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Antioxidant, antibacterial, and antifungal activity of *Hymenochaete rubiginosa*

Şule İnci[1]*, Mustafa Sevindik[2], Sevda Kırbağ[1] and Hasan Akgül[3]

[1]Department of Biology, Faculty of Science, Fırat University, Elazığ 23119, Turkey

[2]Department of Food Processing, Bahçe Vocational School of Higher Education, Osmaniye Korkut Ata University, Osmaniye 80500, Turkey

[3]Department of Biology, Faculty of Science, Akdeniz University, Antalya 07058, Turkey

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The use of fungi in alternative medicine and their use as pharmacological agents is gradually increasing today. *Hymenochaete rubiginosa* (Dicks.) Lév, locally known as Crust, is a species that belongs to the Polyporales family. In this study, it was aimed to determine the antimicrobial and antioxidant activity of *H. rubiginosa* extracts obtained from ethanol solvent. Its antimicrobial activity has been determined using the microdilution (MIC) method. In this study, bacteria such as *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 and *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135 and *Candida glabrata* ATCC 9003 have been used as fungi. Total antioxidant level (TAS), total oxidant level (TOS) and oxidative stress index (OSI) values were calculated for antioxidant activity. According to the results obtained, it has been determined that the ethanol extract of *H. rubiginosa* showed antimicrobial effect against test microorganisms at concentrations of 25-200 µg/mL. The TAS value of *H. rubiginosa* has been determined as 6.313±0.050 mmol/L, TOS value as 14.358±0.202 µmol and OSI value as 0.227±0.002. As a result, it has been determined that *H. rubiginosa* can be used as an antioxidant and antimicrobial agent in pharmacological designs.

Keywords: Antimicrobial, Antioxidant, *Hymenochaete rubiginosa*, Medicinal mushroom, Oxidant.

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Introduction

Human has always benefited from nature in the treatment of diseases and used many natural products such as mushrooms and herbs in the treatment[1]. In particular, in recent years, people have turned to alternative foods with strong antimicrobial and antioxidant effects as well as healthy nutrition[2,3]. Fungi, and especially Basidiomycota species, are known as valuable natural products, thanks to some of their bioactive components[4]. Some mushroom species have shown important pharmacological effects such as antimicrobial, antioxidant, immunomodulatory, and anticancer thanks to these components[5-9]. For this reason, interest and research on natural products or their derivatives still continues in drug use[10]. The increasing resistance of bacteria to antibiotics brings with it the need for alternative antibiotics. It is known that some antibiotics were produced from fungi in 1995[11]. Antioxidants are important compounds that inhibit the oxidation of cell biomolecules, prevent cell damage and prevent the formation of free radicals in our body[12-15]. Unlike synthetic antioxidants, natural antioxidants can neutralize free radicals without toxic and mutagenic effects[15-17]. *Hymenochaete rubiginosa* (Dicks.) Lév., which belongs to the Polyporales family and is locally known as Crust, is a type of non-edible mushroom[18-20]. However, it is known that it was collected and eaten by the people in some regions[12]. Nearly always associated with dead oak trees, this easily-overlooked crust fungus varies considerably in its appearance, sometimes mainly resupinate beneath fallen logs but usually in bracket form when on dead stumps. The specific epithet rubiginosa means rusty and refers to the reddish-brown colour of the hymeneal (fertile) surface of this crust fungus[18-19]. Polyporales have a long history of medical use in hemostatic dressings and bandages. In addition, it has been reported that primary and secondary metabolites exhibit a wide variety of biological activities such as antioxidant, antimicrobial, anticancer, cardiovascular, antiviral,

*Correspondent author
Email: sule.inci@hotmail.com

anti-inflammatory, nematocidal, and immune stimulating[21]. However, it is noteworthy that there are almost no studies on the medicinal effects of *H. rubiginosa*, which belongs to this family. Therefore, in this study, it was aimed to determine the antimicrobial and antioxidant activity of *H. rubiginosa* extract obtained from ethanol solvent.

