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ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF *LAETIPORUS SULPHUREUS* (BULL.: FR.) MURILL

ABSTRACT: Wood-rotting basidiomycete, *Laetiporus sulphureus* (Bull.: Fr.) Murill., also known as chicken of the woods, is known for its nutritional value. In this study, aqueous extract obtained from *L. sulphureus* was investigated for its antimicrobial properties using microdilution *in vitro* assay. Plant, animal and human pathogens, as well as food spoilage agents, were tested. Aqueous extract obtained from *L. sulphureus* showed strong activity against the tested microorganisms in a dose dependent manner. Considering that there is a constant emerging of pathogen resistance to the known synthetic agents, there is an undeniable need for new therapeutical drugs and preservatives in food industry. Thus, these results that indicate activity of natural products may be of practical use.

KEY WORDS: antimicrobial activity, aqueous extract, *Laetiporus sulphureus*

INTRODUCTION

Researches in the past decade suggest that significant number of people is involved in various forms of alternative medicine. Estimates for using complementary and alternative medicine in adults worldwide have increased in the last twenty years (Barnes et al., 2004.). The main reason for this lies in the fact that many people are to a certain extent dissatisfied with the conventional treatment (Astin, 1998). Also, constant emerging resistance of bacterial and fungal species, known for the ailments they cause in humans, to commercial synthetic medicines is probably the most important reason why people turn to alternative medicine. While the use of herbs is rather common in the Western hemisphere, medicinal use of mushrooms, which has a long tradition in the Asian countries, has been slightly increased in Europe since the last decades (Lindequist et al., 2005). Although there have been extensive researches on the use of medicinal mushrooms, their true potential is yet to be revealed.

Excessive use of antibiotics and antimycotics in the treatment of infectious diseases caused by human pathogenic microorganisms led to multiple drug resistance. This motivated scientists to search for new substances with antimicrobial activity in natural products. Plants and mushrooms turned out to be an excellent source of novel chemotherapeutic agents (K a r a m a n et al., 2003). A number of compounds that have been proved to posses significant antimicrobial activities were isolated from polypore fungi. They provide a rich variety of active secondary metabolites and polysaccharides. Polysaccharides emerged as an important class of bioactive substances, and many medicinal and therapeutic properties are attributed to them (A l q u i n i et al., 2004). *Trametes versicolor*, *Laetiporus sulphureus* and *Ganoderma lucidum* are just some of the known mushrooms with this potential. This fact alone made them good candidates for critically needed new antibiotics and antimycotics (Z j a w i o n y, 2004).

Laetiporus sulphureus (Bull.: Fr.) Murill is a wood-rotting basidiomycete, growing on several tree species and producing shelf-shaped fruit bodies with a bright yellow fleshy margin. This recognizable pigmentation along with the fruit body form is responsible for the trivial name under which this fungus is known, and that is sulfur shelf (W e b e r et al., 2003).

Even though it is known as a source of active compounds, and is widely used as food among Anatolian people, literature reports on antimicrobial activity of *L. sulphureus* extracts are scarce (Z j a w i o n y, 2004; T u r k o g l u et al., 2007).

The potential barrier to everyday use of medicinal mushrooms as therapy is the manner in which the mushroom is consumed. Most of the researches on fungi as potential antimicrobial agents are based on ethanolic and methanolic extracts of the fungal fruit body (T u r k o g l u et al., 2007; B a r r o s et al., 2007). Consumption of products on this basis is of no practical use.

Therefore, the aim of the present study is to evaluate the antimicrobial potentials of aqueous extracts of *L. sulphureus* fruit bodies on several microorganisms of medicinal importance. This would perhaps allow daily use of mushrooms that are available in various forms.

MATERIALS AND METHODS

Mushroom

Fruiting bodies of *L. sulphureus* were collected in the woods near Belgrade. Identification and classification were carried out and all specimens were deposited at the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. Mature basidiocarps of *L. sulphureus*, were collected in *Salix alba* in 2009. Fresh mushrooms were randomly divided, dried in an oven at 40 °C before analysis. Dried mushroom material was grounded to a fine powder with blender. The sample (30 mg) was dissolved in 1ml of distilled water and left for 24 h at room temperature. The aqueous solution was then centrifuged at 5000 rpm for 10 minutes; the supernatant was poured off and used in the *in vitro* assay.

Antimicrobial activity

Antibacterial activity

The following Gram negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *and Salmonella typhimurium* (ATCC 13311), *and Enterobacter cloacae* (human isolate), as well as the following Gram positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The antibacterial assay was carried out by modified microdilution method (Daouk et al., 1995; Hanel and Raether 1988; Espinel–Ingroff, 2001) in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria.

The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x 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 check the validity of the inoculum.

Antifungal activity

For the antifungal bioassays, seven fungi were used: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (plant isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures were stored at + 4 °C and sub-cultured once a month (Booth, 1971). In order to investigate the antifungal activity of the tested aqueous extract, a modified microdilution technique was used (Daouk et al., 1995; Hanel and Raether, 1988; Espinel–Ingroff, 2001.). The fungal spores were washed from the surface of 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 x 10^5 CFU in a final volume of 100 μl per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and check the validity of the inoculum.

Microdilution test

The minimum inhibitory, bactericidal and fungicidal concentrations (MICs, MBCs and MFCs, respectively) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 x 10^5 CFU/ml. The microplates were incubated for 24 h at

37°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as 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 and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC value, indicating 99.5% killing of the original inoculum. Streptomycin was used as a positive control (1 mg/ml DMSO).

