Journal of Applied Pharmaceutical Science Vol. 13(04), pp 096-104, April, 2023
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2023.51227
ISSN 2231-3354

Optimal liquid culture conditions and bioactivity of *Pycnoporus sanguineus*

Janice S. Aguilar[1,2], Rich Milton R. Dulay[1*], Sofronio P. Kalaw[1], Renato G. Reyes[1]

[1]Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Philippines
[2]Science Education Institute, Department of Science and Technology, Bicutan, Taguig City, Philippines

ARTICLE INFO

Received on: 30/10/2022
Accepted on: 05/02/2023
Available Online: 28/03/2023

Key words:
Submerged cultivation, *Pycnoporus sanguineus*, mycelial biomass, biological activity.

ABSTRACT

Pycnoporus sanguineus is a species of mushroom in the Philippines with promising nutritional and pharmacological potentials. In this study, the optimum nutritional and physical growth preferences of *P. sanguineus* in submerged conditions were established and the biological activities of ethanol extract of its mycelia were determined. The highest yield (100.13 mg/30ml) was produced in rice bran broth with a wide range of pH concentration. Biomass production was favored under 28°C, in lighted and agitated conditions with 147.20 mg/30 ml, 168.47 mg/30 ml, and 413.30 mg/30 ml, respectively. The extract inhibited the growth of *Staphylococcus aureus* while no antibacterial activity was observed in *Escherichia coli*. It was found toxic to brine shrimp with an LC_{50} value of 190.55 µg/ml. Exposure of zebrafish embryos to the extract caused delayed growth resulting in a lower hatching rate. Growth retardation was the most noticeable teratogenic effect of the extract after 48 hours and high mortality rates were observed in high extract concentrations. This study suggests that the optimum biomass yield of *P. sanguineus* can be achieved when incubated under the most favorable conditions. Mycelia of *P. sanguineus* could be a valuable resource of antibacterial and cytotoxic compounds.

INTRODUCTION

Pycnoporus sanguineus, a species of mushroom under the order Polyporales is normally found on decaying hardwood (Fig. 1). It is characterized by its bright red-orange basidiocarp with no distinct stipe. *P. sanguineus* has various industrial and medicinal uses;including pharmaceutical wastewater treatment (Watanabe *et al*., 2012), detoxification of textile dye, and degrading a wide variety of organic pollutants (Pointing and Vrijmoed, 2000). Cinnabarin and other compounds present in this mushroom have antioxidant (Tuong *et al*., 2020), antibacterial (Jaszek *et al*., 2015), and anticancer properties (Piet *et al*., 2021). Laccase, a protein produced by this mushroom, exhibited strong cytotoxic activity against colon cancer (Piet *et al*., 2021). Moreover, its heavy metal absorbance ability is currently being studied as a potential absorber of heavy metals in the bloodstream (Yahaya and Don, 2014). Accordingly, this mushroom contains beneficial bioactive compounds that can be used in various fields. Therefore, there is a need to establish efficient production technology for this mushroom to continually harness its potential.

Various researchers have successfully grown this mushroom in submerged fermentation using both commercial and indigenous materials. Pointing *et al*. (2000) enhanced its laccase production by modification of the nutrient content of the broth medium. Stirred tank bioreactor with optimized temperature and pH concentrations is also applicable for the efficient production of its biomass (Souza *et al*., 2021). Mendoza *et al*. (2020) utilized coconut water as a growth medium for the production of compounds with strong antioxidant activity. However, carbon and nitrogen sources are the major factors that directly influence the production and bioactivity of this mushroom (Eugenio *et al*., 2009). Aside

*Corresponding Author
Rich Milton R. Dulay, Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Philippines.
E-mail: richmiltondulay @ clsu.edu.ph

Figure 1. Wild fruiting bodies of *P. sanguineus* growing on forest log.

from the nutrient source, temperature, light intensity, aeration, and agitation influence the successful biomass production of mushrooms in submerged culture (Dulay *et al*., 2015a). Thus, submerged fermentation is an efficient production technique that can be used to produce mycelial biomass with promising functional activities.

Submerged cultivation can be used as an alternative to the existing mushroom production technology wherein mycelial biomass is produced in liquid media. Flasks, stirred tanks, airlift bioreactors, and fed-batch fermentation are the most common methods for submerged cultivation (Bakratsas *et al*., 2021). Various species have already been successfully grown in submerged cultures including *Pleurotus ostreatus* (Velioglu and Uzek, 2015), *Lentinula edodes* (Garcia-Cruz *et al*., 2020), and *Cordyceps sinensis* (Chen *et al*., 2016). In the Philippines, Dulay *et al*. (2015a) and Dulay *et al*. (2015b) were able to optimize liquid culture conditions of different *Lentinus* species, *Pleurotus cystidiosus*, *Ganoderma lucidum*, *Schizophyllum commune*, and *Volvariella volvacea* using the conventional shake flask culture. Thus, this technique can be used to produce biomass as other species of mushrooms are not capable of producing fruiting body outside their natural habitat.

Reports regarding the optimum submerged culture conditions of wild Philippine mushrooms are very limited. However, with the aim of utilizing mycelia as a source of functional food and bioactive compounds, the Center for Tropical Mushroom Research and Development in Central Luzon State University (CTMRD-CLSU) is continuously conducting studies to establish biomass production technology of wild mushrooms using indigenous materials. For example, *Lentinus swartzii* mycelia grown in the optimized liquid culture conditions possess strong antioxidant and antidiabetic activities (Austria *et al*., 2021). In addition, the antioxidant potential of both *Lentinus sajor-caju* and *Lentinus tigrinus* is reported to be influenced by the culture medium (Dulay *et al*., 2015b). Mycelial biomass of *G. lucidum*, *V. volvacea*, *S. commune*, and *P. cystidiosus* grown in submerged conditions was found to contain amounts of polar lipids, triglycerides, and free fatty acids (Dulay *et al*., 2015a). Accordingly, mushroom mycelia demonstrated excellent potential as a source of bioactive compounds that are beneficial to mankind.

This study was conducted in our interest to determine the optimum submerged culture conditions for *P. sanguineus* with reference to its nutritional and physical growth preferences. The establishment of an efficient cultivation technique for this mushroom allows effective utilization of its biomass. This was the first report in the country regarding the optimum liquid culture conditions for the mycelia biomass production of *P. sanguineus*. The potential bioactivity of its mycelia biomass was also determined.

