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*Research article*

# Submerged fermentation of mycelial biomass and exopolysaccharide of Philippine *Lentinus tigrinus*: FTIR spectroscopy and bioactivity profiling

**Rich Milton R. Dulay[1,2], Eduard M. Manzano[1]**

[1]Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines
[2]Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

Corresponding author e-mail: richmiltondulay@clsu.edu.ph

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**Abstract**
*Lentinus tigrinus* (tiger sawgill mushroom) is an edible mushroom known for its high nutritional value. This study highlights the optimized submerged fermentation conditions for *L. tigrinus* to enhance mycelial biomass and exopolysaccharide (EPS) production, identifying mango puree as the most suitable culture medium. Optimal parameters were established at pH 7.0, 100 rpm agitation, and 10–15 days of incubation. Fourier transform infrared (FTIR) analysis revealed major absorption peaks at 3291.66, 2927.90, 1614.83, 1411.92, 1257.60, and 1043.35–416.27 $cm^{-1}$, indicating the presence of hydroxyl, carbonyl, amino, and ether groups typical of β-D-glucans and protein–polysaccharide conjugates. Enzyme-based assays showed low inhibitory activity against α-glucosidase, acetylcholinesterase, and cyclooxygenase 1 and 2. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay demonstrated strong radical scavenging activity in the mycelial (62.69%) and EPS (50.76%) extracts. Brine shrimp lethality tests revealed dose-dependent toxicity in the mycelial extract but benign toxicity in EPS. Zebrafish embryo assays confirmed concentration-dependent embryotoxicity in the mycelial extract, while EPS exhibited minimal developmental effects, underscoring its biocompatibility. The mycelial extract also showed antibacterial activity against *Staphylococcus aureus* (8.83 mm). Overall, *L. tigrinus* demonstrates promise as a sustainable Philippine source of bioactive metabolites and polysaccharides with potential nutraceutical and pharmaceutical applications.

**Keywords**
Antibacterial, antioxidant, cytotoxicity, enzyme-based assay, teratogenicity

## Introduction

Macrofungi, a diverse group of filamentous fungi with well-developed fruiting bodies, play essential roles in terrestrial ecosystems and have growing significance across scientific and applied fields. Ecologically, they contribute to nutrient recycling through the decomposition of complex organic matter, enhance soil structure and fertility, and form symbiotic associations with plants (Sivanandhan


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et al., 2017; Niego et al., 2022). Beyond their ecological roles, macrofungi are valued for nutrition, traditional medicine, and biotechnological applications. Their fruiting bodies and mycelia contain polysaccharides, phenolics, and terpenoids, which underlie documented antioxidant, antimicrobial, antitumor, and immunomodulatory activities (Ooi and Liu, 2000; Wasser, 2002; Zaidman et al., 2005; Petrova et al., 2008; De Silva et al., 2012; Dulay et al., 2014a). These bioactive and nutritional properties have driven the incorporation of macrofungi into functional foods and nutraceuticals, as well as their use in environmental and industrial processes such as bioremediation, enzyme production, and the creation of sustainable biomaterials (Elsacker et al., 2020; Joshi et al., 2020).

Within this group, the genus *Lentinus* (Polyporaceae, Basidiomycota) is notable for its global distribution, edibility, and biotechnological relevance. Several species are commercially cultivated for culinary purposes and are valued for pharmacological properties, including antioxidant, anticancer, antibacterial, hypolipidemic, and immune-enhancing activities, largely attributed to polysaccharides and secondary metabolites (Dulay et al., 2017; Fabros et al., 2022). Additionally, *Lentinus* species are increasingly explored for bioconversion applications, particularly due to their ability to degrade lignocellulosic substrates, enabling the transformation of agricultural residues into valuable biomass products (De Leon et al., 2013).

The species *Lentinus tigrinus* (Bull.) Fr., commonly referred to as the tiger sawgill mushroom, remains relatively understudied but exhibits promising biological and biotechnological properties. This white-rot fungus typically colonizes fallen logs and decaying plant material in forest ecosystems, contributing to wood decay and nutrient cycling (Dulay et al., 2012a). Although naturally distributed across tropical and temperate regions, recent studies have focused on wild strains from the Philippines. These local strains have been successfully cultivated using agro-industrial wastes such as rice straw, sawdust, and rice bran, as well as liquid media derived from coconut water and cucurbit extracts, demonstrating the species' adaptability to sustainable cultivation systems (Dulay et al., 2012a; De Leon et al., 2013; Liwanag et al., 2020).

Experimental evidence has shown that *L. tigrinus* holds significant bioactive properties. Aqueous and ethanolic extracts of its fruiting bodies and mycelia have exhibited notable antioxidant activity and total phenolic content measurements (Dulay et al., 2014a; Dulay et al., 2015a; Sevindik, 2018). In addition, its antibacterial activity has been documented, along with hypoglycemic effects in diabetic murine models, suggesting pharmacological relevance (Dulay et al., 2014a; Dulay et al., 2017). Toxicity tests have also demonstrated the safety of its consumption, further supporting its value as a functional food. Importantly, *L. tigrinus* mycelia have shown capacity for heavy metal accumulation, which may lend itself to environmental applications such as bioremediation (Liwanag et al., 2020). From a nutritional perspective, the species is a rich source of protein, dietary fiber, and various macro- and micronutrients, underscoring its potential role in improving dietary health (Wang et al., 2014; Kumar et al., 2021). Despite these encouraging findings, the current literature on *L. tigrinus* remains relatively limited. Most existing studies have focused on cultivation protocols, proximate composition, and initial bioactivity screens, with few investigations delving into its metabolite diversity, enzymatic capabilities, or mechanisms of biological action. Comprehensive toxicological evaluations, structural analyses of its active compounds, and studies assessing its broader industrial applicability are still lacking (Dulay et al., 2012b; Fabros et al., 2022). Furthermore, genetic diversity assessments and large-scale production trials are necessary to determine its suitability for commercial-scale functional food or pharmaceutical development. Geographic records

on its domestication and ecological function outside specific regions in the Philippines also remain sparse (Kalaw et al., 2021).

In light of these research gaps, the present study aims to expand the scientific foundation concerning *L. tigrinus* by investigating its growth physiology, nutritional attributes, and enzymatic activity under varied culture conditions. A key focus of this research is the evaluation of its bioactive potential, with the aim of informing practical utilization. By shedding light on the multifaceted value of *L. tigrinus*, this work supports its integration into food systems, therapeutic applications, and environmentally sustainable technologies. Ultimately, the findings may contribute not only to advancing fungal biotechnology but also to biodiversity conservation and resource utilization strategies in the context of Philippine and global mycology.

## Materials and Methods

*Mushroom source*

The fungal strain *L. tigrinus* DQS75 (ITS GenBank accession number OM102521) was sourced from the official culture repository of the Center for Tropical Mushroom Research and Development (CTMRD) at Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Mycelial blocks were aseptically inoculated onto potato dextrose agar (PDA) plates and incubated at 30 °C for a period of seven days. Following incubation, 10 mm diameter mycelial discs were obtained using a flame-sterilized cork borer and subsequently employed as inoculants for the optimization studies.

*Optimization of intrinsic and extrinsic factors of mycelia and exopolysaccharide*

To improve the production of mycelial biomass and exopolysaccharides (EPS) by *L. tigrinus* under submerged fermentation, key intrinsic and extrinsic parameters were systematically optimized using a one-factor-at-a-time (OFAT) approach. The intrinsic parameters included the type of culture medium and initial pH, while the extrinsic parameters involved agitation speed and fermentation time.

Three liquid culture media namely: mango puree (MP), mature coconut water (CW), and a combination of both, were initially evaluated for their capacity to support mycelial growth and EPS synthesis. For each medium, 100 mL was dispensed into 250 mL Erlenmeyer flasks, sterilized at 121 °C and 15 psi for 30 min, inoculated with 10 mm mycelial discs under aseptic conditions, and incubated at 30 °C in a rotary shaker at 100 rpm for 10 days. Post-incubation, mycelial biomass was collected through filtration, washed with distilled water, air-dried, and quantified as dry weight (mg 100 $mL^{-1}$). The remaining spent liquid culture was used for EPS extraction and quantification. The medium yielding the highest biomass and EPS yield was selected for subsequent optimization. To determine the optimal pH, the selected medium was adjusted to pH levels of 4, 5, 6, 7, and 8 using 1 M NaOH or 1 M HCl. Following pH optimization, agitation effects were evaluated by incubating cultures at static (0 rpm), 50, 100, 150, and 200 rpm for 10 days. Lastly, fermentation periods of 5, 10, 15, and 20 days were assessed using the previously optimized medium, pH, and agitation speed. All experiments were conducted at 30 °C in a rotary shaker incubator. Mycelial dry weight and EPS yield were measured for each treatment. Each experimental condition was performed in triplicate to ensure reproducibility and statistical reliability.

*Mass production*

Mycelial biomass and EPS of the mushroom were mass produced using the previously optimized culture parameters. This approach facilitated the subsequent extraction of bioactive compounds from the mycelia and enabled efficient recovery of EPS.

*Preparation of mycelial extracts*

Bioactive constituents from *L. tigrinus* mycelia were extracted using ethanol as the solvent. The extraction protocol was based on the methods described by Dulay et al. (2014a) and Boukes et al. (2017), with slight modifications. Briefly, 5 g of finely powdered mycelia were soaked in 200 mL of 95% ethanol and incubated for 48 h. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was evaporated to dryness using a rotary evaporator. The resulting dried extracts were stored and subsequently used for bioactivity assessments.

*Isolation of exopolysaccharide*

The isolation of EPS was conducted following the procedure described by Angelova et al. (2021), with minor modifications to accommodate the specific experimental conditions. The method began with the separation of mycelial biomass from the culture broth through filtration using Whatman No. 1 filter paper. The resulting filtrate was subjected to centrifugation, and the supernatant was carefully collected. EPS precipitation was induced by gradually adding cold 95% ethanol to the supernatant in a 2:1 volume ratio. The mixture was incubated at 3 °C for 24 h to ensure complete precipitation. The obtained precipitate was rinsed thoroughly with distilled water, dried at 30 °C for 24 h, and subsequently weighed to determine the EPS yield.

*Fourier transform infrared spectroscopy*

Fourier Transform Infrared (FTIR) Spectroscopy was employed to characterize the chemical composition of the EPS obtained from *L. tigrinus*. The spectral analysis was outsourced and performed at the Nanotech Research and Development Facility, Central Luzon State University, located in the Science City of Muñoz, Nueva Ecija, Philippines.

