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Research article

Bioactivity profiling of mycelial extract of *Volvariella volvacea* (Bull.) Singer (paddy straw mushroom) from the Philippines

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Abstract
Volvariella volvacea (paddy straw mushroom) is an edible and nutritious mushroom. This study highlights the chemical compositions, antioxidant, antibacterial, and teratogenic activities of mycelial extracts of the *V. volvacea* (PQ671094 and PQ671093) isolates from San Jose and La Union, Philippines. The molecular identities of mushrooms were confirmed using the ITS region of rDNA. Mycelia of both isolates contained essential oil, sugar, and coumarin. Phenol, fatty acid, anthrone, and alkaloid were detected only in La Union isolate, whereas flavonoid was only found in San Jose isolate. San Jose mycelial extract recorded radical scavenging activity (RSA) of 81.34%, whereas La Union mycelial extract had 79.69% RSA. In disc-diffusion assay, only La Union isolate extract exhibited antibacterial activity with a diameter zone of inhibition of 10.49 mm against *Staphylococcus aureus*. In both extracts, 100% mortality of zebrafish embryos was registered at 10000 ppm extract at 12-hour post-treatment exposure (hpte), and at 1000 ppm extract at 48-hpte, indicating that extract concentration and time exposure dependent. Embryos at 10 ppm to 100 ppm extract of both isolates significantly decreased the hatchability, while embryos exposed to 1 ppm to 100 ppm extract showed lower heartbeat rates. Teratogenic effects, including tail malformation, head malformation, yolk deformity, pericardial oedema, reduced pigmentation, and delayed growth, were observed at 10 ppm and 100 ppm of both extracts. Altogether, *V. volvacea* mycelia contain mycochemicals and exhibit antioxidant, antibacterial and teratogenic properties.

Keywords
Antioxidant, mycochemical, antibacterial, teratogenicity, mortality

Introduction

Mushrooms are known for their significant potential as a source of bioactive compounds and health-promoting foods for human consumption worldwide. According to Cardwell et al. (2018), mushrooms have gained popularity as a source of bioactive substances that have shown positive

effects on health. In addition, these substances improve general health and strengthen the body's immunity. Dulay et al. (2012) reported the pharmacological ingredients extracted from *Ganoderma lucidum* and its capacity to boost immunity and decrease inflammation. Spelman et al. (2017) showed that using *Cordyceps* mushroom can improve athletic performance, increase energy levels, and preserve respiratory health. The anti-inflammatory, anti-tumour, and immune-boosting effects of *Cordyceps* have also been discovered. Moreover, fermented mycelia of mushrooms have been reported to produce different bioactive substances, including polysaccharides, proteins, terpenoids, and phenolic compounds (Thongbai et al., 2015). The biological activities and physiological attributes of *Morchella esculenta* have been explored, with outcomes revealing significant antioxidant, antibacterial, as well as cytotoxic effects. Ethanolic extracts from wild edible mushrooms demonstrated notable antioxidant activity due to their significant potential against oxidative stress (Eraslan et al., 2021).

Furthermore, the presence of cytotoxic activity observed highlights the potential as a long-established entity for pharmaceutical studies (Eraslan et al., 2021). Fungi are rich in biochemicals such as vitamins, proteins, ascorbic acid, carbohydrates, minerals, unsaturated fatty acids, phenolic compounds, polysaccharides, tocopherols, and terpenoids (Kim et al., 2008; Sevindik et al., 2021). Studies have shown that mushrooms are endowed with numerous bioactive compounds that have shown encouraging pharmacological functions. They exhibit immunomodulatory, antitumor, anti-inflammatory, hypoglycemic, antithrombotic, lipid-lowering, antihypertensive, and antimicrobial activities (Menaga and Ayyasamy, 2013). The function of wild mushrooms including *Helvella leucopus* Fr., *Laeticutis cristata* (Sohaeff) Audet, *Pleurotus ostreatus*, and *Hericium erinaceus* has been studied for their potential role as natural antioxidants. These mushrooms exhibit antioxidant, antimicrobial and antiproliferative properties used in pharmaceuticals as well as for food preservation. Strain-specific studies are useful to clarify the influence of genetic and environmental variability. Hence, there is an urgent need for area-based studies to improve the yield and efficacy of these extracts (Sevindik et al., 2021; Krupodorova et al., 2024; Sevindik et al., 2024). Among the numerous edible mushrooms, *V. volvacea*, commonly referred to as straw mushrooms, is distinct due to its potential bioactive compounds and their positive impact on organisms.

