



(RESEARCH ARTICLE)



PHYTOCHEMICAL EVALUATION AND ANTI-ANXIETY ACTIVITY OF ETHANOLIC-AQUEOUS EXTRACT OF *STACHYTARPHETA JAMAICENSIS*

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ABSTRACT

Medicinal plants have long been utilized in various traditional medicine systems to address neurological and psychological disorders. One such plant, *Stachytarpheta jamaicensis*, commonly referred to as Snakeweed, is recognized for its potential therapeutic benefits. This study aims to assess the phytochemical constituents and the anti-anxiety effects of the ethanolic-aqueous extract derived from *Stachytarpheta jamaicensis*. The extraction process involved three methods: cold extraction, hot extraction, and ethanolic-aqueous extraction, utilizing a 70:30 ratio of ethanol to water. A preliminary phytochemical screening was conducted to identify significant chemical components, including alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, and phenolic compounds. The findings indicated that various bioactive compounds are likely involved in the anxiolytic effects of the plant. This research implies that *Stachytarpheta jamaicensis* may have potential anti-anxiety properties, positioning it as a promising natural source for herbal therapeutic agents.

Keywords: *Stachytarpheta jamaicensis*, Snakeweed, Phytochemical Evaluation, Anti-Anxiety Activity, Ethanolic-Aqueous Extract, Flavonoids, Herbal Medicine, Anxiolytic Activity, Medicinal Plants.

1 INTRODUCTION

Medicinal plants serve as significant sources of bioactive compounds and have a long-standing history in traditional medicine for addressing a range of diseases. Compared to synthetic drugs, herbal remedies are often regarded as safer and more cost-effective options. Numerous medicinal plants are noted for their neuroprotective and anxiolytic properties, largely attributable to their phytochemical constituents. Anxiety is a psychological condition marked by feelings of fear, stress, nervousness, and emotional instability. Synthetic anxiolytic medications frequently come with side effects, such as drowsiness, dependence, and withdrawal symptoms. As a result, there has been a growing interest in exploring natural plant-based alternatives that may present fewer adverse effects. *Stachytarpheta jamaicensis*, commonly referred to as Snakeweed, is a member of the Verbenaceae family. This plant is found extensively in tropical and subtropical regions and has been traditionally utilized to treat conditions such as fever, inflammation, pain,

digestive disorders, and issues related to the nervous system. Phytochemical investigations have identified various compounds in the plant, including alkaloids, flavonoids, tannins, terpenoids, steroids, and phenolic compounds, all of which play a role in its medicinal characteristics. This study seeks to assess the phytochemical constituents found in the ethanolic-aqueous extract of *Stachytarpheta jamaicensis* and to explore its potential anti-anxiety effects.



Figure 1: *Stachytarpheta jamaicensis*

1.1 Organoleptic Characteristics

Organoleptic evaluation was carried out using sensory characteristics of the powdered leaves of *Stachytarpheta jamaicensis*.

Parameter	Description
Colour	Green to dark green
Odour	Characteristic herbal odor
Taste	Slightly bitter
Texture	Coarse powder

1.2 Chemical Constituents

Phytochemical studies on *Stachytarpheta jamaicensis* have revealed the presence of various bioactive constituents such as alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, steroids, carbohydrates, proteins, and fixed oils. These constituents are known to possess antioxidant, anti-inflammatory, antimicrobial, and anxiolytic activities, contributing to the therapeutic potential of the plant.

2 MATERIALS AND PROCESS OF EXTRACTION

2.1 Plant Material Collection

Fresh flowers of *Stachytarpheta jamaicensis* were gathered from a healthy plant and verified for authenticity. The gathered leaves were rinsed thoroughly using distilled water to eliminate dirt and impurities. The flowers were dried in the shade for several days before being ground into powder using a mechanical grinder. The powdered material was kept in an airtight container to be analysed later.

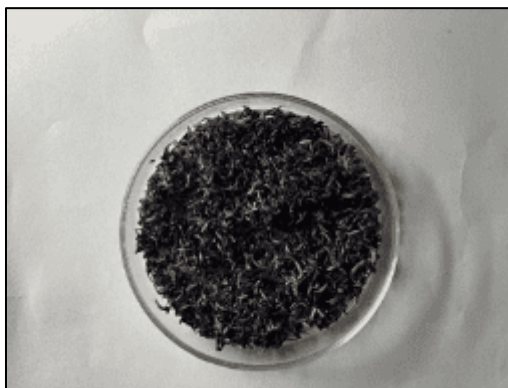


Figure 2: Flowers of *Stachytarpheta jamaicensis*

3 EXTRACT PREPARATION

3.1 Cold Water Extraction (Maceration)- Procedure:

Collection & Preparation: Fresh flowers of *Stachytarpheta jamaicensis* are collected, washed, dried and ground.

Soaking (Maceration): Dried flowers are crushed and taken in a container and added in distilled water within a ratio of 1:10. Covered for 24–48 hours at room temperature. Stirred occasionally.

Filtration: Filter using filter paper.

Concentration: Filtrate is concentrated (if needed) at low temperature. Storage: Stored in an airtight container, refrigerated.



Figure 3: Cold water extraction Hot

3.2 Water Extraction (Decoction)-Procedure

Collection and preparation: Fresh flowers of *Stachytarpheta jamaicensis* are collected and washed, dried under shade.

