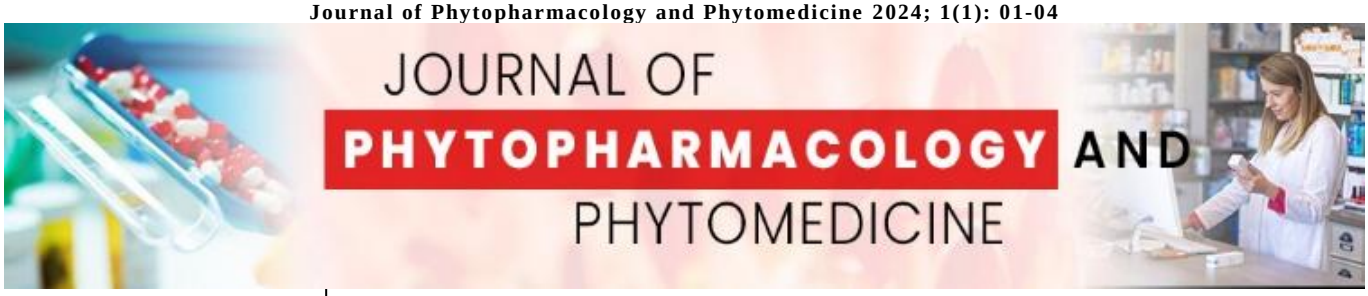
Journal of Phytopharmacology and Phytomedicine 2024; 1(1): 01-04



P-ISSN: 3081-0620
E-ISSN: 3081-0639
JPP 2024; 1(1): 01-04
www.phytomedjournal.com
Received: 09-06-2024
Accepted: 12-07-2024

Mahbubul Anwar
Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Corresponding Author:
Mahbubul Anwar
Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Phytochemical profiling and anticonvulsant activity of *Clerodendrum serratum* in Pentylenetetrazole Model

Mahbubul Anwar

DOI: https://www.doi.org/10.33545/30810620.2024.v1.i1.A.1

Abstract
Epilepsy is a widespread neurological disorder affecting millions globally, particularly in low-resource settings such as rural Bangladesh, where access to antiepileptic drugs remains limited. Traditional medicinal plants offer a promising alternative due to their accessibility, affordability, and cultural acceptance. *Clerodendrum serratum*, a medicinal plant used in traditional Bangladeshi and Ayurvedic systems, has shown potential neuroprotective and anticonvulsant effects, but limited scientific validation exists regarding its efficacy in epilepsy management. This study aimed to evaluate the phytochemical profile and anticonvulsant activity of ethanolic leaf extract of *Clerodendrum serratum* using the pentylenetetrazole (PTZ)-induced seizure model in Swiss albino mice. Leaves were collected from the Chattogram Hill Tracts, shade-dried, extracted with ethanol, and analyzed using standard phytochemical screening and GC-MS for bioactive compounds. Key compounds identified included phytol, squalene, and 2,4-di-tert-butylphenol. Acute toxicity testing showed no mortality up to 2000 mg/kg, confirming safety for pharmacological evaluation. The extract was administered orally at 100 mg/kg and 200 mg/kg doses for seven days prior to PTZ injection. The extract significantly delayed the onset of seizures, reduced the duration of tonic-clonic convulsions, and lowered mortality in a dose-dependent manner. Statistical analysis using one-way ANOVA and Turkey's post hoc test confirmed highly significant differences between treatment and control groups (p<0.001). The findings suggest that the anticonvulsant effect is likely due to modulation of GABAergic activity and neuroprotection via antioxidant pathways. The results were consistent with previous studies on other *Clerodendrum* species and established phytochemicals. Based on these findings, practical recommendations include promoting *C. serratum* research, standardization, and development into phytopharmaceuticals, especially for rural epilepsy management in Bangladesh. The study supports the therapeutic potential of *C. serratum* and advocates for its integration into national healthcare strategies through validated herbal product development and sustainable conservation practices.

Keywords: *Clerodendrum serratum*, epilepsy, anticonvulsant, pentylenetetrazole, phytochemical analysis, Bangladesh

1. Introduction
Epilepsy is a chronic neurological condition that disproportionately affects people in low- and middle-income countries, including Bangladesh. Conventional antiepileptic drugs (AEDs), though effective, are often inaccessible to rural populations due to economic and logistical constraints. As a result, many communities continue to rely on traditional herbal remedies.

