



Evaluation of anti-anxiety activity of *Pothos scandens* using zebra fish (*Danio Rerio*) tail fin and caudal fin model

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Abstract

Pothos scandens belongs to family Araceae, and it is a medicinal climbing plant. It has been traditionally used for treating various health conditions, including inflammation, wounds, convulsions, and nervous disorders. The potential role of *Pothos scandens* in reducing the anxiety has not been studied. Therefore, the present study was conducted to evaluate the anti-anxiety activity of *Pothos scandens* using its leaves extracts performing on zebrafish and Wistar rat models.

The leaves were collected, shade-dried, powdered, and extracted. Qualitative phytochemical evaluation showed the presence of chemical constituents such as flavonoids, tannins, phenolic compounds, steroids, and terpenoids. These compounds consist various pharmacological properties and they may posses' effect on the nervous system.

The anti-anxiety activity was analysed by observing behavioral changes such as swimming pattern, freezing behavior, locomotor activity, and time spent in different zones. Animals are ingested with the extracts indicated anti-anxiety-like responses compared with the control group. Among all the extracts ingested, the alcoholic extract produced the most significant anti-anxiety effect and showed activity comparable to the standard drug diazepam.

The results indicating that *Pothos scandens* exhibiting potential anti-anxiety properties and supports its traditional medicinal use. Further studies are required to identify the active compounds responsible for this activity and to understand their mechanism of action. The results indicate that the *Pothos scandens* may be a potential natural source for the development of safer herbal medicines for anxiety management.

Keywords: Anxiety, *Pothos scandens*, anxiolytic activity, herbal medicine, zebra fish, wistar rats

Introduction

Anxiety is a common mental health disorder which occurs when a person experiences stress and fear. Nowadays anxiety disorders are the most common mental health problems worldwide affecting people of all ages. Various medicines such as benzodiazepines and antidepressants are commonly prescribed to manage anxiety disorders. Although these drugs are effective, their prolonged use may cause several unwanted side effects, which includes drowsiness, dizziness, memory problems, dependence, and withdrawal symptoms^[1].

For ages, medicinal plants have played a vital role in healthcare systems around the world. Many plants contain biologically active compounds that can influence the central nervous system and help manage conditions such as stress, anxiety, insomnia, and depression. Scientifically the plant-derived compounds, particularly flavonoids and phenolic substances, can interact with neurotransmitters involved in regulating mood and emotional behavior^[2].

Pothos scandens is a medicinal climbing plant belonging to the family Araceae. It is widely distributed in tropical regions of Asia and Madagascar and has been used in traditional medicine for many years. Different parts of the plant are employed for the treatment of wounds, inflammation, fractures, convulsions, diarrhea, and various skin disorders. In several traditional systems of medicine, the plant is also used for conditions related to the nervous system, suggesting that it may possess neuroprotective or calming properties^[3].

Previous studies on *Pothos scandens* have reported a variety of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, wound-healing, and antipyretic effects. These activities are mainly attributed to the presence

of important phytochemicals such as flavonoids, tannins, phenolic compounds, terpenoids, and steroids. Many of these compounds have been shown to possess neuroprotective and anxiolytic properties in experimental studies. Since oxidative stress and inflammation are known to contribute to the development of anxiety and other neurological disorders, the medicinal properties of *Pothos scandens* suggest that it may have beneficial effects on anxiety-related conditions^[4].

Despite its traditional use and reported biological activities, very little scientific information is available regarding the anti-anxiety potential of *Pothos scandens*. Therefore, it is important to investigate its effects using suitable experimental models. In recent years, zebrafish and Wistar rats have become valuable animal models for studying anxiety-related behavior because their responses closely resemble several aspects of human anxiety. These models provide reliable and reproducible methods for evaluating the anxiolytic activity of medicinal plants and natural products^[5].

Hence, the present study was undertaken to evaluate the anti-anxiety activity of different extracts of *Pothos scandens* leaves using zebrafish and Wistar rat models. The study also aimed to identify the major phytochemical constituents present in the extracts and assess their possible role in reducing anxiety-like behavior. The findings may provide scientific evidence for the traditional use of the plant and contribute to the development of safer herbal medicines for the management of anxiety disorders^[6].

Pothos scandens Linn., belongs to the family Araceae, it is a climbing plant and used traditionally to treat various health problems like Swelling, wounds, inflammation and skin conditions. It contains various phytochemical compounds

like Alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds. *Pothos scandens* is an evergreen climbing shrub which contains green stems that grow by the support of aerial roots. The leaves are simple, broad, and heart-shaped with a smooth surface. They are dark green in color and leathery in texture with an entire margin and pointed apex. The plant produces small flowers arranged in a spadix type of inflorescence surrounded by a spathe, which is a characteristic feature of the Araceae family. The fruits are small berry-like structures containing seeds [7].