Boiling: 1g of dried flowers are crushed and added in 10ml of water (ratio 1:10) and boiled for 30–60 minutes.

Concentration: Evaporated to get required volume of 2ml. Cooling & Filtration: Allowed to cool, filtered with filter paper. Storage: Stored in a clean, airtight container.



Figure 4: Hot water extraction

3.3 Ethanolic Extraction (Soxhlet)-Procedure

Fresh flowers were collected, shade-dried, and coarsely powdered.

About 15-20g of the powdered material was packed into a thimble and placed in a Soxhlet apparatus. Extraction was carried out using 90-100mL of ethanol (95%) as the solvent for 4-6 hours (approximately 6-8 cycles) until the solvent in the siphon tube became colorless.

The extract obtained was filtered and concentrated under reduced pressure using a rotary evaporator at 40–45 °C to remove the solvent. The concentrated extract was further dried to obtain a semi-solid mass. The final extract was weighed, transferred into an airtight container, and stored at 4 °C until further use.



Figure 5: Ethanolic extraction

Ethanol is commonly used as a solvent in Soxhlet extraction because it can dissolve a wide range of phytochemicals, including both polar and moderately non-polar compounds such as flavonoids, phenolics, alkaloids, and glycosides. Another advantage of ethanol is that it has a relatively low boiling point (around 78 °C), making it easy to evaporate after extraction without damaging heat-sensitive compounds. It is also less toxic compared to many other organic solvents, making it safer to handle and suitable for extracts intended for biological studies. The Soxhlet extraction method proved to be an efficient technique for obtaining the ethanolic extract of *Pongamia pinnata* flowers. Continuous extraction with ethanol ensured better penetration of the solvent and maximum recovery of bioactive compounds. The method yielded a concentrated extract rich in phytoconstituents such as flavonoids and phenolic compounds. Overall, Soxhlet extraction was found to be a reliable and effective method for preparing plant extracts for further pharmacological evaluation.

4 PHYTOCHEMICAL SCREENING

Table 1: Phytoconstituents of *Stachytarpheta jamaicensis*

Test name	Constituent	Procedure	Observation	Inference
Schinoda test	Flavonoids	2 ml extract+ 2-3 small Mg turnings+0.5 ml concentrated HCl (dropwise)	Pink / red / orange color	Presence of Flavonoids
Ferric chloride test	Tannins	2 ml extract+2-3 drops of 5% FeCl ₃ solution	Green colour	Presence of Condensed tannins
Keller-Kiliani test	Glycosides	2 ml extract+1 ml glacial acetic acid+1-2 drops of 1% FeCl ₃ + carefully 1 ml concentrated H ₂ SO ₄ along the side of test tube.	No Brown ring at the junction.	Cardiac glycosides absent.
Salkowski Test	Steroids & Terpenoids	2 ml extract+2 ml chloroform+carefully 1-2 ml concentrated H ₂ SO ₄ along the side.	Reddish-brown layer Yellow-green fluorescence	Terpenoids Steroids
Ferric Chloride Test	Phenols	2 ml extract in a test tube+2-3 drops of 5% Ferric Chloride (FeCl ₃) solution	Deep blue / green / violet color	Presence of Phenols
Mayers test	Alkaloids	2 ml extract+2-3 drops of Mayer's reagent	No Cream / pale yellow precipitate	Alkaloids absent
Dragendorffs test	Alkaloids	2 ml extract+2-3 drops of Dragendorff's reagent.	No Orange / reddish-brown precipitate	Alkaloids absent

