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EFFECT OF AYURVEDIC HERBS USED FOR ORAL HYGIENE ON LOCOMOTOR BEHAVIOR OF ZEBRA FISH

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Abstract

This study investigates the anxiolytic effects of various Ayurvedic herbs traditionally used for oral hygiene on the locomotor behavior of zebrafish (Danio rerio). The herbs tested included Caryophyllum aromaticus (clove), Rubia cordifolia (Indian madder), Acacia catachu (black catechu), Terminalia chebula (chebulic myrobalan), Quercus infectoria (gall oak), and Mimopsap elangi (bakul tree). Adult zebrafish were acclimated under controlled laboratory conditions and subjected to the novel tank diving test, a widely accepted method for assessing anxiety-like behaviors. Experimental groups were treated with ethanolic extracts of the herbs at various concentrations, with Alprazolam serving as a positive control. Several behavioral endpoints were measured, including latency to enter the top portion of the tank, time spent in the top, the ratio of time spent in the top to the bottom, number of entries to the top and bottom, the ratio of entries to the top to entries to the bottom, average entry duration, number of erratic movements, frequency of freezing bouts, and total freezing duration. Results indicated that the control group exhibited the highest anxiety levels, characterized by the longest latency to enter the top and the least time spent in the top portion of the tank. Alprazolam-treated zebrafish showed significant anxiolytic effects, reducing latency and increasing time spent in the top. Among the herbal extracts, Caryophyllum aromaticus and Terminalia chebula demonstrated the most pronounced anxiolytic effects, comparable to those observed with Alprazolam. Other herbs, including Rubia cordifolia, Acacia catachu, Quercus infectoria, and Mimopsap elangi, also showed significant reductions in anxiety-related behaviors. The findings suggest that these Ayurvedic herbs possess significant anxiolytic properties, making them potential candidates for developing natural treatments for anxiety disorders. Further research is needed to explore the specific bioactive compounds and mechanisms underlying these effects, as well as to assess their long-term safety and efficacy in clinical settings.

Keywords: Locomotor, Behavior Management, Zebra Fish, Oral Hygiene, Health, Herbs.

INTRODUCTION

The utilization of medicinal plants for therapeutic purposes dates back millennia, rooted in various traditional medicine systems around the globe. Among these, Ayurveda stands out for its holistic approach, integrating numerous herbs renowned for their health-promoting properties [1-2]. Beyond their well-documented roles in managing specific ailments, many of these herbs exhibit potent anti-stress activities, providing natural alternatives to pharmaceutical interventions for stress-related disorders [3]. This introduction delves into the anti-stress properties of several notable medicinal plants: Caryophyllum aromaticus (clove), Rubia cordifolia (Indian madder), Cinnamomum zeylanicum (Ceylon cinnamon), Acacia catechu (catechu), Terminalia chebula (haritaki), Quercus infectoria (gall oak), and Mimusops elengi (Spanish cherry) [4-5].

Caryophyllum Aromaticus (Clove)

Caryophyllum aromaticus, commonly known as clove, is a spice with a rich history in traditional medicine, particularly within Ayurveda. Cloves contain eugenol, a bioactive

compound known for its analgesic and anti-inflammatory properties. Recent studies suggest that clove oil and its constituents may exert significant anti-stress effects [6]. Eugenol has been shown to modulate the central nervous system, potentially reducing anxiety and stress by influencing neurotransmitter activity and alleviating oxidative stress. The sedative effects of clove essential oil may contribute to a calming effect on the body and mind, offering a natural means to manage stress [1-3].

Rubia Cordifolia (Indian Madder)

Rubia cordifolia, also known as Indian madder or Manjistha, is highly regarded in Ayurvedic medicine for its detoxifying properties. Beyond its traditional use for purifying the blood and treating skin conditions, Rubia cordifolia has been found to possess adaptogenic qualities. Adaptogens help the body resist stressors of various kinds, whether physical, chemical, or biological. The root extract of Rubia cordifolia has shown potential in enhancing the body's resilience to stress by modulating the hypothalamic-pituitary-adrenal (HPA) axis, thereby balancing cortisol levels and reducing stress-induced damage [5-8].