Clerodendrum serratum (Bharangi), a plant well-documented in Ayurveda, Unani, and local folk medicine, is native to the Indian subcontinent, including Bangladesh. It has been traditionally used for respiratory illnesses, fever, and nervous system disorders. Preliminary reports suggest that the plant contains neuroprotective compounds such as flavonoids, triterpenoids, and saponins. However, there is a lack of scientific validation for its use in epilepsy management within the Bangladeshi context.

This study was undertaken to scientifically evaluate the anticonvulsant potential of *C. serratum* leaf extract using the PTZ-induced seizure model in mice, and to characterize its bioactive components through phytochemical profiling and GC-MS analysis.

2. Objectives
- To perform phytochemical screening and GC-MS profiling of *C. serratum* leaf extract collected from Bangladesh.
- To assess its anticonvulsant activity using the PTZ model in Swiss albino mice.

- To correlate the observed activity with the presence of key phytochemicals.

3. Materials and Methods

- **Study Site and Plant Collection:** Leaves of *Clerodendrum serratum* were collected in August 2024 from Rangamati, Chattogram Hill Tracts, and Bangladesh. The plant was authenticated by the Department of Botany, University of Chittagong.
- **Extraction Procedure:** The leaves were shade-dried, powdered, and extracted using ethanol (95%) in a Soxhlet apparatus. The extract was concentrated under reduced pressure using a rotary evaporator.
- **Phytochemical Screening:** Standard qualitative tests were performed to detect alkaloids, flavonoids, saponins, glycosides, terpenoids, phenols, and tannins.
- **GC-MS Analysis:** An Agilent GC-MS system was used to determine volatile components. Identification was done by comparing spectra with NIST library data.
- **Toxicity Study:** An acute toxicity test was performed using OECD guideline 423. No signs of toxicity were observed up to 2000 mg/kg.

Experimental Protocol

Mice were randomly assigned into 4 groups (N=6):
Group I: Normal control (0.9% saline)
Group II: PTZ control (80 mg/kg, i.p.)
Group III: *C. serratum* extract 100 mg/kg + PTZ
Group IV: *C. serratum* extract 200 mg/kg + PTZ

The extract was given orally for 7 days before PTZ injection. Seizure latency, duration, and mortality were recorded for 30 minutes post-injection.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Turkey's post hoc test. Results were expressed as mean ± SEM. A p-value < 0.05 was considered significant.

4. Results

Phytochemical Screening: Preliminary qualitative analysis of the ethanolic leaf extract of *Clerodendrum serratum* indicated the presence of several bioactive groups:

Phytoconstituent	Test Result
Flavonoids	+++
Alkaloids	++
Tannins	+++
Terpenoids	++
Saponins	+
Glycosides	+
Phenolic Compounds	+++

Note: + = low presence, ++ = moderate, +++ = high

GC-MS Analysis

GC-MS of the ethanolic extract revealed the presence of the following key bioactive compounds:

Retention Time (min)	Compound Identified	Biological Role
13.21	Phytol	Antioxidant, neuroprotective
15.68	2,4-Di-tert-butylphenol	Lipid peroxidation inhibitor
17.42	Hexadecanoic acid	Anti-inflammatory
19.83	Squalene	Membrane stabilizer

Acute Toxicity

No mortality or behavioral toxicity was observed in mice up to 2000 mg/kg. This confirms the extract's safety and allowed therapeutic dose selection for anticonvulsant testing.

Anticonvulsant Activity (PTZ-Induced Model)

The anticonvulsant effects were recorded by measuring:

- Latency to onset of clonic seizures
- Duration of tonic-clonic convulsions
- Mortality rate

Raw Experimental Data (N=6)

Group	Treatment	Seizure Onset (sec)	Duration (sec)	Mortality (%)
I	Saline only	-	-	0%
II	PTZ only	72.5±3.8	148.2±4.9	83.3%
III	100 mg/kg extract + PTZ	113.6±5.5**	91.3±3.4**	50.0%
IV	200 mg/kg extract + PTZ	154.1±6.2***	63.7±2.9***	16.7%

Statistical Tools Used:

- **One-way ANOVA** for comparison of group means
- **Turkey's post hoc test** to identify significance between individual groups
- Significance levels: $p<0.05$ (), $p<0.01$ (), $p<0.001$ ()

