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Evaluation of anti-epileptic action of hydro-methanolic extract of *Plumeria acuminata*

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Abstract
Epilepsy is a major neurological disorder characterized by recurrent seizures, and current antiepileptic drugs are often limited by adverse effects and resistance. The present study aimed to evaluate the anticonvulsant activity of hydro-methanolic flower extract of *Plumeria acuminata*. The extract was prepared using Soxhlet extraction and subjected to phytochemical screening, which confirmed the presence of alkaloids, flavonoids, phenolics, terpenoids, and sterols. Acute toxicity studies revealed the extract to be safe up to 1000 mg/kg. Antiepileptic activity was assessed using the maximal electroshock seizure (MES) model in Wistar albino rats. The extract at doses of 100 and 200 mg/kg significantly delayed the onset of hind limb extension and completely abolished clonus, indicating protection against generalized tonic-clonic seizures. These findings suggest that *Plumeria acuminata* flowers possess promising anticonvulsant potential, likely attributable to their phytoconstituents, and warrant further investigation for development as a natural antiepileptic agent.

Keywords: Epilepsy, Anticonvulsant, *Plumeria acuminate*, Hydro-methanolic extract, Maximal electroshock seizure (MES)

Introduction
Epilepsy is a chronic neurological disorder characterized by recurrent seizures resulting from abnormal neuronal activity in the brain [1]. Despite the availability of several synthetic antiepileptic drugs, their use is often limited by adverse effects, drug resistance, and high cost, which necessitates the search for safer and more effective alternatives [2]. Medicinal plants have long been explored as potential sources of bioactive compounds with anticonvulsant properties, owing to their diverse phytochemical constituents such as alkaloids, flavonoids, terpenes, and phenolics that can modulate neurotransmission and neuronal excitability [3].

Plumeria acuminata, commonly known as temple tree or frangipani, is widely cultivated in tropical regions and has been reported to possess multiple pharmacological activities including anti-inflammatory, antioxidant, and antimicrobial effects [4, 5]. Phytochemical investigations of *Plumeria* species have revealed the presence of flavonoids, terpenoids, sterols, and phenolic compounds, many of which are known to influence central nervous system function. However, despite these promising phytoconstituents, there is a paucity of scientific literature evaluating the antiepileptic potential of *Plumeria acuminata* flowers [6, 7].

Given this gap, the present study was undertaken to prepare a hydro-methanolic extract of *Plumeria acuminata* flowers and to investigate its anticonvulsant activity using animal models. By assessing its effect on maximal electroshock (MES)-induced seizures in Wistar albino rats, the study aims to provide experimental evidence supporting the traditional use of plant-derived compounds in epilepsy management and to highlight *Plumeria acuminata* as a potential candidate for further pharmacological development.

The objective is to prepare hydro-methanolic flower extract of *Plumeria acuminata* and to assess the anticonvulsant action of the same by evaluating its effect on animal models.

Material and Method
Collection and identification of the plant material
The flower of *Plumeria acuminata* were collected from the local surroundings of Gwalior, Madhya Pradesh in the month of march and authenticated by botanist at RB Science, Bhopal. The authenticated plant flowers were washed with distilled water and were dried under shade. The dried flowers were powdered using a hand blender at low speed.

Extraction of flower [8]
As previous studies have indicated the presence of flavonoids in the alcoholic extract, hydro-methanolic extraction was carried out using soxhlet apparatus. The powdered leaves were used for the extraction process. 78 g of powder was evenly packed in the extractor of the soxhlet apparatus and extracted using ethanol by hot continuous extraction process for about 11 h. The extract was filtered while hot using Whatman filter paper to make it free from impurities. The volume of solvent was reduced using a rotary vacuum evaporator. The remaining solvent was evaporated on water bath to obtain the resinous extract which was dried in desiccator to obtain dry extract [9]

Preliminary phytochemical screening [9]
The extract was evaluated by phytochemical qualitative reactions for identifying the presence or absence of usual plant secondary metabolites. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

Total flavonoid Content [10]
The determination of total flavonoids content was carried out by aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. The absorbance of

these solutions at 420 nm was determined after mixing each with 1 mL of 2% $AlCl_3$ solution and standing for 1 hour. Calibration curve was plotted for concentration against absorbance and the regression equation was used for calculation of the flavonoid content of the extract equivalent to quercetin. To determine flavonoid in extract, 10 mg extract dissolved in 10 ml methanol and filter. 3 ml of this extract solution was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract and allowed to stand for 60 min at room temperature. The absorbance was measured at 420 nm.

Animals

Healthy adult Wistar albino rats of either sex weighing 180-250g were selected for the study. The animals were housed in large, spacious, hygienic cages during the course of experimental period. The animal house was maintained properly and the animals had 12 ± 1 hour day and night schedule with a temperature [64-79°F] maintained at standard experimental condition. The animals were been fed with standard rodent pellet feed and water ad libitum. The animals were fasted 12 hours prior to the experiment with free access to only water.

