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Phytochemical Screening And Antiepileptic Activity Of Hydroalcoholic Extract Of Clerodendrum Serratum

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Abstract
The present study aimed to investigate the phytochemical profile and antiepileptic potential of the hydroalcoholic extract of Clerodendrum serratum. *The extract was prepared and subjected to preliminary phytochemical screening, revealing the presence of flavonoids, phenols, proteins, carbohydrates, and diterpenes. Quantitative analysis showed total flavonoid and phenol contents of 0.860 mg/100 mg and 0.572 mg/100 mg of dried extract, respectively. The antiepileptic activity was evaluated using pentylenetetrazole (PTZ)- and maximal electroshock (MES)-induced seizure models in albino rats. The extract exhibited a dose-dependent protective effect against PTZ-induced seizures, delaying seizure onset and providing 66.67% and 74.36% protection at 100 mg/kg and 200 mg/kg doses, respectively. In the MES model, it significantly delayed seizure onset, indicating anticonvulsant potential. The observed activity may be attributed to the presence of flavonoids and phenolic compounds, which possess antioxidant and neuroprotective properties. These findings support the traditional use of* Clerodendrum serratum *in neurological disorders and suggest its potential as a source of novel antiepileptic agents.*

Keywords: Clerodendrum serratum, *Hydroalcoholic extract, Phytochemical screening, Antiepileptic activity, Pentylenetetrazole, Maximal electroshock, Flavonoids, Phenols.*

INTRODUCTION

Clerodendrum serratum (L.) Moon, commonly referred to as Bharangi, is a perennial medicinal plant belonging to the Verbenaceae family. This plant has long been recognized in traditional medicine systems, including Ayurveda, Siddha, and Unani, for its wide spectrum of therapeutic applications (Dongare et al., 2020).

Traditionally, *C. serratum* has been employed in the management of respiratory disorders such as asthma, cough, bronchitis, and dyspnea. In addition, it has been utilized for the treatment of inflammation, fever, pain, and various neurological disorders (Smruti; 2021).

The therapeutic potential of *C. serratum* is largely attributed to its diverse phytochemical composition. Phytochemical studies have revealed the presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, phenolic compounds, and tannins (Almubayedh and Ahmad; 2020).

These bioactive constituents are known to exert multiple pharmacological effects, including antioxidant, anti-inflammatory, antipyretic, hepatoprotective, and neuroprotective activities (Dar et al., 2023). Recent pharmacological investigations have focused on evaluating the anticonvulsant and neuroprotective potential of *C. serratum*. Epilepsy is a chronic neurological disorder characterized by recurrent seizures, affecting millions of people worldwide. Despite the availability of conventional antiepileptic drugs, many patients experience side effects, drug resistance, or inadequate seizure control. As a result, there is a growing interest in identifying natural products that can serve as safer and effective alternatives (Potnis et al., 2020).

Experimental studies using rodent models have demonstrated that hydroalcoholic extracts of *C. serratum* leaves exhibit significant anticonvulsant activity. In particular, the pentylenetetrazole (PTZ)-induced seizure model in mice revealed that the extract increases seizure latency, reduces seizure duration, and decreases mortality in a dose-dependent manner (Anwar et al., 2024).

Phytochemical screening and analytical studies of *C. serratum* extracts have identified key secondary metabolites, including flavonoids such as quercetin and rutin, terpenoids like squalene, and other compounds such as phytol and 2,4-di-tert-butylphenol (Anwar; 2024). These constituents are believed to contribute synergistically to the observed pharmacological effects. Additionally, the antioxidant properties of flavonoids and phenolics may offer neuroprotective benefits by scavenging free radicals and preventing oxidative damage to neuronal tissues (Teleanu et al., 2019).

Given its rich phytochemical profile and promising preclinical evidence, the hydroalcoholic extract of *Clerodendrum serratum* presents itself as a potential candidate for developing natural antiepileptic agents.

Further research is necessary to isolate and characterize the active principles, elucidate their mechanisms of action, and evaluate the safety and efficacy of these extracts in clinical trials. This study aims to perform detailed phytochemical screening and assess the antiepileptic potential of hydroalcoholic extracts of *C. serratum*, contributing to the development of safer, plant-based therapeutics for epilepsy.

