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A comprehensive study on acute toxicity of *Cyclosorus extensus* on *Heteropneustes fossilis* of Satajan wetland: a crucial biodiversity hotspot of Assam, India.

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Abstract

This research presents a comprehensive investigation into the acute toxicity of Cyclosorus extensus aqueous extract on the freshwater air breathing catfish Heteropneustes fossilis. The study was conducted during 2025 in Satajan wetland, an important Bird Sanctuary and crucial biodiversity hotspot of Assam, India, with latitude 27Ú12

23.7 to 27Ú12 40.00 N and longitude 94Ú03 08.5 to 94Ú03 08.8 E. It is situated in North Lakhimpur district. The wetland is now under many threats. It is weed-infested and needs conservation attention. The objective was to determine the short-term lethal effects of varying concentrations of the plant extract, contributing to the broader understanding of phytochemical influences on aquatic organisms and potential ecological risks. A static bioassay was conducted under controlled laboratory conditions, with fish subjected to three graded concentrations on the plant extract- high, medium and low, over a 24 hour exposure period.

Findings revealed a definitive dose dependent mortality response. Exposure to high concentrations resulted in 100% mortality of the test organisms within the 24 hour period, indicating severe acute toxicity and the probable presence of potent phytochemicals that disrupt the vital physiological processes. In contrast, fish exposed to medium concentration exhibited no mortality, suggesting a possible sub-lethal threshold that does not compromise the immediate survival of Heteropneustes fossilis. At the lowest tested concentration, 40% mortalitry was observed, signifying that even relatively diluted exposure can have marked lethal effects, though to a lesser extent.

Behavioral alterations such as erratic swimming, air gulping and loss of equilibrium were noted predominantly in all the concentration groups. The absence of mortality at medium concentration may provide insights into potential safe limits or thresholds for further toxicological exploration.

This study emphasizes the ecological and environmental relevance of bioactive plant species like Cyclosorus extensus, particularly in aquatic ecosystems where such extracts might be used traditionally or inadvertently introduced. The outcomes underscore the need for rigorous assessment of botanical substances for their unintended toxicological impacts on non-target aquatic fauna. Furthermore, the findings contribute foundational data for regulatory frameworks concerning the use of natural compounds in aquaculture and water management systems. Future research should expand on chronic exposure effects, histopathological analyses, and the specific phytochemicals responsible for toxicity.

Keywords: Acute toxicity, Cyclosorus extensus, Heteropneustes fossilis, dose dependent mortality response, behavioral alterations.

