

Bioactivity Guided Discovery, Phytochemical Characterization and Mechanistic Validation of plant derived Bioactive Compounds for Anticonvulsant and Neuroprotective Activity

Authors:

Bandaru Chandana¹, Prof. Kumaraswamy Gandla^{*2}

¹Research Scholar, Department of Pharmacy, Chaitanya Deemed to be University, Gandipet, Himayathnagar (Vill), Moinabad (Mandal), Ranga Reddy (District), Hyderabad-500075, Telangana, India.

²Professor, Department of Pharmacy, Chaitanya Deemed to be University, Gandipet, Himayathnagar (Vill), Moinabad (Mandal), Ranga Reddy(District), Hyderabad-500075, Telangana, India.

***Corresponding Author:**

Dr. Kumaraswamy Gandla, Dean and Professor,

Department of Pharmacy, Chaitanya Deemed to be University, Gandipet, Himayathnagar (Vill), Moinabad (Mandal), Ranga Reddy (District), Hyderabad-500075, Telangana, India

DOI: <https://doi.org/10.5281/zenodo.23336956>

Received: 10 September 2026, Revised: 30 September, Accepted: 07 October 2026, Online First: 13 October 2026

ABSTRACT

Epilepsy is a chronic neurological disorder associated with recurrent seizures, neuronal hyperexcitability, oxidative stress, and neuroinflammation. Despite the availability of antiseizure medications, limitations in efficacy and adverse effects highlight the need for alternative therapeutic candidates. Medicinal plants are potential sources of bioactive compounds with anticonvulsant and neuroprotective properties. This proposed study aims to investigate standardized plant-derived extracts, fractions, and isolated compounds using phytochemical screening, chromatographic profiling, and bioactivity-guided fractionation. Anticonvulsant activity will be evaluated using maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) models, subject to ethical approval. Neuroprotective potential will be investigated through appropriate neuronal injury models, biochemical markers, and histopathological assessment. Molecular docking may help identify potential targets, with experimental validation where feasible. The study is expected to provide a scientific basis for identifying plant-derived compounds for further preclinical investigation in seizure control and neuronal protection.

Keywords: *Epilepsy; Anticonvulsant Activity; Neuroprotection; Medicinal Plants; Phytochemicals; Bioactivity-Guided Fractionation; Oxidative Stress; Neuroinflammation; GABAergic and Glutamatergic Pathways; Molecular Docking.*

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures resulting from abnormal and excessive neuronal activity. Despite the availability of several antiseizure medications, some patients continue to experience inadequate seizure control or adverse drug effects. These limitations have encouraged research into new therapeutic candidates with improved efficacy and safety profiles.

Medicinal plants represent a potential source of biologically active compounds that may possess anticonvulsant and neuroprotective properties. Various phytochemicals, including flavonoids, alkaloids, terpenoids, and phenolic compounds, have been

investigated for their possible effects on neuronal excitability, oxidative stress, and neuroinflammation.

The development of plant-derived therapeutic candidates requires systematic botanical authentication, phytochemical characterization, and evaluation of biological activity. Bioactivity-guided fractionation can help identify the constituents responsible for observed pharmacological effects. Furthermore, experimental models of seizures and neuronal injury can provide evidence regarding anticonvulsant activity and potential neuroprotective mechanisms.

Oxidative stress, neuroinflammation, and disturbances in the balance between inhibitory GABAergic and excitatory glutamatergic neurotransmission may

contribute to seizure-associated neuronal damage. Investigating these pathways may help explain the mechanisms underlying the effects of plant-derived compounds. Analytical techniques, biochemical investigations, histopathological assessment, and molecular approaches can be combined to strengthen mechanistic evaluation.

Therefore, the proposed research aims to integrate phytochemical standardization, bioactivity-guided discovery, anticonvulsant evaluation, and mechanistic investigation to identify potentially active plant-derived compounds. This approach may provide a scientific basis for further preclinical investigation of natural compounds as potential candidates for seizure control and neuronal protection.

Aim

To investigate the anticonvulsant and neuroprotective potential of standardized plant-derived bioactive fractions/compounds and to elucidate their possible pharmacological and molecular mechanisms using suitable experimental models.