MATERIALS AND METHODS

Optimization of submerged culture conditions

Source of culture

Pure culture of *P. sanguineus* (CTMRD 7137) was obtained from the culture collection of Bioassay Laboratory, Department of Biological Science, College of Science, CLSU, Science City of Muñoz, Nueva Ecija, Philippines.

Influence of nutritional factors

Prior to the evaluation of the nutritional and physical factors, culture inoculants were prepared. Using an inoculating needle, the agar block was transferred into plates containing media. Then, the plates were incubated at room temperature. After full ramification, mycelial discs were prepared using a cork borer.

The influence of four indigenous liquid culture media (potato sucrose broth, corn grit broth, coconut water, and rice bran broth) on the mycelial biomass production of *P. sanguineus* was determined. The culture broths were prepared following the method of Dulay *et al*. (2015a). About 30 ml of each media was dispensed in bottles and subjected to sterilization at 121°C, 15 psi for 30 minutes. A 10 mm mycelial disc was aseptically inoculated into each bottle containing a liquid culture medium. The culture bottles were incubated for 7 days at room temperature, then, the mycelia were harvested and the mycelial dry weight was recorded. This was followed by the evaluation of pH. Using 1 M NaOH or 1 M HCl, the pH concentration of the most suitable liquid culture medium was adjusted to 4.0–8.0 with an interval of 1.0. This was followed by sterilization, inoculation of mycelial discs, and incubation for 7 days.

Influence of physical factors

The most suitable liquid culture medium adjusted to the best pH was used to determine the optimum physical factors for the mycelial biomass production of *P. sanguineus*. These include temperature, illumination, and agitation. To determine the optimum temperature, the culture bottles inoculated with mycelial discs were incubated at refrigerated (8°C), air-conditioned (21°C), and room temperature (28°C). It was followed by the evaluation of the influence of illumination wherein culture bottles with the mycelial disc were incubated at two different illumination conditions, namely, lighted and dark. Lastly, culture bottles were incubated in static and agitated conditions to determine the influence of agitation. The optimum nutritional and physical

factors were determined in terms of mycelial dry weight after 7 days of incubation. Each setup was carried out in triplicate.

Determination of bioactivity of mycelial extract

Production of mycelial bBiomass

Using the optimum submerged culture conditions, mycelial biomass was produced. This was done by aseptically inoculating mycelial discs into sterile bottles containing media. Mycelial biomasses were harvested after 7 days of incubation and air-dried until constant weight is achieved.

Ethanolic extraction

Dried mycelia were powdered using a blender. Powdered mycelia were soaked in 95% ethanol with a ratio of 1g:10 ml ethanol for 48 hours prior to filtration. The solvent was removed using a rotary evaporator. The crude extract was placed in a sterile vial with a cover and stored in the refrigerator prior to use.

Source of bacterial cultures

Bacterial cultures of *Staphylococcus aureus* **(**UPLB BIOTECH 1582) and *Escherichia coli* (UPLB BIOTECH 1634) were obtained from the culture collection of Philippine National Collection of Microorganisms, BIOTECH, University of the Philippines, Los Baños, Laguna.

Antibacterial assay

The antibacterial potential of the ethanol mycelial extract was tested against *S. aureus* and *E. coli* using the disc diffusion method. Microbial cultures were prepared in a test tube containing nutrient broth and were incubated for 12 to 24 hours. Prior to use, the turbidity of the broth medium was compared to 0.5 McFarland standard. Using a cotton swab, each bacterial culture was swabbed into sterile plates containing media. Then, 6-mm sterile paper discs soaked in the mycelial extract were placed on the surface of the medium. The zone of inhibition formed around the disc was measured after 24 hours of incubation. The test was carried out in triplicate.

Brine shrimp lethality assay

The cytotoxic activity of the extract was assessed using a brine shrimp lethality assay. The eggs were hatched in artificial seawater (25 g rock salt in 1l dH2O) for 24–48 hours. Varying concentrations of *P. sanguineus* extract were prepared (1, 10, 100, 1,000, and 10,000 µg/ml). Five ml of each extract concentration was placed in vials and ten brine shrimp larvae were exposed into each vial. Artificial seawater served as the control and the test was performed in triplicate. The number of dead larvae was recorded 24 hours after treatment application (hpta). Percentage mortality was computed and the LC50 value was determined using probit analysis. The computed LC_{50} value was interpreted based on the interpretation established by Bastos *et al*. (2009), wherein LC_{50} values greater than one thousand (≥1,000 µg/ml) were considered nontoxic, and greater than 500 but less than 1,000 (≥500 ≤1,000 µg/ml) were weakly toxic, while LC_{50} values less than 500 (≤500 µg/ml) were regarded as toxic.

Toxicity and teratogenicity assay

The toxic and teratogenic properties of the extract were determined using zebrafish embryo following the method of Dulay *et al*. (2012b). Five concentrations of the extract were prepared (1 µg/ml, 10 µg/ml, 100 µg/ml, 1,000 µg/ml, and 10,000 µg/ml) in vials and embryo water was used as the control. Each vial containing different extract concentrations was replicated three times. Three embryos at the segmentation phase were introduced into each vial and incubated at room temperature. The observation was done using a microscope 12, 24, and 48 hours after treatment application and the number of dead embryos was recorded. Teratogenic effects and percentage hatchability were determined after 48 hours of exposure. Coagulation, absence of heartbeat, tail not detached, and no somite were considered toxic effects of the extract, while malformation of the head, heart and tail, scoliosis, growth retardation, and yolk deformity were regarded as teratogenic effects (Nagel, 2002).

Statistical analysis

All treatments were laid out in a complete randomized design. ANOVA was used to analyze the data and Tukey's Honestly Significant Difference was used to compare treatment means at a 5% level of significance. Paired *t*-test was used to compare the treatment means of illumination and agitation conditions.

