Total Phenolic Content, Antioxidant and Antimicrobial Properties of *Pleurotus ostreatus* Grown on Lime (*Tilia Tomentosa*) Leaves

Ihlamur (*Tılıa tomentosa*) Yapraklarında Üretiilen *Pleurotus ostreatus* Mantarının Toplam Fenolık Madde Mıktarı, Antioksidan ve Antimikrobiyal Özellikleri

Research Artıcle

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ABSTRACT

In this study, the posibility of using of waste lime leaves in *Pleurotus ostretatus* cultivation was investigated. After a successful harvest, total phenolic content, antioxidant and antimicrobial properties of mushrooms was determined. According to the test results; yield was 15%, biological efficiency was 30%. The total phenolic content was 151.4±0.001 mg GAE/100 g, the antioxidant activity was 2,508±0.056 μmol $FeSO_4.7H_2O$/g. Additionally; methanolic extract of *Pleurotus ostreatus* mushroom showed inhibitory effect against *Klebsiella pneumonia* and *Acinetobacter haemolyticus* bacteria. As a result, *Tilia Tomentesa*'s leaves are suitable raw material with satisfactory medical properties for the *Pleurotus ostretaus* cultivation.

Key Words

Antimicrobial, Antioxidant, Lime leaves, *Pleurotus Ostreatus*.

ÖZET

Sunulan bu çalışmanın amacı, atık ıhlamur yapraklarının *Pleurotus ostreatus* mantarı kültivasyonunda kullanılabilirliğini araştırmaktır. Başarılı bir hasattan sonra, mantarların toplam fenolik madde miktarları, antioksidan ve antimikrobiyal özellikleri belirlenmiştir. Sonuçlara göre, verim %15, biyolojik etkinlik %30, toplam fenol miktarı 1.514±0.001 mg GAE/g, antioksidan aktivitesi 2.508±0.056 μmol $FeSO_4.7H_2O$/g olarak hesaplanmıştır. Ayrıca *Pleurotus ostreatus* mantarı metanolik özütleri *Klebsiella pneumonia* ve *Acinetobacter haemolyticus* bakterilerine karşı inhibitor etki göstermiştir. Sonuç olarak, *Tilia tomentosa* yaprakları tıbbi özelliklere sahip *Pleurotus ostreatus* kültivasyonu için uygun bir hammaddedir.

Anahtar Kelimeler

Antimikrobiyal, antioksidan, ıhlamur yaprakları, *Pleurotus ostreatus*.

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INTRODUCTION

There are some mushroom species which cultivated, especially production and comsuption of *Pleurotus ostretaus* rises day to day [1]. *P. ostreatus* is a prospective source of valuable food protein, and an organism with the ability to utilize various lignocellulosic materials [2,3]. Soybean, sorghum, peanut and wheat straw [4], leaves of hazelnut, leaves of European leaves, waste paper [3], cotton straw, lentil straw,rice bran [5] etc. can be used as substrate for cultivation on *Pleurotus spp*.

Mushrooms [6-8] and plants (fruits, vegetables, medicinal herbs, etc.) [9-14] may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases including cancer, vascular diseases, diabetes, arthritis, and ageing process [15,16]. There are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [17,18] and mushrooms is reported that natural antioxidans [19-22].

In recent years, multiple drug resistance in human pathogenic microorganisms has developed, due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various plants which are good sources of novel antimicrobial chemotherapeutic agents [23,24] Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer [25-27].

The main objectives of this study were: (i) to investigate the posibility of using of waste lime leaves in cultivation of *Pleurotus ostretatus* and calculate yield and biological efficiency, (ii) to determine total phenolic content by Folin-Ciocalteu reagent and antioxidant properties by FRAP essay and (iii) to investigate the antimicrobial activity of methanolic extract of cultivated *P. ostreatus* by agar well diffusion method against some of bacteria and one yeast.

MATERIALS and METHODS

Materials

P. ostreatus spawn was obtained from Agroma mycel, Denizli. *Tilia tomentasa* waste leaves were collected from Karadeniz Technical University Campus. Beech sawdust (control sample) were obtained from workshop of Forest Industry Engineering, Karadeniz Technical University.

Substrate Preparation

Tilia tomentasa's leaves and beech sawdust moistened with water until 70-80% and autoclaved 121°C for 1.5 h. After cooling the substrates to 20°C, they were placed in nylon bags of 1 kg (40x60cm) and inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 3% of the wet weight of compost. Subsrate condition was carried out in four replications. Each nylon bags were inoculated in mushroom growing laboratuary (10-15°C, 70-80% relative humidity). After 13 days, beech sawdust (control subsrate) and after 19 days lime leaves were completely colonized by the mycelium. Harvesting was done in fifth week and the fruit bodies's stipe and cap was calculated and weighed.

