Antimicrobial potential of mushrooms *Macrolepiota procera* and *Chlorophyllum rhacodes*

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Abstract: The aim of this study is to investigate the *in vitro* antimicrobial properties of the acetone extracts of the mushrooms *Macrolepiota procera* and *Chlorophyllum rhacodes*. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and assess the antimicrobial effects against three bacterial and ten fungal species. Overall, the tested mushroom extracts had relatively strong antimicrobial activity against the tested microorganisms. The minimum inhibitory concentration for both extracts related to the tested bacteria and fungi was 0.625 - 20 mg/mL. Extract of *C. rhacodes* exhibited more powerful antimicrobial properties, with ranged MIC values from 0.625 mg/mL to 10 mg/mL. The Acetone extract of *C. rhacodes* has shown the most antibacterial activity against *S. aureus*, while the extract of *M. procera* has not shown activity against *S. aureus* as against *G. candidum*. In comparison to the standard antibiotics as positive controls, the antimicrobial activity of studied extracts was less expressed. The results suggest that mushroom species may be used for pharmaceutical purposes in treating various diseases.

Keywords: mushrooms, acetone extract, antimicrobial activity

1. Introduction

Presently, the global community is confronting noteworthy difficulties in contemporary healthcare provisions as numerous antimicrobial substances have become less potent in combatting infectious diseases predominantly attributable to the emergence of microbial resistance [1]. Searching for bioactive compounds that can effectively treat drug-resistant pathogenic microorganisms is incredibly beneficial. At present, there is an increasing focus on the exploration of novel antimicrobial agents derived from natural sources like bacteria, fungi, and plants [2]. Mushrooms release a range of bioactive compounds, including terpenoids, flavonoids, tannins, alkaloids, and

polysaccharides. They possess abundant bioactive compounds that remain largely unexplored but hold significant potential as valuable natural resources.

Only a few studies describe the antimicrobial properties of mushrooms *Macrolepiota procera* (Scop.) Singer 1948., and *Chlorophyllum rhacodes* (Vittad.) Vellinga 2002. could be found in literature [3, 4, 5].

Thus, the aim of the present work is to evaluate the antimicrobial potential of the acetone extract of mushrooms *M. procera* and *C. rhacodes*.

2. Materials and Methods

Fungal samples of *M. procera* and *C. rhacodes* were collected from Kragujevac, Serbia in October 2020. The demonstration samples are preserved in the facilities of the Department of Biology and Ecology, Faculty of Science, Kragujevac. The fresh fungal material was ground using an electrical mill and then subjected to extraction with acetone. After filtration, the extracts were concentrated under reduced pressure using a rotary evaporator. Before the test, the extracts were dissolved in 5% dimethyl sulphoxide (DMSO).

The antibacterial activity of acetone extracts of *M. procera* and *C. rhacodes* was tested on three species of bacteria: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Proteus mirabilis* (ATCC 12453), and ten species of fungi: *Trichophyton mentagrophytes* (ATCC 9533), *Fusarium oxysporum* (ATCC 62506), *Geotrichum candidum* (ATCC 34614), *Trichoderma viride* (ATCC 13233), *Cladosporium cladosporoides* (ATCC 11680), *Penicillium italicum* (ATCC 10454), *Mucor mucedo* (ATCC 20094), *Aspergillus flavus* (ATCC 9170), *Aspergillus niger* (ATCC 16888) and *Candida albicans* (ATCC 10259).

Cultures of tested species of microorganisms are provided from the American Type Culture Collection (ATCC). Bacterial cultures were maintained on Müller-Hinton agar. Suspensions were prepared with sterile distilled water and were adjusted to contain approximately 10^8 CFU/mL. Fungal cultures were maintained on potato dextrose (PD) agar and Sabourad dextrose (SD) agar (Torlak, Belgrade). Fungal spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and then further diluted to approximately 10^6 CFU/mL according to the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [6].

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method using 96-well microtiter plates [7]. A series of dilutions with concentrations ranging from 40 to 0.019 mg/mL for extracts were used in the experiment against every microorganism tested. Resazurin was used for the evaluation of bacterial growth. The MIC was determined by establishing the visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration. The MIC for antifungal activity was determined by establishing the visible growth of microorganisms.

Standard antibiotics (streptomycin for bacteria and ketoconazole for fungi) were used as positive controls and DMSO was used as negative control.

3. Results and Discussion

The results of testing the antimicrobial activity are presented in Table 1.

Table 1. Minimum inhibitory concentration of acetone extracts of M. *procera* and *C. rhacodes*.

Mushroom species	*M. procera*	*C. rhacodes*	Streptomycin	Ketoconazole
S. aureus	-	0.625	0.039	-
E. coli	20	20	0.039	-
P. mirabilis	20	20	0.078	-
T. mentagrophytes	5	2.5	-	0.078
F. oxysporum	10	2.5	-	0.078
G. candidum	-	5	-	0.078
T. viride	10	10	-	0.078
C. cladosporoides	10	5	-	0.039
P. italicum	5	5	-	0.156
M. mucedo	10	5	-	0.156
A. flavus	20	10	-	0.156
A. niger	20	20	-	0.078
C. albicans	10	10	-	0.039

*Values given as mg/mL for extract and as μg/mL for antibiotics.

MIC values for *M. procera* ranged from 5 to 20 mg/mL, while for *C. rhacodes* they ranged from 0.625 to 20 mg/mL. However, *C. rhacodes* exhibited stronger and more effective antimicrobial activity compared to *M. procera*. *M. procera* has not shown activity against *S. aureus* and *G. candidum*. In general, gram-positive bacteria *S. aureus* was more susceptible than Gram-negative bacteria. Overall, fungi were more resistant than bacteria. The lowest MIC values for fungal species were for *C. rhacodes* against *T. mentagrophytes* (2.5 mg/mL), and against *F. oxysporum* (2.5 mg/ mL). Our results showed that in comparison to standard antibiotics as positive controls, the antimicrobial activity of studied extracts was less expressive.

In our experiments, the tested mushroom extracts showed significant antimicrobial activity. The strength of the antimicrobial effect depended on the species of mushroom, its concentration, and the tested organism. Interestingly, when compared at the same concentrations, the examined mushroom displayed stronger antifungal than antimicrobial activity. The reason for the different sensitivity between the fungi and bacteria can be found in the different transparency of the cell wall [8].

While there is limited information available regarding the antimicrobial activity of *M. procera* and *C. rhacodes*, some authors have previously confirmed their antimicrobial properties [3, 4, 5].

The dissimilarities in our results, when compared to theirs, can be attributed to the disparity in the extraction technique, extraction solvent, and tested organisms.

4. Conclusion

In conclusion, it can be affirmed that mushroom extracts that were tested exhibit potent antimicrobial properties *in vitro*. These findings suggest that mushrooms can serve as valuable natural antioxidants and may have implications in the treatment of human ailments, as well as diseases in animals and plants. Additional research is needed to investigate the extraction and identification of novel compounds from mushrooms that are responsible for their antimicrobial effects.

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