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UNIVERSAL WISER PUBLISHER

Research Article

# A Study on Developmental Toxicity and Behavioral Safety Using Ethanolic Extract of *Pedalium murex* L. on Zebrafish Embryos

**Murugesan R.[1], Vasuki K.[2], Kaleeswaran B.[2*], Ramadevi S.[3]**

[1]Department of Zoology, Annai Vailankanni Arts and Science College, Thanjavur, Tamil Nadu, India
[2]PG and Research Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India
[3]PG and Research Department of Biotechnology, Bon Secours College for Women, Thanjavur, Tamil Nadu, India
E-mail: zookaleesh@gmail.com

**Received:** 21 September 2022; **Revised:** 1 November 2022; **Accepted:** 14 November 2022

**Abstract:** Many are returning to nature from synthetics in search of safety and stability. Herbs are the sole way to combat synthetic drugs' side effects. Toxicity testing may reveal herb hazards, allowing for the prevention of potentially hazardous side effects. Due to the short time needed for investigations, transparency of embryos, short life cycle, high fertility, and similarity of genetic data, the zebrafish embryotoxicity model is at the forefront of toxicology research. The zebrafish animal model is utilised to enhance aquaculture output and biomedicine. *Pedalium murex* L. is a key traditional medicinal herb. Future drug development reference studies must evaluate toxicity factors. In this study, we tested the ethanolic extract of *P. murex* (EEPM) on zebrafish embryos up to 72 hours post-fertilisation (hpf). This is the first study of EEPM on zebrafish embryos. At 24 hpf, embryos grow and move normally. With more plant extract, the heart rate was normal at 48 hpf. At 72 hpf, no afflicted embryos had a tail, eye, head, or heart development. This is the first study to investigate the visible impact of EEPM on zebrafish embryos. The present research found that the higher dose of EEPM significantly raised the heartbeat in the embryo of zebrafish. It happened due to the small oedema in the heart muscle. Besides these, there is no other damage to the muscle. As a result, the current investigation demonstrated that the prospective use of *P. murex* is risk-free and has the potential to be used for the development of novel drugs in a clinical trial.

***Keywords*:** zebrafish, toxicology, *Pedalium murex* L., oedema, fertilisation

## 1. Introduction

The widespread activity of many synthetic pesticides may have many unintended consequences for the ecosystem, including the elimination of useful agents like natural predators and pollinators [1]. Herbs have been used to manage health and illness for ages. Indian Ayurveda, ancient traditional Chinese, and Greek Unani medicine used herbs [2-4]. About 200,000 natural compounds are produced from plants, with many more in higher plants and microbes [5, 6].

The zebrafish (*Danio rerio*) is a Cyprinidae. They have more human-like biology than worms and insects. Invertebrates are superior for cellular or molecular comparisons. Zebrafish are easy to maintain, manipulate, and observe, but no model is perfect. In addition, their embryos contain fewer cells and are small. Tracking individual cell growth is easier [7]. Zebrafish are used in toxicity and biomedical investigations [8, 9]. This method is used to improve aquaculture productivity [10]. Improving zebrafish husbandry, survival, immune response, nutrition, and growth might

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benefit commercial fish [11, 12]. Transparent zebrafish larvae allowed for real-time organ, cell, and tissue analysis [13, 14]. Ecotoxicology and environmental science employ zebrafish as a model organism, and it has numerous potential applications in chemical risk assessment. Zebrafish are used to investigate acute, chronic, and early life-stage toxicity. The Organisation for Economic Co-operation and Development (OECD) and U.S. Environmental Protection Agency (EPA), recommend protecting this species [15, 16]. Since zebrafish endocrine and hormonal signalling pathways are similar to those of other vertebrates, this model's results may be generalised [17]. Zebrafish are used to examine the development and functioning of the nervous system, as well as behavioural, genetic, and biochemical components of movement [18-22]. It's a reliable model for evaluating toxicological effects on reproduction and embryonic development. Direct chemical addition to embryo growth fluid simplifies drug delivery and enables high-throughput screening. Zebrafish are a better research model.