RESULTS AND DISCUSSION

Influence of liquid culture media

Liquid culture media is one of the important factors to be considered for the luxurious and rapid growth of mushroom mycelia. Mycelial biomass production of *P. sanguineus* as influenced by the different liquid culture media after 7 days of incubation was investigated. Among the four media tested, rice bran broth produced the highest mycelial biomass of *P. sanguineus* with a 100.13 mg/30 ml yield (Table 1). This could be explained by the nutritional composition of rice bran that favors the efficient mycelial growth of this mushroom. According to Kalpanadevi *et al*. (2018), it contains protein, fat, carbohydrates, fibers, ash, amino acids, minerals, and lipids. Some micronutrients essential for the mycelial growth of fungi like oryzanol, tocopherols, tocotrienols, and phytosterol are also present in rice bran (Nagendra *et al*., 2011). Therefore, these nutritional components of rice bran make it more favorable for the efficient biomass production of *P. sanguineus* than the other media used. A similar result was obtained by Quiroz-Castañeda *et al*. (2011) for this mushroom which showed excellent mycelial growth in a rice husk medium. Marim *et al*. (2016) concluded that the laccase production of *P. sanguineus* is greatly influenced by nitrogen concentration in the culture media. According to Narh *et al*. (2018), rice bran can be used as an efficient alternative source of nitrogen for growing most mushrooms. In this study, rice bran was used as a source of nitrogen and sucrose as a source of carbon. The

Table 1. Mycelial biomass yield of *P. sanguineus* grown in liquid culture media after seven days of incubation.

Liquid culture media	Biomass yield (mg/30 ml)
PSB	39.65 ± 8.19^{b}
CGB	20.02 ± 6.19^{c}
CW	35.83 ± 7.47^{bc}
RBB	100.13 ± 10.88^{a}

Means with the same superscript are not significantly different from each other at 5% level of significance.

presence of these two primary nutrient requirements of fungi in the substrate favored the efficient biomass production of this mushroom. The same result was observed by Eugenio *et al*. (2009) wherein a high amount of laccase was produced in media with sucrose than in other sources of carbon used. Other species of mushrooms which produced optimum mycelial growth in rice bran include *Trametes elegans* (Dulay *et al*., 2021b) and *L. tigrinus* (Dulay *et al*., 2015b). *Pleurotus eryngii* also showed excellent mycelial growth in media supplemented with rice bran (Peng *et al*., 2000). Contrastingly, other mushrooms such as various species of *Lentinus*, *G. lucidum*, and *A. polytricha* showed excellent mycelial growth in coconut water gulaman (Dulay *et al*., 2021a; Kalaw *et al*., 2016). Among the four media used, corn grit broth has the lowest biomass yield with 20.02 mg/30ml. Dulay *et al*. (2012a) gathered similar results for this media which produced the lowest germination of basidiospores of *L. tigrinus*. On the other hand, corn grit produced very thick mycelia and showed the shortest incubation period of *L. strigosus* (Dulay *et al*., 2017).

Influence of pH

The pH of the media affects the growth and metabolism of most mushrooms. In this study, it was observed that a wide range of pH supports the mycelial biomass production of *P. sanguineus* (Table 2). Biomass production peaked at pH 7 with 162.77 mg/30 ml. However, the statistical analysis showed that there is no significant difference between the mean biomass yields. According to Shu and Lung (2004), the pH of the medium directly affects cell growth, which could possibly be due to the nutrient intake of the cell membrane. This result is congruent with the findings of Quiroz-Castañeda *et al*. (2009) for *P. sanguineus* which showed maximum enzyme activity production at pH 5. Moreover, it was found that these enzymes exhibit strong activity and stability in pH 2 to 8. On the other hand, in the study conducted by Teoh *et al*. (2011), the highest biomass yield of this mushroom is obtained at pH 4.7. Falkoski *et al*. (2012) observed that the enzyme activities of this mushroom peaked at 3.5–4.5. Interestingly, this finding is comparable to the results obtained by Dong *et al*. (2017) for *Irpex lacteus* which produced optimum biomass at pH 3 to 9. On the other hand, some mushrooms like *Cordyceps militaris* have a specific pH preference wherein biomass and exobiopolymer production were favored at pH 6 (Park *et al*., 2001). *P. sanguineus* is not pH sensitive wherein optimum mycelial biomass can be produced from pH 4–8. These results imply that the pH preferences of mushrooms vary depending on the species wherein beyond optimum pH, the growth rate of some mushrooms is decreased (Basu *et al*., 2015).

Influence of temperature

One of the most critical factors to be considered in mushroom cultivation is temperature since growth rate of organism decreases beyond the optimum level (Wang and Zong, 2007). In this study, the influence of temperature on mycelial biomass production of *P. sanguineus* was determined using the most suitable liquid culture media adjusted to the best pH. Table 3 presents the mean biomass yield after 7 days of incubation. Culture bottles incubated at 28°C recorded the highest biomass yield of 147.20 mg/30 ml. Biomass yield was significantly reduced at lower temperatures (21°C) whereas no biomass was produced at 8°C. A similar result was obtained by Quiroz-Castañeda *et al*. (2009) wherein 28°C favored laccase production of *P. sanguineus* in liquid media. These results are comparable with the temperature requirements of *Chlorophyllum molybdites* (Garcia *et al*., 2020), *P. sanguineus* and *Pycnoporus cinnabarinus* (Sharma and Jaitly, 2017), *G. lucidum*, *P. cystidiosus*, *V. volvacea*, *S. commun*e, *L. tigrinus*, and *L. swartzii* (Dulay *et al*., 2015a; Dulay *et al*., 2015b). Moreover, Dulay *et al*. (2021a) found out that room temperature favors luxuriant mycelial growth of *various Lentinus* species. Contrastingly, Smania *et al*. (1997), Alberti *et al*. (2021), and Lee *et al*. (2004) observed that the optimum mycelial growth and antibiotic production of *P. sanguineus*, *Oudemansiella canarii*, and *Grifola frondosa* were favored under 25°C. According to Lin (2004), the temperature preferences of fungi can be used to classify them into three groups; these are temperate, tropical, or semitemperate. Results gathered in this study suggest that *P. sanguineus* is a tropical fungus.

Influence of illumination

Illumination or the intensity of light is one of the environmental factors that affect the growth and development of some fungi. Table 3 shows the mean biomass yield of *P. sanguineus* incubated under lighted and dark conditions. A significantly higher yield was produced in lighted condition with 168.47 mg/30 ml compared to dark (87.6 mg/30 ml). This result is in congruence with

Table 2. Mycelial biomass yield of *P. sanguineus* grown in liquid culture media at varying pH levels after seven days of incubation.

pH concentration	Biomass yield (mg/30 ml)
4	135.30 ± 11.30[a]
5	149.30 ± 7.25[a]
6	135.20 ± 7.26[a]
7	162.77 ± 16.57[a]
8	153.30 ± 5.12[a]

Maens with the same supre script are not significantly different from each othre at 5 % level of significance.

