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Bioprospecting for propitious, safe antiepileptic agents from the leaf and root extracts of *Calotropis procera* (Ait) R. Br.

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Abstract

Epilepsy is a prevalent non-communicable neurological disorder causing recurring seizures, affecting 50 million people globally. Treatments often have limited therapeutic ranges and adverse effects, making it difficult to differentiate from the underlying neurologic condition. *Calotropis procera* has drawn attention for its potential medicinal uses, including anticancer, anti-convulsant, and other therapeutic properties. This study aimed to evaluate the antioxidant, toxicity, and anti-epilepsy profile of *Calotropis procera.* Plant materials were pulverized and extracted sequentially with hexane, ethyl acetate, methanol, and water to yield a series of solvent fractions while another portion was extracted with ethanol using Soxhlet apparatus, and extracts were concentrated using a rotary evaporator. *C. procera* extracts show potent antioxidant activity, with root extract (IC50: 0.56 mg/ml) surpassing leaf extract (IC50: 0.38 mg/ml), suggesting therapeutic potential in neurodegenerative diseases. Following OECD guidelines, a dosing regimen was established for acute toxicity testing. *Calotropis procera* extracts were well-tolerated in mice. Notably, a 600 mg/kg dose of ethyl acetate extract delayed seizure onset, indicating promising antiepileptic potential. The antiepileptic effect was assessed using the Pentylenetetrazole (PTZ) induced seizure model, a standard test for evaluating anticonvulsant activity. The ethyl acetate root extract had a sedative effect, while, n-hexane, methanol, and aqueous extracts of both leaf and root produced no observable toxic signs. The plant extracts were well tolerated orally in laboratory mice. *Calotropis procera* ethyl acetate extract showed promising antiepileptic potential, as a 600 mg/kg dose delayed seizure onset in mice and was well-tolerated when administered orally. The result showed a promising antiepileptic potential of the *Calotropis procera* extract.

Keywords: Anticonvulsant, *Calotropis procera,* Epilepsy, Pentylenetetrazole, Toxicity

1. Introduction

Epilepsy is a neurological disorder that disrupts the brain's normal electrical activity, affecting communication between brain cells (Scharfman, 2007; Jefferys, 2010; Mondal et al., 2023). The prevalence of epilepsy is increasing globally, particularly in the third world, where it is stigmatized, leading to unemployment, increased medical costs, isolation, and discrimination (Engel, 2005; Gooch et al., 2017; Mondal et al., 2023). This highlights the need for effective and alternative treatment options.

2. Literature review

Neurodegenerative disorders, including epilepsy, are linked to free radical damage, which makes antioxidants valuable for neuroprotective strategies (Lalkovičová & Danielisová, 2016; Singh et al., 2019). There is potential for antioxidant compounds to act as neuroprotective agents. While antiepileptic drugs (AEDs) can reduce seizure frequency, they have side effects, drug interactions, and individual variations in response (Zaccara et al., 2007; Tatum, 2010; Cramer et al., 2010). Medicinal plants have the potential to boost cerebral blood flow and tolerance to hypoxia, as well as reduce abnormal brain electrical activity (Jalalpure, 2009; Hosseini & Hosseini, 2018; Bellavite, 2022). *Ficus platyphylla,* Ginseng, and *Pimpinella anisum* have all been demonstrated to reduce seizures and improve cerebral blood flow in studies (Pourgholami et al., 1999; Liu et al., 2017). ***Calotropis procera*** is an indigenous plant that has been used for treating epilepsy and offers a promising alternative to conventional AEDs (Sharma et al., 2013). There are various pharmacological properties of this plant (Jain *et al.,* 1996; Ali-Seyed & Ayesha, 2020), however, it also contains certain toxic components that can cause gastrointestinal problems (Tripathi & Singh, 2003; Farooq et al., 2017). The purpose of this research is to examine *Calotropis procera's* potential as an alternative epilepsy treatment by examining its effects on seizures, cerebral blood flow, and safety compared to conventional epilepsy treatments.

Plate 1: *Calotropis procera*

3. Research methodology

3.1. Materials

All reagents were of analytical grade and used without further purification. Also, solutions were prepared using distilled water.

