*wjpmr, 2023, 9(8), 169-174*

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

# ANTIEPILEPTIC ACTIVITY OF CALOTROPIS PROCERA LINN FLOWERS

**Madhulatha B.[1], Shiva Nandini D.[2], Meghana D.[3], Madhulikha B.[4] and Kotresh Yaligar*[1]**

[1]Associate Professor, Department of Pharmacognosy, Marri Laxman Reddy Institute of Pharmacy, Hyderabad 500043, Telangana, India.

[2,3,4]B.Pharmacy, Department of Pharmacy, Marri Laxman Reddy Institute of Pharmacy, Hyderabad 500043, Telangana, India.

***Corresponding Author: Kotresh Yaligar**

Associate Professor, Department of Pharmacognosy, Marri Laxman Reddy Institute of Pharmacy, Hyderabad 500043, Telangana, India.

**Article Received on 29/05/2023** **Article Revised on 19/06/2023** **Article Accepted on 09/07/2023**

## ABSTRACT

The present study aimed to investigate the phytochemical screening and pharmacological evaluation of the ethanolic extract of *Calotropis procera* Linn. (EEFCP) and petroleum ether extract of the flower of *Calotropis procera Linn*. (PEFCP). The plant materials were collected, authenticated, andsubjected to shade drying then made into coarse powder. The powder plant material is subjected to cold maceration and extracted with soxhlet apparatus by using ethanol (90%) & petroleum ether to get crude extracts. These extracts are subjected to preliminary phytochemical investigation and pharmacological evaluation of antiepileptic activity on *Swiss albino* mice. The acute toxicity studies were carried out to evaluate the drug's toxicity and it shows toxicity ata dose of 4500 mg/kg b.w. The antiepileptic activity of flower extracts of *Calotropis procera Linn*. Was evaluated by using Maximum Electric Shock (MES) induced epilepsy & Chemical Induced Model (Strychnine Induced Epilepsy) in *Swiss albino* mice have been conducted at a dose of 100 mg/kg b.w which shows a significant level of *p<0.05 & **p<0.01 was observed in the test drug at when compared with a standard & control group.

**KEYWORDS:** *Calotropis procera* Linn. MES, Antiepileptic activity, Flowers, Strychnine, etc.

## INTRODUCTION

Herbal medicines not only provide nutrition, but also when needed by the by they also strengthen and support the action of the digestive system, speeding up rate of processing food and improving the absorption of nutrients once taken in the body, nutrients are medicines are carried to the body's estimated 3 trillion cells. The circulatory system has a remarkable ability to adapt to an endlessly shifting pattern of demand.[1]

India is one of the world's important biodiversity center with the presence of over 45,000 different plant species. India's diversity is unmatched due to presence of 16 different climatic zones, 10 with vegetation zones, 25 biotic provinces find 426 biomes [habitats of specific species]. Of these, about 15,000 to 20,000 plants have good medicinal value, only 7000 to 75,000 species are used for their medicinal values by traditional communities. The ayurvedic, unani, sidda, amchi and modern systems of medicine 700, 700, 600, 600, 30 species of plants respectively.

About 8000 herbal remedies have been codified in Ayurvedic system of medicine. The rigveda has recorded 67 medicinal plants, yajurveda 81 plant, species, Athravavedha 290 species, charak Samhita and Sushrut. Samhita had described the properties and uses of 1100 and 1270 species respectively, in the compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine.[2]
Our country is perhaps the largest producer of medicinal herbs and is so-called Botanical Garden of the world. It is estimated that, today the plant materials are known to provide the models for 50% of allopathic drugs.[3] Today there are at least 120 distinct chemical substances derived from the plants that are considered as important herbal drugs.[4]

Epilepsy is one of the most common diseases of the brain, affecting at least 50 million persons worldwide. There is a group of CNS disorders characterized by proximal cerebral dysrhythmia, manifesting as brief episodes [seizures] of loss of disturbances of consciousness, with or without characteristic body movements [called convulsions], sensory or psychiatric phenomena as well as excessive ECG electroencephalogram discharge. It is also characterized by violent spasmodic contractions and relaxations skeletal muscle rapidly and repeatedly and autonomic hyperactivity call conversion stop it has a focal origin in the brain. Manifestations depend on the side of the focus,

region into which he discharges spread and postictal depression of these regions. The patients with epilepsy all conversions may experience a warning signal called 'aura' if a queer feeling known only to him and reveals him the time attack is impending. This is usual colors, smells of songs. Some people may utter a cry please stop the term conversion is sometimes used as a synonym for season however not are epileptic seizures lead to convulsions and not all conversions are caused by epileptic seizures.[5-8]

