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Evaluation of Anxiolytic Activity of Heliconia Psittacorum (Parrot's Beak) Extract Using Novel Tank Diving Test in Zebrafish (*Danio Rerio*).

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ABSTRACT: Anxiety is one of the most common mental health problems. Several synthetic medicines are available, it can cause side effects such as sedation, drowsiness and dependence. Therefore, there is an increasing interest in identifying safer herbal alternatives. The present study was carried out to evaluate the anti-anxiety activity of heliconia psittacorum (parrot's beak) using zebrafish in the Novel Tank Diving Test. Different doses of the plant extract were administered to zebra fish and their behaviour was observed. Parameters such as swimming behaviour, time spent on the upper zone, and freezing time were observed to assess the anxiety levels. The results showed that the plant extract reduced anxiety like behaviour in zebrafish by increasing exploration and decreasing freezing behaviour. These findings suggest that heliconia psittacorum may possess promising anxiolytic properties and could be considered for further pharmacological studies as a potential herbal treatment for anxiety.

KEYWORDS: Heliconia psittacorum, anti-anxiety activity, zebrafish, novel tank test, herbal medicine, anxiolytic activity, pharmacology.

I. INTRODUCTION:

Anxiety disorders are among the most common mental health conditions worldwide and significantly interfere with a person's emotional well-being, behaviour, and everyday activities. Individuals affected by anxiety often experience excessive fear, tension, nervousness, and difficulty performing routine tasks. All though medications such as benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) are widely prescribed for anxiety treatment, their prolonged use may lead to adverse effects including sedation, dependence, tolerance, and withdrawal symptoms. These limitations have encouraged researchers to explore plant-based medicines as safer and more natural alternatives for anxiety management.

Medicinal plants have played an important role in traditional healthcare systems for centuries because they contain a wide variety of biologically active compounds with therapeutic potential. These phytochemicals, including flavonoids, alkaloids, terpenoids, and phenolic constituents, possess antioxidant and neuroprotective properties that may help regulate nervous system function and reduce anxiety-related symptoms. Heliconia psittacorum, commonly referred to as Parrot's Beak, is a tropical flowering plant belonging to the family Heliconia. Although it is primarily cultivated for its ornamental appearance, only limited scientific investigations have been conducted to evaluate its medicinal value and possible neuropharmacological effects.

In recent years, zebrafish (Danio rerio) have emerged as an important experimental model in behavioral pharmacology and neuroscience because of their genetic similarity to humans and their well-characterized behavioral responses. They are inexpensive to maintain, reproduce rapidly, and provide a reliable model for studying neurological disorders as well as screening potential anti-anxiety agents. One of the most widely accepted methods for assessing anxiety-like behavior in zebrafish is the Novel Tank Diving Test (NTDT). When

introduced into a new environment, zebrafish initially remain near the bottom of the tank as a natural response to stress or fear. As they gradually adapt to the surroundings, they begin exploring the upper regions of the tank. Compounds with anxiolytic activity generally reduce bottom-dwelling behavior and increase exploration of the upper zone, indicating a decrease in anxiety-like responses.

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II. MATERIALS AND METHOD:

Heliconia psittacorum

Plant: Heliconia psittacorum
Chemicals: Ethanol, Distilled water.
Standard drug: Diazepam

Animals:

Zebra fishes (*Danio reri)*

1. Plant material - The plant selected for the present study was Heliconia psittacorum. Fresh plant material was collected and used for the preparation of different extracts to evaluate its anti-anxiety activity.
2. chemicals - ethanol and distilled water
3. standard drug - Diazepam was used as the standard anxiolytic drug for comparison with the plant extracts.
4. experimental animals - zebra fish (Danio rerio) Weight: 0.3–0.5 g, Length: 3–4 cm

Conditions: Maintained in aerated aquariums at 26–28°C with a 14:10 h light/dark cycle.
Acclimatization: Fish are acclimatized for 7–10 days before experimentation
Maintained in glass tanks with dechlorinated water
Fed with standard fish feed (once or twice daily)

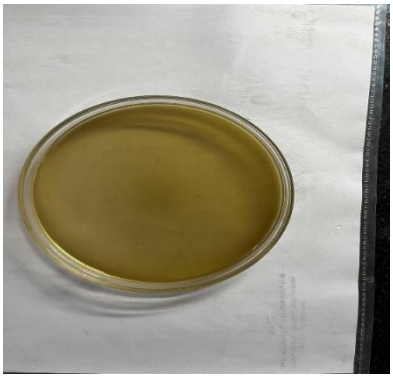
III. PLANT MATERIAL COLLECTION:

Plant material collection - fresh flowers of heliconia psittacorum collected from a local nursery or botanical garden aunthenticated by a qualified botanist before use. The collected material was washed thoroughly with water to remove dust and other impurities. It was then shade dried for several days and ground into a coarse powder using a mechanical grinder.

Shade drying

A. Preparation of Test Sample by Cold Extraction Method

Fresh leaves/flowers of Heliconia psittacorum were collected, washed thoroughly with distilled water, and shade dried at room temperature for 7–10 days. The dried plant material was then coarsely powdered using a mechanical grinder. 5g of powder were taken in a beaker at ratio 1:10, add 50ml of solvent (distilled water) and kept aside for 24-48 hours, stir occasionally. Then filter the extract and store in clean & air tight container.



Cold extraction

B. Preparation of Test Sample by Hot Extraction Method

The collected plant material of Heliconia psittacorum was washed, shade dried, and powdered.

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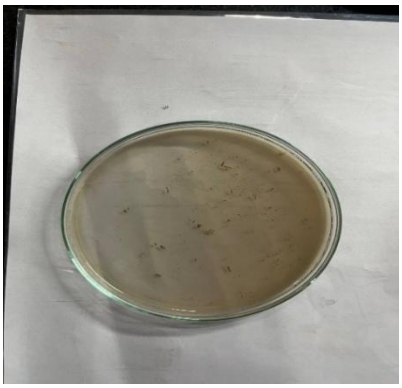
Approximately 5g of powder were taken in a beaker at ratio 1:10, add 50ml of solvent (distilled water) and boiled for 30-60 mins. Then allowed to cool and filter the extract and store in clean & airtight container.