Materials and Methods

Sample collection, identification and extract preparation

H. rubiginosa samples used in the study were collected in Antalya province (2017). The mushroom identification was carried out by Dr. Hasan AKGÜL from Akdeniz University. After the identification of the collected mushroom samples, they were dried at 40 °C. Then, they were pulverized in a mechanical grinder. Pulverized mushroom samples were extracted with ethanol (EtOH) in a Soxhlet apparatus at 50 °C (Gerhardt EV 14). The mushroom extracts were then concentrated under pressure at 40 °C in a rotary evaporator (HeidolphLaborator 4000 Rotary Evaporator) and stored at +4 °C.

Antimicrobial activity

Antimicrobial activity tests were conducted on the mushroom EtOH extracts using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimal inhibitor concentrations (MIC) for each extract were determined against standard bacterial and fungal strains. The following microorganisms were used for this purpose: *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, and *Enterococcus faecalis* ATCC 29212. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606 were used as gram-negative bacteria. Furthermore, *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, and *C. glabrata* ATCC 90030 were used as fungi and were obtained from the American culture collection. Bacteria strains were pre-cultured in Muller Hinton Broth medium and fungal strains were pre-cultured in RPMI 1640 Broth medium. To obtain standard inoculum, the turbidity of the bacteria and fungi was set based on the McFarland 0.5 scale. All extracts were tested at 800-12.5 µg/mL concentrations and distilled water was used in all dilutions. The solvents used in extracts were also individually tested for antimicrobial activity. Fluconazole and Amphotericin B were used as reference drugs for the fungi. Amikacin, ampicillin, and ciprofloxacin were used as reference drugs for the bacteria. The lowest concentration that prevented the proliferation of bacteria and fungi was determined as the minimal inhibitor concentration (MIC)[22].

Antioxidant and oxidant activity

Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of mushroom extracts were determined with Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). TAS value was expressed as mmol Trolox equiv./L and Trolox was used as the calibrator[23]. The TOS value was expressed as µmol H_2O_2 equiv./L and hydrogen peroxide was used as the calibrator[24]. The OSI (Arbitrary unit) was calculated with the formula below.

$$\text{OSI (AU)} = \frac{\text{TOS, µmol } H_2O_2 \ ^{\text{Equiv.}}/_{\text{L}}}{\text{TAS, mmol Trolox } ^{\text{Equiv.}}/_{\text{L}} \times 10}$$

Results and Discussion

Antimicrobial activity

In many developing countries, people use natural products in the treatment of diseases. The use of natural products is gradually increasing, especially in the fight against microorganism-based diseases. Due to the increase in resistant forms of microorganisms in recent years, drugs used against these microorganisms are inadequate. For this reason, it is very important to identify natural products to be used in combating microorganisms[25,26]. In this study, the antimicrobial activity of *H. rubiginosa* against some bacterial and fungal strains was investigated. The findings obtained are shown in Table 1.

According to the results obtained, the ethanol extract of *H. rubiginosa* against *S. aureus, S. aureus* MRSA, and *A. baumannii* at concentration of 25 µg/mL, against *C. glabrata, C. albicans* and *C. krusei* at a concentration of 50 µg/mL, against *E. faecalis*, *E. coli* at concentration of 100 µg/mL and against *P. aeruginosa* at a concentration of 200 µg/mL was determined to be effective. Many studies have reported that fungi have antimicrobial activities[6,27-29]. In the present study, it was found to be effective against test microorganisms at concentrations of 25-200 µg/mL. In this context, it has been determined that *H. rubiginosa* has antimicrobial potential.

