World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 3
Page: 747-758

IN-VITRO INVESTIGATION OF *Psidium guajava* LEAF EXTRACT AGAINST EPILEPSY

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Article Received: 11 February 2026 | | Article Revised: 03 March 2026 | | Article Accepted: 24 March 2026

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DOI: https://doi.org/10.5281/zenodo.19234289

How to cite this Article: Dr. Rajasekaran S., Dr. S. V. Rekha, Amana Jasly, Amina Suhila N., Fathima Shamila, Mirfa Bathool P. P. (2026) IN-VITRO INVESTIGATION OF *Psidium guajava* LEAF EXTRACT AGAINST EPILEPSY. World Journal of Pharmaceutical Science and Research, 5(3), 747-758.

ABSTRACT

This study investigates the in-vitro antiepileptic potential of *Psidium guajava* leaf extract. Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and current antiepileptic drugs often cause adverse effects and incomplete seizure control. Guava leaves contain bioactive compounds such as flavonoids, tannins, and phenolics with antioxidant and neuroprotective properties. In this study, dried and powdered leaves were extracted using a suitable solvent and subjected to preliminary phytochemical screening. The antiepileptic activity was evaluated using neuronal cell line models, PC12 (rat pheochromocytoma) and N2a (mouse neuroblastoma), with glutamate-induced excitotoxicity. Cytotoxicity and neuroprotective effects were assessed using the MTT cell viability assay. Results showed that *Psidium guajava* leaf extract exhibited protective effects against glutamate-induced neuronal damage, indicating potential antiepileptic and neuroprotective activity in vitro.

KEYWORDS: *Psidium guajava*, Epilepsy, Antiepileptic activity, Neuroprotection, Glutamate-induced excitotoxicity, PC12 cells, N2a cells, MTT assay, Phytochemicals.

INTRODUCTION

The word *epilepsy* is from Ancient Greek Epilambanein (to seize, possess, or afflict). Epilepsy is a long term (chronic) disease that causes repeated seizures due to abnormal electrical signals produced by damaged brain cells. A seizure is a sudden burst of abnormal electrical activity in the brain that can cause a variety of symptoms, ranging from brief lapses of awareness or muscle jerks to prolonged convulsions.[1] Seizures can include changes to your awareness, muscle control (your muscles may twitch or jerk), sensations, emotions, and behavior. Epilepsy is also called a seizure disorder.

Epilepsy affects people of all ages and represents one of the most common neurological disorders worldwide. Epidemiological studies suggest that approximately 5–10% of individuals may experience at least one unprovoked seizure during their lifetime, and the risk of recurrence after a first seizure is nearly 40%. The diagnosis of epilepsy generally requires the occurrence of two or more unprovoked seizures separated by at least 24 hours. Clinical evaluation may involve neurological examination, neuroimaging techniques, and electroencephalography to identify abnormal brain activity associated with seizures.[2,3]

Epileptic seizures are broadly classified into generalized seizures, focal seizures, combined generalized and focal seizures, and seizures of unknown onset.[4] Generalized seizures involve widespread neuronal networks across both hemispheres of the brain, whereas focal seizures originate from a specific region and may subsequently spread to other areas.[5] The pathophysiology of epilepsy is complex and involves abnormal neuronal excitability and hypersynchronization of neuronal populations. One of the major mechanisms involved in seizure generation is the imbalance between excitatory and inhibitory neurotransmission within the brain. The excitatory neurotransmitter Glutamate promotes neuronal activation, whereas the inhibitory neurotransmitter Gamma-aminobutyric acid suppresses neuronal firing. An increase in excitatory signaling or a reduction in inhibitory control can lead to abnormal neuronal discharges and seizure activity.[6]

Several factors may contribute to the development of epilepsy, including genetic predisposition, structural abnormalities of the brain, central nervous system infections, traumatic brain injury, metabolic disorders, and autoimmune conditions.[5] However, in nearly half of epilepsy cases, the exact cause remains unidentified. Although various antiepileptic drugs are available for the management of seizures, approximately one-third of patients continue to experience uncontrolled seizures despite appropriate pharmacological treatment.[8] Moreover, long-term administration of conventional antiepileptic medications is often associated with adverse effects such as sedation, cognitive impairment, behavioral changes, and drug interactions. These limitations highlight the need for the discovery of safer and more effective therapeutic agents.[9]

Many plant-derived phytochemicals possess antioxidant, anti-inflammatory, and neuroprotective properties that may contribute to the management of epilepsy. Among these plants, *Psidium guajava*, commonly known as guava, has attracted significant interest due to its diverse pharmacological activities. *Psidium guajava* belongs to the Myrtaceae family and is widely cultivated in tropical and subtropical regions.[10] The plant has been traditionally used in various systems of medicine for the treatment of gastrointestinal disorders, infections, inflammation, and metabolic diseases.