This study used *Pedalium murex* L. to assess zebrafish embryo toxicity and behavioural changes. *P. murex* is called Yanai Nerunji in Tamil Nadu, India. It is grown in India, Pakistan, Sri Lanka, Tropical Africa, and Mexico. It's a natural shrub used to treat colds, coughs, and infections. *P. murex* alkaloids include pedalitin, diosmetin, dinatin, and pedalin dinatin-7-glucuronide. The seeds' glycosides showed no diuretic impact. It treats leucorrhea, gonorrhoea, nocturnal discharges male reproductive issues blood purification and bladder stone removal. According to the Unani School of Medicine, it treats diuretics, enriches the blood, improves menstruation flow, and gargles sore lips and gums [23]. *P. murex* is used to treat lumbago, stranguria, as a tonic, and as an appetiser.

*P. murex* is used to treat urinary issues such as incontinence, gonorrhoea, and dysuria [24]. Root decoction has antibilious qualities, whereas fruit juice promotes lochial discharge. Making a leaf decoction regulates white discharge from excessive body heat. In the case of zebrafish, no reports of developmental toxicity or behavioural safety have been found in the literature despite the use of an ethanolic extract of *P. murex*. As a result, the purpose of this research was to investigate the level of toxicity that *P. murex* posed to zebrafish embryos.

# 2. Materials and methods

## 2.1 *Collection and identification of plant*

The species of the *P. murex* plant was gathered in Tamil Nadu, in the city of Thanjavur (District). It was certified and taxonomically identified by Rev. Dr. S. John Britto SJ, Director of The Rapinat Herbarium and Centre for Molecular Systematics at St. Joseph College in Tiruchirapalli, Tamil Nadu, India.

## 2.2 *Preparation of solvent extracts*

To carry out the extraction procedure, dried plants of the genus *P. murex* were harvested, cleaned, and then crushed into powder. To execute the ethanol extraction, a thimble was fashioned, 50 g of the substance in powdered form was distributed evenly inside, and the temperature ranged from 60 to 80 °C [25]. The extracts were dried out using a rotatory evaporator (Model RE 801, Yamato, Japan), and after they were dried, they were stored in a steam bath to maintain their thick, paste-like consistency. We used concentrations of the extract at 50 and 100 g/mL to evaluate the toxicity as well as the behavioural effects that the extract had on the zebrafish embryos.

## 2.3 *Fish care and egg collection*

The adult zebrafish were bought and used in research conducted in the laboratory on the toxicity of embryos. Both the temperature and the photoperiod were carefully controlled and kept at the same levels (temperature: 28 °C; photoperiod: 12 hours). The bloodworm zebrafish were fed twice a day, once in the morning and once in the evening.

A ratio of two fishes to one is utilised in each of the four distinct tanks, which are each 3 litres in capacity. It stays dark for a short while, making it an excellent environment for egg-laying. The lights were switched off after 12 hours, and the fish didn't start laying eggs for another 16 to 18 hours after that. After carefully removing the eggs, they are put in a Petri dish that has fresh water which has been heated to a temperature of 28 °C. Following the final stage, 256-cell stages may be found in spherical and dividing embryos between 2 and 5 hours post-fertilisation (hpf), while oblong stages can be found between 3 and 7 hpf. These stages are often chosen and used for research (Figure 1).