*Alpha-glucosidase inhibition assay*

The alpha-glucosidase inhibitory activity was evaluated following the method described by Naing et al. (2019). Each test sample was initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 10,000-µg $mL^{-1}$ stock solution, from which a 300-µg $mL^{-1}$ working solution was subsequently prepared. Solutions were homogenized using a vortex mixer, sonicated, and centrifuged to ensure complete dissolution and clarity. The assay was conducted in 96-well quartz microplates, with each well containing a final volume of 250 µL: 190 µL of 50 mM sodium phosphate buffer (pH 6.8) with 100 mM NaCl, 50 µL of a 120 mU $mL^{-1}$ alpha-glucosidase enzyme solution, and 10 µL of the working test sample. All treatments were performed in duplicate with two independent trials (n = 2, t = 2). Acarbose at a final concentration of 1000 µg $mL^{-1}$ per well was used as the positive control, while the negative control consisted of 5% DMSO in PBS, resulting in a final DMSO concentration of 3.33% per well. Following a 10-minute incubation at 37 °C, 50 µL of 1.86 mM p-nitrophenyl-α-D-glucopyranoside was added to initiate the reaction. The production of p-nitrophenol was measured at 405 nm every 30 seconds for 30 minutes using a Multiskan Go® UV-Vis spectrophotometer. The

degree of enzyme inhibition was determined from the average slope of absorbance change over time. Percent inhibition was calculated using the formula:

$$\% \text{ Inhibitory Activity} = [(\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}) / \text{Slope}_{\text{uninhibited}}] \times 100$$

where $\text{Slope}_{\text{uninhibited}}$ corresponds to the negative control and $\text{Slope}_{\text{inhibited}}$ corresponds to the slope obtained for either the test samples or the positive control.

*Cyclooxygenase inhibition assay*

Cyclooxygenase inhibitory activity was determined using the method of Opog and Amor (2019). Each sample was initially dissolved in dimethyl sulfoxide (DMSO) to yield a 10,000-µg $mL^{-1}$ stock solution, from which a 200 µg $mL^{-1}$ working solution was prepared. Homogenization was performed sequentially using a vortex mixer, sonicator, and centrifugation to ensure uniform dispersion of the test materials. An enzyme-cofactor solution was prepared by combining 5184 µL of 100 mM Tris-HCl buffer (pH 8.0), 96 µL of COX enzyme solution (containing 250 U $mL^{-1}$ of either human recombinant COX-2 or sheep-derived COX-1), and 480 µL of 20 µM Hematin in a clean scintillation vial. The mixture was gently mixed to achieve homogeneity. In a 96-well microplate, 50 µL of Tris buffer was first dispensed into each well, followed by the addition of 120 µL of the enzyme-cofactor solution. Subsequently, 10 µL of the test sample, 160 mM indomethacin (positive control), or DMSO (negative control) was introduced. All test conditions maintained a final solvent concentration of 5% DMSO. Each treatment was evaluated in duplicate across two independent experiments (n = 2, t = 2). The plate was incubated at 25 °C for 15 min to facilitate enzyme-inhibitor interactions. Thereafter, the reaction was initiated by the sequential addition of 10 µL of 200 µM Amplex Red and 10 µL of 2000 µM arachidonic acid. Wells were gently mixed and purged with nitrogen gas to reduce oxidative interference. Fluorescence was measured over a 2-min interval using a CLARIOstar microplate reader, with excitation at 535 nm and emission at 590 nm, recording data every 12 s. COX inhibitory activity was determined by calculating the mean reaction slopes from fluorescence measurements. Percent inhibition was computed using the equation:

$$\% \text{ Inhibitory Activity} = [(\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}) / \text{Slope}_{\text{uninhibited}}] \times 100$$

where $\text{Slope}_{\text{uninhibited}}$ represents the average slope from the negative control group, and $\text{Slope}_{\text{inhibited}}$ corresponds to the slope observed in wells treated with either the test sample or indomethacin.

*Acetylcholinesterase inhibition assay*

Test solutions were initially prepared by dissolving the samples in methanol to yield a stock concentration of 10,000 µg $mL^{-1}$. From this, a 2,000-µg $mL^{-1}$ working solution was obtained. To ensure uniform dispersion, the solutions underwent vortexing, sonication, and centrifugation. The assay was performed in a 96-well microplate format. Each well was loaded with 180 µL of 100 mM phosphate buffer (pH 8.0), 10 µL of *Electrophorus electricus* acetylcholinesterase (AChE) at a final enzymatic activity of 1.0 U $mL^{-1}$, and 80 µL of Ellman's reagent, which comprised 10.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 17.9 mM sodium bicarbonate in buffer adjusted to pH 7.0. Subsequently, 15 µL of either the test sample, a 100 µg $mL^{-1}$ galantamine solution (positive

control), or methanol (negative control) was added. The final methanol concentration was standardized to 5% in all wells. Each condition was tested in duplicate across two independent experimental runs (n = 2, t = 2). Following the assembly of the reaction mixtures, the plate was incubated at 25 °C for 15 min to facilitate enzyme-inhibitor interaction. The reaction was initiated by the addition of 15 µL of 2.87 mM acetylthiocholine iodide (AChI). Absorbance readings were captured at 420 nm using a Multiskan microplate reader (Thermo Scientific), with measurements taken at 25-second intervals for a total of 31 readings. The inhibitory effect was determined by calculating the average slope of the absorbance over time for each replicate group. The percentage of inhibition was computed using the formula:

$$\% \text{ Inhibitory Activity} = [(\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}) / \text{Slope}_{\text{uninhibited}}] \times 100$$

where $\text{Slope}_{\text{uninhibited}}$ refers to the rate derived from the negative control, and $\text{Slope}_{\text{inhibited}}$ corresponds to that of the test samples or the reference inhibitor.

*DPPH radical scavenging assay*

The antioxidant potential of the samples was evaluated using the DPPH radical scavenging assay, following the protocol established by Kolak et al. (2006), with slight modifications. Stock solutions were prepared by dissolving 50 mg of either mycelial or EPS extracts in 50 mL of ethanol, resulting in a concentration of 1 mg $mL^{-1}$. A standard solution of ascorbic acid was also prepared in the same manner and concentration. The DPPH reagent was prepared by dissolving 6 mg of DPPH in 100 mL of ethanol. For each assay, 1.5 mL of the extract or standard solution was mixed with 2.5 mL of the DPPH solution in individual plastic cuvettes. All reactions were conducted in triplicate. The reaction mixtures were incubated at 37 °C for 30 min in the dark. Subsequently, the absorbance was measured at 517 nm using a HALO SB-10 Single Beam UV-Visible spectrophotometer. The radical scavenging activity (%RSA) was calculated using the formula:

$$\% \text{ RSA} = [(Ac - As) / Ac] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

*Brine shrimp lethality assay*

The initial evaluation of the cytotoxic activity of the mushroom extract was conducted using the Brine shrimp lethality assay. Artificial seawater was prepared by dissolving 25 g of non-iodized salt in 1 L of distilled water and filtering the solution to obtain a clear saline medium. Brine shrimp (*A. salina*) eggs were then added to this solution, and continuous illumination was provided to promote hatching. After a 36-h incubation period, viable nauplii were collected for use in the toxicity test, as described by Baraza et al. (2009). To prepare the test solutions, the sample extracts was dissolved in dimethyl sulfoxide (DMSO) to produce a stock concentration of 100,000 µg $mL^{-1}$, achieved by dissolving 0.1 g of the extract in 9.9 mL of the saline solution. From this stock, serial dilutions were made to obtain the following concentrations: 10,000, 1,000, 100, 10, and 1 µg $mL^{-1}$. Each concentration, along with a negative control (saline solution), was dispensed into individual wells of a sterile ELISA plate. All treatments were performed in triplicate to ensure experimental reliability. Following the modified

method of Baravalia et al. (2012), ten *A. salina* nauplii were transferred into each well. The plates were then incubated at room temperature (26–28 °C) under static conditions for 24 h. After incubation, mortality was assessed by counting the number of dead nauplii per well. Percent mortality was calculated using the formula:

$$\% \text{ Mortality} = (\text{Number of dead nauplii} / \text{Total number of nauplii}) \times 100.$$

*Zebrafish embryotoxicity and teratogenicity assay*

The embryotoxic and teratogenic effects of the test extracts were assessed using zebrafish (*Danio rerio*) embryos, in accordance with the protocol established by Dulay et al. (2012c). Serial dilutions of the extract were prepared in embryo water to achieve final concentrations of 0.1, 1, 10, 100, 1,000, 10,000, and 100,000 µg $mL^{-1}$. Two control groups were included: a negative control (embryo water only) and a positive control (ethanol-treated). Each test and control solution (200 µL) were dispensed into separate wells of a sterile ELISA plate.

Embryos at the segmentation stage were carefully transferred into each well, with four embryos assigned per treatment. The plates were maintained at 26 °C for 48 h. After incubation, embryo hatchability and mortality were documented, and $LC_{50}$ values were computed using probit analysis. Teratogenic evaluations were subsequently performed 72 h post-treatment exposure (hpte). Morphological observations were examined under a compound microscope and classified based on the criteria described by Nagel (2002). Embryos were categorized as (1) lethal: displaying coagulation, incomplete tail detachment, absence of somites, or lack of heartbeat; (2) teratogenic: exhibiting head, heart, or tail malformations, spinal deformities (e.g., scoliosis), yolk deformities, or growth retardation; or (3) normal development. Heartbeat rates were also recorded. The assay was considered valid only when all embryos in the negative control group developed normally throughout the observation period.

*Disc diffusion assay*

A modified disc diffusion method based on the protocol established by Bauer et al. (1966) was utilized to assess the antibacterial activity of the extracts. *Staphylococcus aureus* and *Escherichia coli* were used as representative Gram-positive and Gram-negative bacterial strains, respectively. Each strain was cultivated in 9 mL of nutrient broth and incubated at 37 °C for 24 h. The bacterial cultures were then adjusted to the 0.5 McFarland standard, corresponding to a cell concentration of approximately $1.5 \times 10^8$ CFU $mL^{-1}$.

