Investigation of Antioxidant Activities of *Pleurotus ostreatus* Grown in Different Composts*

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ABSTRACT

In many countries of the world, the use of organic waste such as tea leaves and wood chips in the cultivation of *P. ostreatus* prevents both these materials from being wasted and contributes to the provided income. In this study, the availability of infused tea leaf in the production of *Pleurotus ostreatus* was investigated. Six different experimental groups and 1 control group were prepared by using "cotton", "siyez", "bran", "infused tea leaf", "poplar sawdust": 1st Group (50% cotton + 5% siyez + 5% bran + 30 %infused tea leaves + 10% poplar sawdust), 2nd Group (50% cotton + 5% siyez + 5% bran + 40%infused tea leaves), 3rd Group (50% cotton + 5% siyez + 5% bran + 10% infused tea leaves + 30%poplar sawdust), 4th Group (50% cotton + 5% siyez + 5% bran + 20% infused tea leaves + 20% poplar sawdust), 5th Group (100% infused tea leaves), 6th Group (90% infused tea leaves + 5% siyez + 5% bran) and Control (7th) Group (50% cotton + 5% siyez + 5% bran + 40% poplar sawdust). In this study, DPPH (1, 1 diphenyl-2-picryl hydrazyl) radical scavenging method, which is widely used for the determination of antioxidant activity of *Pleurotus ostreatus* grown in different compost media, was used. Antioxidant activity of mushroom extracts were expressed as percentage of DPPH radicals inhibition and IC_{50} values (mg/ml). Percentage of inhibition ranged from 16.54 to 91.34 % and IC_{50} values ranged from 0.592 to 1.133 mg/mL for mushroom samples in different composts. The total phenolic content ranged from 84.79 to 169.58 mg/g for mushroom extracts. The content of phenols in methanolic extracts expressed in gallic acid equivalents (GAE) varied between 84.79 ±1.15 and 169.58 ±2.10 mg/g. It was also observed that the antioxidant activity of the specimens grown especially in tea compost (100%) was highest. According to the IC_{50} values, the order of antioxidant activity was determined as 5th Group> 3rd group> 2nd Group> 6th Group> 1st Group> 4th Group> 7th Group from large to small.

Keywords: **Antioxidant activity, *Pleurotus ostreatus*, Different composts, Tea compost, Phenolic substances**

I. INTRODUCTION

The utilization of mushroom as an alternative food contributed to food supply in the market, which has been affected due to the urbanization and population growth prevalent nowadays. *Pleurotus ostreatus* is one of the cultivated mushrooms that has been produced at the commercial level in many countries. This study utilized the waste materials from organic waste from tea leaves and wood chips in the cultivation of *P. ostreatus*. Tea, one of the most popular beverages in the world, is gaining expanding and increasing consumer markets at present in various product forms. However, after extracting water-soluble components from tea leaves, a massive amount of tea waste is left, which results in solid waste problems. Utilization of these materials for mushroom production prevents both these materials from being wasted and, as importantly, contributes to the income.

In recent years, the cultivation of oyster mushrooms has become widespread in the world. However, choosing an efficient and eco-friendly substrate material to reduce production costs has been an important issue in mushroom cultivation. In most cases, cotton waste has been a popular substrate; however, with the increased price of cotton, its utilization for mushroom production has declined. Alternatively, tea waste is a good substrate for mushroom production, which is even higher than cotton in terms of reusable nutrients. Because the wastes from tea production are treated as waste material, the cost of acquisition of material is low, hence, it is a good alternative material that can be utilized, wholly or partially, as a mushroom substrate.

In this study, the availability of infused tea leaf in the production of *Pleurotus ostreatus* was investigated.

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II. METHODS AND MATERIALS

All chemicals were acquired from analytical purity Sigma-Aldrich Co. LLC. Company. Deionized purity water was used at each stage. Absorbance was measured using a pair of 1cm thick quartz tubes, at 517 nm with SHIMADZU UV mini-1240 UV-Visible spectrophotometer (Schimadzu Corp., Kyoto, Japan manufactures).

Preparation of Mushroom Compost

In this study, the availability of infused tea leaf in the production of *Pleurotus ostreatus* was investigated. Six (6) different experimental groups and one (1) control group were prepared by using "cotton", "siyez", "bran", "infused tea leaf", "poplar sawdust" as shown in **Table 1.**

Table 1. Compost contents used in experiment

	Compost Content				
	Cotton %	Siyez %	Bran %	Infused Tea Leaves %	Poplar Sawdust %
1st Group	50	5	5	30	10
2nd Group	50	5	5	40	-
3rd Group	50	5	5	10	30
4th Group	50	5	5	20	20
5th Group	-	-	-	100	-
6th Group	-	5	5	90	-
7th Group	50	5	5	-	40

Preparation of Mushroom Extracts

The mushrooms to be extracted were pulverized and weighed 5.0 g. Then the extract or mixture was dissolved in 30 mL, 80% methanol solution. After standing at room temperature for 3 hours, the mixture was filtered. The solid materials were then redissolved in 20 mL of an 80% methanol solution. After standing for 2 hours, the mixture was once again filtered. The resulting 50 mL homogenate was centrifuged at 5000 rpm for 10 minutes (at 4°C). [1, 2]

Preparation of DPPH Calibration Solutions

About 0.01824 g ($C_{18}H_{12}N_5O_6$) was taken, dissolved in 100 mL of methanol (4.625x10-5 M). Then, it was diluted in fine concentrations for the calibration graph from this solution, in which the 1.542x10-5 M was used as the control group.

