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Growth Performance, Antioxidant, and Teratogenic Activity of *Panaeolus antillarum* Submerged-cultured in Coconut Water Enriched with Ascorbic Acid

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Abstract. *Panaeolus antillarum* is a mushroom commonly growing in animal dung, which has been identified to possess promising medicinal properties. This present study investigated the mycelial growth performance and bioactivities of *Panaeolus antillarum* in coconut water supplemented with varying concentrations of ascorbic acid. Methanolic extract of the culture-spent supplemented with the different amounts of ascorbic acid (0 mg, 5 mg, 10 mg, 15 mg) was used to determine the total phenolic contents (Folin-Ciocalteu), antioxidant (DPPH radical scavenging activity), cytotoxic activity, and teratogenic effects in zebra fish embryo. Statistical analysis revealed the highest mycelial yield in 0 mg (control) and 5 mg with 2.06 g and 1.92 g, respectively, while the lowest was recorded in 20 mg with 0.98 g. The highest volume loss was also registered in 0 mg (18.17 mL), which was significantly different from other concentrations. *Panaeolus antillarum* with 20 mg of ascorbic acid obtained the highest phenolic content (313.76 mg AAE/g sample) and maximum radical scavenging activity (83.08%). Regardless of the presence of ascorbic acid, all culture-spent of *P. antillarum* exhibited a toxic effect after 36 hours, with 100% mortality. Furthermore, the varying amounts of *P. antillarum* affected zebra fish embryos hatchability. Growth retardation was the only noted teratogenic effect of the spent material. Hence, *P. antillarum* could be a promising source of natural bioactive compounds that can be further studied for the possible development of pharmaceutical drugs. With these beneficial effects, this study particularly supports the United Nations' Sustainable Development Goals (SDGs) 3 and 15. Mushrooms are ecologically significant species in the forest and other land areas and are significantly utilized for the promotion of good health. Thus, conservation and protection of these species is necessary.

Keywords: antioxidant; hatchability; radical scavenging activity; teratogenic effects

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INTRODUCTION

Macrofungi or mushrooms have long been prized for their ecological, economic, and medicinal values. They have been recognized as an important component of a forest and land ecosystem. Typically, they participate in decomposition and nutrient cycling, which provides healthy substrates to other species (Bustillos et al. 2025). Ultimately, mushrooms are considered a healthy food and a dietary supplement due to their nutritious content and bioactivity. As food, they are grown and consumed extraordinarily owing to their desirable taste and aroma. Fortunately, over the years, mushrooms have continued to generate interest in medicine due to their reported medicinal and therapeutic values. Mushrooms provide substances that constitute what a food supplement is, such as ascorbic acid, carbohydrates, carotenoids, ergosterols, minerals, proteins, unsaturated fatty acids, and polysaccharides. They are regarded as extraordinary functional foods because they naturally contain compounds that could potentially improve health. Thus, physiological effects can be taken advantage of to promote synergistic and cumulative benefits of their valuable compounds (De Leon et al. 2020a; Jeitler et al. 2020).

Mushrooms are essential in the development of novel medications. It is well documented that both mycelia and fruiting bodies are a good source of secondary metabolites that naturally aid in several human health illnesses, including antimicrobial, anti-inflammatory, anti-tumor, and antidiabetic properties (Dulay et al. 2020; Fukunang et al. 2022). In addition to this, mushrooms also display a wide array of pharmacological effects such as antioxidant activity and teratogenicity (Quintero-Cabello et al. 2021). Nevertheless, it is significant to distinguish these maximum potentials from both edible and non-edible mushrooms. Throughout the years, edible mushrooms have provided an outstanding bioactive metabolite, including their antioxidant effects. Antioxidant and teratogenic tests are a prelude to anticancer discovery. Mushroom intake is reported globally to contribute to the relief of oxidative stress. In fact, the ethanolic extract of several mushrooms in the Philippines has been recognized for its potential antioxidant and teratogenic properties. These include *Lentinus swartzii* (Austria et al. 2021), *Cyclocybe cylindracea* and *Pleurotus cornucopiae* (Landingin et al. 2021), *Lentinus sajor-caju* and *Pleurotus ostreatus* (De Castro & Dulay, 2015), *Lentinus strigosus* (Dulay et al. 2018), *Ganoderma lucidum* and *Pleurotus djamor* (Bustillos et al. 2018), *Pleurotus cystidiosus*, *Xylaria papulis* (De Leon et al. 2020), *Pycnoporus sanguineus* (Aguilar et al. 2023). However, the ability to produce considerable concentrations of metabolites reflects the type of species, cultivation process, and substrates, as they influence the overall production of these important compounds.

