

Anticonvulsant and Neuroprotective Activity of Ethanolic Leaf Extract of *Costus pictus* D. Don in PTZ-Induced Epilepsy in Mice

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Abstract—Epilepsy is a chronic neurological disorder characterized by recurrent seizures arising from abnormal neuronal activity. This study evaluated the anticonvulsant and neuroprotective potential of ethanolic leaf extract of *Costus pictus* D. Don in pentylenetetrazol (PTZ)-induced epilepsy in mice, with supporting phytochemical characterization, UV/FTIR fingerprinting, SH-SY5Y MTT testing, biochemical evaluation and hippocampal histopathology. Leaves were collected from Talegaon, Pune and authenticated at the Botanical Survey of India, Western Regional Centre, Pune (voucher BSI/WBC/Tech./2025/PSB-70). Preliminary screening detected tannins, flavonoids, terpenoids, alkaloids, saponins, reducing sugars, steroids, glycosides, phenols and amino acids. Extract treatment at 125 and 250 mg/kg increased seizure latency, reduced seizure duration and shortened recovery time. The 250 mg/kg dose produced the more consistent response. GABA and dopamine-associated measurements increased toward normal values, while IL-6 and TNF- α were reduced. Calcium and glucose abnormalities also moved toward control values, whereas sodium showed no significant treatment effect. Histopathology showed dose-related preservation of hippocampal architecture. SH-SY5Y viability remained above 69% across tested concentrations, although higher concentrations generally produced lower viability. The findings support further mechanistic and toxicological investigation of *C. pictus* as a potential multi-target anticonvulsant and neuroprotective candidate.

Index Terms—*Costus pictus*; epilepsy; pentylenetetrazol; anticonvulsant; GABA; neuroinflammation.

I. INTRODUCTION

Epilepsy is a major neurological disorder characterized by recurrent unprovoked seizures. Seizures arise when neuronal excitation becomes excessive or inhibitory control is insufficient. GABA is the principal inhibitory neurotransmitter in the brain, whereas glutamatergic transmission provides major excitatory drive. Disturbance of this balance can lower seizure threshold and promote abnormal synchronized firing.

Experimental epilepsy models are used to identify anticonvulsant activity before clinical translation. PTZ is a widely used chemical convulsant because it produces reproducible seizure activity and is particularly useful for screening agents that influence inhibitory neurotransmission. A protective treatment is expected to increase latency, reduce seizure severity or duration and accelerate recovery. The PTZ model is valuable for screening but does not reproduce every feature of chronic human epilepsy.

Seizure activity can also activate inflammatory and oxidative pathways. Cytokines such as IL-6 and TNF- α can participate in neuroinflammatory responses, while altered calcium handling may contribute to neuronal injury. Hippocampal structures, particularly CA1 and CA3, are vulnerable to seizure-associated cellular stress. A multi-endpoint study can therefore provide stronger biological interpretation than a behavioral endpoint alone.

II. LITERATURE RATIONALE

Costus pictus D. Don belongs to Costaceae and is commonly called the insulin plant. The submitted thesis describes traditional and experimental interest in its leaves, particularly for metabolic effects, and reports phytochemical groups including phenolics, flavonoids, terpenoids and alkaloids. Such chemical diversity makes a crude plant extract a plausible source of multiple interacting pharmacological effects, although the present study does not identify a single active molecule.

The rationale of the present investigation was therefore to determine whether the ethanolic leaf extract modifies seizure behavior and whether the behavioral response is accompanied by changes in neurotransmitter-associated markers, inflammatory biomarkers and hippocampal morphology.

2.1 OBJECTIVES

- Evaluate anticonvulsant activity using PTZ-induced seizures.
- Assess modulation of GABA and dopamine.
- Evaluate inflammatory markers including TNF- α and IL-6.
- Investigate neuroprotection using biochemical, SH-SY5Y MTT and histopathological endpoints.

III. PLANT MATERIAL AND EXTRACTION



Fig. 1. *Costus pictus* D. Don plant image reproduced from the submitted thesis.

Leaves were collected in October from Talegaon, Pune. The thesis reports authentication at the Botanical Survey of India, Western Regional Centre, Pune, with voucher number BSI/WBC/Tech./2025/PSB-70. Leaves were washed, shade-dried for approximately 7–10 days, powdered and stored in an airtight container.

