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Zebrafish Embryotoxicity and Teratogenic Effects of *Christia vespertilionis* Leaf Extract

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ABSTRACT

Christia vespertilionis or butterfly wings is a traditional medicinal plant used to treat, among others, colds and bronchitis. The plant was also reported to be a remedy for cancer, with several products based on the plant becoming commercially available, raising some safety concerns on its consumption. The present study was carried out to assess the toxic and teratogenic effects of the plant on the embryonic development of zebrafish (*Danio rerio*) as the animal model. Zebrafish embryos were exposed to 50, 100, 200, 400, and 800 µg/mL of the methanolic leaf extract of *C. vespertilionis*, starting from 5 to 120 hours post-fertilization (hpf). The median lethal concentration (LC_{50}) value of the extract was determined to be 419.84 µg/mL, which is within the safety limit stipulated by the Organisation for Economic Co-operation and Development (OECD) guideline. However, results from the teratogenicity evaluation revealed multiple signs of developmental defects in embryos exposed to 200 µg/mL and higher concentrations of the extract. The magnitude of the defects was observed to be concentration-dependent. Moreover, no hatching and spontaneous movement of tail coiling were observed at 400 and 800 µg/mL concentrations due to the delayed growth and early mortality, respectively. A significant reduction in heartbeat rate was also reported for the surviving embryos at the 400 µg/mL test concentration. The present study has provided preliminary results on the potentially toxic and teratogenic effects of the extract at high concentrations.

Keywords: *Christia vespertilionis*, embryotoxicity, methanolic extract, teratogenic effects

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INTRODUCTION

Plants have served as a valuable source of chemical constituents with a broad spectrum of pharmacological properties, many of which have been translated into clinically used drugs (Ghasemzadeh et al., 2015). The promising potential of plants, especially those with a history of ethnomedicinal uses in curing various diseases and ailments, has also led to the growth of a wide variety of herbal products and supplements globally. However, despite the beneficial effects on human health, herbs and products derived from them have also been associated with cases of adverse side-effects resulting from their ingestion (Hussin, 2001). Thus, the toxicological assessment of herbal products is an essential step within the framework of herbal product development to protect and ensure consumer safety.

By convention, various mammalian models such as mice, rats, and rabbits have been widely used in toxicological studies (Caballero & Candiracci, 2018). Owing to the fact that the whole animal system is typically closely related to human toxicity, the use of animal models is considered a gold standard in toxicological testing (Jayasinghe & Jayawardena, 2019). However, in recent years, the use of zebrafish (*Danio rerio*) as an alternative to the classical higher vertebrate models has gained increasing attention. The wide usage of zebrafish is mainly attributed to its high genetic similarity to humans; zebrafish possess approximately 70% homology with humans, and about 84% of its genes appear to be related to human disease (Howe et al., 2013). Presently, compared to adult zebrafish, embryos are more increasingly being used for toxicological evaluations due to their optical transparency, which permits direct visualization of the model's developmental stages without a need for surgical procedure (Jayasinghe & Jayawardena, 2019). In addition, teratogenic effects upon exposure to chemical substances can be easily observed in zebrafish, giving the excellent predictive ability of the bioassay in evaluating developmental toxicity in mammals (Gao et al., 2014). Moreover, testing on the zebrafish model can also be completed in a short timeframe, which is extremely valuable, and the embryos exhibit a good dose-response to toxicity (Zhang et al., 2003).

Christia vespertilionis, popularly known as 'butterfly wing' or 'rerama,' is a plant of the *Christia* Moench genus in the Fabaceae family. This species is widespread in tropical Southeast Asia and exists in two varieties: red and green-leafed. Traditionally, *C. vespertilionis* has been reported to be used in treating colds, bronchitis, tuberculosis, muscle weakness, poor blood circulation, bone fractures, snake bites, and scabies (Dash, 2016). Pharmacological properties reported on the plant leaves included anti-proliferative (Hofer et al., 2013), cytotoxicity (Abd Latip & Abd Mutalib, 2019; Lee et al., 2020; Nguyen-Pouplin et al., 2007), antimalarial (Nguyen-Pouplin et al., 2007), antidiabetic (Murugesu et al., 2020), and antioxidant properties (Abd Latip & Abd Mutalib, 2019; Lee et al., 2020; Murugesu et al., 2020). Individual bioactive constituents

responsible for these biological properties have yet to be identified. However, in our molecular network-based dereplication of the chemical constituents of the plant, it is shown to be rich in flavonoids and phenolic acids (Norazhar et al., 2021).