Table 3. Mycelial biomass yield of *P. sanguineus* grown in liquid culture media as affected by temperature, illumination and agitation after seven days of incubation.

Physical factors	Biomass yield (mg/30 ml)
Temperature	
8°C	0.00 ± 0.00[c]
21°C	56.30 ± 6.30[b]
28°C	147.20 ± 11.75[a]
Illumination	|168.47 ± 1.75[a]
Lighted	87.6 ± 5.52[a]
Dark	
Agitation	|413.3 ± 50.4[a]
Agitated	134.50 ± 41.00[b]
Static	
	Zone of inhibition (mm)

Means with the same superscript are not significantly different from each other at 5% level of significance.

the findings of Smania *et al*. (1997) wherein *P. sanguineus* showed higher biomass yield under lighted condition. It was reported that light has a positive impact on the fruiting body development of king oysters (Zadrazil, 1978). Poyedinok *et al*. (2008) conveyed that a certain range of light wavelength causes alteration of the essential composition of mushroom spore, aside from this, disturbances on the important enzymes responsible for the growth processes during the vegetative stage occur. Since *P. sanguineus* produced a higher yield in lighted conditions, it indicates that the presence of light has a positive impact on the biomass production of this mushroom. However, other mushrooms like *Coprinus comatus*, *L. tigrinus*, *L. strigosus*, and *L. swartzii* exhibited better mycelial growth in dark conditions (Duarte *et al*., 2012; Dulay *et al*., 2020; Mendoza *et al*., 2020). Meanwhile, Landingin *et al*. (2020) and Jacob *et al*. (2015) reported that mycelial growth performances of *Cyclocybe cylindracea, Pleurotus citrinolipeatus, Pleurotus djamor*, and *Pleurotus salmoneostramineus* were not directly affected by light. These results indicate that different species of mushrooms have different light preferences.

Influence of agitation

Agitation provides better gas supply, mass, and heat transfer to the growing mycelia (Cui *et al*., 1997). In this study, the culture bottles were incubated under two agitation conditions: agitated (100 rpm) and static. The biomass yield of *P. sanguineus* was improved under agitated conditions compared to cultures incubated in static conditions (Table 3). A relative increase in biomass yield of this mushroom (413.3 mg/30 ml) can be explained by the shaking condition of the medium. It allows the mycelia to efficiently utilize the nutrients present in the media as there is enough supply of oxygen (Peng *et al*., 2000). This is also proved by Pointing and Vrijmoed (2000) for the *P. sanguineus* Thailand strain wherein biomass production increased when incubated in agitated conditions compared to static. Aside from biomass production, the enzymatic activity of this mushroom improved as a result of agitated conditions (Marim *et al*., 2016). Other mushrooms with the same agitation preference include *G. lucidum* (Dulay *et al*., 2015a), *Polyporus tricholoma* (Vieira *et al*., 2008), and *Pleurotus pulmonarius* (Abdullah *et al*., 2013). However, some species of mushroom such as *S. commune*, *P. cystidiosus*, and *V. volvacea* did not show sensitivity to agitation (Dulay *et al*., 2015a). In some cases, higher intensity of agitation produced higher yield, so it is also important to evaluate the influence of varying speed of the agitator since only 100 rpm was used in this study. For instance, *Aspergillus niger* produced higher biomass and glucose oxidase at 700 rpm than at lower rpm (460) (Zetelaki and Vas, 1968). The mycelial biomass production of *P. sanguineus* in its optimized submerged culture conditions is shown in Figure 1.

Antibacterial activity of mycelial extracts

Mushrooms naturally produce bioactive compounds as part of their defense mechanism in order to survive. In this study, the antibacterial potential of *P. sanguineus* ethanolic extract was evaluated against *E. coli* and *S. aureus*. The mean diameter zone of inhibitions after 24 hours of incubation is presented in Table 4. *P. sanguineus* ethanol extract showed a 7.47 mm diameter zone of inhibition against *S. aureus* while no zone of inhibition was observed in *E. coli* (Fig. 2). This confirms the findings of Rosa *et al*. (2003) wherein most gram-positive bacteria show more sensitivity to Cinnabarin from *P. sanguineus* than gram-negative bacteria. Other related studies confirmed that ethanolic, acetonitrile, and hexane extracts of *L. tigrinus* were found to be active against *S. aureus* but inactive against *E. coli* (Dulay *et al*., 2014; Dulay *et al*., 2017). Similarly, ethyl acetate extracts of *P. sanguineus* showed inhibitory activity against *Candida krusei*, *Listeria monocytogenes*, and *S. aureus*, but no zone of inhibition was observed in *E. coli* (Rosa *et al*., 2003). However, the ethanolic mycelial extract of this mushroom grown in coconut water demonstrated promising antibacterial potential against both *S. aureus* and *E. coli* (Mendoza *et al*., 2020). The observed differences in the response of bacterial pathogens to the extract can be explained by the findings of Tamboli and Lee (2013) who disclosed that gram-positive and gram-negative bacteria have different cell wall structures. *E. coli* can resist various foreign materials from entering the cell due to the high level of lipid material present in its cell wall which serves as a barrier (Agatemor, 2009). It is also possible that the amount of the compound responsible for its antibacterial activity is not enough since it is crude. Meanwhile, Mendoza *et al*. (2020) reported that *P. sanguineus* mycelia contain novel secondary metabolites including triterpenes, anthrones, tannins, flavonoids, phenols, steroids, alkaloids, anthraquinones, and fatty acids. Among the above-mentioned compounds, triterpenes, anthrones, and alkaloids act as the most effective antimicrobial agents

Table 4. Antibacterial activity of *P. sanguineus* extract against *E. coli* and *S. aureus*.

	Zone of inhibition (mm)	
	E. coli	***S. aureus***
P. sanguineus	6.00 ± 0.00^{b}	7.47 ± 0.20^{b}
Streptomycin	32.33 ± 0.45^{a}	33.13 ± 0.15^{a}
Ethanol	6.00 ± 0.00^{b}	6.00 ± 0.00^{c}

Means with the same superscript are not significantly different from each other at 5% level of significance.

Figure 2. Mycelial biomass of *P. sanguineus* in its optimized submerged culture conditions after seven days of incubation.