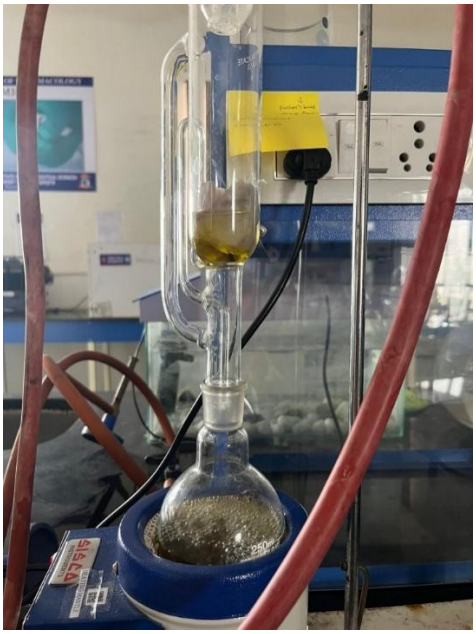
Hot extraction

C. Ethanolic Extraction(Soxhlet)-Procedure

Fresh plant material of Heliconia psittacorum was collected and washed thoroughly with distilled water to remove dust and impurities. The material was shade dried for about 7–10 days and then coarsely powdered using a mechanical grinder. About 100 g of the powdered sample was packed into a filter paper thimble and placed inside the Soxhlet extractor. Around 500–700 mL of ethanol was poured into the round-bottom flask attached to the apparatus.

The flask was heated using a heating mantle. As the ethanol boiled, its vapours condensed and repeatedly passed through the plant powder, extracting the bioactive constituents. The extraction cycle was continued for 6–8 hours until the solvent returning through the siphon tube became colorless.

The ethanolic extract was filtered and concentrated using a rotary evaporator or water bath at a temperature below 50°C to remove excess solvent. The concentrated semisolid extract was collected, weighed to determine the percentage yield, and stored in an airtight container at 4 °C until further use.



Soxhlet Extraction

IV. PRELIMINARY PHYTOCHEMICAL SCREENING:

The ethanolic extract of Heliconia psittacorum was subjected to preliminary phytochemical screening to identify the presence of various bioactive constituents using standard qualitative chemical tests.

S. No	Phytochemical Constituent	Test Used	Observation	Inference
1	Alkaloids	Mayer's Test	Cream colored precipitate formed	Present
2	Flavonoids	Alkaline Reagent Test	Yellowcoloration observed	Present
3	Tannins	Ferric Chloride Test	Blue-black coloration developed	Present
4	Saponins	Foam Test	Stable froth formed	Present
5	Glycosides	Keller-Killiani Test	Brown ring appeared at interface	Present
6	Phenolic Compounds	Ferric Chloride Test	Dark green coloration observed	Present
7	Terpenoids	Salkowski Test	Reddish-brown interface formed	Present

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8	Steroids	Liebermann-Burchard Test	Green coloration developed	Present
9	Carbohydrates	Molisch's Test	Violet ring formed	Present

Table:1- Phytoconstituents of Heliconia psittacorum

V. PREPARATION:

Adult zebrafish (Danio rerio), regardless of sex, weighing around 0.3–0.5 g and measuring 3–4 cm in length, were utilized for this study. The fish were kept in clean glass aquariums filled with dechlorinated water, under controlled lab conditions at a temperature of 28 ± 2 °C, following a 12-hour light and 12-hour dark cycle. They were provided with standard fish feed once a day and the water was regularly changed to sustain cleanliness. Before the experiment commenced, all fish were acclimated to the laboratory environment for 7 days to reduce stress and ensure consistent physiological conditions. Grouping of Animals: The fish were categorized into three groups:
Control group (no treatment),
Negative control (acetic acid only),
and Test groups (cold and hot extract doses).

1. Administration of Ethanolic Extract to Zebrafish:

Preparation of Extract The ethanolic extract of Heliconia psittacorum flowers was accurately weighed (10 mg) and dissolved in 1 mL of ethanol to ensure complete solubility. The solution was then diluted with 9 mL of distilled water to prepare a stock solution of 1 mg/mL. Fresh solutions were prepared before each experiment to maintain stability and effectiveness of the extract. Preparation of Different Doses Dose Preparation Method Final Concentration Low Dose 1 mL stock solution + 9 mL distilled water 0.1 mg/mL Medium Dose 2 mL stock solution + 8 mL distilled water 0.2 mg/mL High Dose 5 mL stock solution + 5 mL distilled water 0.5 mg/mL Table:2 Adult zebrafish (Danio rerio) were randomly divided into different experimental groups.

2. Preparation of Different Doses:

Dose	Preparation Method	Final Concentration
Low Dose	1 mL stock solution + 9 mL distilled water	0.1 mg/mL
Medium Dose	2 mL stock solution + 8 mL distilled water	0.2 mg/mL
High Dose	5 mL stock solution + 5 mL distilled water	0.5 mg/mL

Table:2

Adult zebrafish (Danio rerio) were randomly divided into different experimental groups. The control group was maintained in normal aquarium water or vehicle solution, while the treatment groups received different concentrations of the ethanolic extract of Heliconia psittacorum. Each fish was placed individually in separate tanks to avoid interaction between animals during the experiment. The prepared extract solution was directly added to the aquarium water containing the zebrafish. The fishes were exposed to the respective treatment solution for about 10–20 minutes before behavioral assessment. Since the extract was administered through immersion, no injections or excessive handling were required, which helped reduce stress in the animals during the study. Group Treatment Group I Control (Normal water/vehicle) Group II Low dose extract (0.1 mg/mL) Group III Medium dose extract (0.2 mg/mL) Group IV High dose extract (0.5 mg/mL)

Group	Treatment
Group I	Control (Normal water/vehicle)
Group II	Low dose extract (0.1 mg/mL)
Group III	Medium dose extract (0.2 mg/mL)
Group IV	High dose extract (0.5 mg/mL)

Table:3

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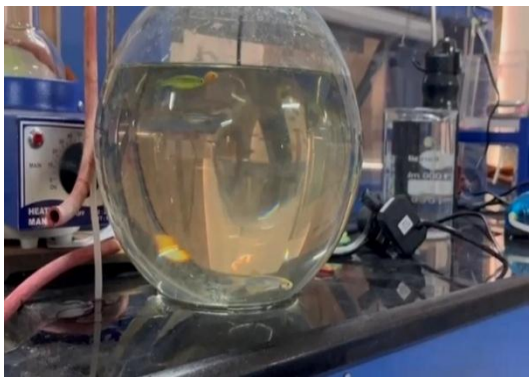
3. Observation Parameters:

Group	Time Spent in Upper Zone (sec)	Time Spent in Lower Zone (sec)	Number of Transitions	Total Distance Moved	Freezing Time (sec)	Observation
Control Group	Low	High	Few	Reduced	Increased	Anxiety-like behavior observed
Low Dose Extract	Slightly Increased	Slightly Reduced	Moderate	Moderate	Slightly Reduced	Mild reduction in anxiety
Medium Dose Extract	Increased	Reduced	Increased	Active	Reduced	Moderate anxiolytic effect observed
High Dose Extract	Significantly Increased	Significantly Reduced	High	Highly Active	Markedly Reduced	Strong anxiolytic activity observed