Sterile filter paper discs of 5 mm in diameter were prepared by punching Whatman No. 1 filter paper, followed by sterilizing at 121 °C and 15 psi for 15 min. These discs were soaked in *L. tigrinus* mycelial or EPS extracts and subsequently air-dried under aseptic conditions. Streptomycin sulfate served as the positive control, whereas 95% ethanol utilized as the extraction solvent was used as the negative control. Mueller-Hinton agar was prepared, sterilized, and poured into sterile Petri dishes to solidify. Bacterial suspensions were evenly spread on the agar surface using sterile cotton swabs, and the plates were allowed to dry for approximately five minutes. Treated and control discs were placed equidistantly on the inoculated agar to avoid overlapping inhibition zones. All treatments were performed in triplicate and incubated at 37 °C for 24 h. After incubation, the diameters of the inhibition zones were measured using a Vernier caliper. Antibacterial activity was inferred by

comparing the inhibition zones of the extracts to those of the control treatments. A clear zone around the disc indicated bacterial susceptibility, while the absence of such a zone suggested resistance or inactivity.

*Statistical analysis*

The data were subjected to analysis of variance (ANOVA) following a completely randomized design at a 5% level of significance using Minitab® 21 Statistical Software. To determine significant differences among treatment means, Tukey's Honest Significant Difference (HSD) test was employed.

## Results

*Intrinsic and extrinsic factors influence in mycelia and exopolysaccharide*

This study systematically evaluated the effects of intrinsic and extrinsic factors on the submerged fermentation performance of *L. tigrinus*, focusing on mycelial biomass and exopolysaccharide (EPS) production. The experimental approach followed a stepwise design, in which intrinsic factors were first assessed before testing extrinsic factors. Among the culture media tested, differences in biomass and EPS yields were observed (Table 1). Mango puree (MP) supported the highest mycelial biomass (1473.90 mg) and EPS production (87.70 mg), whereas coconut water (CW) resulted in lower biomass (678.33 mg) and EPS (18.37 mg). A 1:1 (v/v) mixture of MP and CW produced intermediate yields. These results demonstrate comparative performance among the tested media, with MP performing relatively better under the experimental conditions, and were used to guide the choice of medium for subsequent experiments.

Following the optimization of the culture medium, the initial pH of the MP medium was evaluated. The data showed that pH 7.0 yielded the highest biomass (1791.00 mg) and EPS production (127.90 mg). Although mycelial growth remained statistically comparable across the pH range of 4.0 to 8.0, EPS production showed greater variability, with pH 6.0 and pH 8.0 supporting moderate yields. These results confirmed that a neutral pH was optimal for enhancing both growth and EPS biosynthesis under the selected culture conditions. Once the intrinsic factors were optimized, the study proceeded to examine the influence of extrinsic factors, namely agitation rate and incubation period. Among the agitation rates tested, 100 rpm proved most favorable, resulting in the highest mycelial biomass (1835.43 mg) and EPS production (130.79 mg). Both lower (0–50 rpm) and higher (200 rpm) agitation rates were less effective, underscoring the importance of moderate agitation for optimal oxygen transfer and nutrient distribution. Finally, the incubation period was evaluated to determine the ideal cultivation duration under the optimized intrinsic and agitation conditions. The highest biomass yield was achieved after 10 days of incubation (1880.29 mg), while the maximum EPS production was recorded at 15 days (135.87 mg). However, extending incubation to 20 days led to reductions in both biomass and EPS yields, likely due to nutrient depletion or accumulation of inhibitory metabolites. This study successfully established the optimal combination of intrinsic (culture medium and pH) and extrinsic (agitation rate and incubation period) factors for maximizing the biomass and EPS production of *L. tigrinus* under submerged fermentation.

**Table 1** – *L. tigrinus* mycelial dry weight and exopolysaccharide under varying intrinsic and extrinsic conditions.

| | | **Biomass yield (mg d.w. 100 mL$^{-1}$)** | **EPS yield (mg d.w. 100 mL$^{-1}$)** |
|---|---|---|---|
| Intrinsic factors | Culture media | | |
| | CW | 678.33±80.25$^{c}$ | 18.37±5.52$^{c}$ |
| | MP | 1473.90±86.56$^{a}$ | 87.70±5.66$^{a}$ |
| | Combination | 1158.13±30.08$^{b}$ | 49.17±4.31$^{b}$ |
| | pH level | | |
| | 4.0 | 1537.83±222.51$^{a}$ | 79.60±7.71$^{bc}$ |
| | 5.0 | 1541.43±98.48$^{a}$ | 62.83±19.45$^{c}$ |
| | 6.0 | 1697.63±243.95$^{a}$ | 109.30±7.99$^{ab}$ |
| | 7.0 | 1791.00±135.98$^{a}$ | 127.90±20.46$^{a}$ |
| | 8.0 | 1538.07±222.93$^{a}$ | 93.47±7.46$^{abc}$ |
| Extrinsic factors | Agitation | | |
| | 0 rpm | 467.60±33.68$^{c}$ | 38.73±5.97$^{bc}$ |
| | 50 rpm | 409.80±54.53$^{c}$ | 21.17±1.66$^{c}$ |
| | 100 rpm | 1835.43±135.98$^{a}$ | 130.79±20.46$^{a}$ |
| | 150 rpm | 1593.47±178.58$^{a}$ | 63.73±8.96$^{b}$ |
| | 200 rpm | 1011.30±110.79$^{b}$ | 60.80±10.14$^{b}$ |
| | Incubation period | | |
| | 5 days | 896.73±41.04$^{d}$ | 59.53±10.61$^{b}$ |
| | 10 days | 1880.29±59.94$^{a}$ | 65.93±9.00$^{b}$ |
| | 15 days | 1329.80±56.91$^{b}$ | 135.87±6.84$^{a}$ |
| | 20 days | 1054.70±38.27$^{c}$ | 73.13±3.98$^{b}$ |

Each value represents the mean ± standard deviation of tests conducted in triplicate (n = 3). Means with the same letters of superscript in each factor are not significantly different according to Tukey's HSD at 5% level of significance. Note: Coconut water (CW), Mango puree (MP), Combination, (1:1 v/v).

*Composition analysis (FTIR spectroscopy)*

Fourier Transform Infrared (FTIR) spectroscopy was employed to qualitatively examine the functional groups present in the EPS produced by *L. tigrinus*. FTIR is a widely used analytical technique for identifying characteristic molecular vibrations associated with major chemical functionalities in complex biological matrices, providing rapid and non-destructive analysis with minimal sample preparation (Dytkiewitz and Morlock, 2008; Agatonovic-Kustrin and Morton, 2020). The FTIR spectrum of *L. tigrinus* EPS is shown in Figure 1, and the corresponding peak positions and tentative functional group assignments are summarized in Table 2.

The broad absorption band observed at 3291.66 cm$^{-1}$ is primarily attributed to O–H stretching vibrations, which are characteristic of hydroxyl groups abundant in polysaccharides. A minor contribution from N–H stretching cannot be excluded but is considered secondary. The peak at 2927.90 cm$^{-1}$ corresponds to C–H stretching of aliphatic groups, commonly associated with carbohydrate backbones. The absorption band at 1614.83 cm$^{-1}$ is assigned predominantly to conjugated carbonyl (C=O) stretching and aromatic C=C vibrations. Although N–H bending has been reported in this region, the absence of a distinct amide II band near ~1540 cm$^{-1}$ suggests that protein or amino acid contributions are limited. Therefore, this band is interpreted mainly as arising from non-amide functional groups rather than indicating a substantial protein fraction.

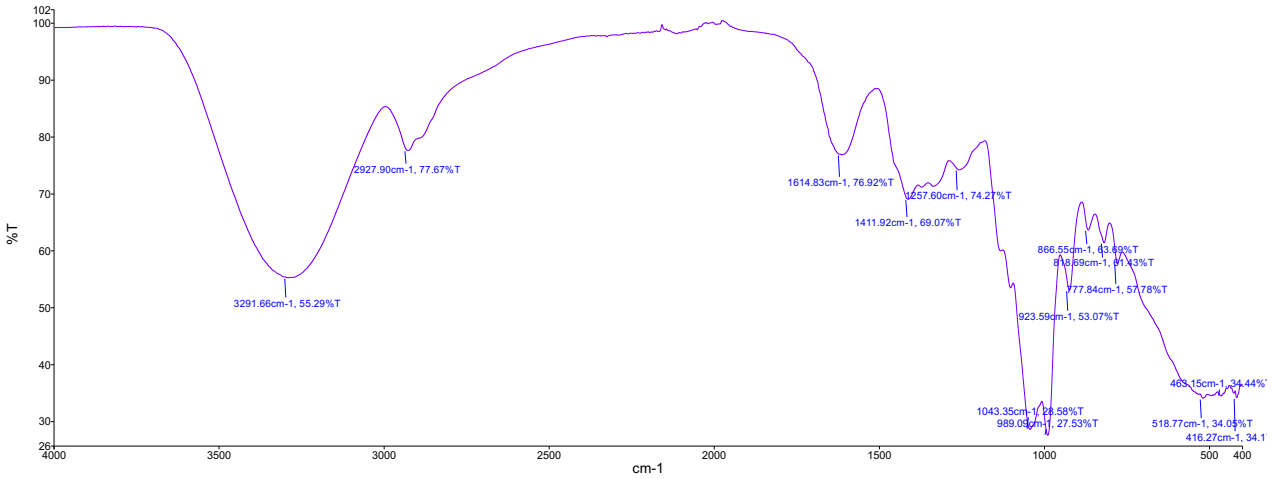


**Fig. 1.** – FTIR spectrum of *L. tigrinus* exopolysaccharide.

**Table 2** – FTIR spectral peak values, functional groups and the possible presence of secondary metabolites of *L. tigrinus* exopolysaccharide.

| Peaks ($cm^{-1}$) | Molecular vibration | Possible presence | References |
|---|---|---|---|
| 3291.66 | O–H/N–H stretching | Polysaccharides, Proteins | [a], [b] |
| 2927.90 | C–H stretching | Polysaccharides | [a], [b] |
| 1614.83 | C=O stretching, N–H bending | Proteins, Amino acids | [a], [b] |
| 1411.92 | Symmetric $COO^-$ stretching | Amino acids, Polysaccharides | [a], [b] |
| 1257.60 | C–O stretching | Polysaccharides | [a], [b] |
| 1043.35 | C–O, C–C stretching | Polysaccharides | [a], [b] |
| 989.09 | C–O stretching | Pyranose | [b] |
| 923.59 | C–O stretching | Pyranose | [b] |
| 866.55 | C–H bending | Pyranose | [b] |
| 818.69 | Ring deformation | Polysaccharides | [b] |
| 777.84 | Ring deformation | Polysaccharides | [b] |
| 518.77 | C–H bending | Proteins, CH groups | [c], [d] |
| 463.15 | C–H bending | CH groups | [c], [d] |
| 416.27 | Ring deformation | β-D-glucans | [b] |

[a] Movasaghi et al. (2008), [b] Yao et al. (2018), [c] Bhat et al. (2013), [d] Eskandari-Nojedehi et al. (2017)

Additional peaks at 1411.92 $cm^{-1}$ and 1257.60 $cm^{-1}$ are attributed to symmetric $COO^-$ stretching and C–O stretching vibrations, respectively, supporting the presence of carboxyl and ether functionalities commonly found in polysaccharides. Strong absorption bands within the region of 1043.35–416.27 $cm^{-1}$ correspond to C–O and C–C stretching, C–H bending, and ring deformation modes, which are typical of carbohydrate structures, including pyranose forms and β-linked polysaccharides. Several absorption bands, particularly those around 3290 and 1615 $cm^{-1}$, are relatively broad, suggesting overlapping contributions from hydroxyl groups, residual moisture, and possibly phenolic components. As comparative spectra were not included in this study, the FTIR results are interpreted as qualitative indicators of dominant functional groups rather than definitive compositional confirmation. Consequently, the assignments reflect the predominant chemical features of the EPS preparation rather than absolute purity. Further comparative or complementary analyses would be valuable to strengthen structural validation in future investigations.

