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Research Article

Anti-epileptic activity of casticin phytoconstituent from *Vitex negundo* on validated animal model

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Abstract

This study investigates the antiepileptic potential of the methanolic extract of *Vitex negundo* leaves in seizure models induced in mice using lithium-pilocarpine, maximal electroshock (MES), strychnine, picrotoxin, and pentylenetetrazol. The extract demonstrated a protective effect against seizures induced by MES and enhanced the efficacy of standard anticonvulsant drugs against chemically-induced seizures. The results suggest that the flavonoids present in the methanolic extract may exert their antiepileptic effects by modulating GABAergic pathways and blocking sodium (Na^+) channels in a dose-dependent manner. These findings highlight the potential of *Vitex negundo* methanolic extract as a promising therapeutic candidate for epilepsy treatment.

Keywords: *Vitex negundo*, methanolic extract, maximal electroshock (MES), antiepileptic, animal models, GABAergic pathway

INTRODUCTION

Epilepsy, a complex neurological disorder characterized by recurrent seizures, represents a significant global health burden affecting millions of individuals across all age groups. This introduction provides an overview of epilepsy, encompassing its epidemiology, etiology, clinical manifestations, impact on patients' lives, current treatment modalities, and ongoing research efforts[1]. Seizures, the hallmark feature of epilepsy, result from abnormal and excessive neuronal activity in the brain, leading to temporary disturbances in behavior, consciousness, sensation, or motor function. Seizures manifest in various forms, ranging from brief episodes of staring or altered consciousness to convulsive movements and loss of consciousness. The diverse clinical manifestations of seizures underscore the heterogeneous nature of epilepsy, with different seizure types and syndromes reflecting underlying differences in the brain's structure and function[2]. Epilepsy exerts a profound impact on the lives of affected individuals, extending beyond the seizures themselves to encompass various physical, psychological, social, and economic challenges. Living with epilepsy entails navigating uncertainties regarding seizure control, managing medication side effects, coping with stigma and discrimination, and addressing limitations in employment, education, and social participation[3]. While AEDs are effective in achieving seizure remission in the majority of patients, approximately one-third of individuals with epilepsy experience inadequate seizure control or intolerable side effects, highlighting the need for alternative therapeutic strategies[4]. Despite the availability of various treatment modalities, a significant proportion of individuals with epilepsy continue to experience seizures and face challenges in achieving optimal seizure control and quality of life. Furthermore, the psychosocial impact of epilepsy, including stigma, discrimination, and socioeconomic disparities, remains a pervasive issue that warrants attention.[5]

In conclusion, epilepsy is much prone and complex neuronal disorder found by repeated seizures, diverse clinical manifestations, & significant psychosocial consequences. Despite advances in diagnosis and treatment, epilepsy remains a challenging condition that requires holistic and individualized management approaches.[6] Ongoing research efforts hold promise for

advancing our understanding of epilepsy, identifying new therapeutic targets, and improving outcomes for individuals living with this condition. By raising awareness, promoting education, fostering collaboration, and advocating for the needs of individuals with epilepsy, we can strive towards a future where epilepsy is better understood, effectively treated, and destigmatized. The term "epilepsy" is used to describe a group of disorders that are characterized by bouts of seizures that occur repeatedly.[7] Epilepsy encompasses each and every one of these individual problems. One of the symptoms that is linked to a wide range of illnesses is the occurrence of a significant number of seizures. The neuronal activity in the brain that causes this kind of seizure is not only abnormal, but it is also triggered by activity. This sort of seizure is cause by both of these factors. These seizures are the result of this action, which is accountable for them. When it comes to giving epilepsy a classification, there are a few main factors that are applied. These features include, in addition to the electroclinical parameters, the type of seizure, the etiology, the age at which the seizures first manifested, and any other characteristics that may be present.[8-10] The epilepsy classification system is underpinned by this collection of traits, which acts as the foundation. Throughout the course of this conversation, we will discuss the fundamental classifications of epilepsy, as well as the clinical symptoms that are associated with each of these classifications, the underlying causes of epilepsy, and the challenges that are associated with the management of epilepsy showing in Fig.no.1