Acute Toxicity Study

Rats were kept overnight fasting prior to drug administration. A total of five animals were used which received a single oral dose (2000mg/kg) of hydro-methanolic extract of the flower of *Plumeria acuminata*. After administration of the test extract, food was withheld further 3–4hr. Animals were observed individually at least once during the first 30min fter dosing, periodically during the first 24hr (with special attention during the first 4hr) and daily thereafter for a period of 14days. Once daily, cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks. LD50 was done as per OECD guidelines for fixing the dose for biological evaluation.

Maximal electroshock seizure [MES] model [11]

Experimental design

Wistar albino rats weighed around 150-250g were used for the study. Rats were divided into four groups of 5 animals each.

Group I - Vehicle control [Equivalent normal saline, oral]
Group II - Standard [Diazepam 5 mg/Kg, oral]
Group III - *Plumaria acuminata* extract (PAE), 75 mg/kg orally
Group III - *Plumaria acuminata* extract (PAE), 150 mg/kg orally

Animals in the control group [Group 1] were been administered equivalent volume of normal saline by oral route. Animals in Group 2 were been administered standard drug Daizepam. In Groups 3 and 4 *Plumaria acuminata* extract (PAE) (75 and 150 mg/kg) was administered by oral route in 1% Sodium lauryl sulphate solution respectively.

After 30 minutes of administration of above drugs, all the rats were been given electroshock with electro convulsiometer through ear electrodes [after moistening the ear of animals with drop of normal saline] at intensity of 150 mA, 60Hz for 0.2 seconds. Thereafter the animal were observed for number of convulsions. The percent protection and duration of tonic hind limb extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension.

Results and Discussion

Extraction Yield

The yield of the dried hydro-methanolic extract was found to be 16.92% (13.2 g) with reference to the weight to the dried flower powder used for extraction. The extract was dark yellow in color and slightly sticky in texture.

Phytochemical Screening

The findings of the phytochemical analysis suggest the presence of alkaloids, phenolics, terpenoids, sterols, and flavonoids in the hydro-methanolic extract of the flowers. The presence of flavonols along with other chemical constituents in the leaves of the *P. acuminata* has also been reported in previous studies (Table 1).

Table 1: Phytochemical screening of *Plumeria acuminata* flower extract

Chemical Tests	Observation	Result	Inference
Mayer's reagent	cream colour precipitate	+	Alkaloid Present
Hager's reagent	yellow colour precipitate	+	
Wagner's reagent	reddish brown precipitate	+	
Dragendorff's reagent	reddish brown precipitate	+	
Froth test	Frothing is seen	-	Glycoside Absent
Kedde's Test	No color	-	
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-	
Keller-Kiliani	No color in acetic acid layer	-	
Ferric chloride	Blue green color	+	Phenolics and Tannins Present
Gelatin Solution	White precipitate	+	
Alkaline reagent test	Yellow to red precipitate	+	
Vanillin HCl test	Purplish red color	+	
Shinoda test	red color	+	Flavonoids Present
Alkaline reagent test	Yellow color that turns red on acidification	+	
Zinc HCl reductino test	red color	+	
Millon's Test	white precipitate, turns red on heating	-	Protein Absent
Ninhydrin Test	Voilet color	-	
Salkowski Test	Yellow color in lower layer	+	Sterol Present

+ indicates a positive observation; - indicates a negative observation

Total flavonoid Content

The total flavonoid content of the extract of *Plumeria acuminata* flower was calculated to be 15.38 mg/100 mg quercetin equivalent.

Acute Toxicity Study

The acute toxicity test was performed by using the hydro-methanolic extract at concentrations 2000 mg/kg and 1000 mg/kg. As it is a natural substance and is not expected to be particularly toxic. Hence 2000 mg/kg of the test animal was administered orally. And 1 animal was found to be dead. As mortality was observed after administration of 2gm/kg body weight, then a lower dose of 1000 mg/kg was administered and no signs of toxicity was observed following administration of 1000 mg/kg. Hence *Plumeria acuminata* extract was found to be safe at 1000 mg/kg.

Antiepileptic Activity

The maximal electroshock induced convulsion in animals represents grand mal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure[12]. The result of the present study shows that the chloroform extract of *Plumeria acuminata* at doses 100 and 200 mg/kg significantly belated the onset of hind limb extension and decreased the duration of extension (table 2).

