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Biological properties of *Dendrobium crumenatum* Sw. leaves

Louise Isaac F. Guevarra[1], Eden S. David[1], Nonnatus S. Bautista[2], and Mary Jhane G. Valentino[1,*]

[1]Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120 Philippines
[2]Plant Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Pedro R. Sandoval Ave, College, Laguna, Philippines

**Corresponding Author: maryjhane.valentino@clsu2.edu.ph*

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ABSTRACT

Dendrobium crumenatum Sw. is one of the native species of orchids in the Philippines, which is known for the aesthetic value of its blossoms. Additionally, it is used in traditional medicine, but few studies have been conducted. The present study was carried out to determine the biological properties of ethanolic extracts from *D. crumenatum* leaves, such as antioxidant, antibacterial, antifungal, cytotoxicity, and teratogenicity. The phytochemical composition was determined using the thin-layer chromatography (TLC) method. Results revealed that essential oils, phenols, fatty acids, anthraquinones, coumarins, anthrones, tannins, flavonoids, and steroids were present in the ethanolic extract of *D. crumenatum* leaves. An antioxidant property of 584.9 ug/mg and a total phenolic content of 496.139 mg GAE/g were also detected. Moreover, antibacterial property as a protectant was recorded against *Escherichia coli* Escherich, Th. 1885 and *Staphylococcus aureus* Rosenbach, 1884. Meanwhile, no antifungal property was observed in the ethanolic extract of *D. crumenatum* leaves. Regarding cytotoxicity, the results revealed a moderately toxic effect with LC_{50} value of 341.680 ppm. Lastly, the ethanolic extract of *D. crumenatum* leaves caused the lethal effect on zebrafish embryo, such as embryo coagulation and absence of heartbeat, while its teratogenic effects included severe growth retardation, yolk deformity, and delayed embryo development. Thus, *D. crumenatum* leaf extract contains phytochemical constituents with moderate toxicity, depicting its pharmaceutical potential.

Keywords: cytotoxicity, eradicant, phytochemical, protectant, teratogenicity

INTRODUCTION

Dendrobium is a diverse and widespread group of flowering plants that belongs to the Orchidaceous family. With a total of 1,556 recognized species, it holds a significant position within this botanical family (Ram et al. 2021; Wang 2021). It is an epiphytic orchid native to the lowland tropics of Southeast Asia. Its natural range extends from India and mainland Southeast Asia through the Malay Archipelago to the Philippines. In the Philippines, this species is one of the most commonly encountered orchids. It can be found growing on host trees at sea level up to about 500 meters in elevation (Kurniawan and Amelia 2021). Its ability to colonize disturbed habitats and its rapid growth contribute to its abundance in both natural

forest settings and urban areas where host trees are abundant (Tabla and Vargas 2004). Due to its attractive and fragrant white flowers, it is widely cultivated and appreciated as an ornamental plant in the Philippines.

It is known in Asian countries as a traditional tonic since time immemorial, and it is also considered one of the 50 fundamental herbs used to treat various ailments (Cakova et al. 2017). Moreover, the stems of *Dendrobium* are beneficial to the stomach and the production of body fluids, nourishing yin, and clearing heat (Hu et al. 2012; Sliwinski et al. 2022). In the Philippines, it is used as a traditional medicine, macerated in coconut oil and applied as an ointment for skin diseases, wounds, ulcers, boils, and burns (Duenas-Lopez 2022). Moreover, Klongkumnuankarn et al. (2015) suggested that *Dendrobium* contains various bioactive compounds, including polysaccharides, alkaloids, flavonoids, and phenols, which may have antioxidant, anti-inflammatory, and immunomodulatory effects. Given the potential health benefits of *Dendrobium*, the study was carried out to address the research gap in exploring the potential of *Dendrobium crumenatum* Sw. leaves as a source of bioactive compounds for the development of novel pharmaceuticals and cosmetics. The present study focused on the antibacterial, antifungal, teratogenic, and cytotoxic properties of *D. crumentaum* leaf extracts.