Volvariella volvacea is grown in tropical and subtropical countries, particularly South Asian countries, and is commonly used in different cuisines. It has gained scientific attention for its functions as well as molecular constituents (Zha et al., 2022). The components of the mushrooms are important for the nutritive value of the mushroom, modulation of the immunity mechanism, microbial action, and reduction of free radicals (Eguchi et al., 2015). Among the food components, the most effective and basic antioxidants have phenolic properties, including phenolic acids and flavonoids, carotenoids, tocopherol, ascorbic acid, and vitamin C, all of which are collectively referred to as mycochemicals (Barros et al., 2008). Antioxidants serve as the body's primary defence against the damage caused by free radicals. Mohammed et al. (2020) reported the phenolic content, antioxidant and antimicrobial potential of *Ferulago platycarpa,* of endemic plant species. The study evaluated the natural resources of bioactive compounds. The plant's phenolic content and antioxidant capacity were tested using common assays (DPPH, ABTS, FRAP) with standard procedures. They also conducted antimicrobial activity of plant extracts against different bacteria and fungi strains. These results indicate that *Ferulago platycarpa* has high antioxidant potential. According to Chennupati et

al. (2012) antioxidants play a crucial part in mitigating various diseases such as cancer, liver and kidney retinal damage, Alzheimer's disease, and diabetes.

Volvariella volvacea is a promising edible mushroom, but there is a need to understand the teratogenicity and toxicity as major factors in the assessment of the safety for use as a natural product Teratogenicity is described as abnormal embryonic development and malformations (De Vera et al., 2016). Regardless of the risks posed by developmental teratogens, some are known to have anti-cancer activity (Blagosklonny, 2005). The zebrafish (*Danio rerio*) embryo is a dependable animal model for most mammals in terms of toxicity and teratogenicity. This is due to its optical transparency, external fertilisation, rapid embryonic development, and proximity to humans genetically (De Luca et al., 2014). Previous studies verified that some mushroom species, such as *Ganoderma lucidum*, *Lentinus tigrinus*, and *Lentinus sajor-caju*, have shown significant period and concentration-dependent impacts on zebrafish embryos, including developmental delays, morphological abnormalities such as bent tails, underdeveloped head and tail, pericardial oedema, and coagulation (Dulay et al., 2012)

Mushrooms have been valued for their unique taste and bioactive compounds, emphasising antioxidants, antibacterial, and teratogenic or toxicity effects. The biological properties and benefits of *V. volvacea* isolates in the Philippines have not been comprehensively investigated for humankind in general. This study evaluated the chemical composition and biological properties of ethanolic mycelial extract of the two *V. volvacea* isolates, from San Jose and La Union, Luzon Island, The Philippines with the intention of harness the promising biological potential of these mushrooms in pharmaceutical and nutraceutical industries.

Materials and Methods

Source of mushroom isolates

Pure cultures of *V. volvacea*, collected from San Jose, Nueva Ecija and La Union, Philippines, were obtained from the Center for Tropical Mushrooms and Research Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The identity of the two mushroom isolates was confirmed using the ITS region (ITS1F and ITS4BR) of rDNA (Gunnels et al., 2020). The consensus sequences were deposited in NCBI GenBank database with accession numbers PQ671094 for San Jose and PQ671093 for La Union.

Mass production of mycelia

Mycelia were mass-produced by inoculating mycelia discs into culture bottles containing 30 mL of sweet potato broth with an initial pH of 7. Fifty culture bottles were prepared for each mushroom isolate. Cultures were incubated at 28 °C for 14 days to allow mycelial biomass production. Mycelia were harvested, oven-dried at 40 °C for three days, and pulverised using a miller. Mushroom samples were prepared for ethanolic extraction.

Ethanolic extraction

Five grams of each powdered mushroom mycelia were soaked in 200 mL of 95% ethanol. After 48 h of soaking, extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated to dryness using a rotary evaporator. The yield of extract was recorded.

Chemical composition analysis

Following the protocol of Guevara (2005), the chemical composition of ethanolic extracts of mycelia was screened. Thin-layer chromatography (TLC) was employed to detect and analyse the presence of mycochemicals in the extracts. TLC was performed in a vertical glass chamber using ethyl acetate. Mycochemicals were detected after showing different spots on TLC layer by using a UV light hot plate and different reagents used for a typical visualization of secondary metabolites. Vanillin sulfuric acid was used to identify sterols and phenols while Dragendorff's reagent and antimony (111) chloride were employed for the detection of alkaloids and flavonoids, respectively. Potassium hydroxide was used to test tannin properties.

Assessment of antioxidant activity of V. volvacea *mycelial extracts*

The DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay protocol of Baliyan et al. (2022) was employed to test the antioxidant property of the ethanolic extracts of *V. volvacea* isolates. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of ethanol. In each test tube, 3 mL DPPH in 100 solutions was mixed with 100 mL of *V. volvacea* extract. A 3 mL of a solution containing DPPH in 100 mL ethanol was used as a standard. The tubes were kept in total darkness for 30 min. The absorbance was then measured at 517 nm. The radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A_{control} - A_{sample}) \div A_{control}] \times 100$$

where: $A_{control}$ —Control reaction absorbance; A_{sample} —Testing specimen absorbance.

