



Evaluation of Anxiolytic Activity of Ethanolic Stem Extract of *Passiflora Edulis* Using Zebrafish (*Danio Rerio*)

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ABSTRACT

Medicinal plants are considered to be rich sources of ingredients that can be used in drug development and synthesis. Anxiety is a complex emotional state characterized by feelings of unease, worry, and tension. The present study highlights the pharmacological evaluation of the in vivo anxiolytic activity of *Passiflora edulis* stem by using an ethanolic extract that is prepared by the cold maceration extraction technique. Zebrafish (*Danio rerio*) have been a prominent model organism in biological research in recent times. It has a lot of physiological and genetic similarities with humans, including the brain, digestive tract, musculature, vasculature, and innate immune system.

The evaluation of anxiolytic activity is carried out in ethanolic extract of the stems of *Passiflora edulis* by using the mirror biting method in zebrafish. Anxiolytics are medications that can treat anxiety symptoms. These drugs act through different pathways to relieve anxiety. Thus, in this study, the ethanolic extract of *Passiflora edulis* shows anxiolytic activity when compared with diazepam. It is found from the response of the ethanolic extract of *Passiflora edulis* and diazepam by mirror biting method in zebrafish using Kinovea software.

Keywords: Anxiolytic; *Passiflora edulis*; ethanolic extract; zebrafish; mirror biting method; diazepam; Kinovea software.

1. INTRODUCTION

Pharmacology is the scientific study of drugs, their origin, composition, effects on the body, and potential adverse effects.^[1] Traditional medicine, also known as folk medicine, has existed for centuries and remains prevalent across diverse cultures globally. From soothing headaches with herbal teas to employing massage techniques for muscle pain relief, traditional medicine offers a holistic approach to health and well-being, emphasizing both preventive and curative measures^[2]. This work is based on the anxiolytic activity of *Passiflora edulis*, belonging to the family Passifloraceae. *Passiflora edulis*, also known as passion fruit, is a climber. Passion fruits and the whole plant are very useful. They have pharmacological activities like antidiabetic, anxiolytic, anti-tumor, anti-oxidant, analgesic,

and anti-inflammatory due to the presence of various chemical constituents like volatile oil, flavonoids, lipids, and triterpenoids, aldehydes, ketones, tridecanone, palmitic acid, stearic acid, linolic acid, quercetin, apigenin, and vitexin. The study is focused on evaluating the anxiolytic activity of *Passiflora edulis* climber due to the presence of a wide range of flavonoids such as isoorientin, orientin, luteolin, apigenin, and chrysin or their glycosides, and so forth^{[3],[4]}.

1.1 Anxiolytics

Anxiety is a complex emotional state characterized by feelings of unease, worry, and tension. It often arises from the anticipation of future threats, whether real or perceived^[5]. Anxiolytics are medications that can treat anxiety symptoms. Medications with this effect span several different drug classes, including medications that mainly treat other conditions.^[6]

There are also multiple ways to reduce anxiety.

1) Increase cell activity: Some drugs tell certain cells to activate. Benzodiazepines, nonbenzodiazepines, and barbiturates are examples of this. They activate a process that makes your body release gamma-aminobutyric acid (GABA), a neurotransmitter your body uses to reduce nervous system activity, which helps with anxiety.

2) Decrease cell activity: Beta-blockers are an example of this, slowing down activity in your sympathetic nervous system (the system that manages your “fight-or-flight” response).

3) Slow reabsorption of neurotransmitters: Some drugs keep your body from reabsorbing certain neurotransmitters. Antidepressants have this effect on neurotransmitters like serotonin and norepinephrine ^[7].

1.2 *Passifloraedulis*

Passifloraedulis, commonly known as Krishna Phal in India, is a well-established plant with significant pharmacological activities and widespread medical applications for treating various diseases. Belonging to the Passifloraceae family, which comprises approximately 500 species, *Passiflora* stands out as one of the largest genera within this family. As a member of the Passifloraceae family, *Passifloraedulis* has garnered attention for its pharmacologically active compounds, making it a valuable asset in the exploration of natural remedies and pharmaceutical applications ^[8]. *Passifloraedulis* includes main components such as dietary fibre, carbohydrates, lipids, carboxylic acids, polyphenols, volatile compounds, proteins and amino acids, vitamins, and minerals. High contents of flavonoids, triterpenoids, and carotenoids are the primary types ^[9].

1.3 Zebra Fish (Experimental Animal)

Zebrafish (*Danio rerio*) has been a prominent model organism in biological research in recent times. Zebrafish was first used as a biological model by George Streisinger (University of Oregon) in the 1970s because it was simpler than mice and easier to manipulate genetically. Zebrafish have a lot of physiological and genetic similarities with humans, including the brain, digestive tract, musculature, vasculature, and innate immune system. Also, 70% of human disease genes have functional similarities with those of zebrafish ^{[10][11]}.

1.4 Plant Profile



Fig 1: *Passifloraedulis* plant



Fig 2: *Passifloraedulis* climber

1.4.1 Synonym: Passion flowers, Passion vines

1.4.2 Vernacular names:

Malayalam: Poonakkai

Hindi : Krishnaphal

Kannada : PyaashanHannu

1.4.3 Taxonomical classification:

Kingdom : Plantae

Phylum : Angiosperms

Class : Eudicots

Order : Malpighiales

Family : Passifloraceae

Genus : *Passiflora*

Species : *edulis*^[12].

1.4.5 Morphology

- ❖ **Stems:** These are cylindrical, woody climbers that can reach up to 25 meters in length. They have a smooth surface with striations and internodes up to 9 cm long.

- ❖ **Leaves:** Arranged alternately, the leaves are trilobate with deeply lobed margins. The leaves are typically 5-10 cm wide and 7-12 cm long.
- ❖ **Flowers:** Appearing solitary at each node, the flowers are large and showy, measuring 5-7.5 cm in diameter. They are hermaphroditic, meaning they have both male and female reproductive organs.
- ❖ **Seeds:** Numerous brown seeds, each about 2.4 mm long, are embedded in the juicy pulp of the fruit.
- ❖ **Fruit:** The fruit has a dark purple rind with fine white specks. It's smaller, weighing around 35 g^[13].