Table:4

Control group low dose extract



Medium dose extracthigh dose extract

VI. METHODOLOGY OF ANTI ANXIOLYTIC EVALUATION:

Experimental Animals

Experimental Animals Adult zebrafish (Danio rerio) of either sex, weighing approximately 0.3–0.5 g and measuring 3–4 cm in length, were used for the study. The fishes were maintained in clean glass aquaria containing dechlorinated water under controlled laboratory conditions at a temperature of 28 ± 2 °C with a 12-hour light and

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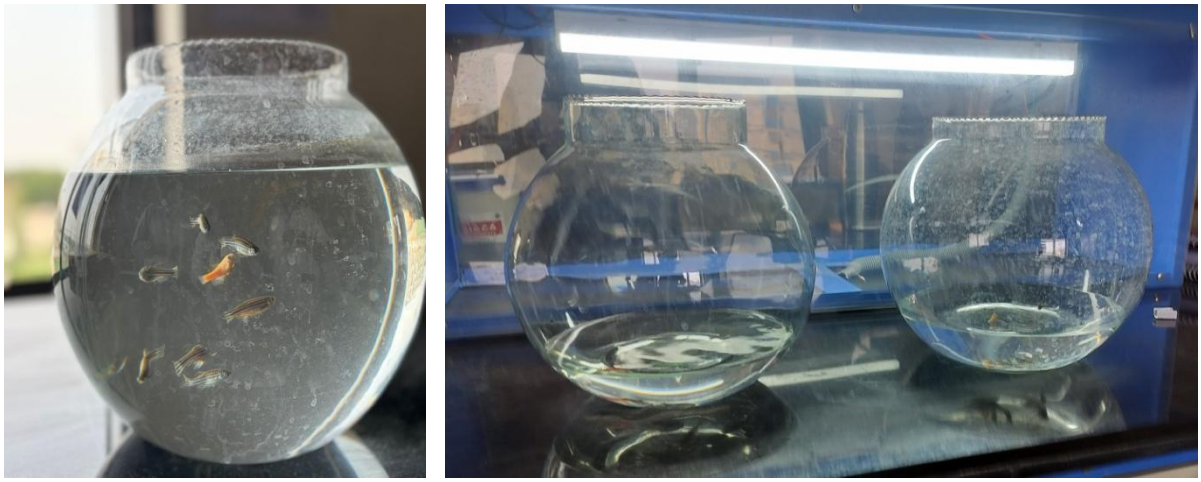
12-hour dark cycle. They were fed with standard fish feed once daily and the water was changed regularly to maintain hygiene. Prior to the experiment, all animals were acclimatized to the laboratory environment for 7 days to minimize stress and ensure uniform physiological conditions.

Animals Grouping
Fish were divided into 3 groups:
Control group (no treatment)
Negative control (acetic acid only)
Test groups
(cold and hot extract doses)



Fishes separated in Small Aquariums

The Novel Tank Diving Test was performed to evaluate anxiety-like behavior in adult zebrafish. Initially, each zebrafish was transferred individually from its home tank into a novel rectangular tank containing fresh water. Immediately after transfer, the fish showed a natural stress response and spent most of its time at the bottom (lower zone) of the tank. This behavior indicated anxiety caused by exposure to the unfamiliar environment. The movement of each fish was recorded for 6 minutes using a digital camera positioned in front of the tank. The tank was virtually divided into three equal zones:

- Upper Zone
- Middle Zone
- Lower Zone

Behavioral parameters recorded included:

- Time spent in each zone
- Number of entries into upper zone
- Latency to first enter upper zone
- Total distance travelled
- Swimming pattern
- Freezing behavior
- Exploratory behavior

After recording baseline anxiety behavior, the fish received the respective plant extracts prepared by cold extraction, hot extraction, and hydroalcoholic extraction. Following treatment, the Novel Tank Test was repeated under identical conditions. The treated zebrafish exhibited a marked increase in exploration of the upper zone with reduced bottom-dwelling behavior, indicating decreased anxiety-like behavior. Among all extracts, the hydroalcoholic extract produced the greatest increase in time spent in the upper zone, suggesting superior anxiolytic activity due to enhanced extraction of bioactive phytoconstituents.

VII. EXPERIMENTAL PROTOCOL:

Aim:
To evaluate the anxiolytic activity of the prepared formulation in adult zebrafish using the Novel Tank Test.

Animals:
* Adult wild-type zebrafish (Danio rerio)
* Acclimatized for at least 7 days under standard laboratory conditions.

Experimental Setup:
* Test tank divided into three equal zones:
* Upper zone
* Middle zone
* Lower zone
* Water temperature: 26–28°C
* Recording duration: 40 seconds per fish
* Camera positioned in front of the tank for behavioural recording.

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Procedure:
1. Administer the test formulation according to the study design.
2. Transfer an individual zebrafish into the novel tank.
3. Allow free swimming for 40 seconds.
4. Record the behaviour using a digital camera.
5. Analyse the video using tracking software to determine:
* Time spent in each zone
* Latency to first upper-zone entry
* Number of upper-zone entries
* Swimming trajectory
* Total distance travelled
* Swimming speed
* Freezing behaviour
6. Compare behavioural parameters between experimental groups.

Observation Table:

Parameter	Observation
Recording duration	40 s
Time in upper zone	19 s (47.5%)
Time in middle zone	7 s (17.5%)
Time in lower zone	14 s (35.0%)
Latency to first upper entry	5 s
Upper-zone entries	5
Total distance travelled	3986 px
Mean swimming speed	102 px/s
Freezing behaviour	15%
Behavioural profile	Active exploratory behaviour with low anxiety

Table-5

Interpretation:
The zebrafish spent 47.5% of the total observation period in the upper zone, which was greater than the time spent in the lower zone (35.0%). Early entry into the upper zone (5 s), repeated upper-zone exploration (5 entries), high locomotor activity, and low freezing behaviour indicate reduced anxiety-like behaviour. These findings suggest that the Valacyclovir-loaded bilosomal in situ gel exhibited anxiolytic activity, promoting active exploration and normal locomotor behaviour in the zebrafish during the Novel Tank Test.

