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LIQUID CULTURE AND ANTIOXIDANT PROPERTIES OF *Ganoderma lucidum* AND *Pleurotus djamor*

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ABSTRACT

Mycelial biomass production of mushrooms is now of considerable interest in order to elucidate their chemical compositions and biological activities. This present work demonstrated the production of mycelial biomass of *Pleurotus djamor* and *Ganoderma lucidum* using different indigenous liquid culture media. Corn grit produced the highest mycelial biomass and volume loss of culture spent of both *P. djamor* (6.12 g and 12.07 ml) and *G. lucidum* (9.10 g and 18.00 ml), respectively. Mycelia of *P. djamor* grown in corn grit recorded the highest radical scavenging activity of 73.29% and total phenolic content of 267.28 mg AAE/g sample. However, *G. lucidum* mycelia grown in coconut water showed the highest radical scavenging activity (77.33%) whereas mycelia in corn grit broth had the highest total phenolic content (402.75 mg AAE/ g sample). Collectively, the production of mycelial biomass and antioxidant activities of *P. djamor* and *G. lucidum* are dependent on the liquid culture media.

Keywords: Anti oxidant properties, *Pleurotus djamor* and *Ganoderma lucidum*

INTRODUCTION

Mushrooms are nutritious and functional food in which they are of considerable interest because of their organoleptic merit, medicinal properties, and economic

significance. In Central Luzon Philippines, fruiting bodies of mushrooms are cultivated in either small or large scale using the developed production technologies utilizing various agro-industrial materials and cellulosic substrates. Some of the commercially cultivated mushrooms are *Pleurotus florida, Pleurotus ostreatus, Pleurotus sajor-caju, Volvariella volvacea,* and *Ganoderma lucidum*. However, submerged cultivation of these mushrooms is less intensively practiced in the region. This cultivation technique is more efficient, fast and could produce large amount of mushroom biomass. Dulay et al. **[1]** reported the successful optimization of liquid culture conditions for production of mycelial biomass and lipid composition of the four Philippine local strains of wild edible mushrooms. This has upgraded the cultivation technology of mushroom biomass as source of bioactives. One of the most important bioactive components of mushrooms is antioxidant. Antioxidants protect the human system against oxidative damage caused by free radicals that may be related to degenerative process of aging and physiologic diseases such as diabetes, cancer, atherosclerosis, and cirrhosis **[2]**. Mushrooms such as *Hericium erinaceus, Lentinula edodes* and *Agrocybe aegerita* extracts showed antioxidant properties **[3]**.

G. lucidum (Ganodermataceae) is a red varnished, soft (when fresh), corky, and flat basidiomycetous mushroom that commonly found growing on trunk of trees and fallen logs. It is considered wonder mushroom due to its numerous biological activities. On the other hand, *Pleurotus djamor* is an exotic species of mushrooms that have been adapted in the tropical condition of the Philippines and cultivated in rice straw-sawdust based substrate formulation. This mushroom plays a vital role in biological activities such as antiviral, antitumor and immunosuppressive activities **[4]**. The present work demonstrated the submerged cultivation of *P. djamor* and *G. lucidum* and established their antioxidant properties as influence by the different liquid culture media.

MATERIALS AND METHOD

Source of Cultures

The cultures of *P. djamor* and *G. lucidum* were obtained from the culture collection of the Center for Tropical Mushroom Research and Development (CTMRD), Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Agar blocks from the cultures were aseptically inoculated onto potato

dextrose agar (PDA) plates. These were incubated for 7 days at 30°C. After incubation, mycelial discs were prepared using a flame sterile 10 mm-diameter cork borer. Mycelial discs were used as inoculant in mycelial growth evaluation.

Evaluation of Mycelial Growth Performance

The effect of different broth culture media namely; coconut water from mature coconut (*Cocos nucifera*), rice bran D1 (class A) broth (50g of *Oryza sativa*/L of water), local yellow corn grit broth (50g of *Zea mays*/L of water) and potato sucrose broth (250g of *Solanum tuberosum*/L of water + 10g of white table sugar) on mycelia growth of the two mushrooms in liquid culture condition was evaluated. Broth media (50 ml) were dispensed in glass bottles, sterilized in an autoclave at 121°C, 15 psi for 30 min. After which, mycelial discs were aseptically inoculated into sterilized bottled liquid media and incubated at 30°C for 15 days to allow fungal growth. Experiments were carried out in triplicate. The fresh weight of the mycelia and volume loss of spent were determined and data were presented as the mean of three replicates. The mycelia were mass produced for analysis and assays.

DPPH Radical Scavenging Activity

Ethyl acetate (10 ml) was added into each sample of mycelia to extract the antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol. The free radical scavenging activity of the samples was estimated using the stable 2,2'-diphenyl11picrylhydrazyl (DPPH) radical following the standard method of Shimada et al. **[5]** with modifications. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated. Triplicate test was done per sample.

Estimation of Total Phenolic

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton **[6]** with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed

as mg of ascorbic acid equivalents (AAEs) per gram of sample. Triplicate test was done per sample.