In Malaysia, the green-leafed variety gained popularity in recent years due to testimonial reports on the therapeutic uses of the plant, which included as an herbal treatment for cancer. According to some patients diagnosed with cancer, consuming a water decoction of the fresh leaves of this plant helped in improving their health and claimed to have 'cured' their cancer (Zakaria, 2015). These have raised public concerns with respect to the validity of the efficacy claims and, more importantly, product safety. Previously, in a study by Nurul et al. (2018), although subacute oral administration of the ethanolic leaf extract to rats showed no mortality, mild to moderate lesions of hepatic necrosis and degeneration, and eventually hepatitis, were observed in all treated groups. Apart from this study, there were no other toxicity reports on the plant. Thus, there is still limited and inadequate toxicity and teratogenicity information on *C. vespertilionis* (green-leafed variety), emphasizing the need for more research to properly establish the toxicity profile of the plant and determine the safe levels for its practical usage for healthcare. The present study was thus carried out to address some aspects of this need by evaluating the toxic and teratogenic effects of the plant extract on the embryonic development of zebrafish (*Danio rerio*).

MATERIALS AND METHODS

Chemicals

Analytical grade methanol and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific, Malaysia.

Plant Material

Christia vespertilionis (green-leafed variety) was obtained from a plant nursery in Skudai, Johor, Malaysia, and taxonomically authenticated by Dr. Mohd Firdaus Ismail, a botanist at the Institute of Bioscience, Universiti Putra Malaysia. A voucher specimen (MFI 0150/20) has been deposited in the herbarium unit of the Institute of Bioscience, Universiti Putra Malaysia, for the record.

Extraction

Fresh leaves of *C. vespertilionis* were washed and dried in a circulating air oven at 40 ºC until constant weight. The dried leaves were then ground into a fine powder using a mechanical blender (HR2056, Philips, Netherlands). Next, 10 g of the ground leaves were mixed with 100% methanol at a solid to liquid ratio of 1:10 (*w/v*) and sonicated for 30 minutes under a frequency of 53 kHz and power of 100 W, bath temperature maintained between 30 ºC to 40 ºC. The extract was filtered with Whatman filter paper No.1 (GE Healthcare, USA), and the solvent was removed using a rotary evaporator under reduced pressure, with the temperature-controlled at 40 ºC. The crude extract was stored at -80 ºC freezer and further lyophilized using a Labconco®

FreeZone Freeze Drier System (USA). The freeze-dried extract was then stored in an airtight container at 4 ºC until further use.

Fish Husbandry

Fish experiments were carried out as approved by the UPM's Institutional Animal Care and Use Committee (IACUC), approval letter number UPM/IACUC/AUP-R045/2019. Adult zebrafish (AB strain), all (> six months old), were maintained under 10:14 h of the dark: light cycle with ambient temperature at 28.5 °C in 3 L aquarium tanks. The adult fishes were originally purchased from the Institute of Molecular and Cell Biology, Singapore. Then, they were maintained and propagated in Bioassay Unit, Natural Medicines and Products Research Laboratory (NaturMeds), IBS, UPM. Adult males and females used for this experiment belong to the F4 generation. Only five fish with a female to male ratio of 3:2 were placed per tank to ensure a stress-free environment for the highly sensitive fish. The tanks were continuously supplied with water by a recirculating water system. The fish were fed with brine shrimps (*Artemia salina*, San Francisco Bay Brand, USA) four times per day to ensure healthy and high fecundity. The volume of brine shrimps fed to the fish was approximately 4 mL/3 L tank for each feeding.

Spawning, Collection, and Selection of Embryos

Healthy (visual assessment of body condition scoring according to Clark et al., 2018), active, and well-fed adult zebrafish (> six months old) were selected for breeding. Five fishes were maintained in a 3 L aquarium equipped with a recirculation water system maintained under 10:14 h of the dark: light cycle at 28.5 °C, with a female to male ratio of 3:2. Artificial aquarium plants were placed in the spawning tank together with a spawn trap for egg collection to stimulate spawning. Three spawning tanks were set up for the experiment to have an adequate supply of fish eggs. Fertilization usually occurs in the morning, within 30 minutes after the light is turned on. Fish eggs were collected, washed with distilled water, rinsed with embryo media [15 mM sodium chloride (NaCl), 0.5 mM potassium chloride (KCl), 1 mM magnesium sulfate ($MgSO_4$), 0.15 mM monopotassium phosphate (KH_2PO_4), 0.05 mM disodium phosphate (Na_2HPO_4), 1 mM calcium chloride ($CaCl_2$), 0.7 mM sodium bicarbonate ($NaHCO_3$), pH 7.0], transferred into clean petri dishes containing embryo media (E3M), and incubated at 28 °C. According to the guideline by Organisation for Economic Co-operation and Development (OECD) (2013), the fertilization rate should be more than 50%, while in our laboratory standard protocol, the experiment will be conducted only when the rate of fertilization is more than 70%. At 4 hpf, normally fertilized embryos that reached the gastrulation stage (50% epiboly) were selected for this experiment. The selection was carried out by examining the collected eggs under a standard dissecting microscope (SZX-12, Olympus, Japan) with magnification set to 3x. The selected fertilized embryos were rinsed with E3M,

and any dead or unfertilized eggs were removed (to eliminate fungal growth).