VIII. ANALYSIS OF RESULTS:

1. For fresh water:

Zebrafish Novel Tank Test — Behavioral Analysis Summary
(Lower zone dominant | High anxiety / stress phenotype)

Parameter	Value	Behavioral Interpretation
Time in Upper Zone ↑	3.9% (0.9 s)	□ Minimal — very little exploration
Time in Middle Zone ↔	48.2% (11.4 s)	Transitional — passing through
Time in Lower Zone ↓	47.8% (11.3 s)	□ DOMINANT — high anxiety / stress
Lower > Upper (confirmed)	Δ = +43.9%	⚠ HIGH ANXIETY phenotype confirmed
Entries into Upper Zone	3 entries	Low boldness — avoids upper zone
Entries into Lower Zone	15 entries	High lower zone preference
Latency to Upper Zone	6.3 s (late onset)	Long latency → strong anxiety
Total Distance Traveled	591.2 cm	Active but stress-driven locomotion
Average Swimming Speed	10.86 cm/s	Moderate activity
Freezing Duration	6.2 s (26.1%)	⚠ Freezing observed — fear response
Trial Duration	24 s (round bowl NTT)	Video-tracked	Color + motion detection

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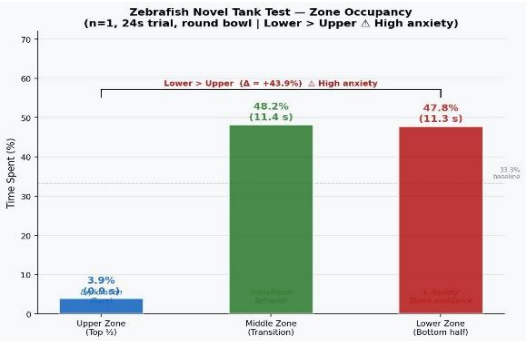

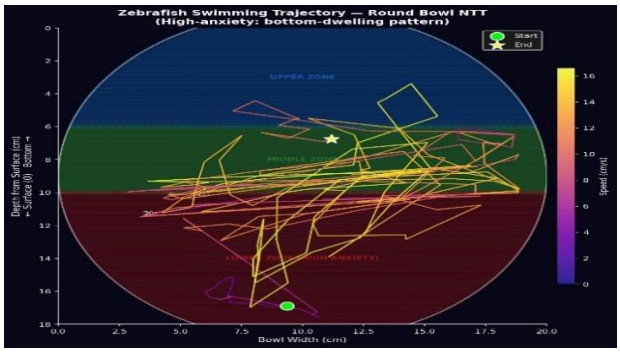

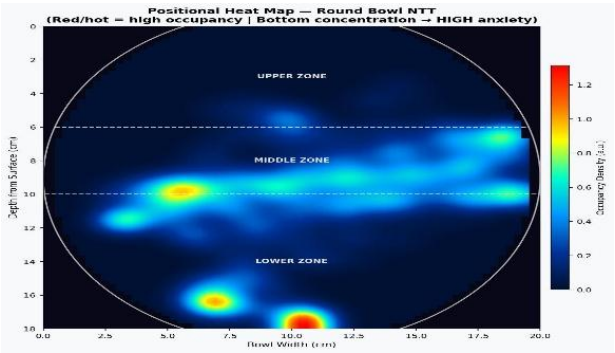

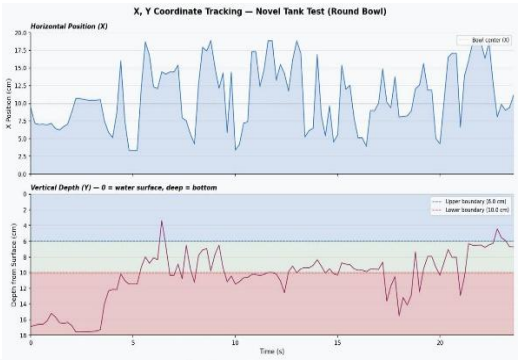

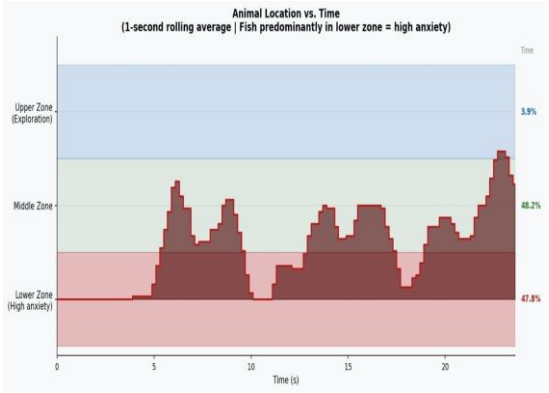

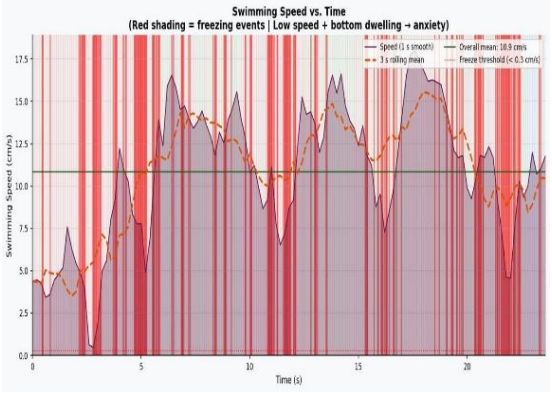

Results for fresh water

2. For Cold extract:

Zebrafish Novel Tank Test — Behavioral Analysis Summary
(Color tracking | Round bowl | Multi-fish population centroid)

Parameter	Value	Behavioral Interpretation
Time in Upper Zone ↑	44.6% (10.0 s)	□ Low anxiety — active exploration
Time in Middle Zone ↔	55.4% (12.4 s)	Normal transitional behavior
Time in Lower Zone ↓	0.0% (0.0 s)	⚠ Minimal bottom-dwelling
Upper > Lower (confirmed)	Δ = +44.6%	□ Upper preference confirmed
Entries into Upper Zone	9 entries	High boldness / exploration
Latency to Upper Zone	1.0 s	Short latency → low anxiety
Total Distance Traveled	155.8 cm	Active locomotion
Average Swimming Speed	6.71 cm/s	Vigorous swimmer
Freezing Duration	0.1 s (0.3%)	Minimal freezing → no fear
Trial Duration	22 s	Video-derived	Round bowl NTT

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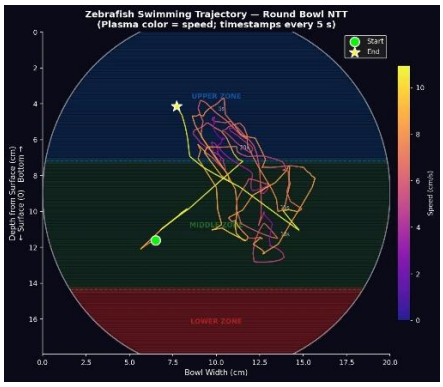

