SFA in total FA content (Yilmaz et al. 2006; Barros et al. 2007b; Pedneault et al. 2007; Kavishree et al. 2008; Ribeiro et al. 2009; Papaspyridi et al. 2013). The linoleic:oleic acid ratio accounted from 3.93 to 4.34 (Table 2) was similar to those reported by Barros et al. (2007a) for *Agaricus arvensis*, higher than those reported by Kavishree et al. (2008) for *C. cibarius*, *C. clavatus*, *G. arinarius*, *R. brevepis*, *S. crispa*, *T. microcarpus* and *T. tylerance*, by Barros et al. (2007a) for *L. deliciosus*, *L. giganteus*, *S. imbricatus* and *T. portentosum* and by Barros et al. (2008a) for *B. edulis*, *C. cornucopioides* and *M. oreades* and lower than those reported by Kavishree et al. (2008) for *L. squarrulosus* and by Barros et al. (2008a) for *A. bisporus*, *A. Silvaticus*, *A. Silvicola*, *C. gambosa* and *C. cibarius*. Kavishree et al. (2008) reported that the linoleic:oleic acid ratio could be considered as a marker for the discrimination between species of the same mushroom genus.

Concerning report by Erkkil et al. (2008) that replacement of SFA with PUFA and MUFA in the diet can decrease cardiovascular risk, the high MUFA:SFA (≥0.78) and PUFA:SFA (≥2.70) ratios presented in *L. sulphureus* total lipids could make this mushroom desirable from consumers, as far as nutrition is concerned.

Antimicrobial activity

The obtained n-hexane and chloroform extracts were submitted to antimicrobial activity evaluation in order

Table 4 Antifungal activity of n–hexane extract of *Laetiporus sulfureus* (MIC/MFC in mg/mL)

Extract		*A.Fumigatus*	*A.Versicolor*	*A.chraceus*	*T. Viride*	*P. Ochrochloron*	*P. Verrucosum*
n–hexane	MIC	2.0±0.00^{b}	2.0±0.20^{b}	2.0±0.03^{b}	1.5±0.03^{c}	2.0±0.00^{c}	2.0±0.20^{c}
	MFC	4.0±0.20^{b}	4.0±0.00^{b}	4.0±0.07^{b}	3.0±0.00^{c}	4.0±0.20^{c}	5.0±0.00^{c}
chloroform	MIC	8.0±0.30^{c}	6.25±0.10^{c}	6.25±0.08^{c}	5.0±0.00^{d}	3.125±0.03^{d}	6.25±0.08^{d}
	MFC	17.0±0.20^{c}	12.5±0.30^{c}	12.5±0.02^{c}	8.0±0.02^{d}	6.25±0.08^{d}	12.5±0.03^{d}
ketoconazole	MIC	0.2±0.02^{a}	0.2±0.02^{a}	0.15±0.00^{a}	1.0±0.07^{b}	1.0±0.07^{b}	1.5±0.00^{b}
	MFC	0.5±0.00^{a}	0.5±0.03^{a}	0.2±0.03^{a}	1.5±0.03^{b}	1.5±0.07^{b}	2.0±0.07^{b}
Bifonazole	MIC	0.15±0.02^{a}	0.1±0.00^{a}	0.15±0.006^{a}	0.15±0.007^{a}	0.2±0.00^{a}	0.2±0.02^{a}
	MFC	0.2±0.03^{a}	0.2±0.02^{a}	0.2±0.003^{a}	0.2±0.02^{a}	0.25±0.02^{a}	0.3±0.08^{c}

Values are mean±SE calculated from three different experiments performed in triplicate. Means with different letters in the same column are significantly different ($p<0.05$)

to compare the results with the standard antibiotics and mycotics. The results of antibacterial activity were presented in Table 3. *L. sulfureus* n-hexane and chloroform extracts were active against all the tested bacteria with minimal inhibitory concentrations of 0.15-2.0 mg/mL and 0.78-1.56 mg/mL, respectively, and bactericidal concentrations was in range 0.5-4.0 mg/mL and 0.78-3.125 mg/mL, respectively. *S. typhimurium* was the most susceptible bacteria to n-hexane extract, while *E. coli* was the most resistant one. Chloroform extract showed the highest activity toward *S. aureus*, and the lowest effect could be seen against *L. monocytogenes*, *E. coli* and P. *aeruginosa*. n-Hexane extract in general possessed slightly highest antibacterial activity compared to chloroform extract. The extracts showed lower antibacterial activity against tested bacteria than the antibiotics amplicillin and streptomycin. The only exception is in the case of n-hexane extract against *S. typhimurium*, where the activity was higher than ampicillin but the same as streptomycin.

Regarding the antifungal activity (Table 4), minimal inhibitory concentrations was in range 1.5-2.0 mg/mL (n-hexane) and 3.125-8.0 mg/mL (chloroform). Fungicidal concentrations of n-hexane and chloroform extracts were ranged between 3.0-5.0 mg/mL and 6.25-17.0 mg/mL, respectively. The highest antifungal potential was verified for *T. viride* (in the case of n-hexane extract) and P. *ochrochloron* (in the case of chloroform extract); while the highest fungicidal activity of hexane extract was expressed on P. *verrucosum* and chloroform extract exhibited the worst activity on *A. fumigatus*. Obtained results showed that hexane extract proved to have better antifungal potential than chloroform, even two times higher. Comparing the antifungal activity of bifonazole and ketoconazole to the investigated extract, it can be seen that commercial antifungal agents expressed higher potential.

As a conclusion, this article presents, for the first time, the antimicrobial properties of the lipid fraction of *L. Sulphureus*. Even though the polar mushroom fractions are usually subjected to antimicrobial tests, mainly due to their rich phenolic content, our results indicated that lipid fractions may also provide mild antibacterial and antifungal properties. Therefore mushrooms could be an excellent alternative healthy food due to the synergistic antibacterial and antifungal properties of both phenolic compounds and lipid ingredients. Moreover, the natural products with antimicrobial activity are safer than synthetic ones in terms of side effects on humans and environment.

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