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Mycelial Biomass Production

Submerged culture is fast and easy process of producing mushroom mycelial biomass in a sterile culture glass container with a liquid medium. The nutritional composition of the media is a very important factor in this cultivation process. Thus, this present work investigated the mycelial biomass production of *P. djamor* and *G. lucidum* in four locally indigenous liquid culture media, namely; rice bran broth, corn grit broth, potato broth and coconut water. Table 1 shows the mean weight of mycelial biomass and volume loss of culture spent of both mushrooms in different liquid media. Apparently, the highest yield of mycelial biomass and volume loss of spent of *P. djamor* was significantly recorded in corn grit broth (6.12 g and 12.07 ml), followed by potato broth with 4.95 g and 8.75 ml, respectively. Coconut water had the lowest mycelial biomass yield and volume loss. On the other hand, *G. lucidum* showed the highest biomass yield of 9.10 g and volume loss of 18.00 ml in corn grit broth. This was followed by potato broth and rice bran broth had the lowest biomass yield and volume loss. It can be observed that yield of mycelial biomass is directly proportional to the volume loss of the culture spent. These results indicate that corn girt broth favors the luxuriant growth of mycelia of both mushrooms. In our previous works, both mycelia of *Lentinus tigrinus* and *Lentinus sajor-caju* efficiently grew on rice bran broth which significantly recorded the highest yield of biomass and volume loss **[7]**. However, the maximum mycelial biomass of *V. volvacea* was significantly achieved in coconut water while *Schizophyllum commune* efficiently grew on rice bran broth **[8]**. Moreover, the maximum mycelial yield of *Panaeolus cyanescens* was achieved in coconut water and rice bran broth **[9]** while potato broth produced the most luxuriant mycelial growth of *Panaeolus antillarium* **[10]**. These findings and the results of the present study demonstrated that the efficient mycelial production of these basidiomycetes depend on the liquid medium used in submerged cultivation.

Antioxidant Properties of Mushrooms

Antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions **[11]**. They play major role in promoting healthful benefits. They reduce the risk for chronic diseases such as cancer, cardiovascular and neurodegenerative diseases **[12]** and physiologic diseases such as diabetes, cancer, atherosclerosis, and cirrhosis **[13]**. In this work, the antioxidant activity of the mycelial biomass of *P. djamor* and *G. lucidum* grown in liquid media was investigated. The DPPH radical scavenging assay was used to determine the antioxidant properties of mycelia of both mushrooms. The percentage radical scavenging activity of the two mushrooms is shown in Table 2. Among the mushroom samples, *P. djamor* mycelia harvested from corn grit broth had the highest scavenging activity of 73.29%. This was followed by mycelia grown in rice bran broth and potato broth. However, *G. lucidum* mycelia from coconut water registered the highest radical scavenging activity of 77.33% among the different media, followed by corn grit broth and potato broth. The lowest activity was noted in rice bran broth with 58.16%. These results indicate that the antioxidant activities of the two mushrooms particularly on their radical scavenging activity are dependent on the types of media they grown. According to Martin and Demain **[14]**, liquid culture provides a more homogenous environment than a solid medium since nutrients or active metabolites are available via diffusion.

Table 1: Mycelial biomass and volume loss of spent of *P. djamor* and *G. lucidum* grown on the different liquid culture media after 15 days of incubation

Liquid Media	*P. djamor*		*G. lucidum*	
	Mycelial biomass (g)	Volume loss of spent (ml)	Mycelial biomass (g)	Volume loss of spent (ml)
Rice bran broth	2.91^{c}	8.34^{b}	2.23^{c}	8.33^{b}
Corn grit broth	6.12^{a}	12.07^{a}	9.10^{a}	18.00^{a}
Potato broth	4.95^{b}	8.75^{b}	6.37^{b}	14.00^{b}
Coconut water	2.78^{c}	7.03^{c}	7.40^{b}	12.33^{c}

In each column, means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.

Table 2. Radical scavenging activity and total phenolic content of *P. djamor* and *G. lucidum* grown on the different liquid culture media.

Liquid Culture Media	*P. djamor*		*G. lucidum*	
	Radical scavenging activity (%)	Total phenolic (mg AAE / g)	Radical scavenging activity (%)	Total phenolic (mg AAE / g)
Rice bran broth	61.76^{c}	259.12^{c}	58.16^{d}	347.07^{c}
Corn grit broth	73.29^{b}	267.28^{a}	71.92^{c}	402.75^{a}
Potato broth	56.12^{d}	263.57^{b}	60.91^{d}	371.45^{b}
Coconut water	52.37^{e}	258.91^{c}	77.23^{b}	398.52^{a}
Cathechin	98.53^{a}	-	98.73^{a}	-

In each column, means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.

The radical scavenging activity of the mushroom species is attributed to the presence of mycochemicals, specifically the phenolic compounds such as alkaloids, flavonoids, and glycosides **[15-17]**. Therefore, this study also analyzed the total phenolic content of the mushroom mycelia grown on the different media (Table 2). In *P. djamor*, the highest phenolic content was also observed in mycelia grown in corn grit broth (267.28 mg AAE/g sample), followed by potato broth (263.57 mg AAE/g sample) and rice bran broth (259.12 mg AAE/g sample). On the other hand, *G. lucidum* mycelia grown in corn grit broth had the highest total phenolic content of 402.75 mg AAE/ g sample. This was followed by coconut water mycelia that showed the highest radical scavenging activity. It can be seen that the radical scavenging activity of *P. djamor* mycelia can be correlated with their corresponding total phenolic content but not in *G. lucidum* mycelia. Therefore, mycelia of the two mushrooms could have promising natural antioxidants. In the study of Jung et al. **[18]**, the antioxidant substances found in the cultured broths of the medicinal fungi *Inonotus xeranticus* and *Phellinus linteus* were identified as hispidin and its dimers, 3,14'-bihispidinyl, hypholomine B, and 1,1-distyrylpyrylethan, which are polyphenols. Moreover, total phenolic contents of *Lentinula edodes* and *V. volvacea* extracts have positive correlation to their antioxidant activities **[19]**.

CONCLUSION

Based on the results of the study, it can be concluded that the mycelial biomass productions and the antioxidant properties of *P. djamor* and *G. lucidum* are dependent on the type of liquid media.

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