



(RESEARCH ARTICLE)



EVALUATION OF SEDATIVE ACTIVITY OF *POTHOS SCANDENS* LEAVES USING ZEBRAFISH (*DANIO RERIO*) MODEL

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ABSTRACT

This experiment was performed to evaluate the main potential of sedative activity of money plant on zebrafish model(*danio rerio*), mainly known as *pothos scandens* leaves. *scandens* leaves are the best alternative to improve the usage of traditional medicines because, sedative drugs have been showing their side effects significantly on zebrafish model. And mainly traditional medicines have therapeutic properties. In this experiment leaves are extracted hot, cold and ethanolic and subjected to the animal model. This experiment is done in different intervals with various doses and concentrations. It shows some parameters like bending movements, tail flicking, slowing down of the zebrafish was observed after the treatment with the leaves extracts indicating the reduction of activity. The change in the behaviour of animal model enables us to understand the cns depressing activity when compared with the control and standard values. Due to presence of bioactive compounds, higher doses and ethanolic extracts produced better results. This study provide findings as scientific support for traditional use of these leaves in the safer and efficient use of sedative drugs

Keywords: *Pothos Scandens*, Zebrafish, Tail Flicking, Bending, Cns Depression, Sedation, Therapeutic Activity.

1 INTRODUCTION

Currently, sleep disorders, anxiety, and stress-related conditions are becoming increasingly common worldwide, affecting the physical and mental health of millions of people. Doctors prescribe sedative drugs to promote relaxation, reduce anxiety, and improve sleep quality. Although conventional sedative agents such as benzodiazepines and barbiturates are effective, their prolonged use can lead to adverse effects such as drowsiness, tolerance, dependence, cognitive impairment, and withdrawal symptoms. Because of these side effects, researchers are looking for safer sedative medicines from medicinal plants that have traditionally been used to treat nervous system disorders.

Pothos scandens is an evergreen climber that belongs to the Araceae family. It grows in many tropical and subtropical regions of Asia. Different parts of this plant have been used to treat fever, inflammation, skin diseases, wounds, and various other ailments. Studies have shown that the leaves of this plant contain flavonoids, phenolic compounds, tannins, terpenoids, and steroids. These compounds possess different medicinal properties. However, the

neuropharmacological potential of *Pothos scandens* leaves, especially their sedative activity, has not been widely investigated.

2 PHARMACOGNOSY OF POTHOS SCANDENS LEAF

2.1 Biological Source

Pothos scandens is a herb which belongs to the family Araceae. For a long time now, the leaves of this herb have been used for medical purposes in herbal medicine.

2.2 Leaf Description

They are simple, glossy and dark green in colour. They are either ovate or elongated and possess smooth leaf margins, an acute apex and cordate base. They are firm and leathery and have a distinct reticulate venation. A petiole connects the leaves to the stem. The stem is slender and green in colour and is made possible by the presence of climbing roots.

2.3 Chemical constituents of the leave

The leaves of *Pothos scandens* contain phytoconstituents like flavonoids, tannins, phenols, saponins, steroids and glycosides. They help protect the body, act against any form of pathogenic microbes, are anti-inflammatory agents and also calm the nervous system.

2.4 Zebrafish (Danio rerio)

Zebrafish are tiny freshwater fish and are commonly used for scientific experiments, especially for brain and nervous system research.

2.5 Maintenance:

- Species: Adult zebrafish (*Danio rerio*)
- Light/Dark Cycle: 14 hours light and 10 hours dark

2.6 Appearance and Features

Zebrafish are small fish with silver bodies and blue horizontal stripes, giving them a zebra-like appearance. Their embryos are transparent, allowing scientists to observe organ development easily. They are inexpensive, easy to maintain in laboratories, and reproduce quickly.

3 MATERIALS AND PROCESS OF EXTRACTION

3.1 Plant Material Collection

Fresh leaves of *Pothos scandens* are collected from the healthy plants and authenticated by qualified botanist. And the collected leaves were washed with distilled water thoroughly to remove the dust and foreign materials. After washing, they were shade-dried at room temperature for at least 10 to 15 days until a constant weight was obtained. And after that leaves are coarsely powered by using a mechanical grinder and that powder stored in an airtight container until any use



Figure 1: Extract Preparation

3.2 Cold Water Extraction (Maceration) – Procedure

Collection and Preparation: Fresh *Pothos scandens* leaves were collected. The leaves were washed with water to remove dirt and dust. After washing, they were shade-dried and ground into a coarse powder.

Soaking (Maceration): About 1 g of the powdered leaves was transferred into a clean glass container. Then, 10 mL of distilled water was added (1:10 w/v ratio). The mixture was covered and kept at room temperature for 24–48 hours with occasional stirring so that the plant compounds dissolved properly in the water.