Evaluation of antibacterial activity of V. volvacea *mycelial extracts*

Staphylococcus aureus and *Escherichia coli* were obtained from the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Each bacterium was inoculated into a test tube with 9 mL of nutrient broth. The tubes were incubated at 37°C for 24 h until the turbidity was comparable to the 0.5 McFarland standard with approximately 1.5×10^8 CFU mL^{-1}.*Volvariella volvacea* extracts (20 µL) were loaded in the sterilised 6-mm filter paper disc. Ethanol (95%) and streptomycin discs were also prepared as controls. In the disc diffusion assay, Mueller-Hinton agar was pour-plated, cooled and solidified. A bacterium was swabbed on the surface of the agar using a cotton swab dipped into bacterial suspension. Treated discs were equidistantly placed on the medium using individual flame-sterilised forceps. Assay plates were sealed and incubated at 37 °C in inverted position. The diameter of inhibition zone of the treated paper discs was measured using a Vernier calliper after 18–24 h.

Evaluation of embryo-toxicity and teratogenicity of V. volvacea *mycelial extracts*

Mature zebrafish at 1 female: 2 male ratio were allowed to spawn by confining them in plastic mesh in an aquarium half-filled with water. The aquarium was covered with black cloth for 12 h to induce spawning, and exposed to light for another 12 h to facilitate fertilisation. Embryos were collected, washed, transferred to a petri plate filled with embryo media, and viewed under the compound microscope to check the uniformity of embryos. Three embryos at the segmentation phase were

placed into each well of ELISA plate, and each treatment was replicated three times. Prior treatment exposure, the embryo water was removed by siphoning out from each well to avoid further dilution of the extract. A 300 µL volume of extract concentration and controls was immediately transferred into the well. The different concentrations of *V. volvacea* extract evaluated were 10,000, 1000, 100, 10, and 1 ppm. Embryo water (0 ppm) and ethanol (95%) were used as controls. The ELISA plate was kept in an incubator at 26 °C ± 1 °C. The toxic and teratogenic activities were examined using a compound microscope after 12, 24, 36, and 48 h of treatment exposure. Coagulation and no visual heartbeat were considered as dead embryo. The percentage hatchability, delayed growth and heartbeat rate were recorded. The gross morphological endpoint of treated embryos was observed up to 72 h of treatment exposure.

Statistical analysis

All treatments were performed based on a Completely Randomized Design (CRD) under laboratory conditions. Data were analysed using analysis of variances (ANOVA) in one-way classification analysis. Treatment means were compared using Tukey's HSD at a 5% significance level in the SAS system version 9.0 (SAS Institute Inc. Cary, NC, USA). In the case of two treatments, a T-test was employed for data comparison.

Results

Chemical composition of V. volvacea *mycelia*

The qualitative chemical composition of the ethanolic extracts of the two *V. volvacea* isolates was analysed. Table 1 presents the chemical composition of the two isolates of *V. volvacea*. San Jose mycelia had four chemicals including essential oils, sugars, coumarins, and flavonoids. On the other hand, seven chemicals were detected in La Union mycelia namely, essential oils, phenols, fatty acids, sugars, coumarins, athrones, and alkaloids. However, anthraquinones, tannins, triterpenes, and steroids were not detected in both mushroom mycelia.

Antioxidant activity of V. volvacea *mycelial extract*

In this study, the DPPH radical scavenging test was conducted to determine the antioxidant activity of the ethanolic extracts of the two *V. volvacea* isolates. The results of the radical scavenging assay are depicted in Table 2. Both mushroom extracts registered high antioxidant activity. Extract of San Jose mycelia recorded a radical scavenging activity of 81.34%, which found to be statistically comparable to catechin ($p > 0.05$). However, La Union mycelia extract recorded a radical scavenging activity of 79.69%, which is significantly lower than the activity value of catechin.

Antibacterial activity of V. volvacea *mycelial extract*

Disc diffusion method was used to assess the antibacterial activity of the mycelial extract of *V. volvacea* isolates against pathogenic bacteria. The diameter zone of inhibition of *V. volvacea* extracts against *E. coli* and *S. aureus* are shown in Table 3. It can be seen that only La Union mycelia extract showed a zone of inhibition in anti- *S. aureus* disc diffusion assay with a diameter of 10.94 mm. Contrastingly, no inhibitory zones were observed for either bacterium treated with San Jose mycelia extract. Statistical analysis showed a significant difference among treatments ($p < 0.05$).

Table 1 - Chemical composition of mycelial extracts of the two *V. volvacea* isolates.

Chemicals	Compostion	
	San Jose mycelia	La Union mycelia
Essential oils	Present	Present
Phenolics	Absent	Present
Fatty acids	Absent	Present
Sugars	Present	Present
Coumarins	Present	Present
Athrones	Absent	Present
Anthraquinones	Absent	Absent
Tannins	Absent	Absent
Flavonoids	Present	Absent
Alkaloids	Absent	Present
Triterpenes	Absent	Absent
Steroids	Absent	Absent

*Data presented are results of triplicate tests (n = 3).