Objectives

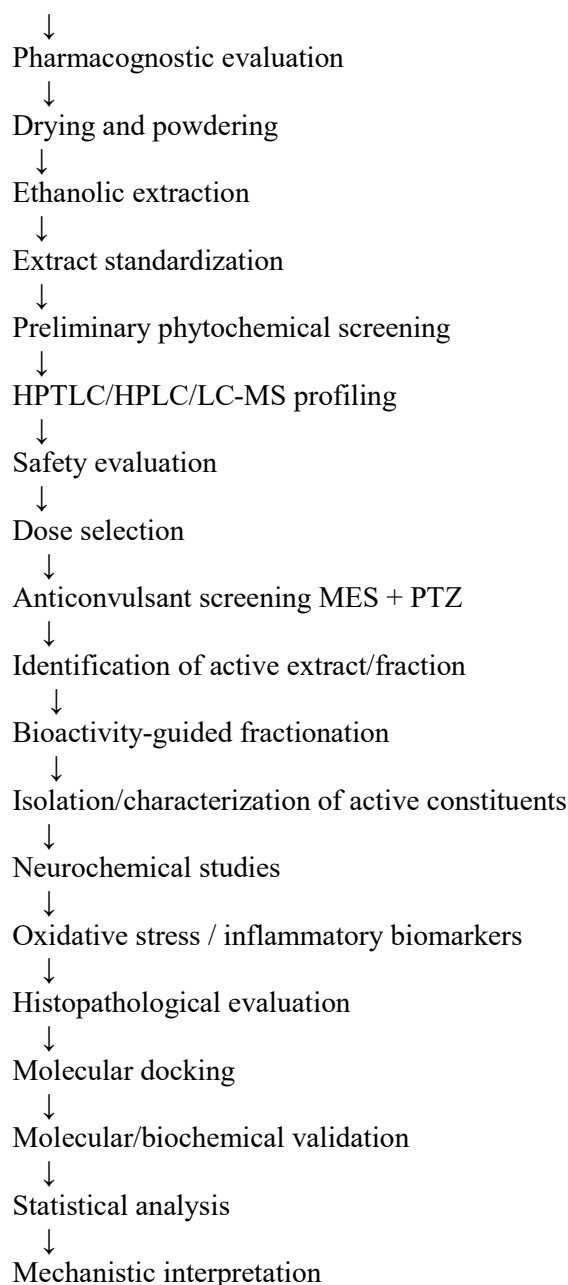
1. Collection and authentication of the selected medicinal plant material.
2. Pharmacognostic evaluation and quality standardization of the crude drug.
3. Preparation of ethanolic extract using a standardized extraction procedure.
4. Preliminary phytochemical investigation of the extract.
5. Chromatographic fingerprinting and chemical standardization.
6. Assessment of preliminary safety/toxicity according to applicable guidelines.
7. Evaluation of anticonvulsant activity using MES and PTZ models.
8. Bioactivity-guided fractionation of the active extract.
9. Identification and characterization of potentially active fractions/compounds.
10. Evaluation of neuroprotective activity.
11. Investigation of oxidative-stress and inflammatory pathways associated with seizure-induced neuronal damage.
12. Investigation of possible GABAergic and glutamatergic mechanisms.
13. Molecular docking/in-silico studies of identified phytoconstituents against relevant molecular targets.
14. Experimental validation of important predicted mechanisms, where feasible.
15. Correlation of phytochemical composition with anticonvulsant activity.

Overall Experimental Design

Plant material



Authentication



Phase I — Pharmacognostic Evaluation

The selected plant material will be authenticated by a qualified taxonomist/herbarium or appropriate botanical institution.

Parameters

Macroscopic evaluation

- * Colour
- * Odour
- * Taste, where appropriate
- * Shape
- * Size
- * Texture
- * Surface characteristics

Microscopic evaluation

- * Transverse section
- * Epidermal characteristics
- * Trichomes
- * Stomata

- * Vascular bundles
- * Calcium oxalate crystals, if present
- * Diagnostic tissues

Powder microscopy

The powdered drug will be examined for characteristic diagnostic structures.