METHODS

Collection and Extraction of *D. crumenatum* Leaves

The *D. crumenatum* plant (Figure 1) was gathered from Antipolo City, Philippines. Leaves free of disease were selected, washed with tap water, and then air-dried to ensure the complete removal of excess moisture. Subsequently, the dried leaves were grinded into a coarse powder using a blender. Twenty grams of the powdered leaves were extracted through a 72-hour maceration process using 100 mL of 95% ethanol at ambient temperature. The mixture was agitated for 15 minutes for every 12 hours. The solution was filtered using Whatman filter paper no. 1, and the solvent was removed via rotary evaporation (Chimsook 2016).

Phytochemical Composition and Antioxidant Property of *D. crumenatum* Leaves

Phytochemical screening was carried out using thin-layer chromatography (TLC) on a 7 × 4 cm plate in a 16 mm ethyl acetate-chloroform (7:3) solution under UV light to visualize separation of

Figure 1. *Dendrobium crumenatum* on its natural habitat.

different compounds. Several reagents were also used for the detection of secondary metabolites, such as vanillin-sulfuric acid reagent (phenols, sterols, triterpenes, and essential oils), metallic potassium hydroxide (anthraquinones, coumarins, and anthrones), ferricyanide-ferric chloride reagent (phenolic compounds and tannins), Dragendorff's reagent (alkaloid), and antimony (III) chloride (flavonoids). The total phenolic content was determined using the Folin–Ciocalteu method, were gallic acid served as the standard. The total phenolic content in the ethanol extracts was quantified and expressed in terms of grams of gallic acid equivalents (gGAE) per 100 g of extract. Lastly, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used for antioxidant detection. For the assay, a standard solution comprising 3 mL of DPPH and 100 µL of methanol was placed in a test tube and incubated in complete darkness for 30 minutes. Absorbance was measured at 517 nm. The percentage of antioxidant activity or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As) \div Ac] \times 100,$$

where Ac represents the absorbance of the control (DPPH solution without extract), and As denotes the absorbance of the test sample (mixture of DPPH, ethanol, and crude extract). Catechin, a synthetic antioxidant, was used as a positive control.

Bioassay of Antimicrobial Property

The antibacterial and antifungal properties of *D. crumenatum* were tested against *Escherichia coli* Escherich, Th. 1885; *Staphylococcus aureus*

Rosenbach, 1884; and *Aspergillus flavus* Link, 1809, following the protocol of Valentino et al. (2015) and Austria et al. (2017). Sterile distilled water served as the negative control, while streptomycin (for the antibacterial test) and nystatin (for the anti-fungal test) served as positive controls through the disc diffusion assay. For the eradicant test, paper discs were soaked in pure crude extracts and were seeded equidistantly in Mueller-Hilton agar plates streaked with cultures of *E. coli* and *S. aureus* in separate plates. For the protectant test, the filter paper discs were soaked with the test pathogens and seeded in plates flooded with leaf extracts. The zones of colonization (protectant) and zones of inhibition (eradicant) were measured at 12-hour and 24-hour observation intervals. For the antifungal test, plates with potato dextrose agar were flooded with crude extracts, and the paper discs were soaked in *A. flavus* with a standardized spore count of 1.5×10^6 spores/mL, grown in potato dextrose broth. The zone of mycelial colonization was observed at 24 and 48 hours of incubation.

Cytotoxicity Assay Brine

The brine shrimp lethality test was performed using 10,000 ppm, 5,000 ppm, 1,000 ppm, and 500 ppm *D. crumenatum* extracts. Each well of an enzyme-linked immunosorbent assay (ELISA) plate contained ten nauplii, and the survival count was recorded at 6-, 12-, 18, and 24-hour intervals. The efficacy of the active component was assessed using Abbot's formula.

Teratogenic Property of *D. crumenatum* Leaves

The methodology for assessing the teratogenicity of *D. crumenatum* leaf ethanolic extracts was adopted from Lindain et al. (2018). Zebrafish were acclimatized at 26 ± 1°C, and spawning was induced in 12 hours of darkness. Fertilized eggs were collected, and morphological uniformity assessment was performed before teratogenicity assay. Three embryos were placed in each well of ELISA plates containing 3 mL of each concentration of *D. crumenatum* leaf ethanolic extract and a control, together with three fertilized eggs. Mortality, hatchability, and morphological abnormalities were examined and determined using a compound microscope at 12-, 24-, 36- and 48-hours post-treatment, and were photo-documented using a digital camera. The number of heartbeats and the LC_{50} were also recorded.