Table 2 - Radical scavenging activities of the mycelial extracts of the two *V. volvacea* isolates.

***V. volvacea* extracts**	**Radical scavenging activity (%)**
San Jose mycelia	81.34 ± 0.67^{ns}
La Union mycelia	79.69 ± 1.94*
Catechin (control)	81.92 ± 0.68

Each value represents mean ± SD of triplicate tests (n=3). Asterisks (*) indicate significant difference between the extract and control at 5% level of significance. ns, not significantly different from the control. The concentration of extracts used was 1000 µg mL^{-1}.

Table 3 - Diameter zone of inhibition exhibited by the mycelial extracts of the two *V. volvacea* isolates against *E. coli* and *S. aureus*.

***V. volvacea* extracts**	**Diameter zone of inhibition (mm)**	
	Escherichia coli	***Staphylococcus aureus***
San Jose mycelia	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}
La Union mycelia	0.00 ± 0.00^{b}	10.94 ± 5.47^{b}
Streptomycin	30.63 ± 1.02^{a}	28.57 ± 1.00^{a}
Ethanol	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}

Each value represents mean ± SD of triplicate tests (n=3). In each column, means with the same letter of superscript are not significantly different from each other at 5% level of significance using Tukey's HSD. A 20 µL of treatment was loaded in a sterilised 6-mm filter paper disc.

Embryo-toxic and teratogenic effects of V. volvacea *mycelial extract in zebrafish*

The embryo-toxic effect of ethanolic extract of mycelia of the two *V. volvacea* isolates was determined using embryos of zebrafish as an animal model. Table 4 shows the mortality of zebrafish embryos after 12, 24, 36 and 48 h of exposure to the different concentrations of *V. volvacea* mycelial extracts. Embryos exposed to 1000 ppm and 10000 ppm of both mushroom extracts all died at 48 h post-treatment exposure (hpte). In contrast, all embryos exposed to embryo water (control) and 1 ppm of either extract survived up to 48 hpte. At 48 hpte, although some embryos exposed to 10 ppm and 100 ppm of San Jose mycelia extract and 10 ppm of La Union mycelia extract died, the percentage mortality was comparable to that in the embryo water ($p > 0.05$). La Union mycelial extract at 100 ppm embryos registered significantly higher percentage mortality than the control embryos at 48 hpte. Noticeably, the embryo-toxic effect of both mushroom extracts was dependent on the extract concentration and exposure time.

The teratogenic effects of the *V. volvacea* mycelial extracts in developing zebrafish embryos were also determined based on the hatchability, delayed growth and heartbeat rate. Results are presented in Table 5. Hatching of all embryos in 1 ppm of both extracts and embryo water was successful after 48 h of treatment exposure. However, those embryos exposed to 10 ppm and 100 ppm of both extracts showed a significant decrease in hatchability ($p < 0.05$), which suggests a growth delay. Delayed growth or growth retardation was also recorded in this study (Table 5). Embryos at 10 ppm or higher concentrations of both mushroom extracts had a significantly higher percentage of delayed growth when compared to those in embryo water. In terms of the heartbeat, the heartbeat rate of extract-treated embryos was noticeably decreasing as the concentration of the extracts increased. Hatchability, delayed growth, and heartbeat rate of embryos are concentration-dependent in zebrafish embryo model.

Table 4 – Mortality of zebrafish embryos after 12, 24, 36 and 48 h of exposure to the different concentrations of mycelial extracts of the two *V. volvacea* isolates.

***V. volvacea* extract**	**Concentration (ppm)**	**Mortality (%)**			
		12 hpte*	**24 hpte**	**36 hpte**	**48 hpte**
San Jose mycelia	10000	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	1000	11.11 ± 19.24^{b}	22.22 ± 19.24^{b}	88.89 ± 19.24^{a}	100.00 ± 0.00^{a}
	100	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.11 ± 19.24^{b}
	10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.11 ± 19.24^{b}
	1	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Embryo water	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Ethanol		100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
La Union mycelia	10000	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	1000	33.33 ± 0.00^{b}	55.56 ± 19.25^{b}	66.67 ± 0.00^{b}	100.00 ± 0.00^{a}
	100	11.11 ± 19.24^{c}	11.11 ± 19.24^{c}	44.44 ± 19.25^{c}	44.44 ± 19.25^{b}
	10	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	11.11 ± 19.24^{c}
	1	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
Embryo water	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
Ethanol		100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Each value represents mean ± SD of triplicate tests (n=3). In each column, means with the same letter of superscript are not significantly different from each other at 5% level of significance using Tukey's HSD.
*hpte hours post-treatment exposure.