MATERIAL AND METHODS

Material

The study employed various analytical and pharmaceutical-grade chemicals and reagents, including Potassium Mercuric Iodide, Iodine, Potassium Iodide, Sodium Nitroprusside, Lead Acetate, and Folin-Ciocalteu reagent, along with solvents such as Methanol, Ethanol, and Chloroform. Other materials used were Pyridine, Gelatin, Nitric Acid, Copper Acetate, Sodium Hydroxide, Sodium Chloride, Fehling's solution, Quercetin, Gallic Acid, Dimethyl Sulfoxide, 3,5-dinitrosalicylic acid, and p-nitro-phenyl-α-D-glucopyranoside, procured from recognized suppliers across Mumbai and New Delhi. These were utilized for phytochemical screening, antioxidant assays, and analytical evaluation of the Clerodendrum serratum extract.

Methods

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Whole plant materials of *Clerodendrum serratum* were collected from Bhopal in the month of February, 2025.

Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs. Whole plant material of *Clerodendrum serratum* were shade dried at room temperature. 50 gram dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted dried powdered of *Clerodendrum serratum* has been extracted with hydroalcoholic solvent (methanol: Water; 75:25v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2002; Kokate, 1991).

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical screening

Phytochemicals encompass a wide range of chemical classes, including alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides, and many others. Each class of phytochemicals may have specific physiological effects and potential health benefits. For example, flavonoids are known for their antioxidant properties, alkaloids often possess antimicrobial or analgesic properties, while terpenoids can have antitumor or anti-inflammatory effects. Phytochemical examinations were carried out for all the extracts as per the standard methods (Garg *et al.,* 2025).

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with methanol to get 100µg/ml. Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared. 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Estimation of total phenol content

10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was

used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vivo* antiepileptic activity of hydroalcoholic extract of *Clerodendrum serratum

Animals

Adult male albino Swiss mice (20–25 g) were group housed (n=6–10) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Toxicity studies were performed following OECD guidelines, specifically focusing on the acute oral toxicity of hydroalcoholic extract from *Clerodendrum serratum*. An acute toxicity assessment was carried out in line with OECD Guideline No. 423, with rats monitored for any signs of toxicity over the subsequent 14 days. The hydroalcoholic extract of *Clerodendrum serratum* was administered orally at a safe dosage. Observations included clinical symptoms such as behavioral changes, alterations in eye appearance, shifts in body weight, and the condition of skin and fur (Kazmi *et al.,* 2023).

Pentylenetetrazole-induced seizures test

Mice were divided into three groups, each with six animals, and were given hydroalcoholic extract of *Clerodendrum serratum* at doses of 100 and 200 mg/kg, along with diazepam at 3 mg/kg. Thirty minutes later, seizures were induced using pentylenetetrazole at a dose of 80 mg/kg via intraperitoneal injection. During the first 30 minutes, the animals were monitored for the number of convulsive episodes, which included latency and duration of myoclonic jerks, the number of fatalities, and the percentage of protection against convulsions and death (Saleem *et al.,* 2024).

Maximal electroshock-induced seizures test

Mice were split into three groups, each consisting of six animals, and were treated with either hydroalcoholic extract from *Clerodendrum serratum* (at doses of 100 and 200 mg/kg) or Phenytoin (25 mg/kg). Thirty minutes later, seizures were triggered using a current stimulus (18 mA, 50 Hz for 0.2 seconds) applied through corneal electrodes connected to a shock generator (Inco, India). We measured the percentage of protection and the duration of tonic hind limb extension, where the hind limbs are fully extended at a 180° angle from the body axis. Protection was defined as the total absence of tonic hind limb extension (Socala and Wlaz, 2021).

RESULTS AND DISCUSSION

The results of the present study demonstrate that the hydroalcoholic extract of *Clerodendrum serratum* possesses significant phytochemical constituents and antiepileptic activity. The extract yielded 16.2% w/w, which indicates efficient extraction of bioactive compounds suitable for pharmacological evaluation. Phytochemical screening revealed the presence of flavonoids, phenols, proteins, carbohydrates, and diterpenes, while alkaloids, glycosides, saponins, and tannins were absent. The presence of flavonoids and phenolic compounds, confirmed by total flavonoid and phenol content estimation (0.860 mg/100 mg and 0.572 mg/100 mg of dried extract, respectively), suggests potential antioxidant and neuroprotective properties, which may contribute to the observed antiepileptic effects.