Materials and Methods

1. Plant



Fig 1: *Pothos scandens*

Chemicals

Ethanol, Distilled water, Acetic acid, Clove oil, Ferric chloride, Hydrochloric acid, Sulphuric acid, Mayer's reagent.

Standard drug

Diazepam

Animals

Zebra fishes

Zebra fish were purchased from Aquarium shop and were maintained under standard environmental conditions (water temperatures of 26–28°C).



Fig 2: Zebra fishes

Plant Material Collecting and drying

Fresh leaves of *Pothos scandens* were collected, kept for drying under shade for 12 to 15 days. After drying, the dried leaves were powdered.



Fig 3: Shade drying

Preparation of Extracts

a. Cold Maceration

1g of powdered *Pothos scandens* leaves were taken in a beaker at ratio 1:10, add 10ml distilled water and kept aside for 24-48 hours, stir occasionally. Then filter the extract and store in clean & air tight container.

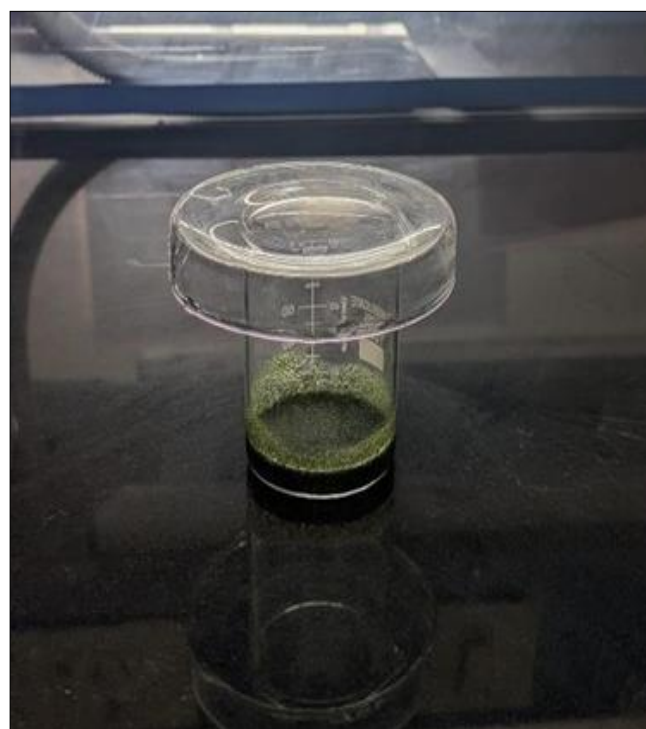


Fig 4: Cold maceration

b. Hot maceration

1g of powdered *Pothos scandens* leaves were taken in a beaker at ratio 1:10, add 10ml distilled water and boiled for 30-60 mins. Then allowed to cool and filter the extract and store in clean & airtight container.



Fig-5: Hot maceration

c. Soxhlet Extraction

About 15-20g of the powdered material was packed into a thimble and placed in a Soxhlet apparatus. Extraction was carried out using 150-250ml of ethanol as the solvent for 24-48 hours 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.



Fig 6: Soxhlet extraction of *Pothos scandens* leaves powder

Qualitative Phytochemical Screening

The *P. scandens* L. extracts were subjected for phytochemical tests to find the presence of major phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, steroids and terpenoids according to standard methods.

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

Preparation

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.

Animals Grouping

Fish were divided into 4 groups:

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

Test groups (cold, hot extract and ethanol doses)



Fig 7: Zebra fishes under test

Experimental Protocol

Zebra fishes

Grouping of Animals

Zebra fishes were divided into 5 groups Group 1: Normal water only

Group 2 (standard): Diazepam

Group 3 (Cold extract): Extract (1ml/L)

Group 4 (Hot extract): Extract (1ml/L)

Group 5 (Ethanol extract): Extract (1ml/L)

Predatory Avoidance Reflex

Parameters Observed

Time spent in upper zone Swimming activity Exploratory behavior Freezing behavior Bottom dwelling