Embryonic Exposure Experiments

The exposure experiment was performed in 24-well plates according to the method described in OECD (2013). After initial range-finding experiments, five concentrations (50, 100, 200, 400, 800 µg/mL) of the extract were selected as the final test concentrations. A stock solution was prepared by dissolving 0.05 g of the sample in 1000 µL DMSO. The highest treatment concentration (800 µg/mL) was first prepared by diluting 240 µL of the stock solution with 14,760 µL of E3M. From this concentration, two-fold serial dilutions were further made to give the subsequent treatment concentrations. The percentage of DMSO in the highest treatment concentration (800 µg/mL) was calculated to be 1.6%, which was well within the safe limit of the organic solvent allowed for zebrafish embryo assay (Maes et al., 2012). Ten embryos at the gastrulation phase were transferred into each well containing the different treatment concentrations. For the control group, embryos were exposed to 1.6% of DMSO in E3M. The maximum volume per well was kept to 2 mL. The plate was incubated at 28 °C for the exposure experiment. Three independent replicates were performed for each treatment concentration.

Evaluation of Toxicity Effects. A series of toxicity parameters such as mortality rate, spontaneous movement of tail coiling behaviour (at 24 hpf), heartbeat rate (at 48 hpf), and hatching rate (at 72 hpf). Upon completion of the early developmental process, a zebrafish larval is normally released from the chorion because of chorion breakdown. Normally, the hatching process is completed by 72 hpf; however, this biological process is interrupted in toxic conditions. The hatching rate was determined by quantifying the number of successfully hatched embryos at 72 hpf. All observations were made and recorded after viewing the embryos under a standard dissecting microscope (SZX-12, Olympus, Japan). The mortality rate data obtained was then used to determine the median lethal concentration (LC_{50}) of the extract by means of probit analysis (Finney, 1971) in Microsoft Excel. The number of tails coiling observed over one minute for the individual embryo was manually counted. The embryo was habituated for five minutes under the microscope before starting the tail coiling count. One complete cycle of coiling is represented by a full-body contraction that brings the tip of the tail to the head, which involves two alternating side to side contractions (left-right) (Saint-Amant & Drapeau, 1998). The heartbeat of the individual embryo was determined by manually counting the embryo's heartbeat over 1 minute. No anaesthetic drug was used while measuring the heartbeat.

Evaluation of Teratogenic Effects. Several parameters of teratogenicity such as the abnormal shape of head, eyes, and heart, bent body axis, growth retardation, uninflated

swim bladder, and deformity of yolk were assessed for 120 hours by viewing under a standard dissecting microscope (SZX-12, Olympus, Japan).

Statistical Analysis

All results obtained were expressed as mean ± standard deviation (SD) from three independent replicates, calculated using Minitab software (Version 16, Minitab Inc., USA). In addition, the *P* values were obtained from analysis of variance (ANOVA) analysis using the post-hoc Tukey's test where *($P \leq 0.05$) was significantly different from the control group.

RESULTS

Effect on Mortality Rate. The effect of the extract on zebrafish embryos mortality rate was evaluated over a range of concentrations (50-800 µg/mL). As shown in Figure 1, zero mortality was recorded for the control and low concentration groups (50 and 100 µg/mL). However, the mortality rate of the embryos was significantly increased with exposure to higher concentrations starting from 200 µg/mL, inducing a significant increment in mortality rate from 10% (200 µg/mL) to 100% (800 µg/mL). In particular, 200 and 400 µg/mL concentrations induced 10% and 50% mortality within 48 hpf, respectively. Meanwhile, it was observed that the highest test concentration of 800 µg/mL induced 56% mortality in the first 24 hpf and 100% mortality before reaching 48 hpf.