The gross morphological endpoints of the extract treated zebrafish embryos were also observed. Table 6 depicts the toxic and teratogenic effects of the different concentrations of mycelial extracts of the two isolates of *V. volvacea*. Coagulated embryo (Fig. 1A, B, C) was the most marked toxic effect of both *V. volvacea* mycelia extracts, and this was observed in embryos exposed to 1000 ppm and 10000 ppm of extracts. However, no visual heartbeat (Fig. 1E, H) was noted in embryos exposed to 10 ppm to 1000 ppm of both extracts at 48 hpte. In terms of teratogenicity, most of the teratogenic effects were observed at 100 ppm of both extracts. These include tail malformation, head malformation, yolk deformity, pericardial edema, reduced pigmentation, and growth delay (Fig. 1). Growth delay was the most marked teratogenic effect of ethanolic mycelial extract of the two isolates of *V. volvacea*.

Table 5 – Hatchability and delayed growth at 48 hpte, and heartbeat rate at pharyngula phase of zebrafish treated with different concentrations of mycelial extracts of the two *V. volvacea* isolates.

***V. volvacea* extract**	**Concentration (ppm)**	**Hatchability (%)**	**Delayed Growth (%)**	**Heartbeat rate (min^{-1})**
San Jose mycelia	100	11.11 ± 19.24^{c}	88.89 ± 19.24^{a}	108.44^{c}
	10	55.56 ± 19.25^{b}	44.44 ± 19.25^{b}	115.34^{c}
	1	100.00 ± 0.00^{a}	0.00 ± 0.00^{c}	148.00^{b}
Embryo water	0	100.00 ± 0.00^{a}	0.00 ± 0.00^{c}	167.78^{a}
La Union mycelia	100	22.22 ± 19.24^{c}	77.78 ± 19.24^{a}	131.33^{c}
	10	55.56 ± 19.25^{b}	44.44 ± 19.25^{b}	137.67^{c}
	1	100.00 ± 0.00^{a}	0.00 ± 0.00^{c}	146.33^{b}
Embryo water	0	100.00 ± 0.00^{a}	0.00 ± 0.00^{c}	152.44^{a}

Each value represents mean ± SD of triplicate tests (n=3). In each column, means with the same letter of superscript are not significantly different from each other at 5% level of significance using Tukey's HSD.

Table 6 – Toxic and teratogenic effects of the different concentrations of mycelial extracts of the two *V. volvacea* isolates in developing embryos in 72 h of exposure.

***V. volvacea* extract**	**Concentration (ppm)**	**Toxic effects**	**Teratogenic effects**					
		CO	**NH**	**TM**	**HM**	**PE**	**YD**	**DG**	**RP**
San Jose	10000	+	-	-	-	-	-	-	-
Mycelia	1000	+	+	-	-	-	-	-	-
	100	-	+	+	-	+	-	+	+
	10	-	+	-	-	-	-	+	-
	1	-	-	-	-	-	-	-	-
Embryo water	0	-	-	-	-	-	-	-	-
Ethanol		+	-	-	-	-	-	-	-
La Union	10000	+	-	-	-	-	-	-	-
Mycelia	1000	+	+	-	-	-	-	-	-
	100	-	+	+	+	-	+	+	+
	10	-	+	+	-	-	-	+	+
	1	-	-	-	-	-	-	-	-
Embryo water	0	-	-	-	-	-	-	-	-
Ethanol		+	-	-	-	-	-	-	-

(+) and (-) indicate the presence and absence of toxic and teratogenic effects of the different treatments, respectively. Coagulated; NH, No heartbeat; TM, Tail malformation; HM, Head malformation; PE, Pericardial edema; YD, Yolk deformity; DG, Delayed growth; RP, Reduced pigmentation.