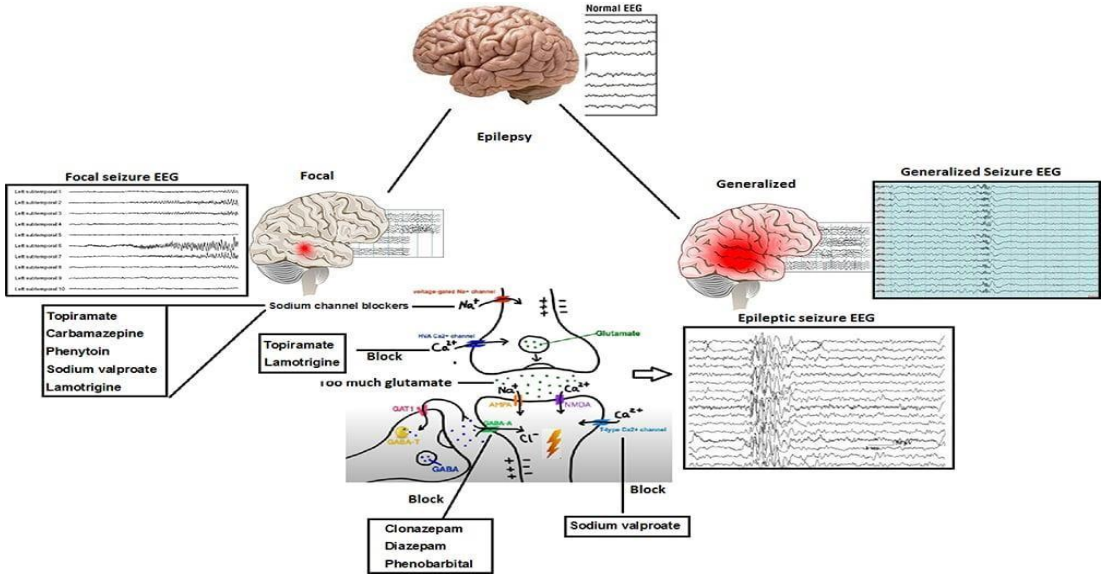


**Fig. 1: Mechanism of epilepsy.**

**Causes of seizures[9]**

Alcohol, barbiturates, intoxication or withdrawal, brain illness or injury, brain tumor [rare], choking, drug abuse, electric shock, epilepsy, fever [particularly in young children], head injury, heart diseases, heat illness, low blood sugar, meningitis, poisoning, stroke, taxemia of pregnancy, uremia related to kidney failure, very high blood pressure [malignant hypertension], venomous bites and stings, withdrawal from benzodiazepines [such as valium].

**Symptoms[9]**

Brief blackout followed by period of confusion, drooling or frothing at the mouth, grunting and snorting, loss of bladder or bowel control, sudden falling, teeth clenching, temporary heart in breathing, uncomfortable much spells with twitching and jerking limbs, and issue behavior like sudden anger sudden laughter or picking up clothing. The person may have warning symptoms before attack, which may consist of fear or anxiety, nausea, vertigo, visual symptoms [such as flashing bright lights, spots or wavy lines before the eyes].

**Types of seizures[10]**

**a. Generalized seizures**

i. Generalized tonic-clonic seizures [major epilepsy, grandmal]: Commonest,1-2 minutes. The usual sequences is aura- cry- unconscious tonic spasm of body musclesclonic jerking followed by prolonged sleep and depression of all CNS functions.
ii. Absence seizures [minor epilepsy, petitmal]: Prevalent in children, lasts about half a minute. Momentary loss of consciousness, patient apparently freezes and stares in one direction, no muscular component or little bilateral jerking. EEG shows characteristic 3 cycles per second spike and wave pattern.
iii. Atonic seizures [Akinetic epilepsy]: Unconsciousness with relaxation of all muscles due to excessive inhibitory discharges. Patients may fall.
iv. Myoclonic seizures: Shock line momentary contraction of muscles of a limb or whole body.

