



## Mycelial Biomass Production and Antioxidant Activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in Indigenous Liquid Culture

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### Abstract

With the increasing demand of functional foods with antioxidant properties, it is necessary to establish new sources such as wild edible mushrooms which could provide beneficial effects to human health. Herein, we evaluated the different indigenous liquid culture media for biomass production and elucidated the free radical scavenging activity and total phenolics of *Lentinus tigrinus* and *Lentinus sajor-caju*. Results revealed that *L. tigrinus* efficiently grew on rice bran decoction which significantly recorded the highest yield of mycelia (11.53 g), volume loss of the medium (24.33 ml), radical scavenging activity (18.94%) and total phenolics (26.59 mg AAE/g sample). Similarly, rice bran decoction significantly registered the highest yield of mycelia (9.75 g), volume loss of the medium (20.95 ml), scavenging activity (16.94%) and total phenolics (25.60 mg AAE/g sample) for *L. sajor-caju*. Both species also showed considerable antioxidant properties when cultured in coconut water, corn grit decoction and potato broth. Hence, it is noteworthy that both studied *Lentinus* species hold promising antioxidants which are influenced by different liquid media.

**Key words** – Mycelial biomass – *Lentinus* – total phenolics – liquid culture – white rot fungi.

### Introduction

Mushrooms have long been considered as effective traditional medicines and now they are being recognized as functional foods because of their several bioactive potentials. Mushrooms have been shown to exhibit hypoglycaemic, hypocholesterolemic, hypotensive, antitumour, antiviral, antibiotic, and anti-inflammatory activities (Hobbs 2003, Ooio & Liu 1999). These properties are due to the variety of potent secondary metabolites including phenolic compounds, polysaccharides, terpenes, polypeptides, steroids and others. Polysaccharide and triterpenes isolated from *Ganoderma lucidum*, *Pleurotus sajor-caju* and *Lactica sativa* are potent compounds against cancer (Kao *et al.* 2013, Moharib *et al.* 2014). Phenolics from mushrooms have been found to be an excellent antioxidant and synergist (Li *et al.* 2005) and have been associated with the inhibition of atherosclerosis and cancer (Martínez-Valverde *et al.* 2000). With these important attributes, mushrooms have become an attractive source for the development of nutraceuticals and drugs.

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Mushroom basidiocarp are commonly collected from the wild or cultivated on the different solid compost or lignocellulosic residues such as rice straw, sawdust, sugarcane bagasse, dried banana leaves and among others. This method of cultivation, however, is a lengthy and complex process. Thus, this present work introduces the growing of mycelia in liquid culture on the different indigenous source of nutrient medium as simple and fast alternative method of biomass production for bioactivity evaluation. Several studies have demonstrated the utilization of submerged culture of mushroom mycelia for various pharmacological properties. For instance, the antioxidant activity of submerged culture of mycelia of *Panaeolus anitllarium* (Dulay *et al.* 2015) and *Agaricus brasiliensis* (Carvajal *et al.* 2012), the antidiabetic and hypolipidemic effect of *Collybia confluens* mycelia produced by submerged culture in streptozotocin-diabetic rats (Yang *et al.* 2006), the antitumor activity of water-soluble intracellular polysaccharides extracted from cultured mycelia of *Phellinus igniarius* (Li *et al.* 2015).

Antioxidants are protective agents 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 (Halliwell & Gutteridge 1999). Bioactive compounds from many natural products with antioxidant activity have been elucidated. These include tocopherol, ascorbic acid, carotenoids, and phenolics (Carvajal *et al.* 2012). Indeed, continues search for new bioactive natural products with antioxidant activity is of great interest by many researchers. Plants and fungi are huge group of organisms with diverse structure and chemical composition that can be tapped for this purpose.

*Lentinus tigrinus* and *Lentinus sajor-caju* are edible wood rotting basidiomycetes that have been successfully optimized on the different culture media and cultivated on the rice straw-sawdust based formulation (Dulay *et al.* 2012, Santos *et al.* 2012). Previously, our team studied the nutritional attributes and functional activities of *L. tigrinus*. The pileus of *L. tigrinus* contained higher amounts of protein (25.9%), fat (2.1%), ash (7.4%) and energetic value (142.1 kcal/100 g) than the corresponding stipe, while its stipe contained higher amounts of total carbohydrates (67.7%), which consists of dietary fiber (63.0%) and reducing sugar (4.7%), than pileus. Aside from these valuable nutrients, 100 mg/kg and 250 mg/kg dosages of lyophilized hot water extract of the fruiting body exhibited anti-diabetic activity in diabetic mice whereas the ethanolic extract of fruiting body and the immobilized secondary mycelia showed high antibacterial activities against *Staphylococcus aureus* (Dulay *et al.*, 2014). In the present work, we investigated the mycelial biomass production of these two species using indigenous liquid culture broth intended for the evaluation of their radical scavenging activity and elucidation of total phenolics.