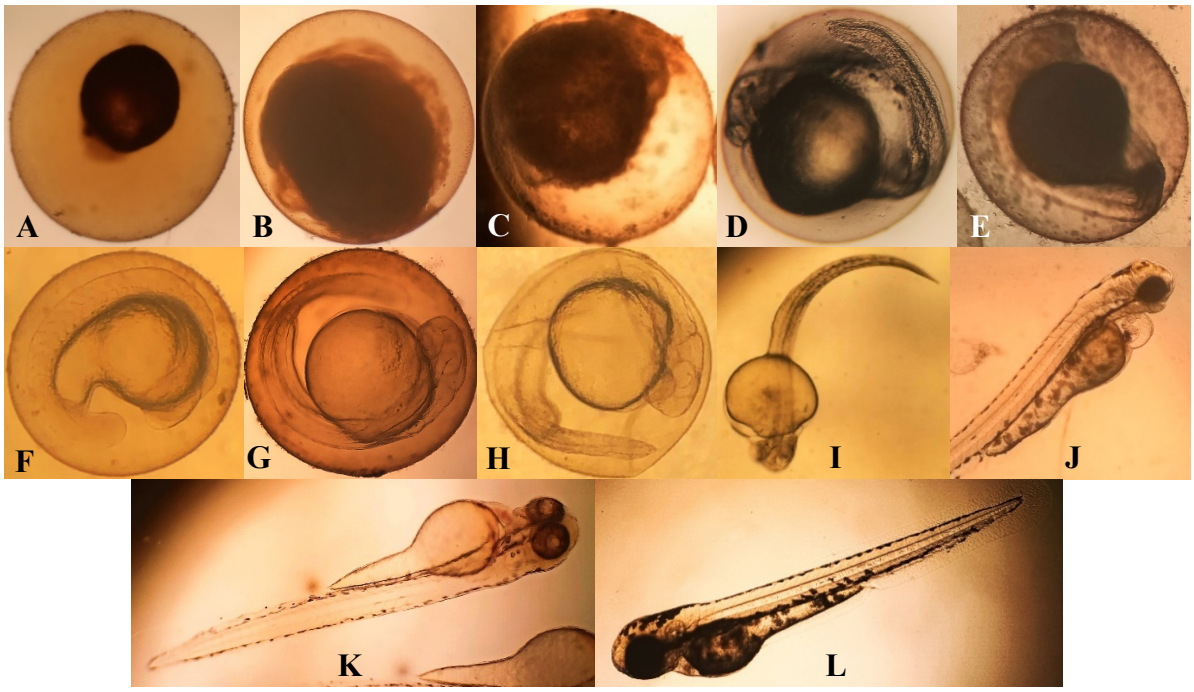

Fig. 1 – Morphological endpoints zebrafish embryos exposed to the different concentrations of ethanol extracts of mycelia of the two *V. volvacea* isolates. Coagulated embryos exposed to ethanol (A), 10,000 ppm of ethanolic extracts of mycelia of the *V. volvacea* San Jose (B) and La Union (C) isolates, tail malformation embryo (D), yolk deformity and no visual heartbeat embryo (E), and head malformation and delayed growth embryo (F) at 100 ppm extract of La Union extract, delayed growth embryo (G), no visual heartbeat embryo (H), tail malformation (I), pericardial edema (J) at 100 ppm extract of San Jose extract, reduced pigmentation emrbyo (K), and normal embryo at embryo water.

Discussion

Mycochemicals are a wide class of chemical substances produced naturally by fungi (Gürgen and Sevindik, 2022). In the present study, it was found out that ethanolic extracts of mycelia of *V. volvacea* contain chemical constituents, and these may vary depending on the isolate. The presence of these chemicals offers valuable insights into the potential biological activities and health benefits of *V. volvacea*. Alkaloids derived from fungi, animals and plants are natural organic bases that contain nitrogen atoms (Adibah and Azzreena, 2019). They have been validated to have strong biological activities, such as prominent anti-tumour activities that arrest cancer cell proliferation and kill them through apoptosis (Kampa et al., 2007; Thu et al., 2020). They are also known to possess antimicrobial properties through interfering with the microbial cellular membranes, thus acting against viruses, bacteria, and fungi (Cushnie et al., 2014). Aside from *V. volvacea*, alkaloids were also detected in other mushrooms such as *Coprinus comatus* and *Pleurotus cystidiosus* (Kalaw and Albinto, 2014; Garcia et al., 2020). Flavonoids are a wide group of polyphenolic compounds known to be effective in acting as antioxidants and anti-inflammatory activities (Rakha et al., 2022). Phenolic compounds are known to exhibit antioxidant effects that assist in the reduction of heart disease, hindering cancer, and cytoprotective effects (Doughari, 2012). They are also known as natural free radical scavengers (Ferreira et al., 2007; Dulay et al., 2016). Phenolic acids and flavonoids have reduced the chance of developing chronic illnesses like cardiovascular problems and cancer (Keles et al., 2011). Essential oils, originating from plants and various fungi, are volatile oils that provide

plant-specific fragrances and are used in pharmaceuticals (Mohammed et al., 2021). Coumarins belong to a group of heterocyclic compounds that possess manifold pharmacological uses, such as antifungal and antibacterial agents (Rathore et al., 2017; Sangthong et al., 2022). Anthrones extracted from fungal sources have antibacterial and antiparasitic impact, inhibit the growth of fungi and bacteria, and prevent oxidative stress (De Silva et al., 2013). Common biomolecules, such as sugars, play vital roles in numerous biochemical reactions. On the other hand, *V. volvacea* basidiocarp is a rich source of terpenes, polypeptides, antioxidant enzymes, sugars, flavonoids, and phenolics that exhibit a wide array of therapeutic properties such as anti-inflammatory, anti-tumor, anti-cancer, anti-microbial, antioxidant, anti-allergic, and anti-malarial effects (Ali et al., 2024). Apart from the above-mentioned chemical constituents, *V. volvacea* also contains zymosan, β-glucan, flammutoxin, selenium, and volvatoxin that demonstrate anti-tumor and anti-oxidant properties, compound 2-pyrrolidinones that exhibit antimicrobial activity, Interleukin-1β, Tumour Necrosis Factor-α, and Interleukin-8 that display anti-cancer and anti-inflammatory activities, and amino acids such as phenylalanine, leucine, and isoleucine that show anti-inflammatory property (De Silva et al., 2013). The presence of these chemicals implies that the nutritional and health benefits associated with paddy straw mushroom are unquestionable.