1.4.6 Chemical constituents

- ❖ **Flavonoids:** These are the most abundant and well-studied compounds in passion fruit. They include isorientin, orientin, quercetin, luteolin, apigenin, and chrysin, which possess antioxidant, anxiolytic, and anti-inflammatory properties.
- ❖ **Alkaloids:** These include harmine and harmaline, which are believed to contribute to the fruit's sedative and anxiolytic effects.
- ❖ **Carotenoids:** These include beta-carotene and lycopene.
- ❖ **Vitamins and minerals:** Passion fruit is a good source of vitamin C, vitamin A, potassium, and magnesium^[14].

1.4.7 Pharmacological action

- ❖ **Anti-anxiety activity:** Studies suggest *Passiflora edulis* extracts display anti-anxiety effects in various animal models. Extracts like aqueous, butanolic, and methanol extracts exhibited anxiolytic properties in tests like elevated-plus-maze and inhibitory avoidance tasks. ^[15]
- ❖ **Anti-inflammatory activity:** Extracts from the leaves significantly reduced inflammation in animal models by hindering the movement of immune cells (leukocytes) towards inflamed tissues and by suppressing the production of inflammatory molecules like myeloperoxidase, nitric oxide, TNF- α , and IL-1 β . ^[15]
- ❖ **Anti-hypertensive activity:** The extracts from passion fruit peel were given to rats with high blood pressure; the extracts lowered their blood pressure; reduced blood vessel parameters, and decreased nitric oxide levels in the blood. This anti-hypertensive effect is thought to be caused by bioactive compounds within the fruit, like polyphenols. ^[16]
- ❖ **Hepatoprotective activity:** The extracts from purple passion fruit peel can protect against liver damage caused by various factors like chloroform, carbon tetrachloride (CCl₄), and ethanol. ^[17]
- ❖ **Antioxidant activity:** The extracts from *Passiflora edulis* leaves possess significant antioxidant properties. This antioxidant action seems to be linked to the presence of polyphenols. ^[18]
- ❖ **Antitumor activity:** In lab studies, various extracts exhibited cytotoxicity against cancer cell lines. These effects might be linked to polyphenols, polysaccharides, and specific fatty acids. ^[19]

1.5 Animal Profile^[20].



Fig 3: Zebrafish

1.5.1 Scientific Name: *Danio rerio*

1.5.2 Taxonomical classification

Kingdom : Animalia
 Phylum : Chordata
 Class : Actinopterygii
 Order : Cypriniformes
 Family : Cyprinidae
 Genus : Danio

Species : rerio

1.5.3 Anatomy

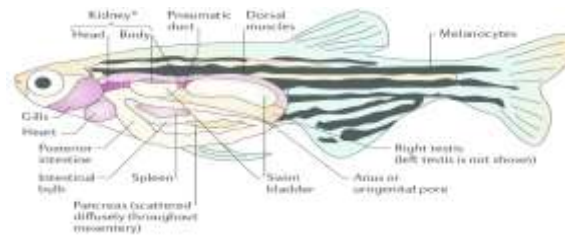


Fig.4: Anatomy of zebrafish

- ❖ **Body:** The zebrafish has a slender, elongated body with a slightly compressed lateral profile. They typically grow to be about 2.5-4 cm (1-1.5 inches) in length.
- ❖ **Eyes:** Zebrafish have well-developed vision and can perceive color, similar to humans.
- ❖ **Brain:** The zebrafish brain is relatively complex and shares many of the same regions as the human brain.
- ❖ **Heart:** The zebrafish heart is a two-chambered structure similar to the human heart.
- ❖ **Gills:** Zebrafish extract oxygen from the water through their gills, similar to other fish.
- ❖ **Digestive system:** The zebrafish digestive system is relatively simple but includes all the major organs involved in digestion, including the stomach, intestines, and liver. ^[21]

1.5.4 Physiology Similarity with Humans

Zebrafish share a surprising degree of physiological similarity with humans. Approximately 70% of human genes have a recognizable counterpart in the zebrafish genome. This genetic homology allows scientists to study the function of genes and the development of diseases in zebrafish, potentially leading to breakthroughs in human health research ^{[22][23]}. The zebrafish displays a high physiological homology to humans, and its neurotransmitter system also exhibits similar neurobehavioral phenotypes. Moreover, these animals have a permeable blood-brain barrier, which leads to a high sensitivity to drugs that owe their mechanism of activity to target sites in the brain ^{[24][25]}.

1.5.5 Mechanism of Action

Anxiolytic and anti-depressive properties have been demonstrated for isolated flavonoids, such as apigenin and quercetin, which selectively bind with high affinity to central benzodiazepines receptors, including GABA_A. These flavonoids can act as anxiolytics due to their high lipophilicity (larger lipid-water partition coefficient and lower topological polarity surface area), which facilitates penetration through the blood-brain barrier ^{[26][27]}.

1.5.6 Advantages of Using Zebrafish as a Model Organism

- ❖ **Rapid development:** Zebrafish embryos develop rapidly, reaching sexual maturity in just a few months, to study multiple generations of fish in a short time frame.
- ❖ **Transparency:** Zebrafish embryos are transparent, allowing researchers to observe the development of organs and tissues in real-time.
- ❖ **High fecundity:** Zebrafish lay large numbers of eggs, making them a readily available source of experimental animals.
- ❖ **Genetic tractability:** Zebrafish are relatively easy to genetically modify, allowing scientists to study the effects of specific genes on development and disease. ^{[28][29][30]}

1.5.7 Types of Mazes used in Zebrafish Behavioural Studies.

Zebrafish are more commonly used to study social behavior because of their tendency to behave in groups.