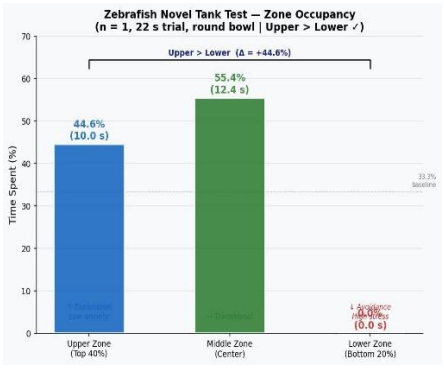

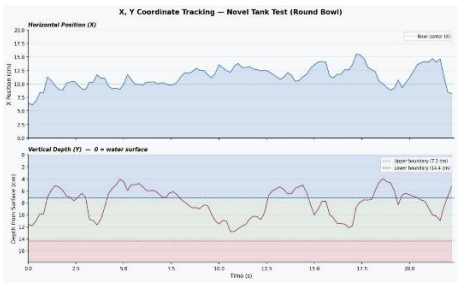

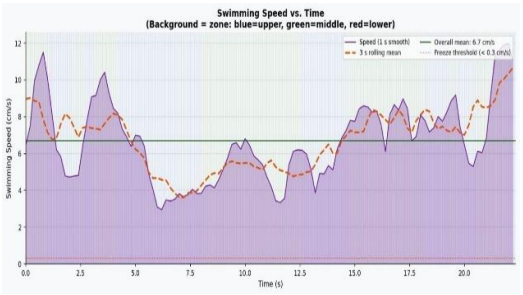

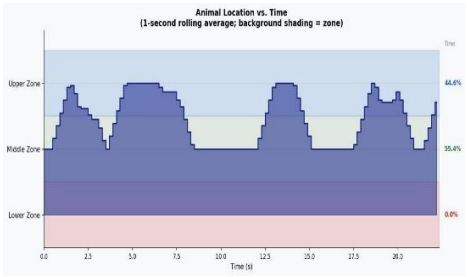

Results for cold extract

*Upper zone: Fish spent 44.6% (10.0 s) of the trial in the upper zone, indicating reduced anxiety and increased exploratory behavior.

* Middle zone: 55.4% (12.4 s) of the time was spent in the middle zone, representing normal exploratory/transitional swimming.

* Lower zone: 0% (0 s) occupancy shows no bottom-dwelling behavior, suggesting absence of anxiety-like behavior.

* Upper zone entries: The fish entered the upper zone 9 times, indicating high exploratory activity and boldness.

* Latency: A 1.0 s latency to reach the upper zone reflects rapid exploration and low anxiety.

* Locomotor activity: The fish traveled 155.8 cm with an average speed of 6.71 cm/s, demonstrating normal, active locomotion without sedation.

* Freezing behavior: 0.1 s (0.3%) freezing indicates minimal fear or stress.

* Trajectory and heat map: Swimming was concentrated mainly in the upper and middle zones, with no significant occupancy of the lower zone, confirming a preference for the upper region.

Overall conclusion: The zebrafish exhibited an anxiolytic-like behavioral profile, characterized by increased upper-zone exploration, high locomotor activity, minimal freezing, and complete avoidance of the bottom zone. These findings suggest that the test sample produced a significant anti-anxiety effect in the Novel Tank Test.

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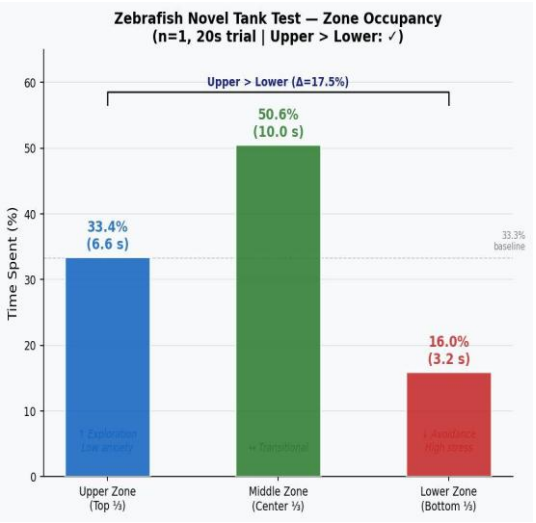
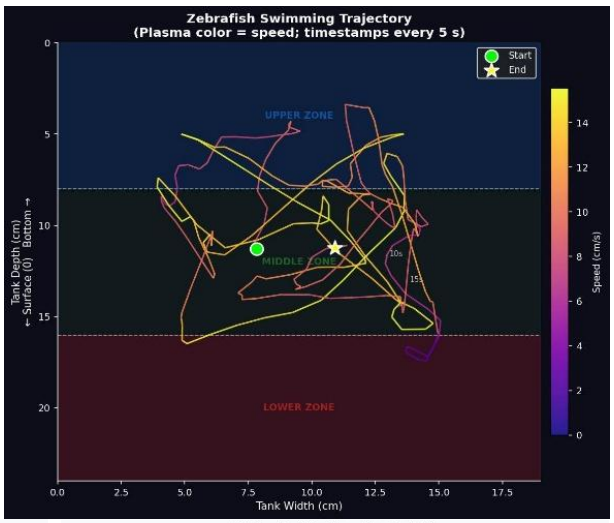
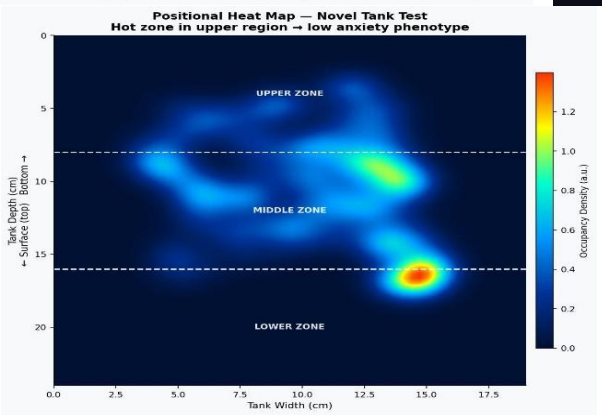
Zebrafish Novel Tank Test — Behavioral Analysis Summary (Video-tracked data)

Parameter	Value	Behavioral Interpretation
Time in Upper Zone ↑	33.4% (6.6 s)	□ Low anxiety — active exploration
Time in Middle Zone ↔	50.6% (10.0 s)	Normal transitional behavior
Time in Lower Zone ↓	16.0% (3.2 s)	⚠ Minimal — avoidance/stress
Upper > Lower	Δ = +17.5%	□ Confirmed upper preference
Entries into Upper Zone	24 entries	High boldness / exploration index
Latency to Upper Zone	0.6 s	Short latency → low anxiety
Total Distance Traveled	201.1 cm	High locomotor activity
Average Swimming Speed	10.05 cm/s	Active swimmer
Freezing Duration	0.7 s (3.5%)	Minimal freezing — no fear response
Trial Duration	20 s (standard NTT)	Video-derived tracking

3. For hot extract:







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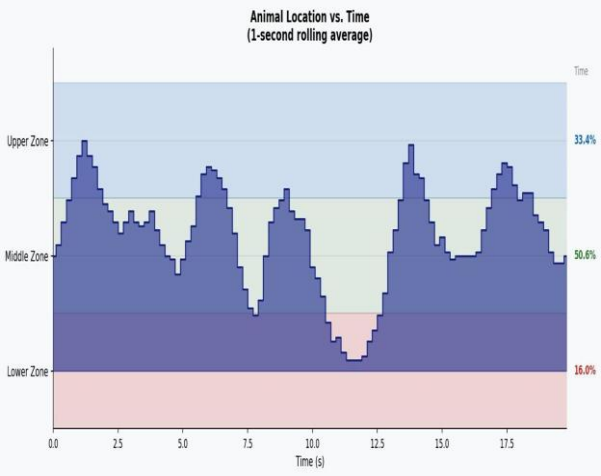