*Assessment of enzyme-mediated inhibition activities*

The enzyme inhibitory potential of *L. tigrinus* mycelial and EPS extract was evaluated at a concentration of 10 µg $mL^{-1}$ against three clinically relevant enzymes: α-glucosidase, acetylcholinesterase (AChE), and cyclooxygenases (COX-1 and COX-2), which are pharmacological targets in the management of metabolic, neurodegenerative, and inflammatory disorders, respectively. As presented in Supplementary Table S1, both mycelial and EPS extracts demonstrated weak inhibitory activity against α-glucosidase, with inhibition rates of 0.83% and 2.30%, respectively. In contrast, the reference drug acarbose, a well-known α-glucosidase inhibitor used in diabetes therapy, exhibited a significantly higher inhibition of 88.76% ($p < 0.05$), confirming assay reliability. In the acetylcholinesterase assay, neither extract showed meaningful inhibition. The mycelial sample exhibited a negligible effect (–0.37%), while the EPS extract had no observable activity (0.00%). In comparison, galantamine, a clinically approved AChE inhibitor for Alzheimer's disease, produced a near-complete inhibition of 98.03% ($p < 0.05$), further validating the assay's sensitivity. Moreover, the mycelial extract showed no significant inhibitory activity against cyclooxygenase enzymes, with inhibition values of –10.82% for COX-1 and 2.36% for COX-2, indicating the absence of meaningful COX inhibition under the assay conditions. The EPS extract showed moderate inhibition of COX-1 at 15.72%, but no inhibitory effect was observed against COX-2 (–13.18%). In contrast, indomethacin, a standard nonsteroidal anti-inflammatory drug (NSAID), markedly suppressed both COX isoforms with 93.98% inhibition for COX-1 and 91.86% for COX-2 ($p < 0.05$).

*Antioxidant Activity*

The DPPH radical scavenging assay was employed to evaluate the antioxidant potential of the ethanolic extracts derived from the mycelia and EPS of *L. tigrinus*. As presented in Supplementary Table S2, both extracts demonstrated notable antioxidant activity, though they varied in efficacy. The mycelial extract exhibited a higher scavenging activity, reaching 62.69%, while the EPS extract displayed a lower value of 50.76%. In contrast, the reference standard ascorbic acid showed significantly stronger radical scavenging activity at 92.31%. Statistical analysis revealed that the differences among treatments were significant at the 5% level. These findings suggest that while both extracts possess antioxidant properties, the mycelial extract exhibits comparatively greater free radical neutralization at the tested concentration.

*Cytotoxicity effects*

The cytotoxic properties of *L. tigrinus* mycelial and EPS extracts were assessed using the brine shrimp lethality assay (BSLA), a well-established model for preliminary toxicity screening. Mortality of *Artemia salina* nauplii was measured after 24 h of exposure to the extract concentrations ranging from 0.1 to 100,000 µg $mL^{-1}$. As shown in Supplementary Table S3, the mycelial extract induced a clear, concentration-dependent toxic response. Complete mortality (100.00%) was observed at concentrations of 1,000 µg $mL^{-1}$ and above. Notably, toxicity decreased at lower doses, with moderate lethality recorded at 100 µg $mL^{-1}$ (60.00%) and 10 µg $mL^{-1}$ (46.67%), followed by significantly reduced mortality at 1 µg $mL^{-1}$ (23.33%) and 0.1 µg $mL^{-1}$ (13.33%). In contrast, the EPS extract exhibited minimal toxicity. The highest mortality observed was 23.33% at 100,000 µg $mL^{-1}$, which declined rapidly as the concentration decreased. From 1,000 µg $mL^{-1}$ down to 0.1 µg $mL^{-1}$, no

mortality was recorded, indicating that the EPS poses low or negligible cytotoxic risk within the tested range. Saline control showed no mortality, while ethanol used as a positive control resulted in complete lethality.

*Embryotoxicity and teratogenic effects*

The embryotoxic and teratogenic properties of *L. tigrinus* mycelial and EPS extracts were investigated using zebrafish (*Danio rerio*) embryos as an in vivo model. Embryos were exposed to various concentrations of each extract, and their viability and hatchability were monitored at intervals up to 48 h post-treatment exposure (hpte). As shown in Table 3, both extracts induced complete mortality (100%) at the highest tested concentration (100,000 µg $mL^{-1}$), with EPS-treated embryos reaching 83.33% mortality as early as 12 hpte. A clear dose-dependent response was observed across lower concentrations. Mycelial treatments at 100 to 10,000 µg $mL^{-1}$ consistently caused full embryo mortality by 36 hpte, while EPS treatments at these levels resulted in partial lethality ranging from 33.33% to 83.33% by 48 hpte. In contrast, exposures at 1 µg $mL^{-1}$ and below yielded markedly lower toxicity. At 0.1 µg $mL^{-1}$, both extracts exhibited minimal to no mortality, with the EPS group showing full hatchability (100%), statistically comparable to the embryo water control, while the mycelial group achieved a slightly reduced hatch rate of 83.33%.

In addition to mortality and hatchability, morphological abnormalities were documented following exposure to various dose levels (Table 4). Embryos exposed to medium and high doses of the mycelial extract exhibited teratogenic outcomes, including head malformations, scoliosis, and growth retardation. Notably, heart malformations were uniquely observed in embryos treated with medium to high concentrations of EPS. These effects were supported by heartbeat rates, wherein high-dose treatments led to bradycardia, especially in mycelial-treated embryos (93 beats $min^{-1}$), compared to controls (120–126 beats $min^{-1}$). Ethanol-treated embryos, used as a positive control, showed complete coagulation and absence of heartbeat. Figures 2 and 3 illustrate representative morphological endpoints observed after 72 hpte. High-dose mycelial treatments induced scoliosis and growth retardation, while medium doses led to head deformities. EPS exposure, on the other hand, caused cardiac malformations in both high- and medium-dose groups. Embryos treated with low doses (0.1–1 µg $mL^{-1}$) remained morphologically normal and comparable to untreated controls. Collectively, these findings indicate that *L. tigrinus* mycelial and EPS extracts elicit embryotoxic and teratogenic effects in a dose-dependent manner.

*Antibacterial Activity*

The antibacterial activity of *L. tigrinus* mycelial extract and its EPS extract was assessed against two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), using the disc diffusion method. The diameter of the resulting zones of inhibition served as the indicator of antibacterial efficacy, as summarized in Supplementary Table S4 and illustrated in Figure 4. Among the tested samples, only the mycelial extract exhibited measurable antibacterial activity, showing a moderate inhibitory effect against *S. aureus*, with an average zone of inhibition of 8.83 mm. In contrast, no inhibition zone was observed for *E. coli* treated with either the mycelial or EPS extracts. Similarly, the EPS extract demonstrated no antibacterial effect against either bacterial strain, indicating its limited bioactivity under the tested conditions. Statistical analysis confirmed

significant differences among the treatments at the 5% level of significance, supporting the observed variations in antibacterial response.

**Table 3** – Mortality of zebrafish embryos at 12, 24, 36, and 48 h, and hatchability at 48 h following exposure to varying concentrations of mycelia and exopolysaccharide extracts of *L. tigrinus*

| Extracts | Conc. | $LC_{50}$ | Mortality (%) | | | | Hatchability (%) |
|---|---|---|---|---|---|---|---|
| | (µg mL$^{-1}$) | (µg mL$^{-1}$) | 12 hpte | 24 hpte | 36 hpte | 48 hpte | 48 hpte |
| Mycelia | 100,000 | | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| | 10,000 | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| | 1,000 | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| | 100 | 19.56 | 0.00±0.00$^{b}$ | 33.33±1.53$^{b}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| | 10 | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 83.33±1.15$^{a}$ | 83.33±1.15$^{a}$ | 16.67±1.15$^{c}$ |
| | 1 | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 33.33±0.58$^{b}$ | 33.33±0.58$^{b}$ | 50.00±0.00$^{b}$ |
| | 0.1 | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 83.33±0.58$^{a}$ |
| | EW | | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 100.00±0.00$^{a}$ |
| | EtOH | | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| EPS | 100,000 | | 83.33±0.58$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |
| | 10,000 | | 75.00±0.00$^{a}$ | 83.33±0.58$^{a}$ | 83.33±0.58$^{ab}$ | 83.33±0.58$^{a}$ | 16.67±0.58$^{c}$ |
| | 1,000 | | 33.33±0.58$^{b}$ | 41.67±0.58$^{b}$ | 66.67±0.58$^{b}$ | 75.00±1.00$^{ab}$ | 25.00±1.00$^{c}$ |
| | 100 | 30.48 | 33.33±0.58$^{b}$ | 33.33±0.58$^{b}$ | 58.33±0.58$^{bc}$ | 66.67±0.58$^{abc}$ | 33.33±0.58$^{bc}$ |
| | 10 | | 16.67±0.58$^{bc}$ | 25.00±0.00$^{bc}$ | 33.33±0.58$^{c}$ | 41.67±0.58$^{bc}$ | 33.33±0.58$^{bc}$ |
| | 1 | | 0.00±0.00$^{c}$ | 16.67±0.58$^{bc}$ | 33.33±0.58$^{c}$ | 33.33±0.58$^{cd}$ | 66.67±0.58$^{ab}$ |
| | 0.1 | | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 100.00±0.00$^{a}$ |
| | EW | | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 100.00±0.00$^{a}$ |
| | EtOH | | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 100.00±0.00$^{a}$ | 0.00±0.00$^{c}$ |

Each value represents the mean ± standard deviation of tests conducted in triplicate (n = 3). Values within the same column that do not share the same superscript letter indicate statistically significant differences at the 5% level of significance using Tukey's HSD. Hours post-treatment exposure (hpte), Embryo water (EW), Ethanol (EtOH).