The percentage mortality data at 200 and 400 µg/mL were used to determine the LC_{50} value of the test extract by means of probit analysis. Consequently, the LC_{50} value of the extract was calculated to be 419.84 µg/mL. The logarithmic estimation

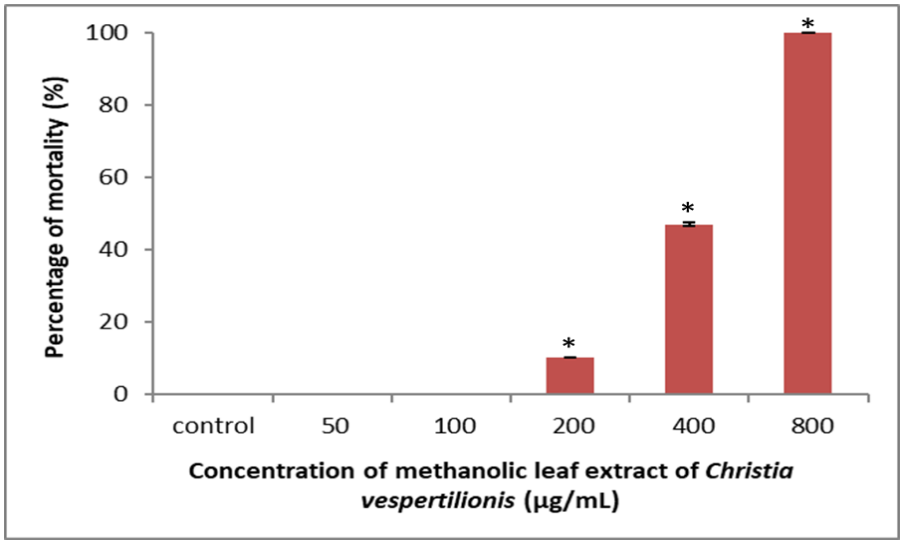


Figure 1. Mortality rate of zebrafish embryos exposed to methanolic leaf extract of *Christia vespertilionis*. Values are expressed as mean ± standard deviation of three biological replicates.

Note. *Significantly different from the control ($P \leq 0.05$)

of the LC_{50} value is displayed in Figure 2. Generally, higher LC_{50} values imply less test chemical toxicity as greater concentrations are required to elicit 50% mortality in the test organisms (Thiagarajan et al., 2019). Meanwhile, according to the OECD (2013), any toxicants are categorized as 'harmful', 'toxic', and 'highly toxic' if the value of LC_{50} ranges between 10–100 mg/L, 1–10 mg/L, and < 1 mg/L, respectively. Since the LC_{50} value of the extract was higher than the OECD values, it could be concluded, at this stage, that this methanolic extract is non-toxic and safe for consumption, at least for concentrations lower than its LC_{50} value. However, the mortality rate is not the final decisive criterion for the safety of a plant extract. Its effect on the overall development of an organism must also be considered.

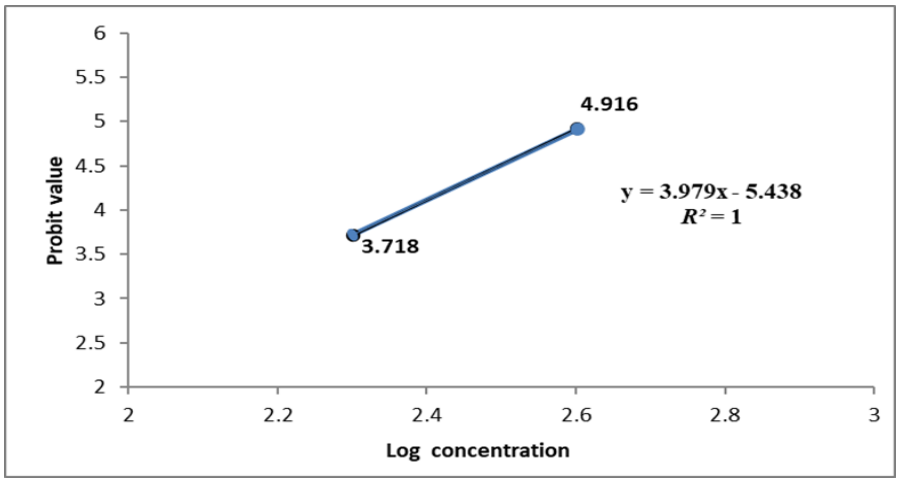


Figure 2. Median lethal concentration (LC_{50}) value of methanolic leaf extract of *Christia vespertilionis* based on probit analysis

Effect on Rate of Heartbeat. The normal heartbeat rate of zebrafish embryos ranges from 120 to 180 beats per minute (bpm) (De Luca et al., 2014). Therefore, the effect of the varying concentrations of the extract on the embryos heartbeat rate was evaluated at 48 hpf; values were expressed as several beats per minute (bpm). The results are shown in Figure 3. There was no significant difference in the mean heartbeat rate between the control group and groups with 50-200 µg/mL concentrations. In contrast, embryos exposed to 400 µg/mL showed a significant decrease in their heartbeat rate with a mean value of 102.067 bpm, compared to the control and the 50-200 µg/mL treatment groups. Meanwhile, no heartbeat was observed in the embryos exposed to the highest 800 µg/mL concentration due to early mortality.