Wild coprophilous mushroom species of *Panaeolus* are non-edible with reported toxicity and psychoactivity. Species such as *P. cyanescens* and *P. cinctulus* are known to contain this property, but another candidate, *P. antillarum,* has no reported psychoactive effects (Strauss et al. 2023). Commonly growing in tropical countries like the Philippines, this type of mushroom is frequently seen in small to medium-sized grey mushrooms, which usually grow on animal dung of carabao, horse, sheep, goat, cow (Amandeep et al. 2015), and elephant (DE & Perry, 2017). It is edible but not commonly eaten. Functionally, *P. antillarum* is found to contain valuable nutrients such as fiber, fat, carbohydrate, and protein in both mycelia and fruiting bodies (Dulay et al. 2015) and secondary metabolites, alkaloids, saponins, and flavonoids, which are commonly attributed to their antioxidants and teratogenic activities (Bustillos et al. 2014). Consequently, several factors may contribute to the low differences in concentrations of secondary metabolites production that affect its quality in response to oxidative homeostasis, limiting its antioxidant potential. In this view, the supplementation of ascorbic acid was employed in this investigation to determine the improvement of the antioxidant activity of *P. antillarum* mycelia grown in liquid culture. Ascorbic acid, a specified antioxidant under phytochemicals, is well-known for its scavenging activity of free radicals. Often known as Vitamin C, this is found in certain foods, and a small amount of it can keep the body functioning normally as it acts as the body's major defense against the damage caused by free radicals by producing stable radicals (Bratovcic 2020; Santos 2022). The addition of ascorbic acid can induce higher antioxidant and teratogenic activity of *P. antillarum* against zebra fish (*Danio rerio*) embryos. Results of this study can provide tangible evidence that this mushroom is an excellent source of natural constituents with potential application in pharmaceuticals and the development of therapeutic drugs in managing human diseases.

METHODS

Source and Revival of Pure Culture

The *P. antillarum's* pure culture was obtained from the NEUST, San Isidro Campus laboratory, Nueva Ecija. From the pure culture, an agar block measuring around 10 mm^2 was meticulously and aseptically inoculated into potato dextrose agar (PDA) plates. Culture plates underwent incubation at room temperature for 7 days to allow mycelia to proliferate. After incubation, the mycelial discs were prepared with the use of a sterile 10 mm cork-borer after 7 days of incubation. Those mycelial discs were used as a culture inoculant to determine the optimal growth conditions of the specimen.

Production of Culture Spent at Different Amounts of Ascorbic Acid

In submerged cultivation of mycelia, the basal medium used was coconut water, whereas a total of 1.2 liters of it was divided into four to prepare the different amounts of ascorbic acid (0 mg, 5 mg, 10 mg, and 20 mg per 100 mL of coconut water), which served as the different treatments. The prepared treatments with 100 mL each were placed into a glass bottle. Three replicates per treatment were prepared. In order to be sterilized, the bottled media were autoclaved for 30 minutes at 121°C, 15 psi. The mycelial discs were inoculated aseptically onto the prepared liquid culture media and cultured at room temperature for the growth of fungi. After ten days of incubation, the mycelial mat was weighed, and the culture spent was collected and measured. All cultures were subjected to antioxidant and teratogenic assay (Dulay et al. 2015b).

