Antimicrobial Activity of Crude Methanolic Extracts from *Ganoderma lucidum* and *Trametes versicolor*

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Abstract
In this paper the antimicrobial activity of crude methanolic extracts obtained from *Ganoderma lucidum* and *Trametes versicolor* were investigated. The antimicrobial activities of the extracts against *E. coli*, *P. aeruginosa*, *S. epidermis*, *E. raffinosus*, *S. cerevisiae* and *C. albicans* were determined by the microbroth dillution method according by EUCAST in 96-well microplates. Microorganisms were obtained from Czech Collection of Microorganisms. Absorbances after and before the experiment were subtracted, converted to binary system and obtained values to Probit analysis were used. Of the two macromycetes extracts tested, not all extracts showed antimicrobial activity in tested MICs range. The highest antimicrobial activity showed the both extracts to *Saccharomyces cerevisiae*. The less antimicrobial effects had the both macromycetes extracts to *Staphylococcus epidermis*. Antimicrobial activity of macromycetes methanolic crude extracts to others tested microorganisms showed no effect or used concentration could be higher.

Keywords: Antimicrobial activity, macromycetes methanolic extracts, MIC, *Ganoderma lucidum*, *Trametes versicolor*

1. Introduction

Nature is a very good source of many medical compounds for thousands of years. In the last decades problem with antibiotic resistant bacteria has emerged. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and nowadays, the need to discover new and more potent of these agents as accessories or alternatives to antibiotic therapy is stronger [1, 2]. Macromycetes are rich sources of biologically active compounds with an enormous variety of chemical structures. Therefore, mushrooms could be useful in the search of new potent antimicrobial agents [3].
There are many different studies about antimicrobial activity of different types of fungi extracts from India [4, 5] and China [6]. Fungi medicine has tradition for many years ago in this country. For example, *Ganoderma lucidum* is one of the most famous traditional medicinal fungi, being used as functional food and in preventive medicine, mostly in the form of extracts with an annual global market [7, 8].
Consumer's life is about changes and development. In some cases, it is question of comeback, in another ones the question of futuristic wishes nevertheless, the only important thing is to satisfy our customer, but nowadays, do not forget sustainability issues in broaden understanding [9].
The present work is focus to antimicrobial activity of wild medicinal mushroms extracts isolated from *Ganoderma lucidum* and *Trametes versicolor* against some selected Gram-positive, Gram-negative bacteria and yeasts.

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2. Materials and methods

Fungi materials

The fungi materials used in this experiment consist of fruit bodies of *Ganoderma lucidum* and *Trametes versicolor*. Fungi were collected from outskirts of Spišská Nová Ves and Gelnica, Slovakia during the summer season 2013. Harvested fungi were identified by Martin Rajtar (Mykoforest). Fungi were initially dried at the room temperature in the dark. More detailed information is showed in Table 1.

Table 1. Additional information about tested fungi

Orig. Latin title	Fungal part	Yield (mg)	Area	Extraction time	Extracted by
Ganoderma lucidum	fruiting body	1029.7	SNV	2 weeks at room temperature	Vacuum evaporator from methanol at room temperature at -800 mbar
Trametes versicolor	fruiting body	1307.5	Gelnica		

Test microorganisms

Six strains of microorganisms were tested in this research. Two Gram-negative bacteria include *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 1960, two Gram-positive bacteria include *Staphylococcus epidermis* CCM 4418, *Enterococcus raffinosus* CCM 4216 and two yeast strains include *Saccharomyces cerevisiae* CCM 819 and *Candida albicans* CCM 8215. All tested strains were collected from the Czech Collection of Microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37 °C and the yeasts suspensions was culture in the malt extract broth (Biomark Pune, India) at 30 °C.

Preparation of extracts

After drying, the fungi materials were crushed, weighed out to 10 g and soaked separately in 100 mL of methanol p.a. (99.5 %, Sigma, Germany) during two weeks at room temperature in the dark. Exposure to sunlight was avoided to prevent the degradation of active components. Then, methanolic fungi extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the methanol (Stuart RE300DB rotary evaporator, Bibby scientific limited, UK, and vaccum pump KNF N838.1.2KT.45.18, KNF, Germany). For the antimicrobial assay, the crude fungi extracts were dissolved in dimethyl sulfoxid (DMSO) (Penta, Czech Republic) to equal 102.4 mg/mL as stock solution, while for chemical analysis methanol was used as solvent. Stock solutions of fungi extracts were stored at -16 °C in freezer until use.

