

Antioxidant and antimicrobial activity of *Cerrena unicolor*

*Mustafa Sevindik

Department of Biology, Faculty of Science, Akdeniz University, Antalya, Turkey

*Corresponding author's email: sevindik27@gmail.com

Abstract

In this study, it was aimed to determine antioxidant and antimicrobial activity of *Cerrena unicolor* (Bull.) Murrill which is a wood decaying mushroom. Mushroom samples were extracted with ethanol, methanol and dichloromethane in soxhlet apparatus. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the extracts were determined by Rel Assay Diagnostics kits. Antimicrobial activity was determined by using the modified agar dilution method against 9 bacterial and fungus strains (*Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *C. krusei*, and *C. glabrata*). TAS value of *C. unicolor* was found 6.706 ± 0.059 mmol L⁻¹, TOS value was 19.308 ± 0.114 μ mol L⁻¹ and OSI value was 0.288 ± 0.003 . Mushroom extracts were found to be effective against microorganisms at concentrations of 25-400 μ g mL⁻¹. In addition, the anti-candidal activity of the extracts were higher. As a result, it was determined that *C. unicolor* has antioxidant and antimicrobial potential.

Keywords: *Cerrena unicolor*, Antioxidant, Oxidant, Antibacterial, Antifungal.

Introduction

Antioxidants are defined as substances that delay or inhibit oxidative damage caused by the presence of high doses of reactive oxygen species (ROS). Exogenous antioxidants play a key role in the delicate balance between oxidation and antioxidant in living systems. Many natural materials such as plants and mushrooms are rich in point of antioxidant compounds (Bouayed and Bohn, 2010; Selamoglu *et al.*, 2016). In this study, antioxidant potential of *C. unicolor* was determined.

The pharmacology industry has produced many new antibiotics over the past three decades, but the resistance of microorganisms to these drugs has increased. The increase in the problem of microbial resistance has revealed uncertainty about the use of antimicrobial drugs in the future. Therefore, to reduce this problem, it is important to control the use of antibiotics to understand the mechanisms of genetic resistance of bacteria and to continue to develop new synthetic or natural drugs (Nascimento *et al.*, 2000; Gonelimali *et al.*, 2018). In this study, antibacterial and antifungal activity of *C. unicolor* was determined in the determination of new natural antimicrobial agents.

Macrofungal species have been reported about 2200 species in Turkey until today (Sesli and Denchev, 2008; Altuntaş *et al.*, 2017). In recent years, biological activities on these macrofungi have accelerated studies in Turkey, which is macrofungi rich in terms of biodiversity. Saprotrophic mushroom species can produce different bioactive secondary metabolites in their bodies. It is in the family Polyporaceae, wood decaying *Cerrena unicolor* causes white decay. *C. unicolor* is cosmopolitan in

regions such as Europe, Africa and South America and is an aggressive wood decay organism. *C. unicolor*, *Aesculus hippocastanum* L., *Fraxinus excelsior* L., *Acer* sp. L., *Betula* sp. L., *Fagus* sp. L. or *Quercus* sp. L. can be found in many deciduous hardwoods. Recently, it has been reported that *C. unicolor* may be the source of bioactive compounds of pharmacological and medical importance (Mizerska-Dudka *et al.*, 2015). In this study, it was aimed to determine the antioxidant and antimicrobial capacity of *C. unicolor* extracts of ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM).

Materials and Methods

C. unicolor samples were collected from Gaziantep (Turkey) in 2017. The collected mushroom samples were extracted with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) at 50 °C for about 6 hours (Gerhardt EV 14). The extracts were concentrated by rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Antioxidant activity tests

TAS and TOS values of mushroom were determined using Rel Assay kits (Assay Kit Rel Diagnostics, Turkey). Trolox as calibrator was used for the TAS value. Results are shown as mmol Trolox equiv. L⁻¹ (Erel, 2004). H₂O₂ (hydrogen peroxide) as calibrator was used for the TOS value. The results were shown as μ mol H₂O₂ equiv.L⁻¹ (Erel, 2005). The following formula was used to calculate the OSI (arbitrary unit: AU) and the percentage was expressed (Erel, 2005). In this study,

6 samples were taken from mushroom samples and 5 replicates were studied.

$$\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol H}_2\text{O}_2 \text{ equiv. L}^{-1}}{\text{TAS, mmol Trolox equiv. L}^{-1} \times 10}$$

Antimicrobial activity tests

The antimicrobial activity tests of the EtOH, MeOH and DCM extracts of *C. unicolor* were determined by the agar dilution method. Minimal inhibitory concentrations (MIC) for each extract were determined against standard bacteria and fungus strains. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as test bacteria. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 ATCC 13803 and *C. glabrata* ATCC 90030 were used as fungus strains. Bacterial strains were cultured on Muller Hinton Broth medium and fungus strains were pre-cultured on RPMI 1640 Broth medium. To obtain a standard inoculum, the blur of bacteria and fungi was prepared according to McFarland 0.5. Standard extract concentrations were prepared with distilled water as 800-12.5 $\mu\text{g mL}^{-1}$. Fluconazole and amphotericin B for fungi, was used as a reference drug, whereas Amikacin, Ampicillin and Ciprofloxacin for bacteria were used as reference drugs. The lowest concentration inhibiting the growth of bacteria and fungi was determined as the minimum inhibitor concentration (MIC) (Bauer *et al.*, 1966; Hindler *et al.*, 1992; CLSI, 2012; EUCAST, 2014; Matuschek *et al.*, 2014; EUCAST, 2015).