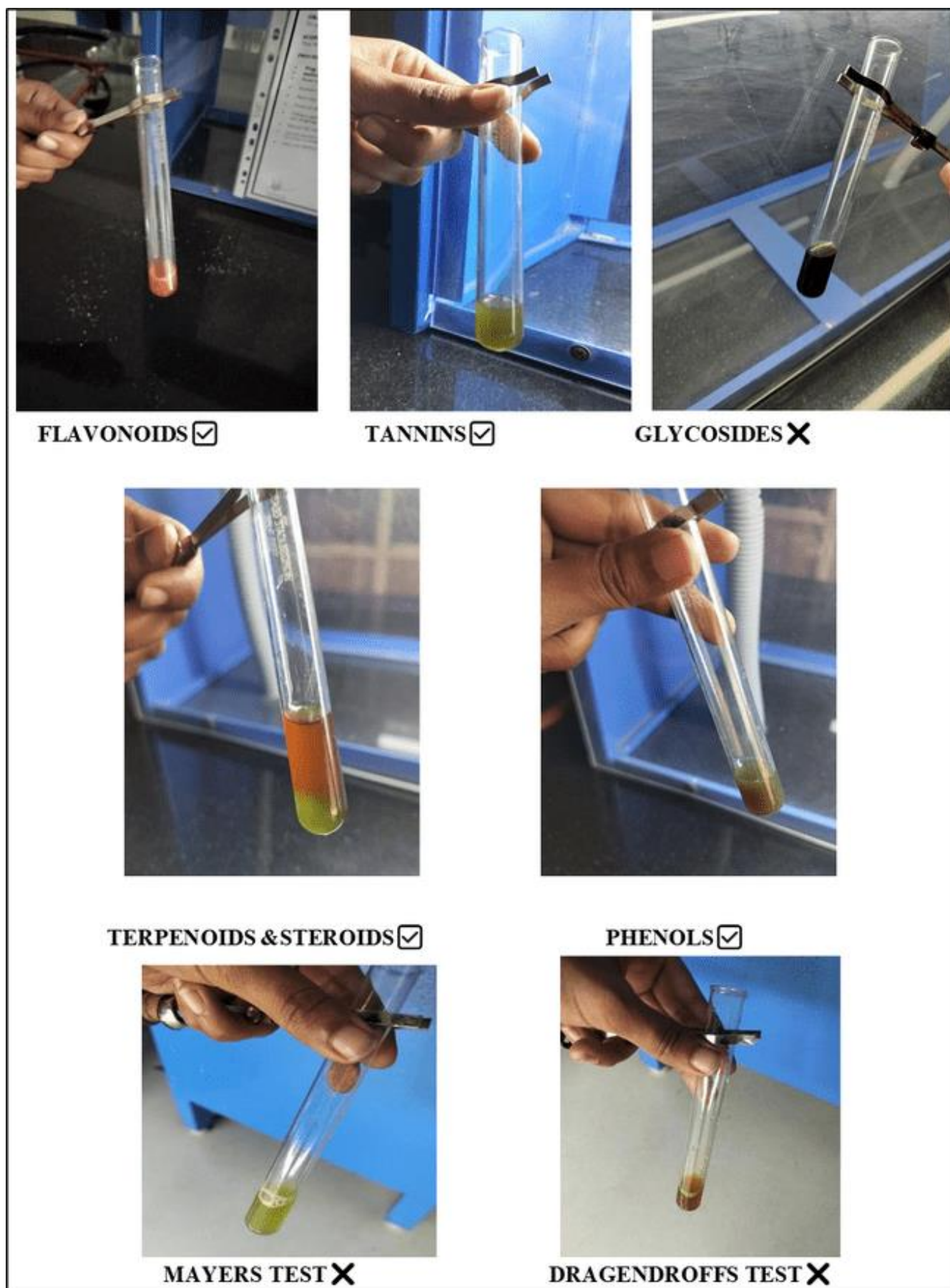


Figure 6: Identification tests of Chemical constituents

5 METHODOLOGY OF ANALGESIC EVALUATION

5.1 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 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.

5.2 Animals Grouping

Fish were divided into 3 groups:

Control group (no treatment) Negative control (acetic acid only)

Test groups (cold and hot extract doses)

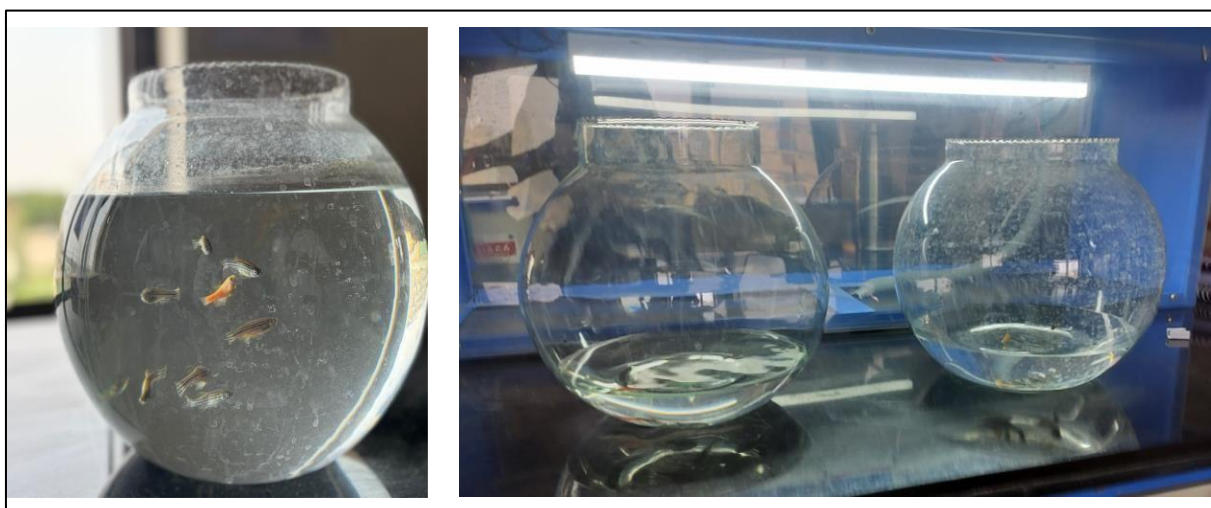


Figure 7: Fishes separated in Small Aquariums

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.

6 EXPERIMENTAL PROTOCOL

6.1 Aim:

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

6.2 Animals:

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

6.2.1 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.

6.2.2 Procedure:

- Administer the test formulation according to the study design.
- Transfer an individual zebrafish into the novel tank.
- Allow free swimming for 40 seconds.
- Record the behaviour using a digital camera.
- 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

Compare behavioural parameters between experimental groups.