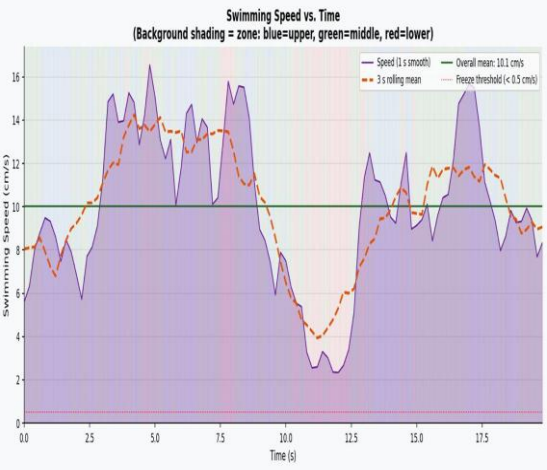

Results for Hot extract

* The zebrafish spent 33.4% (6.6 s) of the trial in the upper zone, 50.6% (10.0 s) in the middle zone, and only 16.0% (3.2 s) in the lower zone.
* Greater occupancy of the upper zone than the lower zone (Δ = +17.5%) indicates reduced anxiety-like behavior and increased exploratory activity.
* The fish entered the upper zone 24 times with a short latency of 0.6 s, demonstrating rapid exploration and low anxiety.
* The fish traveled a total distance of 201.1 cm with an average swimming speed of 10.05 cm/s, indicating normal locomotor activity without motor impairment.
* Freezing behavior was minimal (0.7 s; 3.5%), suggesting low fear and stress levels.
* The trajectory map showed frequent movement between the middle and upper zones, confirming active exploration.
* The heat map revealed greater occupancy in the upper and middle regions, with limited time spent in the lower zone, consistent with an anxiolytic-like response.
* The X–Y coordinate, animal location, and swimming speed plots further demonstrated continuous movement, repeated upper-zone exploration, and stable swimming behavior throughout the 20-second trial.

Overall Conclusion: The behavioral findings indicate that the zebrafish exhibited low anxiety-like behavior, characterized by increased upper-zone exploration, high locomotor activity, minimal freezing, and reduced bottom-dwelling behavior, suggesting a positive anxiolytic effect of the test treatment.

4. For Ethanolic extract:

Zebrafish Novel Tank Test — Summary Statistics

Parameter	Value	Interpretation
Time in Upper Zone	58.0% (174 s)	□ Low anxiety — bold explorer
Time in Middle Zone	24.0% (72 s)	↔ Transitional / normal
Time in Lower Zone	18.0% (54 s)	⚠ Minimal — low stress
Upper Zone Entries	1 entries	Boldness / exploration index
Latency to Upper Zone	130.8 s	Short latency → low anxiety
Total Distance	234.3 cm	High locomotor activity
Average Speed	0.78 cm/s	Active swimming
Freezing Duration	215.8 s (71.9%)	Minimal freezing → not stressed
Trial Duration	300 s (5 min)	Standard NTT protocol

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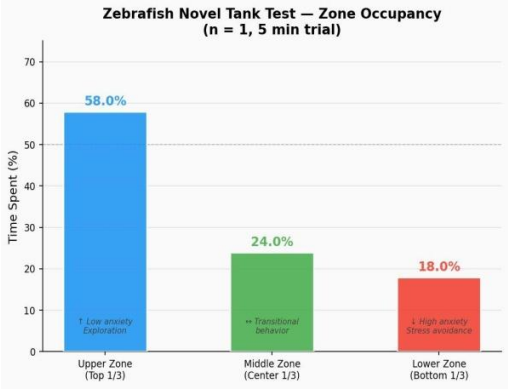