Results and Discussion

Antioxidant activity

It is known that a wide range to pathological damages such as carcinogens and rheumatoid arthritis may be caused by oxygen free radicals. Nearly all organisms are well protected against free radical damage by many antioxidant compounds such as superoxide dismutase, catalase, ascorbic acid, tocopherols and glutathione. However, sometimes the antioxidant protection mechanism becomes unstable and then its oxidative damage can be reduced with many reinforcing antioxidants (Mujić *et al.*, 2010). In this study, TAS, TOS and OSI values of *C. unicolor* were determined for the first time. The results are shown in Table 1.

In this study, the TAS value of *C. unicolor* was reported 6.706±0.059 mmol L⁻¹, the TOS was 19.308±0.114 $\mu\text{mol L}^{-1}$ and the OSI value was 0.288±0.003. In previous studies, TAS value of *Fomitopsis pinicola*, which is wild wood decaying mushrooms, was reported 1.44 mmol L⁻¹, TOS value

was 14.21 $\mu\text{mol L}^{-1}$ and OSI value was 0.99 (Sevindik *et al.*, 2017a). TAS value of *Ompholatus olearius* was reported 2.836 $\mu\text{mol L}^{-1}$, TOS value was 8.262 $\mu\text{mol L}^{-1}$ and OSI value was 0.291 (Sevindik *et al.*, 2017b). TAS value of *Tricholoma terreum* was reported 0.38 $\mu\text{mol L}^{-1}$, TOS value was 16.76 $\mu\text{mol L}^{-1}$ and OSI value was 4.41 (Akgül *et al.*, 2016). Compared to these studies, it was determined that TAS and TOS values of *C. unicolor* used in our study were higher than *F. pinicola*, *O. olearius* and *T. terreum* mushrooms. In this context, it has been determined that it has higher antioxidant potential of *C. unicolor*. However, it has been observed that higher oxidant compounds produced by the metabolic activities due to environmental and structural factors. When compared to OSI values, *C. unicolor* was found to be lower than *T. terreum*, *F. pinicola* and *O. olearius*. In addition, the differences in these TAS, TOS and OSI values among the fungi are thought to be due to the living environment, lifestyle, substrate and stress factors of habitat of mushroom. It was previously reported that *C. unicolor* had antioxidant activity (Jaszek *et al.*, 2013). In our study, it was determined that *C. unicolor* has high antioxidant potential.

Antimicrobial activity

Nowadays, many diseases with microorganism factors have been defined and continue to be defined day by day. In this context, synthetic antibiotics used today against infectious diseases are insufficient. Also, the resistance against these synthetic antibiotics of bacteria creates serious problems (Peres-Bota *et al.*, 2003; Lima *et al.*, 2016). In this context, new antimicrobial sources to be obtained are very important. In this study, antimicrobial activity of EtOH, MeOH and DCM extracts of *C. unicolor* against 9 bacteria and fungus strains was determined and the results are shown in Table 2.

In previous studies, the ethanol extracts of *C. unicolor* have been reported to be effective against *Bacillus cereus*, *B. subtilis*, *Listeria monocytogenes*, *Staphylococcus epidermidis* and *Streptococcus agalactiae* in different concentrations (Altuner *et al.*, 2012). In a different study, polysaccharides of *C. unicolor* have been reported to have antibacterial effects on *Escherichia coli* and *Staphylococcus aureus* (Jaszek *et al.*, 2013). In our study, EtOH, MeOH and DCM extracts of *C. unicolor* were used and it has been found to have effects against *Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *C. krusei* and *C. glabrata* in 25-400 $\mu\text{g mL}^{-1}$ concentrations. EtOH extracts of the mushroom were determined to have higher activity than MeOH and DCM extracts. In addition, the anticandidal activity of EtOH, MeOH and DCM extracts was found to be higher. In this context, *C. unicolor* can be used as an antimicrobial agent

against test microorganisms used in the study.

Conclusion

In this study, total antioxidant capacity, total oxidant capacity, oxidative stress index and antimicrobial activity of *C. unicolor* mushroom collected from Gaziantep (Turkey) province were determined. *C. unicolor* has been found to have high antioxidant potential. In addition, the mushroom

extracts had high anticandidal activity. As a result, it was determined that *C. unicolor* has antioxidant and antimicrobial potential.

Acknowledgment

We would like to express thanks to Dr. Ilgaz AKATA for their contributions to the present study.