**b. Partial seizures**

i. Simple partial seizures [SPS, cortical focal epilepsy]: Last half a minute to one minute often secondary. Convulsions are confined to a group of muscles or localized sensory disturbance depending on the area of cortex involved in the seizure, without loss of consciousness.
ii. Simple partial or complex partial seizures second secondarily generalized: The partial seizure occurs first and evolves into generalized tonic clonic seizures with loss of consciousness. Most of cases are primary [idiopatic], some maybe secondary to trauma/surgery on the head, intra-cranial tumour, tuberculoma, cysticercosis, cerebral isehaemia, etc. treatment is symptomatic and the same whether epilepsy is primary or secondary.

**Risk factors[11]**

Sleep depression, Missed doses of anti-epileptic drugs (AED'S), Alcohol withdrawals, recreation drug misuse, Physical and mental exhaustion, Flickering lights (includes TV, computer screeners, comes under generalized epilepsy), Interconnect infections, Metabolic disturbances, uncommon reasons like loud noises, very hot baths etc.

**Side effects**[11]
Tiredness, Stomach upset, Dizziness, Blurred vision, Urinary retention, Sexual dysfunction, Nausea, and fatigue, etc.

## MATERIALS AND METHODS

**a. Collection, Authentication, and Drying of plant material**
The Calotropis procera Linn flowers were collected from in and around the dundigal region, Hyderabad. The herbarium of *Calotropis procera* Linn. with voucher specimen (MLRIP/P.COG/HERBARIUM/CP/2023) was submitted and preserved in the Marri Laxman Reddy Institute of Pharmacy. The collected plant material is washed thoroughly with purified water, dried under shade at room temperature, and powdered using a hand mill to make a coarse powder. Then they are stored in a well-closed light-resistant container until further use.

**b. Extraction & Preparation of the extracts**[12-13]
**i. Cold maceration:** 200 gm of powdered plant material is subjected for cold maceration for 5 days with 600ml of alcohol (ethanol 90%). The solvent was then separated by filtration and the marc is air-dried.
**ii. Soxhlet Extraction:** The air-dried marc was subjected to extraction with petroleum ether using the Soxhlet apparatus at 50 degrees centigrade. Materials were extracted until liquid in the side arm of the Soxhlet apparatus became colorless. The extract is then dried in reduced pressure using a vacuum. Then marc extract is collected and should be subjected to extraction with ethanol (90%) for 6-8 hours.
**c. Preparation of plant extracts for biological screening:** Alcohol is the moderately polar solvent utilized to extract various groups of compounds present in the crude drug. In this process, Ethanol (90%) and Petroleum ether are used to obtain Ethanolic and petroleum ether extracts respectively.
**d. Preliminary phytochemical screening**[14]**:** The above obtained ethanolic extract and petroleum ether extracts were screened for the presence of phytoconstituents.

**e. Pharmacological evaluation**
**Experimental animals**
*Swiss albino* mice of either sex between 18-22 g were used for the experimental work. Institutional Animal Ethical Committee approved the experimental protocol. Animals were maintained under standard conditions husbandry, room temperature of 24±2 degree centigrade, relative humidity of 45-55%, 12 hours dark-light cycle, in an animal house approved by the The Committee for the Control and Supervision of Experimental Animals (CCSEA). Animals were obtained from the central animal house, Marri Laxman Reddy Institute of Pharmacy, Dundigal. The animals had free access to standard diet and water and were housed in the poly-propylene cages. All the animals were kept fasting 12 hours prior to the experiment but allowed to free access to water. Following permission from the IAEC (Institutional Animal Ethical Committee) and CCSEA standards for the use and care of experimental models, all experimental procedures were carried out (1567/PO/RE/S/11/CPCSEA).

**Toxicity studies**[15]
The acute toxicity study was performed according to the OPPTS (Health Effect Test Guideline 2004, Office of prevention, pesticide and toxic substance) by Up and Down procedure (OECD 423) using *Swiss albino* mice of either sex. The petroleum ether and ethanolic extracts were suspended in Tween 80 (1%w/v) and administered orally. The safe dose was found to be up to 4500mg/kg.