RESULTS

Phytochemical Composition and Antioxidant Property of *D. crumenatum* Leaves

The examination of phytochemical composition involved TLC analysis for the identification of secondary metabolites within the ethanolic extract of *D. crumenatum* leaves. As presented in Table 1, essential oils, phenols, fatty acids, anthraquinones, coumarins, anthrones, tannins, flavonoids, and steroids were detected in the *D. crumenatum* leaf extract. For the total phenolic content, *D. crumenatum* leaf ethanolic extract contained 496.139 mg GAE/g and an antioxidant property of 584.9 μgmL^{-1} (Table 2).

Table 1. Phytochemical composition of *Dendrobium crumenatum* leaf ethanolic extract.

Phytochemicals	Ethanolic Extract
Essential oils	+
Triterpenes	-
Sterols	-
Phenols	+
Fatty acids	+
Sugars	-
Anthraquinones	+
Coumarins	+
Anthrones	+
Tannins	+
Flavonoids	+
Steroids	+
Alkaloids	-
Amino acids	-

Antimicrobial Potential of *D. crumenatum* Leaves

For the eradicant test against *E. coli* (Table 3), no zone of inhibition was observed, while against *S. aureus,* zones of inhibition measuring 10.17 mm and 9.80 mm at 12 and 24 hours, respectively, were recorded. As a protectant (Table 4 and Figure 2), reduction in zones of colonization was recorded for both *E. coli* (10.45 mm and 16.22 mm at 12 and 24 hours) and *S. aureus* (8.15 mm and 8.47 mm at 12 and 24 hours, respectively). For the antifungal property, no inhibition of mycelial growth was observed in *A. flavus* after 24 to 48 hours of incubation (Figure 3).

Table 2. Total phenolic content and radical scavenging *Dendrobium crumenatum* leaf ethanolic extract. *Wavelength 517 nm BK UV 1000 Spectrophotometer *Abs DPPH = 0.212.

Sample Description	Total Phenolic Content (mg GAE/g)	Radical Scavenging Activity (ug/mL)
D. crumenatum	496.139	584.9
Catechin (control)	-	781.8

Cytotoxicity of *D. crumenatum* Leaves

The cytotoxicity of the ethanolic extract of *D. crumenatum* leaves was assessed via the brine shrimp lethality assay. The mean and percentage mortality of brine shrimp (*Artemia salina* Linnaeus, 1758) nauplii exposed to various extract concentrations for 24 hours are detailed in Table 5. Maximum mean mortality

(100%) occurred at 10,000 ppm and 5,000 ppm, whereas the minimum mean mortality (66.67%) was observed at 500 ppm. Statistical analysis revealed no significant differences between 10,000 ppm and 5,000 ppm, while 1,000 ppm was comparable to both higher concentrations. Significant differences were noted among all concentrations compared to the control. The LC_{50} was estimated at 341 ppm.

Teratogenicity of *D. crumenatum*

For the teratogenicity of *D. crumenatum*, several parameters were evaluated which include hatchability, heartbeat rate, morphological defects, teratogenic effects, and mortality rates of *Danio rerio* (Hamilton, 1822) embryos.

Hatchability of *D. rerio* embryos. Hatching usually occurs within 48 to 72 hours post-treatment application (hpta). Table 6 shows the mean hatchability percentages of embryos exposed to different concentrations of leaf extract after 48 hours. Embryos in embryo water and 500 ppm solutions had the highest mean hatchability at 100%, while in 5,000 ppm and 10,000 ppm, embryos did not hatch. This may be due to premature embryo arrest and delayed development, indicating sublethal effects of the extract.