- ❖ Different mazes are used in the following fields:
- ❖ Social Development and Behavioral Research
- ❖ Stress and Anxiety Research
- ❖ Learning and Memory Research

- ❖ Pharmacology and Toxicology Research
- ❖ Vision and Retina Research ^{[31][32]}

Generally, the following types of mazes are used in behavioural assays:

1. **Mirror Biting Pham test:** a popular method used in studies of agonistic interaction, especially in fish aggression studies as they require fewer participants and avoid pseudo-replication. Mirrors also provoke a strong, aggressive response in the subject without endangering them ^[33].
2. **Zebrafish Plus Maze:** a “+” shaped maze that contains four end compartments and one central compartment. It is used to analyse associative learning behaviour in zebrafish. ^[34].
3. **Latent Learning Apparatus:** used to analyse the learning and memory function in zebrafish. The apparatus contains a start box and a goal box connected via tunnels ^[35].
4. **Light Dark Tank for Zebrafish:** an acrylic tank (15 cm × 10 cm × 45 cm height × width × length) that is divided equally into one-half black and one-half white. Walls and bottom are either black or white, so as to create a similar experimental paradigm to the rodent light dark box ^[36].
5. **Zebrafish Environmental Enrichment Chamber:** this is a widely used task to evaluate spatial and nonspatial learning as well as memory in zebrafish ^[37].
6. **Zebrafish Y Maze:** used for memory task and uses a simple and rapid training session for novelty exploration ^[38].
7. **Place Preference Chamber:** is created to allow for maximum flexibility for your experiments. The chamber comes with 2 divider slots that allow the zebrafish to choose a chamber or retain the zebrafish in the centre area. ^[39].
8. **Zebrafish T Maze:** allows for choice experiments. Arms are baited with colours or cues and zebrafish are allowed to make choices in experiments ^[40].
9. **Vertical Tank Array:** is used for anxiety experiments in zebrafish ^[41].

2. MATERIALS AND METHODS

2.1 Plant collection and drying

The plant *Passiflora edulis* of the family Passifloraceae was collected and dried under shade for about 15 days. It was then powdered with a pulverizer and stored in an airtight container.

2.2 Extraction of stem

The extraction of dried powder from the stem of *Passiflora edulis* was carried out by maceration using ethanol. Around 90 grams of dried powder were weighed. Moistened with ethanol in a conical flask and then extracted with ethanol. The extract was filtered, the solvent was evaporated, and the dried extract was obtained and stored in a desiccator until used ^[42].



Fig.5: Cold maceration of *P. edulis* stem

2.3 Phytochemical screening

2.3.1 Test for alkaloids

- (a) Dragondroff's reagent test
- (b) Wagner's reagent test
- (c) Mayer's reagent test
- (d) Hager's reagent test

2.3.2 Test for carbohydrates

The minimum amount of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to testing for carbohydrates.

- (a) Molisch's test
- (b) Fehling's test

2.3.3 Test for proteins

The extract was dissolved in a few ml of water and subjected to test for proteins.

- (a) Millon's test
- (b) Biuret test
- (c) Ninhydrin test

2.3.4 Test for flavonoids

- (a) Aqueous sodium hydroxide test
- (b) Lead acetate test

2.3.5 Test for phenolic compounds and tannins

- (a) Lead acetate test
- (b) Ferric chloride test
- (c) Decolorization

2.3.6 Test for sterols and terpenoids

Salkowski reaction: 2 ml of extract was mixed with 2 ml of chloroform and 2 ml concentrated sulfuric acid. Shake well. The chloroform layer appears red, and the acid layer shows greenish-yellow fluorescence.

2.3.7 Test for saponin

Foam Test: The extract and powder were vigorously mixed with water. Persistent foam was observed.

2.3.8 Test for glycosides

A small portion of the extract was hydrolyzed with dil. HCL for a few hours in a water bath, and a hydrolysate was later subjected to the following test to detect the presence of different glycosides.

- (a) Legal's test
- (b) Baljet's test
- (c) Liebermann-Burchard's test
- (d) Borntrager's test

2.4 Anxiolytic study

Drugs

1. Test drug: Ethanolic stem extract of *Passifloraedulis*.
2. Standard drug: Diazepam (Brand name: CALMPOSE; Manufactured by: Sun pharma laboratories; Batch No.GT02710A) ^{[44][45]}

Procedure

Mirror biting method using zebrafish ^{[46][47]}.

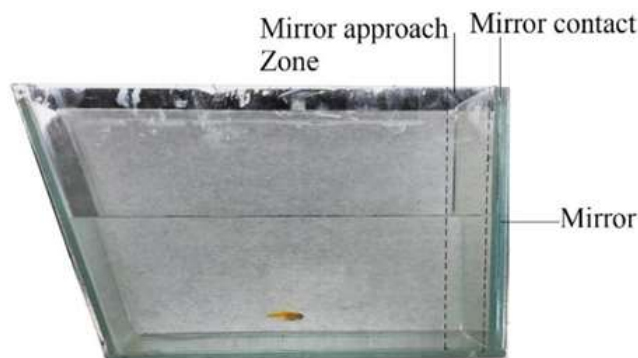


Fig 6: Trapezoid tank

- ❖ Zebrafish were collected from a nearby aquarium and kept in a fish tank of the required size.
- ❖ Placed the fish in a small tank (1.5-L trapezoidal tank 15 height × 28 top × 23 bottom × 7 cm width) and left undisturbed for a long period of time for acclimation.
- ❖ The normal movement of fish was noted by using the video recording software Kinovea for 5 minutes.
- ❖ Drawn a light line on the tank with a marker 0.5 cm from the mirror, to represent the zone of “contacting the mirror” (Mirror bite). Another line is drawn 2.5 cm from the first line to represent the zone of “approach to the mirror” (Mirror approach).
- ❖ A mirror was introduced into the tank for performing the mirror biting test.
- ❖ The path of fish in the control (anxiety-induced) was recorded using the same software for 5 mins. Number of mirror approaches and mirror bites, latency to mirror approach and mirror bite were calculated in control.
- ❖ A 5 mg/L dose of the standard drug diazepam was administered by transferring the fish into a beaker. The path was recorded for 5 mins. The number of mirror approaches and mirror bites, latency to mirror approach and mirror bite are calculated.
- ❖ A 20 mg/L dose of the test drug (extract of *P.edulis*) was administered to anxiety-induced fish by transferring it into a beaker. The path was recorded for 5 mins. The number of mirror approaches and mirror bites, the latency to mirror approach and mirror bite are calculated.
- ❖ Mirror biting of the test group was compared with standard and control by using **Kinovea software**
- ❖ Using the same software, the speed and distance travelled by zebrafish in the control, standard, and test were compared. ^{[48][49][51]}

3.RESULTS

3.1 Extraction of stem

The dried stem of *Passifloraedulis* was powdered and subjected to extraction by cold maceration method using ethanol. After extraction, the percentage yield of the aqueous extract was calculated with reference to the air-dried drug used in the study. The percentage yield of the extract was found as 9.73 % w/w.

