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Antimicrobial and antioxidant activities of medicinally important Lactarius deliciosus

Emre Avcı[1], Gulcin Alp Avci[2]

[1]*Department of Molecular Biology and Genetics, Biochemistry, Hitit University, Corum, Turkey*
[2]*Department of Molecular Biology and Genetics, Biotechnology, Hitit University, Corum, Turkey*

Abstract: *Fungi, which are of particular importance in the ecosystem because of their biodegradable properties, are known to be an important source of biologically active components of both food and medical value. Fungal extracts are used in the treatment and prevention of many diseases. Lactarius deliciosus mushroom is also known as Kanlica mushroom and is an edible mushroom with high nutritional value. The aim of this study was to determine the antioxidant and antimicrobial activities of water, ethanol and chloroform extracts of Lactarius deliciosus. Antioxidant activities of the extracts were determined by DPPH (2,2-diphenyl-1-picrylhydrazase) method. In addition, total antioxidant status of mushroom extracts was determined spectrophotometrically. Antimicrobial activities against Escherichia coli, Candida albicans, Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa were determined by disc diffusion method. The water and ethanol extracts of Lactarius deliciosus were found to have total antioxidant and DPPH activity. And also antimicrobial activity was determined on microorganism. The most susceptible strain was P. aeruginosa and the most resistant strain was E. coli. Mushrooms are highly important due to their properties such as fat, vitamins, carbohydrates and proteins. It is important to evaluate these fungi in terms of antioxidant and antimicrobial activity.*

Keywords: *Antimicrobial Activity, Antioxidant Activity, Lactarius deliciosus*

I. INTRODUCTION

Fungi, which have a certain importance in the classification of living organisms and in the ecosystem, have been consumed as food in many parts of the world for centuries and are known as an important source of biologically active components of medicinal value. Because of these properties, it is extracted and used in the treatment and prevention of many diseases. In addition, fungi; fat, calories and carbohydrates, such as substances that can be harmful to the body low, vitamins, minerals, protein-rich foods are preferred in terms of nutrition [1-3].

Mushrooms have antioxidant, antihypertensive, cholesterol-lowering, liver protective, antifibriotic, antiinflammation, antidiabetic, antiviral and antimicrobial effects as well as immunological and anticancer properties [4]. The antimicrobial effects of macrofungi are caused by certain phenolic compounds, purines, pyrimidines, quinones, terpenoids and phenyl proponoid-derived antagonistic substances, which are synthesized in the fungal structure and are usually organism-specific. The most important substances showing antitumoral effect are calvine, volvotoxin, flammutoxin, lentinan and poricin which are isolated only from macrofungi and also antiviral compounds. More than 50 of these antagonistic agents are known in one or more species of the genera *Alerodiscus, Cjitocybe, Coprinus, Cortinarius, Marasmius, Pleurotus* and *Polyporus* [5].

The other compound that is found in the structure of fungi and which has recently attracted attention is β-glucan. β- glucan, a polysaccharide showing a β- (1 → 3) linkage, can be found in the fungal cell wall and show β- (1 → 6) branching. Cap fungi also have antimicrobial, antioxidant, anti-carcinogenic, cholesterol lowering, antiviral, antifungal, immune system stimulating properties [6-9].

There are more than 12000 fungus species registered in the world, but about 2000 of them are identified as edible. In these estimates, the presence of up to 15,000 corked mushrooms and 650 of these are considered medically important [10-13]. It is fairly uncommon in Britain and Ireland and this kind of milkcap is found throughout Europe and in parts of North America and Australia *Lactarius deliciosus* among the mushrooms grow in a natural environment in Turkey. *Lactarius* literally means' milky 'and delicatessen' delicious. The popular *Lactarius delicatessen*, also known as Kanlıca 'or Çıntar' mushroom, grows extensively in the forests of the Black Sea region and in many regions of our country. They usually occur in autumn rains in forests and forests, rural areas, old cultivated areas. [14-15]. Natural antimicrobial agents in foods have been increasingly studied in recent years to extend quality and shelf life. As a result, the chemical status of plant-derived components related to antimicrobial activity remains to be elucidated [15,16]. Fungi need properties such as antifungal, antibacterial, antioxidant to survive in natural environment. Edible fungi, which secrete extracellular metabolites to combat pathogenic microorganisms and the presence of a cell wall with immunomodulatory activities, are rich in natural antibiotics [15-18]. Fungal extracts are used in many medical studies. In these studies, fungi were found to be antioxidant compounds such as phenolic, flavonoid, polysaccharide, glycoside, tocopherol, carotenoids and ascorbic acid. The heteropolysaccharide isolated from *L. deliciosus* fungus species (LDG-A) has been reported to have antitumor activity [19-22].

The aim of this study was to determine the antioxidant and antimicrobial activities of water, ethanol and chloroform extracts of *Lactarius deliciosus*.