**Table 4** – Toxic and teratogenic effects, and alterations in heartbeat rate induced by mycelia and exopolysaccharide extracts at varying dose levels relative to the $LC_{50}$ of *L. tigrinus* in zebrafish embryos after 48 to 72 h of exposure

| Extract | Dose level | Toxic effects | | | Teratogenic effects | | | | | | | Heartbeat |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (µg mL$^{-1}$) | C | T | NH | NS | HM | TM | MHr | SC | DY | GR | (min$^{-1}$) |
| Mycelia | HD (1.96) | – | – | – | – | – | – | – | + | – | + | 93 |
| | MD (0.98) | – | – | – | – | + | – | – | + | – | – | 102 |
| | LD (0.49) | – | – | – | – | – | – | – | – | – | – | 117 |
| | EW | – | – | – | – | – | – | – | – | – | – | 120 |
| | EtOH | + | – | – | – | – | – | – | – | – | – | 0 |
| EPS | HD (3.05) | – | – | – | – | – | – | + | – | – | – | 99 |
| | MD (1.52) | – | – | – | – | – | – | + | – | – | – | 99 |
| | LD (0.76) | – | – | – | – | – | – | – | – | – | – | 111 |
| | EW | – | – | – | – | – | – | – | – | – | – | 126 |
| | EtOH | + | – | – | – | – | – | – | – | – | – | 0 |

(+) and (–) symbols denote the presence and absence, respectively, of toxic and teratogenic effects across the different dose levels. Dose levels (based on $LC_{50}$): High dose (HD), Medium dose (MD), Low dose (LD). Embryo water (EW), Ethanol (EtOH). Toxic effects: Coagulation (C), Tail not detached (T), No heartbeat (NH), No somites (NS). Teratogenic effects: Head malformation (HM), Tail malformation (TM), Malformation of heart (MHr), Scoliosis (SC), Deformity of yolk (DY), Growth retardation (GR).

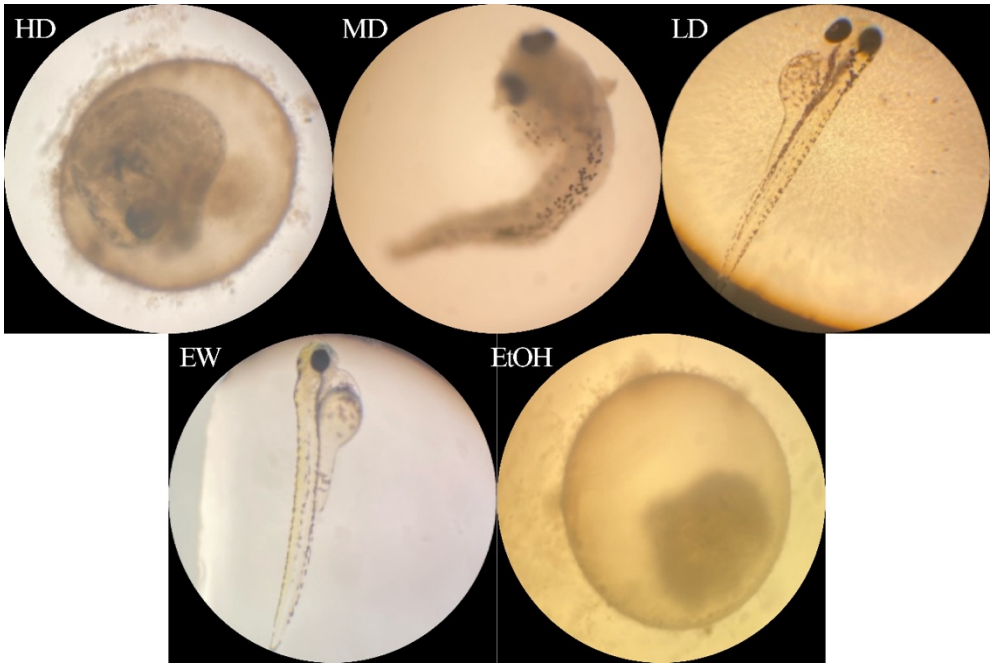


**Fig. 2.** – Morphological endpoints in zebrafish embryos exposed to different dose levels (based on $LC_{50}$) of ethanol extracts from the mycelia of *L. tigrinus* after 72 h of exposure. High dose (HD): scoliosis, and observed growth retardation, Medium dose (MD): head malformation, and scoliosis, Low dose (LD): no observed abnormalities, Embryo water (EW): normal embryo, Ethanol (EtOH): coagulated.

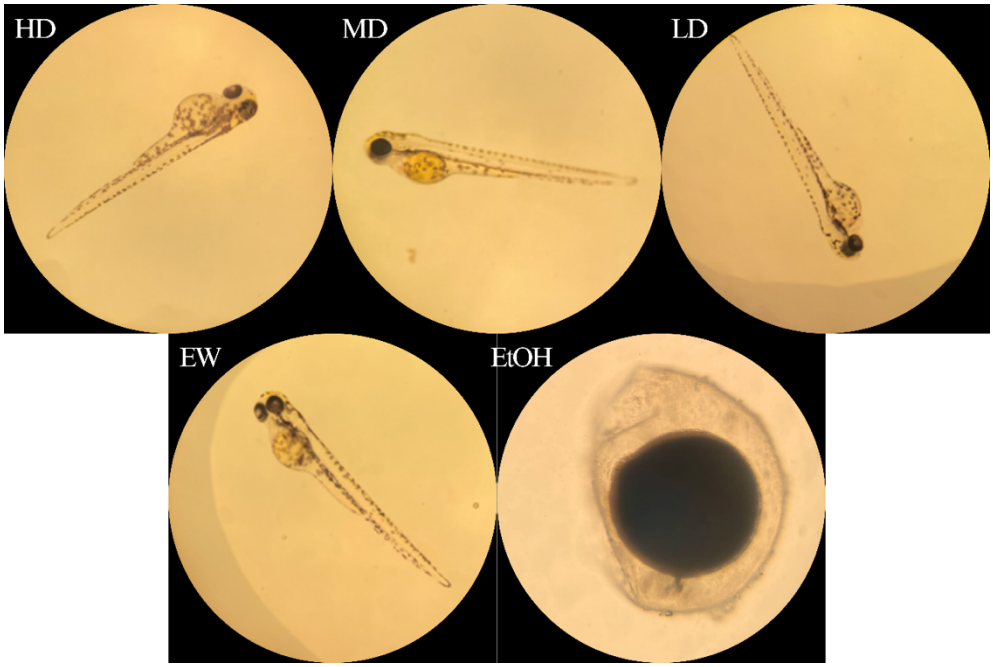


**Fig. 3.** – Morphological endpoints in zebrafish embryos exposed to different dose levels (based on $LC_{50}$) of ethanol extracts from the exopolysaccharide of *L. tigrinus* after 72 h of exposure. High dose (HD): Malformation of heart, Medium dose (MD): Malformation of heart, Low dose (LD): No observed toxic and teratogenic abnormalities, Embryo water (EW): normal embryo, Ethanol (EtOH): coagulated.

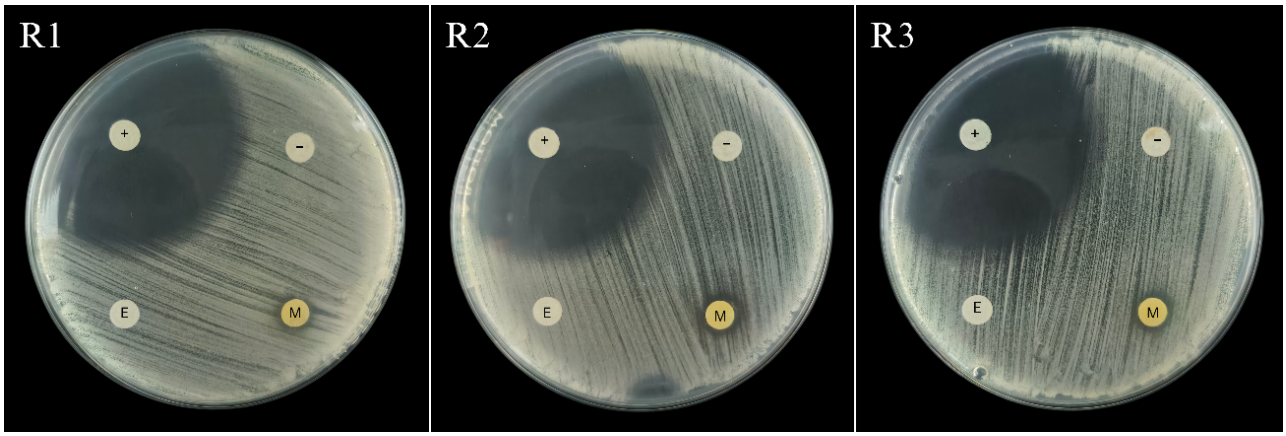


**Fig. 4.** – Zones of inhibition exhibited by *L. tigrinus* mycelial and exopolysaccharide extracts against *S. aureus*. Symbols: (+) positive control (streptomycin sulfate), (–) negative control (ethanol), E – EPS extract, M – mycelial extract.

## Discussion

Mushrooms are increasingly recognized for their diverse biological activities, which include antioxidant, antimicrobial, anticancer, prebiotic, antidiabetic, and anti-inflammatory functions. These health-promoting properties are largely associated with their rich composition of bioactive molecules, such as polysaccharides, protein–polysaccharide complexes, peptides, proteins, terpenoids, and phenolic compounds (Kour et al., 2022). In the present study, the Philippine *L. tigrinus* was cultivated under submerged fermentation, with growth parameters carefully optimized to maximize mycelial biomass and exopolysaccharide (EPS) production. The resulting extracts were subjected to bioactivity assessments to explore their potential pharmacological applications. Overall, this investigation provides valuable insights into the therapeutic prospects of *L. tigrinus* and emphasizes the sustainable use of Philippine macrofungi as promising sources of biologically active metabolites.