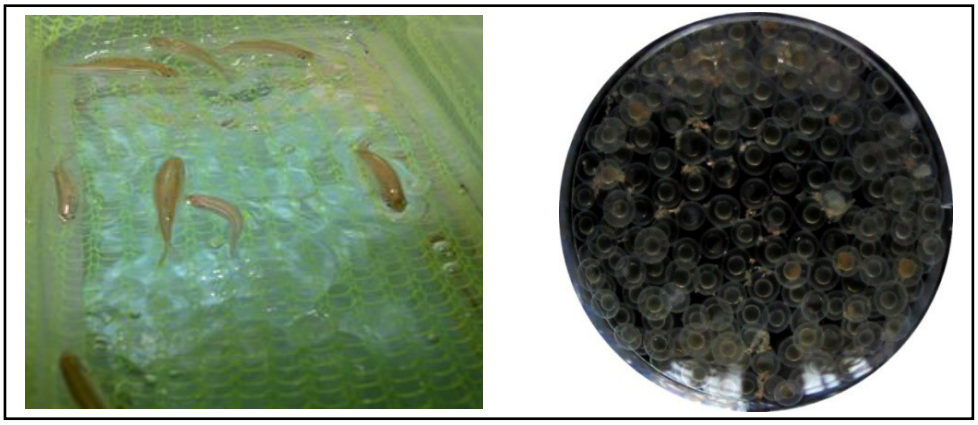

**Figure 1.** Rearing and collection of the egg from zebrafish

### 2.4 *Determination of lethal concentration ($LC_{50}$)*

At 1 hour after fertilisation, the embryo was given ethanolic extract of *P. murex* (EEPM) (up to 2,000 mg/mL in 0.1%) to determine lethal concentration ($LC_{50}$) values. The effects of this treatment were recorded at 24 and 48 hpf (number, n = 20). In the group that had been pre-tested, 1% dimethyl sulfoxide (DMSO) was shown to be safe [26].

### 2.5 *Preparation of test solutions*

Standard water was produced by mixing deionized water with potassium chloride (KCl) at a concentration of 0.23 mg/L; calcium chloride dihydrate ($CaCI_2.2H_2O$) at 11.76 mg/L; sodium bicarbonate ($NaHCO_3$) at 2.59 mg/L; and magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) at 4.93 mg/L. The solution for the EEPM experiment was prepared four hours before the consistent water was aerated. To set up the experiment solutions, the concentrations of EEPM that were employed were 50 g/mL and 100 g/mL, both of which included 0.1% DMSO.

### 2.6 *Treatment of EEPM with chorion*

Eggs and many different amounts of plant extracts were mixed in a glass Petri dish. Each of the selected eggs was then placed in a 96-well plate that contained 250 mL of the test solution. EEPM was given at concentrations of 50 and 100 g/mL to be treated with. At 24, 48, and 72 hours after fermentation, the plates were covered and later the eggs were observed under a light microscope. Triplicates were performed in each experiment.

### 2.7 *Statistical analysis*

All of the data are shown as the mean along with the standard deviation (SD), and the statistical significance (p) is determined using a one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test, and the significant level at $p < 0.05$.

### 2.8 *Determination of $LC_{50}$*

It was determined that embryos were secure up to a level of 2,000 mg/mL.

## 3. Results

Embryos of zebrafish were subjected to an investigation in which we evaluated the developmental toxicity of *P. murex* as well as its behavioural safety. Following the administration of EEPM to zebrafish embryos that have been

treated with chorion for varying amounts of time, one may do calculations. At the dosage of 50 µg/mL, there was no morphological development that could be seen at 24 hpf, including the creation of the tail, embryonic movement, head, or eye. There was no substantial development took place in the embryonic development of the 36 embryos when the concentration was 100 µg/mL.

In the first 24 hours after fertilisation, there are no modifications in the development process that suggest there has been any coagulation between the embryos. The normal sequence of development occurs in the embryo's tail, head, eye, and embryonic mobility. When compared to the group that served as a control, the development of the tail was typical in size and growth, and there was a rise in higher concentration. When compared to the group that did not get treatment, the head developed properly after receiving it. When the concentration of EEPM was increased to 50 and 100 µg/mL, no additional alterations were seen in the eye development, which continued to progress normally in terms of growth and size. This was likewise the case when the concentration of plant extract was increased.

The average number of embryonic movements per minute was at mean of 7.80, SD of 1.037 for the control treatment; mean of 7.55, SD of 1.132 for the 50 µg/mL group; and mean of 7.52, SD of 1.081 for the 100 µg/mL group (Table 1).