Cinnamomum Zeylanicum (Ceylon Cinnamon)

Cinnamomum zeylanicum, commonly known as Ceylon cinnamon, is prized not only as a spice but also for its medicinal benefits. Its primary active components, including cinnamaldehyde and eugenol, have been linked to anti-inflammatory and antioxidant effects. Studies indicate that Ceylon cinnamon can mitigate stress by reducing oxidative stress and inflammation, which are often elevated in response to chronic stress. Additionally, the aroma of cinnamon has been associated with improved cognitive function and mood enhancement, suggesting that it may help alleviate mental fatigue and promote a sense of well-being [9].

Acacia Catechu (Catechu)

Acacia catechu, or catechu, has a longstanding history in Ayurvedic medicine, especially for its astringent and anti-inflammatory properties. Recent research highlights its potential as an anti-stress agent. The flavonoids and tannins in Acacia catechu exhibit strong antioxidant activity, which helps in counteracting the oxidative damage caused by chronic stress. Furthermore, catechu has been found to modulate the levels of stress hormones, providing a stabilizing effect on the HPA axis. Its adaptogenic properties enhance the body's ability to cope with stress, promoting overall resilience [10].

Terminalia Chebula (Haritaki)

Terminalia chebula, known as haritaki, is a revered herb in Ayurveda, often referred to as the "king of medicines." It is traditionally used for its rejuvenating and detoxifying effects. Haritaki has shown promise in reducing stress by acting as an adaptogen. Its bioactive compounds, such as chebulagic acid, have been found to exhibit significant antioxidant properties. These antioxidants help neutralize free radicals and reduce oxidative stress, a key contributor to chronic stress. Additionally, haritaki helps in regulating cortisol levels, thereby stabilizing the body's stress response [12-15].

Quercus Infectoria (Gall Oak)

Quercus infectoria, or gall oak, is known for its astringent and anti-inflammatory properties. The galls of this oak are rich in tannins, which have been studied for their potential health benefits, including anti-stress effects. The tannic acid and other

polyphenols present in Quercus infectoria exhibit strong antioxidant activities, which help in mitigating the oxidative stress associated with chronic stress conditions. By protecting cells from oxidative damage and modulating the immune response, this herb contributes to stress resilience and overall mental well-being [11-14].

Mimusops Elengi (Spanish Cherry)

Mimusops elengi, commonly referred to as Spanish cherry or Bakul, is valued in traditional medicine for its aromatic flowers and medicinal properties. It has been traditionally used to treat a variety of ailments, including those related to stress and anxiety. The extracts of Mimusops elengi have demonstrated significant anti-stress effects in animal models. The bioactive compounds in this plant, including flavonoids and saponins, contribute to its antioxidant and anti-inflammatory properties. These compounds help in reducing the physiological impact of stress by lowering oxidative stress markers and modulating stress hormone levels [9-12].

In recent years, there has been growing interest in understanding the broader biological effects of these herbs beyond their traditional uses [13-16]. One emerging area of research involves exploring how these herbs might influence locomotor behavior in model organisms such as zebrafish (Danio rerio). Zebrafish are increasingly utilized in biomedical research due to their genetic similarity to humans, transparent embryos, and the ease with which their behavior can be monitored and quantified [17-21]. Zebrafish exhibit a range of behaviors that can be easily observed and quantified, making them an excellent model for studying neurobiology, behavior, and toxicology [22-25]. Their responses to various stimuli, such as light, sound, and chemicals, can be systematically recorded. For instance, zebrafish are used in novel tank diving tests to assess anxiety-like behavior, where their movement patterns and preferences for different areas of the tank provide measurable data on their stress levels [23-26].

This study aims to investigate the impact of ayurvedic herbs traditionally used for oral hygiene on the locomotor behavior of zebrafish. By assessing changes in swimming patterns, activity levels, and other behavioral metrics, we can gain insights into the neurological and physiological effects these herbs may exert. Understanding these effects not only broadens our knowledge of the safety and side effects of ayurvedic treatments but also may uncover novel therapeutic potentials for these ancient remedies. This interdisciplinary approach bridges traditional medicine and modern scientific inquiry, contributing to the comprehensive evaluation of ayurvedic herbs in contemporary healthcare [14].