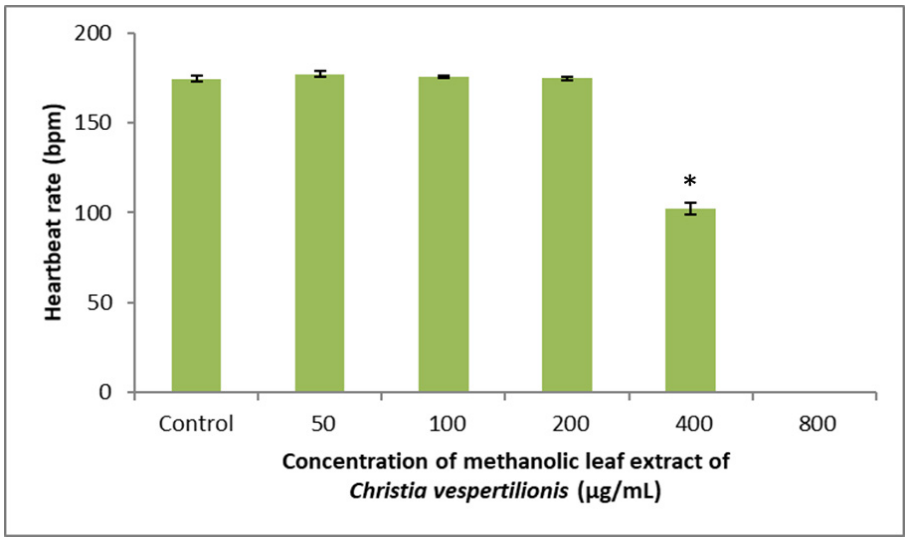


Figure 3. Heartbeat rate of zebrafish embryos at 48 hpf exposed to methanolic leaf extract of *Christia vespertilionis*. Values are expressed as mean ± standard deviation of three biological replicates.

Note. *Significantly different from the control ($P \leq 0.05$)

Effect on Hatchability. During normal embryogenesis of zebrafish, the hatching process is characterized by the breakdown of the chorion, releasing the free-living larvae. This process usually occurs within 48-72 hpf (Thiagarajan et al., 2019). Therefore, the hatchability rate of zebrafish embryos exposed to varying concentrations was evaluated. As presented in Figure 4, the hatchability rate of the exposed embryos was strongly dependent on the concentration of the test extract. At higher concentrations of 400 and 800 µg/mL, no hatching was observed at 72 hpf due to the delayed growth and 100% mortality were recorded even before 48 hpf, respectively. In contrast, 100% hatching was recorded for concentrations of 50, 100, and 200 µg/mL, which was comparable to the control group.

Effect on Spontaneous Movement of Tail Coiling. Spontaneous motor activity is an ideal behavioural test for neuronal function. This parameter is commonly used to evaluate the neurotoxic potential of chemical substances (Moser, 2011). The spontaneous movement of tail coiling in zebrafish embryos at 24 hpf was evaluated to determine the motor deficit potentially induced by the varying concentrations of the test extract. The results, as depicted in Figure 5, showed that there was the absence of spontaneous movement of tail coiling at the concentrations of 400 and 800 µg/mL due to their delayed growth and early mortality, respectively. In contrast, no significant changes in the spontaneous movement of tail coiling were observed for the concentrations of 50 to 200 µg/mL compared to the control.

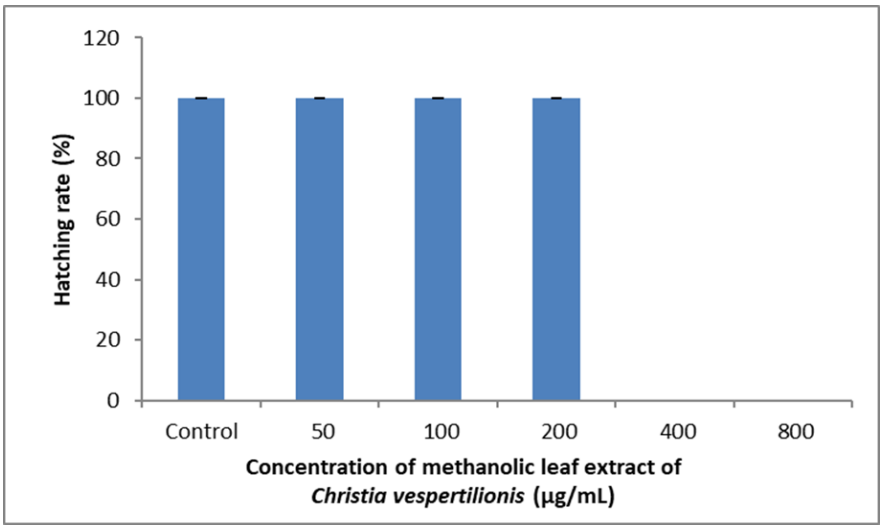


Figure 4. Hatching rate of zebrafish embryos exposed to methanolic leaf extract of *Christia vespertilionis*. Values are expressed as mean ± standard deviation of three biological replicates