The leaves of *Psidium guajava* contain a variety of bioactive phytochemicals, including flavonoids, tannins, polyphenols, and other phenolic compounds such as quercetin and kaempferol. These compounds have been reported to exhibit antioxidant, anti-inflammatory, antimicrobial, and neuroprotective activities.[11] Certain phytochemicals present in guava leaves are believed to modulate neuronal signaling pathways and may influence neurotransmitter systems involved in seizure activity. Therefore, *Psidium guajava* leaves may represent a promising natural source of compounds with potential therapeutic applications in neurological disorders.[14]

Excessive activation of excitatory neurotransmission plays a critical role in neuronal damage associated with epilepsy. Under pathological conditions, elevated levels of glutamate can overstimulate glutamate receptors, resulting in excessive influx of calcium ions into neurons. This process, known as excitotoxicity, leads to neuronal injury and cell

death.[7] Glutamate-induced excitotoxicity is widely used as an experimental model to study neuronal damage and to evaluate the neuroprotective potential of pharmacological agents.

In vitro neuronal models provide an effective platform for investigating the cellular mechanisms of neurotoxicity and for screening potential neuroprotective compounds. Neuronal cell lines such as PC12 (rat pheochromocytoma) cells and N2a (mouse neuroblastoma) cells are commonly used experimental models for studying neuronal function, differentiation, and neurotoxicity.[12] These cell lines are particularly useful for evaluating the protective effects of natural compounds against glutamate-induced neuronal damage under controlled laboratory conditions.

The study was designed to evaluate the in vitro neuroprotective and potential antiepileptic activity of *Psidium guajava* leaf extract against glutamate-induced excitotoxicity using neuronal cell line models, including PC12 and N2a cells.

Taxonomic history of *Psidium guajava*

Guava (*Psidium guajava* L., Myrtaceae) is a Neotropical fruit that is widely consumed around the world. However, its evolutionary history and domestication process are unknown. Here we examine available ecological, taxonomic, genetic, archeological, and historical evidence about guava. Guava needs full sunlight, warm temperatures, and well-distributed rainfall throughout the year to grow, but tolerates drought. Zoochory and anthropochory are the main forms of dispersal.[15] Guava's phylogenetic relationships with other species of the genus *Psidium* are unclear. A group of six species that share several morphological characteristics are tentatively accepted as the *Psidium guajava* complex. A significant amount of archeological information exists, with a greater number and older records in South America than in Mesoamerica, where there are also numerous historical records. From this information, we propose that: (1) the guava ancestor may have originated during the Middle or Late Miocene, and the savannas and semi-deciduous forests of South America formed during the Late Pleistocene would have been the most appropriate ecosystems for its growth, (2) the megafauna were important dispersers for guava, (3) dispersal by humans during the Holocene expanded guava's geographic range, including to the southwestern Amazonian lowlands, (4) where its domestication may have started, and (5) with the European conquest of the Neotropics, accompanied by their domestic animals, new contact routes between previously remote guava populations were established. These proposals could direct future research on the evolutionary and domestication process of guava.[15]

Standardization and Phytochemical Profile of *Psidium guajava*

Standardization of medicinal plants is an essential step to ensure the identity, purity, quality, and reproducibility. *Psidium guajava L.*, commonly known as guava, is widely used in traditional medicine and has been reported to possess several pharmacological activities.

- Pharmacognostic evaluation of *P. guajava* leaves includes the examination of macroscopic and microscopic characteristics. The leaves are typically elliptical to oblong in shape, with entire margins and a rough surface, measuring about 5–15 cm in length. Microscopic studies reveal characteristic features such as anomocytic stomata, multicellular trichomes, vascular bundles, and calcium oxalate crystals, which serve as diagnostic markers for the identification of the plant material.[11]
- Physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, extractive values, and moisture content are commonly evaluated during the standardization process.[10] These parameters help determine the purity, quality, and presence of inorganic contaminants in the plant material. Establishing these values provides a reference for the quality control of *P. guajava* leaves used in herbal formulations.