Physicochemical parameters

- * Foreign matter
- * Moisture content/loss on drying
- * Total ash
- * Acid-insoluble ash
- * Water-soluble ash
- * Alcohol-soluble extractive
- * Water-soluble extractive

These parameters will establish preliminary quality-control specifications for the crude drug.

Phase II — Extraction

The authenticated leaves will be:

Collection → Cleaning → Drying → Pulverization → Extraction → Filtration → Concentration → Drying → Storage

An appropriate hydroalcoholic/ethanolic extraction method will be selected based on the research objective and laboratory facilities.

The following will be documented:

- * Weight of starting material
- * Solvent used
- * Extraction method
- * Extraction time
- * Extraction temperature
- * Extract yield
- * Physical characteristics of extract

Percentage yield

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered drug}} \times 100$$

Phase III — Phytochemical Investigation

The extract will undergo preliminary phytochemical screening for groups such as:

- * Alkaloids
- * Flavonoids
- * Phenolics
- * Tannins
- * Terpenoids
- * Steroids
- * Glycosides
- * Saponins
- * Carbohydrates
- * Proteins/amino acids

The screening will be followed by chemical profiling rather than relying only on qualitative tests.

Phase IV — Standardization and Chemical Fingerprinting

HPTLC

To develop a characteristic fingerprint of the extract/fractions.

HPLC/UHPLC

For quantitative or semi-quantitative estimation of selected marker compounds.

LC-MS/MS

To identify major and potentially bioactive constituents.

GC-MS

If volatile/semi-volatile constituents are relevant.

NMR

If sufficient purified compounds are obtained.

The final objective is to establish a chemical fingerprint/marker profile that can be correlated with pharmacological activity

Phase V — Safety Evaluation

Before efficacy studies, appropriate safety evaluation should be performed according to the applicable institutional and regulatory requirements.

The study should include:

- * Appropriate dose-range assessment
- * Observation of general behaviour
- * Changes in locomotor activity
- * Food/water intake where relevant
- * Body-weight changes
- * Mortality/morbidity
- * Gross pathological observations
- * Relevant biochemical/hematological parameters where justified

Animal experiments should only be conducted after approval from the appropriate Institutional Animal Ethics Committee (IAEC) and in accordance with applicable CPCSEA requirements and institutional SOPs

Phase VI — Anticonvulsant Evaluation

Two complementary experimental models can provide a stronger pharmacological evaluation.

A. Maximal Electroshock Seizure (MES) Model

The MES model is commonly used to investigate protection against electrically induced generalized seizures and is particularly useful for assessing protection against seizure spread.

Parameters

Depending on the validated institutional protocol:

- * Seizure occurrence
- * Seizure duration
- * Tonic hind-limb extension
- * Protection against tonic extension
- * Mortality/survival
- * Latency to seizure

A suitable standard antiseizure drug will be included as a positive control.

PTZ-Induced Seizure Model

Pentylentetrazole is commonly used to induce chemically mediated seizures and is useful for investigating mechanisms related to neuronal inhibitory/excitatory balance.

Parameters

- * Latency to first seizure
- * Seizure severity
- * Seizure duration
- * Incidence of seizures
- * Mortality
- * Percentage protection

The findings from MES and PTZ can then be compared to determine whether the extract shows activity across different seizure mechanisms

Proposed Experimental Groups

The exact number of animals and doses should be finalized following statistical power calculation, institutional SOPs and IAEC approval.

Group	Treatments
Group-1	Normal vehicle control
Group-2	Standard antiseizure drug
Group-3	Test extract -lower dose
Group-4	Text extract-intermediate dose

For mechanistic experiments, additional appropriately controlled groups may be required.

Animal numbers should not be fixed arbitrarily; they should be determined using an appropriate power calculation and the 3Rs principle.

Dose Selection

The doses should be selected based on:

- * Preliminary safety data
- * Existing literature
- * Pilot pharmacological data
- * Maximum feasible concentration
- * Pharmacokinetic considerations, where available

The final doses should be scientifically justified rather than selected only by convention.