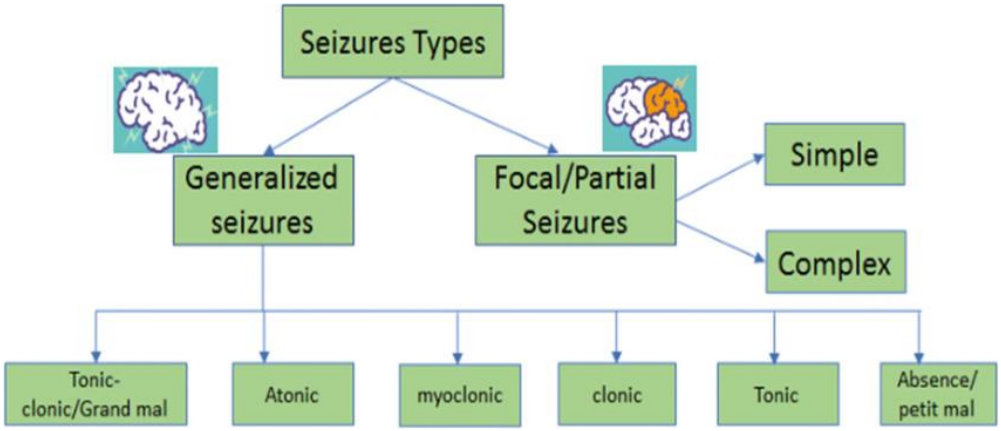

Figure 1: depicted in the flow diagram in types of epilepsy

In conclusion, epilepsy encompasses a wide range of disorders that are distinguished by the occurrence of seizures for an extended period of time. The type of seizure and the electroclinical characteristics of the individual are the two primary factors that identify the two primary categories of epilepsy, which are focal epilepsy and generalized epilepsy.[11,12] When it comes to the classification, diagnosis, and treatment of epilepsy, a multidisciplinary approach is essential. It is necessary for this strategy to take into account clinical expertise, neuroimaging investigations, information obtained from electroencephalograms, and the preferences of the healthcare provider. It is possible for medical professionals to tailor treatment strategies to the specific needs of individual patients if they have a basic awareness of the many types of epilepsy and the factors that contribute to the development of each kind.[13] Those who are living with epilepsy are able to experience an improvement in their quality of life and the opportunity to manage their seizures in the most effective manner possible.[14]

Table 1: Medications for Common Epilepsy in Children

Seizure Type	Commonly Prescribed Antiepileptic Medications
focal seizures	carbamazepine, clobazam, lamotrigine, levetiracetam, oxcarbazepine, phenytoin, sodium valproate, topiramate, lacosamide, zonisamide
generalised tonic clonic seizures	carbamazepine, clobazam, lamotrigine, levetiracetam, oxcarbazepine, phenytoin, sodium valproate, topiramate, lacosamide, zonisamide
absence seizures	ethosuximide, lamotrigine, sodium valproate
myoclonic, tonic and atonic seizures	clobazam, clonazepam, lamotrigine, levetiracetam, sodium valproate, topiramate
infantile spasms	prednisolone, vigabatrin, ACTH, nitrazepam
neonatal seizures	phenobarbitone, phenytoin, clonazepam, levetiracetam, topiramate

Advancement of herbal sources of anti-epileptic agents[16]

Herbal remedies have been utilized for centuries in the management of various health conditions, including epilepsy. While the scientific evidence supporting the efficacy of herbal treatments for epilepsy is limited compared to conventional anti-epileptic drugs, some herbal remedies have shown promise in preclinical and clinical studies. Flow diagram showing the antiepileptic drugs in herbal sources. Here are a few herbal remedies that have been explored for their potential anti-epileptic properties.