- Phytochemical investigations have shown that *P. guajava* leaves are rich in several bioactive constituents, including flavonoids, tannins, terpenoids, saponins, and phenolic compounds.[10] Among these, flavonoids such as quercetin, kaempferol, and myricetin are considered important marker compounds responsible for many of the plant's biological activities. These compounds possess antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties, which may contribute to potential anticonvulsant effects.
- Advanced analytical techniques such as Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), and High Performance Liquid Chromatography (HPLC) are frequently employed to develop chemical fingerprints and identify marker compounds in *P. guajava* leaf extracts[11] Such chromatographic profiling helps ensure the consistency and reliability of the herbal extract used in experimental studies.
- Furthermore, quality assessment also includes the evaluation of microbial contamination, heavy metals, pesticide residues, and other toxic impurities, which is essential to ensure the safety of herbal preparations.[11] Through these standardization procedures, the medicinal potential of *P. guajava* leaves can be reliably assessed and utilized for pharmacological investigations.

Pharmacological Review of *Psidium guajava*

Antioxidant activity

Guava leaves exhibit significant antioxidant activity due to the presence of flavonoids and phenolic compounds such as quercetin, catechin, and gallic acid. These compounds help neutralize free radicals and reduce oxidative stress.[13] Antioxidant activity is particularly important in neurological disorders, as oxidative stress has been implicated in neuronal damage and seizure development.

Antimicrobial activity

Extracts of P. guajava leaves have shown strong antimicrobial effects against several bacterial and fungal pathogens.[11] The presence of tannins, flavonoids, and essential oils contributes to its ability to inhibit microbial growth. This property supports the traditional use of guava leaves in the treatment of infections, diarrhea, and wound healing.

Anti-inflammatory activity

Guava leaf extracts have demonstrated significant anti-inflammatory effects in various experimental models. Flavonoids such as quercetin inhibit inflammatory mediators and reduce the production of prostaglandins and cytokines.[13] This activity may contribute to the plant's therapeutic effects in inflammatory conditions.

Antidiabetic activity

Several studies have reported that *Psidium guajava* leaf extracts possess antidiabetic properties. The plant has been shown to reduce blood glucose levels and improve insulin sensitivity.[11] These effects are mainly attributed to polyphenols and flavonoids that regulate carbohydrate metabolism and glucose absorption.

Anticancer activity

Certain phytochemicals present in *P. guajava*, particularly flavonoids and carotenoids, have been reported to exhibit anticancer and cytotoxic activities.[11] These compounds may inhibit the proliferation of cancer cells and induce apoptosis in tumor cells.

Neuroprotective and anticonvulsant potential

Recent studies suggest that the phytochemicals present in guava leaves may possess neuroprotective properties.[13] Flavonoids and antioxidants can protect neurons from oxidative damage and modulate neurotransmitter activity in the brain. Since oxidative stress and neuronal excitability are associated with seizure disorders, these bioactive compounds may contribute to potential anticonvulsant activity.

MATERIALS AND METHODS

Chemicals and Reagents

The leaves of *Psidium guajava L.*, was collected from the Palakkad district, Kerala, India. The plant material was identified and authenticated by Dr. Vivek. PJ, Associate Professor and Head, Post Graduate, and Research Dept. of Botany, Sree Neelakanta Govt. Sanskrit College, Pattambi, Palakkad.

Ethyl alcohol, distilled water, dilute hydrochloric acid (HCl), ammonium nitrate (saturated solution), chloroform water (0.1%), □-naphthol, concentrated sulphuric acid, Fehling's solutions A and B, Benedict's reagent, Barfoed's solution, Dragendroff's reagent, Wagner's reagent, Mayer's reagent, Hager's reagent, chloroform, acetic anhydride, pyridine, sodium nitroprusside solution, sodium picrate solution, dilute sulfuric acid, ether, ammonia, acetic acid, ferric chloride, magnesium turnings, tin granules, thionyl chloride, lead acetate solution, gelatin solution, bromine water, dilute iodine solution, dilute nitric acid , Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic-antimycotic solution, trypsin-EDTA, phosphate-buffered saline (PBS), glutamate, MTT reagent (5 mg/mL), and dimethyl sulfoxide (DMSO).