3.2. Collection of *Calotropis procera*

Calotropis procera leaves and roots were collected from the Federal Housing Area, Lugbe, Abuja, Nigeria, and identified at the National Institute of Pharmaceutical Research and Development (NIPRD) Herbarium unit of Medicinal Plant Research & Traditional Medicine (MPR&TM)

Department, Idu, Abuja FCT, Nigeria with identification number NIPRD/H/7259. The plant leaves and roots were thoroughly cleaned, air-dried, pulverized, and safely kept for further use.

3.3. Extraction

The pulverized leaves and root of *C. procera* were each successively extracted using the soxhlet extraction method with solvents of increasing polarity viz, n-hexane, ethyl acetate, methanol, and distilled water. Other portions of the plant leaves and roots were extracted with ethanol using the maceration method. The different extracts were concentrated using a rotatory evaporator.

3.4. DPPH radical scavenging assay

The methanol extract's antioxidant activity was assessed using a UV-Visible Spectrophotometer at 517 nm (Olajide et al., 2013). The radical scavenging activity (RSA) was determined by measuring the percentage inhibition of DPPH (Sigma-Aldrich) discoloration, calculated using the following equation

$$\% \, Inhibition = \frac{Ab - Aa \; x \; 100}{Ab}$$

Ab is the absorption of the blank sample (without the extract) and Aa is the absorption of the extract.

3.5. Animals

Adult Swiss mice (both sexes), weighing 22±2 g, mice were obtained from NIPRD's Toxicology and Pharmacology Department, Abuja. They were housed in polypropylene cages under standard conditions, with *ad libitum* access to food and water. The mice were maintained on a standard rodent diet and water ad libitum, ensuring optimal conditions for the study. The experimental protocol was duly approved by the National Institute of Pharmaceutical Research and Development Committee on Animal Use and Care (ACE number: NIPRD. 05. 03. 43).

3.6. Acute toxicity study

Acute toxicity (OECD 2001 Guidelines). Ten groups of four mice were formed. They were fasted for three to four hours before being treated with the various extracts. The extract-treated mice were given different doses of extract at 150, 300, 600, and 2000 mg per kg body weight, whereas the control group of 4 animals received normal saline. Prior to administration, the extracts were dissolved with a solvent (3% tween 20). For four hours after administration, they were constantly monitored for poisoning symptoms such as tremors, sedation, ataxia, convulsions, hypnosis, and muscle spasms. The mice were further observed for 48 h to detect any death. The observation was extended for another 14 days to determine delayed toxicity.

3.7. Pentylenetetrazole (PTZ) induced seizure

The animals in each group were pretreated with 100, 200, and 400 mg/kg of *Cs procera* extract orally. After 30 mins, Pentylenetetrazole (PTZ)was administered at a dose of 80 mg/kg subcutaneously and observed for the convulsive behavior for 30 min. There were five parameters measured: onset, latency, clonus, tonic flexion (tonic hind limb extension), and mortality rate. In comparison with the respective control group, the efficacy of the test extract was measured as a prolonged latency at

the first mention period and decreased mortality percentage of each group. The latency to the onset of seizures is recorded in each mouse after PTZ administration. Additionally, the duration of seizures is recorded by observing the animal's behavior during seizures (Loscher, 1991).

Statistical analysis

Data are presented as mean ± SEM. One-Way ANOVA and Dunnett's post-hoc test were used for analysis. Statistical significance was set at $p < 0.05$.

4. Data analysis

Table 1: Antioxidant properties of crude extract of *C. procera* leaf and root

Conc mg/ml	% inhibiton root	% I nhibitionleaf	% Inhibition Vit C
1.0	**61.70**	**61.92**	**81.92**
0.5	**55.02**	**50.29**	**77.88**
0.25	**47.57**	**39.57**	**77.69**
0.125	**43.16**	**41.13**	**72.50**
0.0625	**43.16**	**32.83**	**60.38**