Antimicrobial assay

The minimum inhibitory concentration (MIC) is the lowest concentration of the sample that will inhibit the visible growth of microorganisms. Fungi extracts dissolved in DMSO were prepared to a final concentration of 1024 µg/mL. Minimum inhibitory concentrations (MICs) were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation [10] in Mueller Hinton broth (Biolife, Italy) for bacteria and Sabouraud broth (Biolife, Italy) for yeasts. Briefly, the DMSO fungi extracts solutions were prepared as serial two-fold dilutions, in order to obtain a final concentration ranging between 0.5 – 1024 µg/mL. Each well was then inoculated with microbial suspension at the final density of 0.5 McF°. After incubation at 37 °C for bacteria and 30 °C for yeasts for 16-20 hours, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The 96 micro-well plates were measured before and after experiment. Differences between both measurements were evaluated as growth. Measurement error was established for 0.05 values from absorbance. Wells without fungi extracts were used as positive controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the minimum inhibitory concentrations of used medical plant extracts.

Statistical analysis

Using obtained absorbances before and after the analysis, we were able to express the differences in absorbance between the measurements as a set of binary values. These values were assigned to exact concentrations. The following formula was created for this specific experiment: value 1 (inhibitory effect) was assigned to absorbance

values lower than 0.05, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05. For this statistical evaluation Probit analysis in Statgraphic software was used.

3. Results and discussion

Antimicrobial activity of fungi crude extracts

The antimicrobial activity (expressed as µg/mL) of two methanolic fungi extracts from *Ganoderma lucidum* and *Trametes versicolor* against various strains of bacteria and yeast are summarized in Table 2. The most effective were tested fungi extracts against *Saccharomyces cerevisiae* with a MIC 50 value 3 ug/mL for *Ganoderma lucidum* extract and MIC 50 value 24 ug/mL for *Trametes versicolor* extract. The lower effect had both extracts against tested Gram positive microorganisms. We did not determined antimicrobial activity of tested fungi extracts against Gram negative bacteria in prepared range of concentrations. Equally we did not determined antimicrobial activity against *Candida albicans*.

Table 2. The minimum inhibitory concentration (MIC) of methanolic fungi extracts on six test microorganisms

Abr.	Tested microorganisms	Antimicrobial activity of macromycetes fungal extract (ug/mL)			
		Ganoderma lucidum		***Trametes versicolor***	
		MIC 50	**MIC 90**	**MIC 50**	**MIC 90**
EC	*Escherichia coli* CCM 3988	> 1024	> 1024	> 1024	> 1024
PA	*Pseudomonas aeruginosa* CCM 1960	> 1024	> 1024	> 1024	> 1024
SE	*Staphylococcus epidermis* CCM 4418	383.65	407.95	766.01	814.26
ER	*Enterococcus raffinosus* CCM 4216	> 1024	> 1024	766.01	814.26
SC	*Saccharomyces cerevisiae* CCM 8191	3.00	3.25	24.00	25.76
CA	*Candida albicans* CCM 8215	> 1024	> 1024	> 1024	> 1024

Legend: Abr. – abreviations,

Discussion

Antimicrobial activity of fungi crude extracts

Authors Heleno et al., [11] tested crude extract from *Ganoderma lucidum* against 8 bacterial pathogens as *S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *P. aeruginosa*, *S. typhimurium*, *E. coli* and *E. cloacae*. They measured MIC results in mg/mL and they determined similar antimicrobial activity for all tested microorganisms. From their results MIC ranged from 0.0125 to 0.75 mg/mL.

Many different species of macrofungi extracts were tested against different bacteria by Suay et al., [12]. These researchers determined and confirmed antimicrobial activity of crude macrofungi extracts. Equally, they tested extracts from *Ganodermataceae* genera and they confirmed antimicrobial activities theirself. However, they didn't tested macrofungi extracts from *Trametes* species.

Many researchers who tested macromycetes crude extracts against bacteria confirmed their antimicrobial activities. From the macromycetes extracts were isolated many types of chemical compounds which showed potential antimicrobial activities. For example, chemical compound as terpenes were isolated from ganoderma species and they had effect against *E. coli*, *P. vulgaris* and *S. marcescens*. Organic acids isolated from *Lentinus edodes* showed activity against *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *P. fluorescens*. Also were isolated benzoic acid derivates which showed antimicrobial activity against *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *P. fluorescens*. Chemical compounds as quinoline isolated from *Leucopaxillus albissimus* showed activity against many different species of bacteria [13-22]. Some protein isolated form macromycetes have also been reported against Gram-negative bacteria. The protein CSAP isolated from *Cordyceps sinensis* and already mentioned above showed activity against *E. coli*, *P. vulgaris* and *S. typhi* [23]. There are many kind of chemical structures which had an antimicrobial activities but the mechanisms of actions of each one of the isolates compounds is not completely clear and described in the available reports.

4. Conclusions

In conclusion, we can state that the methanolic fungi extracts of both fungi *Ganoderma lucidum* and *Trametes versicolor* showed the strongest antimicrobial activity against *S. cerevisiae*. Equally, lower antimicrobial activity of fungi extracts against Gram-positive microorganisms was detected. We didn't find antimicrobial activity of fungi extracts against Gram-negative bacteria and *Candida albicans*. We think that more studies and experiments and more range of concentration of fungi extracts are needed for better information about antimicrobial activity of macro fungi extracts. Nature and macromycetes are a rich sources of antimicrobial compounds awaiting for discover.

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