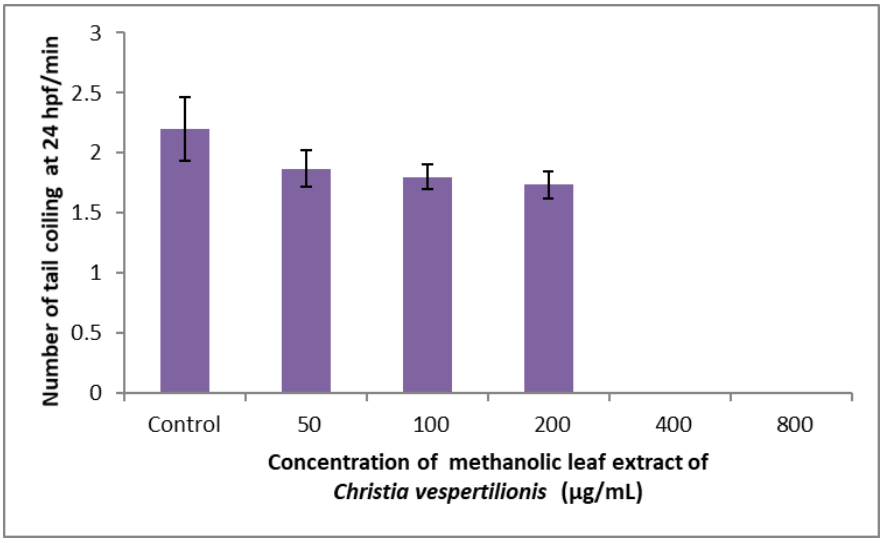


Figure 5. Spontaneous tail coiling rate of zebrafish embryos exposed to methanolic leaf extract of *Christia vespertilionis*. Values are expressed as mean ± standard deviation of three biological replicates

Teratogenic Effects

As shown in Figures 6 and 7, embryos exposed to high concentrations exhibited multiple signs of developmental abnormalities, including delay in development, bent or undetached tail, spinal column curving, pericardial sac oedema, yolk sac oedema, small eyes, abnormal head shape, and uninflated swim bladder. Delayed growth (stage delay) was noted at 24 hpf in the surviving embryos at 400 and 800 µg/mL concentrations (Figure 6), which showed that the embryos were still at 14-somite and 5-somite stages, respectively. In contrast, active embryos with complete detachment of tail from the yolk sac were observed at the concentrations of 50, 100, and 200 µg/mL, comparable with the normal embryos in the control group. After 72 hpf, it was observed that hatched larvae exposed to 200 and 400 µg/mL exhibited severe morphological abnormalities (Figure 7).

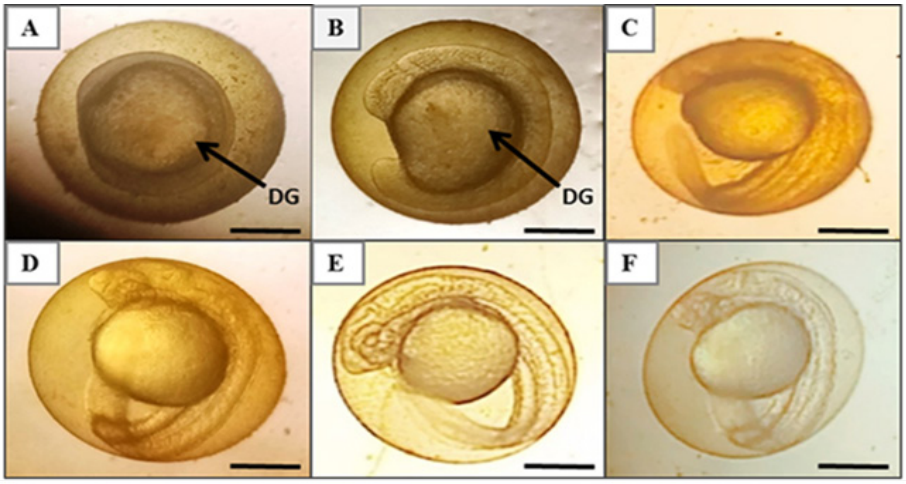
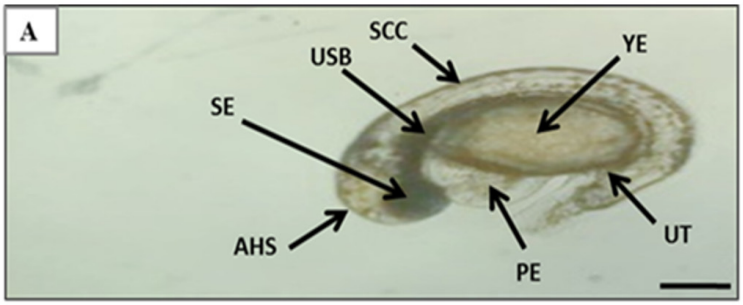