In seizure models, the hydroalcoholic extract of *Clerodendrum serratum* demonstrated a dose-dependent protective effect. In the pentylenetetrazole (PTZ)-induced seizure model, the extract delayed the onset of seizures and offered 66.67% and 74.36% protection at 100 mg/kg and 200 mg/kg doses, respectively, compared to 81.13% protection by diazepam. Similarly, in the maximal electroshock (MES)-induced seizure model, the extract significantly delayed seizure onset in a dose-dependent manner, though less potent than phenytoin, suggesting that the extract may exert its antiepileptic action through modulation of both GABAergic and excitatory neurotransmission.

The presence of flavonoids and phenolic compounds likely plays a key role in the observed anticonvulsant activity by scavenging free radicals, reducing oxidative stress, and modulating neuronal excitability. Overall, the study confirms that the hydroalcoholic extract of *Clerodendrum serratum* exhibits promising antiepileptic activity, supporting its traditional use in neurological disorders and highlighting its potential for development into therapeutic agents for seizure management.

Table 1: % Yield of extract of *Clerodendrum serratum*

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	16.2

Table 2: Phytochemical screening of extract of *Clerodendrum serratum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline reagent test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
	Folin-ciocalteu test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Benedict's Test	+ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	-ve
	Foam Test	-ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

[+ve= positive; -ve= negative]

Total flavonoids content estimation (TFC)

Table 3: Preparation of Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.235
2	10	0.465
3	15	0.716
4	20	0.945
5	25	1.165

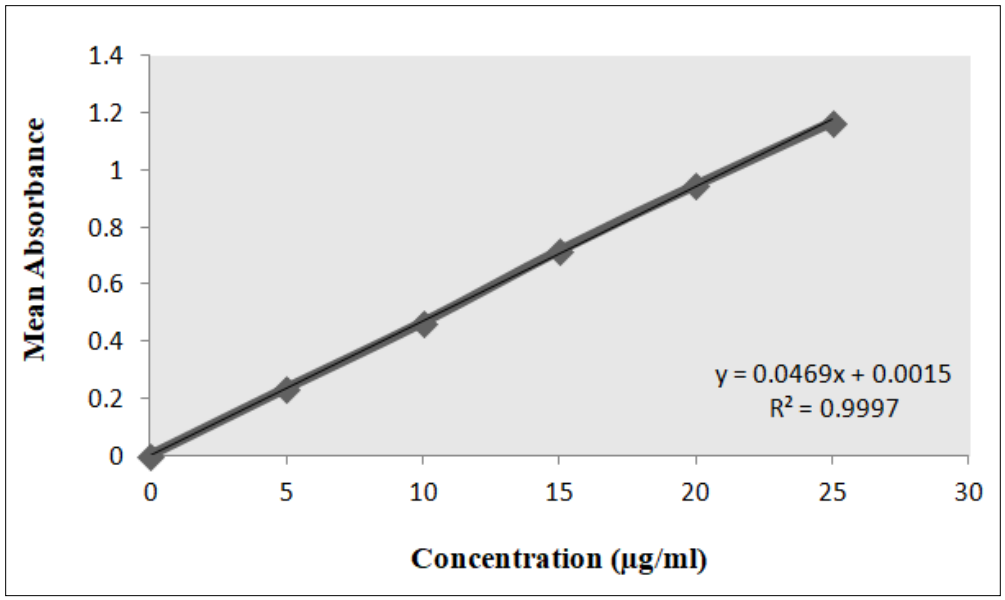

Figure 1: Graph of calibration curve of Quercetin

Table 4: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean absorbance
1	10	0.335
2	20	0.658
3	30	0.912
4	40	1.253
5	50	1.562