(Asamenew *et al*., 2011; Chudzik *et al*., 2015; Saxena *et al*., 2013). These compounds need to be isolated and purified in order to determine the exact concentration needed to inhibit the growth of bacteria. Thus, based on the results gathered in this study, the ethanolic extract of *P. sanguineus* is a good source of compounds with antibacterial potential against *S. aureus*.

Cytotoxic activity of mycelial extract

Mushrooms contain agents with anticancer activity. It was reported that *P. sanguineus* contains a high amount of phenols, carbohydrates, and protein compounds such as laccase that oxidize and degrade cancer-inducing compounds (Piet *et al*., 2021). In this study, the cytotoxic activity of *P. sanguineus* mycelia ethanol extract was assessed using a brine shrimp lethality assay. The computed LC_{50} value is equal to 190.55 µg/ml which indicates that the extract is toxic. Results showed that the mortality rate of brine shrimp larvae is dose-dependent; it can be observed that as the concentration increases, the mortality rate also increases. Similarly, Mendoza *et al*. (2020) gathered similar results for mycelial extracts of *P. sanguineus* grown on coconut water wherein an LC_{50} value of 16.02–154.83 µg/ml was obtained. It produces a valuable amount of triterpene, a secondary compound that is known for its anticancer activity (Chudzik *et al*., 2015). Moreover, Piet *et al*. (2021) found that *P. sanguineus* demonstrated high cytotoxicity toward colon cancer cells which is correlated with the presence of phenolic compounds. The findings of this study clearly suggest that *P. sanguineus* contain bioactive compounds with the cytotoxic property.

Teratogenic effects of *P. sanguineus* extract

Teratogenicity is defined as the ability of a certain extract or drug to cause morphological malformations in a developing organism (Elefant *et al*., 2020). This work demonstrated the teratogenicity of *P. sanguineus* extracts in zebrafish embryos. Table 5 shows the mean percentage hatchability of embryos exposed to the different concentrations of the extract at 48 hours after treatment application. Figure 4A shows the normal hatched embryo in the control group after 48 hours. A significantly lower hatching rate was observed in embryos exposed to 1 µg/ml, 10 µg/ml, and 100 µg/ml compared to the control group. Accordingly, these results clearly indicate that the hatchability of embryos is influenced by varying concentrations of *P. sanguineus* extract. It was observed that the hatching rate decreases as the extract concentration increases. Exposure of the embryo to certain chemicals during the early stages of development can cause morphological abnormalities that affect the developmental processes, which further leads to delayed hatching (Dulay *et al*., 2012b). Growth retardation was found to be the most noticeable teratogenic effect (Figure 4C and G) of the extract after 48 hours. Miao *et al*. (2022) claimed that the compound responsible for the teratogenicity of mushrooms works by interrupting some biochemical processes, resulting in reduced blood flow and locomotor activity of the embryo. Insufficient amount of glucose, triglyceride, and cholesterol in the bloodstream was also observed since the mushroom extract disrupts the genes responsible for lipid and cholesterol metabolism. Observations in this study indicate that exposure of zebrafish embryos to *P. sanguineus* extract can cause growth retardation suggesting that it contains substances with teratogenic properties.

Lethal effects of *P. sanguineus* extract

The toxic or lethal effect of *P. sanguineus* extract was assessed using zebrafish embryo. Table 6 presents the mean percentage mortality observed after 12, 24, and 48 hours of exposure to different concentrations of the extract. It was found that the lethal effects of *P. sanguineus* extract were dose and time of exposure dependent. In all observation periods, embryos exposed to lower concentrations of the extract survived whereas high mortality rates were observed in embryos exposed to higher concentrations (10,000 µg/ml and 1,000 µg/ml). Coagulation (Fig. 4H) was the most obvious lethal effect of *P. sanguineus* extract. Embryos with observed growth retardation caused by 1,000 µg/ml extract showed a complete absence of heartbeat which confirmed their mortality after 48 hours. Aside from *P. sanguineus*, other species of mushrooms such as *P. ostreatus*, *L. sajor-caju*, *L. tigrinus*, *Trichaleurina celebica*, and *G. lucidum* (De Castro and Dulay 2015; Dulay *et al*., 2012b; Dulay *et al*., 2014; Sogan *et al*., 2018) exhibited toxicity to zebrafish embryo. Mendoza *et al*. (2020) revealed that the mycelia of this mushroom produce triterpenes, phenols, and anthraquinones all of which possess anticancer activity. The presence of these secondary metabolites, therefore, act together causing adverse effects that lead to the death of the embryo. Lima *et al*. (2012) disclosed that mushrooms contain aromatic hydrazines which are considered carcinogenic. They also observed that patients diagnosed with failures in the renal system can develop cryptogenic encephalopathy when exposed to mushroom compounds with similar characteristics

Table 5. Hatchability rate embryos at different concentrations of *P. sanguineus* extract after 48 hours.

Concentration (µg/ml)	% Hatchability
0	100 ± 0.00^{a}
1	55.6 ± 19.2^{b}
10	55.6 ± 19.2^{b}
100	33.33 ± 0.00^{b}
1,000	0.00 ± 0.00^{c}
10,000	0.00 ± 0.00^{c}

Means with the same superscript are not significantly different from each other at 5% level of significance.

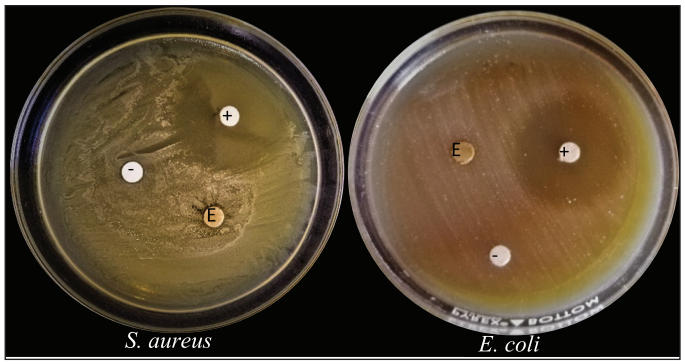

Figure 3. Antibacterial assay plates showing the zones of inhibition exhibited by the mycelial extract of *P. sanguineus* against *S. aureus* and *E. coli.* (E) mycelial extract, (+) streptomycin and (-) ethanol.

Table 6. Mortality rate at 12, 24, 48 hpta of embryos at different concentrations of *P. sanguineus* extract.