6.3 Observation Table

Table 2: Observation table for response of drug

Parameter	Observation
Recording duration	40 s
Time in upper zone	19 s (47.5%)

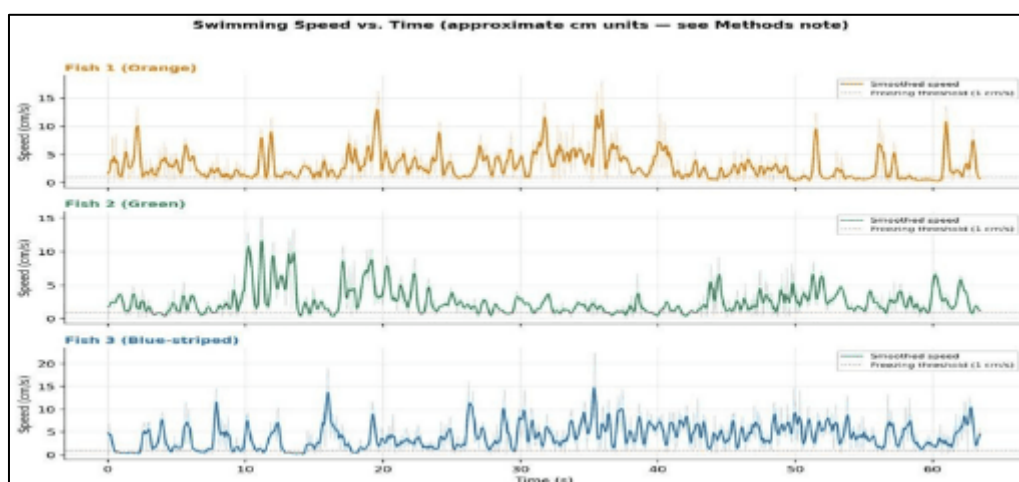
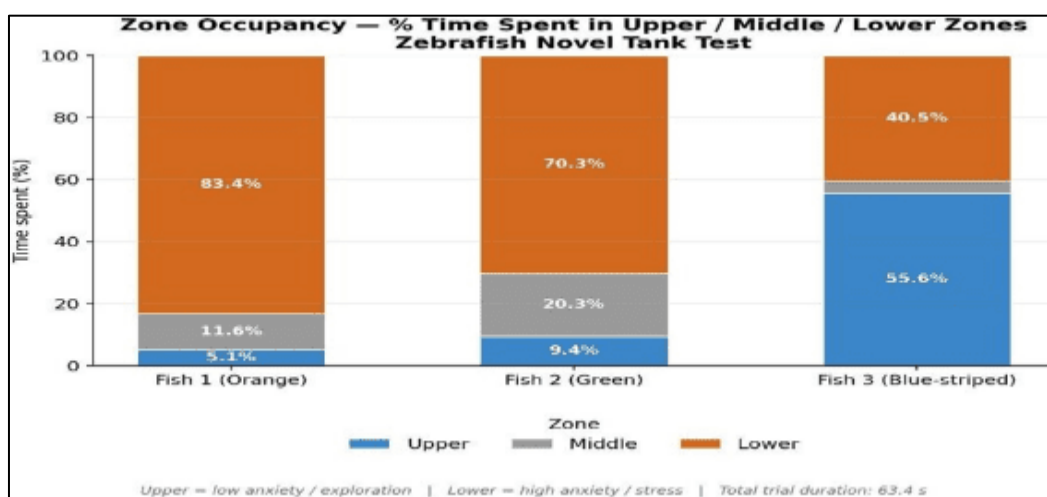
Time in middle zone	7 s (17.5%)
Time in lower zone	14 s (35.0%)
Latency to first upper entry	5 s
Upper-zone entries	5
Total distance travelled	3986 px
Mean swimming speed	102 px/s
Freezing behaviour	15%
Behavioural profile	Active exploratory behaviour with low anxiety