For the principal experimental extract, the thesis describes maceration of powdered leaves with 70% ethanol at a 1:10 w/v ratio. The mixture was maintained in a sealed container at room temperature for 72 hours with occasional stirring, filtered and concentrated under reduced pressure. A separate preparation described for spectral analysis used 10 g powder in 100 mL of 95% ethanol with shaking for 24–48 hours. These two preparation descriptions should not be merged during final editing.

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

Class	Result	Possible relevance
Tannins	Present	Phenolic/antioxidant contribution
Flavonoids	Present	Antioxidant and signaling effects
Terpenoids	Present	Broad pharmacological potential
Alkaloids	Present	Detected by Wagner/Mayer/Dragendorff tests
Saponins	Present	Bioactive secondary metabolites
Steroids	Present	Steroidal fraction detected
Glycosides	Present	Glycosidic constituents

Phenols	Present	Supports phenolic contribution
Amino acids	Present	Qualitative detection
Proteins	Absent	No positive qualitative reaction reported

3.2 SPECTRAL CHARACTERIZATION

The thesis reports UV absorption maxima near 227 and 278 nm. FTIR bands were reported near 3393, 2923, 1616, 1446, 1078 and 619 cm^{-1} . The broad 3393 cm^{-1} region is compatible with O–H-containing constituents, while the remaining bands provide a fingerprint of mixed functional groups. Because the extract is chemically complex, FTIR should be described as preliminary fingerprinting rather than definitive compound identification.

IV. EXPERIMENTAL DESIGN AND METHODS

The thesis reports healthy adult female Swiss albino mice weighing approximately 25–30 g and aged 8–10

weeks. Animals were housed under controlled laboratory conditions with food and water available ad libitum. The animal protocol was approved by the Institutional Animal Ethical Committee under reference KBIPER/IAEC/2024-25/04.

4.1 PTZ MODEL

The reported disease-control dose of PTZ was 85 mg/kg, administered subcutaneously. *C. pictus* extract was administered orally at 125 and 250 mg/kg, 30 minutes before PTZ. Sodium valproate 200 mg/kg intraperitoneally was used as the reference anticonvulsant. Animals were observed after induction for seizure expression and recovery.

Group	Treatment	Dose	Purpose
Normal	0.9% saline	—	Baseline control
Disease control	PTZ	85 mg/kg, s.c.	Seizure model
Treatment 1	<i>C. pictus</i> extract	125 mg/kg, p.o.	Low dose
Treatment 2	<i>C. pictus</i> extract	250 mg/kg, p.o.	High dose
Standard	Sodium valproate	200 mg/kg, i.p.	Reference drug

4.2 SEIZURE SCORING

The thesis describes a 0–6 behavioral scale: 0, normal behavior; 1, immobilization/lying on the abdomen; 2, head nodding or focal myoclonus; 3, continuous whole-body myoclonus; 4, rearing/tonic seizure/falling; 5, tonic-clonic seizure with falling, rushing or jumping; and 6, death.

4.3 BIOCHEMISTRY AND HISTOLOGY

GABA, dopamine, AChE, IL-6 and TNF- α were measured together with glucose, calcium and sodium. Hippocampal tissue was examined histologically. The thesis reports preserved architecture in normal animals, neuronal loss and pyknosis in disease control, and progressively better preservation at 125 and 250 mg/kg.

V. RESULTS: ANTICONVULSANT ACTIVITY

Group	Latency (s)	Duration (s)	Recovery (min)
Disease control	137.60 $\pm$ 13.240	180.80 $\pm$ 11.300	17.02 $\pm$ 3.781
<i>C. pictus</i> 125 mg/kg	169.00 $\pm$ 12.610	155.60 $\pm$ 10.526	11.12 $\pm$ 3.271
<i>C. pictus</i> 250 mg/kg	172.74 $\pm$ 12.818	148.00 $\pm$ 16.446	9.66 $\pm$ 2.666
Sodium valproate	189.00 $\pm$ 25.632	146.40 $\pm$ 15.436	8.97 $\pm$ 2.722

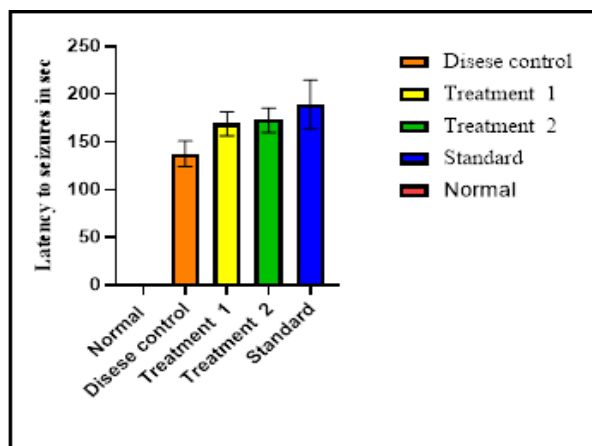


Fig. 3. Mean latency to seizure. Values are reproduced from thesis results.