Table 1 — MIC values (µg/mL) of *Hymenochaete rubiginosa* against test microorganisms

	A	B	C	D	E	F	G	H	J
EtOH	25	25	100	100	200	25	50	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

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei;* *200, 100, 50 and 25 µg/mL extract concentrations

Antioxidant activity

Living organisms produce different levels of oxidant compounds in their bodies by environmental influences. While these oxidant compounds show beneficial effects on metabolic activities at low levels, they have detrimental effects at high levels[30]. With the increase of oxidant compounds, antioxidant compounds enter the circuit. Antioxidant compounds play a role in suppressing or reducing the effect of oxidant compounds[31]. Oxidative stress occurs when antioxidant compounds are insufficient. In a result of oxidative stress, different diseases such as Alzheimer's, Parkinson's, cancer, and cardiological disorders can occur in humans. Supplementary antioxidants are used to reduce the effect of oxidative stress[32,33]. In this context, determining new natural sources of antioxidants is very important in terms of supplemental antioxidants. In the present study, EtoH extract of *H. rubiginosa* was used and TAS, TOS, and OSI values were determined. The results obtained are shown in Table 2.

Table 2 — TAS, TOS, and OSI values of *H. rubiginosa*

	TAS	TOS	OSI
H. rubiginosa	6.313±0.050	14.358±0.202	0.227±0.002

Values are presented as mean±SD; Experiments were made in 5 parallel

In the literature review, it was seen that the TAS, TOS, and OSI values of *H. rubiginosa* were not determined. But, studies have been conducted on different types of wild mushrooms. The TAS value of *Lepistanuda* has been reported as 3.102 mmol/L, TOS value as 36.920 µmol/L, and OSI value as 1.190[34]. The TAS value of *Cantharellus cibarius* has been reported as 5.268 mmol/L, TOS value as 6.380 µmol/L, and OSI value as 0.121[35]. *L. cristata* demonstrated significant antioxidant potential, with a TAS value of 3.623, TOS of 27.476[36]. TAS value of *T. hirsuta* was determined to be 3.466 mmol/L, TOS value as 13.482 µmol/L, and OSI value as 0.39038[37]. TAS value is an indicator of the whole of the endogenous antioxidants produced by the mushroom[38]. Compared to these studies, it was observed that the TAS value of *H. rubiginosa* was higher than *L. cristata*, *T. hirsuta*, *L. nuda,* and *C. cibarius*. The difference in TAS that occurs between fungi is thought to be due to the mushroom's potential to produce antioxidant compounds. Also, it has been previously reported that *H. rubiginosa* has high DPPH free radical scavenging activity[21,39]. In this study, the TAS value of *H. rubiginosa* was determined for the first time and it was observed that it has a high potential to produce compounds with antioxidant properties. The TOS value indicates the whole of the oxidant compounds produced by the fungus as a result of environmental effects and metabolic activities[38]. It was determined that the TOS value of *H. rubiginosa* was higher than *T. hirsuta* a nd *C. cibarius*, but lower than *L. cristata* and *L. nuda*. In this context, it is seen that the TOS value of *H. rubiginosa* is at normal levels compared to other mushrooms. The OSI value shows how much endogenous oxidants produced by mushroom are suppressed by endogenous antioxidants. It is seen that the higher the OSI value, the less effective the antioxidant defense system[38]. It was determined that the OSI value of *H. rubiginosa* was higher than that of *C. cibarius*, but lower than that of *T. hirsuta* and *L. nuda*. In this context, it is seen that the antioxidant defense system of *H. rubiginosa* is more active.

Conclusion

In this study, the antioxidant, oxidant, and antimicrobial potentials of *H. rubiginosa* were investigated. As a result of the studies, it is seen that its antimicrobial activity increases depending on the increase in concentration. Also, it has been determined that the mushroom has important antioxidant potential. In addition, it was determined that EtOH extract of mushroom has antimicrobial potential. As a result, it is thought that *H. rubiginosa* can be used as an antioxidant and antimicrobial agent in pharmacological designs.

Conflict of interest

The authors declare no conflict of interest.

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