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1. Introduction

Aquatic ecosystems are intricately connected to the terrestrial environments, leading to a constant exchange of materials. Plants play a vital role in maintaining ecological balance and sustaining life on earth. However, their growth and development can be adversely affected by various biotic and abiotic factors, including exposure to toxic compounds. Aquatic ecosystems are dynamic environments that are highly sensitive to external influences, including chemical and biological pollutants. Interestingly, some toxic compounds originating from plants in the form of a variety of secondary metabolites, such as alkaloids, tannins, saponins and phenols serve as defense mechanism against herbivores, pathogens and environmental stress. 325 fish- poisoning plants spread among 71 families with 183 genera (Newinger, 2004). The effects of plant toxicity vary depending on the type and concentration of toxins, the sensitivity of the affected plants and environmental conditions. The plants used in treating human ailments and animal diseases may be considered poisonous and their beneficial affects often occur at lower doses whereas overdose can induce poisoning (Botha and Penrith, 2008). Fish being at various trophic levels in aquatic food webs, are particularly vulnerable to some toxicants. Plant derived toxins can affect fish behavior, growth, reproduction and survival, leading to both ecological and economic consequences. Study reveals 73 species of plants belonging to 54 generas and 28 families of pteridophytes, gymnosperms and angiosperms used as piscicide in the northeastern states. (Bokolial and Nath, 2014). Plants may produce and release toxins directly into the water, impacting fish through ingestion, absorption through gills or contact with the skin. This is called direct toxicity. The most commonly found classes of fish poisons are triterpenoid saponins and rotenoids with rotenone being the most widely used commercially. Some other poisons include sesquiterpenes, diterpenoids including diterpenoid orthoesters, triterpenes, furanocoumarins, 2-hydroxy-5-methoxy-3-undecyl-1,4- benzoquinone, prenyl phenyl propanoid and sterol acylglucosides (Cannon et al., 2004). Besides, indirect toxicity occurs when plant decomposition in water can lead to the depletion of dissolved oxygen, the release of harmful metabolites and the reaction of anoxic conditions.Those can stress and harm fishes. Poisonous nature of a plant may be due to production of toxic substances such as alkaloids, glucosides, amines, toxalbumins, picrotoxins, resins, saponins, tannins, essential oils, etc. many of which are harmful to man and animal life, at least under certain conditions (Katewa et al 2008). The eastern Himalayan region of India is rich in plant bio resources having immense economic, cultural and medicinal values. This region is exceptionally diverse in topography, varied ecosystems, climate, vegetation pattern, traditional cultural heritage and ethnobotanical knowledge base (Rao and Hajra, 1986). Besides having many positive values, there are several plants studied which have been traditionally used as fish poison in several areas. Most widely used plants for fishing in small streams include *Aesculus assamica, Derris scandens, Gymnocladus burmanicus, Persicaria hydropiper* and *Zanthoxylum rhesta* belonging to the tropical vegetation and subtropical mixed forest of the six districts of Arunachal Pradesh (Tag H et al., 2015). One such piscicidal plant is the *Cyclosorus extensus* which is a synonym of *Thelypteris opulenta* is a species of fern belonging to the family Thelypteridae. It is commonly found in tropical and subtropical regions particularly in Asia and parts of Pacific. The fern thrives in moist shaded environments such as wetlands, riverbanks and forest understories. The leaves of this plant are pinnate, elongated fonds with a leathery texture and it reproduces through spores found on the underside of its fronds. *Cyclosorus extensus* often contain secondary metabolites such as alkaloids, tannins, flavonoids and saponins. Some of these compounds may be toxic to aquatic organisms by affecting their metabolism, growth or survival. If ingested by a fish certain phytochemical could cause irritation or damage to the digestive tract. *Heteropneustes fossilis,* also known as Asian stinging catfish or fossil cat, is a species of air sac catfish found in India, Bangladesh, Pakistan, Nepal, Srilanka, Thailand, Myanmar and Bhutan. Their bodies are elongated and compressed with greatly depressed heads. Outstanding anatomical feature is a pair of accessory respiratory organ (air sacs) which extends backwards from the gill chamber on either side of vertebral column. Caudal rounded. These fishes are very common in pond, ditches, swamps and marshes but found in muddy river also. They feed mainly on Mollusks 15.5%, Cypris 15.6%, Chironomoid larvae 13.4% ; fish and its scales 11.1%, eggs of invertebrates 13.2%, copepod 16.5%, weeds 20.3%, detritus 41.0% and fungus and others 16.3% (Shafi and Quddus, 2001). Each 100 g of this fish contains-protein 23.0 g, fat 0.6 g, calcium 670 mg, phosphorus 650 mg and water 79.3% (Siddique, 2010).

This study aims to test the phytochemical agents present in the *Cyclosorus extensus* plant and evaluate the toxic effects of *Cyclosorus extensus* leaf extract on *Heteropneustes fossilis***,** a

fresh water catfish species widely used in aquaculture. The research aimed to evaluate the potential of the plant extract as an alternative to synthetic pathogens or toxins in aquaculture systems.

2. Materials and Methods

Study area: The study was conducted during 2025 in Satajan wetland (Latitude 27Ú12 23.7 to 27Ú12 40.00 N and Longitude 94Ú03 08.5 to 94Ú03 08.8 E), an important Bird Sanctuary and Biodiversity hotspot of Assam, India. It is situated in North Lakhimpur district (Fig 1). The wetland is now under many threats. It is weed-infested and needs immediate conservation attention.