Antioxidant Activity, Assay for DPPH Radical Scavenging Activity

The DPPH radical scavenging activity assay, following the protocol of Baliyan et al. (2022), was utilized to measure the ability of the *P. antillarium's* extracted culture spent to scavenge free radicals. To attain the desired final concentration of 1000 ppm, methanol was used to dissolve the crude culture extract. The 3.49 mg DPPH in 10 mL methanol, which makes up the 1 mL of DPPH stock solution, was also diluted to 100 mL of methanol to freshly prepare 0.1mM DPPH in methanol. At 37˚C for 30 minutes in the dark, 4 mL of DPPH solution and 1 mL of each of the crude culture extracts were then mixed and incubated. The reaction was performed three times on each crude culture extract. The UV-Vis spectrophotometer (APEL-100) was utilized in measuring the absorbance reading and was monitored at 517 nm.

Lastly, the equation below was utilized to measure the specimen's capacity to scavenge the DPPH radical:

$$\%\text{DPH scavenging effect} = [(\text{Acontrol} - \text{Asample})/\text{Acontrol}] \times 100$$

Where the absorbance of the control, which is the DPPH solution without crude culture extract, is known as Acontrol, while the absorbance of the test sample comprising the DPPH and crude culture extract mixture is known as the Asample. As a positive control, synthetic antioxidant catechin was also utilized (Baliyan et al. 2022).

Total Phenolic Content

The Folin-Ciocalteu technique was used to identify the crude culture extracts' total phenolic content De Leon et al. (2020a). Initially, a calibration curve was created using an APEL-100 UV-Vis spectrophotometer (PD-303UV) at various ascorbic acid concentrations (0.25, 0.5, 1.0, 2.0, and 4.0 mg/mL). These 20 µL ascorbic acid solutions were put into vials. Then, a reagent known as Folin-Ciocalteu with 200 µL was added to each vial, and each of them was incubated for five minutes at room temperature. A 20 mL solution of sodium carbonate with a concentration of 1 mg/mL was produced as well. Subsequently, 1 mL of sodium carbonate was added to the ascorbic acid and FC reagents' mixture. This was then poured into cuvettes and was analyzed at a wavelength of 680 nm using a UV-Vis spectrophotometer. Following the standardization of the calibration curve, distilled water (3 mL) was utilized to dissolve 3 mg of the crude extracts. Then, 200 µL of each crude extract was put into vials (three times over), and 200 µL of FC reagent was added to these before being incubated at room temperature for five minutes. Subsequently, 1 mL of sodium carbonate was mixed into the combined solution of crude extracts and FC reagent, and was read at 680 nm wavelength with the use of the APEL-100 UV-Vis spectrophotometer (PD-303UV). Using ascorbic acid, the comparison between the calibration curve and the absorbance values of the crude extracts was done. The ascorbic acid standard curve and its linear regression were used to compute the total phenolics, as indicated by the following equation:

$$y=mx+b$$

In this case, y stands for the OD, m for the ascorbic acid slope, and b for the y-intercept. The unit of measurement for Ascorbic Acid Equivalent (AAE) was mg AAE/gram sample.

Toxic and Teratogenic Activity: Zebrafish spawning and fertilization

To guard against egg predation or cannibalism after they were laid, adult fish were enclosed behind a plastic mesh. A 12-hour/night light system was used for the spawning of zebra fish. After 12 hours of dark conditions, an additional 12 hours of exposure to light conditions were done to the eggs. During the onset of light, the fertilization of the eggs took place. Mature zebrafish in a screen were transferred to another aquarium (De Leon et al. 2020a).

Collecting Embryos

Using a hose tube, embryos at the segmentation phase were removed from the aquarium following spawning and fertilization. To verify the homogeneity of the embryo phase, these were put in a petri dish with embryo water and were examined under a compound microscope. The unfertilized eggs were thrown away (De Leon et al. 2020a).