3.2 Phytochemical screening of extract

The phytochemical studies on the stem of *Passifloraedulis* were carried out. The ethanolic extract of *Passifloraedulis* shows the presence of flavonoids, alkaloids, proteins, sterols, saponin, phenolic compounds, tannins, glycosides, and terpenoids.

3.3 Pharmacological screening

ANXIOLYTIC ACTIVITY

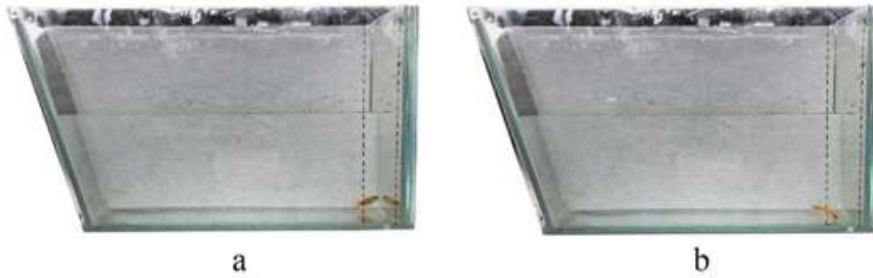


Fig 7: a) Mirror bite b) Mirror approach

The results obtained from the mirror biting method of various tests by zebrafish using Kinovea software are as follow

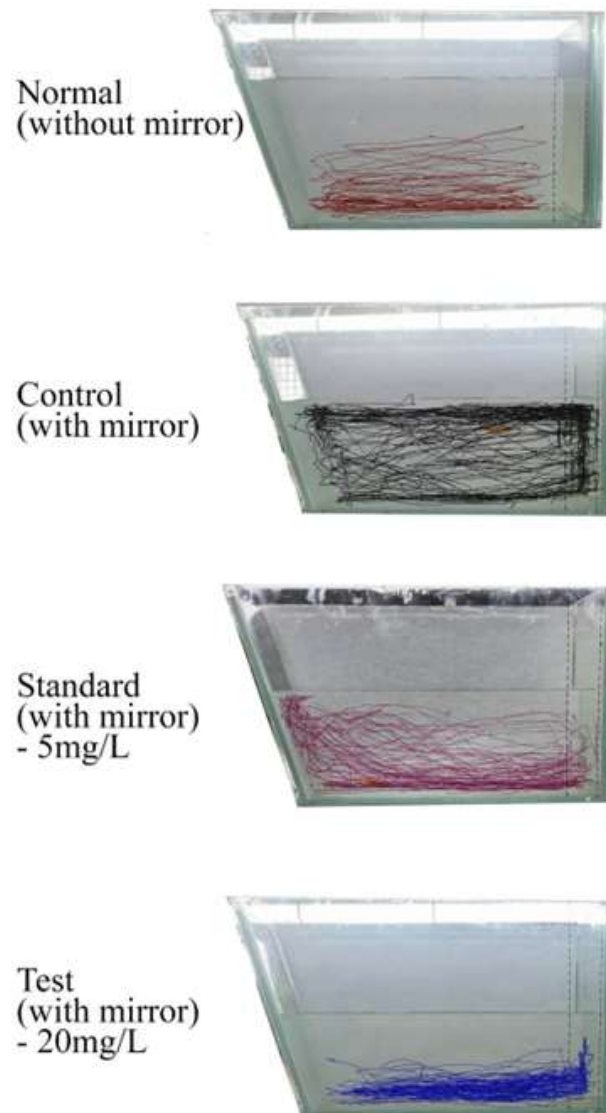


Fig.8: Video-graphic analysis showing path of zebrafish travelled in different tests in mirror biting methods using Kinovea software

SL.NO	PARAMETERS	CONTROL	STANDARD	TEST
1	MIRROR APPROACHES	50	32	40
2	MIRROR BITES	161	33	45

Table 3: Number of mirror approaches and mirror bites in various groups

TIME	MIRROR APPROACHES		
	CONTROL	STANDARD	TEST
1	11	5	4
2	23	13	13
3	33	18	24
4	42	26	32
5	50	32	40

Table 4: Comparative response of mirror approaches by zebra fish in control, diazepam, and ethanolic stem extract of *P. edulis*

TIME	MIRROR BITES		
	CONTROL	STANDARD	TEST
1	27	7	7
2	72	16	17
3	105	22	27
4	133	28	33
5	162	33	45

Table 5: Comparative response of mirror bites by zebra fish in control, diazepam, and ethanolic stem extract of *P. edulis*

Table 3-5 shows the mirror approaches and mirror bites in various tests done in zebra fish using mirror biting method.

It was compared with the control, standard, and test drug respectively.

A decrease in the frequency of mirror approaches and mirror bites in the diazepam and extract treated groups was observed, as shown in Fig.10-12

Sl.No	PARAMETERS	CONTROL	STANDARD	TEST
1	LATENCY TO MIRROR APPROACHES	2	9	16
2	LATENCY TO MIRROR BITES	4	29	18

Table 6: Comparative response of latency to mirror approaches and mirror bites by zebrafish in various groups

Table 6 shows the latency to mirror approaches and mirror bites in various tests done in zebrafish using the mirror biting method. It was compared with the control, standard, and test drug respectively.

An increase in the latency of mirror approach and mirror bite in the diazepam and extract treated group was observed, as shown in Fig.13.