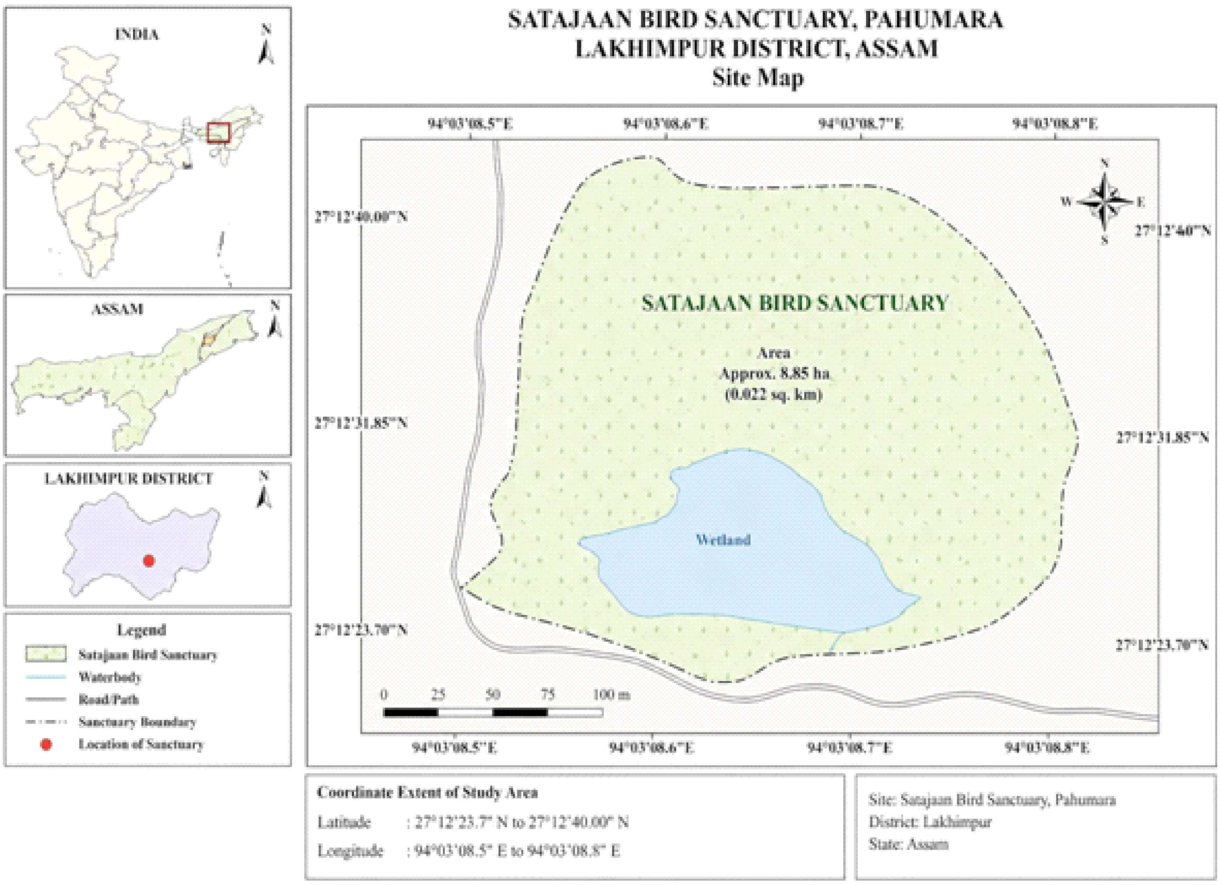

Figure-1 : Map of the study area

Collection of plant for the preparation of plant extract and for the phytochemical testing: The whole plants of *Cyclosorus extensus* was gathered from the study area.

Preparation of fish poison tree powder: The collected plant was shadow dried to a constant weight until the moisture was lost completely. After it lost its complete moisture and was dried it was ground in the mixture grinder. Finally a fine powder of the plant was prepared and was stored. The chemicals used for the preparation of the *Cyclosorus extensus* plant extract and for the testing of phytochemical agents present in the extract are- Methanol, 10% FeCl3, Fehling's solution, Molisch's reagent, Concentrated HSO4, Magnesium chunk, HCl, Chloroform, Meyer's reagent, Dragendroff's reagent, NaOH *a*nd Glacial acetic acid.

Preparation of the plant extract: About 25 g of powdered sample was mixed with 200 ml of methanol. The mixture was mixed with the help of magnetic stirrer to get a fine paste of the powder.

Phytochemical tests: The prepared extract was used to test various phyto constituents present in them. The tests (Tannin/polyphenol, reducing sugar, glycosides, flavonoids, Dilute NH3, Terpenoids, Meyer's test, Dragendroff's reagent test, Test for saponins, Test for volatile oils, Test for cardiac glycosides, Test for steroids) were done based on the references from Alamzed et al, 2013, Thusa and Mulmi, 2017, Sharma et al, 2020 and Talukdar et al., 2010.