Minimum inhibitory concentration (MIC) determinations for fungi were performed using 96-well microtiter plates. The microplates were incubated for 7 days at 25 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μl into microtiter plates containing 100 μl of broth per well and further incubation for 5 days at 25 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Commercial fungicide ketoconazole was used as positive control (1mg/1ml DMSO).

RESULTS

The antimicrobial effect of aqueous extract of *L. sulphureus* was tested against four species of Gram negative bacteria, four species of Gram positive bacteria, and seven species of fungi. The extract generally exhibited better antifungal than antibacterial activity (Figure 1). Among the tested bacteria, *L. sulphureus* extract strongly inhibited *M. flavus* and *L. monocytogenes*, with MIC values of only 1.50 mg/ml. As for the remaining bacterial strains, in the tested concentrations (0.15-1.50 mg/ml) aqueous extract showed activity, but insufficient to be considered as inhibitory. Antibiotic streptomycine (1mg/1ml DMSO) was used as the positive control. In the tested concentrations, streptomycine showed inhibitory activity in the range of 0.63-12.50 x 10^{-3} mg/ml, and bactericidal activity on the tested bacteria in the range of 1.25-25.00 x 10^{-3} mg/ml. The bacteria most resistant to the effect of streptomycine was proved to be *L. monocytogenes*, which could be of practical use, because it was also proved to be the most susceptible to the effects of extract of *L. sulphureus*.

DISCUSSION

Presented results of antibacterial activity are consistent with the results for *M. flavus* published by T u r k o g l u et al. It proved to be the most susceptible species, though it should be noted that in the mentioned study agar-well diffusion method was used, and the extract was methanolic (T u r k o g l u et al., 2007).

It should be mentioned that there are no previous reports on the antifungal activity of extract of *L. sulphureus*. In the present study, aqueous extract

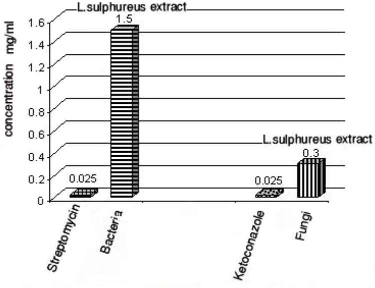

Fig. 1 – Antibacterial and antifungal effect of *L. sulphureus* extract in comparison to streptomycin and ketoconazole

showed excellent fungicidal activity against all the tested fungi, with 0.30 mg/ml as MFC value. In comparison with antifungal activity of antimycotic ketoconazole (1mg/ml DMSO), the tested extract showed lower activity, but still good enough to be considered as a promising antifungal agent. Ketoconazole exhibited inhibitory activity against all the tested fungi in the range of 0.63-25.00 x 10^{-3} mg/ml, and fungicidal activity in range of 1.25-25.00 x 10^{-3} mg/ml.

Resistance to antibiotics and antifungal agents is emerging in a wide variety of organisms, and multiple drug resistant organisms pose a serious threat to the treatment of infectious diseases. Hence, medicinal mushrooms, or mushroom derived antimicrobial substances must receive proper attention, especially when they include a harmless basis, which is water in this case.

Terpenes, lectins, polysaccharides etc. are known to have an effect on bacterial cytoplasmatic membrane, making it vulnerable (L i n and C h o u, 1998; Y a n g et al., 2002). *L. sulphureus* is rich source of these compounds, which are potentially responsible for its antimicrobial activity.

C o w a n (1999) reported that the most active components are generally water-insoluble, but O l e n n i k o v et al. (2009) determined the existence of water-soluble polysaccharides in *L. sulphureus* that could be bioactive principles playing an important role in the activity of mushroom (C o w a n, 1999; O l e n n i k o v et al., 2009). It is expected that low polarity organic solvents would yield more active extracts, but at the same time organic solvent is what makes these extracts unsafe and impractical for everyday use (C o w a n , 1999).

Reports on antimicrobial activity of *L. sulphureus* are very scarce, and this is the first report on the antibacterial and antifungal activity of aqueous extract of this mushroom.

Results of this study confirm once more the true potential of natural products as antimicrobial agents. *L. sulphureus* presents a valuable source in the fight against the tested pathogens, as a sole therapy, which requires further clinical trials, or in combination with other chemotherapeutical agents.

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АНТИМИКРОБНА АКТИВНОСТ ВОДЕНОГ ЕКСТРАКТА *L. SULPHUREUS* (BULL.: FR.) MURILL

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Резиме

Гљива мрке трулежи, *Laetiporus sulphureus* (Bull.: Fr) Murill., позната и као шумско пиле, већ је позната по својој нутритивној вредности. У овој студији испитивана је антимикробна способност воденог екстракта *L. sulphureus*, микродилуционом *in vitro* методом. Биљни, животињски и хумани патогени, као и проузроковачи кварења хране су тестирани. Водени екстракт добијен од *L. sulphureus* показао је снажну активност на тестиране микроорганизме уз дозну зависност. С обзиром да постоји константни пораст резистентности патогена на познате синтетичке агенсе, потреба за новим терапеутским лековима и конзервансима у прехрамбеној индустрији је неопходност. Стога, резултати који указују на активност природних продуката могу бити од великог практичног значаја.