## Methods

### Culture and inoculant

Pure cultures of *L. tigrinus* and *L. sajor-caju* from the culture collections of the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines were aseptically revived into sterilized potato dextrose agar (PDA) plates and incubated at 30 °C for 7 days. Mycelial discs were prepared using flame sterile cork borer (10 mm diameter) and served as inoculant.

### Mycelial production in different culture broth

The broths of different indigenous nutrient resource were used in the evaluation of mycelial biomass production of the two *Lentinus* species. These include coconut water from mature coconut (*Cocos nucifera*), rice bran decoction (50g of *Oryza sativa*/L of water), local yellow corn grit decoction (50g of *Zea mays*/L of water) and potato sucrose broth (250g of *Solanum tuberosum*/L of water+10g of white table sugar). Culture broth (100 ml) were dispensed into microwavable plastic container and sterilized in an autoclave at 121C, 15 psi for 30 minutes. There were five replicates per treatment. After cooling, each culture container was inoculated with mycelial disc and

incubated at 32°C for 10 days to allow fungal growth. The mycelial mats were harvested and weighed and the volume loss of culture spent was measured.

### **Extraction of Antioxidant Compounds**

Culture spent and mycelia were homogenized using a food processor and then ethyl acetate (10 ml) was added into each cultured broth to extract antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol for antioxidant analysis.

### **Radical scavenging activity assay**

The free radical scavenging activity of the samples was estimated using the stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical following the standard method of Shimada *et al.* (1992) with minor 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 microtiter 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.

### **Analysis of total phenolics**

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard & Singleton (1977) 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.

### **Statistical Analysis**

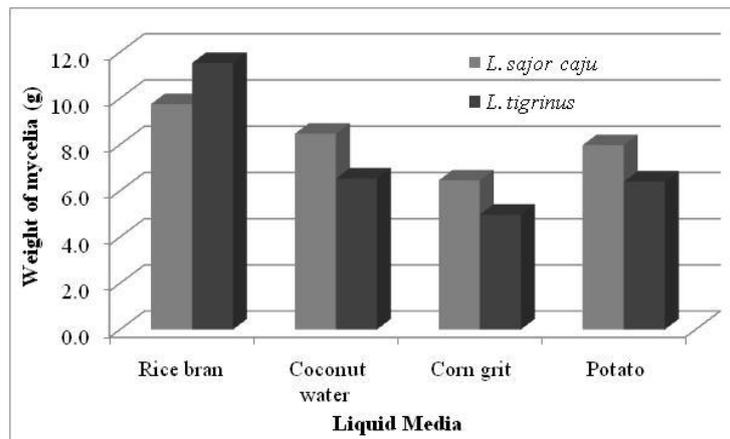
Data were analyzed using Analysis of Variance (ANOVA) in SAS Statistical Program. Means were compared using Least Significant Difference (LSD) at 5% level of significance.