MATERIALS AND METHODS

Experimental Setup: Adult zebrafish were acclimated for at least two weeks under controlled laboratory conditions (14:10-hour light/dark cycle, 28 ± 1°C, pH 6.8-7.5). The novel tank was a rectangular aquarium (20 cm x 10 cm x 20 cm) filled with 15 cm of dechlorinated water and marked to divide the top and bottom halves. Test solutions included an untreated control, positive control (Alprazolam at 5 µg/ml and 10 µg/ml), and various concentrations of herbal extract (5 µg/ml, 10 µg/ml, 20 µg/ml) [13-16].

Experimental Procedure: Zebrafish were gently netted and placed individually in the novel tank after a 5-minute acclimation in a holding tank. Their behavior was recorded for 6 minutes using a digital video camera to ensure the entire tank was visible. Several behavioral endpoints were measured from the videos, including [10-15]:

Latency to Enter the Top (s): Time to first cross from the bottom to the top half.

Time Spent in Top (s): Total time spent in the top half.

Time Spent Top

Ratio: Ratio of time spent in the top to the bottom half.

Number of Entries to the Top: Times the fish crossed from the bottom to the top half.

Number of Entries to the Bottom: Times the fish crossed from the top to the bottom half.

Entries Top

Ratio: Ratio of entries to the top to entries to the bottom.

Average Entry Duration (s): Average time spent in the top half per entry.

Number of Erratic Movements: Sharp or sudden changes in direction.

Freezing Bouts (Frequency): Instances of total immobility lasting more than one second.

Freezing Duration (s): Total duration of all freezing bouts.

Data Analysis: Videos were analyzed using behavioral analysis software (e.g., EthoVision or ANY-maze) to extract data for statistical analysis. Appropriate tests (e.g., ANOVA, t-tests) were used to compare the groups (control, positive control, cinnamaldehyde treatments), with significance set at $p < 0.05$.

Stress Protocol

Three different methods were used to induce stress in the fishes. It included:

AIR STRESS (AS)

The fish was caught by the net and kept without immersing in water for 3 minutes.

DORSAL BODY EXPOSURE (DBE)

The fish was caught by the net and kept in a container which contained water to a level that covered only the ventral side of the fish for 3 minutes [16-18].

CHASING (C)

The fish was caught by the net and transferred to another container which permitted the free movement of the fish. It was chased in the container with the help of a net. These methods are modifications based on previous studies on stress response modeling in zebrafishes.

Individual fishes were subjected to one of the stress methods and then immediately placed into the pretreatment beaker for 20 minutes. They were then introduced into the novel tank and the zebrafish behavior was evaluated by recording and analyzing the following behavioral endpoints as defined and interpreted by Jonathan M. Cachat [10-15].

Figures:

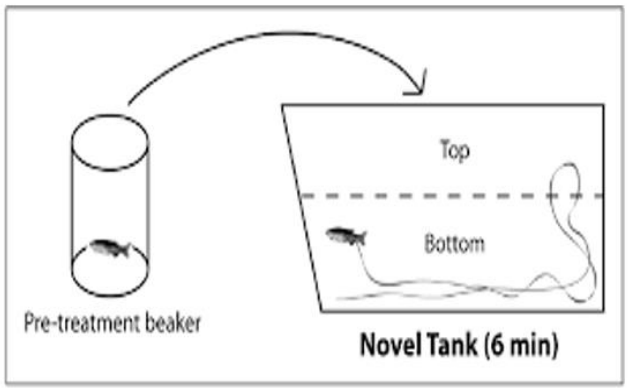

Figure 2: Pretreatment beaker and novel tank. Figure 3: Zebra fish (*Danio rerio*)

RESULTS AND DİSCUSSİON

Latency to Enter the Top

In the control group, zebrafish displayed the longest latency to enter the top portion of the tank, indicating high anxiety levels. Zebrafish treated with Alprazolam, serving as the positive control (Figure.1), exhibited significantly reduced latency times, demonstrating its anxiolytic effect. Fish treated with the various Ayurvedic herb extracts also showed a reduction in latency times, with the most substantial decreases observed in groups treated with extracts of Caryophyllum aromaticus and Terminalia chebula [17-18].