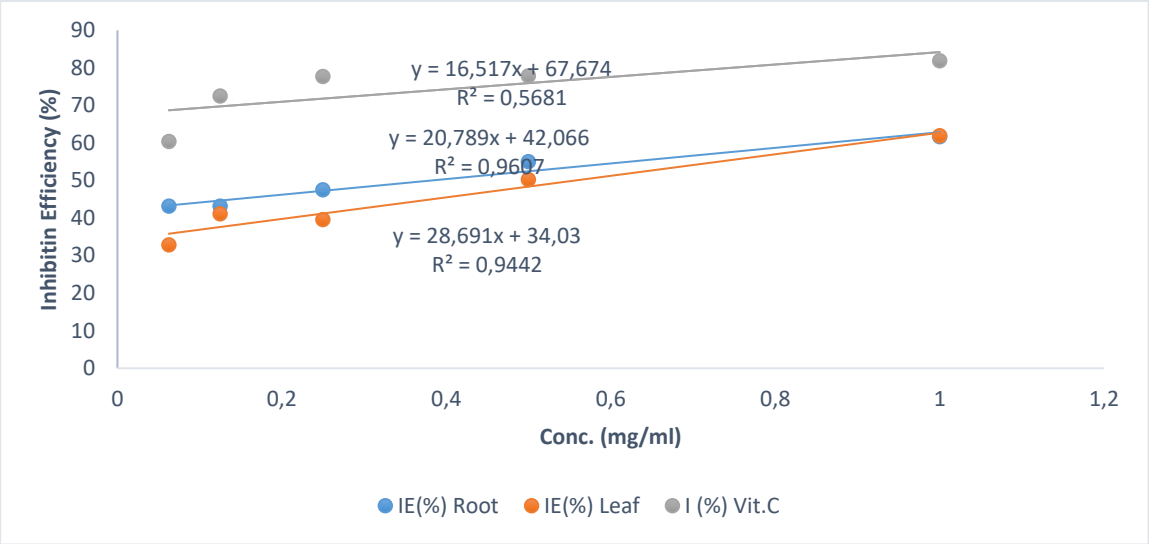

Figure 1: Graphical representation of antioxidant activity for methanolic crude leaf and root bark extracts of *C.procera*

Table 2: Toxicity and safety activity of *C.procera* leaves and root bark at dose of 2000 mg/kg

Sample	Observed Toxicity	Death
Aqueous root	No observed toxicity	0/4
Methanol root	No observed toxicity	0/4
Ethylacetate root	Decrease motility and sedation. active after 1 hour	0/4
n- Hexane root	No observed toxicity	0/4
Aqueous leaf	No observed toxicity	0/4
Methanol leaf	No observed toxicity	0/4
Ethylacetate leaf	No observed toxicity	0/4

n- Hexane leaf	No observed toxicity	0/4
leaf	No observed toxicity	
Ethanol root	Tremor, seizure, convulsion within 1 hour of administration	2/4
Ethanol leaf	Tremor,seizure,convulsion within 1 hour of administration	1/4

Oral toxicity using the OECD Limit dose of 2000 mg/kg. n=4

Table 3: Toxicity result of ethanol crude root extract of *C. procera* at reduced dosage

	Onset	time to THLE	survival
	41.00±4.28	81.60±4.85	0
150	47.20±4.75	123.80±16.22*	0
300	45.40±3.91	109.00±26.55	0
600	43.00±1.95	85.80±3.31	0

Table 4: Toxicity result of the ethanol crude leaf extract of *C. procera* at reduced dosages

	Onset	time to THLE	survival
	41.00±4.28	81.60±4.85	0
150	67.00±18.88	427.20±34.23	1
300	45.50±2.66	60.50±25.54	0
600	59.00±19.71	135.40± 29.59	0

Table 5: Anticonvulsant activity of *C.procera* methanolic crude leaf and root

Sample	Onset of seizure (s)	Time to THLE (s)	Survival
Control	41 ± 4.278	81.6 ± 4.854	0
Aqueous root	50 ± 3.661	100 ± 25.10	0
Methanol root	47.4 ± 4.179	170 ± 17.22	0
Ethylacetate root	69.4 ± 21.93	171.5 ± 54.21	2
n- Hexane root	62.4 ± 8.835	138. 8 ± 17.76	0
Aqueous leaf	46±4.528	158 ± 28.92	0
Methanol leaf	45.8±4.116	117.8±24.29	0
Ethylacetate leaf	46.2±4.532	130.2±25.72	0
n- Hexane leaf	41.6±4.885	109±17.19	0