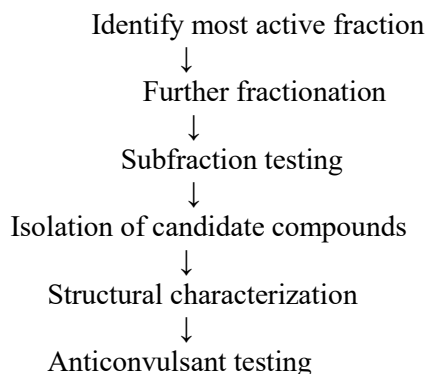
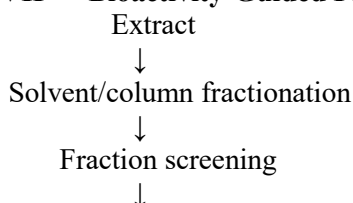
Behavioural Evaluation

Depending on the study design, behavioural parameters may include:

- * Seizure severity
- * Latency
- * Locomotor activity
- * Motor coordination
- * General neurological behaviour

This helps distinguish anticonvulsant activity from nonspecific sedation or motor impairment.

Phase VII — Bioactivity-Guided Fractionation



This creates a direct connection between:

Phytochemistry → Fraction → Compound → Biological activity

Isolation and Characterization

Potential active compounds/fractions can be investigated using:

- * Column chromatography
- * Preparative TLC
- * Preparative HPLC
- * LC-MS/MS
- * FTIR
- * ¹H-NMR
- * ¹³C-NMR
- * 2D-NMR where necessary

The isolated compounds should be evaluated for purity and structurally characterized using appropriate analytical techniques.

Neurochemical Investigation

To investigate the mechanism of anticonvulsant action, relevant neurotransmitter systems can be examined.

Inhibitory pathway

GABAergic system

Possible parameters:

- * GABA concentration
 - * Relevant GABA-related enzymes/receptors, depending on facilities
- Excitatory pathway
Glutamatergic system

Possible parameters:

- * Glutamate
- * Relevant glutamate receptors/pathways

The overall objective is to determine whether the treatment alters the excitatory–inhibitory balance associated with seizure generation.

Oxidative Stress Investigation

Seizure activity can be associated with oxidative stress and neuronal injury.

Possible biochemical parameters:

Oxidant markers

- * Lipid peroxidation/MDA
 - * Reactive oxygen species, where validated
- Antioxidant defence
- * Superoxide dismutase
 - * Catalase

- * Reduced glutathione
- * Glutathione peroxidase

This will help determine whether neuroprotection is associated with modulation of oxidative stress.

Neuroinflammatory Investigation

If supported by the research facilities and preliminary findings, inflammatory pathways can be investigated.

Potential biomarkers include:

- * TNF- α
- * IL-1 β
- * IL-6
- * NF- κ B-related signalling

The selection should be hypothesis-driven rather than measuring every available marker.

Histopathological Investigation

Brain tissues can be examined for seizure-associated neuronal alterations.

Potential regions include:

- * Hippocampus
- * Cerebral cortex

Depending on the experimental model, parameters may include:

- * Neuronal degeneration
- * Cellular changes
- * Inflammatory changes
- * Tissue architecture

Appropriate staining and blinded histopathological assessment should be used.

Molecular Investigation

A strong PhD component can investigate potential molecular targets.

Possible approaches:

In-silico

Phytoconstituent identification

↓

Target prediction

↓

Molecular docking

↓

Binding interaction analysis

↓

Candidate target selection

↓

Experimental validation

Potential target classes may include:

- * GABA-related targets
- * Glutamate receptors
- * Ion channels
- * Oxidative-stress pathways
- * Neuroinflammatory pathways

Importantly, docking results alone should not be presented as proof of mechanism. Where feasible, important computational predictions should be experimentally validated.

Correlation Analysis

One of the strongest aspects of the proposed PhD is to correlate:

Phytochemical profile

with

Fraction/compound concentration

with

Anticonvulsant activity

with

Neurochemical changes

with

Oxidative-stress parameters

with

Histopathological findings

This may help identify which constituents or chemical fingerprints are associated with biological activity.