**Evaluation of antiepileptic activity**
The antiepileptic activity was evaluated using Petroleum ether and Ethanolic flower extracts of *Calotropis procera* Linn.

**i. Maximum electric shock induced epilepsy**[16-18]
The Maximal electroshock convulsion model was used to evaluate the antiepileptic activity of the extract. Seizures was induced in mice by transauricular electroshock of 30 mA for 0.2 sec by means of an Electroconvulsiometer through a pair of crocodile ear clips. Four groups of mice (n=6), were treated with varying extracts and 60 min after injecting were subjected for MES seizure response. Duration of tonic hind limb extension was noted in all groups.
Group 1recieved 0.9% NaCl solution (1ml/100gm, b.w, p.o)-served as control.
Group 2 received phenytoin (25mg/kg, b.w, i.p)-served as standard.
Group 3 received (EEFCP) Ethanolic extract of flower of *Calotropis procera* Linn. (100mg/kg, b.w, p.o)-served as test.
Group 4 received (PEFCP) Petroleum ether flower extract of *Calotropis procera* Linn. (100mg/kg, b.w, p.o)-served as test.

**ii. Strychnine induced epilepsy**[19,20]
The mice in this study were categorized into 4 groups (n=6). The mice received strychnine only (2mg/kg, i.p) for the induction of seizures.
Group 1 received 0.9%NaCl solution (1ml/100mg, b.w, p.o)-served as control.
Group 2 received diazepam (2mg/kg, b.w, i.p)-served as standard.
Group 3 received (EEFCP) Ethanolic extract of flower of *Calotropis procera* Linn. (100mg/kg, b.w, p.o)-served as test.
Group 4 received (PEFCP) Petroleum ether flower extract of *Calotropis procera* Linn. (100mg/kg, b.w, p.o)-served as test.

## RESULTS

The present study attempts the extraction, preliminary phytochemical studies and pharmacological evaluation of antiepileptic activity of flowers of *Calotropis procera* Linn. In this study an attempt is made to evaluate the antiepileptic activity of petroleum ether and ethanolic

extract by using Maximal Electric shock induced epilepsy *(in-vivo)* and strychnine-induced epilepsy model in *Swiss albino mice*.

**Collection and Authentication**

The flowers of Calotropis procera Linn. collected from in and around dundigal region, Hyderabad. In India, the plant is found mostly on the dry and barren hills occurrence of the species is between 8 degrees N and 31 degrees and 600 to 800 mts respectively. The plant material was authenticated by B.Madhulatha, Associate Professor, Department of Pharmacognosy. The plant material was subjected for shade-drying and powdered. The herbarium of *Calotropis procera* Linn. with voucher specimen was submitted and preserved in Marri Laxman Reddy Institute of Pharmacy.

**Extraction & Preparation of extracts**

Alcohol is the moderately polar solvent utilized to extract various groups of compounds present in the crude drug. In this extraction process, the cold maceration and soxhlet extraction method by using ethanol (90%) and petroleum ether as solvents are used to obtain ethanolic and petroleum ether extracts respectively.

**Preliminary Phytochemical Screening:** Preliminary Phytochemical Screening reveals the presence of alkaloids, cardiac glycosides, flavanoids, tannins, triterpeniods, carbhohydrates, and saponins in ethanolic extract and steroids, triterpenoids, saponins, tannins in petroleum ether extract.

**Evaluation of antiepileptic activity**

Epilepsy is one of the CNS disorder affecting brain, which is characterized by paroxysmal cerebral dysrhythmia, brief episodes of loss or disturbances of consciousness with or without characteristic body movements with excessive EEG discharge. Epilepsy occurs due to imbalance between excitatory and inhibitory influences in the brain. Inhibitory influence involve GABA as the neurotransmitter and increases the extra-cellular K+ and excitatory neurotransmitters involve opening of voltage-dependent Na+ channels.[21]

**Maximal electric shock induced epilepsy**

The Maximal electroshock convulsion model was used to evaluate the antiepileptic activity of the extract. Seizures was induced in mice by transauricular electroshock of 30 at a duration of 0.2 sec by means of electroconvulsiometer through a pair of corneal ear clips. Four groups of mice (n=3), were treated with varying extracts and 60 min after injecting were subjected for MES seizure response. Duration of tonic hind limb extension was noted in all groups.