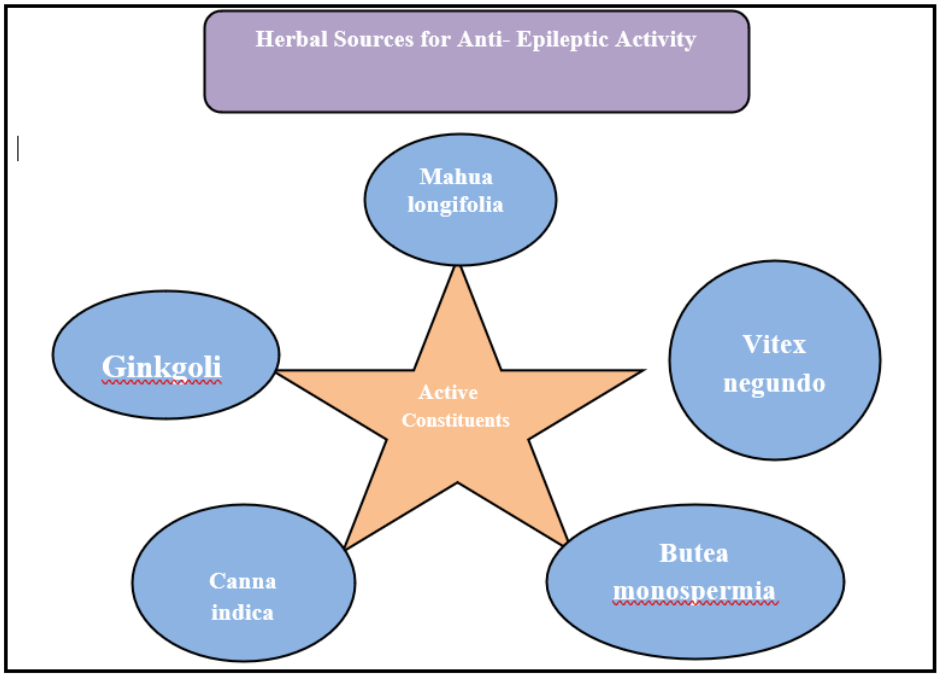

Figure 2: Antiepileptic Activity of Herbal Sources

MATERIALS AND METHODS

Plant profile of Vitex Negundo

Vitex Negundo commonly known as the five-leaved chaste tree, is a large aromatic shrub or small tree native to the Indian subcontinent and Southeast Asia. It typically grows up to 10 meters in height and has compound leaves with five lance-shaped leaflets. The flowers are small and bluish-purple in color, arranged in spikes. In traditional medicine systems like Ayurveda, different parts of Vitex negundo are used for various medicinal purposes. Its leaves, seeds, and roots are believed to have therapeutic properties and are used to treat a wide range of ailments such as fever, inflammation, respiratory disorders, skin diseases, and menstrual disorders. The plant contains various phytochemicals such as flavonoids, alkaloids, terpenoids, and essential oils, which contribute to its medicinal properties. Scientific studies have shown that extracts from Vitex negundo possess various pharmacological activities including anti-inflammatory, analgesic, antimicrobial, antioxidant, antidiabetic, and immunomodulatory effects.

Preliminary phytochemical analysis of *Vitex negundo*[18,19]

Samples of plant extract were subjected to preliminary phytochemical analysis, which revealed the presence of a number of phytochemical components. These components included carbohydrates, phenols, flavonoids, steroids, alkaloids, glycosides, and saponins. Mucilage, terpenes, sterols, proteins, tannins, and gums were not present at all. Terpenes were also not present.