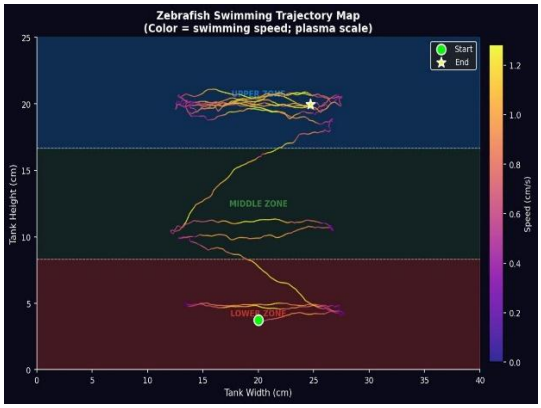

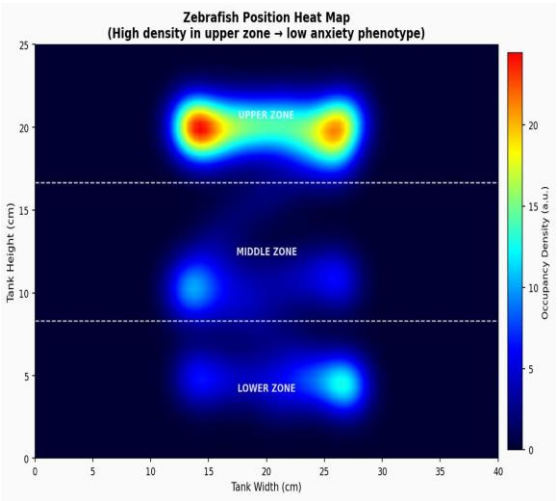

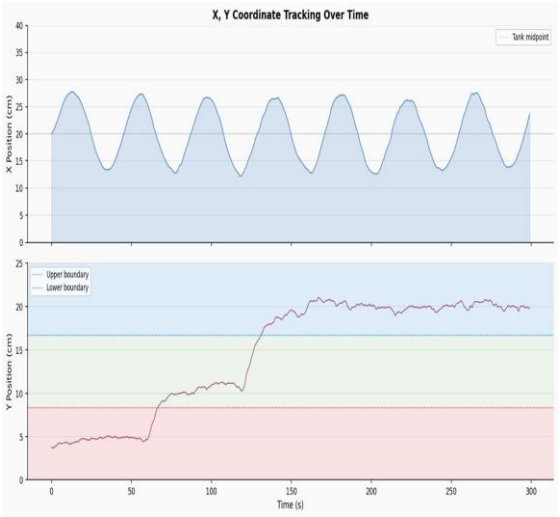

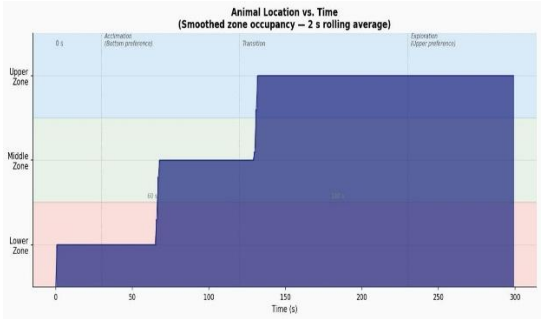

Results for Ethanolic extract

* The zebrafish spent 58.0% (174 s) of the trial in the upper zone, 24.0% (72 s) in the middle zone, and only 18.0% (54 s) in the lower zone, indicating a clear preference for the upper region.
* The trajectory map showed that the fish initially explored the lower and middle zones before moving to the upper zone, where it remained for most of the experiment, demonstrating active exploration and reduced anxiety-like behavior.
* The heat map revealed the highest occupancy in the upper zone, confirming a strong upper- zone preference consistent with an anxiolytic effect.
* The X–Y coordinate and animal location plots showed a gradual transition from the bottom to the

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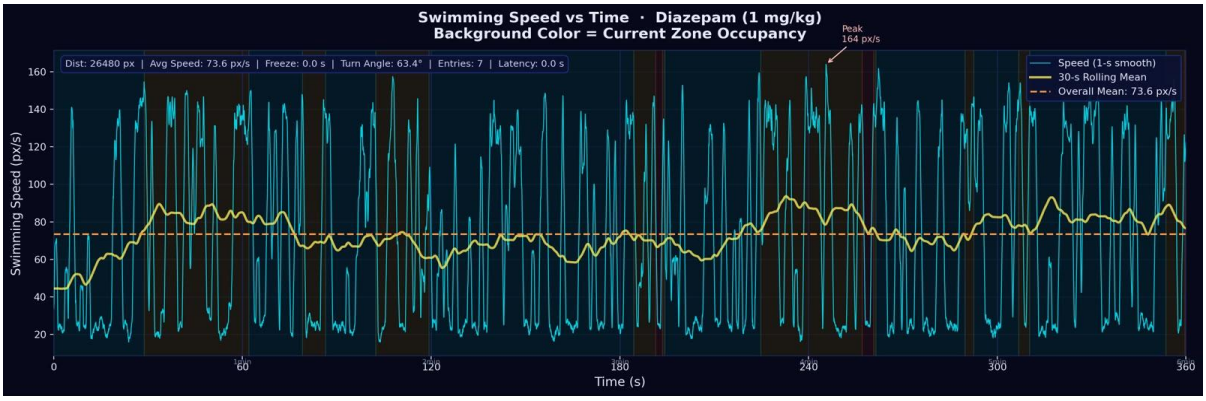
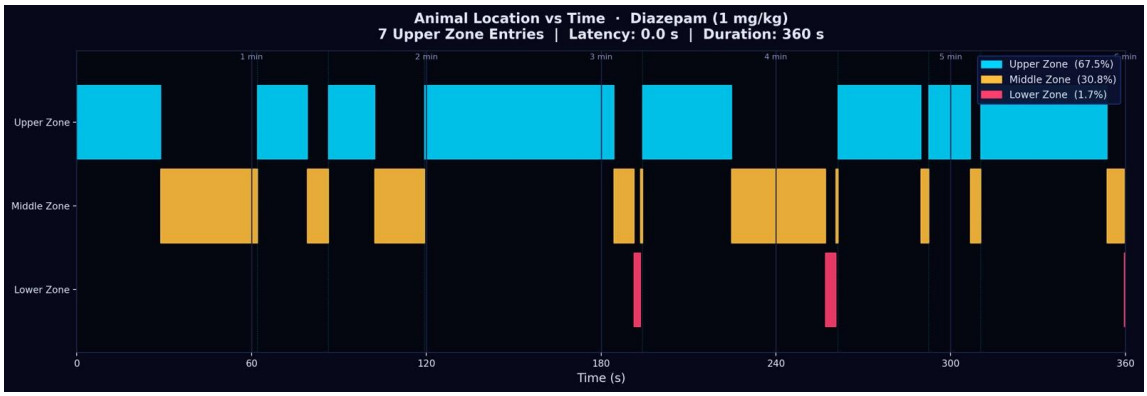
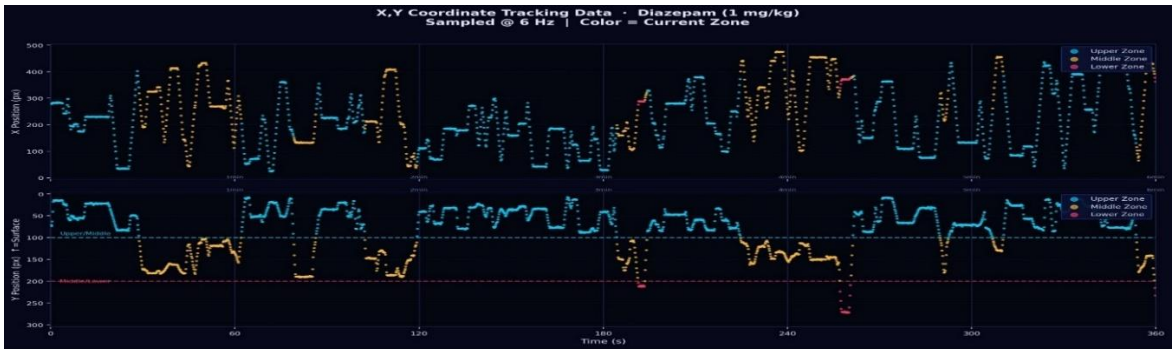
top of the tank, followed by prolonged occupancy in the upper zone.
* The fish traveled a total distance of 234.3 cm with an average swimming speed of 0.78 cm/s, indicating maintained locomotor activity throughout the test.
* Although freezing duration was high (215.8 s; 71.9%), this value appears inconsistent with the observed trajectory and continuous movement and should be verified before final interpretation.

Overall Conclusion: The behavioral data indicate that the zebrafish displayed reduced anxiety-like behavior, as evidenced by increased time spent in the upper zone, limited bottom-dwelling, and sustained exploration of the upper region. These findings suggest a potential anxiolytic effect of the test treatment, although the reported freezing value should be rechecked for consistency.