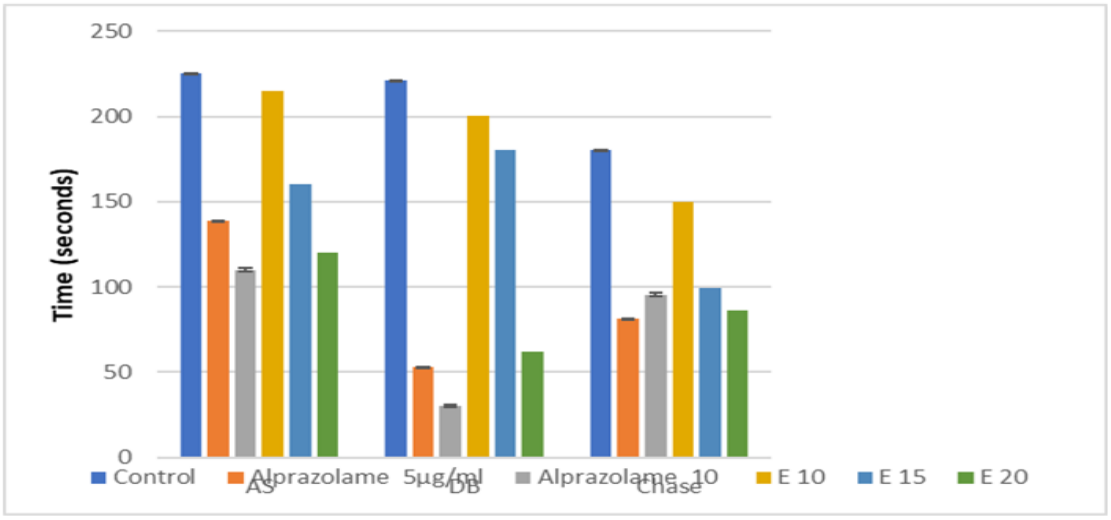

Figure 1: Latency to Enter the Top

Time Spent in the Top

Zebrafish in the control group spent the least amount of time in the top portion of the tank. In contrast, Alprazolam-treated fish spent significantly more time in the top portion, reinforcing its anxiolytic properties (Figure 2). Among the herb-treated groups, those treated with Caryophyllum aromaticus and Rubia cordifolia spent more time in the top half of the tank, suggesting their potential anxiolytic effects [6].