GRAPHICAL REPRESENTATION

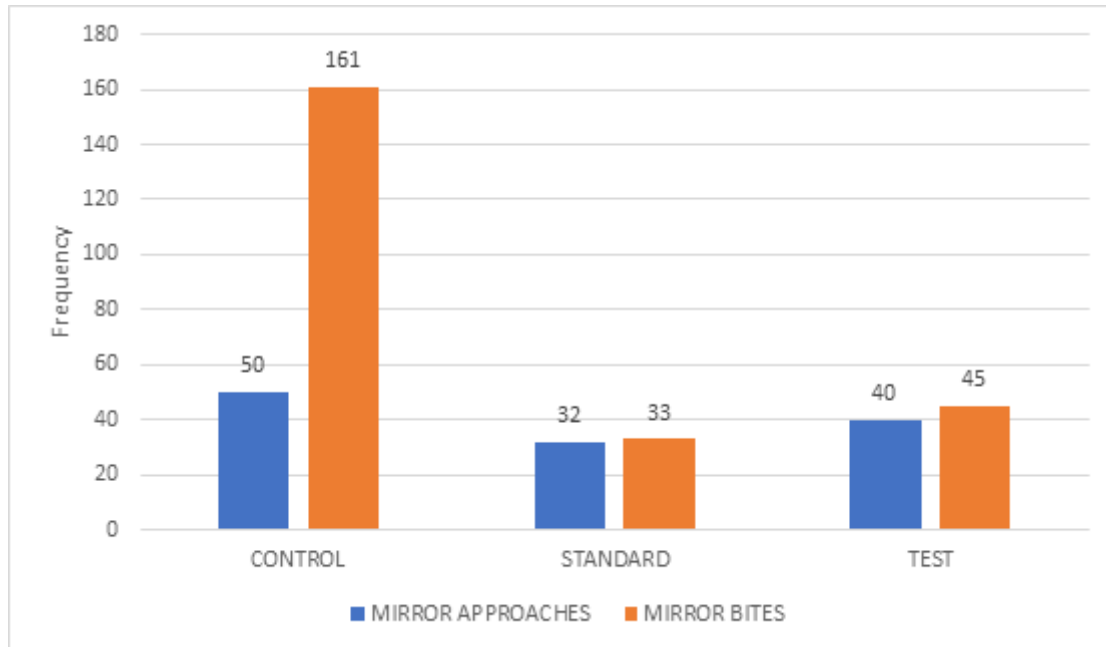


Fig.10: Bar graph representing number of mirror approaches and mirror bites in various tests

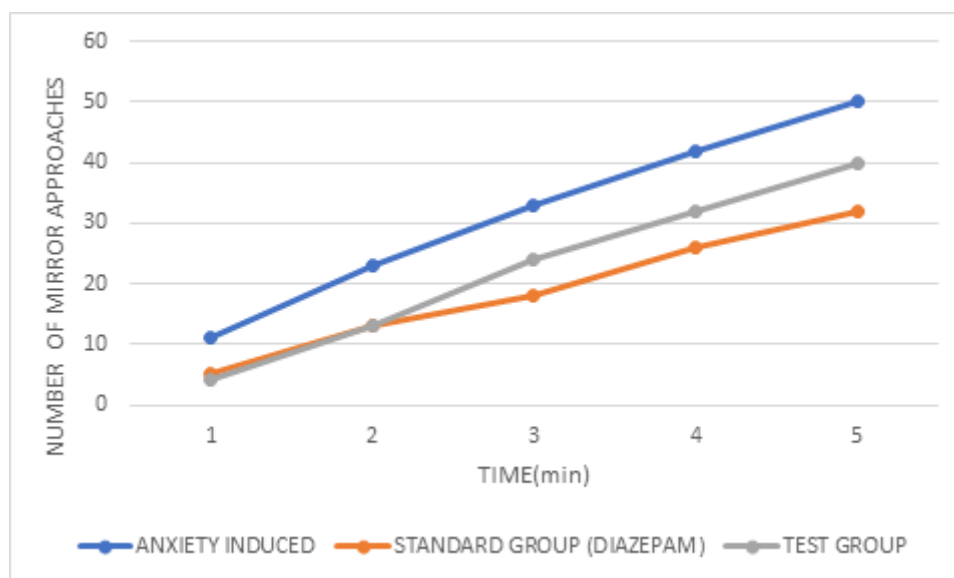


Fig.11: Comparative response of mirror approaches by zebra fish in control, diazepam, and ethanolic stem extract of *P. edulis*

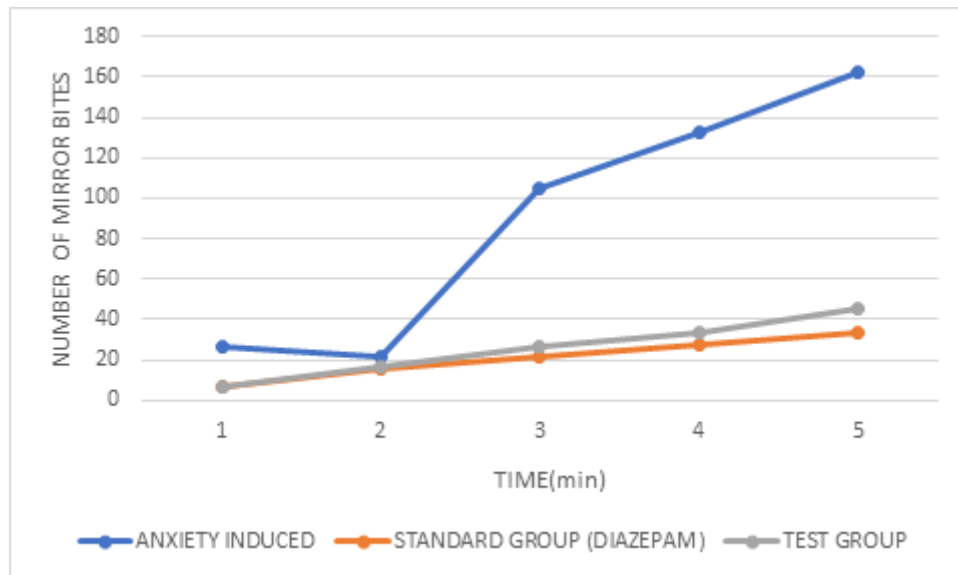


Fig.12: Comparative response of mirror bites by zebra fish in control, diazepam and, ethanolic stem extract of *P. edulis*