**Table 1.** Treatment of *P. murex* with chorion at 24 hpf

| | | | *P. murex* | | |
|---|---|---|---|---|---|
| No. | Observation | | Control | 50 µg/mL | 100 µg/mL |
| 1 | Number of embryo (n) | | 36 | 36 | 36 |
| 2 | Coagulated (n) | | 0 | 0 | 0 |
| 3 | Tail development (n) | | 0 | 0 | 0 |
| 4 | Eye development (n) | | 0 | 0 | 0 |
| 5 | Head development (n) | | 0 | 0 | 0 |
| 6 | Sum of affected (n) | | 0 | 0 | 0 |
| 7 | Embryonic movement (n/min) | Mean | 7.80 | 7.55 | 7.52 |
| | | SD | 1.037 | 1.132 | 1.081 |

Following the development of the embryo, the heart began to form between 24 and 48 hpf. After 48 hours of observation, there was no morphological change seen. The heart rate that was observed was determined to be within the normal range when the concentration was 50 µg/mL, and when the concentration of dosages was raised to 100 µg/mL, there were also no alterations in the heart rate. At 48 hpf, there was no evidence of oedema since there was no discernible change in heart rate. The average number of embryonic movements per minute was at mean of 129.83, SD of 3.0752 for the control treatment; mean of 130.13, SD of 3.163 for the 50 µg/mL group; and mean of 130.44, SD of 3.272 for the 100 µg/mL group (Tables 2 and 3). The first dosages were administered at 24 hpf, and observations were carried out at 48 and 72 hpf after that. In Figure 2, it was discovered that there was an increase in the heart rate that was precisely proportionate to the increase in dosage.

**Table 2.** Treatment of *P. murex* with chorion of heart rate at 24 to 48 hpf

| No. | Observation | | *P. murex* | | |
|---|---|---|---|---|---|
| | | | Control | 50 µg/mL | 100 µg/mL |
| 1 | Number of embryo (n) | | 36 | 36 | 36 |
| 2 | Coagulated (n) | | 0 | 0 | 0 |
| 3 | Tail development (n) | | 0 | 0 | 0 |
| 4 | Eye development(n) | | 0 | 0 | 0 |
| 5 | Head development (n) | | 0 | 0 | 0 |
| 6 | No heartbeat (n) | | 0 | 0 | 0 |
| 7 | No circulation (n) | | 0 | 0 | 0 |
| 8 | Oedema (n) | | 0 | 0 | 0 |
| 9 | Heart rate (beats/min) | Mean | 129.83 | 130.13 | 130.44 |
| | | SD | 3.0752 | 3.163 | 3.272 |

**Table 3.** Treatment of *P. murex* with chorion at 72 hpf

| No. | Observation | *P. murex* | | |
|---|---|---|---|---|
| | | Control | 50 µg/mL | 100 µg/mL |
| 1 | Number of embryo (n) | 36 | 36 | 36 |
| 2 | Coagulated/dead (n) | 0 | 0 | 0 |
| 3 | Tail development (n) | 0 | 0 | 0 |
| 4 | Eye development (n) | 0 | 0 | 0 |
| 5 | Head development (n) | 0 | 0 | 0 |
| 6 | No heartbeat (n) | 0 | 0 | 0 |
| 7 | Oedema (n) | 0 | 0 | 0 |
| 8 | Sum of affected (n) | 0 | 0 | 0 |
| 9 | Sum of survival (n) | 36 | 36 | 36 |

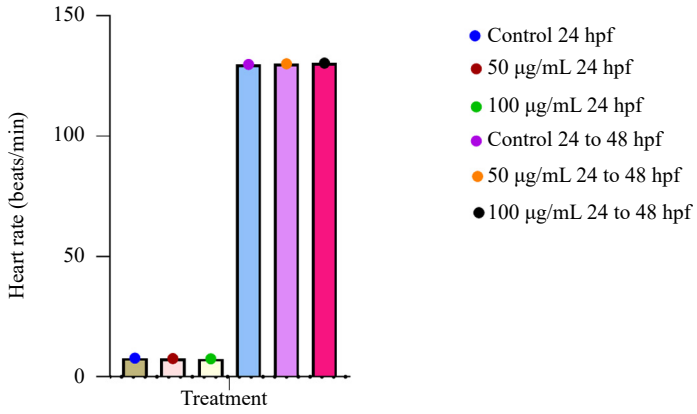


**Figure 2.** Comparison of heat rate for embryos with 24 hpf and 24 to 48 hpf. Values are expressed as mean ± SD, $p < 0.05$