Concentration (µg/ml)	% Mortality		
	12	24	48
0	0.00 ± 0.00[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
1	0.00 ± 0.00[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
10	0.00 ± 0.00[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
100	0.00 ± 0.00[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
1,000	11.1 ± 19.2[b]	77.78 ± 19.2[b]	100 ± 0.00[a]
10,000	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]

Means with the same superscript are not significantly different from each other at 5% level of significance.

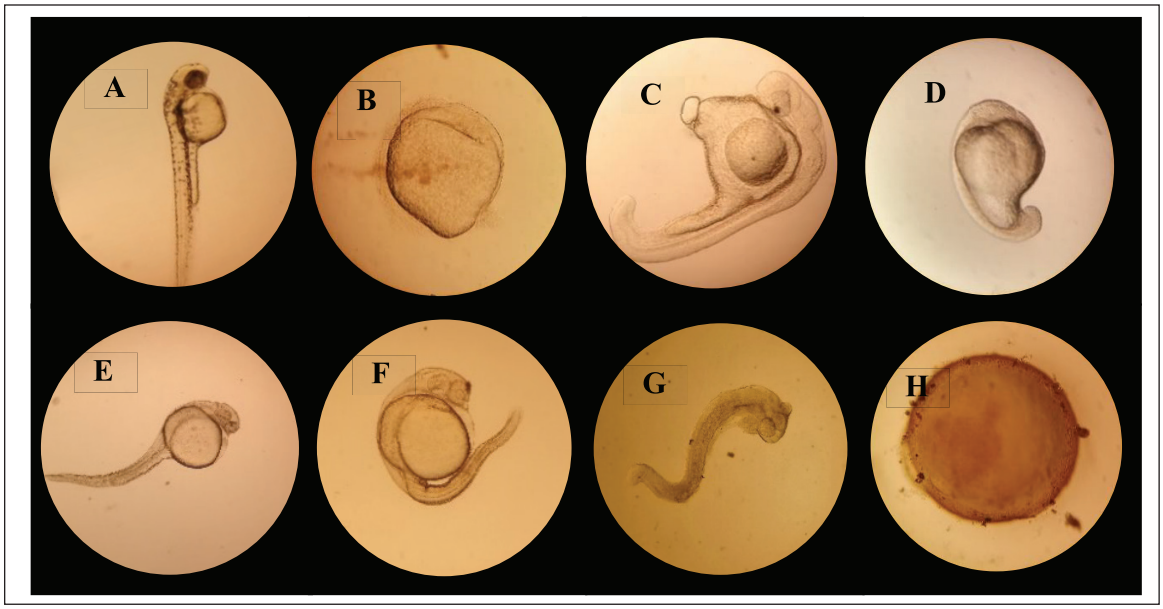

Figure 4. Teratogenic and toxic effects of *P. sanguineus* extract on zebrafish embryos at 48 hpta. (A) normal hatched embryo (embryo water). (B-G) Growth retardation at 1 µg/ml to 1,000 µg/ml extract concentration (H) Coagulated embryo incubated at 10,000 µg/ml and 1,000 µg/ml extract concentration.

as vitamin D. These results suggest that high concentrations of *P. sanguineus* can cause lethal effects to zebrafish embryos. Thus, it could be a remarkable resource of toxic substances that can be used for the formulation of effective anticancer drugs.

CONCLUSION

Results gathered in this study suggest that a high biomass yield of *P. sanguineus* in liquid culture can be obtained when provided with the most suitable growth medium and pH. Light, temperature, and agitation are important physical factors that directly improve the mycelia growth of this mushroom during incubation. The observed antibacterial and cytotoxicity of *P. sanguineus* extract imply that it could be used as a valuable resource of antibacterial and cytotoxic compounds.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

This study was funded by the Academic Research Council of Central Luzon State University.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

Abdullah N, Ismail R, Johari NM, Annuar MS. Production of liquid spawn of an edible grey oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quel by submerged fermentation and sporophore yield on rubber wood sawdust. Sci Hortic, 2013; 161:65–9.

Agatemor C. Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. Elec J Environ Agricu Food Chem, 2009; 8(3):195–200.

Alberti MM, Perez-Chavez AM, Niveiro N, Alberto E. Towards an optimal methodology for basidiomes production of naturally occurring species of the genus *Oudemansiella* (Basidiomycetes). Curr Microbiol, 2021; 78(4):1256–66.

Asamenew G, Bisrat D, Mazumder A, Asres K. In vitro antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana* Reynolds. Phytother Res, 2011; 12:1756–60.

Austria AB, Dulay RM, Pambid RC. Mycochemicals, antioxidant and anti–diabetic properties of Philippine sawgill mushroom *Lentinus swartzii* (higher basidiomycetes). Asian J Agricu Biol, 2021; 10 :202006365.

Bakratsas G, Polydera A, Katapodis P, Stamatis H. Recent trends in submerged cultivation of mushrooms and their application as a source of nutraceuticals and food additives. Future Foods, 2021; 4:100086.

Bastos ML, Lima MR, Conserva LM, Andrade VS, Rocha EM, Lemos RP. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Ann Clin Microbiol Antimicrob, 2009; 8(1):1–6.

Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S. Evolution of bacterial and fungal growth media. Bioinformation, 2015; 11(4):182.

Chen X, Wu JY, Gui X. Production and characterization of exopolysaccharides in mycelial culture of *Cordyceps sinensisfungus* Cs-HK1 with different carbon sources. Chin J Chem Eng, 2016; 24(1):158–62.

Chudzik M, Korzonek-Szlacheta I, Krol W. Triterpenes as potentially cytotoxic compounds. Molecules, 2015; 20:1610–25.

Cui YQ, Van der Lans RG, Luyben KC. Effect of agitation intensities on fungal morphology of submerged fermentation. Biotechnol Bioeng, 1997; 55(5):715–26.

De Castro M, Dulay RM. Toxic and teratogenic effects of *Lentinus sajor-caju* and *Pleurotus ostreatus* ethanolic extracts in *Danio rerio* embryo model. Int J Biol Pharm Allied Sci, 2015; 4:2261–9.

Dong X, Song X, Dong C. Nutritional requirements for mycelial growth of milk-white toothed mushroom, *Irpex lacteus* (Agaricomycetes), in submerged culture. Int J Med Mushrooms, 2017; 19(9):829–38.

Duarte LT, Tiba JB, Santiago MF, Garcia TA, Bara MT. Production and characterization of tyrosinase activity in *Pycnoporus sanguineus* CCT-4518 crude extract. Braz J Microbiol, 2012; 43(1):21–9.