Antioxidants can be obtained from edible mushrooms and act by mildly inhibiting the negative effects of free radicals through oxidation prevention. Previous studies have shown that certain mushroom species possess natural antioxidant properties (Hung and Nhi, 2012; Krupodorova and Sevindik, 2020). The present study demonstrates that *V. volvacea* extracts exhibit notable DPPH radical scavenging activity. This activity could be attributed to the chemical compositions detected, such as flavonoids and phenolics. Flavonoids are effective antioxidants through chelation of ferrous ions, scavenging of free radicals, and prevention of lipid peroxidation (Dinesh, 2021). Phenolic compounds act as single oxygen quenchers, hydrogen donors, and free radical scavenger (Sahu and Saxena, 2013). In addition to these two important antioxidants, *V. volvacea* has high concentrations steroids, ascorbic acid, tocopherols, lycopene, and carotenoids, which are also reported to demonstrate anti-oxidant properties (Ferreira et al., 2009). Recently, isolated and identified five novel antioxidant peptides from *V. volvacea* coded as DWPGFK, SFDWTGFK, FDWPGFKT, DWPTFKAF, and SGPSFDWPGFK, of which DWPGFK and DWPTFKAF have shown strong antioxidant properties *in vitro*, enhance antioxidant enzyme activity, protect human skin fibroblasts from oxidative damage, and inhibit matrix metalloproteinase activity (Xu et al., 2025). These findings add to the understanding of the mechanisms of antioxidant capabilities of *V. volvacea* and their potential applications in the pharmaceutical industry. This mushroom may serve as a potential remedy for disorders associated with oxidative stress (Sangthong et al., 2022). Anti-oxidative rich foods prevent inflammation, cancer, Alzheimer's, Parkinson's, cardiovascular, and neurodegenerative diseases (Ali et al., 2024). In addition, Sangthong et al. (2022) reported that *V. volvacea* extract in skincare products may offer anti-ageing benefits and be used for skin health. Interestingly, high free radical scavenging activity has potential applications in environmental remediation, neutralising free radicals to mitigate oxidative damage in polluted water bodies.

Antimicrobial compounds are one of the important attributes of mushrooms that enable them to survive in a microbiologically diverse natural environment. Mushrooms represent a valuable reservoir of potent substances capable of impeding the growth of various microbial organisms in a

cross-spectrum (Alves et al., 2012). In this study, La Union mycelia extract showed a zone of inhibition against *S. aureus*. This antibacterial activity could be attributed to the chemical constituents detected in the thin layer chromatography. La Union mycelia contains essential oils, phenols, fatty acids, sugars, coumarins, anthrones, and alkaloids, which are reported to exhibit antibacterial properties. Moreover, the activity could also be attributed to the simpler cell wall structure of *S. aureus* (Dulay et al*.,* 2017). Results corroborate with the results of Rosa et al. (2003), who reported that most Gram-positive bacteria show more sensitivity to cinnabarin from *Pycnoporus sanguineus* (L.) Murrill than Gram-negative bacteria. Moreso, Dulay et al. (2017) demonstrated the inhibitory activity of acetonitrile extracts of *L. trigrinus* and *Pleurotus djamor* (Rumph. ex Fr.) Boedijn mushrooms against *S. aureus*. The discovery of this natural antibacterial property in *V. volvacea* could have significant implications in the field of natural medicine and the quest for new strategies against antibiotic-resistant bacteria. With the increasing prevalence of multidrug-resistant pathogens, there is a growing need for novel antimicrobial substances from sustainable sources, like *V. volvocea* might result in the development of new antibiotics or adjuvants for existing treatments, offering a promising avenue for future antimicrobial research. In addition, the utilisation of mushroom-delivered compounds in antimicrobial therapy aligns with the principle of sustainable and eco-friendly medicine, as mushrooms can be cultivated and harvested without causing harm to the environment (Agarwal et al., 2023).

Zebrafish is a reliable and ideal vertebrate model in examining the embryotoxic and teratogenic effects of extracts or compounds being tested. This work also considered the determination of the toxic and teratogenic effects of the ethanolic extracts of mycelia of the two isolates of *V. volvacea* in developing embryos of zebrafish. It was found out that the embryo-toxic effect of both mushroom extracts is dependent on the extract concentration and exposure time, and the most marked toxic effect is coagulation of the embryo, which is a result of inhibited development. Similarly, high mortality of embryos due to coagulation is observed in embryos exposed to extracts of other mushrooms. The mycelial extract of *V. volvacea* increased mortality rates in zebrafish embryos in a stepwise fashion up to 48 h. High concentrations led to mortality due to cytotoxic impacts of bioactive compounds, damaging the chorion and interfering with organ growth, affecting cardiovascular or neural systems. Mortality was concentration- and exposure time-dependent, with 100% mortality at higher concentrations as early as 12 h post-treatment application (hpta), while lower concentrations showed no mortality. These findings align with previous studies where mushroom extracts exhibited dose-dependent toxicity effects on zebrafish embryos. De Castro and Dulay (2015) reported 100% mortality at 2.5% and 5% concentrations starting from 12 h to 48 h, with coagulation as the major toxic manifestation. Similarly, Dulay et al. (2014) observed a significant increase in lethal effects at concentrations of 5% or higher after 72 h. Rapid mortality at high concentrations suggests disruption of critical developmental processes. These results reinforce the concentration-dependent toxicity of natural extracts on aquatic organisms and the need for careful dosage consideration. Water extracts of *Schizophyllum commune* Fr. and *Lentinus tigrinus* significantly increased zebrafish embryo mortality with higher concentrations and prolonged exposure (Dulay et al., 2014). *Ganoderma lucidum* water extract also exhibited concentration- and time-dependent toxic effects (Dulay et al., 2012). The toxic effects could be attributed to biologically active compounds present in mushrooms