Zebrafish (*D. rerio*) toxicity and teratogenicity assay

Using a pipette, two (2) mL of each culture was placed in an ELISA plate well. Each treatment was replicated three times. Four embryos were placed in each well with the spent medium. The room temperature was maintained while the plates were covered and incubated. A compound microscope was also used to scan the specimens for toxic and teratogenic activity after 36 hours of incubation (De Leon et al. 2020a). The Nagel criteria (Nagel, 2002); lethal (coagulation, no somites, tail not detached, and no heartbeat) and teratogenic (head and/or tail malformation, stunted tail growth retardation, scoliosis, and limited movement) were used to evaluate the morphological endpoints of zebrafish (Table 3). Mortality was defined as the absence of visible heartbeats and coagulated embryos. Hatchability, deformity, and mortality rates were documented.

Statistical Analysis

A complete randomized design (CRD) was used in all tests under laboratory conditions. Data was analyzed using analysis of variance (ANOVA) in one-way classification. A Duncan Multiple Range Test (DMRT) was employed to determine the significant treatment comparisons at 5% level of significance of mycelial growth, volume loss, total phenolic contents, radical scavenging activity, and cytotoxicity.

RESULTS AND DISCUSSION

Volume loss of the Culture Spent

The volume loss of *P. antillarum* submerged cultured in coconut water enriched with ascorbic acid was determined in this study, as it can be used as a parameter for determining the growth response of the culture organism. It is directly proportional to the biomass of the mycelia of a fungal organism. The highest mycelial biomass was recorded at 0 mg of ascorbic acid (control), with 2.06 mg, which was significantly comparable to 1 mg with 1.92 mg. The lowest mycelial growth was registered in the media supplemented with 20 mg of ascorbic acid, with 0.98 mg. The results of volume loss of *P. antillarum's* culture grown on coconut water with varying amounts of ascorbic acid were recorded and shown in Table 1. It can be gleaned that coconut water without ascorbic acid significantly showed the highest volume loss of 18.17 mL. This was followed by coconut water with 5 mg ascorbic acid, which is statistically comparable with coconut water with 10 mg ascorbic acid. The lowest volume loss was found in coconut water with 20 mg of ascorbic acid. The results strongly suggest that the addition of ascorbic acid to the medium affects the growth of *P. antillarum*. Volume loss decreased in increasing amounts of ascorbic acid.

Moreover, the coconut water liquid culture media is another contributory factor to aggressive mycelial production and accumulation as a result of volume loss in spent media. In fact a 100 g of coconut water may contain 3.71g carbohydrates, 2.61 g sugars, 1.1 fiber, 0.72 g protein, and 0.2 g fat. Its mineral contents also include 250 mg potassium, 105 mg sodium, 25 mg magnesium, 24 mg calcium, 20 mg phosphorus, 0.29 mg iron, 0.14 manganese, 0.1 mg zinc, 0.04 mg copper, and vitamins such as ascorbic acid, thiamine, niacin, panthothenic acid, pyridoxine, cobalamin, folate and choline (Food Data Central USDA, 2019).

Antioxidant Activity (DPPH Radical Scavenging Activity)

The DPPH free radical scavenging activity assay was used in this study to determine the antioxidant activity, where free radicals are generated by DPPH. The absorbance reaction, radical scavenging activity, and total phenolic content were measured and shown in Table 2. The highest scavenging activity is found in the positive control (catechin) with 98.10%. Among all concentrations used, the one with 20 mg of ascorbic acid obtained the highest scavenging activity (83.08%) and the lowest at 5 mg ascorbic acid (72.64%). A trend, however, is observed that as the concentration of ascorbic acid increases, the ability of the extract to scavenge free radicals also increases. Thus, antioxidant activity is highly attributed to ascorbic acid supplementation.

Table 1. Volume loss of culture of *P. antillarum* grown in coconut water with ascorbic acid.

Amount of Ascorbic Acid (mg) in Coconut Water	Mycelial Dry Weight (g)	Volume loss (mL)
0 mg	2.06^{a}	18.17^{a}
5 mg	1.92^{a}	17.17^{b}
10 mg	1.40^{b}	17.00^{b}
20 mg	0.98^{c}	16.17^{c}

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

Table 2. Scavenging activity (RSA) and total phenolic composition of the culture spent of *P. antillarum* grown in coconut water supplemented with ascorbic acid.