Dulay RMR, Alcazar AA, Kalaw SP, Reyes RG, Cabrera EC. Nutritional and physical requirements for mycelial growth and basidiocarp production of *Trametes elegans* from the Philippines. Asian J Agric Biol, 2021a; 1: 1–9.

Dulay RM, Cabrera EC, Kalaw SP, Reyes RG. Optimization of submerged culture conditions for mycelial biomass production of fourteen *Lentinus* isolates from Luzon Island, Philippines. Biocatal Agricu Biotechnol, 2021b; 38:102226.

Dulay RMR, Cabrera EC, Kalaw SP, Reyes RG. Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus* (Bull.) Fr. Mycosphere, 2012a; 3(6):926–33.

Dulay RMR, Cabrera EC, Kalaw SP, Reyes RG, Hou CT. Cultural conditions for basidiospore germination of *Lentinus swartzii* and *Lentinus strigosus* and their morphogenesis. Asian J Agric Biol, 2020; 8(4):377–85

Dulay RMR, Flores KS, Tiniola RC, Marquez DHH, Dela Cruz AG, Kalaw SP, Reyes RG. Mycelial biomass production and antioxidant activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in indigenous liquid culture. Mycosphere, 2015b; 6(6):659–66.

Dulay RMR, Garcia EJB. Optimization and enriched cultivation of Philippine (CLSU) strain of *Lentinus strigosus* (BIL1324). Biocatal Agric Biotechnol, 2017; (12):323–8.

Dulay RM, Kalaw SP, Reyes RG, Alfonso NF, Eguchi F. Teratogenic and toxic effects of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (higher Basidiomycetes), on zebrafish embryo as model. Int J Med Mushrooms, 2012b; 14(5):507–12.

Dulay RM, Kalaw SP, Reyes RG, Cabrera EC. Embryo-toxic and teratogenic effects of Philippine strain of *Lentinus tigrinus* (tiger sawgill basidiomycetes) extract on zebrafish (*Danio rerio*) embryos. Ann Biol Res, 2014; 5(6):9–14.

Dulay RM, Miranda LA, Malasaga JS, Kalaw SP, Reyes RG, Hou CT. Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*. Biocatal Agric Biotechnol, 2017; 9:141–4.

Dulay RM, Ray K, Hou CT. Optimization of liquid culture conditions of Philippine wild edible mushrooms as potential source of bioactive lipids. Biocatal Agric Biotechnol, 2015a; 4(3):409–15.

Elefant E, Hanin C, Cohen D. Pregnant women, prescription, and fetal risk. Handb Clin Neurol, 2020; 173:377–89.

Eugenio ME, Carbajo JM, Martín JA, Gonzalez AE, Villar JC. Laccase production by *Pycnoporus sanguineus* under different culture conditions. J Basic Microbiol, 2009; 49(5):433–40.

Falkoski DL, Guimarães VM, de Almeida MN, Alfenas AC, Colodette JL, de Rezende ST. Characterization of cellulolytic extract from *Pycnoporus sanguineus* PF-2 and its application in biomass saccharification. Appl Biochem Biotechnol, 2012; 166(6):1586–603.

Garcia BL, Undan JR, Dulay RM, Kalaw SP, Reyes RG. Molecular identification and optimization of cultural conditions for mycelial biomass production of wild strain of *Chlorophyllum molybdites* (G. Mey) Massee from the Philippines. J Appl Biol Biotechnol, 2020; 8(6):1–6.

Garcia-Cruz F, Duran-Paramo E, Garin-Aguilar MA, del Toro GV, Chairez I. Parametric characterization of the initial pH effect on the polysaccharides production by *Lentinula edodes* in submerged culture. Food Bioprod Process, 2020; 119:170–8.

Jacob JK, Kalaw SP, Reyes RG. Mycelial growth performance of three species of *Pleurotus* on coconut water gelatin. Curr Res Environ Appl Mycol, 2015; 5(3):263–8.

Jaszek M, Osinska-Jaroszuk M, Sulej J, Matuszewska A, Stefaniuk D, Maciag K, Polak J, Matuszewski L, Grzywnowicz K. Stimulation of the antioxidative and antimicrobial potential of the blood red bracket mushroom *Pycnoporus sanguineus* (higher Basidiomycetes). Int J Med Mushrooms, 2015; 17(8):701–12.

Kalaw SP, Alfonso DO, Dulay RM, De Leon AM, Undan JQ, Undan JR, Reyes RG. Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. Curr Res Environ Appl Mycol, 2016; 6(4):277–87.

Kalpanadevi C, Singh V, Subramanian R. Influence of milling on the nutritional composition of bran from different rice varieties. J Food Sci Technol, 2018; 55(6):2259–69.

Landingin HR, Francisco BE, Dulay RM, Kalaw S, Reyes R. Optimization of culture conditions for mycelial growth and basidiocarp production of *Cyclocybe cylindracea* (Maire). CLSU Int J Sci Technol, 2020; 4(1):1–7.

Lee BC, Bae JT, Pyo HB, Choe TB, Kim SW, Hwang HJ, Yun JW. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. Enzyme Microb Technol, 2004; 35(5):369–76.

Lima ADL, Costa Fortes R, Garbi Novaes MRC, Percario S. Poisonous mushrooms; a review of the most common intoxications. Nutr Hosp, 2012; 27(2):402–8.

Lin Z. Grass (Juncao). mushroom grower's handbook 1: oyster mushroom cultivation. MushWorld-Heineart Incorporated, Seoul, South Korea, pp 107–13, 2004.

Marim RA, Oliveira AC, Marquezoni RS, Servantes JP, Cardoso BK, Linde GA, Colauto NB, Valle JS. Use of sugarcane molasses *by Pycnoporus sanguineus* for the production of laccase for dye decolorization. Genet Mol Res, 2016; 15(4) :1–9.

Mendoza WC, Dulay RM, Valentino MJ, Reyes RG. Mycelial biomass and biological activities of Philippine mushroom *Pycnoporus sanguineus* in time-course submerged culture. J Appl Biol Biotechnol, 2020; 8(05):88–93.

Miao W, He L, Zhang T, Li C. Lentinan impairs the early development of Zebrafish embryos, possibly by disrupting glucose and lipid metabolism. Processes, 2022; 10(1):120.