The study identified mango puree (MP) as the most suitable basal culture medium for the submerged fermentation of *L. tigrinus*, supporting the highest yields of both mycelial biomass and EPS ($p < 0.05$). This result is likely due to the nutrient composition of mango. Ripe mango fruits are rich in sugars (glucose, fructose, and sucrose) as well as other carbohydrates such as starch and pectins, providing diverse carbon sources essential for fungal metabolism and growth (Bello-Pérez et al., 2007). According to the USDA Nutrient Database, the total carbohydrate and sugar contents of cultivars such as Tommy Atkins, Haden, Kent, and Keitt are 14.98 g and 13.66 g per 100 g of fruit, respectively, with sucrose (6.97 g), fructose (4.68 g), and glucose (2.01 g) as the major individual sugars, and 1.6 g of dietary fiber (USDA, 2018). Similarly, Colombian table of food composition report that mango pulp contains 3.9 g glucose, 1.0 g fructose, 8.8 g sucrose, 1.8 g starch, and 8.2 g pectin per 100 g of pulp, along with 2.6 g of dietary fiber (ICBF, 2015). In African cultivars, total sugar content varies between 10.5 % and 32.4 %, while in Sudanese varieties it ranges from 10 % to 12 % (Othman and Mbogo, 2009; Nour et al., 2011). Across cultivars, sucrose, fructose, and glucose are generally present in descending order of abundance (Bello-Pérez et al., 2007).

Previous studies have demonstrated that *Lentinus* species exhibit distinct preferences for specific carbon sources. For instance, *Lentinus strigosus* Fr., *L. tigrinus*, and *Lentinus swartzii* Berk. were reported to favor fructose, sucrose, and starch, respectively (Dulay et al., 2020a). Similarly, *Lentinus edodes* (Berk.) Singer achieved maximum growth in media containing glucose, maltose, and starch (Azuma and Kitamoto, 1994), whereas *Lentinus subnudus* Berk. favored fructose, maltose,

dextrin, and glucose (Kadiri and Fasidi, 1994). Moreover, *Lentinus tuberregium* (Fr.) Fr. exhibited enhanced growth in media supplemented with dextrose, maltose, sucrose, mannitol, and starch compared to controls (Manjunathan and Kaviyarasan, 2010). Accordingly, the suitability of mango as a culture medium for *L. tigrinus* likely derives from its sugar-rich composition, which enhances both mycelial biomass and, consequently, EPS yield. It is important to note, however, that while biomass establishes the fungal basis for EPS biosynthesis, EPS secretion is strongly influenced by environmental and physiological factors. Thus, the relationship between biomass and EPS production is condition-dependent and can often be decoupled under optimized fermentation conditions. Moreover, the cultivation medium composition, especially nutrient availability (e.g., carbon and nitrogen sources), plays a crucial role in determining both mycelial growth and EPS secretion. Even when biomass is high, EPS production may not necessarily increase if nutrient balance or environmental conditions are suboptimal (Joshi et al., 2012).

The pH of the culture medium plays a crucial role as an intrinsic factor because it influences the ionic balance of the medium, cellular morphology, structural integrity, and physiological processes of fungi, including nutrient assimilation and metabolite biosynthesis (Deacon, 2006). In this study, a pH of 7.0 supported the highest mycelial growth, although no significant difference was observed across the tested pH levels. Conversely, EPS production was significantly higher at pH 7.0 compared with acidic conditions (pH 4–5), but not significantly different from neutral to slightly alkaline conditions (pH 6 and 8). These findings are in line with Dulay et al. (2021), who reported that *Lentinus* isolates generally thrived in slightly acidic to neutral media (pH 4–7), though the preferred range varied by species. For instance, *L. tigrinus* and several other isolates exhibited enhanced growth at pH 6, while *Lentinussajor-caju* (Fr.) Fr. achieved greater biomass at pH 4–5. Such variation underscores that pH tolerance and preference differ among *Lentinus* spp., yet efficient mycelial biomass production typically occurs in mildly acidic to neutral conditions. EPS production in mushrooms, on the other hand, is highly species-specific and responsive to different pH levels. For instance, Wu et al. (2006) demonstrated that *Auricularia auricula* (L.) Underw. produced the highest EPS at pH 5.5, with further improvements achieved through a two-stage pH control (pH 5.0 for 48 h followed by pH 5.5). Similarly, EPS synthesis in *Phellinus linteus* (Berk. & M.A.Curtis) Teng KCTC 6190 was optimal at an initial pH of 4.0 under shake-flask conditions (Hwang et al., 2003). These findings confirm that EPS biosynthesis is strongly regulated by pH in submerged fermentation systems.

Agitation was identified as an important extrinsic factor influencing both mycelial growth and metabolite yield. In the present study, agitation at 100 rpm supported the highest biomass production and EPS yield of *L. tigrinus*. Comparable results have been reported in other fungi: *Lentinus squarrosulus* Mont., *Pleurotus albidus* (Berk.) Pegler, *Pleurotus ostreatus* (Jacq.) P.Kumm., *Ganoderma lucidum* (Curtis) P.Karst., and *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo all achieved maximum mycelial biomass under shake-flask conditions at agitation rates ranging from 70 to 180 rpm (Dulay et al., 2015b; Wahyudi et al., 2015; de Souza Kirsch et al., 2016; Dulay et al., 2016b). Agitation enhances oxygen availability, ensures homogeneous nutrient distribution, and maintains concentration gradients between the cell interior and exterior (Dulay et al., 2015b). However, excessively high agitation (> 250 rpm) may exert detrimental shear stress, leading to smaller, irregular pellets and fragmented hyphae, ultimately lowering biomass yield, as observed in *Pisolithus* sp. (da Costa et al., 2020). Elisashvili (2012) emphasized that an optimum

agitation rate reflects a balance between oxygen transfer (positive effect) and shear stress (negative effect). Nevertheless, increased agitation has been shown to favor the biosynthesis of secondary metabolites. For instance, Kim et al. (2010b) reported that the highest EPS yield in *Tricholoma matsutake* (S.Ito & S.Imai) Singer was achieved at 300 rpm. Similarly, Agudelo-Escobar et al. (2017) noted that 200 rpm enhanced polysaccharide production in *G. lucidum* during 5-L bioreactor fermentation. Finally, the present work identified the optimal fermentation period for *L. tigrinus* in submerged fermentation. A 10-day incubation period maximized mycelial biomass production, whereas EPS yield was highest at 15 days. Previous studies likewise highlight that the optimal incubation period for *L. tigrinus* varies with the culture medium employed. For instance, Dulay et al. (2015a) reported that rice bran broth supported the highest mycelial biomass after 10 days of incubation at 32 °C, yielding an average of 11.53 g dry weight. Moreover, when cucurbit-based broths such as squash and luffa decoctions were used, the optimal biomass yield was observed after 15 days at 30 °C, particularly at higher substrate concentrations (Liwanag et al., 2020). These findings suggest that the composition of the basal medium strongly influences fungal growth kinetics and the duration of optimal incubation. On the other hand, the observed result for EPS production is consistent with the general principle that secondary metabolites, including EPS, are often synthesized in later stages of culture, once biomass accumulation has plateaued. This has been demonstrated in *G. lucidum*, where EPS yield peaked in the stationary growth phase, distinct from the biomass maximum, and was further enhanced using a two-stage fermentation strategy (Kim et al., 2006). Similarly, studies in *A. auricula* showed that pH-controlled fermentation extended into later incubation days improved EPS productivity (Wu et al., 2006). These results indicate that EPS secretion is typically decoupled from biomass accumulation and is condition-dependent, favoring prolonged incubation periods under optimized parameters. Taken together, these results highlight the critical roles of both intrinsic and extrinsic parameters in improving submerged fermentation efficiency and establishing favorable conditions for biomass and metabolite production in *L. tigrinus*.

The FTIR spectral analysis of the EPS obtained from *L. tigrinus* demonstrated the presence of multiple functional groups, reflecting the chemical complexity of this biomolecule. Distinct vibrational peaks confirmed that the EPS is not a uniform polymer but a heterogeneous matrix composed of hydroxyl, amino, carbonyl, and ether groups. Such structural diversity, which is also evident in other fungal polysaccharides, can arise from differences in monosaccharide composition, branching patterns, molecular weight, and glycosidic linkages. These variations are important because they influence solubility, molecular conformation, and biological interactions, thereby supporting activities such as immune modulation, antioxidant defense, and antimicrobial action through mechanisms including receptor engagement with toll-like receptors (Ferreira et al., 2015; Wu et al., 2025).

A broad absorption band appeared at 3291.66 $cm^{-1}$, which corresponds to O–H and N–H stretching vibrations. This signal typically indicates the presence of hydroxyl groups in polysaccharides and hydrogen-bonded amino groups in proteins, suggesting that the EPS of *L. tigrinus* contains both carbohydrate and protein components. Comparable findings were reported for polysaccharides from *G. lucidum*, where glycosidic linkages, branching features, and protein conjugation were directly linked with antioxidant, antitumor, and antimicrobial properties, emphasizing the significance of structure–activity relationships in fungal EPS (Ferreira et al., 2014). Another relevant peak was recorded near 2927.90 $cm^{-1}$, corresponding to aliphatic C–H stretching.

This feature is typical of polysaccharides with extended carbon backbones and highlights the carbohydrate-dominant nature of the EPS. The band observed at 1614.83 $cm^{-1}$ is attributed to C=O stretching and N–H bending, signaling the presence of amide and carbonyl groups, which point toward proteinaceous fragments integrated into the EPS structure. Previous studies suggest that such protein–polysaccharide conjugates can enhance immunostimulatory and anti-inflammatory activity (Agatonovic-Kustrin et al., 2020). Additional peaks at 1411.92 $cm^{-1}$ and 1257.60 $cm^{-1}$, which correspond to symmetric $COO^-$ and C–O stretching, indicate the presence of acidic and ether functionalities, characteristic features of acidic polysaccharides associated with antioxidant and immunomodulatory effects (Shen et al., 2024). The fingerprint region, spanning 1043.35–416.27 $cm^{-1}$, provided further confirmation of the polysaccharide-rich profile of the EPS. Strong absorption bands in this range were associated with C–O and C–C stretching, ring vibrations, and β-D-glucan linkages. In particular, peaks between 1000 and 400 $cm^{-1}$ are often linked with β-D-glucans and the pyranose form of glucose (Yao et al., 2018). β-D-glucans are especially noteworthy due to their established immunomodulatory properties and their application in both functional foods and pharmaceuticals (Ruthes et al., 2015). Overall, the FTIR spectrum of *L. tigrinus* EPS points to a structurally diverse biopolymer built on polysaccharide backbones and enriched with protein residues and secondary metabolites. The identification of hydroxyl, amino, carbonyl, and ether groups corresponds well with earlier FTIR reports of EPS derived from mushrooms such as *Trametes versicolor* (S.Ito & S.Imai) Singer, where similar chemical signatures were associated with bioactive polysaccharides of therapeutic relevance (Angelova et al., 2022). Particularly, the detection of β-D-glucans and pyranose structures provides a molecular explanation for the observed biofunctional activities of the EPS and supports its potential value as a nutraceutical or pharmaceutical ingredient.