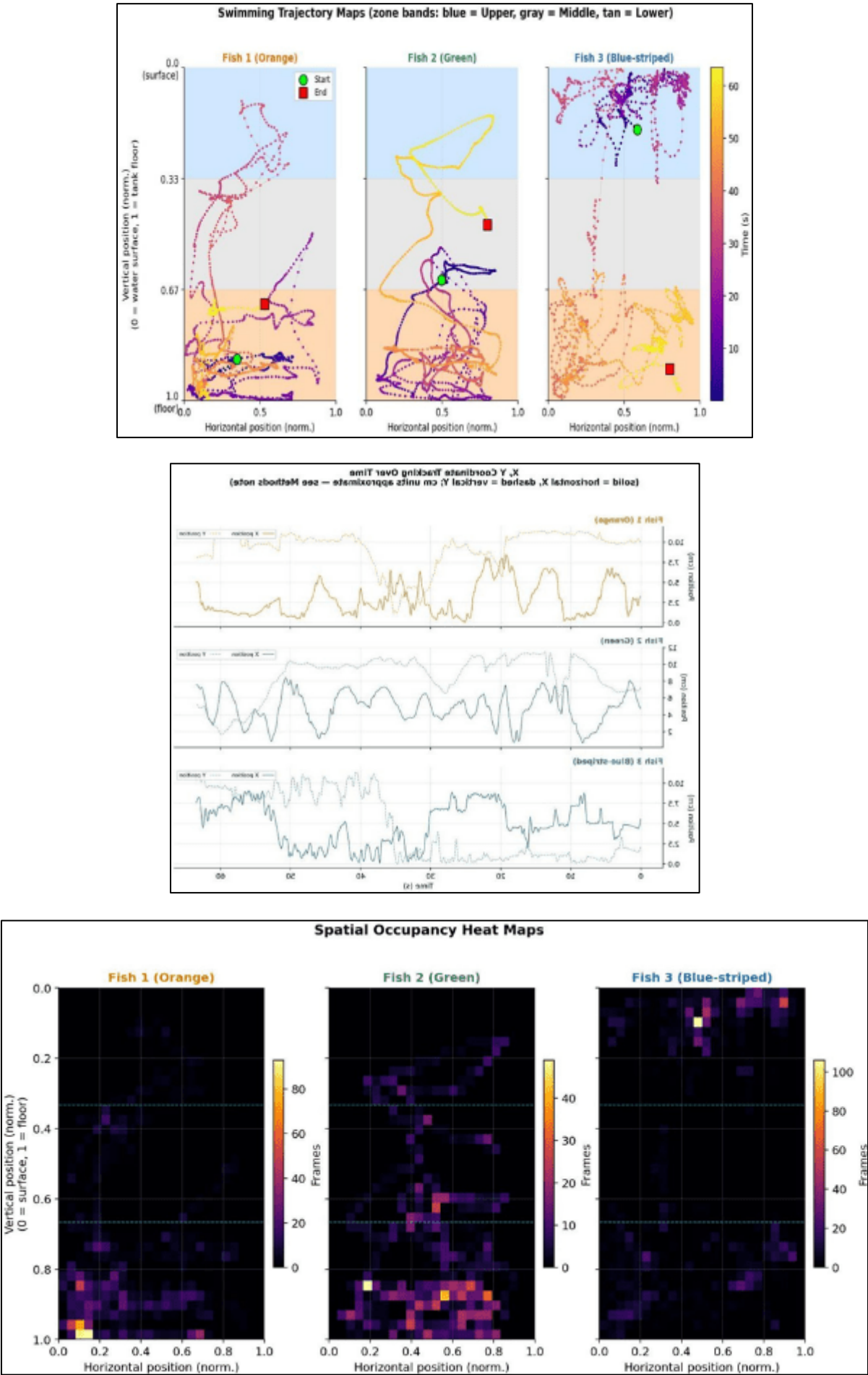
6.4 Interpretation

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.

7 ANALYSIS OF RESULTS

7.1 For cold extract



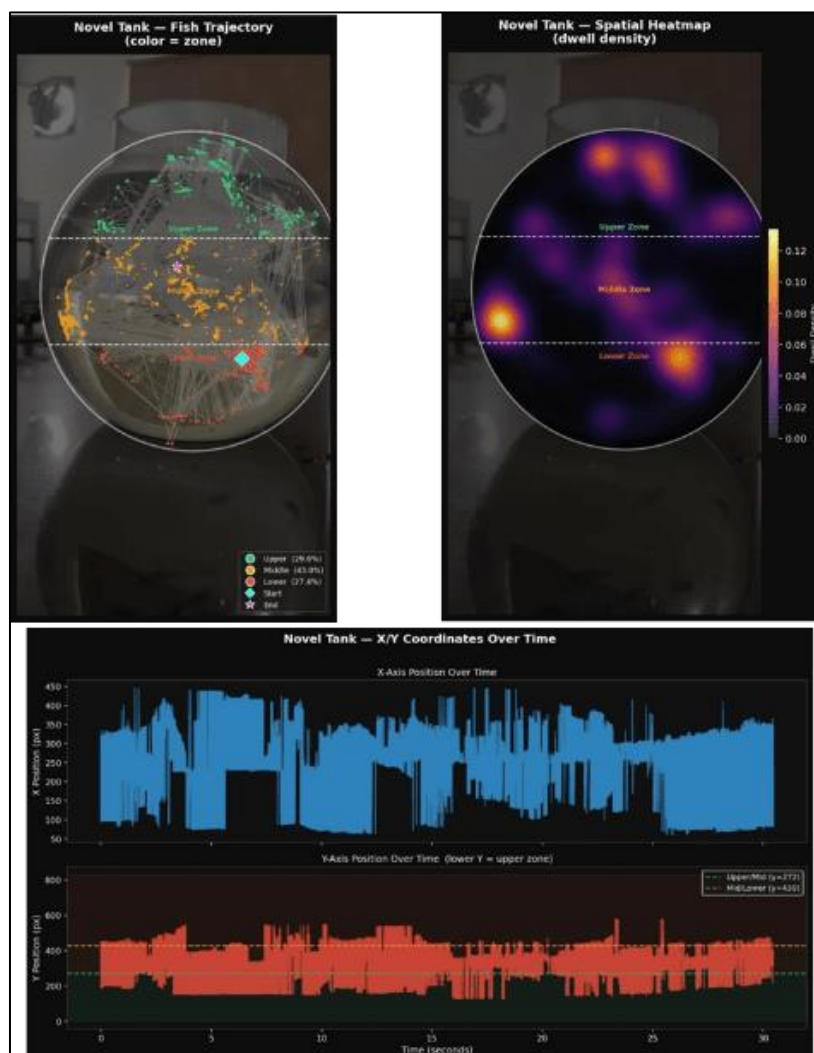


Summary of Novel-Tank Behavioral Metrics (per fish)			
Trial duration: 63.4 s See Methods note on calibration assumptions			
Behavioral Parameter	Fish 1 (Orange)	Fish 2 (Green)	Fish 3 (Blue-striped)
Time in Upper zone (%)	5.1%	9.4%	55.6%
Time in Middle zone (%)	11.6%	20.3%	3.9%
Time in Lower zone (%)	83.4%	70.3%	40.5%
Entries into Upper zone (n)	2	1	1
Entries into Lower zone (n)	3	3	7
Total zone transitions (n)	10	8	16
Latency to first Upper entry (s)	30.8	55.8	0.0
Total distance traveled (cm, approx.)	188.4	164.1	259.6
Average speed (cm/s, approx.)	2.97	2.59	4.09
Max speed (cm/s, approx.)	18.04	15.18	22.35
Freezing duration (s)	3.2	1.2	3.5
Freezing (% of trial)	5.1%	1.8%	5.6%
Freezing bouts (n, >=1s)	2	1	2
Erratic movement events (n)	0	2	0
Mean turn angle (deg/frame)	25.7	16.0	32.9
Mean angular velocity (deg/s)	768	479	982