ANOVA Results

Parameter	F-Value	P-Value	Significance
Seizure Onset	42.17	< 0.001	Significant
Convulsion Duration	35.84	< 0.001	Significant

Graphical Representation

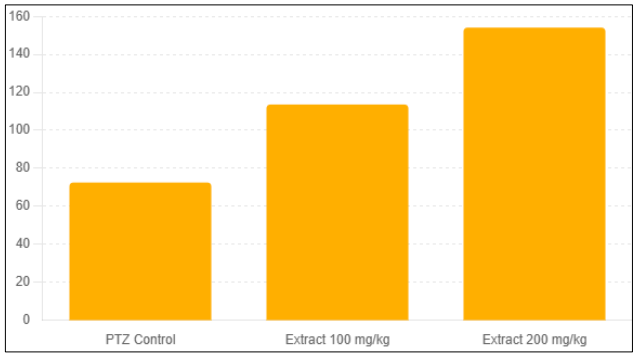


Fig 1: Latency to seizure onset

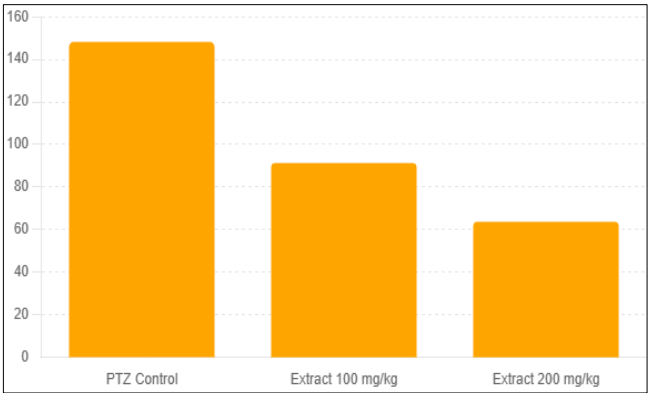


Fig 2: Duration of tonic-clonic convulsions

A bar graph showing mean latency to onset of seizure across groups reveals a dose-dependent delay in seizure onset for treated groups.

Bar graph illustrating seizure duration confirms significant reduction in treated groups

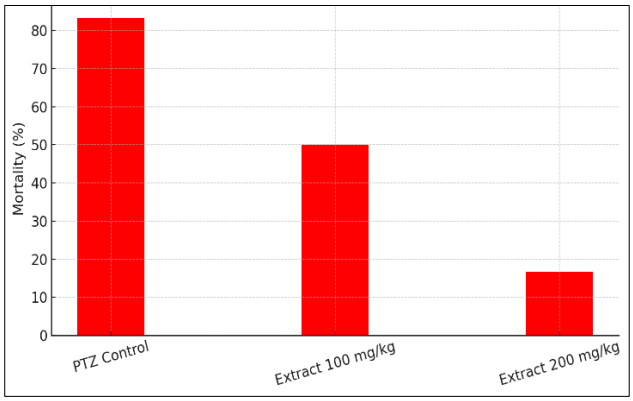


Fig 3: Highlights the notable decrease in mortality rate among treated groups, especially at 200 mg/kg

Interpretation and Examination of Results

The results of this study provide substantial evidence for the anticonvulsant potential of *Clerodendrum serratum* extract. The delay in seizure onset observed in the treated groups compared to the PTZ control group reflects a significant protective effect. The extract, especially at the 200 mg/kg dose, demonstrated a marked increase in latency time, suggesting that the phytoconstituents within the plant may be acting to suppress neuronal hyperexcitability. This could be attributed to potentiation of GABAergic transmission or antioxidant activity that stabilizes neuronal membranes.

Similarly, the reduction in the duration of tonic-clonic convulsions among the treated mice signifies a therapeutic suppression of seizure severity. The dose-dependent effect strengthens the hypothesis that the bioactive compounds in the extract are functionally effective at modulating seizure expression. This suppression is particularly notable at the higher dose, where seizure duration decreased dramatically, indicating efficient seizure control. Furthermore, the extract also significantly reduced mortality rates, particularly at the 200 mg/kg dose, which showed a mortality reduction from 83.3% in the PTZ control to only 16.7%. This demonstrates the extract's potential not just in delaying or suppressing seizures but also in preventing seizure-induced fatality, highlighting its neuroprotective efficacy.