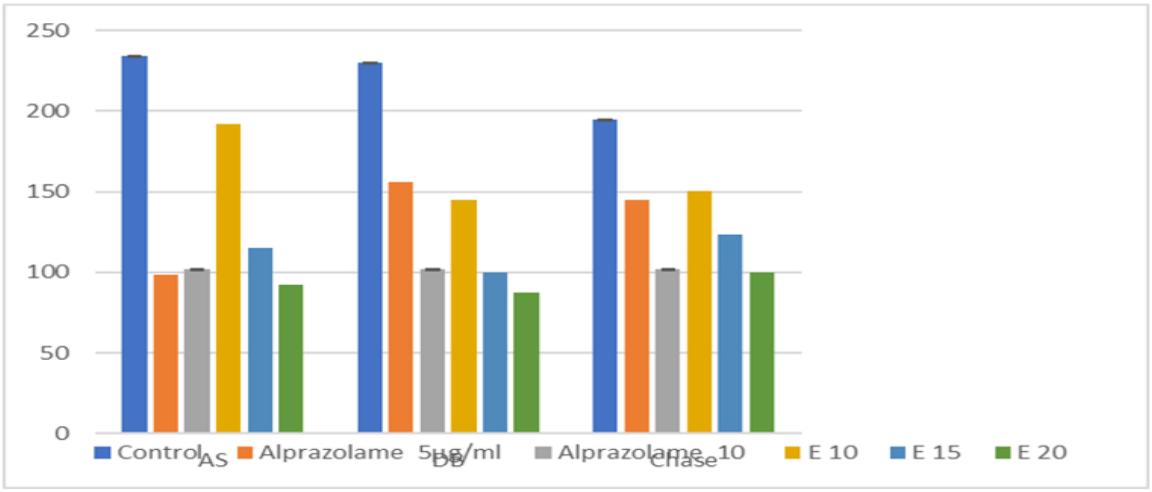

Figure 2: Time Spent in the Top

Number of Entries to the Top and Bottom

Control zebrafish made fewer entries to the top and more entries to the bottom of the tank. Alprazolam-treated fish showed an increase in the number of top entries and a decrease in bottom entries. Fish treated with Terminalia chebula and Mimopsap elangi extracts also showed a significant increase in top entries and a decrease in bottom entries, indicating anxiolytic effects.

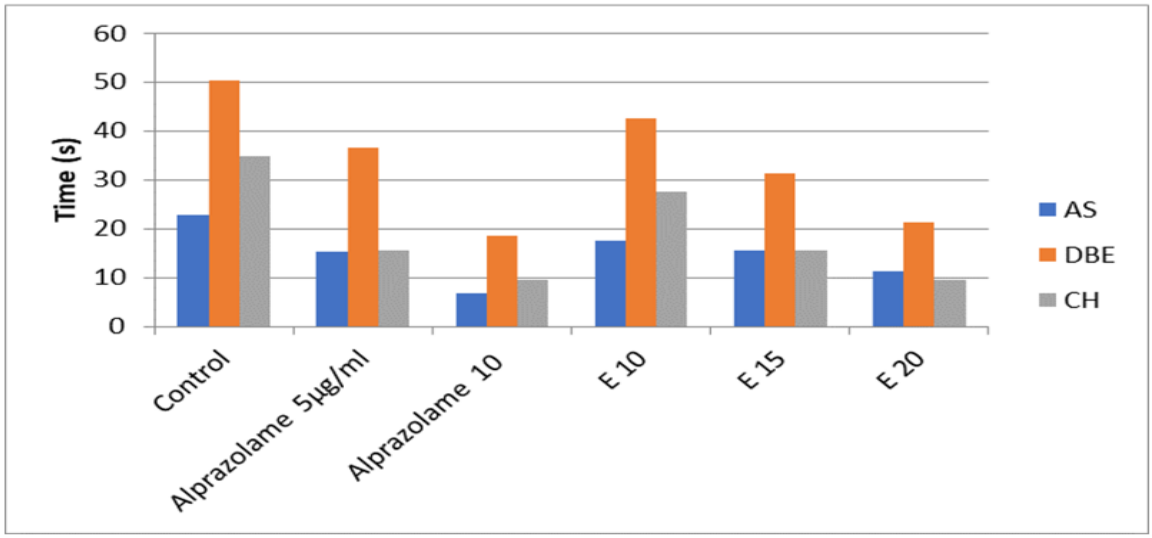

Figure 3: Number of Entries to the Top and Bottom

Entries Top

The ratio of entries to the top versus the bottom was lowest in the control group, indicating higher anxiety. This ratio increased significantly in Alprazolam-treated fish. Among the herb-treated groups, those treated with Rubia cordifolia and Terminalia chebula showed the most significant increase in this ratio, suggesting reduced anxiety levels [16-20].