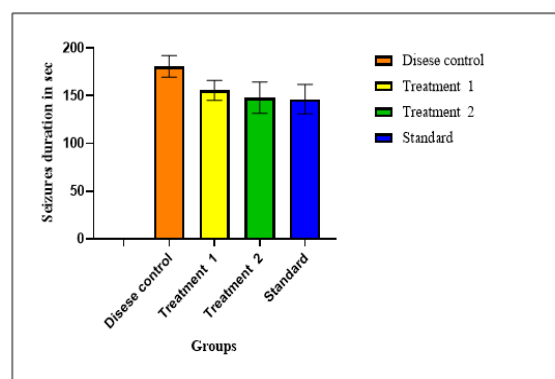


Fig. 4. Mean seizure duration. Values are reproduced from thesis results.

Interpretation: compared with disease control, the 125 and 250 mg/kg doses increased mean seizure latency by approximately 22.8% and 25.5%, respectively. Seizure duration decreased by approximately 14.0% and 18.1%, while recovery time decreased by approximately 34.7% and 43.2%. These calculations are derived from the reported means and are descriptive; the original inferential statistics should remain the authority for significance.

VI. RESULTS BIOCHEMICAL MARKERS

Group	IL-6 (pg/mL)	TNF- α (pg/mL)	GABA (pg/mL)
Normal	112.258 $\pm$ 9.806	89.358 $\pm$ 9.455	212.000 $\pm$ 6.426
Disease control	148.504 $\pm$ 1.999	122.155 $\pm$ 8.845	174.042 $\pm$ 1.035
C. pictus 125 mg/kg	107.642 $\pm$ 7.757	89.116 $\pm$ 7.422	203.060 $\pm$ 9.650
C. pictus 250 mg/kg	114.270 $\pm$ 8.094	81.376 $\pm$ 7.121	215.652 $\pm$ 8.531
Sodium valproate	99.904 $\pm$ 13.351	76.852 $\pm$ 7.669	221.516 $\pm$ 9.506

Group	Dopamine (pg/mL)	AChE (pg/mL)	Glucose (mg/dL)	Calcium (mg/dL)
Normal	182.96 $\pm$ 6.154	335.20 $\pm$ 8.023	207.44 $\pm$ 10.731	6.52 $\pm$ 0.579
Disease control	147.56 $\pm$ 3.487	243.69 $\pm$ 13.666	155.828 $\pm$ 7.089	9.78 $\pm$ 0.931
C. pictus 125 mg/kg	173.18 $\pm$ 1.544	315.62 $\pm$ 8.804	207.18 $\pm$ 12.880	6.04 $\pm$ 0.785
C. pictus 250 mg/kg	182.96 $\pm$ 4.788	340.36 $\pm$ 10.333	217.50 $\pm$ 7.547	5.32 $\pm$ 0.775
Sodium valproate	180.00 $\pm$ 6.348	342.56 $\pm$ 22.513	219.892 $\pm$ 7.680	5.00 $\pm$ 0.838

Sodium values were reported as 141.56 $\pm$ 8.608, 139.884 $\pm$ 7.864, 139.0 $\pm$ 2.204, 140.0 $\pm$ 8.204 and 141.88 $\pm$ 8.150 mmol/L for normal, disease, 125 mg/kg, 250 mg/kg and standard groups, respectively; the thesis reports no significant treatment effect.

6.1 BIOLOGICAL INTERPRETATION

PTZ was associated with higher IL-6/TNF- α and lower GABA/dopamine/AChE-associated measurements. Extract treatment shifted these measures toward normal values. The 250 mg/kg dose produced the strongest overall biochemical normalization. These observations are consistent with a combined inhibitory-neurotransmission and anti-

inflammatory effect, but they do not prove a direct molecular target.