Figure 8: Results for cold extract

The Novel Tank Test demonstrated differences in anxiety-like behavior among the zebrafish. Fish 1 and Fish 2 spent most of their time in the lower zone (83.4% and 70.3%, respectively), indicating higher anxiety. In contrast, Fish 3 spent 55.6% of the time in the upper zone and only 40.5% in the lower zone, suggesting reduced anxiety. Fish 3 also showed immediate entry into the upper zone, greater swimming distance (259.6 cm), higher average speed (4.09 cm/s), and increased zone transitions (16), indicating enhanced exploratory behavior. The swimming trajectory and heat maps further confirmed increased occupancy of the upper zone following treatment.

7.2 For hot extract



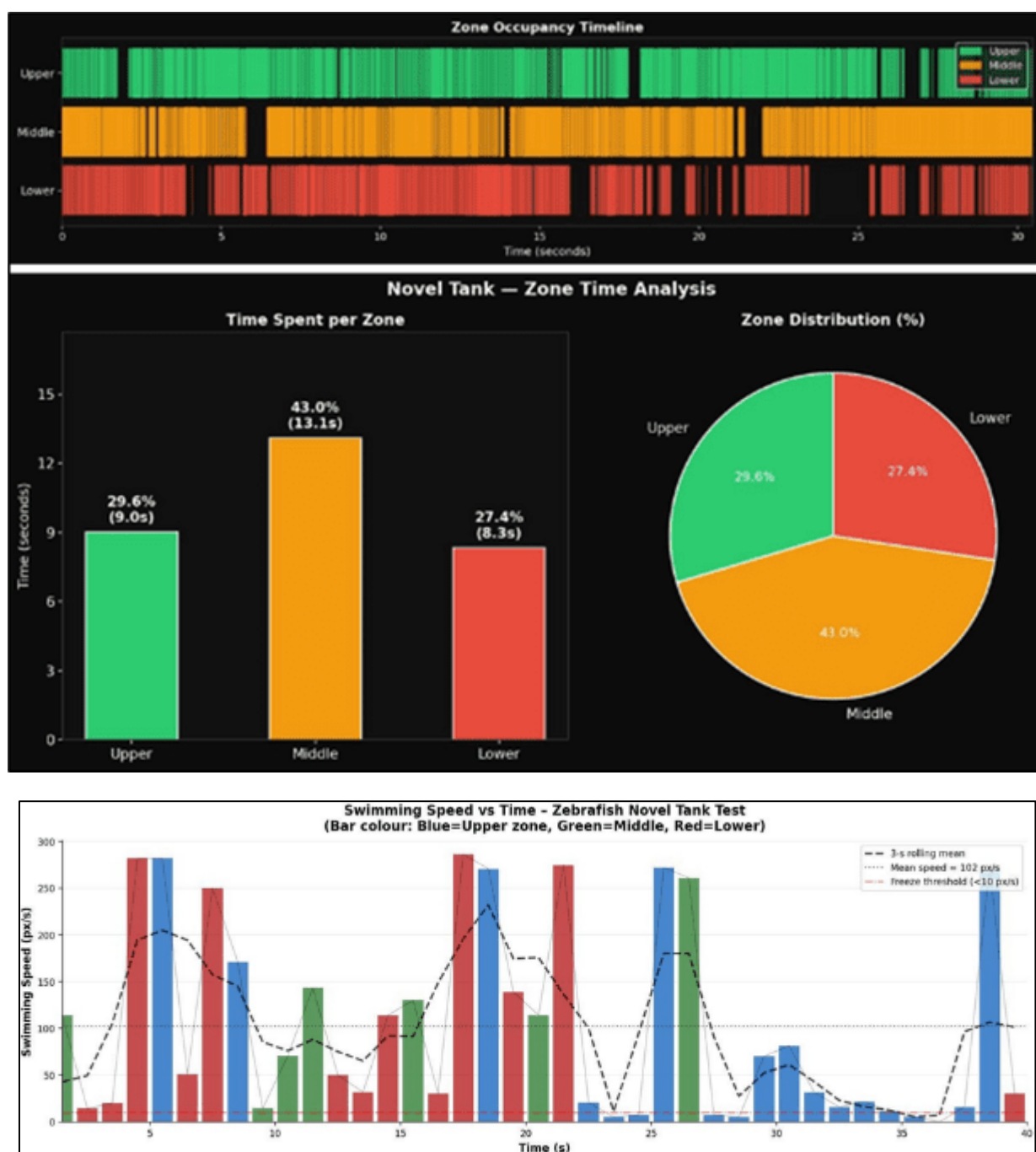


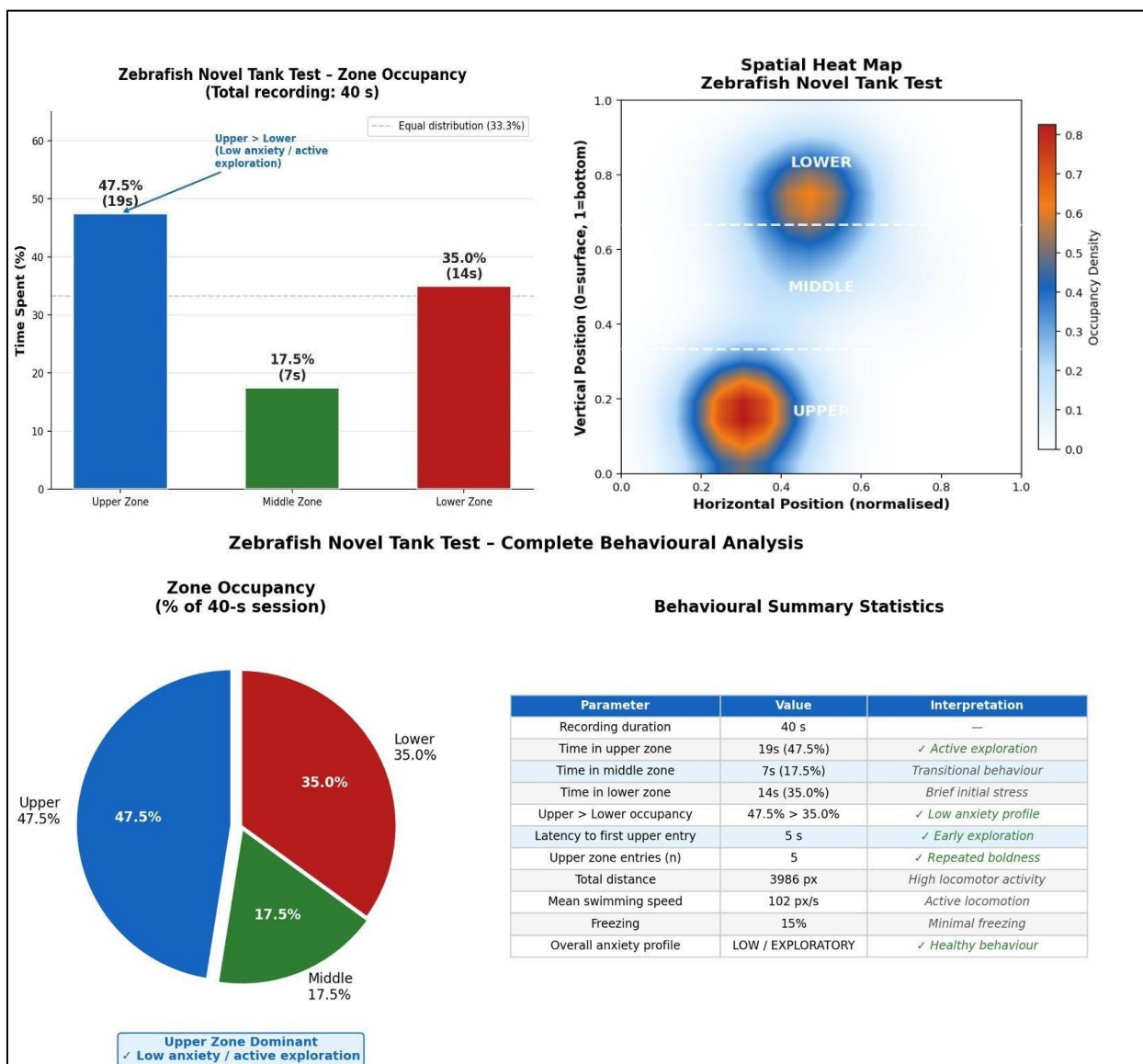
Figure 9: Results for Hot extract

The Novel Tank Test demonstrated that the zebrafish explored all three zones of the tank, with the maximum time spent in the middle zone (43.0%, 13.1 s), followed by the upper zone (29.6%, 9.0 s) and lower zone (27.4%, 8.3 s). The relatively higher occupancy of the upper zone indicates reduced anxiety-like behavior, as zebrafish naturally prefer the bottom of a novel environment when stressed. The trajectory map shows frequent movement throughout the tank with repeated entries into the upper zone, indicating active exploration. The heatmap reveals higher dwell density in the middle and upper regions, confirming that the fish did not remain confined to the lower zone. The zone occupancy timeline also demonstrates regular transitions between zones without prolonged bottom-dwell.

7.3 For ethanolic extract

The Novel Tank Test (40 s) showed that the zebrafish spent the highest time in the upper zone (47.5%, 19 s), followed by the lower zone (35.0%, 14 s) and the middle zone (17.5%, 7 s). Greater occupancy of the upper zone compared to the lower zone indicates reduced anxiety-like behaviour and enhanced exploratory activity. The trajectory map demonstrates

repeated movement between the upper and lower zones, with five entries into the upper zone and the first upper-zone entry occurring within 5 seconds, suggesting rapid adaptation to the novel environment. The heat map shows higher occupancy density in the upper region, confirming sustained exploration of the surface area. The X–Y coordinate and animal location plots further indicate active locomotion with minimal freezing and frequent transitions between zones.