Filtration: After soaking, the mixture was first filtered through muslin cloth and then filtered again using Whatman No. 1 filter paper to obtain a clear extract.

Concentration: The filtrate was heated gently using a heating mantle to remove excess water and obtain a more concentrated extract.

Storage: Finally, the concentrated extract was transferred into a clean, airtight container and stored in a refrigerator at 4°C until it was used for further pharmacological evaluation.



Figure 2: Hot Water Extraction (Decoction) – Procedure

Collection and Preparation: First, *Pothos scandens* leaves were collected and washed with clean water to remove dirt and dust. After washing, the leaves were shade-dried and then ground into a fine powder.

Boiling: About 1 g of the powdered leaves was taken and mixed with 10 mL of distilled water (1:10 w/v ratio). The mixture was boiled gently for 30–60 minutes so that the plant compounds could dissolve into the hot water.

Concentration: After boiling, the extract was concentrated to one-fourth of its original volume by gentle heating.

Cooling and Filtration: The extract was allowed to cool to room temperature and then filtered using Whatman No. 1 filter paper to remove the remaining plant particles and obtain a clear extract.

Storage: Finally, the filtered extract was transferred into a clean, airtight container and stored in a refrigerator at 4°C until it was used for further studies.



Figure 3: Ethanolic Extraction (Soxhlet) – Procedure

Collection and Preparation: First, *Pothos scandens* leaves were collected and washed thoroughly to remove dirt and dust. The leaves were then shade-dried and ground into a coarse powder.

Packing of the Sample: About 20 g of the powdered leaves was taken and placed inside a cellulose thimble. The thimble was then placed in the Soxhlet extraction apparatus.

Extraction: Approximately 200 mL of 95% ethanol was used as the extraction solvent. The extraction was carried out for about 6–8 hours. During the process, the hot ethanol repeatedly passed through the leaf powder and extracted the plant compounds. The process was continued until the solvent in the siphon tube became nearly colourless, indicating that most of the plant compounds had been extracted.



Figure 4: Soxhlet extraction

After the extraction process was completed, the extract was filtered to remove any plant residue. After filtration, the extract was concentrated using a rotary evaporator at a temperature of 40-45°C which helped to evaporate the ethanol without any damage to the phytochemical compounds from the plant. Finally, drying continued until the semisolid extract was achieved.

The extracted sample was then weighed and transferred to an amber airtight container. The extract was kept at the temperature of 4°C in a refrigerator until further analysis and biological screening tests were performed.

In this experiment, ethanol was chosen as a solvent because it is able to dissolve all types of phytochemicals including flavonoids, phenols, alkaloids, glycosides, tannins, terpenoids, and steroids. Its low boiling point allows for easy evaporation of the solvent without damaging any heat-sensitive phytochemicals. Moreover, ethanol is a safe and efficient solvent for making a plant extract for biological study.

Soxhlet extraction method was chosen because it allows solvent to go through the plant materials several times and, thus, it results in higher yields of plant compounds. Extract prepared using this method is ready to use for phytochemical analysis and testing of sedative properties of *Pothos scandens* leaves using zebrafish model.

Table 1: Phytochemical screening

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	Terpenoids

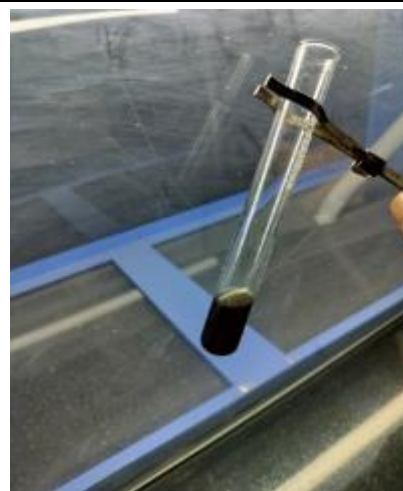
			Yellow-green fluorescence	Steroids
Ferric Chloride Test	Phenols	2 ml extract in a test tube+2-3 drops of 5% Ferric Chloride (FeCl_3) solution	Deep blue / green / violet color	Presence of Phenols
Mayers test	Alkaloids	2 ml extract+2-3 drops of Mayer's reagent	No Cream / pale yellow precipitate	Alkaloids absent
Dragendorffs test	Alkaloids	2 ml extract+2-3 drops of Dragendorff's reagent.	No Orange / reddish-brown precipitate	Mild Alkaloids present.