- Group 1recieved 0.9% NaCl solution (1ml/100gm, b.w, p.o) served as control.
- Group 2 received phenytoin (25mg/kg, b.w, i.p) served as standard.
- Group 3 received EEFCP Ethanolic extract of flower of *Calotropis procera* Linn. (100mg/kg, b.w, p.o) served as test.
- Group 4 received PEFCP Petroleum ether flower extract of *Calotropis procera* Linn. (100mg/kg, b.w, p.o) served as test.

**Table 1: Effect of ethanolic flower extract and petroleum ether extract of *Calotropis procera* Linn. on Maximal Electric shock induced epilepsy in mice after 60 minutes.**

| S.NO | Groups | Flexion (in sec) | Extension (in sec) | Clonus (in sec) | Stupor (in sec) | Recovery/Death (in sec) |
|---|---|---|---|---|---|---|
| 1. | Control | 3.47±0.49 | 18.10±1.87 | 28.82±3.38 | 53.24±2.50 | 116.67±0.47 |
| 2. | Phenytoin (25mg/kg) | 5.95±0.56** | 0.00±0.56** | 0.00±1.03 ** | 0.00±1.03** | 16.04±3.59** |
| 3. | EEFCP (100mg/kg) | 2.78±0.89** | 5.58±0.45** | 18.03±0.8 4** | 30.41±1.46** | 39.61±1.17** |
| 4. | PEFCP (100mg/kg) | 2.51±4.41** | 7.14±3.95** | 21.02±29.16* | 35.63±11.04* | 72.75±10.35** |

Values represent the mean±SD of animals (Calculated by using one-way ANNOVA software)
EEFCP –Ethanolic flower extract of Calotropis procera Linn,
PEFCP-Petroleum flower extract of *Calotropis procera* Linn.
*=p<0.05, **=p<0.01 (the mean difference was considered significant at 0.01 & 0.05 level).

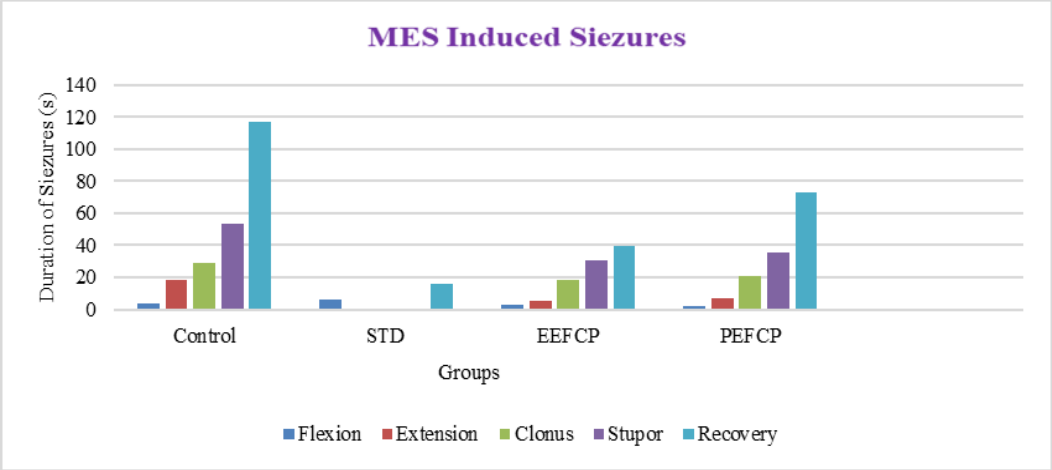


**Fig. 2: Histograph showing effect of petroleum ether and ethanolic flower extracts of *Calotropis procera* Linn. on Maximal Electro shock induced epilepsy in mice after 60 minutes.**

**Strychnine induced epilepsy**

The mice in this study were categorized into 4 groups (n=6).

Group 1 received 0.9% NaCl solution (1ml/100mg, b.w, p.o) served as control.