## **Results and Discussion**

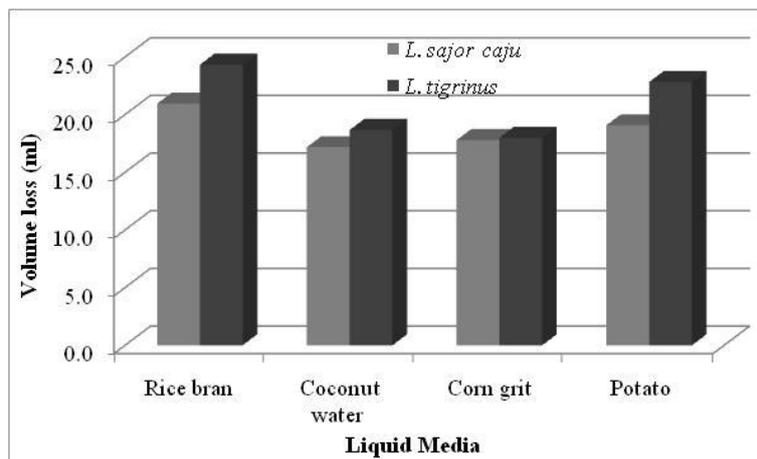
### **Mycelial biomass production**

Evaluation of the culture conditions is an important technique in order to establish mushroom biomass production technology for pharmacological purposes. One of the most important factors in evaluating culture condition is the nutrient source or medium being used. The effect of the different culture broth on the mycelial biomass production the two *Lentinus* species was determined in this present work and the results are shown Figure 1. It can be seen that in both evaluated species the maximum mycelial biomass was significantly achieved in a rice bran broth having means of 9.8 g (for *L. sajor-caju*) and 11.5 g (for *L. tigrinus*). The superiority of rice bran broth could be attributed to the variety of chemical components of rice bran that support the efficient mycelial growth. Rice bran is a valuable source of carbohydrates (49.69 g), dietary fiber (21 g), sugar (0.9 g), sucrose (0.5 g), glucose (0.2 g), fructose (0.2 g), protein (13.35 g), and fat (21 g) (USDA 2012). However, in our previous works, the secondary mycelia of *L. tigrinus* grew best on solid medium with coconut water gulaman (local crude agar) at a pH range of 7.0–8.0 (Dulay *et al.*, 2012). In some mushrooms, glucose was the best carbon source followed by molasses, starch and sucrose, while yeast extract was found to be the best nitrogen source followed by skim milk, soybean and fish powder for efficient mycelial growth of *Grifola umbellata* (Huang & Liu 2008). In addition, the maximum mycelial growth of *Pleurotus ostreatus* was achieved in glucose as source of carbon and peptone as source of nitrogen (Nwokoye *et al.* 2010). However, this preference of glucose over other carbon source observed in a number of basidiomycetous fungi would exert a negative effect when the medium has higher concentrations of glucose that might be detrimental to mycelial physiology and metabolites biosynthesis (Fang & Zhong 2002).

Aside from the important nutritional components, minerals (calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, manganese and selenium), and vitamins (riboflavin, thiamin, niacin, pantothenic acid, folate, choline, alpha-tocopherol, vitamin B6 and vitamin K) are also found in rice bran (USDA 2012). Minerals at certain concentration stimulate the mycelial growth of mushroom. Iron is essential for vegetative growth of the mycelia, whereas calcium performs an important physiological role in the medium. Calcium supports maximum growth of *Pleurotus* that may be attributed to its role in the metabolic process such as glycolysis and respiration (Fasidi & Olorunmaiye 1994). Moreover, good yield of *P. florida* can be obtained from medium with 5:1 carbon/nitrogen ratio, thiamine, gibberellin, calcium, and zinc. In addition, the efficiency of biomass production in rice bran broth could also be explained by the buffering capacity of this liquid medium, thus the effect of pH using this medium on the mycelia biomass production of these two *Lentinus* species must be evaluated. In contrast, low mycelial biomass production was observed when using corn grit broth as liquid medium.



**Fig. 1** – Effect of different culture broth on the mycelial biomass of the two *Lentinus* species after 10 days of incubation.



**Fig. 2** – Volume loss of the culture spent of the two *Lentinus* species after 10 days of incubation.

Volume loss of the culture spent was also determined in this study (Figure 2). It is obvious that the medium which produced the highest mycelial biomass recorded the greatest volume loss. In both species, the maximum volume loss was observed in rice bran broth followed by potato broth. These results clearly indicate the efficient utilization of the liquid media to convert it to mycelial biomass by the two mushrooms. This finding is congruent to the observed weight loss of the lignocellulosic substrate after fruiting of cultivated mushrooms in artificial logs.