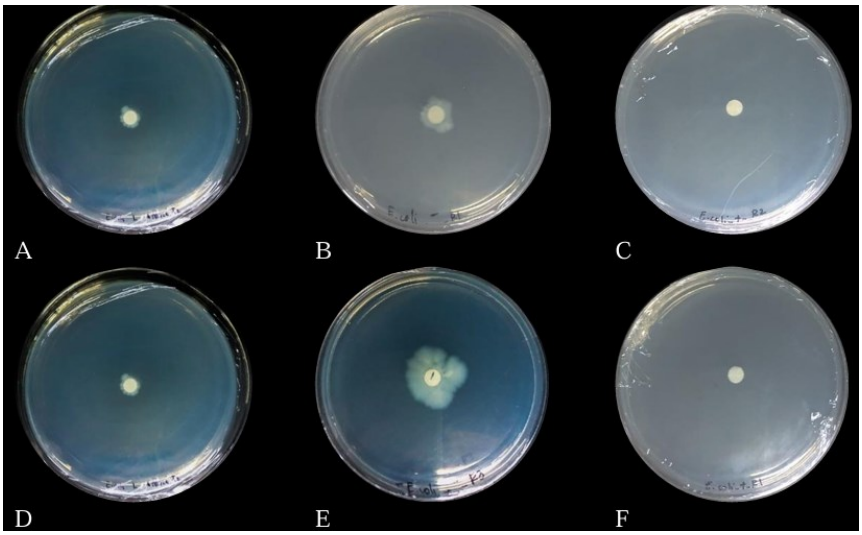

Figure 2. Antibacterial activity of *Dendrobium crumenatum* as protectant (A) 12 hours of incubation, ethanolic extract; (B) 12 hours of incubation, negative control; (C) 12 hours of incubation, positive control; (D) 24 hours of incubation, ethanolic extract; (E) 24 hours of incubation, negative control; and (F) 24 hours of incubation, positive control against *Escherichia coli.*

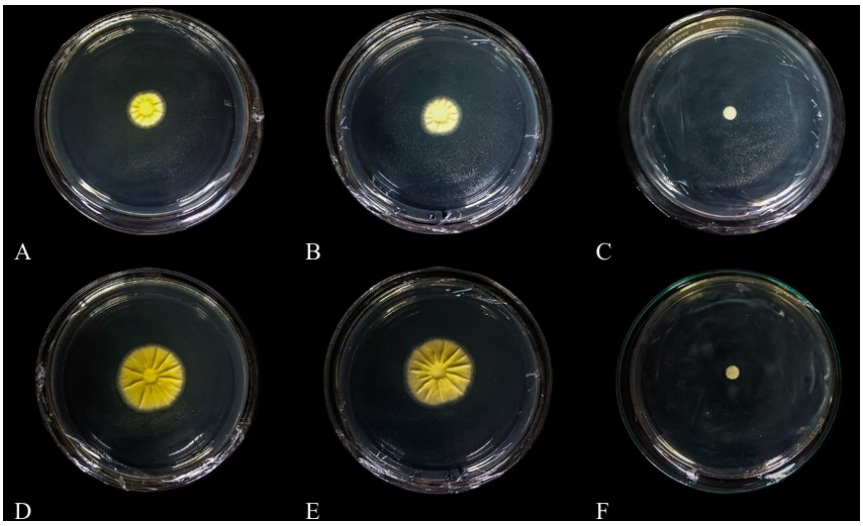

Figure 3. Antifungal activity of *Dendrobium crumenatum* (A) 24 hours of incubation, ethanolic extract; (B) 24 hours of incubation, negative control; (C) 24 hours of incubation, positive control; (D) 48 hours of incubation, ethanolic extract; (E) 48 hours of incubation, negative control; and (F) 48 hours of incubation, positive control against *Aspergillus flavus.*

Table 3. Mean diameter of zone of inhibition (mm) affected by the treatments against *Escherichia coli* and *Staphylococcus aureus* after 12 and 24 hours of incubation. Values are presented as mean ± sd, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Treatment	Diameter of Zones of Inhibition			
	Escherichia coli		*Staphylococcus aureus*	
	12 hours	24 hours	12 hours	24 hours
Ethanol extract	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$10.17^{b} \pm 4.49$	$9.80^{b} \pm 4.16$
Positive control	$29.05^{a} \pm 0.80$	$27.8^{a} \pm 0.99$	$33.20^{a} \pm 3.34$	$31.78^{a} \pm 0.71$
Negative control	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$