Samples	Radical Scavenging Activity (%)	Total Phenolic Content (mg AAE/g sample)
Spent without ascorbic acid	71.90 [c]	206.92 [c]
Spent with 5 mg ascorbic acid / 100 mL media	72.64 [c]	257.58 [b]
Spent with 10 mg ascorbic acid / 100 mL media	77.37 [b]	285.19 [ab]
Spent with 20 mg ascorbic acid / 100 mL media	83.08 [ab]	313.76 [a]
Cathechin (positive control)	98.10 [a]	

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

Mushrooms are consumed as part of the diet due to their nutraceutical attributes. Overabundance of free radicals as a result of the body's daily wear and tear is damaging to tissues and cells and causes mortality. Ascorbic acid or vitamin C is one of the basic exogenous vitamins, known for having tremendous antioxidant properties. Additionally, ascorbic acid interacts synergistically with other molecules such as polyphenols to activate enzymatic activity that protects DNA and protein against oxidation (Gęgotek & Skrzydlewska, 2023). Antioxidants are substances that scavenge these free radicals and prevent their harmful effects of oxidation (Goyal et al. 2025). One appropriate method for determining antioxidant activity is through the radical scavenging assay. The phenolic chemicals, an important bioactive compound, have antioxidant activity and are known to prevent cardiac problems, combat cancer, and even have anti-inflammatory properties (Chun et al. 2021).

On the other hand, the ethanolic extract phenolic content was determined using the Folin-Ciocalteu method. Results indicated significant amounts of phenolics in *P. antillarum*. Among all concentrations of ascorbic acid used, the highest phenolic concentration was recorded at 20 mg ascorbic acid/100mL media (313.76 mg AAE/g sample). The lowest content was recorded at 0 concentration. A similar trend was observed from the DPPH result, as seen in Table 2. This result showed that the phenolic composition of *P. antillarum* can be improved by the addition of ascorbic acid to the media. Significantly, the considerable amount of phenolics found in this study played a role in antioxidative activity via radical scavenging. This result is in conformity with Phan et al. (2014) on *P. giganteus, which* documented high antioxidant activity, which was correlated to the high amounts of phenols found in the extracts. Moreover, the total phenolics obtained from this are relatively higher compared to the recorded results of *Panaeolus cyanescens* (Bustillos et al. 2014), *Panaeolus antillarum* (Dulay et al. 2015), *Pleurotus cystidiosus* (Garcia et al. 2020), and *Xylaria papulis* (De Leon et al. 2020a). Contrastingly, the total phenolics obtained from this investigation are lower than those of *G. lucidum* and *P. djamor* using similar coconut as a liquid culture substrate (Bustillos et al. 2018).

In this investigation, *P. antillarum* submerged coconut water with ascorbic acid supplementation is considerably higher than previous results in other liquid cultures, such as potato broth. This is evident in other studies, like *L. tigrinus* mycelia mat that grew using rice bran decoction has only a 26.59 mg AAE/g sample mean of total phenolics and has a radical scavenging activity of only 18.94%. Additionally, *L. sajor-caju* has only a total phenolic of 25.60 mg AAE/g sample and a scavenging activity of 16.94% (Dulay et al. 2015). Moreover, *Cyclocybe cylindracea, Pleurotus eryngii, Leccinum duriusculum, and Flammulina velutipes* were found to be highest in gills and peels. Consummately, the mushroom mycelia, fruiting body, and spent media can all have different levels of phenolics and antioxidant activity. It is interesting, therefore, to investigate the mycelial mat and fruiting body parts in determining the potential difference between *P. antillarum* mushrooms.