Figure 6. Representative optical image of zebrafish embryo exposed to (**A**) 800 µg/mL, (**B**) 400 µg/mL, (**C**) 200 µg/mL, (**D**) 100 µg/mL, (**E**) 50 µg/mL, and (**F**) control at 24 hpf. Malformations are indicated by arrows. DG–delayed growth. Scale bar = 1mm



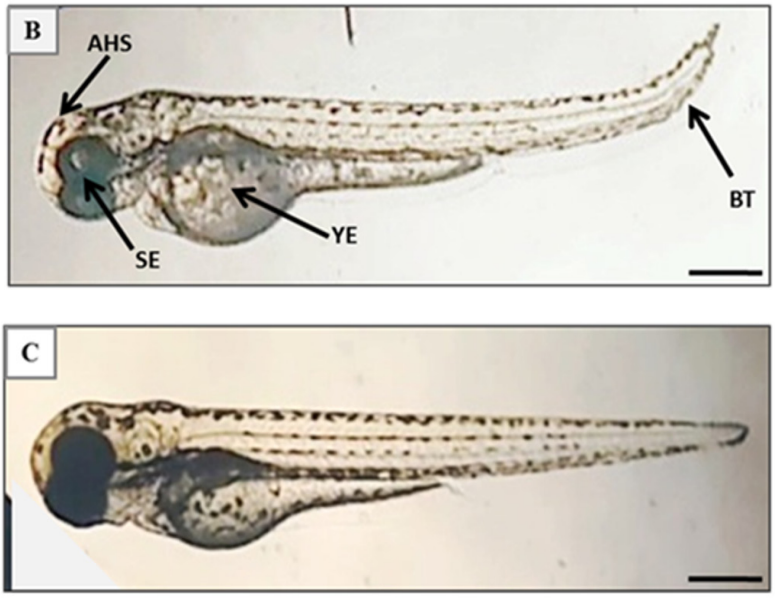


Figure 7. Representative optical image of zebrafish larvae after 72 hpf. Malformations were indicated by arrows. Larvae with (**A**) spinal column curving (SCC), uninflated swim bladder (USB), pericardial sac oedema (PE), yolk sac oedema (YE), abnormal head shape (AHS), small eyes (SE), and undetached tail (UT) at 400 µg/mL; (**B**) bent tail (BT), yolk sac oedema (YE), small eyes (SE), and abnormal head shape (AHS) at 200 µg/mL; and (**C**) normal morphology (control). Scale bar = 1mm

DISCUSSION

According to the OECD guidelines (2013), the leaf extract of *C. vespertilionis* may be considered non-toxic, based strictly on the high LC_{50} value of 419.84 µg/mL. However, the overall embryonic development of the exposed groups indicated that the embryos are affected acutely by a high concentration of the extract. At high concentrations, the extract was lethal and induced a significant decrease in heartbeat and hatchability rates and caused various teratogenic effects on the embryos. The delayed hatching observed at 400 µg/mL indicated growth retardation of the embryos. The delayed hatching may be due to developmental abnormalities in the developing embryos, as evidenced by a severe spinal column curvature in the treated embryos, which limited their ability to break the chorion (Murugesu et al., 2019). The decreased heartbeat rate observed in all surviving embryos at 400 µg/mL suggested that high extract concentrations may cause cardiotoxicity. Consistent with this was the occurrence of oedema in the pericardial sac of the hatched larvae exposed to 400 µg/mL of the extract, which reflected the embryos failed to develop into the normal morphology as observed in the control group. In general, proper function of the heart is crucial for growth and development in the later stages of life since abnormal heart function is known to cause severe developmental effects (Chen et al., 2018).