Statistical Analysis

Data will be analyzed using an appropriate statistical software package.

Depending on the experimental design, methods may include:

- * Descriptive statistics
- * One-way ANOVA
- * Two-way ANOVA
- * Appropriate post-hoc tests
- * Non-parametric tests where assumptions are not met
- * Dose-response analysis
- * Correlation analysis

The statistical method should be selected before the experiment, based on the study design and distribution of the data.

A predefined significance level such as $p < 0.05$ may be used where appropriate.

Expected Outcomes

The study is expected to:

1. Establish pharmacognostic quality parameters for the selected plant material.
2. Develop a standardized ethanolic extract.
3. Generate a reproducible phytochemical fingerprint.
4. Identify potentially important bioactive constituents.
5. Establish whether the extract/fractions demonstrate anticonvulsant activity.
6. Determine whether activity occurs in both MES and PTZ models.
7. Identify potentially active fractions or isolated compounds.
8. Establish possible neuroprotective effects.
9. Determine the association between anticonvulsant activity and oxidative-stress modulation.
10. Investigate possible GABAergic/glutamatergic mechanisms.
11. Identify potential molecular targets.
12. Establish correlations between phytochemistry and pharmacological activity.
13. Generate evidence for further development of selected natural compounds as potential lead molecules.

Novelty of the Proposed Research

The novelty should not simply be claimed as “first study” unless supported by a systematic literature search.

The proposed work can nevertheless be positioned around the integration of:

Pharmacognostic standardization

+

Chemical fingerprinting

+

Bioactivity-guided fractionation

+

Anticonvulsant evaluation

+

Neuroprotection

+

Neurochemical investigation

+

Molecular validation

This integrated approach gives the project substantially greater depth than a conventional crude-extract anticonvulsant screening study

In-vivo Models for Anticonvulsant Research

1. MES-induced seizure model

Purpose

The Maximal Electroshock Seizure (MES) model is a classical model for evaluating protection against electrically induced generalized seizures.

What it evaluates

- * Seizure susceptibility
- * Tonic hind-limb extension
- * Seizure duration
- * Mortality
- * Percentage seizure protection

Concept

Test extract/fraction

↓

MES challenge

↓

Observation of seizure response

↓

Protection against tonic extension

↓

Anticonvulsant activity

2. PTZ-induced seizure model

Purpose

The pentylenetetrazole (PTZ) model is useful for evaluating chemically induced seizures and investigating mechanisms involving the balance between neuronal inhibition and excitation.

Parameters

- * Latency to first seizure
- * Seizure incidence
- * Seizure severity
- * Seizure duration

* Mortality

* Percentage protection

Mechanistic relevance

PTZ-related seizure susceptibility is particularly useful when investigating GABAergic mechanisms.

Concept

Extract/fraction

↓

PTZ challenge

↓

Altered seizure susceptibility

↓

Changes in latency/severity

↓

Possible anticonvulsant effect

3. 6-Hz seizure model

The 6-Hz model provides an additional seizure phenotype and is commonly used in research involving focal seizures and pharmacoresistant seizure phenotypes.

It can therefore complement MES and PTZ.

Possible endpoints

- * Seizure incidence
- * Seizure severity
- * Seizure duration
- * Protection

Basic concept

Repeated subconvulsive seizure-inducing stimulation

↓

Progressive increase in seizure susceptibility

↓

Development of increasingly severe behavioural seizures

↓

Assessment of whether treatment modifies this progression

Parameters

- * Seizure-stage progression
- * Seizure latency
- * Seizure duration
- * Seizure severity
- * Seizure threshold
- * Long-term behavioural effects

In-vitro Models for Anticonvulsant Research

GABA-T inhibition Anticonvulsant mechanism

Purpose

The main purpose is to determine whether the test extract/fraction inhibits GABA transaminase (GABA-T) and thereby provides evidence for a GABAergic anticonvulsant mechanism.

Specific purposes:

- * To assess GABA-T enzyme inhibitory activity of the plant extract.
- * To determine the percentage inhibition of GABA-T.