Yield and Biological Efficiency

Mushroom yield (g) was calculated by division of fresh weight of fruit bodies obtained from each one bag to dry weight of 1-kg compost [28]. Biological efficiencies was defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrateas [29].

Total Phenolic Content and Antioxidant Activity

Sample Preparing

P. ostreatus divided into five samples and dried 40°C before analysis. 4 g dried sample was extracted with 40 mL methanol and shaked 150 rpm for 24 h then filtered through Whatman No. 4 filter paper. Extracts were stored at 4°C for further use.

Total Phenolic Content

Total phenolic contents of the methanolic extracts were determined by Folin–Ciocalteu method using gallic acid standard [30]. The Folin assay was also based all phenolic contents including phenolic acids, flavonoids, and anthocyanins in the aquatic solution gives a blue colour complex whose maximum absorbance can be read at 760 nm. 680 μL distilled water and 20 μL methanolic extract and 400 μL of 0.5 N Folin-Ciocalteu regents were mixed with in a tube, vortexed for 2 min, then 400 μL Na_2CO_3 (10%) was added and incubated for 2 hour. Following the incubation at room temperature, absorbance of the mixtures were measured at 760 nm on an ATI-Unicam UV-2 UV-VIS spectrophotometer (Cambridge, U.K.). The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents per g of dry weight.

Determination of Antioxidant Activity

Antioxidant activity of all mushrooms was determined using the following one method.

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay [31,32] with some modifications. Working FRAP reagent was prepared as required by mixing 300 mM acetate buffer, pH 3.6 with 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. 3 mL freshly prepared FRAP reagent and 100 μL of the samples were mixed and incubated in 4 min at 37°C and the absorbance was read at 593 nm against reagent blank containing distilled water. $FeSO_4.7H_2O$ was used a positive control. The ferric-reducing antioxidant power of the antioxidants in the extracts was calculated by comparison with $FeSO_4.7H_2O$ as μmol $FeSO_4.7H_2O$/ g dry weight of mushrooms.

Antimicrobial Activity

P. ostreatus extracts were tested for antimicrobial activity by agar-well diffusion method in accordance with the Clinical & Laboratory Standards Institute (CLSI). Tested microorganisms were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumoniae* ATCC 13883, *Salmonella Typhimirium* ATCC 14028 and *Candida albicans* ATCC 10231. Microorganisms were obtained from Karadeniz Technical University, Department of Medical Microbiology, Faculty of Medicine Trabzon, Turkey.

Microbial Suspension and Test Technique

Colonies of bacteria were mixed with 5 mL of sterile isotonic sodium chloride solution for moderate opalescence. Microbial suspension turbidity was adjusted to a density equivalent to a 0.5 McFarland standard. The microbial suspension was distributed on Mueller Hinton agar in three different directions using sterile cotton swabs, then extarct, positive and negative (pure methanol) control 100 μl was filled into the wells of agar plates directly. The petri dishes were leaved for diffusion for 15 min then incubated at 37°C for 24 hours. Activity was determined by visual inspection and measurement of the diameter of clear inhibition zones around the agar-well.

Statistical Analysis

Total phenolic content and antioxidant analyses were performed in triplicates. The data were recorded as means ∔ standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 16.0)

RESULTS and DISCUSSION

Yield and Biological Efficiency

After the harvest, mushrooms morphological properties were calculated. Diameter of fruit bodies and stipe, length of stipe are given Table 1.

Table 1. Morphological properties (cm) of mushrooms.

Mushroom substrate	Diameter of fruit bodies	Diameter of stipe	Length of stipe
Lime leaves	5±2.3	0.30±0.1	0.95±0.3
Beech sawdust	5.2±1.2	0.30±0.1	0.9±0.25

Avarege diameter of mushrooms cap was 5.0 cm, diameter of stipe was 0.30 cm, length of stipe was 0.95 cm. The results were founded similar with other studies [36,37].

Yield and biological efficiency was calculated as 15%, and 30% for *T. tomentosa* leaves, and for beech sawdust as 28% and 77%, respectively and presented in Table 2.

Table 2. Yield (%) and biological efficiency (%) of subsrates.