VII. IN VITRO NEURONAL CELL-VIABILITY ASSESSMENT

Concentration ($\mu\text{g/mL}$)	Viability (%)
Control	100.00

1	98.91
5	88.53
25	92.47
50	77.97
100	87.97
250	71.57
500	69.95

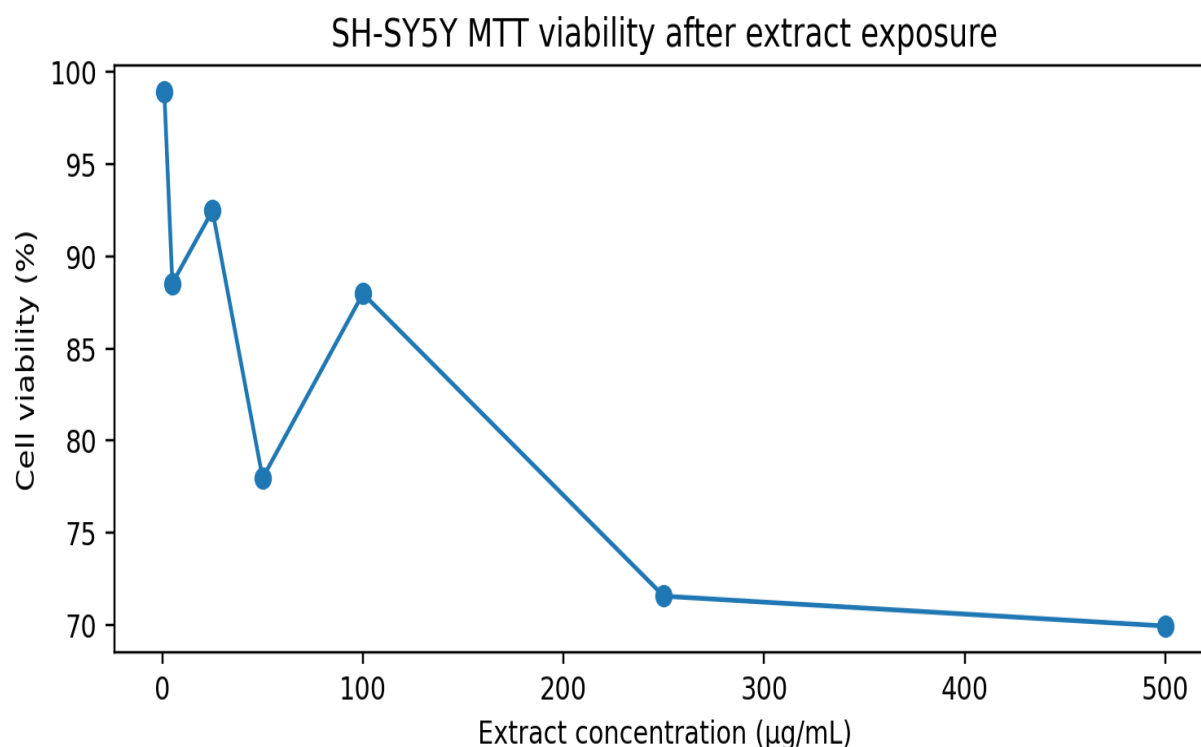


Fig. 5. SH-SY5Y MTT viability after exposure to *C. pictus* ethanolic extract.

The SH-SY5Y assay was used as a cytocompatibility screen. The reported viability remained relatively high across the tested concentrations, but the response was not strictly monotonic. Viability was 98.91% at 1 $\mu\text{g/mL}$ and 69.95% at 500 $\mu\text{g/mL}$. The intermediate fluctuations should not be interpreted as a simple linear dose-response relationship.

7.1 INTERPRETATION

MTT measures cellular metabolic activity rather than directly measuring cell number or long-term survival. Therefore, the findings support the statement that no severe loss of metabolic viability was observed under most tested conditions, but they do not establish complete safety. Higher concentrations produced

lower viability overall, making concentration selection important for future mechanistic studies.

7.2 QUALITY-CONTROL RECOMMENDATIONS

- Include untreated and vehicle controls in every independent experiment.
- Report number of biological replicates and technical replicates.
- Confirm extract interference with MTT absorbance, particularly for colored plant extracts.
- Use a complementary assay such as LDH release or apoptosis markers for confirmation.
- Report concentration-response statistics from independent experiments.