## 4. Discussion

The basic goals of the toxicological examination of any herbal medication are to identify any harmful effects and determine the upper limits of exposure levels at which these effects may occur. When deciding whether a herbal treatment is safe to use, the kind and severity of any possible adverse effects, as well as the dose at which they become evident, are two of the most essential considerations. Toxicology testing may uncover specific potential dangers linked with the use of herbs, especially in locations where such concerns are already prevalent [27]. The zebrafish embryo toxicity test (ZET) is a simple and effective culture technique that allows researchers to examine the effects of medicinal herbs on a whole vertebrate embryo throughout its development.

Several different assays for determining whether a substance is hazardous during development have been developed over a substantial period. These assays range from cell-line-based testing to varied organ cultures and entire embryo cultures of rats or zebrafish [28]. The zebrafish embryo develops very rapidly. The toxicologist has also taken into account the myriad of benefits that come with the development of the zebrafish embryo. Because of the embryo's transparency and the fact that it develops independently from the mother, determining whether a substance is teratogenic or embryotoxic is quite straightforward.

The embryo of the zebrafish is considered to be one of the most promising vertebrate systems for the study of mechanistic toxicity [29, 30]. In addition to investigating gene function, the zebrafish may be used as a model for high-throughput chemical screening, target identification and validation, assay development, and studies into the toxicity of medications [7]. This study aimed to evaluate if the plant *P. murex* was a developmental hazard at early life stages by using zebrafish embryos as a useful model. Additionally, the researchers wanted to elucidate the likely mechanism behind this effect. Previous findings reported that the zebrafish embryos are sensitive to developmental damage and behavioural modifications in toxicological research.

The research concluded that this animal was hypersensitive to the plant since it caused a number of developmental difficulties in the animal, including abnormalities in the tail and cardiovascular system, as well as behavioural changes that had not been observed before. It has been discovered that the EEPM is hazardous to the development of zebrafish. According to the findings, there is no discernible difference between the experimental group and the control group with regard to morphological characteristics such as the length of the tail, the size of the head, the number of eyes, or the mobility of the embryos. According to toxicology and behavioural tests of plants, there is no change in the morphological or cardiac activity. Due to the relatively short amount of time needed for investigations, the transparency of embryos, the relatively short life cycle, the high fertility, and the genetic data similarity, the zebrafish embryotoxicity model is now at the forefront of toxicology research [31].

## 5. Summary and conclusion

To effectively treat a broad range of medical conditions, plants may be successfully employed to produce both traditional and contemporary pharmaceuticals. This call for the discovery of novel antimicrobial drugs, such as those derived from medicinal plants, to combat these infectious diseases. A zebrafish embryo model was used to investigate the toxicity and behavioural changes caused by *P. murex* ethanol extracts. No coagulation occurs, and the tail, head, and eye remain the same across all EEPM concentrations. Increases in plant extract content are associated with no increase in pulse rate. The plant showed no signs of toxicity or morphological changes even at the maximum and recommended extract dose. To summarise, we have shown that *P. murex* when administered to zebrafish embryos at the optimal dosage level, is not hazardous. On the other hand, a minor oedema of the heart muscle was noticed when the dosage was increased. For the sake of future study, it is necessary to first explain the species-specific effects of *P. murex* and then come to some conclusions on the human risk assessment. This study provides preliminary evidence that zebrafish embryos are safe when exposed to a higher concentration of plant extract and optimal environmental conditions.

## Acknowledgement

The authors are grateful to A. Veeriya Vandayar Memorial (AVVM) Sri Pushpam College (An Autonomous

Institution Affiliated with Bharathidasan University), Poondi, Thanjavur district, Tamil Nadu, India, for providing the necessary facilities.

## Conflict of interest

The authors of this study have stated unequivocally that they do not have any known financial or interpersonal conflicts of interest that may be interpreted as affecting the research that was provided in this study.