Interpretation

Increased upper-zone exploration = Reduced anxiety

Reduced freezing = Anxiolytic activity

Increased swimming exploration = Anxiolytic activity

Observation

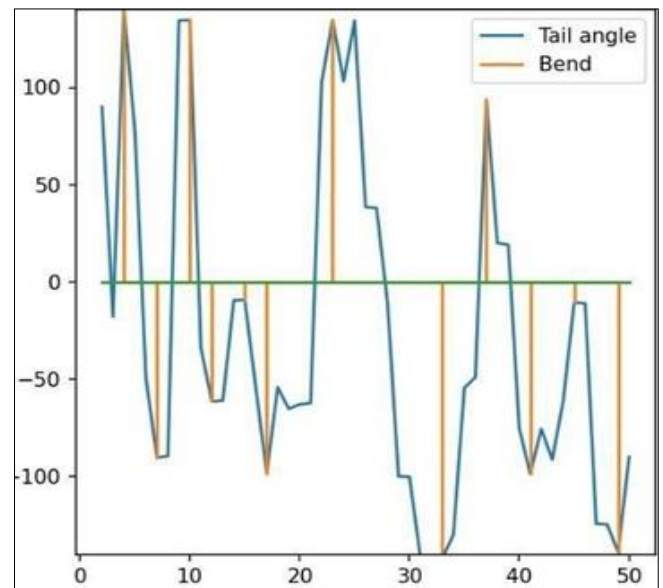


Fig 8: Normal

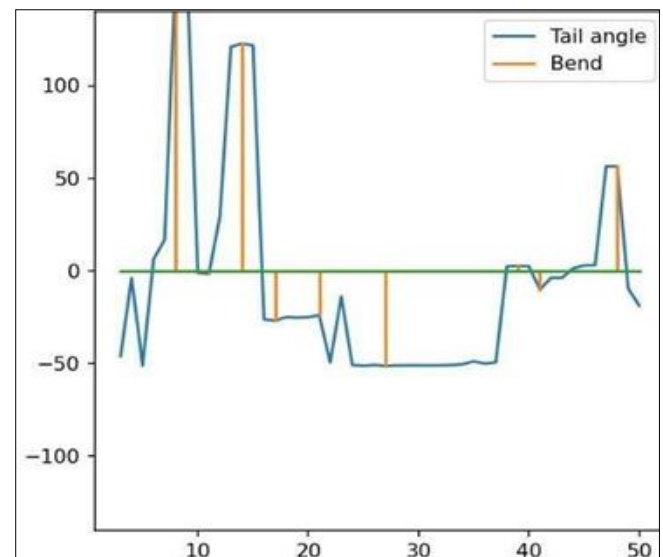


Fig 9: Cold extract

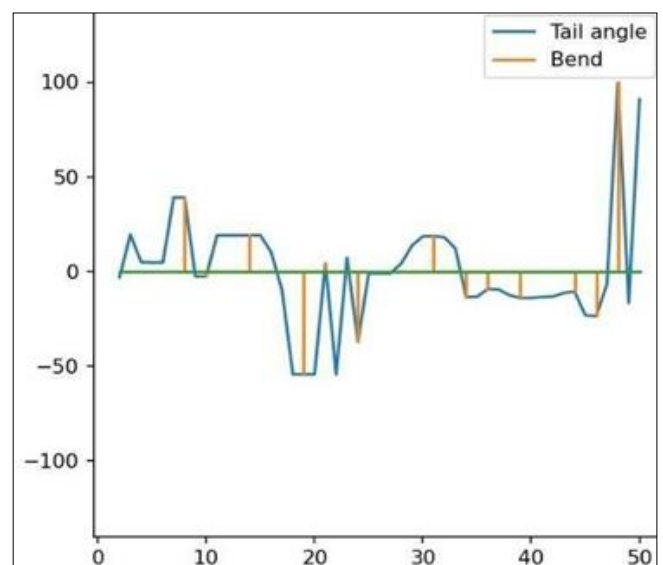


Fig 10: Hot extract

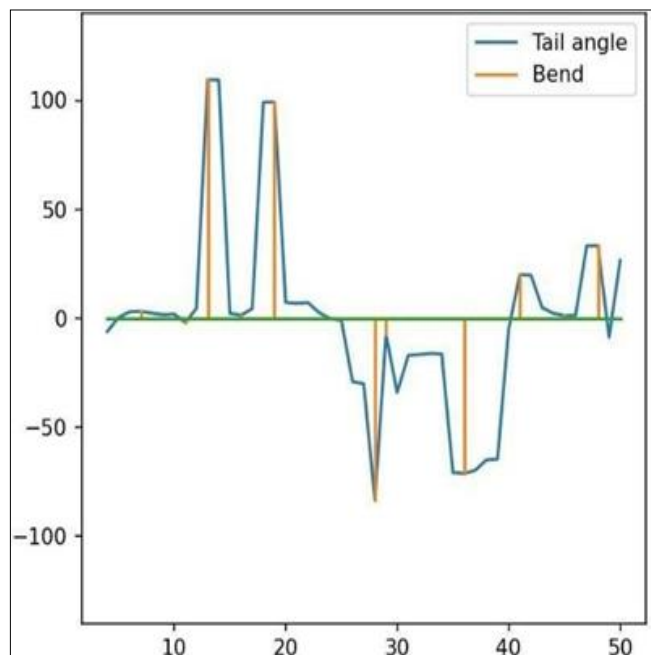


Fig 11: Ethanolic extract

Results

The present study demonstrated significant anti-anxiety activity of *Pothos scandens* extracts in zebra fish. Animals treated with the extracts exhibited increased exploratory behavior, reduced freezing responses, and greater locomotor activity compared with the control group. Among all extracts tested, the ethanolic extract produced the strongest anxiolytic effect and showed results comparable to diazepam. The hot extract demonstrated moderate activity, while the cold extract exhibited mild to moderate effects. The behavioral observations indicate that *Pothos scandens* possesses significant anti-anxiety properties.

Discussion

In this study, the anxiolytic activity of *Pothos scandens* was tested on valid animal models. The behavioral results showed that the extracts possessed anxiolytic activity and facilitated exploratory activity.

Such anxiolytic activity can be ascribed to flavonoids, phenolics, tannins, steroids, and terpenoids found in the plant. These phytochemicals were shown to modulate various neurotransmitters, such as GABAergic and serotonergic ones, playing an essential role in the regulation of anxiety.

The ethanolic extract had better anxiolytic activity than other extracts as it was capable of extracting a variety of biologically active substances from the plant.

These data indicate that *Pothos scandens* affects the pathways of central nervous system that regulate emotional processes.

This study confirms the folk use of this plant for treatment of diseases of the nervous system.

More research is needed to identify and isolate biologically active compounds and understand the mechanism of their action.

Conclusion

This current research has confirmed that there is a very high level of anti-anxiety effects from the *Pothos scandens* on zebrafish model.

From all the extracts used in the study, the ethanolic extract demonstrated the highest level of anxiolytic effect, which is similar to diazepam.