Nagel R. Dart: the embryo test with zebrafish *Danio rerio*- a general method in eco-toxicology and toxicology. Altex, 2002; 19(1/02):38–48.

Nagendra Prasad MN, Sanjay KR, Shravya Khatokar M, Vismaya MN, Nanjunda Swamy S. Health benefits of rice bran-a review. J Nutr Food Sci, 2011; 1(3):1–7.

Narh C, Frimpong C, Mensah A, Wei Q. Rice bran, an alternative nitrogen source for *Acetobacter xylinum* bacterial cellulose synthesis. Bioresources, 2018; 13(2):4346–63.

Park JP, Kim SW, Hwang HJ, Yun JW. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. Lett Appl Microbiol, 2001; 33(1):76–81.

Peng J, Lee C, Tsai Y. Effect of rice bran on the production of different king oyster mushroom strains during bottle cultivation. J Agric Res China, 2000; 49(3):60–7.

Piet M, Zając A, Paduch R, Jaszek M, Frant M, Stefaniuk D, Matuszewska A, Grzywnowicz K. Chemopreventive activity of bioactive fungal fractions isolated from milk-supplemented cultures of *Cerrena unicolor* and *Pycnoporus sanguineus* on colon cancer cells. 3 Biotech, 2021; 11(1):1–3.

Pointing SB, Jones EB, Vrijmoed LL. Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid culture. Mycologia, 2000; 92(1):139–44.

Pointing SB, Vrijmoed LP. Decolorization of azo and triphenylmethane dyes by *Pycnoporus sanguineus* producing laccase as the sole phenoloxidase. World J Microbiol Biotechnol, 2000; 16:317–8.

Poyedinok NL, Mykhailova OB, Shcherba VV, Buchalo AS, Negriyko AM. Light regulation of growth and biosynthetic activity of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae), in pure culture. Int J Med Mushrooms, 2008; 10(4) :369–78.

Quiroz-Castañeda RE, Balcazar-Lopez E, Dantan-Gonzalez E, Martinez A, Folch-Mallol J, Martinez Anaya C. Characterization of cellulolytic activities of *Bjerkandera adusta* and *Pycnoporus sanguineus* on solid wheat straw medium. Electron J Biotechnol, 2009; 12(4):5–6.

Quiroz-Castaneda RE, Perez-Mejia N, Martinez-Anaya C, Acosta-Urdapilleta L, Folch-Mallol J. Evaluation of different lignocellulosic substrates for the production of cellulases and xylanases by the basidiomycete fungi *Bjerkandera adusta* and *Pycnoporus sanguineus*. Biodegradation, 2011; 22(3):565–72.

Rosa LH, Machado KM, Jacob CC, Capelari M, Rosa CA, Zani CL. Screening of Brazilian basidiomycetes for antimicrobial activity. Mem Inst Oswaldo Cruz, 2003; 98(7):967–74.

Sharma V, Jaitly AK. Optimization of growth of two wild species of *Pycnoporus* collected from foothill of Uttarakhand. Int J Agric Innov Res, 2017; 6:2319–1473.

Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. J Pharmacogn Phytotherapy, 2013; 1(6):168–82.

Shu CH, Lung MY. Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. Process Biochem, 2004; 39(8):931–7.

Smania EF, Smania Jr A, Loguercio-Leite C, Gil ML. Optimal parameters for cinnabarin synthesis by *Pycnoporus sanguineus.* J Chem Technol Biotechnol Int Res Process Environ Clean Technol, 1997; 70(1):57–9.

Sogan MM, Maslang JA, Dulay RM. Myco-Chemicals and teratogenic activity of wild mushroom *Trichaleurina celebica* from Mt. Palali, Quezon, Nueva Vizcaya, Luzon Island, Philippines. CLSU Int J Sci Technol, 2018; 3(2):17–23.

Souza EF, Passos DF, Souto F, Calado V, Pereira N. Enhancement of kraft lignin molecular relaxation based on laccases from *Pycnoporus sanguineus* produced in instrumented bioreactors. Biomass Convers Biorefin, 2021; 28:1–2.

Tamboli DP, Lee DS. Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram-positive and gram-negative bacteria. J Hazard Mater, 2013; 260:878–84.

Teoh YP, Don MM, Ujang S. Media selection for mycelia growth, antifungal activity against wood-degrading fungi, and GC-MS study by *Pycnoporus sanguineus*. BioResources, 2011; 6(3):2719–31.

Tuong TD, Chu DX, Dieu BT. Antioxidant activity of fruiting body extracts from *Pycnoporus sanguineus* (L.: Fr.) Murrill mushroom. Vietnam J Sci Technol, 2020; 58(2):143–54.

Velioglu Z, Urek Ro. Biosurfactant production by *Pleurotus ostreatus* in submerged and solid-state fermentation systems. Turk J Biol, 2015; 39(1):160–6.

Vieira GR, Liebl M, Tavares LB, Paulert R, Smania Júnior A. Submerged culture conditions for the production of mycelial biomass and antimicrobial metabolites by *Polyporus tricholoma* Mont. Braz J Microbiol, 2008; 39:561–8.

Wang SJ, Zhong JJ. Bioreactor engineering. In: Yang ST (ed.). Bioprocessing for value-added products from renewable resources. Elsevier, Amsterdam, The Netherlands, pp 131–61, 2007.

Watanabe RA, Sales PD, Campos LC, Garcia TA, Valadares MC, Schimidt F, Santiago MF. Evaluation of the use of *Pycnoporus sanguineus* fungus for phenolics and genotoxicity decay of a pharmaceutical effluent treatment. Rev Ambiente Agua, 2012; 7:41–50.

Yahaya YA, Don MM. *Pycnoporus sanguineus* as potential biosorbent for heavy metal removal from aqueous solution: a review. J Phys Sci, 2014; 25(1):1.

Zadrazil F. Cultivation of *Pleurotus*. The biology and cultivation of edible mushrooms by ST Chang and WA Hayes. Academic press Incorporated, Orlando, FL, vol. 1, pp 521–58, 1978.

Zetelaki K, Vas K. The role of aeration and agitation in the production of glucose oxidase in submerged culture. Biotechnol Bioeng, 1968; 10(1):45–59.

How to cite this article:
Aguilar JS, Dulay RMR, Kalaw SP, Reyes RG. Optimal liquid culture conditions and bioactivity of *Pycnoporus sanguineus*. J Appl Pharm Sci, 2023; 13(04):096–104.