- * To establish a concentration-dependent response.
- * To calculate the IC₅₀ of the extract/fraction.
- * To compare activity with a standard GABA-T inhibitor.
- * To identify fractions or constituents potentially responsible for GABAergic activity.

Basic concept

GABA-T normally breaks down GABA:

GABA + α -ketoglutarate

↓ GABA-T

Succinic semialdehyde + glutamate

↓

GABA-T inhibition means:

↓

GABA-T activity

↓

GABA degradation ↓

↓

GABA availability ↑

↓

GABAergic inhibitory neurotransmission ↑

↓

Neuronal excitability ↓

↓

Anticonvulsant effect

So, the basic principle of the assay is:

If the test extract inhibits GABA-T, the measured GABA-T enzymatic activity will decrease compared with the untreated control.

Neuronal excitotoxicity Direct neuronal protection

Purpose

The main purpose is to determine whether the test extract:

- * Protects neuronal cells against excitotoxic damage.
- * Reduces neuronal cell death caused by excessive excitatory stimulation.
- * Preserves neuronal viability and membrane integrity.
- * Provides evidence for a neuroprotective mechanism relevant to epilepsy.
- * Helps determine whether the anticonvulsant activity involves mechanisms beyond GABAergic enhancement.

Basic concept

Excitotoxicity occurs when excessive excitatory neurotransmission—particularly glutamate-mediated signaling—causes excessive intracellular Ca²⁺ entry and neuronal injury.

Basic pathway

Excessive glutamate/excitatory stimulation

↓

Activation of NMDA/AMPA receptors

↓

Ca²⁺ influx ↑

↓

Intracellular Ca²⁺ overload

↓

Mitochondrial dysfunction

↓

ROS/RNS generation ↑

↓

Oxidative stress

↓

Membrane and protein damage

↓

Mitochondrial energy failure

↓

Apoptotic/necrotic neuronal death

↓

Neuronal injury

If the test extract is neuroprotective:

Test extract

↓

Reduction of excitotoxic injury

↓

Ca²⁺ overload ↓ / oxidative stress ↓ / mitochondrial dysfunction ↓

↓

Neuronal viability ↑

↓

Direct neuronal protection

Oxidative-stress neuronal model Neuroprotective mechanism

Purpose

The purpose of this model is to evaluate whether a test extract has direct neuroprotective activity against oxidative-stress-induced neuronal damage.

Specifically, it aims to determine whether the extract:

- * Reduces excessive ROS generation
- * Prevents oxidative damage
- * Enhances endogenous antioxidant defense
- * Protects mitochondrial function
- * Prevents neuronal apoptosis
- * Improves neuronal cell survival
- * Identifies possible mechanisms contributing to its anticonvulsant/neuroprotective activity

Basic concept

Neurons normally maintain a balance between ROS production and antioxidant defense.

Oxidative stress

Oxidative insult

↓

ROS ↑

↓

Antioxidant defense ↓

↓

Lipid/protein/DNA damage

↓

Mitochondrial dysfunction
 ↓
 ATP ↓ + membrane potential ↓
 ↓
 Apoptotic signaling ↑
 ↓
 Neuronal death

Neuroprotective effect

Test extract
 ↓
 ROS ↓
 +
 Antioxidant defense ↑
 ↓
 Lipid/oxidative damage ↓
 ↓
 Mitochondrial function preserved
 ↓
 Apoptosis ↓
 ↓
 Neuronal survival ↑

Neuroinflammation model Inflammatory mechanism

Purpose

The purpose is to determine whether the extract:

- * Reduces neuroinflammatory responses.
- * Suppresses excessive activation of microglia and/or astrocytes.
- * Decreases pro-inflammatory cytokines.
- * Reduces inflammatory signaling pathways.
- * Protects neurons from inflammation-associated damage.
- * Provides mechanistic evidence for neuroprotective and potentially anticonvulsant activity.

Basic concept

The CNS has resident immune cells, particularly microglia and astrocytes.