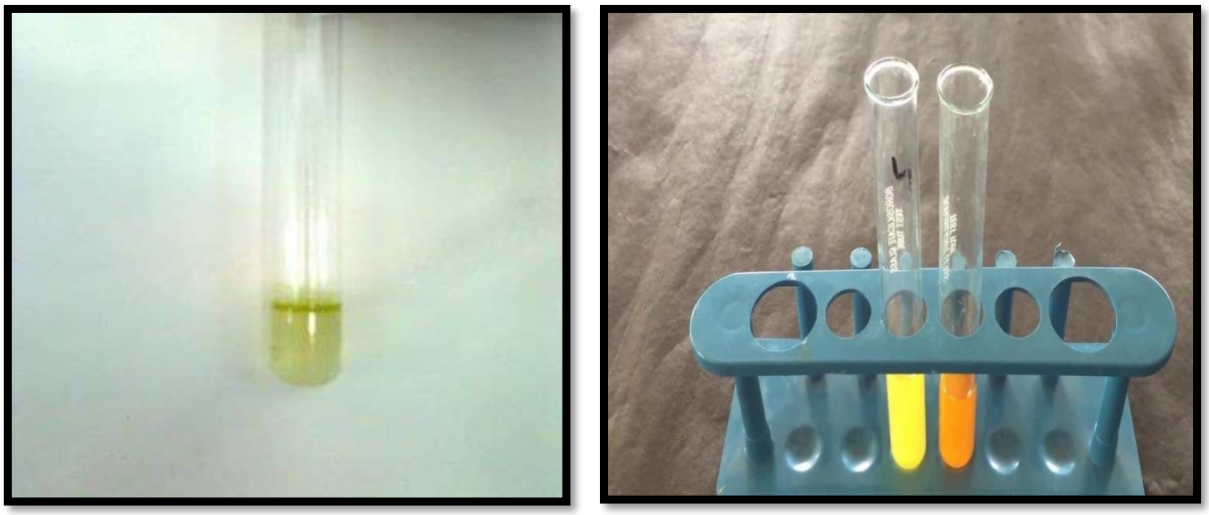

Figure 3: Presence of Flavonoids and Saponin

Table 1: Presence of phytoconstituents presence of Vitex Negundo

S. No	Compounds	Presence/Absence
1	Carbohydrate	---
2	Flavanoids	+++
3	Saponin	+++
4	Tannin	---
5	Glycosides	+++
6	Alkaloid	+++

UV-Visible Spectroscopy analysis of Vitex negundo extracts

The UV-Vis spectrophotometer is utilized for spectroscopy involving photons in the UV-visible region. UV-Vis spectroscopy employs light within the visible range or its neighboring range. The color of the chemicals directly impacts absorption within the visible range. Molecules experience electronic transitions within these regions of the electromagnetic spectrum. In the current investigation, the UV-Vis spectral profile displayed peaks at 330 nm.

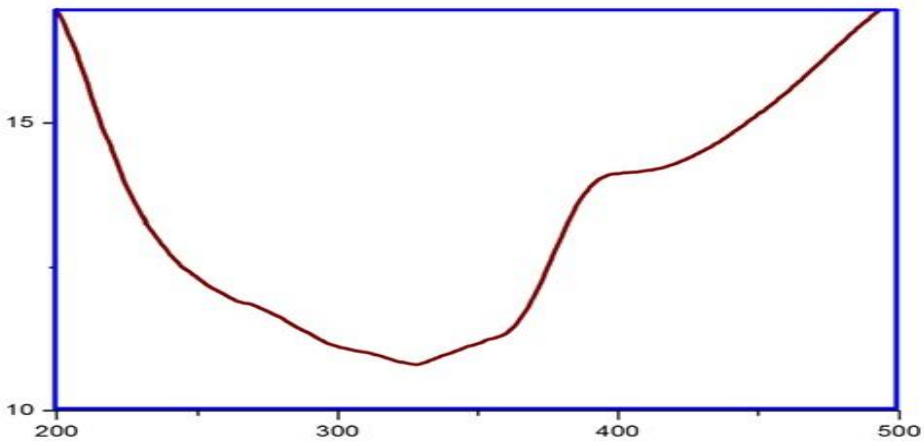

Figure 4: UV spectrum of plant extract of Vitex negundo

Chromatographic investigation by TLC Methods

To illustrate the thin layer chromatogram (TLC) that was carried out on the ethyl acetate extract of Vitex negundo that was utilized in the experiment, the figure 5.2 is presented here. After the use of a solvent phase that was composed of chloroform, ethanol, ethyl acetate, hexane, and acetic acid in the proportions of 10:2:5:1:1, the technique of thin-layer chromatography (TLC) analysis indicated the presence of a spot that had an Rf value of 0.80.