Sample (Mushroom Extract + Methanol + DPPH) System Solution Preparation

About 2 mL (Stock 4.625x10-5 M DPPH) + X mL Mushroom Extract + (3-X) mL methanol and a total volume = 6 mL reaction mixture were used in this study.

DPPH Measurements

DPPH calibration solutions were prepared with methanol and incubated for 15 minutes at room temperature and in the dark, after which they absorbed the absorbance at 517 nm against the blanket formed from methanol. In the same way, a methanol-DPPH solution was prepared for control, and was used as a standard for sample studies. The reduced absorbance values of the samples prepared with mushroom extracts gave the remaining amount of DPPH solution, hence, free radical scavenging activity. The radical scavenging activity of the different concentrations of mushroom extracts was calculated by the following formula and expressed as percent inhibition [3, 4].

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Here A_0 is the control absorbance and absorbance in the presence of A_1 samples. [3].

Determination of Total Phenolics

The total phenolic component of the methanol extracts was determined using Folin-Ciocalteu reagent and gallic acid as standard. And then, about 4.5 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent were added. After 3 minutes, a solution of 0.3 ml of Na_2CO_3 (2%) and 0.1 extract solution were added and shaken vigorously. After 2 hours of waiting, the absorbance was measured at 760 nm [5, 6, 7].

The concentrations of the phenolic compounds were calculated using the following equation obtained from the standard gallic acid graph:

Absorption = 0.401 Gallic Acid (µg) + 0.144 , R_2 = 0.961

Statistical Analysis

Calculation was performed using Microcal Origin Pro 8.5.1 (Origin Lab. Corp., Northampton, MA, USA) for descriptive statistical analysis.

III. RESULTS AND DISCUSSION

In this study, antioxidant activity and total phenolic substance determination of *Pleurotus ostreatus* grown in different compost environments were made in order to optimize compost conditions in mushroom cultivation. It was observed that the highest inhibition percentage at the concentration of 1.33 mg/mL was the composted mushrooms (3rd Group [50% cotton + 5% chrysanthemum + 5% bran + 10% infused tea leaves + 30% poplar sawdust]), the lowest inhibition percentage Control (7th) Group (% 50 cotton + % 5 siyez + 5% bran +% 40 poplar sawdust) (**Figure 1**).

Amount of total phenolic content was found to range from high to low; group 4>3>6>1>2>5>7. These results showed that the total amount of phenolic compounds of mushroom species was not a sufficient parameter to change the antioxidant properties (**Table 2**). Changes in the amount of phenolic substances in the fungus is not the same as the order of antioxidant activity. For this reason, the phenolic values should not be solely taken into account, and suitable mixtures should be created for optimum conditions.

Moreover, some samples showed differences between the presence of total phenolics and their antioxidant capacities in the mushroom samples obtained. This may be related to the solubility of some phenolic substances in the examined samples [8, 9]. Therefore, it will be important to improve the solubility of phenolic acids in compost to enhance the usefulness of phenolics as antioxidant components.

Table 2. IC_{50} and total phenolic values of *Pleurotus ostreatus* grown in different types of compost

Compost Types	IC_{50} (mg/mL)	Total phenolics (mgGA/g)
1. 50% cotton + 5% siyez + 5% bran + 30% infused tea leaves + 10% poplar sawdust	0,957	134,66
2. 50% cotton + 5% siyez + 5% bran + 40% infused tea leaves	0,616	112,22
3. 50% cotton + 5% siyez + 5% bran + 10% infused tea leaves + 30% poplar sawdust	0,601	167,08
4. 50% cotton + 5% siyez + 5% bran + 20% infused tea leaves + 20% poplar sawdust	0,965	169,58
5. 100% infused tea leaves	0,592	92,26
6. 90% infused tea leaves + 5% siyez + 5% bran	0,806	152,12
7. (**Control**) 50% cotton + 5% siyez + 5% bran + 40% poplar sawdust	1,133	84,79

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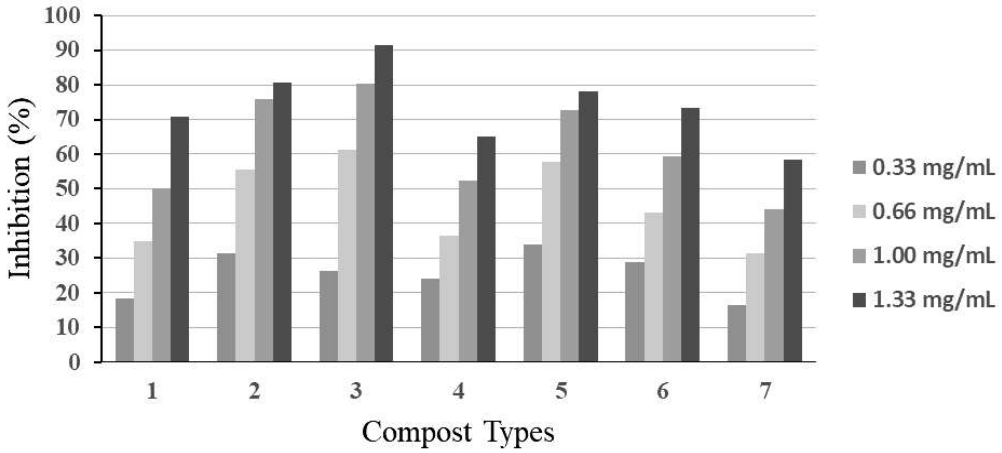

Figure 1. Inhibition changes of Pleurotus ostreatus grown in different types of compost were 0.33; 0.66; 1.00; % Inhibition changes at 1.33 mg / mL

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