When activated excessively:

Neuronal stress/injury
 ↓
 Microglial activation
 ↓
 Pro-inflammatory signaling ↑
 ↓
 TNF- α , IL-1 β , IL-6 and other mediators ↑
 ↓
 ROS/RNS generation ↑
 ↓
 Mitochondrial dysfunction + neuronal injury
 ↓
 Synaptic dysfunction
 ↓
 Neuronal hyperexcitability / neuronal damage

A neuroprotective extract may suppress excessive inflammatory activation:

Test extract
 ↓
 Microglial/astrocyte activation ↓
 ↓
 Pro-inflammatory cytokines ↓
 ↓
 Oxidative/inflammatory injury ↓
 ↓
 Neuronal damage ↓
 ↓
 Neuroprotection

Strengths of the Proposed Research

The proposed research integrates pharmacognostic standardization, phytochemical characterization, bioactivity-guided fractionation, and pharmacological evaluation. The use of complementary anticonvulsant models may provide a broader assessment of potential biological activity. The inclusion of neurochemical, oxidative-stress, neuroinflammatory, and histopathological investigations may help explore possible mechanisms underlying the observed effects. Combining phytochemical profiling with biological activity may also assist in identifying potentially active plant-derived compounds for further preclinical investigation.

Limitations of the Proposed Research

The proposed study has several anticipated limitations. The plant species and specific plant material have not yet been clearly identified, which may affect the reproducibility and interpretation of the findings. The availability of advanced analytical instruments, neuronal injury models, and molecular validation facilities may influence the scope of the investigation. Plant-derived extracts may also contain multiple compounds with variable concentrations and potentially interacting effects. Furthermore, results obtained from in-vitro and animal models cannot be directly extrapolated to clinical efficacy or safety in humans.

Future Directions

Future research should focus on the isolation and detailed characterization of promising bioactive compounds, followed by independent confirmation of their anticonvulsant and neuroprotective effects. Further studies may investigate pharmacokinetic properties, dose-response relationships, toxicity, bioavailability, and reproducibility across experimental models. Promising compounds may subsequently undergo more advanced preclinical evaluation before any consideration of clinical development.

CONCLUSION

The goal of the proposed study, "Bioactivity-Guided Discovery, Phytochemical Characterization and Mechanistic Validation of Plant-Derived Bioactive Compounds for Anticonvulsant and Neuroprotective Activity," is to offer a methodical scientific analysis of compounds derived from medicinal plants as possible candidates for neuroprotective and anticonvulsant uses. Pharmacogenetic examination, phytochemical screening, chemical fingerprinting, in-vivo anticonvulsant evaluation, bioactivity-guided fractionation, and mechanistic research are all integrated into the study. The goal of using pentylenetetrazol (PTZ) and maximal electroshock seizure (MES) models is to offer complementary evidence about anticonvulsant capability. Direct neuroprotective effects may be further investigated using oxidative stress models and in vitro neuronal excitotoxicity.

Understanding the processes underlying the reported biological effects may be aided by biochemical studies of GABAergic and glutamatergic neurotransmission, oxidative-stress indicators, and specific inflammatory mediators. Relationships between phytochemical makeup and pharmacological activity may be established through chromatographic and spectroscopic analysis of separated ingredients and active fractions.

Additional information about possible molecular targets may be obtained by molecular docking and related computational techniques, which, where possible, are validated experimentally.

In general, it is anticipated that the planned study will create a scientific framework for locating and describing potentially active substances derived from plants and examining their neuroprotective and anticonvulsant qualities. The evidence supporting the further development of particular natural compounds as possible lead molecules for neurological research may be strengthened by combining phytochemical standardization, bioactivity-guided discovery, and mechanistic validation.

The experimental results, such as the reproducibility of biological activity, identification of active ingredients, confirmation of pertinent mechanisms, and assessment of safety, will determine the study's final relevance. Before any identified molecule could be taken into consideration for clinical development, additional pharmacokinetic, toxicological, and preclinical research would be required.

DECLARATIONS

Ethical Considerations

All animal experiments, if undertaken, will be initiated only after approval from the appropriate Institutional Animal Ethics Committee (IAEC) and in accordance with applicable CPCSEA requirements and institutional guidelines. The study will follow the

principles of replacement, reduction, and refinement (3Rs).