5. Results and discussions

Epilepsy is associated with oxidative stress, which can result in chronic brain damage and increase the risk of developing neurodegenerative diseases like Alzheimer's and Parkinson's. Managing epilepsy effectively requires reducing oxidative stress to prevent these complications and improve quality of life (Parsons et al., 2022; Neri et al., 2022). *C.procera* exhibited antioxidant properties and

antiinflammatory effects, which may contribute to its role in reducing seizures (Sivapalan et al., 2023). Its antioxidant properties could aid in diminishing the production of pro-inflammatory molecules in the brain, potentially preventing seizure activity. The antioxidant activity of *C. procera* leaf and root extracts have been found to effectively scavenge free radicals, as shown in Table 1 and Figure 2, with the root extract demonstrating higher antioxidant activity (IC50: 0.56 mg/ml) compared to the leaf extract (IC50: 0.38 mg/ml). This suggests potential therapeutic benefits in neurodegenerative diseases, consistent with study (Rani et al., 2019). Notably, the root extract of *C. procera* demonstrated higher antioxidant activity compared to the leaf extract (Table 1 and Figure 1), attributed to bioactive compounds such as terpenoids, flavonoids, and phenolic acids (Kumar et al., 2013). Moreover, in the investigation relating to toxicity profile of the plant (Table 2), the experiments with rats indicated that *C.procera* varied extracts (methanol, n-hexane, and aqueous showed no toxic effects. This suggested the plant is well tolerated orally up to a dose of 2000 mg/kg with reduced motor function, tremors, convulsions, and mortality observed with ethylacetate extract.

However, *Calotropis procera* leaves and root bark at a dose of 2000 mg/kg indicated the presence of toxicity with ethanol crude extract. The ethylacetate root extract indicated more promising activities as the crude extracts greatly reversed the effects of Pentyltetrazole compared to the rest. Further activities involving ethanol leaf and root extracts at reduced dosages of 150, 300, and 600 mg/kg (Table 3 and Table 4). The plant exhibited no toxicity except the leave extract at 300 mg/kg. Consequently, *Calotropis procera* may mitigate the side effects of anti-epileptic drugs, potentially enhancing the quality of life for epileptic patients. In a further examination utilizing animal models induced with pentylenetrazole (PTZ) (Table 5), *Calotropis procera* leaf and root were found to delay the onset, shorten the duration of the PTZ effect, and improve survival rates of seizures. The potential anticonvulsant properties of *C. procera* suggests its suitability for medical intervention in seizures. Previous phytochemical analysis revealed the presence of alkaloids, sugars, cardiac glycosides, saponins, phenols, tannins, terpenoids, and flavonoids in *Calotropis procera* leaves and roots (Al-Snafi, 2015; Akindele, 2017). The extraction of flavonoids and phenolic compounds is facilitated by polar solvents like ethanol and methanol, while nonpolar solvents like n-hexane extract facilitate terpenes and fatty acids (Tiwari et al., 2011). Several phytochemical classes, including alkaloids, flavonoids, terpenes, and phenolics, are known to cross the bloodbrain barrier, suggesting their potential efficacy in neurological conditions (Kavitha et al., 2020; Yadav et al., 2021). *Calotropis procera* extracts show potential antiepileptic properties, antioxidant and anti-inflammatory effects, and anticonvulsant properties in animal models.

6. Recommendations

Further research is essential to unlock the full potential of *Calotropis procera* as an alternative treatment for epilepsy. This includes elucidating its mechanisms of action, evaluating its safety and efficacy, and conducting rigorous clinical trials in human subjects. Only through such thorough investigations can we consider *Calotropis procera* as a viable source for the development of new anti-epileptic drugs, ultimately improving the lives of individuals living with epilepsy.

7. Conclusion

A study of *Calotropis procera's* effects on epilepsy is necessary to clarify the mechanisms underlying its effects and to determine the optimal dosage and formulation of the drug. While this study has shown promising results in reducing seizure frequency and severity in animal models, additional pharmacological investigations using isolated active ingredients are necessary to confirm efficacy and mechanism of action. Additionally, safety and toxicity considerations must be thoroughly addressed before considering Calotropis procera as a potential source for a new anti-epileptic drug for pharmaco-resistant epilepsy.

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