II. METHODOLOGY

2.1. Material

Lactarius deliciosus specimens used in the study were collected from 8 different locations in Bolu province (Turkey) in suitable season conditions between 2018-2019. The collected samples were brought to the laboratory under appropriate conditions and identified by the macrofungus specialist. Fresh mushroom samples were divided into several pieces and dried in the oven at 39-40 ° C. The dried mushroom samples were weighed to 25 g. Twenty-five g of fungus were placed into 250 ml of solvent in a Soxhlet apparatus and allowed to extract for about 8 hours. Distilled water, ethanol and chloroform were used as solvent in the extraction. The extracts were evaporated in a rotary evaporator. The remaining 0.1 g samples were added into 1000 ml solvent. Then samples were filtered and stored at + 4 °C

Figure 1: Drying of Mushroom Samples

2.2. Methods

2.2.1. Extracting extracts using Soxhlet extractor;

The dried samples were ground to a powder. 10 grams were weighed for extraction of the mushroom samples and extracted into the soxhlet apparatus in 200 ml of solvent for 6 hours. Ethanol, chloroform and purified water were used as solvent in the extraction.

2.2.2. Evaporator Extract with Extraction;

The water extracts were evaporated at 45-50 °C and ethanol and chloroform extracts were evaporated at 40-45 °C until a final volume of 5 ml remained. The extract was incubated at room temperature for 24 hours to evaporate the remaining solvent.

Figure 2: Soxhlet extraction of fungi samples

Figure 3: Evaporator Extract with Extraction

2.2.3. Sterilization of Extracts;

The extracts were filtered using Wathman No. 6 and sterilized by passing through 0.45 µl filters. All extracts were stored at + 4 °C.

2.3.4. Determination of antioxidant activity;

Total antioxidant status was determined by spectrophotometric method. A fully automated method developed by Erel was used to measure the body's total antioxidant capacity against strong free radicals [23].

Calculation

$A2 - A1 = \Delta Abs$ of standard or sample or H_2O

$$\text{Results} = \frac{[\Delta Abs\ H_2O - \Delta Abs\ Sample]}{[\Delta Abs\ H_2O - \Delta Abs\ Standard]}$$

2.2.5. Determination of Antimicrobial Activity;

In this study, two Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), two Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) and one fungus (*Candida albicans* ATCC 90028) were used. Disc diffusion method was preferred for the determination of antimicrobial activity. Bacterial strains were activated two times and the bacterial density in 5 ml potassium buffer solution (PBS) was adjusted according to McFarland 0.5 turbidity. Mueller Hinton Agar was used as medium. 100 µl of the sample taken from the suspension was added in media. For the disc diffusion

method, the prepared extracts were impregnated with micropipette to 10 µl of discs made of 6 mm diameter empty sterile Wathmann no: 6 papers. Plates were incubated 37°C 18-20 h. After incubation, zone diameters were measured as millimeters.

2.2.6. DPPH Activity Determination

The DPPH method developed by Blois (1958) and Khalaf (2008) was modified and determined spectrophotometrically. DPPH value was calculated according to the following formula.

Percent (%) inhibition of DPPH activity = Ablank-Sample / Ablank X 100

III. RESULTS

The antimicrobial and antioxidant activities of *Lactarius deliciosus* fungi were examined using extracts obtained with distilled water, ethanol and chloroform solvents. In antioxidant activity studies, it was observed that *Lactarius deliciosus* ethanol extracts (7.06 mmol Trolox equivalent / l) had total antioxidant status (Table 1).

TABLE 1. Total antioxidant status and DPPH activity of ethanol extracts of fungi.

	Total Antioxidant status (mmol Trolox Equiv./L)	**DPPH IC_{50} (µg)**
Lactarius deliciosus (Ethanol Extracts)	7.06±0.95	58.57± 4.23
Lactarius deliciosus (Water Extracts)	6.10±0.56	36.68± 8.62

Values were expressed as mean ± standard deviation.

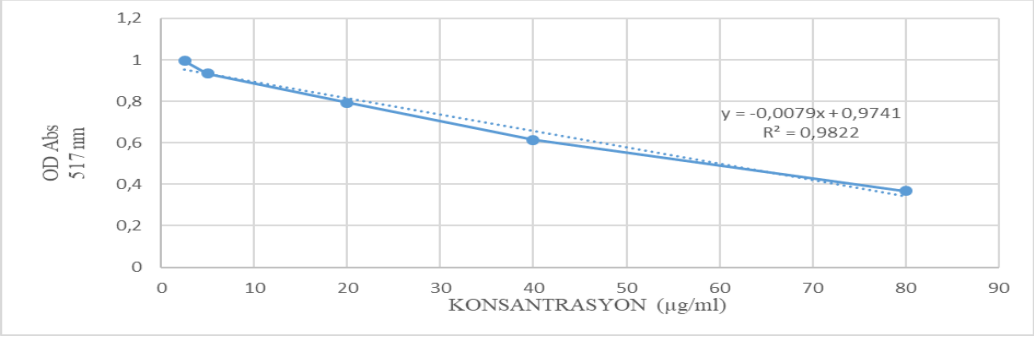

Figure 4: DPPH Standard graphic

Antimicrobial activity; Fungal samples were observed to be effective on all microorganisms in varying proportions. In the antimicrobial activity studies, *S. aureus* and *P. aeruginosa* and *E. coli* were the most susceptible strains for the sample (Table 2).