VIII. HISTOPATHOLOGY AND NEUROPROTECTION

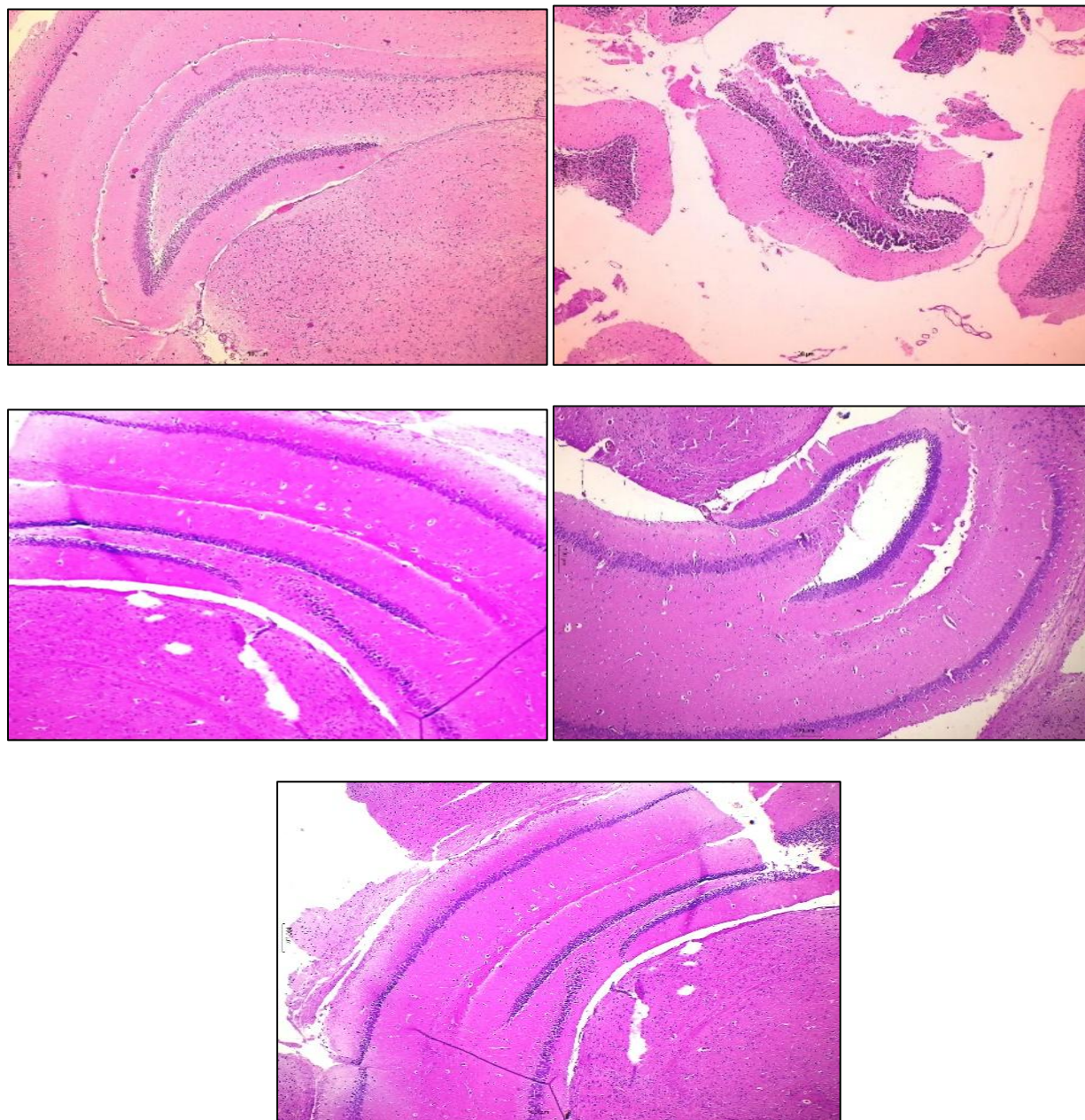


Fig. 6. Hippocampal histopathology across experimental groups, reproduced from the submitted thesis. Arrows identify representative regions described in the thesis.

The normal-control hippocampus showed preserved architecture and clearly defined layers. PTZ disease-control animals displayed neuronal loss, nuclear pyknosis and disruption of hippocampal cytoarchitecture, particularly in the CA1 and CA3 regions. The 125 mg/kg extract group showed partial preservation with some degenerative change, whereas

the 250 mg/kg group demonstrated more marked preservation.

8.1 SIGNIFICANCE OF THE HISTOLOGICAL FINDINGS

The histological pattern provides an important tissue-level correlate for the behavioral and biochemical findings. A treatment that delays seizures and

simultaneously preserves hippocampal structure has a stronger neuroprotective interpretation than a behavioral effect alone. The present findings are compatible with reduced seizure-associated neuronal stress, although the study did not quantify neuronal density or use blinded morphometric scoring.