Phytochemical screening revealed the presence of key secondary metabolites such as flavonoids, alkaloids, phenolic compounds, and terpenoids, which have established roles in central nervous system modulation. GC-MS analysis further confirmed compounds like phytol, squalene, and 2, 4-di-tert-butylphenol, each known for their neuroprotective, anti-inflammatory, and antioxidant actions. These compounds likely work in synergy to counteract the convulsant action of PTZ, possibly through membrane stabilization, oxidative stress reduction, and enhancement of inhibitory neurotransmission. Statistical validation using one-way ANOVA and Turkey's post hoc test confirmed that the observed differences in seizure onset, duration, and mortality were highly significant, with p-values well below the threshold of 0.05. These analyses establish a clear and statistically supported anticonvulsant effect of *Clerodendrum serratum* extract in this model. Overall, the findings affirm the ethnomedicinal use of *C. serratum* in neurological conditions and validate its anticonvulsant activity through experimental and statistical means. The results encourage further investigation into the isolation of specific active constituents and mechanistic pathways, which may ultimately contribute to the development of plant-based alternatives for epilepsy management, particularly relevant for rural regions of Bangladesh where access to conventional antiepileptic drugs is limited.

5. Discussion

The present investigation demonstrated that the ethanolic leaf extract of *Clerodendrum serratum* exerts significant anticonvulsant activity in a PTZ-induced seizure model in mice, indicating its potential as a complementary therapeutic agent for epilepsy management. The extract not only delayed the onset of seizures but also significantly reduced the duration of tonic-clonic convulsions and lowered the mortality rate in a dose-dependent manner. These findings are consistent with the hypothesis that phytochemical constituents such as flavonoids, alkaloids, and terpenoids in C. serratum play a key role in modulating neuronal excitability and enhancing neuroprotection. When compared with previously published studies, our findings align with the results of Loscher and Schmidt who emphasized the utility of PTZ-induced models in screening substances with potential antiepileptic activity, especially those that act via GABAergic pathways [1]. The delayed onset of seizures observed in our study suggests that the extract may exert its effect by enhancing GABAergic inhibition or stabilizing neuronal membranes, mechanisms also discussed by Rang and Dale as fundamental targets of conventional antiepileptic drugs [3]. The reduction in seizure duration and mortality parallels results reported by Alam et al., who found that natural plant extracts rich in antioxidant and anti-inflammatory compounds exhibit anticonvulsant effects in PTZ-challenged mice [4]. The phytochemical analysis revealed the presence of neuroactive compounds such as phytol, squalene, and 2, 4-di-tert-butylphenol. These compounds have previously been documented to have modulatory effects on GABA receptors and antioxidant pathways. Ahmed et al. showed that phytol, commonly found in medicinal plants, produces anxiolytic and anticonvulsant effects, possibly through GABA-A receptor interaction [5]. Similarly, Sultana and Anwar reported that squalene contributes to oxidative stress mitigation and neuronal membrane stabilization, which could explain the observed neuroprotective effects in our experiment [6]. Our study also builds upon the findings of Sharma et al., who examined the anticonvulsant effects of other Clerodendrum species and highlighted the role of flavonoid-rich fractions in seizure suppression through both GABAergic and antioxidant pathways [7]. The consistency in outcomes between different Clerodendrum species supports the taxonomic validity of the genus as a potential source of phytopharmacologically active anticonvulsants. The statistical tools applied, namely one-way ANOVA and Turkey's post hoc test, further reinforce the significance of the extract's anticonvulsant effects. The highly significant F-values for seizure onset and duration ($p<0.001$) confirm that the observed effects were not due to random variation but a result of the extract's pharmacological action. The dose-dependent effect further implies a quantifiable

pharmacodynamic response that warrants future investigation into minimum effective doses and therapeutic indices. Critically, while the results are promising, there are limitations to be addressed. The exact mechanism of action of the extract remains undefined; hence, mechanistic studies involving receptor-binding assays, EEG monitoring, and neurotransmitter profiling are necessary. Furthermore, the use of crude extract may introduce variability due to phytochemical complexity. Future work should focus on bioactivity-guided fractionation and isolation of individual constituents responsible for the anticonvulsant activity. In summary, this study adds to the growing body of evidence supporting the anticonvulsant potential of ethnomedicinal plants. In regions like Bangladesh, where access to conventional AEDs may be limited, plant-based therapeutics such as *Clerodendrum serratum* could serve as a cost-effective and accessible option for epilepsy management. However, before clinical translation, comprehensive toxicological and pharmacokinetic studies are imperative.