Table 1: TAS, TOS and OSI values of *C. unicolor*

	TAS (mmol/L)	TOS (µmol/L)	OSI
<i>C. unicolor</i>	6.706±0.059	19.308±0.114	0.288±0.003

Values are presented as mean±SD.

Table 2: MIC values of different extracts of *C. unicolor* and standard antibiotics against test microorganisms

	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
EtOH	200	200	100	50	100	400	25	25	50
MeOH	200	200	100	50	200	400	25	25	50
DCM	200	200	200	50	200	400	25	50	50
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

The MIC values are presented in units of µg mL⁻¹.

References

- Akgül H, Nur AD, Sevindik M, Doğan M, 2016. *Tricholoma terreum* ve *Coprinus micaceus*' un bazı biyolojik aktivitelerinin belirlenmesi. *Artvin Çoruh Üniversitesi*, **17**: 158-162.
- Altuner EM, Akata I, Canli K, 2012. *In vitro* antimicrobial screening of *Cerrena unicolor* (Bull.) Murrill. *Fresen Environ. Bull.*, **21**: 3704-3710.
- Altuntaş D, Allı H, Akata I, 2017. Macrofungi of Kazdağı National Park (Turkey) and its close environs. *Biol. Divers. Conserv.*, **10**: 17-25.
- Bauer AW, Kirby WM, Sherris, JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, **45**: 493-96.
- Bouayed J, Bohn T, 2010. Exogenous antioxidants - double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxid. Med. Cell. Longev.*, **3**: 228-237.
- CLSI, 2012. Ethods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition (M11-A8). The Clinical and Laboratory Standards Institute.
- Erel O, 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.*, **37**: 277-285.
- Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, **38**: 1103-1111.
- EUCAST, 2014. Breakpoint tables fungal isolate for interpretation of MICs. 2014; Version 7.0. European Committee on Antimicrobial Susceptibility Testing.
- EUCAST, 2015. Breakpoint tables for Bacteria interpretation of MICs and zone diameters, Version 5.0. European Committee on Antimicrobial Susceptibility Testing.
- Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR, 2018. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front. Microbiol.*, **24**:1639.
- Hindler J, Hochstein L, Howell A. 1992. Preparation of routine media and reagents used in antimicrobial susceptibility testing. Part 1.

- McFarland standards, In: Isenberg HD (ed) Clinical Microbiology Procedures Handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Jaszek M, Osińska-Jaroszuk M, Janusz G, Matuszewska A, Stefaniuk D, Sulej J, Polak J, Ruminowicz M, Grzywnowicz K, Jarosz-Wilkolazka A. 2013. New bioactive fungal molecules with high antioxidant and antimicrobial capacity isolated from *Cerrena unicolor* idiophasic cultures. *BioMed Res. Int.*, Article ID 497492.
- Lima VN, Oliveira-Tintino CD, Santos ES, Morais LP, Tintino SR, Freitas TS, Geraldo YS, Pereira RL, Cruz RP, Menezes IR, Coutinho HD. 2016. Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol. *Microb. Pathog.*, **99**: 56-61.
- Matuschek E, Brown DF, Kahlmeter G. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin. Microbiol. Infect.*, **20**: 255-266.
- Mujić I, Zeković Z, Lepojević Ž, Vidović S, Živković J. 2011. Antioxidant properties of selected edible mushroom species. *J. Central Eur. Agric.*, **11**: 387-392.
- Nascimento GG, Locatelli J, Freitas PC, Silva GL. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.*, **31**: 247-256.
- Mizerska-Dudka M, Jaszek M, Błachowicz A, Rejczak TP, Matuszewska A, Osińska-Jaroszuk M, Stefaniuk D, Janusz G, Sulej J, Kandfer-Szerszeń M. 2015. Fungus *Cerrena unicolor* as an effective source of new antiviral, immunomodulatory, and anticancer compounds. *Int. J. Biol. Macromol.*, **79**: 459-468.
- Peres-Bota D, Rodriguez H, Dimopoulos G, DaRos A, Mélot C, Struelens MJ, Vincent JL. 2003. Are infections due to resistant pathogens associated with a worse outcome in critically ill patients?. *J. Infec.*, **47**: 307-316.
- Selamoglu Z, Akgul H, Dogan H. 2016. Environmental effects on biologic activities of pollen samples obtained from different phytogeographical regions in Turkey. *Fresen Environ. Bull.*, **25**: 2484-2489.
- Sesli E, Denchev CM. 2008. Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. *Mycotaxon*, **106**: 65.
- Sevindik M, Akgul H, Akata I, Alli H, Selamoglu Z. 2017a. *Fomitopsis pinicola* in healthful dietary approach and their therapeutic potentials. *Acta Aliment.*, **46**: 464-469.
- Sevindik M, Akgül H, Bal C. 2017b. Determination of Oxidative Stress Status of *Ompholatus olearius* Gathered from Adana and Antalya Provinces in Turkey. *Sakarya Univ. J. Sci.*, **16**: 153-156.