FLAVONOIDS



CONDENSED TANNINS



GLYCOSIDES



TERPENOIDS



PHENOLS



MAYERS TEST



DRAGENDROFF TEST

Figure 5: Phytochemical evaluation

4 METHODOLOGY - ADMINISTRATION OF EACH EXTRACTS

4.1 Preparation of Extract Concentrations:

The required concentrations of *Pothos scandens* extracts (10, 50, and 100 mg/L) can be prepared by using the stock solution method. This process contain a known amount of each extract (hot aqueous, cold aqueous, and ethanolic) was accurately weighted and dissolved in a suitable solvent. Distilled water was used for the aqueous extracts, while a small amount of ethanol or DMSO was used to ethanolic extract. Then the solution is diluted with system water to obtain a stock solution of 1000 mg/L.

The working concentrations were prepared from the stock solution as follows:

- 1)10 mg/L: 1 mL stock solution diluted to 100 mL with water
- 2)50 mg/L: 5 mL stock solution diluted to 100 mL with water
- 3)100 mg/L: 10 mL stock solution diluted to 100 mL with water

All test solutions are freshly prepared before the experiment.

4.2 Experimental Conditions-

- 1)Exposure duration: 30 minutes
- 2)Number of fish per group: 6
- 3)Number of replicates: 3
- 4)Observation time: Immediately after exposure

The behavioral parameters recorded were distance travelled, swimming velocity, freezing duration, and time spent in the upper zone.

4.3 Cold Extract Administration Procedure-

Since the cold extract was found to be hydrophilic in nature, the cold extract produced through maceration procedure was immediately dissolved in system water. Zebrafish were transferred to treatment tanks having different concentrations of the extract. The process of behavioral analysis was done via immersion approach where zebrafish were exposed to water extract for 30 minutes.

5 RESULTS

Table 2: Cold Extract Effects

Parameter	Control	10mg/L	50mg/L	100mg/L
Distance(cm)	120	130	145	155
Velocity(cm/s)	2.1	2.3	2.5	2.7
Freezing(sec)	60	55	40	35
Top time(sec)	80	90	110	120

There was an improvement in behavioral parameters in a progressive manner with increasing dose. Presumably, the maintenance of heat-sensitive substances by maceration may have been responsible for moderate pharmacological action. Reduced freezing and increased exploratory behavior indicate the possibility of an anxiolytic effect.

5.1 Administration of Hot Extract

The hot extract obtained maceration was reconstituted in distilled water. Due to possible concentration of certain compounds, the solution was filtered to remove insoluble residues before use. Zebrafish were exposed to the extract solution under identical conditions as the cold extract group. Care was taken to maintain temperature stability, as thermal extraction may alter compound stability.

6 RESULTS

Table 3: Hot Extract Effects

Parameter	Control	10mg/L	50mg/L	100mg/L
Distance(cm)	120	115	110	105
Velocity(cm/s)	2.1	2.0	1.9	1.8
Freezing(sec)	60	65	70	75
Top time(sec)	80	75	770	65

The locomotor activity was found to decrease with an increase in dose in the hot extract. This could be due to the thermal breakdown of bioactive components during the extraction process. An increase in freezing behavior denotes stress or lack of stimulation of the CNS.