Group 2 received diazepam (2mg/kg, b.w, i.p) served as standard.

Group 3 received (EEFCP) Ethanolic extract of flower of *Calotropis procera* Linn. (100mg/kg, b.w, p.o) served as test.

Group 4 received (PEFCP) Petroleum ether flower extract of *Calotropis procera* Linn. (100mg/kg, b.w, p.o) served as test.

The animals in all groups received strychnine at a dose of 2 mg/kg, i.p after 60min for the induction of epilepsy.

**Table 2: Effect of petroleum ether and ethanolic flower extracts of *Calotropis procera* Linn. on strychnine induced epilepsy in mice after 60 minutes.**

| S.NO | Groups | Onset of convulsions (in sec) | Duration of convulsions (in sec) | Recovery/death |
|---|---|---|---|---|
| 1. | Control | 4.71±0.58 | 36.09±0.58 | Recovered |
| 2. | Diazepam (5mg/kg) | 22.06±0.57** | 12.71±0.57** | Recovered |
| 3. | EEFCP (100mg/kg) | 13.52±1.47** | 30.40±1.47** | Recovered |
| 4. | PEFCP (100mg/kg) | 8.62±0.67** | 16.31±0.67** | Recovered |

Values represent the mean±SD of animals (Calculated by using one-way ANNOVA software

EEFCP –Ethanolic flower extract of *Calotropis procera* Linn.

PEFCP-Petroleum flower extract of *Calotropis procera* Linn.

*=p<0.05 **= p<0.01(the mean difference was considered significant at 0.01 & 0.05 levels)

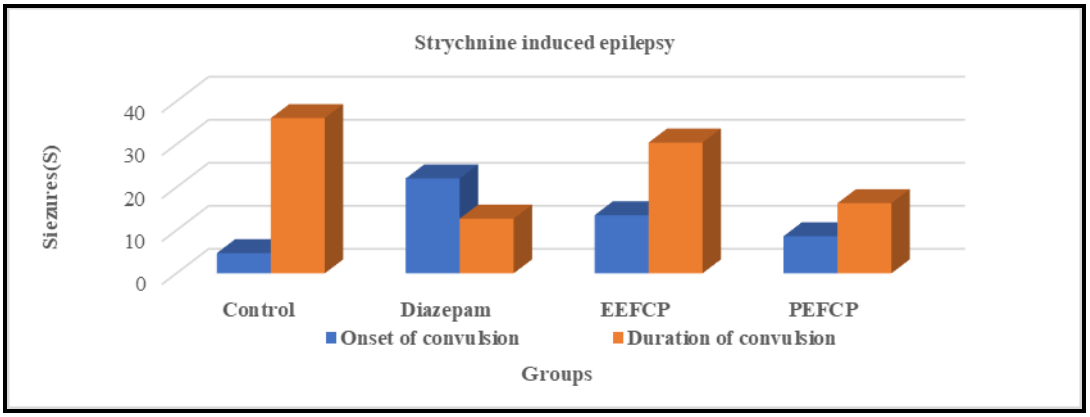


**Fig. 3: Histograph showing effect of petroleum ether and ethanolic flower extracts of *Calotropis procera* Linn. on strychnine induced epilepsy in mice after 60 minutes.**

## CONCLUSION

In the present study preliminary phytochemical investigation and anticonvulsant activity of the petroleum ether and ethanolic extract of *Calotropis procera* Linn. flowers has been evaluated. In preliminary phytochemical analysis presence of alkaloids, glycosides, flavonoids, tannins, triterpenoids, carbohydrates and saponins were observed in petroleum ether and ethanolic flower extracts. Antiepileptic activity was performed using Maximal Electroshock (MES) induced epilepsy and chemical induced epilepsy i.e.Strychnine induced epilepsy model. All the treated groups showed reduction in duration of flexion, extension, clonus, and stupor phases. The petroleum ether and ethanolic extract of *Calotropis procera* Linn. showed significant ($p<0.01$) and ($p<0.05$) activity at 100mg/kg b.w. respectively. In strychnine model antiepileptic activity was confirmed by the reaction in the onset of seizures and duration of convulsions as well as by the restoration towards the normal condition of the mice compared to the normal & standard groups.