1. COLLECTION OF THE PLANT

Fresh leaves of Psidium guajava L. (2 kg) were collected and shade-dried to remove moisture.

2. PHYSICOCHEMICAL EVALUATION

Loss on drying

The Loss on Drying (LOD) was calculated to determine the moisture content of the fresh plant material. The percentage of loss was calculated using the weight of the material before and after drying until a constant weight was achieved.

Determination of foreign matter

Approximately 100g of the dried plant material was spread in a thin layer and examined macroscopically for the presence of foreign materials, including molds, insects, and other animal contaminants. Any foreign matter was separated and weighed to calculate the percentage of contamination relative to the initial sample weight.

Determination of moisture content

An accurately weighed quantity of the sample was placed in a tared evaporating dish and dried in an oven at 105ºC for a duration of five hours. The sample was weighed until a constant weight was achieved, and the percentage of volatile matter was calculated based on the weight loss.

Determination of Total ash value

To determine the total ash content, 2g of the ground air-dried plant material was accurately weighed into a previously ignited and tared crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 500-600º C until it became white, indicating the absence of carbon. For samples where carbon-free ash could not be

obtained, the residue was moistened with a saturated solution of ammonium nitrate before final ignition. The crucible was cooled in a desiccator, weighed, and the total ash percentage was calculated relative to the air-dried material.

Determination of acid insoluble ash value

The acid-insoluble ash was determined by boiling the total ash residue with 25ml of dilute hydrochloric acid for five minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited in the original crucible until a constant weight was reached. This value was used to estimate the amount of silica and siliceous earth present in the sample.

Determination of Extractive Values

The extractive values were determined to estimate the amount of active constituents soluble in specific solvents. For the alcohol-soluble extractive, 5g of the air-dried drug was macerated with 100ml of alcohol in a closed flask for 24 hours, followed by filtration and evaporation of the filtrate to dryness. A similar procedure was followed for the water-soluble extractive using 0.1% chloroform water as the solvent. The percentage of each extractive was then calculated based on the weight of the air-dried drug.

3. PREPARATION OF EXTRACT

Plant material and extraction

The granulated dried leaves of *Psidium guajava* (6g) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for using 250 ml of ethanol (95 % v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator.

The ethanolic extract of the dried leaves of *Psidium guajava* was stored in air-tight container and stored in desiccator.

4. PRELIMINARY PHYTOCHEMICAL SCREENING

The alcoholic extract of the leaves of *Psidium guajava* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc.

Test for carbohydrates

1. **Molisch Test:** It consisted of treating the compounds of α-naphthol and concentrated sulphuric acid along the sides of the test tube. Reddish violet ring was produced at the junction between two liquids.
2. **Fehling's Test:** Equal quantity of Fehling's solution A and B were added. Heated gently, no brick red precipitate was obtained.
3. **Benedict's test:** To the 5ml of Benedict's reagent, were added 8 drops of solution under examination. Mixed well, the mixture was boiled vigorously for two minutes and then cooled. Red precipitate was obtained.
4. **Barfoed's test:** To the 5ml of the Barfoed's solution added 0.5ml of solution under examination, heated to boiling, no red precipitate of copper oxide was obtained.

Test for Alkaloids

1. **Dragendroff's Test:** To the extract, 1ml of Dragendroff's reagent was added Orange red precipitate was produced.
2. **Wagner's test:** To the extract Wagner reagent was added. Reddish brown precipitate was not produced.
3. **Mayer's Test:** To the extract 1ml or 2ml of Mayer's reagent was added. Dull white precipitate was not produced.
4. **Hager's Test:** To the extract 3ml of Hager's reagent was added. Yellow precipitate was produced.

Test for Steroids and Sterols

1. **Liebermann Burchard test:** The test sample was dissolved in 2ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.
2. **Salkowski test:** The sample of test solution was dissolved in chloroform and equal volume of conc. sulfuric acid was added. Bluish red, cherry red and purple color is noted in chloroform layer, whereas acid assumed marked green fluorescence.