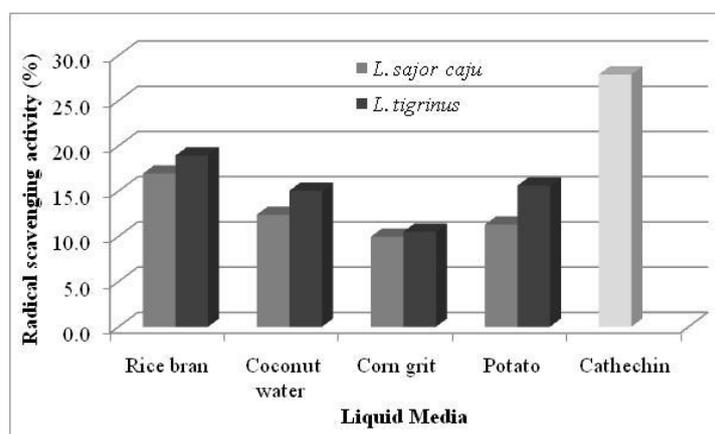
### DPPH radical scavenging activity

Antioxidant in food plays a major role in promoting healthful benefits. It reduces the risk for chronic diseases such as cancer, cardiovascular and neurodegenerative diseases (Prakash *et al.* 2001). In this work, the antioxidant activity using DPPH radical scavenging assay was investigated. The radical scavenging activity of the two *Lentinus* species grown in different indigenous liquid culture is depicted in Figure 3. Apparently, both mushrooms exhibited radical scavenging activity. However, *L. tigrinus* showed higher activity than *L. sajor-caju* in all liquid culture types. Regardless of the species, among culture types, mushroom mycelia grown in rice bran recorded the highest scavenging activity of 16.9% (for *L. sajor-caju*) and 18.9% (for *L. tigrinus*), whereas those grown in corn grit showed the lowest activity 11.3% (for *L. sajor-caju*) and 15.6% (for *L. tigrinus*). This clearly indicates that the antioxidant activity is species and culture medium dependent. Several studies have established that many mushrooms possess antioxidant property. For instance, *Lentinula edodes*, a relative of *Lentinus* species, showed antioxidant activity of 39.0% at a concentration of 1 mg/ml with an EC<sub>50</sub> value of 4.4 mg/mL. Its total phenolic content was estimated to be 70.83 mg gallic acid equivalent (GAE) per gram of dry extract (Sasidharan *et al.*, 2010). Moreover, *Schizophyllum commune* (3.0 mg/ml), *Pleurotus djamour var. djamour* (5.5 mg/ml), *Pleurotus pulmonarius* (6.0 mg/ml), *Pleurotus djamour var. roseus* (7.5 mg/ml) and *Pleurotus ostreatus* (12.0 mg/ml) inhibit DPPH free radicals (Gan *et al.* 2013). In addition, among the mushrooms evaluated by Wong *et al.* (2013), *Auricularia polytricha* showed the highest scavenging activity of 79% followed by *Pleurotus eryngii* had 52%, while *Flammulina velutipes* and *Pleurotus florida* both have 23%. These findings establish the great potential of mushrooms to neutralize free radicals. There are several substances responsible for antioxidant potentials of many mushrooms. These include  $\beta$ -glucan, tocopherols, niacin, flavin, pyridoxine, ascorbate, shikimate, malate, fumarate, monoterpene, diterpene, lipids, hydrophobins and trace elements such as selenium (Yim *et al.* 2010, Aggarwal *et al.* 2012).

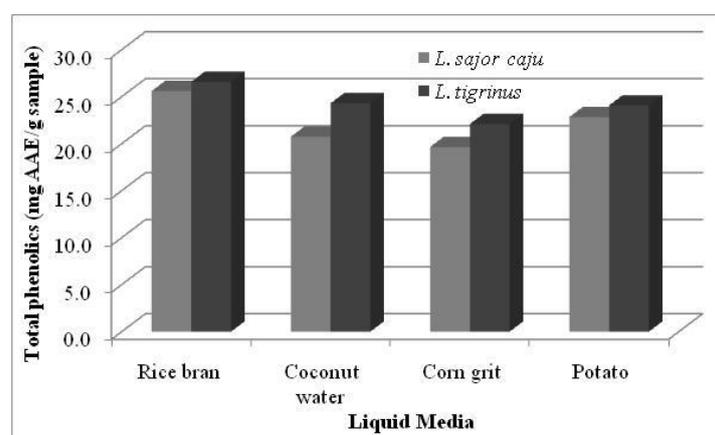
### Total phenolic content

Phenolics are one of the groups of nonessential dietary components present in plants and mushrooms as secondary metabolites. Figure 4 presents the total phenolic content of the two *Lentinus* species as influenced by the different liquid culture media. *L. tigrinus* mycelia had higher phenolic content than *L. sajor-caju* mycelia. Regardless of the species, rice bran broth supports the efficient production of not only mycelial biomass but most importantly its phenolic content (25.6 mg AAE/g sample for *L. sajor-caju* and 26.6 mg AAE/g sample for *L. tigrinus*). In contrast, corn grit broth had the lowest phenolic content (19.6 mg AAE/g sample for *L. sajor-caju* and 22.1 mg AAE/g sample for *L. tigrinus*). These result gathered strongly dictates that the phenolic content of mushroom is species and culture media dependent. Phenolics exhibited antioxidant activities and may interfere physiological processes of cancers, leading to cancer risk reduction (Hollman 2001). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxigenase, and scavenge free radicals (Mallavadhani *et al.* 2006).

In conclusion, we established the mycelial biomass production of the two *Lentinus* species in indigenous liquid culture. The mycelial biomass production and antioxidant activity of both mushrooms showed species and culture media dependent. Rice bran broth produced the maximum mycelial biomass and recorded the highest antioxidant properties by DPPH radical scavenging activity and total phenolic content. Thus, this medium could be used in efficient production of mushroom biomass and valuable bioactive metabolites. With the significant attributes of these two mushrooms when grown on rice bran broth in liquid culture, several potential biological activities is highly expected. In the future, it is recommended the elucidation, characterization and identification of novel compounds responsible for these bioactivities must be investigated.



**Fig. 3** – Radical scavenging activity of the two *Lentinus* species in different indigenous liquid culture.



**Fig. 4** – Phenolic contents of the two *Lentinus* species in different indigenous liquid culture.

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