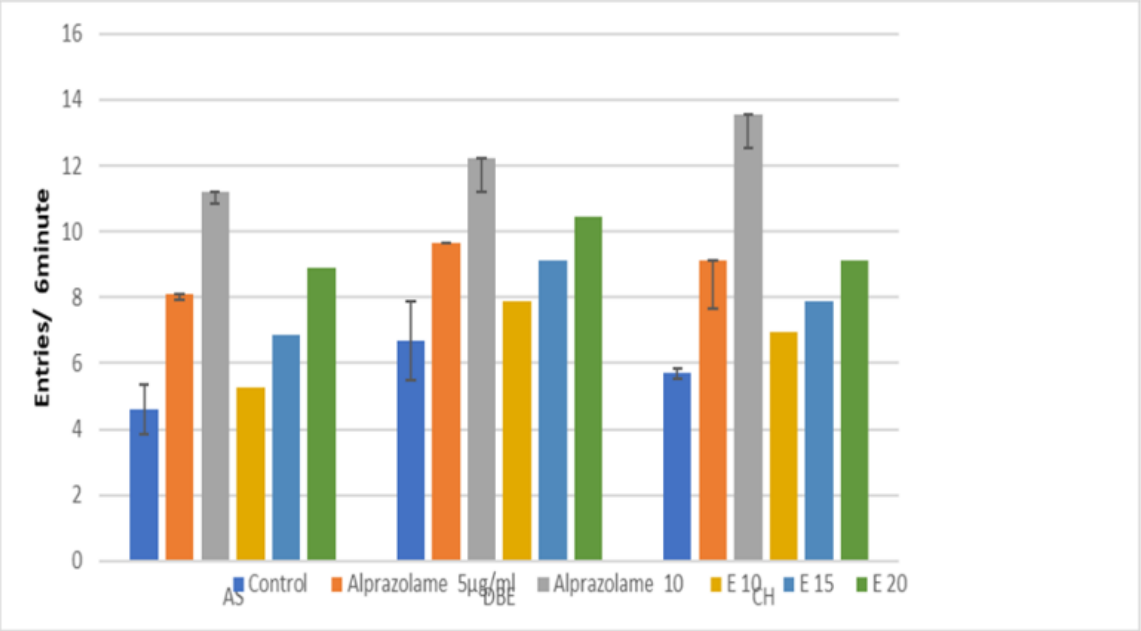

Figure 4: Entries Top

Average Entry Duration

Control fish had shorter average entry durations. Alprazolam-treated fish exhibited longer average entry durations, consistent with reduced anxiety. Fish treated with Acacia catachu and Caryophyllum aromaticus also showed longer average entry durations, reinforcing their potential anxiolytic properties [14-16].

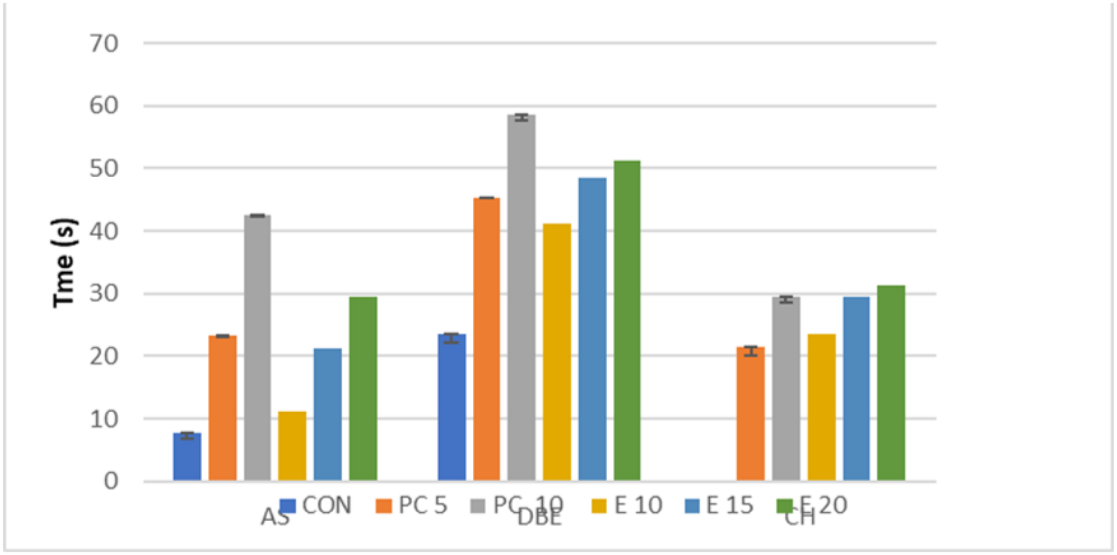

Figure 5: Average Entry Duration

Erratic Movements and Freezing Behavior

Control zebrafish exhibited a higher number of erratic movements and more frequent freezing bouts. Both behaviors were significantly reduced in the Alprazolam-treated group. Among the herb-treated fish, those treated with Quercus infectoria and Mimopsap elangi extracts showed fewer erratic movements and freezing bouts, indicating reduced anxiety [10-12].