Preparation of Aqueous Extract for Fish Toxicity Experiment: For the fish toxicity study, fresh whole plant of *Cyclosorus extensus* (including leaves, stems and tender parts) were chopped into small pieces and ground thoroughly using an electric mixer grinder along with clean running tap water to uniform slurry. The prepared plant-water mixture was then strained using a strainer and the aqueous extract from the plant residue was obtained.

Experimental Setup: The fishes were treated in accordance with guielines of the local ethics committee. Healthy specimens of *Heteropneustes fossilis,* collected from the study area were exposed to varying concentration of *Cyclosorus extensus* aqueous extract placed in four separate containers:

Low concentration: 1950 ml water + 50 mL plant extract

- Medium concentration: 1900 ml water + 100 mL plant extract
- High concentration: 1800 ml water + 200 mL plant extract
- Control group: 2000 ml water without extract.

Each container housed 5 fish, and the exposure period was 24 hours under controlled laboratory conditions.

3. Results

Phytochemical test results: Out of the ten phytochemical components tested, seven were present in the sample which includes reducing sugar, dilute NH3 test, terpenoids, saponin, volatile oils, cardiac glycosides and steroids. Tannin, glycosides and flavonoids were absent.

Sl no	Test of Components	Remarks
1	Tannin	-
2	Reducing sugar	+
3	Glycosides	-
4	Flavonoids	-
5	Dilute NH3	+
6	Terpenoids	+
7	Saponin	+
8	Volatile oils	+
9	Cardiac glycosides	+
10	Steroids	+

(+) = present; (-) = absent

Table 1: Phytochemical constituents present in Cyclosorus extensus powder

Toxicity experiment on *Heteropneustes fossils*:

The results of the experiment demonstrated a clear concentration-dependent response in *Heteropneustes fossilis* to the *Cyclosorus extensus* aqueous extract, with significant effects observed at higher concentrations (Table 1).

Toxicity High Concentration (200 ml extract/1800 ml water):

Four among the five fishes exposed to the high concentration of the aqueous extract died within 12 hours of exposure, and the remaining one dies within 24 hours of exposure, resulting in a 100% mortality rate.

The mortality was rapid, with all fish showing signs of distress by the 6-hour mark, followed by death within 12 hours and 24 hours. No recovery was observed after 24 hours.

Medium Concentration (100 ml extract/1900 ml water):

No mortality was observed in the medium concentration group at both the 12-hour and 24- hour observation points.

Fish in this group remained alive throughout the experiment with no signs of death or severe distress.

Low Concentration (50 ml extract/1950 ml water):

Two fishes out of the five fishes were found dead in the low concentration group during the 12-hour and 24- hour exposure periods.

The remaining fishes in this group remained alive throughout the experiment with little signs of distress.

Control Group (no extract):

The control group exhibited no mortality or abnormal behavior throughout the experimental period.

- The fish maintained normal swimming activity and displayed typical behavior.