Toxic and Teratogenic Effects on *Danio rerio* Embryos

Embryos were directly exposed to the different cultures spent of *P. antillarum*. Table 3 shows the mortality percentage of *D. rerio* embryos at different cultures after 36 hours of exposure. All doses of *P. antillarum*, regardless of the presence of ascorbic acid, significantly showed a toxic effect after 36 hours, with 100% mortality. The coagulated embryos at the different culture spent of *P. antillarum* are shown in Figure 1. No mortality was noted in the control embryos. However, hatchability (Table 3) of embryos was also affected by the different spent of the *P. antillarum*. Although a low percent hatchability was noted in the control embryos after 36 hours, unhatched embryos in the control showed the hatching process after 48 hours. In this result, the sole teratogenic effect noted was growth retardation. Some embryos from the spent developed but coagulated after 36 hours. A similar result was obtained by De Castro & Dulay (2015) in their investigation on *L. sajor-caju* and *P. ostreatus,* where mortality is evident after 36 hours of exposure to higher concentrations. This is also in congruence with the varying concentrations of *X. papulis,* where mortality increases as the concentration increases with time (De Leon et al. 2020a). In contrast, *Fomitopsis feei* did not record mortality after 36 hours, but prolonged exposure up to 48 hours resulted in mortality (De Leon et al. 2020b).

The culture spent of *P. antillarum* exhibited a toxic effect on developing embryos with a hundred percent mortality rate. Teratogenic effects, on the other hand, as characterized by growth retardation, are mainly due to compounds that interrupt biochemical processes that results blood flow and locomotion malfunction (Miao et al. 2022). This proves that although mushrooms provide benefits to humankind, some of their compounds can be toxic and teratogenic, which may cause deformities to developing embryos, especially the genera of *Panaeolus, Amanita, Russulaceae,* and *Inocybe* species (Bustillos et al. 2016). Moreover, although *Trichaluerina celebica* is edible, it must be eaten with precaution because it was found to be a source of toxic and teratogenic compounds, as it has shown a high mortality rate, tail malformations, head deformation, delayed growth, and a low percentage of hatchability to zebrafish embryos (Sogan, 2018).

Mushrooms are now popular as 'super sustainable foods' since they grow easily and can be produced on a variety of agro-industrial wastes. They contain more nutrients than animal-based foods and plant-based foods combined, making them a significant part of our diet. The diverse range of their medicinal and pharmacological properties also made them promising candidates for the development of new biologically active chemicals (Muszynska et al. 2019; Kulshreshtha 2024; Pinar 2024). Regardless of being non-edible or being harmful when eaten in large amounts, psychedelic fungi are also rising in popularity for their bioactivity, their potential in nutraceuticals, and as a treatment for many medical and psychological disorders. (Strauss, 2023; Voto, 2024).

Panaeolus antillarum is a type of coprophilous species that grows in the manure of ruminants that are not commonly eaten macrofungi due to its reported toxicity (Bustillos et al. 2014, 2016). Their occurrence is supported by the nitrogen content of animal dungs and help on the ecological process of rejuvenating the nutrients available for plants, animals, and insects. However, few studies of this species in the Philippines are mainly due to the reported hallucinogenic effects of the genus *Panaeolus*. The compound psilocybin and its related metabolite psilocin are both derivatives known to cause this are exploited and found to have pharmacological benefits, reason for their demand in modern medicine. They have been commercialized as alternative therapeutic substances for the treatment of major ailments and disorders, including depression, anorexia, obsessive-compulsive disorder, stress, and relief of cluster of pain (Carhart-Harris and Goodwin 2017; Reichi et al. 2018). Despite having these beneficial compounds, only a few strains have been given priorities and few studies have been initiated in the Philippines.

Table 3. Percentage mortality and hatchability of *Danio rerio* after 36 hours of treatment application.