8.2 RECOMMENDED QUANTITATIVE HISTOLOGY

- Count viable neurons in CA1 and CA3 per defined microscopic field.
- Use blinded scoring by an independent observer.
- Report a predefined degeneration/pyknosis score.
- Where possible, add neuronal markers such as NeuN and apoptosis-associated markers.
- Use consistent magnification and representative fields for every group

IX. DISCUSSION

9.1 Anticonvulsant response

The behavioral pattern is internally coherent: both extract doses delayed seizure onset, reduced seizure duration and accelerated recovery, with 250 mg/kg generally outperforming 125 mg/kg. The reference drug produced the greatest latency and slightly shorter duration/recovery values, so the extract should be described as active but not equivalent to sodium valproate.

9.2 GABA and neuronal excitability

The increase in GABA-associated measurements after extract treatment is particularly relevant to the PTZ model because PTZ is strongly associated with impairment of GABAergic inhibition. Restoration of GABA may therefore contribute to the observed increase in seizure latency. However, the current study cannot distinguish between increased synthesis, reduced metabolism, altered transport, receptor-level modulation or indirect network effects.

9.3 Inflammation

The disease-control group showed higher IL-6 and TNF- α than normal control, while treatment reduced both markers. This supports the possibility that anti-inflammatory activity contributes to neuroprotection. IL-1 β was listed as an objective in the thesis but numerical results were not available in the reported

tables; it should therefore not be presented as a demonstrated finding.

9.4 Calcium, glucose and sodium

Calcium was elevated in disease control and moved downward after treatment, which is directionally compatible with reduced excitotoxic stress. Glucose was reduced in disease control and returned toward normal after treatment. Sodium remained broadly similar among groups and was reported as non-significant. These variables should be treated as supportive biochemical observations rather than primary evidence of anticonvulsant mechanism.

9.5 Phytochemical basis

The presence of flavonoids, phenols, tannins and terpenoids provides a plausible basis for antioxidant and anti-inflammatory effects, but qualitative screening cannot establish which compound produced the response. Chromatographic standardization and compound-level quantification are required before attributing activity to a particular phytochemical.

9.6 Comparison with standard

At 250 mg/kg, the extract approached sodium valproate for seizure duration and recovery and showed substantial biochemical normalization. Nevertheless, sodium valproate remained superior for seizure latency. The comparison is useful for contextualizing magnitude, not for claiming therapeutic equivalence.

X. CONCLUSION, LIMITATIONS AND FUTURE SCOPE

Conclusion: Ethanolic leaf extract of *Costus pictus* D. Don demonstrated anticonvulsant and neuroprotective potential in the reported PTZ-induced seizure model. The 250 mg/kg dose produced the most consistent improvement across behavioral, biochemical and histological endpoints. The pattern suggests a multi-factorial effect involving restoration of inhibitory neurotransmission, reduction of inflammatory markers and preservation of hippocampal structure.

10.1 LIMITATIONS

- The thesis reports initial group allocation of six animals but the result tables use $n=5$; exclusions/attrition should be documented.

- Exact extract yield and chemical standardization should be added if available.
- AChE units should be verified against the assay-kit documentation.
- Exact p-values should be taken from the original statistical software output because narrative significance statements are inconsistent in places.
- IL-1 β numerical results were not reported.
- Acute PTZ testing does not model all features of chronic or drug-resistant epilepsy.
- Histopathology was primarily descriptive rather than quantitatively scored.
- MTT alone cannot establish long-term safety or neuroprotective efficacy.

10.2 FUTURE RESEARCH

- Standardize the extract by HPLC/LC-MS fingerprinting and quantify total phenolics/flavonoids.
- Test the extract in additional seizure models such as maximal electroshock and chronic kindling.
- Investigate GABA_A receptor modulation, glutamate signaling and calcium-channel pathways.
- Measure oxidative-stress markers and microglial/neuroinflammatory pathways.
- Perform pharmacokinetic and brain-penetration studies.
- Conduct repeated-dose toxicity and safety-pharmacology studies.
- Use blinded quantitative hippocampal morphometry and neuronal immunohistochemistry.

10.3 PUBLICATION CHECKLIST

Requirement	Action before submission
Abstract	Keep within 150–250 words.
Keywords	Use 4–6 focused terms.
Figures	Cite every figure in text; use descriptive captions.
Tables	Number and cite each table.
Statistics	Verify exact p-values and n.
Units	Verify all assay units and kit details.
References	Cross-check every bibliographic record.

Ethics	Retain IAEC reference KBIPER/IAEC/2024-25/04.
Originality	Run plagiarism/similarity screening and disclose any required AI assistance.

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