Funding

The funding status of the proposed research should be stated accurately. Any institutional, departmental, or external financial support should be acknowledged.

Conflict of Interest

The authors should disclose any financial or non-financial conflicts of interest relevant to the proposed research.

Author Contributions

Bandaru Chandana: Conceptualization, investigation planning, methodology development, and preparation of the initial manuscript.

Prof. Kumaraswamy Gandla: Supervision, methodology review, critical revision, and approval of the final manuscript.

The contribution statement should be revised to reflect the authors' actual contributions.

Data Availability

No experimental dataset has been generated or analyzed in this proposed study. Data availability will be addressed when experimental data become available, where applicable.

Acknowledgements

The authors may acknowledge the institution, laboratory facilities, technical staff, or other individuals who provided substantial assistance, where applicable.

REFERENCES

1. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: A practical clinical definition of epilepsy. *Epilepsia*. 2014;55(4):475–482. doi:10.1111/epi.12550.
2. Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure*. 2011;20(5):359–368. doi:10.1016/j.seizure.2011.01.003.
3. White HS, Johnson M, Wolf HH, Kupferberg HJ. The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous pentylenetetrazol seizure models. *Epilepsia*. 1995;36(1):1–6. doi:10.1111/j.1528-1157.1995.tb01656.x.
4. Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs. *Nature Reviews Drug Discovery*. 2010;9(1):68–82. doi:10.1038/nrd2997.

5. Löscher W, Klein P. The pharmacology and clinical efficacy of antiseizure medications: From bromide salts to cenobamate and beyond. *CNS Drugs*. 2021;35:935–963.
6. Treiman DM. GABAergic mechanisms in epilepsy. *Epilepsia*. 2001;42(Suppl 3):8–12.
7. Meldrum BS. Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *Journal of Nutrition*. 2000;130(4 Suppl):1007S–1015S.
8. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of γ -aminobutyric acid(A) receptors: Classification on the basis of subunit composition, pharmacology, and function. *Pharmacological Reviews*. 2008;60(3):243–260. doi:10.1124/pr.108.00505.
9. Fabisiak T, Patel M. Crosstalk between neuroinflammation and oxidative stress in epilepsy. *Frontiers in Cell and Developmental Biology*. 2022;10:976953. doi:10.3389/fcell.2022.976953.
10. Pearson-Smith JN, Patel M. The role of oxidative stress in epileptic seizures. *Neurochemistry International*. 2017;107:65–75.
11. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nature Reviews Neurology*. 2011;7(1):31–40. doi:10.1038/nrneuro.2010.178.
12. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall; 1998.
13. Evans WC. *Trease and Evans Pharmacognosy*. 16th ed. Edinburgh: Saunders Elsevier; 2009.
14. Heinrich M, Barnes J, Gibbons S, Williamson EM. *Fundamentals of Pharmacognosy and Phytotherapy*. 2nd ed. Edinburgh: Churchill Livingstone Elsevier; 2012.
15. Claasen FW, et al. Recent developments in the rapid analysis of plants and tracking of their bioactive constituents. *Phytochemistry Reviews*. 2010;9:1–14.
16. Sarker SD, Nahar L. An introduction to natural products isolation. In: Sarker SD, Nahar L, editors. *Natural Products Isolation. Methods in Molecular Biology*. New York: Humana Press.
17. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*. 2020;18(7):e3000410. doi:10.1371/journal.pbio.3000410.
18. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th ed. Washington, DC: National Academies Press; 2011.
19. Indian National Science Academy. *Guidelines for Care and Use of Animals in Scientific Research*. New Delhi: Indian National Science Academy; 2000.
20. Ellman GL. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*. 1959;82(1):70–77. doi:10.1016/0003-9861(59)90090-6.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 1979;95(2):351–358. doi:10.1016/0003-2697(79)90738-3.
22. Aebi H. Catalase in vitro. *Methods in Enzymology*. 1984;105:121–126. doi:10.1016/S0076-6879(84)05016-3.