Thus, the 100% mortality recorded at the highest 800 µg/mL concentration could be related to the test organism's severe cardiac malfunction. Other observed abnormal developments could also have resulted from altered functions of multiple genes during embryonic development. For example, a phenotype with a bent tail malformation has been linked to a disruption of the *cysteine-rich motor neuron1* (*crim1*) gene, specifically affecting vasculature and somites development (Kinna et al., 2008). In the case of spinal column curving, the phenotype could be due to a decrease in collagen synthesis in the spinal column, changes in amino acid composition, or resulting from inhibition or downregulation of *protein tyrosine kinase 7* (*PTK7*) gene, a critical regulator of Wnt signalling (Pamanji et al., 2015).

Despite the prolonged use of plants as a valuable source of pharmacologically active constituents, the phytochemicals it contains could also be potential toxins for humans and animals (Chandra et al., 2012). Similarly, the adverse effects experienced by the embryos upon exposure to high concentrations of the leaf extract of *C. vespertilionis* may be attributable to its phytochemical composition. Phytochemical analysis of the extract revealed it to contain high amounts of polyphenolic constituents, comprising of flavonoids as the major class (mono- and di-hydroxyflavones, *C*-glycosylflavone derivatives, flavone-*C*, *O*-diglycoside, and flavonol-3-*O*-glycosides) and followed by phenolic acids, among other classes of minor constituents (Norazhar et al., 2021).

Previous studies have mostly focused on the beneficial effects of polyphenolic compounds on a broad spectrum of pharmacological properties. However, several studies have reported that high doses of polyphenolic-rich foods can potentially cause adverse effects through pro-oxidative effects (Martin & Appel, 2009). Instead of exhibiting powerful antioxidant activities, high concentrations of polyphenolic compounds can also increase oxidative stress at a cellular level, and thus, increase the risk of diseases. From this perspective, the toxic and teratogenic effects of the leaf extract of *C. vespertilionis* on the embryonic development of zebrafish observed in this study could also be due to the accumulation of high amounts of the flavonoids and phenolic acids constituents in the exposed embryos.

Our findings are similar to the study by Alafiatayo et al. (2019), who reported that high concentrations of methanolic extract of *Curcuma longa*, containing an abundance of the flavonoids catechin, epicatechin, and naringenin, caused mortality and developmental abnormalities in zebrafish embryos. Ismail et al. (2017) also demonstrated that zebrafish embryos exposed to the phenolics-rich aqueous extracts of *Cinnamon zeylanicum* and *Eugenia polyantha* showed a significant toxicity effect after 48 hpf, evidenced by a decrease in survival rate, organ malformations, abnormal heartbeat rates, and delayed hatchability. In another study by Gaitan et al. (1989), the *C*-glycosylflavones-enriched fractions and several purified

C-glycosylflavones (glucosylvitexin, glucosylorientin, and vitexin) of pearl millet were shown to inhibit thyroid peroxidase (TPO) *in vitro*. Furthermore, they caused a significant increase in thyroid weight of female Sprague-Dawley rats—these findings demonstrated a strong correlation between high amounts of *C*-glycosylflavones and the genesis of goitre. Doerge and Divi (1995) further proposed that inhibition of TPO, the enzyme responsible for the thyroid hormone production, could be associated with the ability of polyphenolic compounds with free resorcinol (metahydroxyphenol) units to react with the enzyme. Bezerra et al. (2016) also reported that the hydroethanolic extract of *Turnera diffusa*, containing flavone-*C*, *O*-diglycoside as the main constituents, was found to be toxic at high concentrations, specifically at 1000 µg/mL, evidenced by increased cell death of the astrocyte culture after 6 and 24 hours of incubation. Further, Du et al. (2017) reported that intravenous injection of high doses of phenolic acids to male Wistar rats led to an imbalance between oxidant and antioxidant mechanisms, boosting the expression level of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, resulting in damage to microvascular endothelial cells.

There are still relatively few reports on the adverse effects of individual polyphenolic compounds. Therefore, at present, it is not possible to link the observed toxic effects of the individual polyphenolic compounds. Deeper studies on the purified compounds will need to be carried out before any suggestions can be made on their safety levels with regard to human consumption.

CONCLUSION

The present study revealed that the methanolic leaf extract of *Christia vespertilionis* (green-leafed variety) is toxic to zebrafish embryos at concentrations of 200 µg/mL and above, causing multiple signs of developmental abnormalities. Results of the present study have provided an initial insight into the potentially toxic and teratogenic effects of the extract. Further substantiation of the results and a deeper understanding of the observed effects will require further investigations on other animal or *in vitro* models. Phenolic constituents of the plant are implicated as the cause of the toxicity and teratogenicity of the plant, but the definite proof will also require more detailed studies on the purified constituents. At this stage, based on the results of the present study, extreme caution is advised in using the plant for healthcare purposes at uncontrolled concentrations.

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