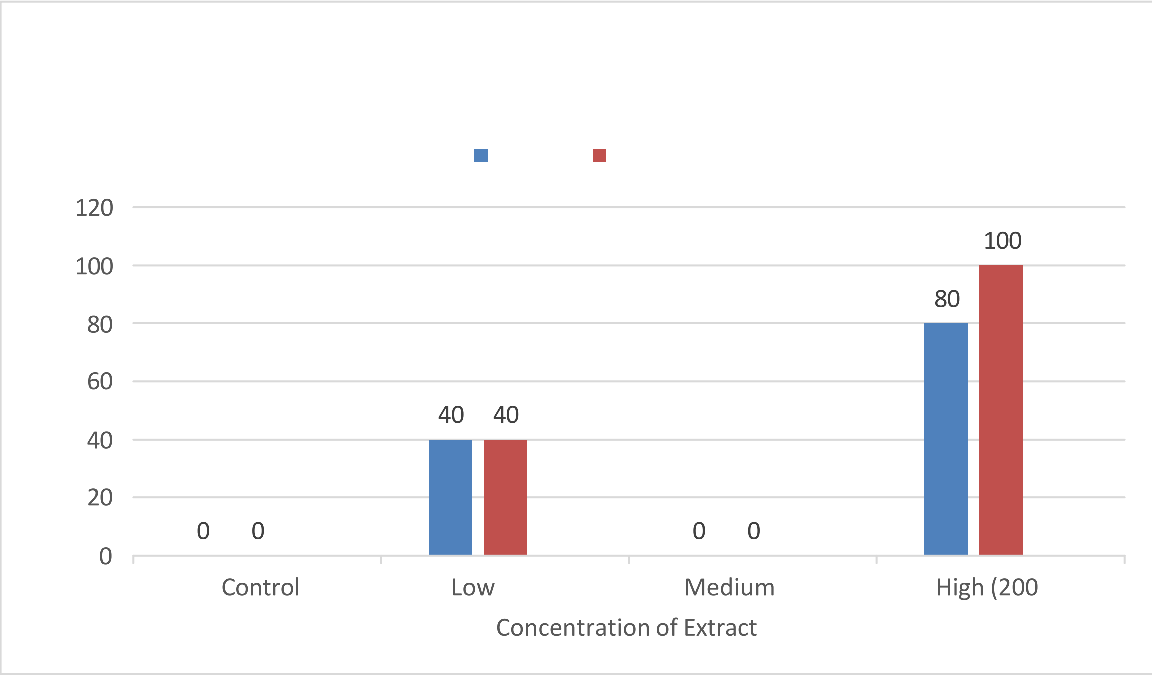

Figure 1: Mortality rate (%) of *Heteropneustes fossilis* after 12 and 24 h exposure to different concentrations of *Cyclosorus extensus* extract under laboratory conditions

3. Behavioral Observations:

High Concentration (200 ml extract/1800 ml water): By 12 hours, four of the five fishes had succumbed to the toxic effects of the extract and died. The remaining one fish in this group survived long enough for further behavioral observations beyond the 12-hour mark but that too died within 24 hours (Fig 2).

Medium Concentration (100 ml extract/1900 ml water): Fish in the medium concentration group exhibited mild behavioral changes by the 12-hour mark, such as reduced swimming activity and slower movement compared to the control group.

By 24 hours, fish in this group continued to show reduced activity levels, with some occasional periods of immobility. No other significant behavioral abnormalities were observed.

Low Concentration (50 ml extract/1950 ml water): Three out of the five fishes in the low concentration group exhibited small effects at both the 12-hour and 24-hour marks. But two fishes died within 12 hours, and a slight reduction in swimming activity was observed, with fish swimming more slowly and spending more time near the bottom of the container in the remaining hours.

Control Group (no extract): Fish in the control group exhibited consistent, typical behavior. They maintained a normal swimming pattern and showing no signs of stress or abnormality

Extract Concentration	**Observed Behavioral Changes**
Control (0 ml)	Normal behavior, no visible changes.
Low (50 ml)	40 % death in 12 hours and same result in24 hour
Medium (100 ml)	Pronounced lethargy
High (200 ml)	80 % death within 12 hours and 100 %Death within 24 hours.

Table 2 : Table showing the behavioral changes in fishes in different concentrations

4. Physiological Observations

High Concentration (200 ml extract/1800 ml water): No necropsy (post-mortem examination) was performed on the dead fish; therefore, internal physiological changes could not be recorded.

Medium Concentration (100 ml extract/1900 ml water): Some fish exhibited minor external physiological changes after 24 hours, such as slight discoloration of body scales and rough texture. No internal examination was conducted (Table 2).

Low Concentration (50 ml extract/1950 ml water): 60% of the fishes were dead and the remaining percent showed no noticeable external physiological changes. Their body coloration, scale condition, and general appearance remained normal throughout the observation period.

Control Group (no extract): Fish appeared healthy with normal body coloration, scale texture, and no visible signs of physiological distress.

5. Discussion

The impact of plant-based ichthyotoxins on aquatic life, particularly on economically important fish species, has gained increasing attention in ecotoxicology and environmental biology. This study evaluates the toxicological effects of *Cyclosorus extensus*, a fern known for its phytochemical richness, on *Heteropneustes fossilis* (commonly known as stinging catfish). The experiment involved exposing the fish to graded concentrations of aqueous extracts of *C. extensus*, revealing a dose-dependent increase in mortality.

1. Toxicity and Phytochemical Composition of *Cyclosorus extensus*

Cyclosorus extensus contains several bioactive compounds, including terpenoids, dilute NH_3, saponin, and steroids. These secondary metabolites, while contributing to the plant's medicinal and pesticidal properties, also possess cytotoxic and neurotoxic characteristics that may adversely affect aquatic organisms. When released into the aquatic environment, these compounds can interact with the physiological systems of fish, leading to stress, behavioral abnormalities, and even death.