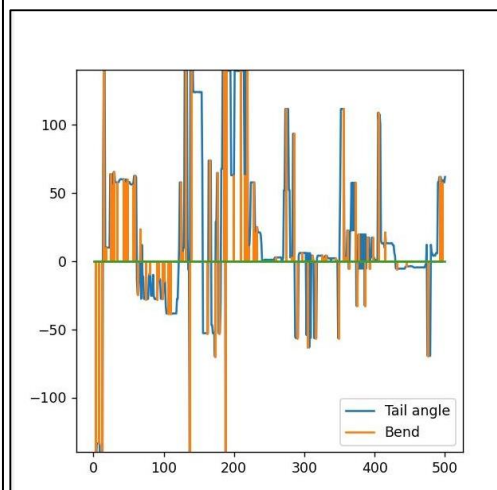
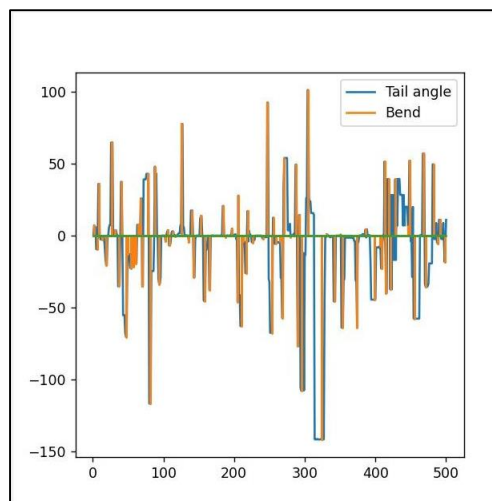
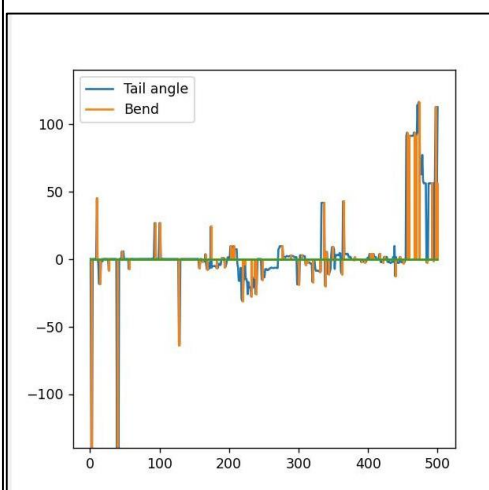
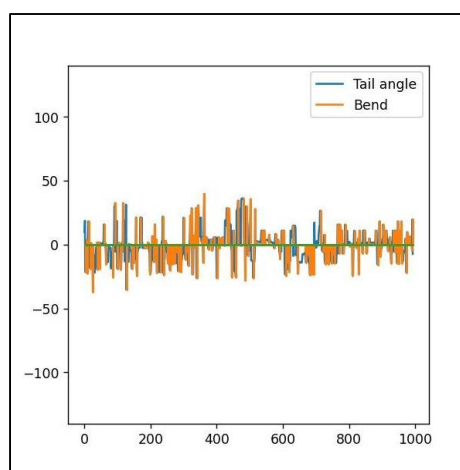
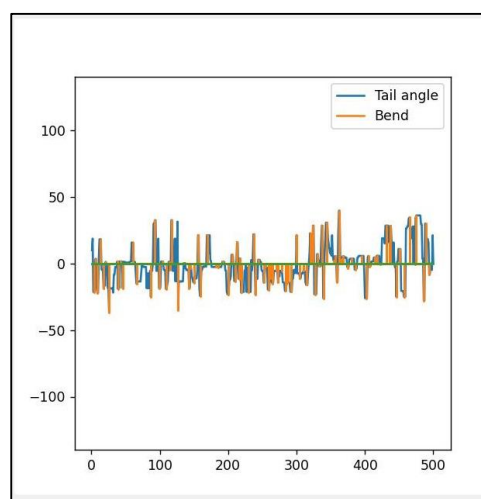
6.1 Administration of Ethanolic Extract:

The ethanolic extract was initially dissolved in a small volume of ethanol and then diluted with system water to achieve the desired concentration. The final ethanol content was kept minimal to prevent solvent-induced behavioral changes. Fish were exposed using the immersion method, similar to other groups. This extract required careful mixing to ensure uniform dispersion in water.

7 RESULTS

Table 4: Ethanolic Extract Effects

Parameter	Control	10mg/L	50mg/L	100mg/L
Distance(cm)	120	150	170	190
Velocity(cm/s)	2.1	2.8	3.1	3.4
Freezing(sec)	60	40	30	20
Top time(sec)	80	120	140	160

GRAPH 1-Freshwater(Control)**GRAPH 2- Coffee extract****GRAPH 3- Cold extract****GRAPH 4- Hot extract****GRAPH 5- Ethanolic extract-****Figure 6: Graphical Interpretation: Tail angle & bend movements**

The ethanolic extract had a pronounced dose-related increase in behavioral performance. The fact that there is increased movement and decreased freezing behavior shows an anxiolytic and CNS stimulant effect. This could be

caused by the effective extraction of flavonoids and phenolic compounds, which are known to affect neurotransmitter systems.

8 CONCLUSION

The study successfully demonstrated that extracts of *Pothos scandens* significantly modulate zebrafish locomotor behavior, confirming its central nervous system (CNS) activity. Among the tested extracts, the ethanolic extract exhibited the most pronounced behavioral alterations, indicating superior extraction of bioactive phytoconstituents. A dose-dependent effect (10, 50, 100 mg/L) was observed, where increasing concentration resulted in progressive reduction in locomotor activity, supporting pharmacological efficacy. Significant decreases in bout speed, distance, and oscillations suggest a sedative or CNS depressant effect of the plant extracts. Changes in tail angle amplitude indicate modulation of motor coordination and possible involvement of motor control pathways. The observed effects may be attributed to the presence of phytochemicals such as alkaloids and flavonoids, which are known to interact with GABAergic and serotonergic systems & produce anxiolytic and sedative effects. The behavioral profile observed in zebrafish is consistent with CNS depressant drugs, suggesting potential therapeutic applications. The use of zebrafish as a model proved effective due to its high sensitivity, genetic similarity to humans, and suitability for neurobehavioral studies. The findings support the traditional medicinal use of *Pothos scandens* and highlight its potential as a source of neuroactive compounds. The study provides a foundation for future pharmacological and toxicological investigations, including: Isolation of active compounds, Mechanistic studies, Clinical relevance. Further studies are required to identify specific molecular targets and confirm long-term safety and efficacy.

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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