Table 4. Mean diameter of zone of bacterial colonization (mm) of *Escherichia coli* and *Staphylococcus aureus* against *Dendrobium crumenatum* leaves ethanol extract after 12 and 24 hours of incubation. Values are presented as mean ± sd, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Treatment	Diameter of Zones of Colonization			
	Escherichia coli		*Staphylococcus aureus*	
	12 hours	24 hours	12 hours	24 hours
Ethanol extract	$10.45^{b} \pm 2.05$	$16.22^{b}1 \pm 2.3$	$8.15^{b} \pm 0.39$	$8.47^{b} \pm 0.23$
Positive control	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
Negative control	$13.13^{c} \pm 0.76$	$22.43^{c} \pm 0.15$	$9.28^{c}9.28 \pm 0.65$	$11.98^{c} \pm 1.98$

Table 5. Mean percentage mortality of *Artemia salina* embryos after 12, 24, 36 and 48 hours of exposure to different concentrations of *Dendrobium crumenatum* Swarts leaves ethanolic extract. Values are presented as mean ± sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	Mortality Rate (%)			
	12 hpta	24 hpta	36 hpta	48 hpta
0.00	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
500	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
1000	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
5000	$25.00^{ab} \pm 50.00$	$25.00^{ab} \pm 50.00$	$25.00^{ab} \pm 50.00$	$25.00^{ab} \pm 50.00$
10000	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$

Table 6. Mean percentage hatchability of *Dendrobium rerio* embryo after 48 hours of exposure to different concentration of *Dendrobium crumenatum* Swarts leaves ethanol extract. Values are presented as mean ± sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	Hatchability	Heartbeat Rate
0.00	$100.00^{a} \pm 0.00$	$168.44^{a} \pm 6.71$
500	$100.00^{a} \pm 0.00$	$171.11^{a} \pm 18.73$
1000	$88.89^{a} \pm 19.25$	$118.67^{b} \pm 20.78$
5000	$0.00^{b} \pm 0.00$	$0.00^{c} \pm 0.00$
10000	$0.00^{b} \pm 0.00$	$0.00^{c} \pm 0.00$

Heartbeat rate of *D. rerio* embryos. The heartbeat, a vital physiological indicator, was monitored in *D. rerio* embryos during the pharyngula stage while exposed to different concentrations of *D. crumenatum* ethanol extract. As presented in Table 6, higher concentrations led to adverse effects, including early coagulation and developmental delays, with no heartbeat observed in embryos exposed to 10,000 ppm after 36 hours of incubation, which may be attributed to early coagulation evident at 12 hpta. Similarly, embryos exposed to 5,000 ppm exhibited delayed developmental growth, precluding heartbeat recording.

As shown in Table 6, lower concentrations resulted in increased heartbeat rates compared to the control, but a reduction was noted at 1,000 ppm. Overall, exposure to *D. crumenatum* ethanol extract resulted in a dose-dependent reduction in heartbeat rate. These underscore the escalating toxicity with increasing concentrations of *D. crumenatum* ethanol extract on *D. rerio* embryos. In contrast, control embryos exhibited a mean heartbeat of 168.44, while those exposed to 500 ppm demonstrated a significantly higher mean heartbeat of 171.1, higher than the control but lower than the 1,000 ppm group, which recorded a mean heartbeat of 118.67. Consequently, exposure to

varying concentrations of *D. crumenatum* ethanolic extract resulted in a reduction in heartbeat rate.

Morphological abnormalities and teratogenicity effects. The ethanolic extract from *D. crumenatum* induced morphological abnormalities and teratogenic effects at various developmental stages (Table 7). Embryos exposed to 1,000 ppm and 500 ppm exhibited yolk sac edema and limited pigmentation, with more severe effects at the higher concentration. Growth retardation was observed at 5,000 ppm, while coagulation, indicating lethal effects, occurred at 10,000 ppm. Control groups showed no abnormal morphological traits (Figure 4).

Mortality of *D. rerio* embryos. The mortality rates of embryos following various durations of exposure are delineated in Table 8, with mortality indicated by embryo coagulation. Findings reveal that concentrations of 10,000 ppm and 5,000 ppm resulted in 100% and 25% mortality, respectively, within 12 hours. Conversely, concentrations ranging from 0 to 1,000 ppm exhibited no mortality.

Utilizing probit analysis, the median lethal concentration (LC_{50}) of the ethanolic extract derived from *D. crumenatum* leaves in zebrafish embryos was 6,984.314 ppm. This outcome is attributed to the presence of phytochemicals, which, despite displaying diverse functional properties such as antibacterial and antioxidant effects, can also exert toxic effects on developing embryos.