2. Behavioral and Physiological Responses in *H. fossilis*

Behaviorally, fish exposed to high doses exhibited hyperactivity, erratic swimming, loss of equilibrium, and excessive mucus secretion, which are typical indicators of acute stress and neurotoxic effects. Low dose groups, while showing milder behavioral changes such as reduced activity and sluggishness, still experienced unexpected mortality. The medium dose group, in contrast, showed minimal behavioral alterations and no mortality, suggesting a possible adaptive and hermetic response at this concentration.

These findings partially contrast with previous research on other fern based phytochemicals. For instance, studies by Singh et al. (2019) on the effects of *Azolla pinnata* extracts reported a dose dependent increase in toxicity in *Oreochromis mossambicus*, with highest mortality seen only at maximum concentrations. Similarly, Ghosh and Mandal (2020) observed linear toxicity patterns when *Nephrolepis exaltata* extracts were used in *Channa punctatus*, indicating a more predictable dose response relationship than what we have observed with *C. extensus.*

3. Concentration-Dependent Mortality

The mortality observed at low doses in this study may indicate the presence of specific phytochemicals in *C. extensus* that exhibit toxic effects at sub lethal concentrations through mechanisms such as endocrine disruption or metabolic interference. Conversely, at moderate concentrations, these compounds might have triggered detoxification pathways or stress resistance mechanisms, which have been documented in other species such as Labeo rohita when exposed to mild herbal stressors (Patra et al., 2021).

The lack of mortality in the medium dose group may also be due to enzymatic adaptation or behavioral tolerance, a phenomenon described in previous toxicological assessments involving herbal extracts and heavy metals (Tripathi and Shukla, 2017). This emphasizes the complexity of plant extract organism interactions and the possibility that certain doses induce a protective response rather than toxicity.

4. Ecological and Practical Implications

The findings of this study have important implications for both aquaculture management and environmental monitoring. Accidental or deliberate introduction of *C. extensus* extracts into water bodies—either through herbal runoff or bio-pesticide usage—could threaten non-target aquatic organisms such as *H. fossilis*. This species plays a significant role in freshwater ecosystems and holds commercial value, particularly in South and Southeast Asia.

Furthermore, these results highlight the potential use of *C. extensus* in piscicidal formulations. In traditional practices, plant extracts are used to stun or kill fish during harvest. However, this must be approached cautiously, considering long-term ecological consequences and bio accumulation in aquatic food chains.

6. Conclusion

The present study provides compelling evidence on the toxicological impact of *Cyclosorus extensus* extract on *Heteropneustes fossilis* (commonly known as stinging catfish). The results demonstrated a clear concentration-dependent effect of the plant extract, where increasing doses corresponded to significantly higher mortality rates among the test fish population. This indicates that *C. extensus*, although a naturally occurring plant, possesses potent bioactive compounds that can adversely affect aquatic organisms when present in sufficient concentrations.

At lower concentrations, *H. fossilis* exhibited minimal behavioral changes and lower mortality, suggesting that the plant's bioactive components may be tolerable or less disruptive at these levels. However, as concentrations increased, the fishes displayed signs of acute physiological stress, abnormal behavior, and ultimately high mortality rates, indicating severe toxicity. The direct correlation between concentration and mortality strongly suggests that the phytochemicals in *C. extensus*—possibly including tannins, flavonoids, alkaloids, and phenolic compounds—interfere with the normal biological functioning of the fish, potentially affecting the respiratory and nervous systems.

This outcome underscores the importance of careful evaluation before employing plant-based extracts in aquatic environments, whether for pest control, traditional practices, or other applications. Although natural products are often considered environmentally friendly, their ecological impact can be profound, especially when applied without regulation. The high mortality observed in *H. fossilis* at elevated concentrations also highlights the plant's potential as a natural piscicide, which may have implications in managing invasive species or in aquaculture pond preparation. However, such applications must be guided by stringent environmental assessments to avoid unintended ecological consequences.

In conclusion, while *Cyclosorus extensus* demonstrates notable biological activity against *Heteropneustes fossilis*, its toxic nature at higher concentrations warrants cautious and controlled use. Further research is needed to isolate the specific toxic components, understand their mode of action, and assess the long-term ecological effects of the extract in aquatic ecosystems. This study contributes valuable data to the growing field of ecotoxicology and supports the call for integrating traditional knowledge with scientific evaluation to ensure environmental sustainability.

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