Test for Glycosides

1. **Legal's test:** Sample was dissolved in pyridine; sodium nitroprusside solution is added to it and made alkaline. Pink red color was not produced.
2. **Baljet test:** To the drug sample, sodium picrate solution was added. Yellow to orange color was produced.
3. **Borntrager test:** Few ml of dilute sulfuric acid was added to the test solution. Boiled filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet color was produced in organic layer.
4. **Killer Killani test:** Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulfuric acid. No characteristic reaction was observed.

Test for Saponins

Foam test: About 1ml of alcoholic sample was diluted separately with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. No persistent foam observed.

Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid were added. Red color was produced.

Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution was added. Pink color was produced which indicated the presence of triterpenoids.

Tests for Tannins and Phenolic Compounds

To 2-3 ml of extract, add few drops of following reagents were added

a). 5% $FeCl_3$ solution **:** Deep blue-black color.
b). Lead acetate solution **:** White precipitate.
c). Gelatin solution **:** White precipitate
d). Bromine water **:** Discoloration of bromine water.
e). Acetic acid solution **:** Red color solution
f). Dilute iodine solution **:** Transient red color.
g). Dilute HNO_3 **:** Reddish to yellow color.

5. *IN-VITRO* SCREENING

MTT ASSAY

Principle

The MTT assay is a colorimetric assay for measuring cell metabolic activity by detecting the conversion of yellow tetrazolium salt (MTT) to purple formazan crystal by mitochondrial enzymes in metabolically active cells. It is to assess cell viability, proliferation, and cytotoxicity in research. It measures the reduction of a yellow tetrazolium salt (MTT) to purple formazan crystals by mitochondrial enzymes in metabolically active cells.

Cell culture and treatment

PC12 and N2a cells were used for the in vitro evaluation of the neuroprotective potential of Psidium guajava leaf extract against glutamate-induced excitotoxicity. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2.

Cells were harvested using trypsin-EDTA and seeded into 96-well plates at a density of 1 x 105 cells/mL in 200 µL of complete medium per well. The cells were allowed to adhere and stabilize for 24 hours. After incubation, the medium was removed and the cells were washed with phosphate-buffered saline (PBS).

To induce excitotoxicity, the cells were exposed to glutamate prepared in serum-free DMEM and incubated for 24 hours. Following glutamate treatment, various concentrations of Psidium guajava leaf extract were added to the respective wells and incubated for an additional 24 hours. Control cells received only culture medium without glutamate or extract.

Cell viability assay

The MTT assay was applied for determination of cell viability. After the treatment period, MTT reagent (10 µL of 5 mg/mL solution) was added to each well and the plates were incubated for 2-4 hours at 37 °C to allow the formation of purple formazan crystals. The medium was the fully aspirated, and the wells were washed with PBS.

DMSO (100 µL) was added to each well to dissolve the formazan crystals. The absorbance at 570 and 620 nm (background) was measured using a Stat FAX 303 plate reader. The percentage of cell viability was calculated, and the IC50 value of the extract was determined using GraphPad Prism software.

Formula

$$\text{Percentage cell viability (\%)} = (\text{Test OD}/\text{Control OD}) \times 100$$

RESULTS AND DISCUSSION

Physico-Chemical Parameters of the Plant

Table 1: Physico-chemical parameters (after shade drying) of the plant.

S. No	Name of the plant	Foreign matter	Moisture content	Total ash	Acid insoluble ash	EtOH soluble extractive value	Water soluble extractive value
1.	*Psidium guajava L.*	0.6%	7%	5%	0.8%	12%	20%

Preliminary Phytochemical Screening

The preliminary phytochemical analysis of *Psidium guajava* leaf extract showed the presence of steroids, alkaloids, flavonoids, glycosides, tannins, and carbohydrate. The results of the data were tabulated in table no:2.

Table 2: Phytochemical screening of ethanolic extract of *Psidium guajava.*

Constituents	Test	*Psidium guajava*
Carbohydrates	Molisch Test Fehling's Test Benedict's test: Barfoed's test:	+ - + -
Alkaloids	Dragendroff's Test Wagner's test Mayer's Test Hager's Test	+ - - +
Steroids and Sterols	Liebermann Burchard test Salkowski test	+ +
Glycosides	Legal's test Baljet test Borntrager test Killer Killani test	- + + -
Saponins	Foam test	-
Flavonoids	Shinoda test	+
Tri-terpenoids	In the test tube, 2 or 3 granules of tin+2ml of thionyl chloride solution and test solution is added.➔Pink color	+

+ Present, - Absent

MTT ASSAY

Effect of *Psidium guajava* leaf extract on glutamate-induced cytotoxicity in PC12 and N2a cells

To evaluate the neuroprotective effect of *Psidium guajava* leaf extract, PC12 and N2a cells were treated with different concentrations of the extract (6–200 µg/ml), and cell viability was assessed using the MTT assay after 24 hours. Treatment with the extract alone did not show any significant cytotoxic effect, indicating that the extract was safe for both cell lines.