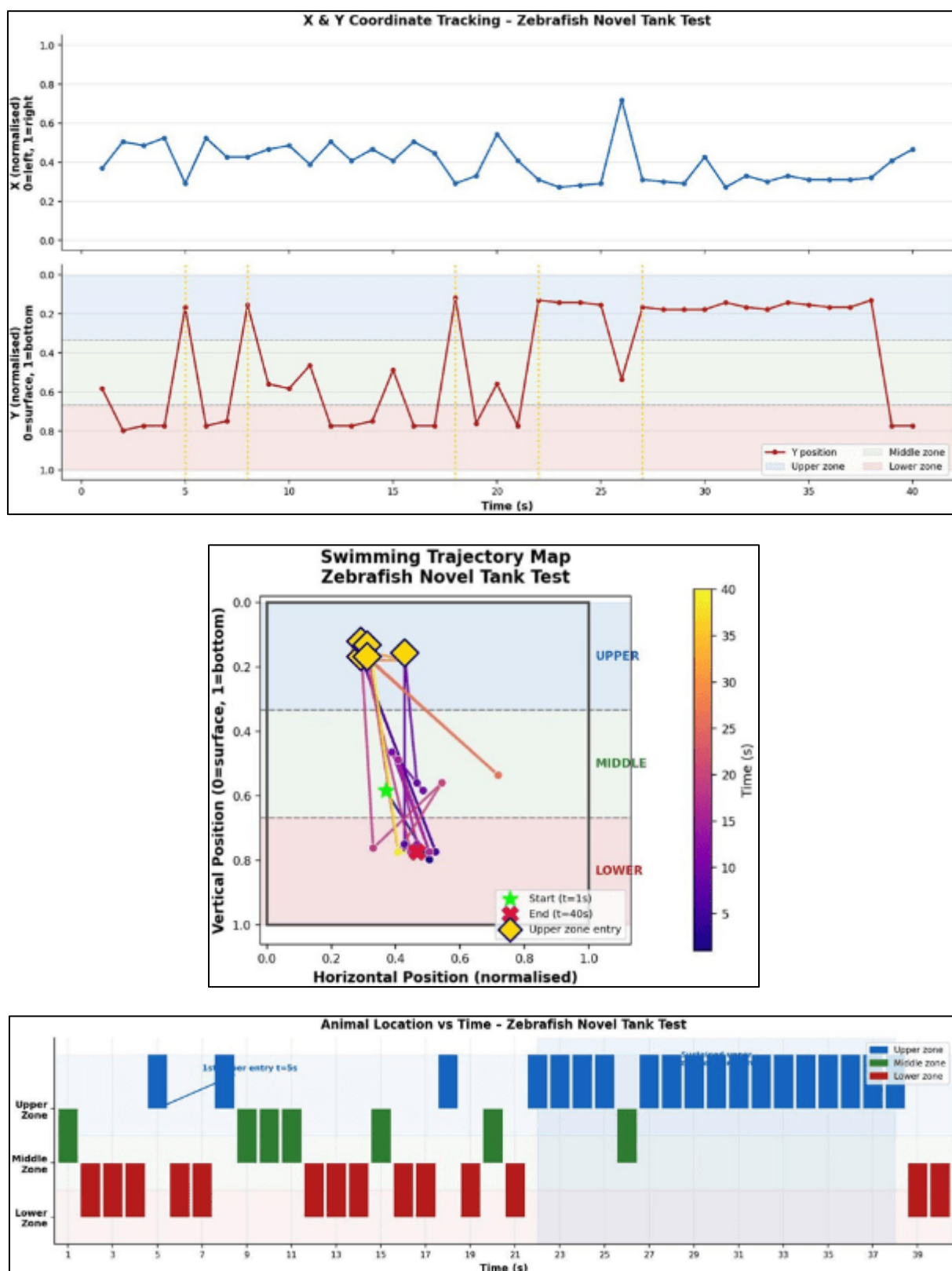


Figure 10: Results for ethanolic extract

8 RESULTS

8.1 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.

8.2 Novel Tank Test:

8.2.1 Before treatment:

- Fish remained mostly in the lower zone.
- Reduced exploration.
- Increased freezing.
- Delayed entry into upper zone.

8.2.2 After treatment:

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

8.2.3 Among the three extracts:

Hydroalcoholic extract > Hot extract > Cold extract in anxiolytic activity.

9 DISCUSSION

The current research highlights that *Stachytarpheta jamaicensis* possesses a variety of bioactive phytochemicals that could have medicinal applications. Specifically, flavonoids and phenolic compounds are recognized for their antioxidant and neuroprotective effects, while alkaloids and terpenoids might play a role in reducing anxiety. The method of ethanolic-aqueous extraction proved to be effective for retrieving both polar and non-polar phytochemicals from the plant material. The observed anti-anxiety effects may stem from the synergistic action of several phytochemical constituents found in the extract. These results lend support to the traditional use of Snakeweed in herbal medicine and suggest its potential for developing anti-anxiety treatments.

10 CONCLUSION

The current research indicates that the ethanolic-aqueous extract of *Stachytarpheta jamaicensis* contains a range of significant phytochemical constituents, such as alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and steroids. Notably, the extract demonstrated potential anti-anxiety activity, suggesting it could be a viable natural source for herbal anxiolytic agents. However, additional pharmacological and clinical investigations are necessary to validate its therapeutic efficacy and safety.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest regarding this manuscript.

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