## References

[1] Sahayaraj K, Sathyamoorthi P. The toxicity and biological effect of *Pedalium murex* L. extracts on the tobacco cutworm, *Spodoptera litura* (Fabr.) larvae. *Archives of Phytopathology and Plant Protection*. 2010; 43(18): 1768-1780. https://doi.org/10.1080/03235400902753626

[2] Alestrom P, Holter JL, Nourizadeh-Lillabadi R. Zebrafish in functional genomics and aquatic biomedicine. *Trends in Biotechnology*. 2006; 24(1): 15-21. https://doi.org/10.1016/j.tibtech.2005.11.004

[3] Bhatnagar VK, Hussain SA, Ali M. A brief history of Ayurveda in Hyderabad. *Bulletin of the Indian Institute of History of Medicine (Hyderabad)*. 1994; 24(1): 63-74. http://www.ccras.nic.in/sites/default/files/viewpdf/jimh/BIIHM_1994/63%20to%2074.pdf

[4] O'Brien KA, Xue CC. The theoretical framework of Chinese medicine. In: Leung PC, Xue CC, Cheng YC. (eds). *A comprehensive guide to Chinese medicine*. New Jersey, USA: World Scientific Publishing Co; 2003. p.47-84. https://doi.org/10.1142/5390

[5] Aly AH, Debbab A, Kjer J, Proksch P. Fungal endophytes from higher plants: A prolific source of phytochemicals and other bioactive natural products. *Fungal Diversity*. 2010; 41(1): 1-16. https://doi.org/10.1007/s13225-010-0034-4

[6] Kinghorn AD, Pan L, Fletcher JN, Chai H. The relevance of higher plants in lead compound discovery programs. *Journal of Natural Products*. 2011; 74(6): 1539-1555. https://doi.org/10.1021/np200391c

[7] Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: A complete animal model for in vivo drug discovery and development. *Current Drug Metabolism*. 2009; 10(2): 116-124. https://doi.org/10.2174/138920009787522197

[8] Roush W. Zebrafish embryology builds a better model vertebrate. *Science*. 1996; 272(5646): 1103. https://doi.org/10.1126/science.272.5265.1103

[9] Bergeron SA, Milla LA, Villegas R, Shen MC, Burgess SM, Allende ML, et al. Expression profiling identifies novel Hh/Gli-regulated genes in developing zebrafish embryos. *Genomics*. 2008; 91(2): 165-177. https://doi.org/10.1016/j.ygeno.2007.09.001

[10] Ribas L, Piferrer F. The zebrafish (*Danio rerio*) as a model organism, with emphasis on applications for finfish aquaculture research. *Reviews in Aquaculture*. 2014; 6(4): 209-240. https://doi.org/10.1111/raq.12041

[11] Oyarbide U, Rainieri S, Pardo MA. Zebrafish (*Danio rerio*) larvae as a system to test the efficacy of polysaccharides as immuno stimulants. *Zebrafish*. 2012; 9(2): 74-84. https://doi.org/10.1089/zeb.2011.0724

[12] Hedrera MI, Galdames JA, Jimenez-Reyes MF, Reyes AE, Avendaño-Herrera R, Romero J, et al. Soybean meal induces intestinal inflammation in zebrafish larvae. *PLoS One*. 2013; 8(7): e69983. https://doi.org/10.1371/journal.pone.0069983

[13] Eisen JS. Zebrafish make a big splash. *Cell*. 1996; 87(6): 969-977. https://doi.org/10.1016/s0092-8674(00)81792-4

[14] Fishman MC. Zebrafish genetics: The enigma of arrival. *Proceedings of the National Academy of Sciences*. 1999; 96(19): 10554-10556. https://doi.org/10.1073/pnas.96.19.10554

[15] Braunbeck T, Boettcher M, Hollert H, Kosmehl T, Lammer E, Leist E, et al. Towards an alternative for the acute fish $LC_{50}$ test in chemical assessment: The fish embryo toxicity test goes multi-species- an update. *ALTEX - Alternatives to Animal Experimentation*. 2005; 22(2): 87-102. https://www.altex.org/index.php/altex/article/view/911

[16] The Organization for Economic Co-operation and Development (OECD). *Guidelines for Testing of Chemicals*. 1992. https://www.oecd.org/chemicalsafety/risk-assessment/1948241.pdf [Accessed 23rd November 2022].