Macrofungi are well-established producers of bioactive compounds that engage multiple enzyme-mediated therapeutic pathways. For instance, polysaccharides derived from *Grifola frondosa* (Dicks.) Gray were shown to significantly reduce fasting serum glucose, insulin levels, and insulin resistance in diabetic models through activation of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1), and by regulating the PI3K/Akt signaling pathway (Xiao et al., 2015). Methanol extracts of *Pleurotus pulmonarius* (Fr.) Quél. have been shown to moderately inhibit acetylcholinesterase and suppress inflammatory mediators like nitric oxide in LPS-stimulated macrophages, indicating both neuroprotective and anti-inflammatory activities (Nguyen et al., 2016). Furthermore, extracts of *Hypholoma lateritium* (Schaeff.) P.Kumm. significantly reduce COX-2 expression in LPS-activated RAW 264.7 cells, pointing to direct anti-inflammatory effects via COX-2 modulation (Ványolós et al., 2020). In the present study, at the screening concentration of 10 µg $mL^{-1}$, *L. tigrinus* extracts exhibited very low α-glucosidase inhibitory activity (mycelia: 0.83%; EPS: 2.30%), in contrast to the positive control acarbose, which produced 88.76% inhibition ($p < 0.05$). This result indicates that neither the mycelial nor the EPS fraction contains appreciable levels of potent α-glucosidase inhibitors at this concentration.

The absence of significant inhibition in this study is consistent with the structural binding requirements of α-glucosidase inhibitors. Acarbose, for example, is a pseudo-tetrasaccharide composed of a valienol moiety linked via nitrogen to isomaltotriose, which confers an exceptionally high binding affinity, approximately $10^4$ to $10^5$ times greater than that of natural oligosaccharides (Rosak and Mertes, 2012). This strong interaction enables acarbose to competitively inhibit α-glucosidase. Similarly, canonical inhibitors such as acarbose and voglibose act as pseudo-

oligosaccharides that closely mimic natural substrates, allowing them to bind at the catalytic site with high affinity. This molecular imitation explains their potent inhibitory activity and clinical efficacy at low micromolar concentrations (Bischoff, 1994). In contrast, mushroom extracts contain a heterogeneous mixture of macromolecules and secondary metabolites, most of which lack the specific structural features required for strong enzyme binding. Consequently, the absence of measurable inhibition at low screening concentrations is consistent with their expected biochemical limitations.

Another possible explanation for the observed relatively low activity is the mode of action of fungal EPS and β-glucans. Activation of innate immunity by β-glucans is primarily mediated through their binding to specific receptors, such as dectin-1 on macrophages (Kim et al., 2010; Batbayar et al., 2011) and complement receptor 3 (CR3) on granulocytes and natural killer (NK) cells (Thornton et al., 1996). These high–molecular-weight polysaccharides typically exert their effects through immunomodulatory mechanisms involving pattern recognition receptors (e.g., dectin-1, CR3), rather than direct enzyme inhibition (Wasser, 2014). Reports of polysaccharides directly inhibiting α-glucosidase typically involve chemically modified derivatives, such as acid-hydrolyzed low–molecular-weight polysaccharides from *Cordyceps militaris* (L.) Fr., which show significantly higher inhibitory activity than their native high-molecular-weight counterparts. This suggests that structural modification enhances enzyme accessibility and potency (Zhu et al., 2016). Therefore, at 10 µg $mL^{-1}$, the EPS fraction is unlikely to provide sufficient low–molecular-weight oligosaccharide structures to effectively interfere with α-glucosidase catalysis. Moreover, many of the most potent α-glucosidase inhibitors derived from fungi are low-molecular-weight compounds such as phenolics, flavonoids, or terpenoids, which are typically enriched within semi-polar or organic solvent extracts rather than aqueous polysaccharide fractions (Ying et al., 2014). Because the present study utilized EPS and ethanolic mycelial extracts without extensive fractionation, the abundance of such small molecules is likely too low to elicit significant inhibition at a single-point concentration. Indeed, extraction solvent polarity significantly influences the enrichment of bioactive metabolites: acetone extracts of *Pleurotus citrinopileatus* Singer exhibit markedly higher α-glucosidase inhibitory activity compared to water extracts, indicating that semi-polar solvents preferentially concentrate small, active phenolic and flavonoid compounds (Yin et al., 2019). Finally, single-point screening at low concentrations (e.g., 10 µg $mL^{-1}$) provides preliminary insight but cannot exclude inhibitory potential that may emerge at higher concentrations or following purification. Dose–response profiling and bioassay-guided fractionation remain essential for detecting low-abundance α-glucosidase inhibitors (Zhu et al., 2016).

The acetylcholinesterase (AChE) inhibitory activity of both *L. tigrinus* mycelial and EPS extracts was absent, with mean values of –0.37% and 0.00% inhibition, respectively. These results indicate an absence of significant inhibition compared to the positive control galantamine, which showed potent activity at 98.03% inhibition. The lack of activity suggests that *L. tigrinus* extracts contain no bioactive compounds that interact with the cholinergic system at the tested concentration.

The absence of significant AChE inhibition in *L. tigrinus* may be explained by the compositional characteristics of its extracts. Although mushrooms are recognized for their richness in polysaccharides and diverse secondary metabolites, acetylcholinesterase inhibition is more commonly associated with alkaloids, terpenoids, and phenolic compounds rather than polysaccharides. Specifically, mushroom-derived AChE-inhibitory effects have been attributed to terpenoids and alkaloids binding to the enzyme's active or peripheral sites, and to phenolic acids and

flavonoid derivatives acting as potent inhibitors (Patocka, 2012; Roseiro et al., 2012). Broader reviews of natural AChE inhibitors likewise emphasize terpenoids, phenolics, coumarins, and particularly alkaloids as dominant classes of inhibitory compounds (Tamfu et al., 2021). Mushrooms are known to contain phenolic acids and flavonoids that contribute to neuroprotective and antioxidant activities, which may indirectly support cholinesterase inhibition (Li et al., 2023). However, direct inhibitory activity is often weak. For instance, Randhawa and Shri (2018) reported that among four *Pleurotus* species, the strongest inhibition was observed in *Pleurotus florida* Eger with an $IC_{50}$ of 59.13 ± 1.37 mg $mL^{-1}$, a potency considered low compared to standard alkaloid-based inhibitors. Similarly, *G. lucidum* has been shown to exert limited direct AChE inhibition; while its polysaccharides provide neurotrophic and antioxidant benefits, its bioactive ganoderic acids primarily modulate pathogenic proteins and autophagy pathways rather than cholinesterase activity (Lian et al., 2024). Overall, these findings suggest that the weak AChE inhibition observed in *L. tigrinus* aligns with previous reports, where polysaccharide-rich mushroom extracts primarily exert antioxidant and immunomodulatory effects but are not major sources of cholinesterase inhibitors.

In addition, cyclooxygenase inhibition assays revealed differential responses between the COX-1 and COX-2 isoforms. EPS extracts exhibited minimal inhibition against COX-1 (15.72%) but no measurable activity against COX-2 (–13.18%). Conversely, mycelial extracts showed negative inhibition toward COX-1 (–10.82%) and only weak inhibition against COX-2 (2.36%). These values were markedly lower than those of the positive control, indomethacin, which displayed potent inhibition of both COX-1 (93.98%) and COX-2 (91.86%) ($p < 0.05$). The relatively weak COX-1 inhibition by EPS may be linked to the presence of specific carbohydrate moieties capable of interacting with inflammatory pathways, though only to a limited extent. By contrast, previous studies have demonstrated that *G. lucidum* contains several triterpenoids, including novel and known lanostane-type compounds, which significantly reduced LPS-induced COX-2 expression as well as NO and IL-1β production in RAW264.7 macrophages. These effects were linked to suppression of NF-κB signaling, highlighting the strong inflammation-modulatory potential of triterpenoid-rich extracts (Wu et al., 2019). Moreover, *G. lucidum* is widely recognized for its triterpenoid profile and anti-inflammatory efficacy. Reviews and phytochemical investigations consistently report that ganoderic acids and related lanostane triterpenoids are the principal anti-inflammatory metabolites, modulating COX pathways and downstream inflammatory mediators (Ko et al., 2007). Similarly, studies on *P. linteus* demonstrated that ethanol and n-butanol (n-BuOH) fractions, rich in lipophilic secondary metabolites suppressed COX-2 expression and reduced the production of pro-inflammatory mediators such as NO and $PGE_2$ in LPS-stimulated RAW264.7 macrophages. The mechanism involved inhibition of NF-κB and MAPK signaling, supporting the role of non-polar compounds (e.g., triterpenoids and phenolics) in mitigating COX-mediated inflammation (Kim et al., 2007). The absence of comparable inhibitory effects in *L. tigrinus* suggests that its extracts are structurally and compositionally distinct, favoring antioxidant and immunomodulatory properties rather than anti-inflammatory action.

The antioxidant potential of both the mycelial extract and the EPS of *L. tigrinus* was assessed. Results showed that the ethanolic extract of the mycelia exhibited a radical scavenging activity of 62.69%, whereas the EPS extract displayed a slightly lower activity of 50.76%. These findings are consistent with those reported by Dulay et al. (2015a), who observed a scavenging activity of 18.94% in *L. tigrinus* mycelia cultivated on rice bran decoction. Similarly, Manzano et al. (2025) documented

a higher scavenging effect of *L. tigrinus*, reaching 28.39%, when distilled water was added at day 0 during mycelial incubation. Other species of the genus *Lentinus* have also demonstrated comparable antioxidant capacity; for instance, Austria et al. (2021) reported that ethanolic extracts of *L. swartzii* mycelia at 1000 µg $mL^{-1}$ exhibited 35.29% scavenging activity against DPPH radicals. Beyond the genus *Lentinus*, polysaccharides of *Agaricus brasiliensis* Wasser, Didukh, de Amazonas & Stamets, predominantly composed of (1→6)-β-D-glucans and obtained through pronase deproteinization, were shown to exert strong antioxidant effects against hydroxyl and superoxide radicals (Siu et al., 2014). Indeed, β-glucans are widely regarded as the major antioxidative constituents in mushrooms, functioning not only as radical scavengers but also as activators of systemic defense mechanisms (Wasser, 2010; Batbayar et al., 2012).