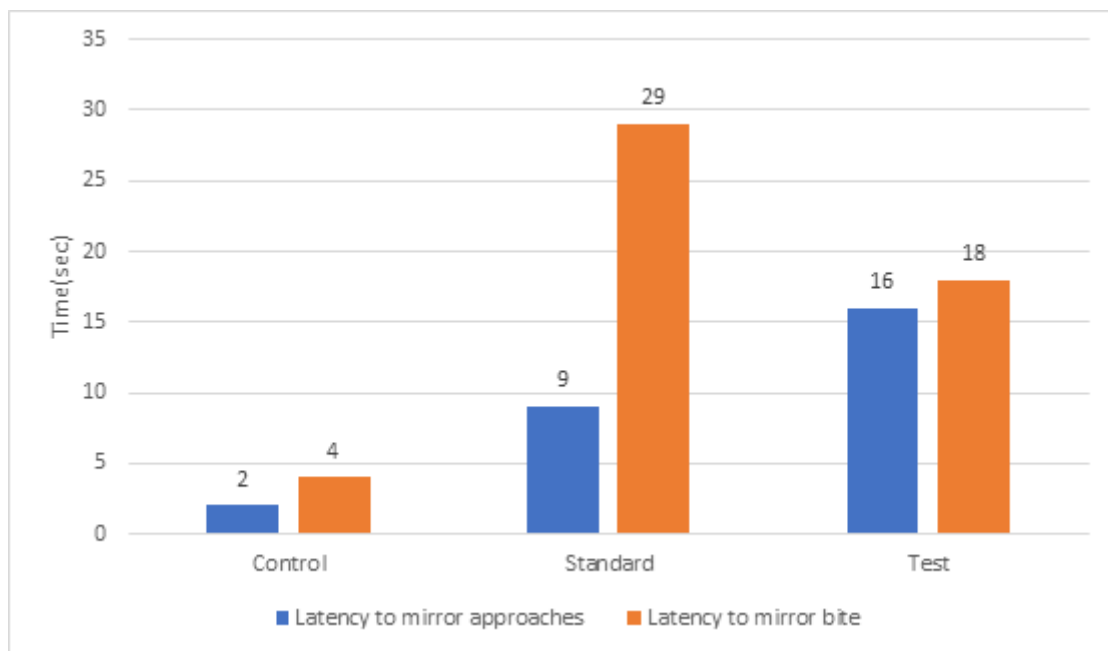
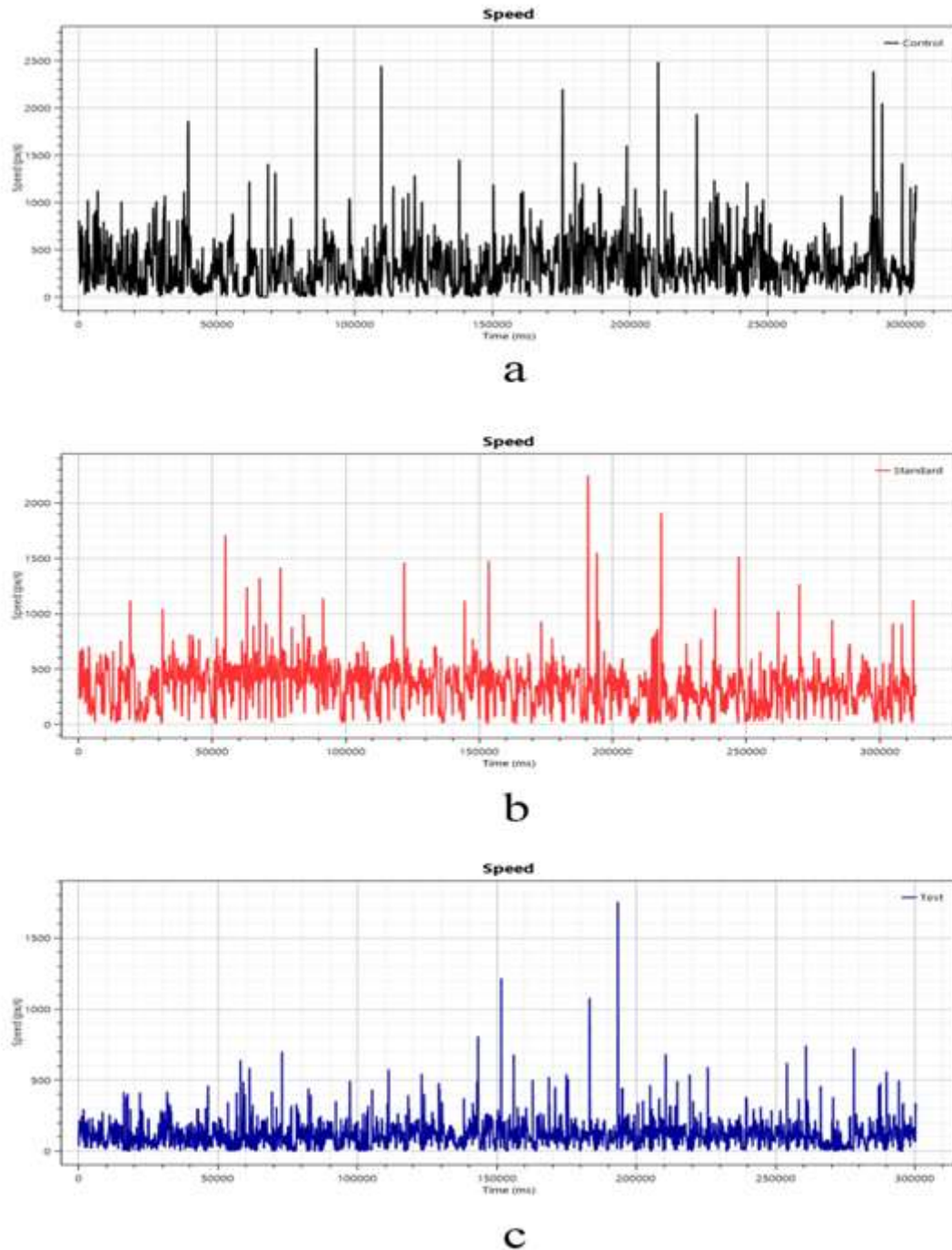


Fig.13: Comparative response of latency to mirror approaches and mirror bite by zebrafish in various groups



**Fig.14: Comparative speed of zebrafish in a) control, b) standard, and c) test.
X axis: time (ms), Y axis: speed (px/s)**

Fig.14 shows speed of zebrafish in various tests which reveals that control shows increased speed due to anxiety. It is then compared to the standard and test group which show a decrease in the speed of zebrafish.