[17] Laan M, Richmond H, He C, Campbell RK. Zebrafish as a model for vertebrate reproduction: Characterization of the first functional zebrafish (*Danio rerio*) gonadotropin receptor. *General and Comparative Endocrinology*. 2002;

125(3): 349-364. https://doi.org/10.1006/gcen.2001.7738
[18] Fetcho JR, McLean DL. Some principles of organization of spinal neurons underlying locomotion in zebrafish and their implication. *Annals of the New York Academy of Sciences*. 2010; 1198(1): 94-104. https://doi.org/10.1111/j.1749-6632.2010.05539.x
[19] Fetcho JR, Higashijima SI, McLean DL. Zebrafish and motor control over the last decade. *Brain Research Reviews*. 2008; 57(1): 86-93. https://doi.org/10.1016/j.brainresrev.2007.06.018
[20] Fetcho JR. The utility of zebrafish for studies of the comparative biology of motor systems. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*. 2007; 308B(5): 550-562. https://doi.org/10.1002/jez.b.21127
[21] Guo S. Linking genes to brain, behaviour and neurological diseases: What can we learn from zebrafish? *Genes, Brain and Behaviour*. 2004; 3(2): 63-74. https://doi.org/10.1046/j.1601-183x.2003.00053.x
[22] Drapeau P, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Brustein E. Development of the locomotors network in zebrafish. *Progress in Neurobiology*. 2002; 68(2): 85-111. https://doi.org/10.1016/s0301-0082(02)00075-8
[23] Rai A, Fazil M, Rasheed A, Ahmad B, Parveen S, Nikhat S. Role of unani medicines in oro-dental diseases: A comprehensive review and scientific evidence. *Fortune Journal of Health Sciences*. 2020; 3(1): 30-54. https://www.fortunejournals.com/articles/role-of-unani-medicines-in-orodental-diseases-a-comprehensive-review-and-scientific-evidence.html
[24] Satyavathi GV, Gupta AK, Tandon N, Seth SD. *Medicinal plants of India*. New Delhi, India: Indian Council of Medicinal Research; 1987.
[25] Murugesan R, Vasuki K, Kaleeswaran B, Santhanam P, Ravikumar S, Alwahibi MS, et al. Insecticidal and repellent activities of *Solanum torvum* (Sw.) leaf extract against stored grain pest, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of King Saud University-Sciences*. 2021; 33(3): 101390. https://doi.org/10.1016/j.jksus.2021.101390
[26] Kumar RBS, Kar B, Dolai N, Haldar PK. Study on developmental toxicity and behavioral safety of *Streblus asper* Lour. bark on zebrafish embryos. *Indian Journal of Natural Products and Resources*. 2013; 4(3): 255-259. http://nopr.niscpr.res.in/bitstream/123456789/22476/1/IJNPR%204(3)%20255-259.pdf
[27] Obidike I, Salawu O. Screening of herbal medicines for potential toxicities. In: Gowder S. (ed.) *New insights into toxicity and drug testing*. Rijeka, Croatia: Intech Open; 2013. p.63-88. https://doi.org/10.5772/54493
[28] Genschow E, Spielmann H, Scholz G, Pohl I, Seiler A, Clemann N, et al. Validation of the embryonic stem cell test in the international ECVAM validation study on three in vitro embryonic tests. *Alternatives to Laboratory Animals*. 2004; 32(3): 209-244. https://doi.org/10.1177/026119290403200305
[29] Aoelsoud NH. Herbal medicine in ancient Egypt. *Journal of Medicinal Plant Research*. 2010; 4(2): 82-86. https://academicjournals.org/journal/JMPR/article-abstract/F90544714963
[30] Langheinrich U. Zebrafish: A new model on the pharmaceutical catwalk. *Bioessays*. 2003; 25(9): 904-912. https://doi.org/10.1002/bies.10326
[31] Chahardehi AM, Arsad H, Lim V. Zebrafish as a successful animal model for screening toxicity of medicinal plants. *Plants*. 2020; 9(10): 1345. https://doi.org/10.3390/plants9101345