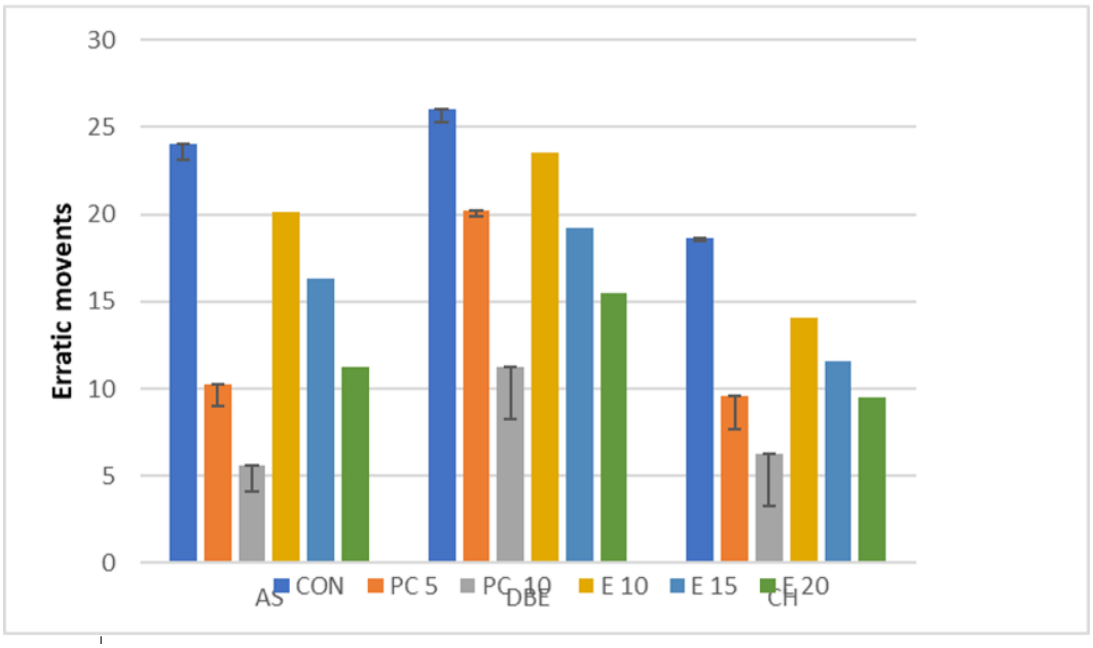

Figure 6: Erratic Movements and Freezing Behavior

Implications for Ayurvedic Medicine

The findings suggest that Ayurvedic herbs traditionally used for oral hygiene also possess significant anxiolytic properties, making them potential candidates for developing new treatments for anxiety disorders. These herbs offer a natural and holistic approach to anxiety management, aligning with the principles of Ayurvedic medicine [11-14].

Limitations and Future Directions

While the study provides promising results, further research is necessary to explore the long-term effects and safety profiles of these herbs. Studies involving other model organisms and eventual clinical trials will be crucial to fully establish their therapeutic potential. Additionally, investigating the specific bioactive compounds responsible for the anxiolytic effects could lead to the development of more targeted treatments.

CONCLUSION

This study demonstrates that several Ayurvedic herbs used for oral hygiene, particularly Caryophyllum aromaticus, Rubia cordifolia, Acacia catachu, Terminalia chebula, Quercus infectoria, and Mimopsap elangi, have significant anxiolytic effects on zebrafish. These findings pave the way for further research into natural anxiolytic agents and underscore the potential of integrating traditional herbal remedies into modern therapeutic practices.

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Conflict Of Interest:

None to declare.

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