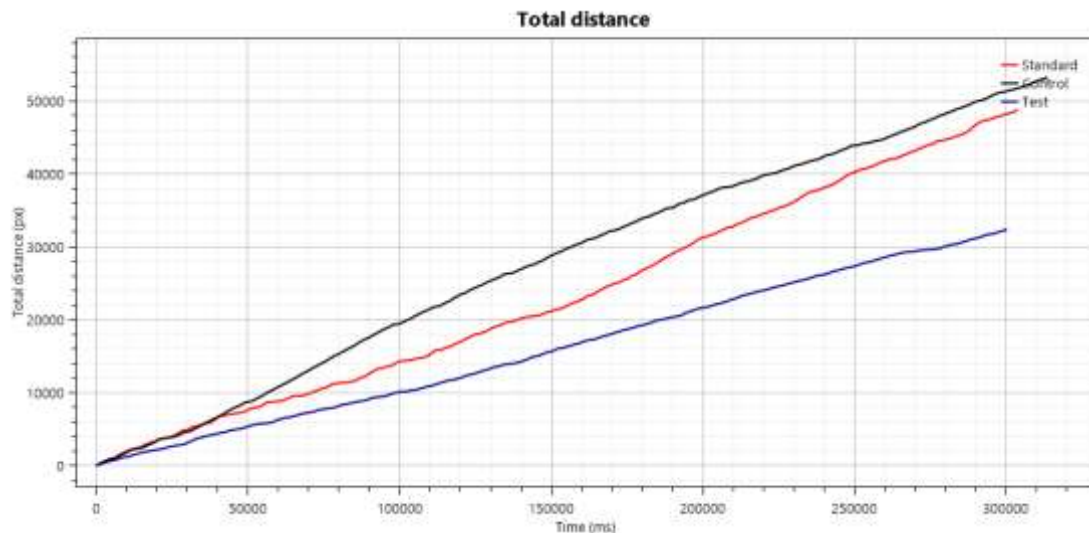


Fig 15: Comparative distance travelled by zebrafish in control, standard, and test

X axis: time (ms), Y axis: total distance (px)

Fig.15 shows the distance travelled by zebrafish in various tests, which reveals that control shows increased distance due to anxiety symptoms.

It is then compared to the standard and test group, which show a decrease in the distance travelled by zebrafish.

4.CONCLUSION

The stems of *P.edulis* were collected, authenticated, dried, and subjected to extraction in ethanol as solvent using the cold maceration method.

The phytochemical screening of the leaves of *Passifloraedulis* indicates the presence of proteins, flavonoids, sterols, saponins, phenolic compounds, tannins, glycosides, and terpenoids.

One of the important pharmacological actions shown by our extract is anxiolytic effect. The study was carried out in zebrafish for ethanolic extract, and it was concluded that the ethanolic extract of *Passifloraedulis* stem has a significant anxiolytic effect as compared to the standard (diazepam). The anxiolytic effect may be due to the presence of flavonoids, sterols, phenolic compounds, tannins, glycosides, and terpenoids in the extract.

The drug, being an herbal, could be a suitable alternative and could be a newer member of the class of anxiolytic drugs.

The present study showed that, ethanolic extract of *P.edulis* is capable of inhibiting the response to a wide range of anxiety symptoms. Therefore, *P.edulis* may be useful in the treatment of anxiety and its symptoms.

Further study with regards to isolation, purification, mechanisms, and pharmacological screening of the active principles responsible for the activity is to be carried out.

5.REFERENCE

1. Rang, H. P., & Dale, M. M. (2023). Rang & Dale's pharmacology (9th ed.). Elsevier.
2. World Health Organization. (2023, September 29). Traditional, complementary and integrative medicine
3. Rai S, Chandra J, Mukim M. Pharmacological and Medicinal Importance of PassifloraEdulis: A Review. (2022, April 4).
4. Passifloraedulis: An Insight into Current Researches on Phytochemistry and Pharmacology: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6115900/>
5. American Psychological Association. (2023, January 11). What is anxiety disorders?
6. Tripathi, K.D. (2008) Essentials of Medical Pharmacology. 6th Edition, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 816-835.
7. Anxiolytic and Hypnotic Drugs. In: Stringer JL, eds. Basic Concepts in Pharmacology: What You Need to Know for Each Drug Class. 6th ed. McGraw Hill; 2022