These effects can be attributed to flavonoids, phenolic components, tannins, steroids, and terpenoids in the plant.

It proves the medicinal importance of the *Pothos scandens* and implies that the plant can be used in the formulation of herbal anxiolytics.

References

1. Gupta S, *et al.* Pharmacological and Phytochemical Updates on *Pothos scandens* L. Pharmacognosy Communications, 2018.
2. Kim J, *et al.* *In vitro* anti-inflammatory activity of *Pothos scandens* extract. Food Science & Biotechnology, 2017.
3. Sajeesh T, *et al.* Antioxidant and antipyretic studies on *Pothos scandens*. Asian Pacific Journal of Tropical Medicine, 2011.
4. *Pothos scandens* pharmacological review article. Pharmacognostic and phytopharmacological review of *Pothos scandens* Linn.
5. *In vitro* anti-inflammatory activity of *Pothos scandens* extract in RAW 264.7 cells.
6. A Review on Ethanomedicinal Claims and spread of *Pothos scandens* L.
7. Adongo DW, Benneh CK, Tandoh A, *et al.* Anxiolytic-like effects of *Pseudospondias microcarpa* hydroethanolic leaf extract in zebrafish: Possible involvement of GABAergic and serotonergic pathways. Natural Products and Bioprospecting, 2023;13(33).
8. Khan KM, Collier AD, Meshalkina DA, *et al.* Zebrafish models in neuropsychopharmacology and CNS drug discovery. British Journal of Pharmacology, 2017;174(13):1925–1944.
9. Sajeesh T, Arunachalam K, Parimelazhagan T. Antioxidant and antipyretic studies on *Pothos scandens* L. Asian Pacific Journal of Tropical Medicine, 2011;4(11):889–899.
10. Phenolic constituents of the Bangladeshi medicinal plant *Pothos scandens* and their biological activities. Phytochemistry, 2016;121:30–37.
11. Kokate CK. Practical Pharmacognosy. 5th Edition. Vallabh Prakashan.
12. Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: from tank to bedside. Trends in Neurosciences, 2014.
13. Jethinlalkhosh JP, Antony A, Praveena P, Doss VA. Phytochemical, antioxidant and free radical scavenging activities of hydroethanolic extract of aerial parts of *Pothos scandens* L. Asian Journal of Pharmaceutical and Clinical Research, 2016.
14. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 23rd Edition. Nirali Prakashan, Pune.
15. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th Edition. Nirali Prakashan.
16. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd.
17. The leaves were collected, washed thoroughly and dried under shade for 6-7 days. Then grind the leaves into fine powder. Store in a airtight container.