TABLO 2: Antimicrobial activities of mushroom extracts on microorganisms tested

	Control						***Lactarius deliciosus***					
	Chloroform		**Ethanol**		**Water**		**Chloroform**		**Ethanol**		**Water**	
	5 µl	**10 µl**	**5 µl**	**10 µl**	**5 µl**	**10 µl**	**5 µl**	**10 µl**	**5 µl**	**10 µl**	**5 µl**	**10 µl**
E. coli	ND	ND	ND	10	ND	ND	ND	ND	ND	10.34±1.07	ND	ND
C. albicans	7.25±0.25	9.25±0.55	6.25±0.23	9.75±0.78	ND	ND	7.12±0.34	9.12±0.40	7.12±0.30	9.87±0.60	ND	ND
S. aureus	7.5±0.33	11±0.91	7.25±0.52	8.75±0.23	ND	ND	6.81±0.50	8.56±0.53	7.94±0.53	10.43±0.37	ND	ND
E. faecalis	ND	ND	ND	9.25±0.87	ND	ND	ND	ND	ND	10.41±0.62	ND	ND
P. aeruginosa	6.75±0.61	8.75±0.44	ND	8.75±0.71	ND	ND	6.81±0.18	8.38±0.55	ND	9.69±0.80	ND	ND

DN: not determined zone. Values were expressed as mean ± standard deviation (SD).

Mushroom extracts showed different antimicrobial activity according to the extraction solvent used and the type of microorganism studied. Generally, activity against test microorganisms was observed, whereas the interaction of the extracts with water showed no activity. The antimicrobial effects were not showed on *E. coli* and *E. faecalis* of chloroform extract.

IV. DISCUSSION

Turkey, as well as the medicinal and aromatic plants also has a rich variety of mushrooms. *Lactarius deliciosus* is another fungus that grows in natural environments in our country and cannot be cultured.

In our study, antioxidant and antimicrobial properties of *Lactarius deliciosus* fungi collected from different localities were determined and their medical importance was planned. The studies show that the high phenolic compounds of *L.deliciosus* mushroom makes this mushroom valuable in terms of medicine.

In literature studies, total phenolic content of water-methanol extract was found to be highest in *Lactarius deliciosus* fungus. This is thought to be due to the fact that *Lactarius deliciosus* fungus has water-soluble phenolic compounds as well as lipophilic carotenoids that are well soluble in methanol [24]. The antioxidant properties of fungi, which are another medicinal value, were examined. In the study of Avcı et al. *Coprinus micaceus* (Bull.: Fr.) collected from Çorum (Turkey). fungi of the species have been found to show strong antioxidant and antimicrobial activity of high medical importance [26].

Kalogeropoulos et al. They determined the antioxidant activity by DPPH method to be the most common species of *L. deliciosus* fungus [27]. In our study, we determined that *Lactarius deliciosus* showed high antioxidant activity. There are studies on antimicrobial activity by many researchers in different parts of the world. They found that some naturally growing and commercially important macrofungi micelle cultures exhibit antimicrobial activity against bacteria, yeast and dermatophyte [28]. Ajith et al. found in their study that *Ganoderma lucidium* contains some antioxidant properties [29]. In the same way, Ofodile et al., Another study of the effect of some ganoderma species on microorganisms, mushrooms on a number of bacteria, but the effect of microorganisms that have not been reported [30].

The antimicrobial activity of phenolic extracts of Portuguese wild edible fungus species (*Lactarius deliciosus, Sarcodon imbricatus, and Tricholoma portentosum)* against pathogens is described by Barros et al. (2007). These fungi have been shown to exhibit a higher inhibition against Gram-positive bacteria, whereas Gram-negative bacteria are resistant [31]. The total phenolic content of water-methanol extract was highest in *Lactarius deliciosus* fungus. This is thought to be due to the fact that *Lactarius deliciosus* fungus has water-soluble phenolic compounds as well as lipophilic carotenoids that are well soluble in methanol.

In a study, the methanol extract of *L. deliciosus*, especially was found to be effective on acid-resistant *Mycobacterium* sp and effective on gram-positive bacteria. However, they could not determine an activity on

yeasts [31]. In our study, *L. deliciosus* shows similar results in terms of both antioxidant and antimicrobial efficacy. It has been found as a fungus with high biological activity and of medical importance

V. CONCLUSION

Fungi are of high importance because of their properties such as fat, vitamins, carbohydrates and proteins. The nutritional value they contain may vary depending on the growing environment and collection times. They can also use as support in the treatment of some diseases, not only as nutrients but also in medical terms. In this study, antimicrobial and antioxidant activities of extracts obtained from extracts of *Lactarius deliciosus* fungus with distilled water, ethanol and chloroform. As conclusion, *L. delicous* fungus did not show a very high antimicrobial effect. The antioxidant activity was highly valued in many fungal species. *L. deliciosus* has a moderate antioxidant activity.

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