DISCUSSION

Phytochemicals are secondary metabolites produced by plants to which their medicinal properties can be attributed (Larayetan et al. 2019; Gorlenko et al. 2020). These include saponins, flavonoids, alkaloids, phenols, essential oils, steroids, lignins, and tannins, all with known biological activities (Lam et al. 2015; Li et al. 2022). In recent years, the antimicrobial, antiparasitic, anticancer, antioxidant, and cytotoxic activities of these phytochemicals have been greatly explored and are continuously being established (Lin et al. 2018; Meitei et al. 2019). Accordingly, phenolic compounds and flavonoids are natural sources of antioxidant activities, eliminating reactive oxygen species that cause various diseases (Mazid et al. 2011; Paudel et al. 2019; Madjid et al. 2020).

Phytochemicals derived from plants constitute integral components in the process of drug development. The utilization of certain medicinal plants has facilitated the identification and isolation of therapeutic agents used in addressing various human ailments (Paudel et al. 2019). Essential oils, from aromatic plants like *Margotia gummifera* (Desf.) Lange, *Schinus areira* L., *Lavandula angustifolia* Mill, *Matricaria chamomilla* L., and *Cordia verbenacea* D.C., serve as valuable resources for aromatherapy, particularly for inhalation therapy, and present potential as complementary and alternative treatments for chronic obstructive pulmonary disease.

Table 7. Teratogenic effects of various concentrations of ethanolic extract of *Dendrobium crumenatum* Sw. leaves at 12, 24, 36 and 48 hours of exposure (+ = present; - = absent).

Dendrobium crumenatum	**Toxological Endpoints**	**Time of Exposure (hours)**	**Concentration (%)**				
Ethanol extract	Lethal		0	500	1000	5000	10000
	Coagulation	12	-	-	-	-	-
		24	-	-	-	-	+
		36	-	-	-	-	+
		48	-	-	-	-	+
	Teratogenic						
	Little pigmentation	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	+	-	-	-
	Growth retardation	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	-	-	+	-
	Yolk sac edema	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	+	-	-
		48	-	-	+	-	-