Samples	Mortality (%)	Hatchability (%)
Spent without ascorbic acid	100.00^{a}	0.00^{b}
Spent with 5 mg ascorbic acid / 100 ml media	100.00^{a}	0.00^{b}
Spent with 10 mg ascorbic acid / 100 ml media	100.00^{a}	0.00^{b}
Spent with 20 mg ascorbic acid / 100 ml media	100.00^{a}	0.00^{b}
Embryo water	0.00^{b}	16.67^{a}

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

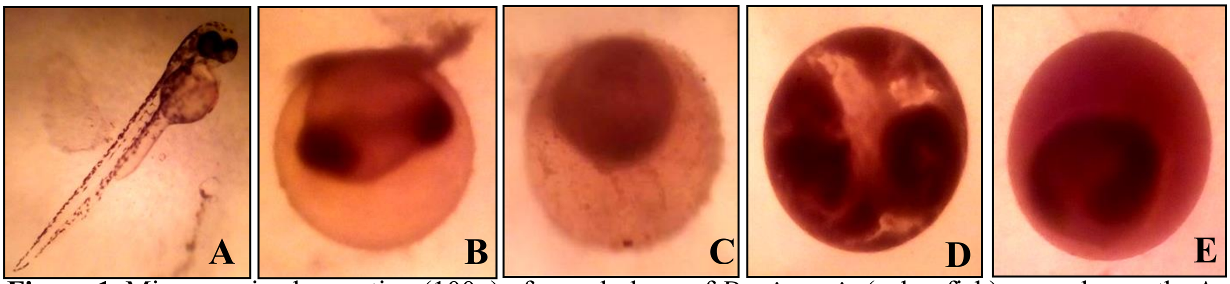

Figure 1. Microscopic observation (100x) of morphology of *Danio rerio* (zebra fish) normal growth- A (embryo water), delayed growth- B (spent without ascorbic acid), C (spent with 5 mg ascorbic acid), coagulated embryo D (spent with 10 mg ascorbic acid), E (spent with 20 mg ascorbic acid) after 36 hours of exposure.

With the increasing interest in macrofungal species, this study *on P. antillarium* contributes to the body of knowledge as the results indicate that spent of *P. antillarum* exhibited a toxic effect on developing embryos, therefore, suggesting its role in pharmacological research. Its antioxidant and teratogenic activity can be attributed to its anticancer property. Teratogenicity of a certain extract can cause morphological malformations in the cellular and tissue integrity of developing organisms. The demonstrated teratogenicity of the *P. antillarum* mycelial culture spent positively correlates its significance in pharmaceutical considerations. Thus, these findings suggest that possible bioactive components can be isolated for further pharmacological studies to alleviate human illness.

This study supports Sustainable Development Goals of the United Nations, including SDGs 3 Good Health and Well-being and SDGs 15 Life on Land. In particular, the utilization of mushrooms posts positive effects on human health as they possess bioactive components that have been beneficial to the pharmaceutical industries. Apart from their human health effects, they are ecologically significant species. The symbiotic relationship they create with various organisms in the forest, grassland, and other terrestrial habitats helps sustain the health of ecosystems. Thus, conservation of their existence and habitats is deemed necessary.

CONCLUSION

This present study showed that the mycelial growth of *P. antillarum* can be affected by the supplementation of ascorbic acid, as observed in the mycelial biomass and volume losses. *P. antillarum* mycelial spent has high phenolic contents and exhibit ability to scavenge free radicals. Mortality of the *Danio rerio* embryo was found to be higher in mycelial spent with higher ascorbic acid concentration. Also, growth retardation was the only observed teratogenic effect of the mycelial spent. The addition of ascorbic acid could be a significant factor in the antioxidant and toxic teratogenic effects of *P. antillarum* spent. It can be recommended to identify the secondary metabolites present in the culture spent contributing to its effective antioxidant and teratogenic activity. Likewise, the mycelia and fruiting bodies can be analyzed for these properties. Altogether, the antioxidant and teratogenic properties of the spent of *P. antillarum* supplemented with ascorbic acid imply promising effects that could be used as a valuable source of pharmaceutical drugs against human diseases, including cancer.

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AUTHOR CONTRIBUTION STATEMENT

The authors were responsible for: RGB (conceptualization, methodology, investigation, resources, original draft preparation, review, and editing) and ESC (investigation and editing).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial

intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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