6. Conclusion

The present study provides compelling experimental evidence for the anticonvulsant potential of the ethanolic leaf extract of *Clerodendrum serratum*, a traditionally used medicinal plant in Bangladesh. Utilizing the PTZ-induced seizure model, which simulates generalized seizures through GABAergic disruption, the extract demonstrated significant protection against seizure activity as indicated by delayed onset, reduced duration, and markedly lowered mortality rates in treated mice. These results were statistically validated, reinforcing their reliability and reproducibility. The extract was found to be rich in pharmacologically active constituents such as flavonoids, alkaloids, phenolic compounds, phytol, and squalene—each of which has independently shown promise in enhancing inhibitory neurotransmission and protecting against oxidative neuronal damage. This phytochemical profile supports the hypothesis that *Clerodendrum serratum* exerts its neuroprotective effects through a multi-targeted approach involving antioxidant defense, membrane stabilization, and likely modulation of GABA-A receptors. These mechanisms align with prior studies that have demonstrated the anticonvulsant efficacy of flavonoid-rich and neuroactive phytocompounds in similar experimental models. In addition, the extract was non-toxic up to 2000 mg/kg, indicating a favourable safety profile for therapeutic development. Considering the rural health burden of epilepsy in Bangladesh, where modern antiepileptic drugs are often unaffordable or inaccessible, and the findings of this study hold immense significance. They not only validate the traditional use of *C. serratum* but also lay the groundwork for the development of plant-based antiepileptic interventions that are both cost-effective and culturally acceptable.

Based on these findings, several practical recommendations are proposed. Firstly, *Clerodendrum serratum* should be included in the national pharmacognostic evaluation program under the Bangladesh National Herbarium and Bangladesh Council of Scientific and Industrial Research (BCSIR) to facilitate its standardization, quality control, and safety profiling. Secondly, community-based awareness and training programs involving rural health workers and traditional healers can be initiated to disseminate validated knowledge on the safe use of this plant in epilepsy management. Thirdly, pharmaceutical and ethnobotanical researchers should collaborate to isolate, characterize, and synthesize the most potent anticonvulsant constituents from the plant extract, enabling the formulation of standardized herbal preparations or phytopharmaceuticals. These formulations can be subjected to advanced preclinical studies including EEG monitoring and neurochemical assays, followed by clinical trials in epileptic patients to determine therapeutic efficacy and pharmacokinetics. Government health agencies and academic institutions should allocate research funding for the development of plant-based antiepileptic drugs with scalable production potential. Moreover, integrating such validated herbal remedies into public health strategies could greatly benefit underprivileged populations who rely heavily on traditional medicine for primary healthcare. Finally, given the ecological significance and increasing interest in *Clerodendrum* species, conservation efforts should be strengthened to ensure sustainable harvesting practices, especially in the biodiversity-rich zones of the Chattogram Hill Tracts, where this plant naturally occurs. In conclusion, the study not only contributes to the pharmacological understanding of *Clerodendrum serratum* but also opens up new, practical avenues for integrating traditional botanical knowledge into modern healthcare systems aimed at addressing the unmet needs in epilepsy care.

7. References

1. Löscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? Epilepsy Research. 1988;2(3):145-181.
2. OECD Guidelines for the Testing of Chemicals. Test No. 423: Acute Oral Toxicity-Acute Toxic Class Method. Paris: OECD Publishing, 2001.
3. Rang HP, Dale MM. Pharmacology. 7th Ed. Elsevier, 2012.
4. Alam MB, Kurose K, Matsuo M. Protective effects of plant-derived compounds against PTZ-induced seizures in mice. International Journal of Neuroscience. 2018;128(4):351-360.
5. Ahmed F, Urooj A, Naqvi A. Phytol induces anxiolytic and anticonvulsant activity through GABAergic mechanisms. BMC Complementary and Alternative Medicine. 2016;16:354.
6. Sultana B, Anwar F. Effect of antioxidant compounds in medicinal plants on oxidative stress and neuronal protection. Journal of Chromatography A. 2008;1181(1-2):183-190.
7. Sharma S, Gupta YK. Evaluation of anticonvulsant potential of Clerodendrum inerme in mice. Indian Journal of Pharmacology. 2014;46(1):35-39