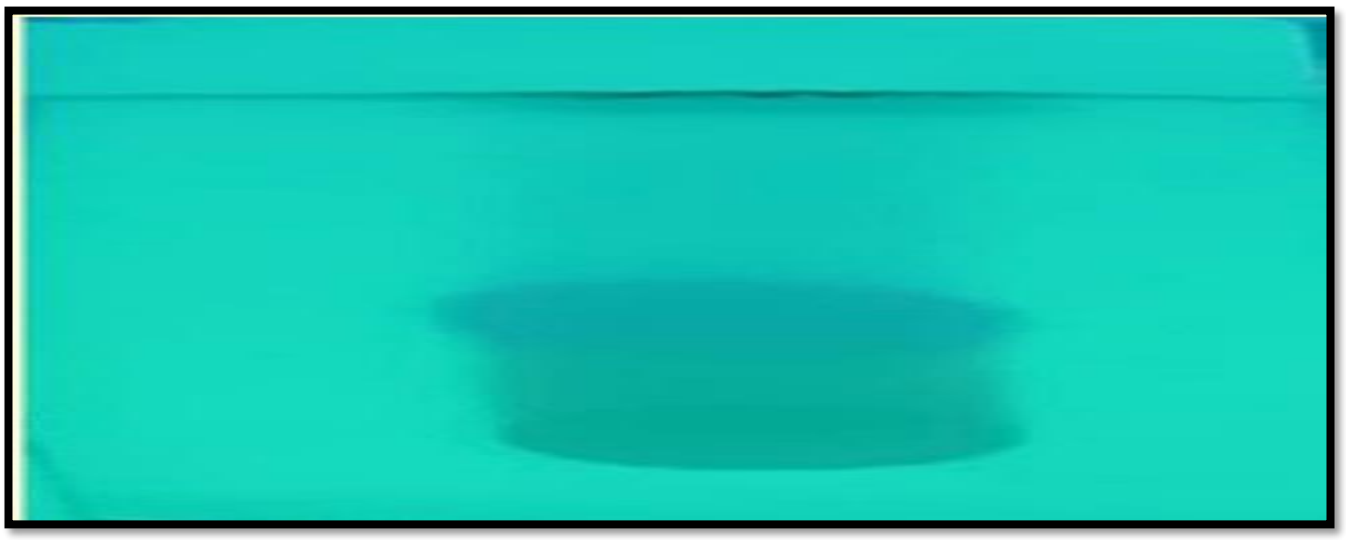

Figure 5: TLC analysis indicated the presence of a spot

Ethical considerations: The experiments conducted in this study strictly adhered to the principles outlined in the CPCSEA Guideline on animal use.

Pharmacological Screening Tests[20,21]

Strychnine induced seizure test

Administering strychnine (4 mg/kg; i.p.) led to tonic-clonic convulsions in mice, with an onset recorded at 3.04 ± 0.13 seconds and resulting in 100% mortality among control subjects. When treated with vitex negundo at the same 4 mg/kg dose, a significant delay in the onset of tonic-clonic convulsions was observed (14.66 ± 0.43 seconds; $p < 0.001$) compared to the control group. However, there was no notable difference in mortality rates between the vitex negundo-treated group and the control group reference by www.tandfonline.com.

Maximal electroshock seizure test

An electrical shock of 56 mA administered for 0.2 s triggered hind limb extension, leading to complete mortality in all subjects. However, animals treated with Vitex negundo (4 mg/kg, i.p.) showed no signs of convulsions and remained fully shielded from mortality. Moreover, the administration of Vitex negundo significantly reduced the duration of hind limb extension (10.25 ± 0.41 at 4 mg/kg) in a dose-dependent manner. Additionally, Vitex negundo treatment effectively safeguarded against mortality resulting from maximal electroshock reference by www.tandfonline.com.

Pentylenetetrazole seizure test

In this investigation, a solitary intraperitoneal injection of pentylenetetrazole (PTZ; 75 mg/kg) was noted to trigger tonic-clonic convulsions, with an onset recorded at 1.79 ± 0.32 minutes, ultimately leading to 100% mortality. Animals administered diazepam (5 mg/kg, i.p.) did not display any convulsive symptoms and were entirely safeguarded from mortality. Interestingly, when administered at varied doses, Vitex negundo notably delayed the initiation of tonic-clonic convulsions (12.35 ± 0.95 at 4 mg/kg, respectively; $p < 0.05$). Notably, treatment with Vitex negundo (4 mg/kg) provided a significant 60% protection against mortality induced by PTZ-induced convulsions**.**

Picrotoxin induced seizure test

Exposure to picrotoxin (4 mg/kg; i.p.) instigated tonic-clonic convulsions in mice , with an onset recorded at 229.80 ± 10.20 seconds and resulting in 100% mortality within the picrotoxin-only group. Interestingly, treatment with vitex negundo at a dosage of 4 mg/kg notably postponed the initiation of tonic-clonic convulsions (1134.60 ± 14.76; $p < 0.001$) compared to the control condition.