Table 2: Effect of *Plumeria acuminata* extract on onset of hind limb extension an clonus in MES model

Group	Onset Time (sec)		Recovery/Mortality
	Extension	Clonus	
I (Control)	4.84 ± 0.167	29.88 ± 1.207	Recovery
II (Standard)	17.54 ± 0.680	0	Recovery
PAE 100	10.53 ± 0.695	0	Recovery
PAE 200	13.64 ± 0.444	0	Recovery

Values represented as average ± standard deviation of 5 readings; 0 represents no occurrence of clonus

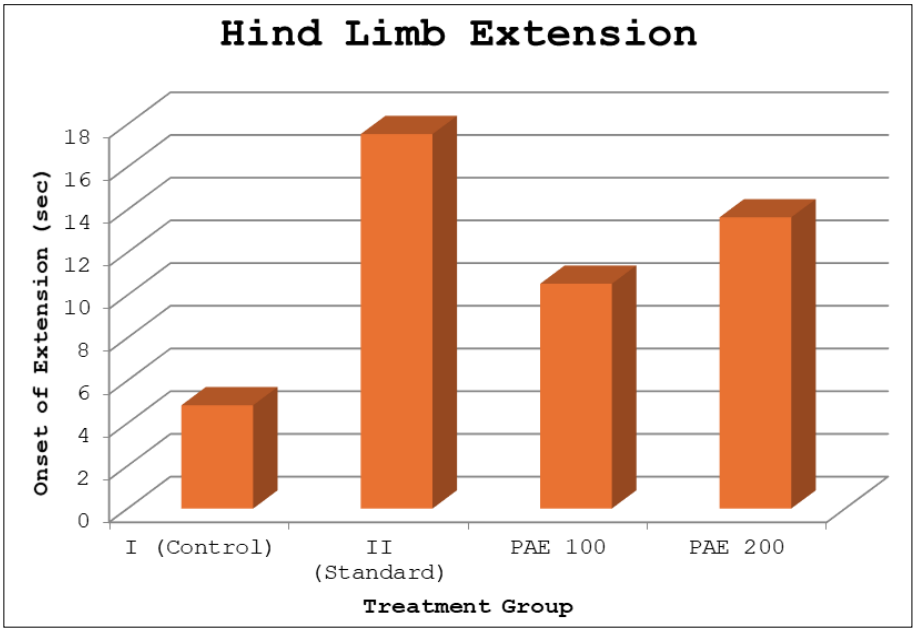

Fig 1: Effect of *Plumeria acuminata* on hind limb extension onset in MES model

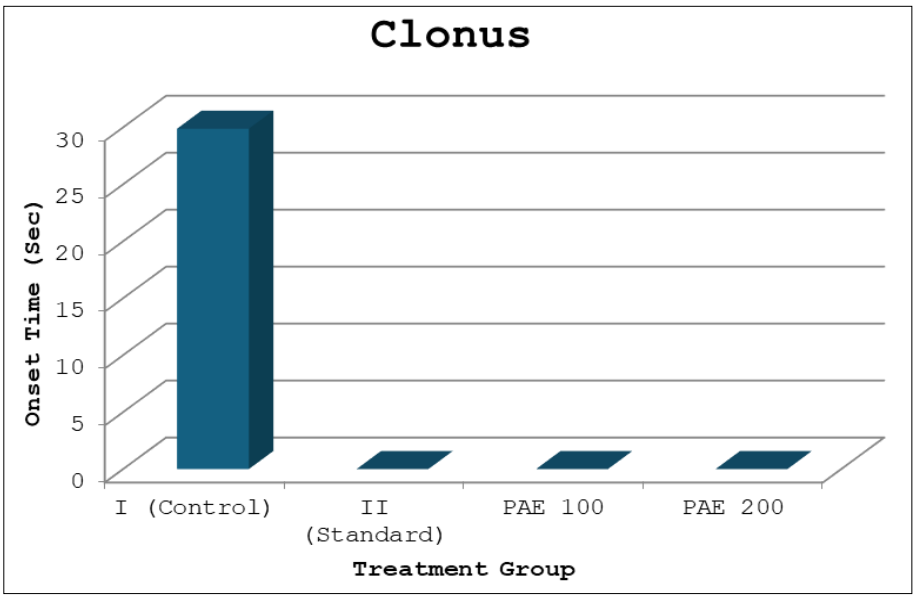

Fig 2: Effect of *Plumeria acuminata* on clonus onset in MES model

As seen from the figure 2 both doses of *Plumeria acuminata* flower extract (hydro-methanolic) were able to completely abolish the phase of convulsion in MES induced convulsion (clonus, involuntary muscle movement) models.

Conclusion

The hydro-methanolic extract of *Plumeria acuminata* flowers demonstrated significant antiepileptic activity in the MES model, as evidenced by delayed onset of hind limb extension and complete abolition of clonus. The phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, and phenolics, which are known to modulate neurotransmission and may contribute to the observed anticonvulsant effects. Acute toxicity studies established the safety of the extract up to 1000 mg/kg, supporting its potential for therapeutic use. Overall, the study provides experimental evidence that *Plumeria acuminata* flowers could serve as a promising natural source for antiepileptic drug development. Further studies involving isolation of active constituents, mechanism of action, and clinical evaluation are recommended to validate and expand upon these findings.

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