The relatively higher activity observed in the mycelial extract of *L. tigrinus* can likely be attributed to its richer composition of secondary metabolites, particularly phenolic compounds and tocopherols. These metabolites are well known for their capacity to neutralize free radicals by donating hydrogen atoms or electrons, thereby mitigating oxidative stress (Barros et al., 2006; Ferreira et al., 2009; Heleno et al., 2009; Kozarski et al., 2015). In contrast, EPS, being primarily carbohydrate in nature, generally exhibit more modest antioxidant activity, which is thought to be mediated by hydroxyl groups that provide only limited radical quenching potential (Kozarski et al., 2015). This fundamental compositional difference, namely the presence of secondary metabolite-enriched mycelia and carbohydrate-dominant EPS, offers a sound explanation for the stronger antioxidant effect of the mycelial extract.

Brine shrimp are widely employed as a model organism in the Brine Shrimp Lethality Assay (BSLA) due to their sensitivity to toxic compounds, ease of culture, and short life cycle, making them a reliable bioindicator for preliminary screening of cytotoxic, pharmacological, and pesticidal activities (Meyer et al., 1982; Carballo et al., 2002). In the present study, the cytotoxic properties of *L. tigrinus* mycelial and EPS extracts were evaluated using this assay. Results revealed a marked difference between the two fractions. The mycelial extract displayed a clear concentration-dependent lethality, with complete mortality (100%) observed at concentrations ranging from 1,000 to 100,000 µg $mL^{-1}$. Mortality declined progressively at lower doses, with 60% at 100 µg $mL^{-1}$, 46.67% at 10 µg $mL^{-1}$, 23.33% at 1 µg $mL^{-1}$, and 13.33% at 0.1 µg $mL^{-1}$. In contrast, the EPS extract exhibited relatively lower toxicity, producing only 23.33% as the highest mortality at 100,000 µg $mL^{-1}$and no observable lethality from 1,000 µg $mL^{-1}$down to 0.1 µg $mL^{-1}$. These findings indicate that the cytotoxic activity of *L. tigrinus* is predominantly associated with its mycelial extract, while the EPS fraction demonstrates relative biocompatibility.

The differential toxicity profiles observed between mycelial and EPS extracts are consistent with previous reports on mushroom bioactivity. Mycelial extracts often harbor bioactive secondary metabolites, including phenolics and terpenoids, that contribute to cytotoxicity, whereas EPS, being polysaccharide-rich and of high molecular weight, are generally non-toxic and exert immunomodulatory rather than cytotoxic effects (Zhang et al., 2006). Supporting this, Dulay (2011) reported developmental toxicities of *L. tigrinus* mycelial extracts in zebrafish embryos and mammalian cell assays, although oral toxicity tests in mice confirmed general biosafety at practical doses. More recently, Dulay (2021) demonstrated that ethanolic mycelial extracts of Philippine *Lentinus* isolates, including *L. tigrinus*, exhibited moderate cytotoxicity against human colorectal carcinoma (HCT-116) cells ($IC_{50}$ values: 242.75–444.79 µg $mL^{-1}$), while showing weak effects on

hepatocellular carcinoma (HepG2) and normal kidney epithelial (HK-2) cells, highlighting the selective toxicity of the mycelial extract.

The study of *Schizophyllum commune* Fr. by Acanto et al. (2022) demonstrated potent cytotoxic activity of its ethanolic extract through the Brine Shrimp Lethality Assay (BSLA), with an $LC_{50}$ value of 55.64 µg $mL^{-1}$, indicating high cytotoxic potential attributed to its diverse phytochemical constituents such as alkaloids, flavonoids, tannins, terpenoids, and saponins. Similarly, EPS from *Hericium coralloides* (Scop.) Pers. exhibited strong cytotoxicity against gastric cancer cell lines, reducing the viability of AGS and MKN-45 cells to 20% and 30%, respectively, after 48 h of exposure (Tabibzadeh et al., 2022). In addition, EPS from *T. versicolor* showed cytotoxic potential linked to their complex heteropolysaccharide structure, with cytokine modulation suggesting growth-inhibitory effects and anticancer relevance (Angelova et al., 2022).

Zebrafish is an established vertebrate model for assessing embryotoxicity and teratogenicity of natural products, owing to its rapid development, optical transparency, and sensitivity to toxicants (Weigt et al., 2011; Howe et al., 2013). In the present study, the embryotoxic and teratogenic effects of *L. tigrinus* mycelial and EPS extracts were examined using this model. Results revealed that both extracts exhibited concentration and time-dependent toxicity. At the highest tested concentration (100,000 µg $mL^{-1}$), complete mortality (100%) was observed within 48 h post-treatment exposure (hpte). Notably, EPS-treated embryos already reached 83.33% mortality by 12 hpte, whereas mycelial treatments from 100 to 10,000 µg $mL^{-1}$ consistently induced full mortality by 36 hpte. At lower concentrations (≤ 1 µg $mL^{-1}$), toxicity was minimal to absent, with EPS-treated embryos displaying complete hatchability (100%), statistically comparable to embryo water controls. Conversely, mycelial-treated embryos at 0.1 µg $mL^{-1}$ showed a slightly reduced hatch rate (83.33%). These findings suggest that both extracts contain bioactive compounds with embryotoxic potential, but the mycelial extract exerts stronger lethality compared to EPS. Morphological assessments revealed that embryonic malformations were also dose-dependent. Medium to high concentrations of the mycelial extract induced head malformations, scoliosis, and growth retardation, while EPS treatments uniquely caused heart malformations only. Bradycardia was a consistent outcome at high doses, with both mycelial and EPS-treated embryos showing reduced heart rates (93–99 beats $min^{-1}$) compared to controls (120–126 beats $min^{-1}$). These effects are consistent with reports on mushroom-derived metabolites disrupting embryonic development. Specifically, *G. lucidum* extract elicited dose- and time-dependent teratogenic responses in zebrafish embryos, including tail malformations, growth retardation, and increased mortality, indicative of impaired organogenesis and cardiovascular development (Dulay et al., 2012c).

These findings corroborate earlier reports on the embryotoxic and teratogenic effects of various edible and medicinal mushrooms. For example, the hot water extract of *L. tigrinus* has been shown to induce marked embryotoxicity in zebrafish, characterized by reduced hatchability, delayed development, impaired heartbeat, and malformations such as tail deformities, pericardial edema, and underdeveloped organs, all in a strongly dose-dependent manner (Dulay et al., 2014b). Such results highlight the concentration-dependent nature of mushroom-induced embryotoxicity, wherein high doses rapidly disrupt essential developmental processes, while sublethal exposures primarily cause growth retardation and morphological abnormalities. The teratogenic effects observed in the present study, such as growth retardation, head malformations, and scoliosis are consistent with those reported for other mushrooms. De Castro and Dulay (2015), for instance, demonstrated that ethanolic

extracts of *P. ostreatus* and *L. sajor-caju* caused concentration- and time-dependent lethality in zebrafish embryos, with *P. ostreatus* inducing complete mortality within 12 h at 2.5–5% concentrations and *L. sajor-caju* increasing mortality up to 83.33% within 24 h at 5%. Similarly, water extracts of *L. strigosus* caused dose-dependent lethality, with complete mortality at 10,000 µg $mL^{-1}$ (24 h) and 1,000 µg $mL^{-1}$ (48 h) of the fruiting body extract, while lower concentrations of mycelial extract were less toxic (Dulay et al., 2018). Additional evidence comes from De Castro et al. (2016), who reported that extracts of *Termitomyces clypeatus* R.Heim significantly reduced hatchability at ≥ 0.05% and induced malformations such as wavy somites, twisted tail tips, and developmental arrest at higher concentrations. Interestingly, Taufek et al. (2020) demonstrated that EPS from *G. lucidum* showed no observable teratogenic effects in zebrafish embryos at concentrations up to 3000 µg $mL^{-1}$, with no alterations in hatching, heartbeat, or morphology. Likewise, Wan-Mohtar et al. (2021) reported that EPS and endopolysaccharides (ENS) from *Ganoderma applanatum* (Pers.) Pat. did not induce hatching delays, malformations, or cardiac abnormalities at concentrations below 1.0 µg $mL^{-1}$. These results suggest that mushroom-derived EPS are largely biocompatible at low concentrations, exhibiting minimal to no embryotoxic effects. Taken together, these comparative findings emphasize the duality of mushroom-derived metabolites: while mycelial extracts from often exert concentration-dependent embryotoxic and teratogenic effects, mushroom EPS may be non-toxic and even suitable for safe applications in food, nutraceutical, and pharmaceutical industries.

Lastly, the antibacterial activity of the mycelial and EPS extracts of *L. tigrinus* was evaluated against *S. aureus* and *E. coli*. The mycelial extract showed moderate inhibition of *S. aureus* with an average zone of inhibition of 8.83 mm, while no activity was detected against *E. coli*. Likewise, the EPS extract exhibited no measurable antibacterial effect against either strain, indicating limited bioactivity under the tested conditions. Statistical analysis confirmed significant differences among treatments at the 5% level. The inhibition of *S. aureus* suggests the presence of bioactive compounds capable of disrupting Gram-positive bacteria, consistent with previous studies attributing such effects to phenolic compounds, terpenoids, and polysaccharide–protein complexes in mushrooms (Alves et al., 2012; Heleno et al., 2013). Similar findings were reported by Dulay et al. (2016b), who observed inhibition zones against *S. aureus* using *L. tigrinus* acetonitrile extracts. In contrast, the absence of activity against *E. coli* supports the well-documented resistance of Gram-negative bacteria, largely due to their lipopolysaccharide-rich outer membrane (Nikaido, 2003; Bajpai et al., 2008), as also observed in other mushroom extracts (Dulay et al., 2014a; Manzano et al., 2025). The lack of EPS activity may be explained by its primarily polysaccharide composition, which is better known for immunomodulatory and antioxidant functions than for direct antibacterial effects (Wasser, 2002; Kozarski et al., 2015). Overall, these results indicate that *L. tigrinus* mycelial extract has moderate potential against Gram-positive bacteria but limited efficacy against Gram-negative species, warranting further investigation of its bioactive constituents.

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**Author Contributions**

Conceptualization, R.M.R.D.; methodology, R.M.R.D. and E.M.M.; validation, R.M.R.D. and E.M.M.; formal analysis, E.M.M.; investigation, R.M.R.D. and E.M.M.; resources, R.M.R.D.; data curation, E.M.M.; writing—original draft preparation, R.M.R.D. and E.M.M.; writing—review and editing, R.M.R.D. and E.M.M.; visualization, E.M.M.; supervision, R.M.R.D.; project administration, R.M.R.D.; funding acquisition, R.M.R.D. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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