Substrate	Yield	B. E.
Lime leaves	15	30
Beech sawdust	28	77

In the literature; yield of *P. ostreatus* was reported between 15% [32], 4.63-45.4% [33] and biological efficiency was reported between 0-61% [34], 48.9- 90.5% [35] on different compost.

Total Phenolic Content and Antioxidant Properties

The Folin-Ciocalteu assay has been used for many years to measure of total phenolic contents in natural products, and the basic mechanism is an oxidation/reduction reaction [38,39].

Total polyphenols were the major naturally occurring antioxidant components found in the methanolic extracts from wild edible mushrooms [40]. It is reported that phenolic compounds are capable of natural antioxidants. Phenolic compounds, stopping or preventing the free radical reactions preventing to the arise of many diseases such as cancer, heart disease and lung diseases [41]. Total phenolic content and antioxidant (FRAP) values of mushroom are given in Figure 1.

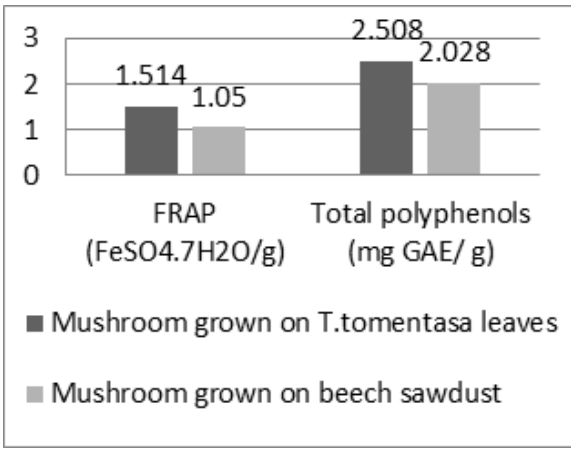

Figure 1. Total phenolic content and antioxidant (FRAP) values of mushroom.

In the prensent study, total phenolic content was determined that 1.514±0.001 mg GAE/g for *P. ostreatus* grown on *Tilia tomentosa* leaves and 1.050±0.013 mg GAE/g for *P. ostreatus* grown on beech sawdust. It can be conluded that substrate can effect total phenolic content of mushrooms. When the antioxidant activity values of the mushrooms determined by the FRAP method where compared with other vegatables, it was observed that mushroom rown on *Tilia tomentosa* leaves presented higher antioxidant activity than those reported for chicory (1.0912 mg GAE/g), *Lepidium sativum* (1.2614 mg GAE/g) [42] and other mushroom's content was reported for *Pleurotus eryngii* (0.634 ±0.004 mg GAE/g), *Cyttaria gunnii* (0.761 ± 0.004 mg GAE/g) [43].

In this study, antioxidant activity was measured by the FRAP method, which measures the capacity of an antioxidant to reduce a Fe^{3+} -TPTZ complex to Fe^{2+} -TPTZ. In this way, a higher Fe^{3+} -TPTZ reduction means a higher antioxidant activity. FRAP assay was used to determine antioxidant activity, as the technique is simple and quick. Higher FRAP values indicate higher antioxidant capacity, because FRAP values are based on reducing ferric ions, where antioxidants are the reducing agent. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [44]. Frap activity of mushrooms grown

on *Tilia tomentosa* leaves and beech sawdust was found as 2.508±0.056 and 2.028 0.009 μmol $FeSO_4.7H_2O$/g, respectively. *P. ostreatus* grown on *Tilia tomentosa* leaves value is higher than the other reported value of *Pleurotus ostreatus* 2.385 μmol/g [45]. Previous studies indicate that concentration, extraction methods and type of solvent affect antioxidant activity [46,47].

Antimicrobial Activity

The tested fungi inhibit two gram-negative bacteria *Klebsiella pneumoniae* and *Acinetobacter haemolyticus*. Compared to other studies, *P. ostreatus* was able to inhibit *E.coli*, *B. cerreus*, *Listeria innocua* [47] and other gram positive or negative bacteria and fungi isolates [48]. Other mushrooms such as *Morchella conica* [49], Ramaria flava [50], *Lycoperdon pusilum* ve *L. Giganteum* [51] have also been reported to have antimicrobial activity against many microbes.

Based on the results of this study, it can be concluded that (i) waste lime leaves can be as substrat for *Pleurotus ostreatus* mushroom cultivation. Additionally; the leaves are also suitable raw material with satisfactory medical properties. *P. ostreatus* growing on linden leaves, (ii) have antioxidant potentials and (iii) antimicrobial activity. Further investigations would try different solvents, concantrations, other microorganisms and methods for assays.

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