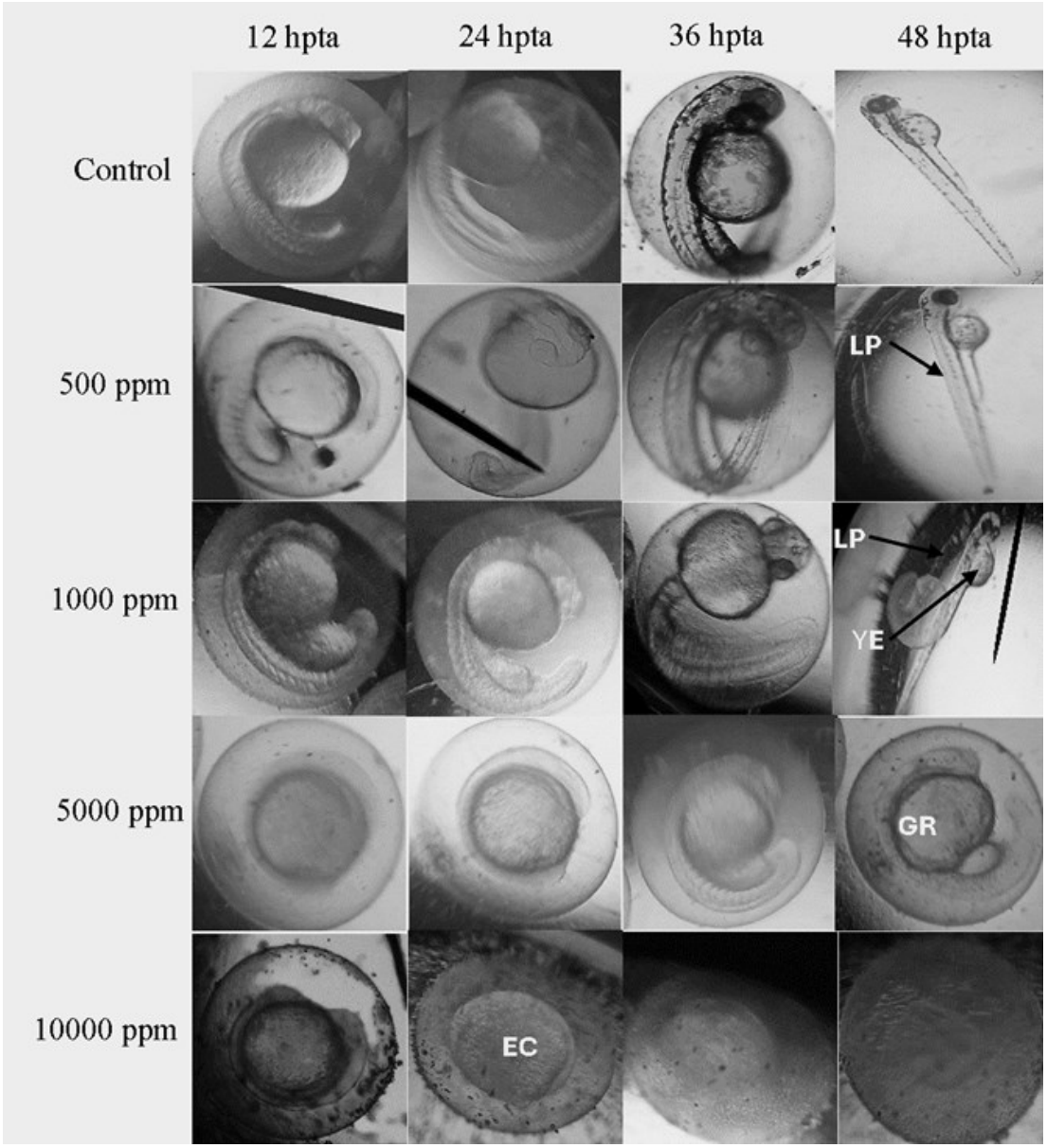

Figure 4. Morphological development of zebrafish embryos exposed to the different stages with different concentrations of *Dendrobium crumenatum* Swarts leaves ethanolic extract after 12, 24, 36 and 48 hours of exposure. Lethal effects: embryo coagulation (EC) 24 hpta in 10000 ppm; growth retardation (GR) 48 hpta in 5000 ppm; teratogenic effects: yolk sac edema (YE) and little pigmentation (LP) at 48 hpta in 1000 ppm and little pigmentation at 500 ppm at 48 hpta.

Table 8. Mean percentage mortality of *Dendrobium rerio* embryos after 12, 24, 36 and 48 hours of exposure to different concentrations of *Dendrobium crumenatum* leaves ethanolic extract. Values are presented as mean ± sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	12 hpta	24 hpta	36 hpta	48 hpta
0.00	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
500	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
1000	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
5000	$25.00^{ab} \pm 0.00$	$25.00^{ab} \pm 0.00$	$25.00^{ab} \pm 0.00$	$25.00^{ab} \pm 0.00$
10000	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$

Their efficacy in managing lung-related inflammation underscores their therapeutic significance (Zhang et al. 2022). Additionally, phenolic compounds, including tannins and flavonoids, are recognized for their antioxidant properties in both medicinal and culinary plants (Dai and Mumper 2010).

Based on the study of Ramesh et al. (2019), several genera of the family Orchidaceae are considered medicinal orchids. The detection of anthraquinones and anthrones in the ethanolic extract of *D. crumenatum* is noteworthy due to their diverse biological activities, including anticancer and antimicrobial properties (Fouillaud et al. 2016). Moreover, the presence of coumarins in *D. crumenatum* suggests therapeutic applications in managing inflammatory conditions, cardiovascular diseases, and oxidative stress-related ailments (Venugopala et al. 2013). The presence of steroids in *D. crumenatum* further underscores the plant's potential in treating inflammatory conditions, autoimmune disorders, and cancer (Sandrasagaran et al. 2014). Phenolic acids are known for their antioxidant properties, both direct and indirect, through the induction of protective enzymes and regulation of signaling pathways (Kumar and Goel 2019; Zhang et al. 2022). Variations in antioxidant activity among *Dendrobium* species are due to differences in species, isolated constituents or fractions, extraction techniques, and assay methodologies (Chimsook 2016).