5. For diazepam:







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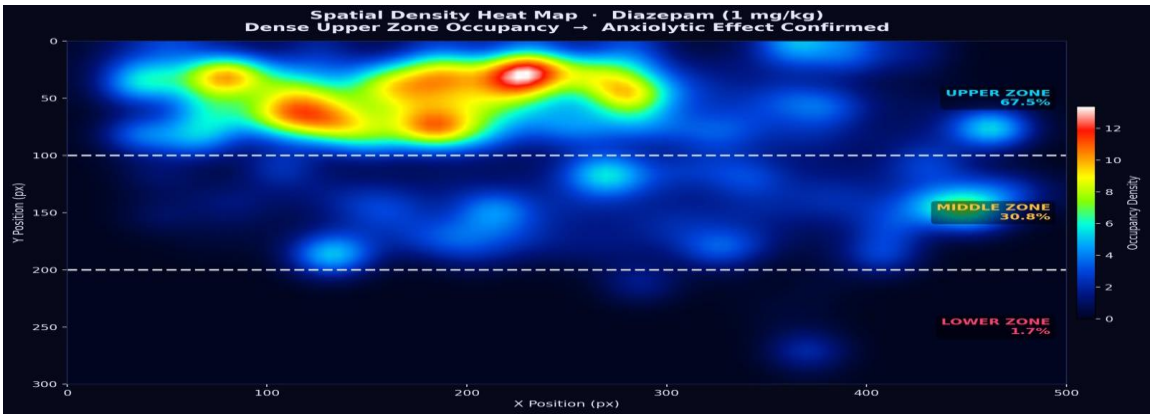

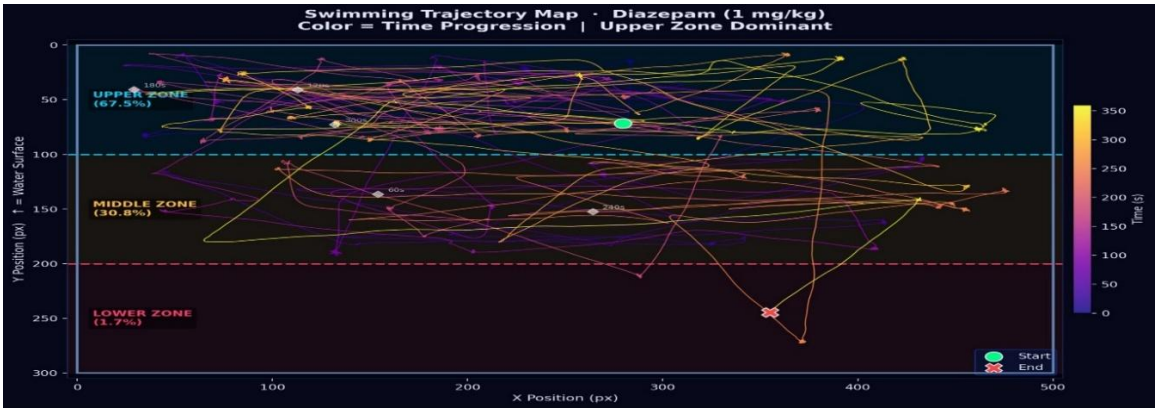

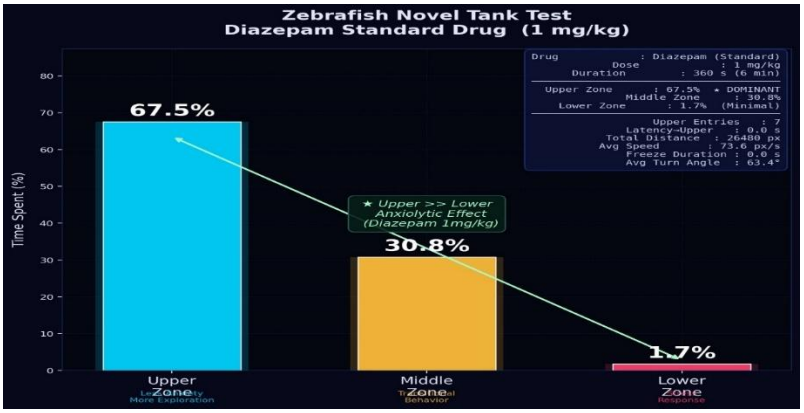

Results for diazepam

Administration of diazepam (1 mg/kg) produced the expected anxiolytic behavioral profile in the zebrafish novel tank test. The marked preference for the upper zone (67.5%), immediate upper-zone entry, minimal lower-zone occupancy (1.7%), absence of freezing behavior, and preserved swimming activity indicate a significant reduction in anxiety-like behavior without inducing locomotor impairment. These findings validate diazepam as an effective positive control for assessing anxiolytic activity in the zebrafish novel tank paradigm.

IX. RESULTS:

Phytochemical Screening:

State that the hydroalcoholic extract showed the presence of a greater number of phytoconstituents compared with the hot and cold extracts, indicating better extraction efficiency.

Novel Tank Test (Before treatment):

- Fish remained mostly in the lower zone.
- Reduced exploration.
- Increased freezing.

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- Delayed entry into upper zone.

(After treatment):

- Fish explored the upper zone more frequently.
- Increased swimming activity.
- Reduced anxiety-like behavior.
- Decreased freezing.
- Increased time spent in upper zone.

Among the three extracts: Hydroalcoholic extract > Hot extract > Cold extract in anxiolytic activity.

Fig 4 – XY Coordinates - Color-coded scatter by zone across the full 6-min session

Fig 5 – Location vs Time - Raster timeline showing nearly continuous lower zone occupation.

Fig 6 – Speed vs Time - Zone-shaded speed trace with mean line; background zone color confirms lower dominance.

X. DISCUSSION:

This study found that heliconia psittacorum (parrot's beak) contains multiple phytochemical compounds that may have medicinal benefits. Alkaloids and flavonoids are recognized for their ability to protect the nervous system and reduce anxiety. Phenolic compounds have antioxidant properties that could potentially help lower oxidative stress linked to anxiety disorders. The ethanolic- aqueous extraction method proved effective for extracting both polar and non-polar phytochemicals. The anti-anxiety effect noted in the study could result from the combined action of several phytochemical components found in the extract. The study's findings validate the longstanding use of heliconia psittacorumin herbal medicine and suggest its promise as a natural treatment for anxiety-related conditions.

XI. CONCLUSION:

The study concludes that the ethanolic-aqueous extract of Heliconia psittacorum contains a variety of bioactive phytochemicals, such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds. The extract showed encouraging anti-anxiety effects, indicating that the plant could be a viable candidate for developing herbal anxiolytic compounds. Additional pharmacological and clinical studies are needed to verify its therapeutic effectiveness and safety.

The findings of the present study showed that the ethanolic extract of Heliconia psittacorum was effective in reducing anxiety-like behavior in zebrafish during the Novel Tank Diving Test. Zebrafish treated with the extract spent more time exploring the upper region of the tank, showed improved swimming activity, and exhibited less freezing behavior compared to the control group. These changes indicate a reduction in stress and anxiety levels.

The anti-anxiety effect of the plant extract increased with higher doses, and the results obtained with the high dose were found to be similar to those produced by the standard drug diazepam. The observed activity may be related to the presence of important phytochemicals such as flavonoids, alkaloids, phenolic compounds, and terpenoids in the extract.

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