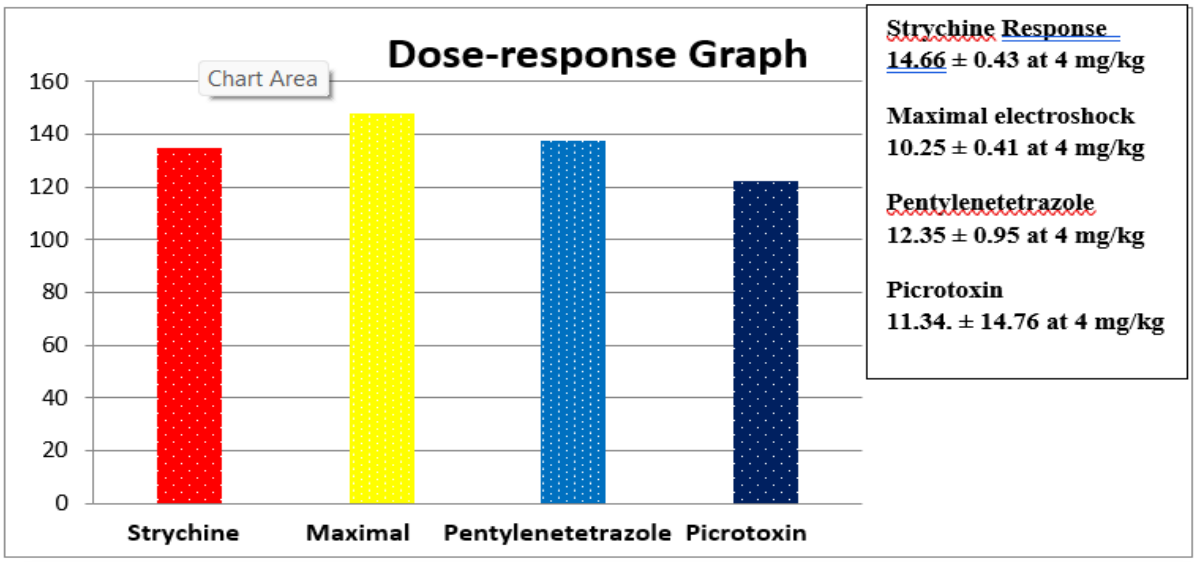

Figure 6: Bar graph showing the Different model and Dose response curve

Figure 7: Experimental photographic images showing the activity of mice after dose

RESULTS AND DISCUSSION

To determine whether or not Vitex negundo possesses anticonvulsant effects, the research included a number of different assays. Particular absorption peaks at 330 nm were discovered by UV-Vis spectroscopy, which indicated the presence of electronic transitions inside the molecule. An ethyl acetate extract of Vitex negundo was subjected to thin layer chromatography (TLC), which revealed the existence of a distinct spot with an Rf value of 0.80. This finding may indicate the presence of specific chemicals. During the strychnine-induced seizure test, the administration of Vitex negundo resulted in a considerable delay in the beginning of convulsions when compared to the control group. However, this did not bring about any changes in fatality rates. Additionally, in the maximal electroshock seizure test, Vitex negundo demonstrated a dose-dependent protective effect against convulsions as well as mortality. According to the results of the pentylenetetrazole seizure test, Vitex negundo slowed down the start of convulsions and offered some degree of protection against death. To conclude, in the test of seizure induced by picrotoxin, the administration of Vitex negundo resulted in a considerable delay in the beginning of convulsions as compared to the control group. Overall, these findings suggest that Vitex negundo possesses anticonvulsant properties, as evidenced by its ability to delay the onset of convulsions in various seizure models. Further research could explore the specific compounds responsible for these effects and their mechanisms of action.

CONCLUSION

The future scope of research on the anti-epileptic activity of phyto-constituents from herbal plants is broad and promising. By advancing phytochemical profiling, optimizing extraction and formulation techniques, developing novel animal models, and elucidating

mechanisms of action, researchers can uncover new therapeutic potentials. Clinical translation, comprehensive safety assessments, economic evaluations, and regulatory considerations will be crucial for bringing these findings to clinical practice. Engaging with the public and professionals, adhering to ethical standards, and respecting cultural contexts will ensure that research is conducted responsibly and effectively. Embracing interdisciplinary approaches, exploring synergistic effects, and focusing on rare and intractable epilepsy forms will further enhance the impact of herbal medicine in epilepsy treatment. The investigation of phyto-constituents for their anti-epileptic properties holds significant promise, yet the research is subject to various limitations that impact its scope, reliability, and applicability. Here, we explore the limitations in depth, spanning methodological, biological, and practical constraints, and their implications for future research and clinical application.

Conflict of Interest: There is no conflict of interest regarding the publication of this paper.

Author Contributions: All authors equally contribute to this work.

Data Availability: All data used and analyzed during the present study will be available from the corresponding author if deemed necessary.

Funding statement: No funding.

Ethical approval: The experiments conducted in this study strictly adhered to the principles outlined in the CPCSEA Guideline on animal use and approval was taken from the ethical committee of our institute.

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