8. TNAU Agritech; <https://agritech.tnau.ac.in/banking/pdf/Passion%20Fruit.pdf>
9. World Flora Online; <https://www.worldfloraonline.org/taxon/wfo-0000479905>
10. Streba L, Gheonea D.I, Scheker M. Current Trends in Cancer Management (2019). IntechOpen.
11. Y. T. B. H. Fero K, "The behavioural repertoire of larval zebrafish," *Zebrafish Models in Neurobehavioral Research Cambridge University Press* Kalueff AV, Cachat JM, 2010.
12. Rizwan HM, Zhimin L, Harsonowati W, Waheed A, Qiang Y, Yousef AF, et al. Identification of Fungal Pathogens to Control Postharvest Passion Fruit (*Passiflora edulis*) Decays and Multi-Omics Comparative Pathway Analysis Reveals Purple Is More Resistant to Pathogens than a Yellow Cultivar. *J. Fungi*. 2021; 7(1), 879-889. <https://doi.org/10.3390/jof7100879>.
13. Nutritional value of passion fruit: <https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102676/nutrients>
14. Wen LJ, Mao HJ, Zhang YC, Li YJ. Compositions and antioxidant activity of *Passiflora edulis* rind. *Food Sci*. 2008; 29, 54–58.
15. Silva RO, Damasceno SR, Brito TV, Dias JM, et al. Polysaccharide fraction isolated from *Passiflora edulis* inhibits the inflammatory response and the oxidative stress in mice. *J. Pharm. Pharmacol*. 2015; 67, 1017-1027. doi: 10.1111/jphp.12399.
16. Lewis BJ, Herrlinger KA, Craig TA, et al. Antihypertensive effect of passion fruit peel extract and its major bioactive components following acute supplementation in spontaneously hypertensive rats. *The Journal of nutritional biochemistry*. 2013; 24(7), 1359-1366. <https://doi.org/10.1016/j.jnutbio.2012.11.003>.
17. Nerdy N, Ritarwan K. Hepatoprotective Activity and Nephroprotective Activity of Peel Extract from Three Varieties of the Passion Fruit (*Passiflora Sp.*) in the Albino Rat. *Open Access Maced J Med Sci*. 2019;7(4):536-542. doi:10.3889/oamjms.2019.153.
18. Sunitha M, Devaki K. Antioxidant Activity of *Passiflora edulis* Sims Leaves. *Indian J Pharm Sci*. 2009;71(3):310-311. doi:10.4103/0250-474X.56038.
19. Amaral RG, Gomes SVF, Andrade LN, et al. Cytotoxic, Antitumor and Toxicological Profile of *Passiflora alata* Leaf Extract. *Molecules*. 2020; 25(20):4814. Published 2020 Oct 20. doi:10.3390/molecules25204814.
20. Zebrafish Model Organism Database: <https://zfin.org/>
21. https://www.researchgate.net/figure/Zebrafish-anatomy-An-adult-zebrafish-is-shown-with-the-anatomical-structures-labelled_fig1_311241166
22. Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders *Trends Pharmacol Sci* 2014;35:63–75
23. (Lillesaar et al., 2007; Shams et al., 2018; Schweitzer and Driever, 2009).
24. Sarris J. Herbal medicines in the treatment of psychiatric disorders: a systematic review. *Phytother Res* 2007;21:703–716.
25. K. Howe, M. Clark, C. T. J. C. Torroja, M. Muffato, J. Collins and S. Humphray, "The zebrafish reference genome sequence and its relationship to the human genome," *Nature*, vol. 496, p. 498–503, 2013.
26. Akabas MH. GABAA receptor structure-function studies: a reexamination in light of new acetylcholine receptor structures. 2004;62:1–43.
27. Sigel E. Mapping of the benzodiazepine recognition site on GABA(A) receptors. *Curr Top Med Chem* 2002;2:833–839
28. Kalueff, M. Gebhardt, A. Stewart, J. Cachat, M. Brimmer, J. Chawla, C. Craddock, E. Kyzar, A. Roth, S. Landsman and e. al., "Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond," *Zebrafish*, vol. 10, p. 70–86, 2013.
29. R. Friedrich and Z. P. Jacobson, "Circuit neuroscience in zebrafish," *Curr. Biol*, vol. 20, p. R371–R381, 2010
30. D. Kokel and R. Peterson, "Chemobehavioural phenomics and behaviour-based psychiatric drug discovery in the zebrafish," *Brief. Funct. Genom. Proteom.*, vol. 7, p. 483–490, 2008.
31. N. a. G. R. Miller, "From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*)," *PLoS One*, p. 7, 2012.
32. M. B. F. D. D. e. a. Barreiros, "Zebrafish automatic monitoring system for conditioning and behavioral analysis," *Sci Rep*, vol. 11, p. 9330, 2021
33. Kalueff, M. Gebhardt, A. Stewart, J. Cachat, M. Brimmer, J. Chawla, C. Craddock, E. Kyzar, A. Roth, S. Landsman and e. al., "Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond," *Zebrafish*, vol. 10, p. 70–86, 2013.
34. L. M. G. S. R. A. Gerlai R, "Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects," *Pharmacol Biochem Behav*, vol. 67, pp. 773-782, 2000.

35. J. R. WolmanMA, "Chemical modulation of memory formation in larval zebrafish," *PNAS*, vol. 108, p. 15468–73, 2011.
36. M. & H. M. Bourin, "The mouse light/dark box test," *European Journal of Pharmacology*, Vols. 1-3, no. 463, pp. 55-65, 2003.
37. M. Wolman, R. Jain, K. Marsden, H. Bell, J. Skinner, K. Hayer, J. Hogenesch and M. Granato, "A genomewide screen identifies papp-aa-mediated igfrsignaling as a novel regulator of habituation learning.," *Neuron*, vol. 85, p. 1200–1211, 2015.
38. F. Zimmermann, K. Gaspary, C. Leite, G. De Paula Cognato and C. Bonan, " Embryological Exposure to Valproic Acid Induces Social Interaction Deficits in Zebrafish (Daniorerio): A Developmental Behavior Analysis," *Neurotoxicol. Teratol*, vol. 52 , p. 36–41, 2015.
39. D. J. Darland T, "Behavioral screening for cocaine sensitivity in mutagenized zebrafish," *Proc Natl AcadSci USA* , vol. 98, pp. 11691-11696, 2001
40. Fernandes, K. Fero, A. Arrenberg, S. Bergeron, W. Driever and H. Burgess, "Deep Brain Photoreceptors Control Light-Seeking Behavior in Zebrafish Larvae," *Curr. Biol.* , vol. 22, p. 2042–2047, 2012.
41. Kalueff, M. Gebhardt, A. Stewart, J. Cachat, M. Brimmer, J. Chawla, C. Craddock, E. Kyzar, A. Roth, S. Landsman and e. al., "Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond," *Zebrafish*, vol. 10, p. 70–86, 2013.
42. Vongsak, B.; Sithisarn, P.; Mangmool, S.; Thongpraditchote, S.; Wongkrajang, Y.; Gritsanapan, W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringaoleifera leaf extract by the appropriate extraction method. *Ind. Crops Prod.* 2013, 44, 566–571
43. R. C Jagessar Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of Passifloraedulis and Viciafaba L. (Fabaceae).
44. Oggier DM, Weisbrod CI, Stoller AM, Zenker AK, Fent K (2010) Effects of diazepam on gene expression and link to physiological effects in different life stages in zebrafish Daniorerio. *Environ SciTechnol* 44: 7685–7691
45. Bencan Z, Sledge D, Levin ED (2009) Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *PharmacolBiochemBehav* 94: 75–80.
46. Ogi A, Licitra R, Naef V, Marchese M, Fronte B, Gazzano A, Santorelli FM. Social preference tests In zebrafish: A systematic review. *Front Vet Sci.* 2021; 7: 590057
47. Oliveira RF, Silva JF, Simoes JM (2011) Fighting zebra fish: characterization of aggressive behavior and winner-loser effects. *Zebra fish* 8(2):73–81
48. Moretz AA, Martins EP, Robinson BD (2007) Behavioral syndromes and the evolution of correlated behavior in zebra fi sh. *BehavEcol* 3:556–562
49. Desjardins JK, Fernald RD (2010) What do fish make of mirror images? *Biol Lett* 6(6): 744–747
50. Pal S, Mandal S, Firdous SM. Neurobehavioral assessment of seed extract of Swieteniamahagoni on zebrafish model. ||| *Bangladesh Journal of Pharmacology*|||. 2023 Dec 17;18(4):130-7.