The potency of antiepileptic activity was found with petroleum ether and ethanolic flower extracts at a dose of 100mg/kg bw. All the results indicate the potency of antiepileptic effect in absence seizures and in tonic-clonic seizures. All the studied parameters in the present work clearly substantiate the traditional claim of antiepileptic property of *Calotropis procera* Linn. moreover, the study clearly shows the petroleum ether and ethanolic extract of *Calotropis procera* Linn. flowers has got a significant effects at a level of *=$p<0.05$, **=$p<0.01$ of antiepileptic activity when compared to control & standard groups.

The present study provides the scientific evidence in support of the antiepileptic activity of herbal extracts. The findings support the traditional claims of medicinal plants. Many phytochemicals present in the plant material are observed to be the reason for their antiepileptic activity. This research work can establish a support the plant material to have antiepileptic activity and the observation of finding phytochemicals can prove a route for the establishment of various phytochemicals present in them which are responsible for the activity. Further isolation and purification of these compounds can be worth to find out compounds specifically useful for their activity can be formulated into a dosage form.

## CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

## ACKNOWLEDGMENTS

The authors would like to thanks to Dr.N.Jyothi, principal & professor, Marri laxman reddy institute of pharmacy, Dr.Arunabha Mallik, vice principal and professor, Marri laxman reddy institute of pharmacy for their kind support during pharmacological studies.

## REFERENCES

1. Sammbamurthy. A.V.S.S. Dictionary of medicinal plants. 1sted. New delhi: Satish kumar Jain for cbs publishers and distributors, 2006; 1.
2. http://www.herbalmedicine.in/herbal-medicine-cures-treatment.html
3. Analgesic. WebMD. Last Updated: February 15, 2006. Retrieved on September 19, 2007.
4. "Analgesic." mediLexicon. (c) 2007. Retrieved on September 19, 2007.
5. Goyal RK. Elements of 0Pharmacology.Sixteenth edition, 2006-2007: 280-283.
6. Tripati KD. Essentials of Medical Pharmacology.5th edition, 2003; 369-380.
7. http://www.Wikipedia.com the free encyclopedia.
8. 8.http://www.nlm.nih.gov/medlineplus/ency/artcle/000021.htm.
9. https://www.mayoclinic.org/diseases-conditions/epilepsy/symptoms-causes/syc-20350093.
10. https://www.myupchar.com/en/disease/convulsions.
11. https://www.ninds.nih.gov/health-information/disorders/epilepsy-and-seizures.
12. Prusti A and Mishra SR, Botanical Leaflets, 2008; 12: 227-230.
13. Wallia TE. Text book of Pharmacognosy. 5thed. New delhi: CBS Publishers and Distributors, 1985.
14. Khandelwal KR, Kokate CK, Pawarv AP, Gokhle SB. Practical pharmacognosy.1st ed. Nirali prakashan,1995.
15. Ashutosh Kumar. Acute and subacute toxicity stud of Ethanolic extract of Calotropis procera (Aiton) Dryand flower in Swiss albino mice. Phytomedicine Plus, May 2022; 2(2): 100-224.
16. Mayur Powal. Anticonvulsant effect of Annona squamosa leaves in mice. Pharmacologyonline, 2011; 44-52.
17. Darpan Kaushik. Anticonvulsant activity of Bacopa monniera in rodents. 07th April 2009.
18. Karunakar Hegdge. Anticonvulsant Activity of Carissa carandas Linn Root Extract in Experimental Mice. Tropical Journal of Pharmaceutical Research, April 2009; 8(2): 117125.
19. Ahmed Salim Mahmood. Antiepileptic effect of Neuroaid on Strychnine-Induced Convulsions in Mice. Pharmaceuticals, 2022; 15: 1468.
20. Balamurugan G, Muralidharan P, Selvarajan S. Antiepileptic activity of poly herbal extract from Indian medicinal plants. Journal of Scientific Research, 2009; 1(1): 153-159.
21. Duraisami R, Srinivasam D, Ramaswamy S. Anticonvulsant activity of Bioflavanoid gossypin. Bangladesh Journal of Pharmacology, 2009; 4: 51-54.