The antibacterial potential is attributed to phytochemicals such as flavonoids, tannins, fatty acids, and alkaloids. These substances destabilize cellular membranes by integrating into lipid bilayers, leading to membrane disruption and potential bacterial inhibition (Desbois et al. 2010; Cushnie et al. 2014; Agustini et al. 2020; Zhang et al. 2022). Tannins exhibit a range of physiological impacts, including anti-inflammatory, secretion-reducing, antibacterial, and antiparasitic properties (Zhang et al. 2022). According to Cushnie et al. (2014), flavonoids and alkaloids have been recognized for their diverse therapeutic benefits, including their roles as antihypertensive, anti-rheumatic agents, antimicrobial, and antioxidants. In a study by Desbois et al. (2010) free fatty acids (FFA) showed potent biological activity, with the ability to kill or inhibit bacterial growth by destabilizing cellular membranes. Moreover, FFAs are used to defend against many parasitic and pathogenic bacteria, with various applications in medicine, agriculture, and food preservation. They function by integrating into and altering lipid bilayers, thus disrupting the membranes' barrier properties. This action can lead to membrane fusion, resulting in the release and aggregation of substances within the membranes. In the present study, the protectant property of *D. crumenatum* against *S. aureus* and *E. coli* was depicted. This indicates that it can be applied to healthy host cells prior to infection to combat microbial colonization (Mehta et al. 2020). Protectant is also known as preventive agent and is commercially used as antibacterial protection for disinfection, often as a component of antibacterial cleansers. They are only effective before the occurrence of bacterial pathogens (Namukobe et al. 2021).

Plant extracts with LC_{50} < 1,000 ppm as toxic and those with LC_{50} > 1,000 ppm as non-toxic. The teratogenic activity of *D. crumenatum* can also be attributed to the anticancer properties of its phytochemical constituents. Some of the compounds in orchids with anticancer properties include erianin (Petpiroon et al. 2017; Liu et al. 2019), phoyunnanin (Phiboonchaiyanan et al. 2018), erianthridin (Boonjing et al. 2021), ephemeranthol (Nonpanya et al. 2020), gigantol (Cai et al. 2021); denbinobin (Wang et al. 2022) and, dendrofalconerol (Petpiroon et al. 2017). Their actions include suppressing the signaling pathways for the growth and regulation of cancer cells (Bhummaphan and Chanvorachote 2015; Treesuwan et al. 2018; Luo et al. 2019; Aksorn et al. 2021), as well as migration and invasion, which lead to cancer cell death (Yang et al. 2023). Erianin was first isolated from *Dendrobium* sp. of Orchidaceae, with known active components with antitumor potentials (Zhang et al. 2019; Li et al. 2023; Deng et al. 2024). Its antitumor property is due to its cell apoptosis-promoting activity, which induces cell cycle arrest, apoptosis and autophagy, while inhibiting angiogenesis (Zhou et al. 2009; Li et al. 2019; Yi and Lan 2020). According to Petpiroon et al. (2017), phoyunnanin, from *D. venustrum*, has anti-migration and epithelial-mesenchymal transition-suppression activities caused by reduced alpha v and beta 3 integrins and focal adhesion kinase/ protein kinase signals. Another active phenol, erianthridin, suppresses migration and invation of all non-small cell lung cancer cells (H460) through actin and matrix metalloproteinase inhibition (Pothongsrisist et al. 2021). Moreover, denbinobin has the ability to downregulate the expression of decoy receptor-3, and it functions together with the Fas ligand, causing a reduction in pancreatic adenocarcinoma (Magwere 2009). Lastly, dendrofalconerol also possesses anti-migratory activity against metastatic cancer cells via expression of integrin β1 and integrin α4 (Pengpaeng et al. 2015).

Further studies are necessary to verify the findings of the study. High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) may be used for the quantification of the phytochemicals detected. Meanwhile, the Minimum Inhibitory Concentration (MIC) for the antibacterial property of *D. crumenatum* must be performed. Lastly, cytotoxicity tests using cancer cell lines can be conducted for further utilization of *D. crumenatum.*

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ETHICAL CONSIDERATIONS

The study is exempted from any ethics guidelines.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests to any authors.

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