that suggest strong inhibitory activity, which warrants further studies particularly on the antiproliferative effect in cancer cells.

Normal hatching is an indicator of successful embryonic development. All embryos exposed to 1 ppm of both extracts and embryonic water successfully hatched after 48 hpte, while low to no hatching was observed at higher concentrations, suggesting a concentration-dependent hatchability. This low to no hatching may be attributed to the delayed growth of embryos, a distinct teratogenic effect of mycelial extracts of the two isolates of *V. volvacea*. Delayed growth could be due to the inhibition of the essential growth factors or enzymes, or the disruption of various physiological processes responsible for growth and development. Another reason for low to absent hatching is the restricted movement of the tail due to tail malformation, which impedes the ability of the larva to rupture the chorion and hatch. Similarly, these hatching disturbances are also observed in embryos exposed to extracts of other mushrooms such as *L. tigrinus* and *G. lucidum* (Dulay et al., 2012, 2014) and other compounds such as warfarin and coumarin (Weigt et al., 2012). Another important teratogenic parameter in zebrafish is the heartbeat rate. Findings of the present study revealed that heartbeat rates of extract-treated embryos significantly decreased as the extract concentration increased. A reduction in heartbeat rate is also observed when the zebrafish embryos are exposed to increasing concentrations of the ethanolic extract of the mushroom *Fomitopsis feei* (De Leon et al., 2020). This significant effect of mushroom extracts on the heartbeat of zebrafish indicates important roles of mushroom extract in the cardiovascular functions, which warrants further study.

Delayed growth of zebrafish embryos was identified as a distinct teratogenic effect of the *V. volvacea* mycelial extract. Similarly, developmental delay is the sole teratogenic effect of 100 µg/ml extract derived from the mycelia and fruiting body of *Lentinus strigosus* mushroom (Dulay et al., 2018). This sub-lethal effect is the underlying cause of various morphological abnormalities of the extract-treated embryos, including tail malformation, head malformation, yolk deformity, pericardial edema, and reduced pigmentation. These growth-delay associated malformations have also been observed in previously studied mushrooms. For instance, embryos treated with *Termitomyces clypeatus* extract exhibited coagulation, tail malformation, twisted tail tip, and wavy somite (De Castro et al., 2016). Moreover, pericardial oedema, underdeveloped organs, perverted tail, hook-like tail, and other tail malformations were documented as growth delay-related endpoints of zebrafish when exposed to the water extract of *L. tigrinus* (Dulay et al., 2014). Additionally, hook-like tails and bent tails are the distinct dysmorphologies of *Lentinus sajor-caju* and *Pleurotus ostreatus* treated embryos (De Castro and Dulay, 2015). However, the teratogenic effect of some mushrooms has also been investigated in other animal models. An exposure of cultured mouse embryos to ribosome inactivating proteins from *Hypsizigus mamoreus*, called hypsin, and from *Lyophyllum shimeji*, called lyophyllin, causes embryonic abnormalities (Chan et al., 2010; Ng et al., 2010). Results obtained in the present study and based on the above-cited previous works provide evidence on the embryotoxic and teratogenic properties of mushrooms that could be attributable to the chemicals they contain, which are promising bioactive compounds with functional activities such as cytotoxic, anticancer, and antitumor, since most anticancer drugs are teratogenic, and most teratogenic agents have anticancer activities (Blagosklonny, 2005).

In conclusion, the two isolates of mushrooms from San Jose and La Union, Philippines identified as *V. volvacea* using the ITS region of rDNA contain chemicals that exhibit a wide array

of therapeutic properties. Both mycelial extracts demonstrate DPPH radical scavenging activity, and embryo toxic and teratogenic properties in developing zebrafish embryos. However, only La Union mycelial extract shows inhibitory activity against *S. aureus*. Elucidation, characterisation and identification of novel bioactive compounds responsible for the observed biological activities of mycelial extracts of the two *V. volvacea* isolates warrant a more detailed mechanistic study. It is also necessary to establish biomass cultivation techniques for the two mushroom isolates to fully realise their mass propagation, which is an ongoing study in our laboratory.

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