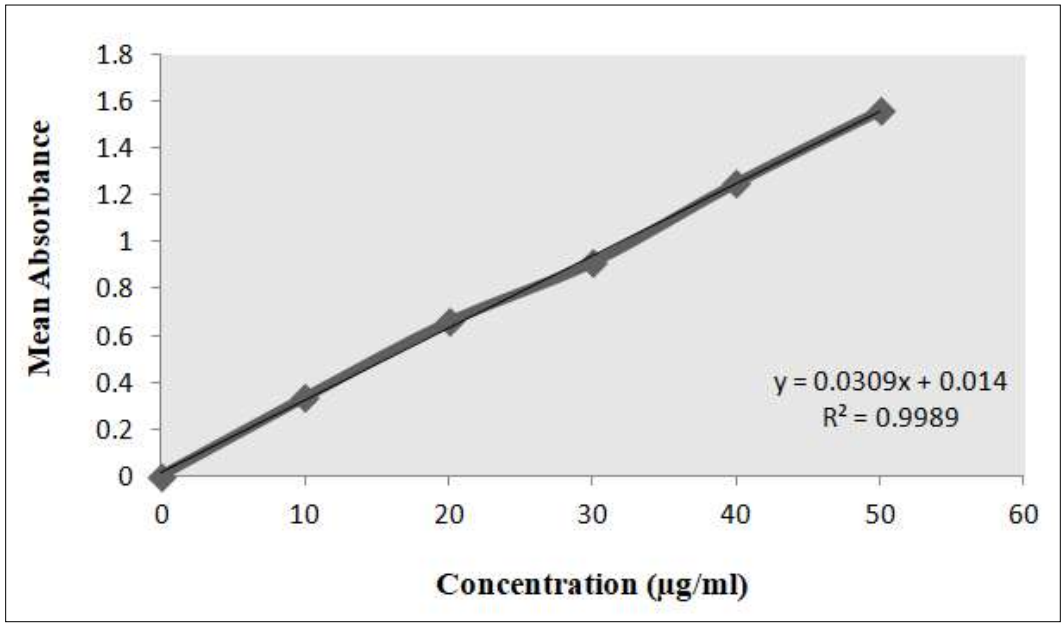

Figure 2: Graph of calibration curve of Gallic acid

Table 4: Estimation of total flavonoids and phenol content of *Clerodendrum serratum*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	

1.	Hydroalcoholic	0.860	0.572

Table 5: Effects of Hydroalcoholic extract of *Clerodendrum serratum* on pentylenetetrazole-induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)	% protection against seizures
Diazepam	3	265 ± 12.5***	81.13%
Hydroalcoholic extract of *Clerodendrum serratum*	100	150 ± 10.3*	66.67%
Hydroalcoholic extract of *Clerodendrum serratum*	200	195 ± 11.1**	74.36%

Values are expressed as the mean ± SEM of six observations. *P<0.05, **P<0.01, ***P<0.001 (One-way ANOVA followed by Dunnett's post hoc test).

Table 6: Effects of hydroalcoholic extract of *Clerodendrum serratum* on MES-induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)
Phenytoin	25	0.00 ± 0.00***
Hydroalcoholic extract of *Clerodendrum serratum*	100	1.95 ± 0.15*
Hydroalcoholic extract of *Clerodendrum serratum*	200	3.85 ± 0.18**

Values are expressed as the mean ± SEM of six observations. *P<0.05, **P<0.01, ***P<0.001 (One-way ANOVA followed by Dunnett's post hoc test).

CONCLUSION

The hydroalcoholic extract of *Clerodendrum serratum* demonstrated significant antiepileptic activity in both PTZ- and MES-induced seizure models, supporting its traditional use in managing convulsive disorders. The extract showed a dose-dependent effect, with higher doses providing greater protection and delayed seizure onset. Phytochemical analysis revealed the presence of bioactive constituents such as flavonoids, phenols, proteins, carbohydrates, and diterpenes, which likely contribute to its neuroprotective and anticonvulsant properties. Quantitative estimations confirmed notable flavonoid and phenol content, suggesting antioxidant mechanisms may underlie the observed pharmacological effects. These results indicate that *Clerodendrum serratum* holds promise as a natural source for developing novel antiepileptic agents, warranting further studies for isolation and characterization of active constituents.

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