Exposure to glutamate resulted in a significant reduction in cell viability compared to control cells ($p < 0.001$), confirming glutamate-induced excitotoxicity. Pre-treatment with *Psidium guajava* leaf extract significantly increased cell viability in a concentration-dependent manner in PC12 cells when compared to the glutamate-treated group ($p < 0.05–0.001$).

Similarly, in N2a cells, the extract markedly attenuated glutamate-induced cell death, as evidenced by a significant improvement in cell viability at higher concentrations ($p < 0.01–0.001$).

These findings suggest that *Psidium guajava* leaf extract exhibits significant neuroprotective activity against glutamate-induced neuronal damage in PC12 and N2a cell lines.

Table 3: Effect of *Psidium guajava* leaf extract on glutamate-induced cytotoxicity in PC12 cells using MTT assay.

Treatment	Concentration (µg/ml)	Cell viability (% ± SEM)	Significance
Control	-	100	-
Glutamate	-	51±0.54	p<0.001
Extract + Glutamate	12	68±1.48	p<0.05
Extract + Glutamate	25	72±2.58	p<0.01
Extract + Glutamate	50	78±2.80	p<0.001
Extract + Glutamate	100	82±4.38	p<0.001
Extract + Glutamate	200	87±2.65	p<0.001

Table 4: Effect of *Psidium guajava* leaf extract on glutamate-induced cytotoxicity in N2a cells using MTT assay.

Treatment	Concentration (µg/ml)	Cell viability (% ± SEM)	Significance
Control	-	100	-
Glutamate	-	50±2.60	p<0.01
Extract + Glutamate	25	65±2.10	p<0.05
Extract + Glutamate	50	72±1.90	p<0.001
Extract + Glutamate	100	80±2.20	p<0.001
Extract + Glutamate	200	86±2.50	p<0.001

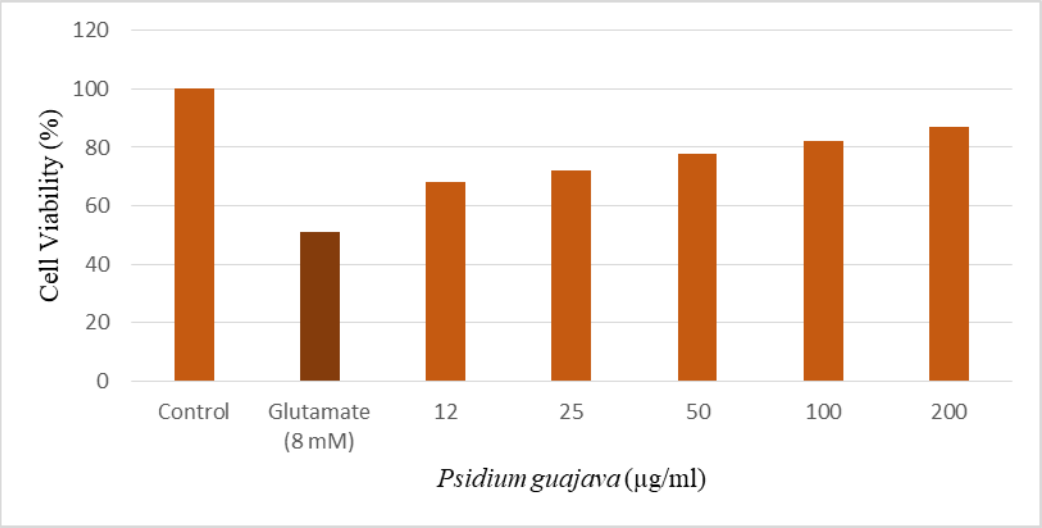

Fig 1: Effect of Psidium guajava on cell viability of glutamate-injured PC12 cells. The cells were pretreated with different concentrations of the extract for 2 h and then exposed to glutamate (8 mM) for 24 h. The cell viability was quantitated by MTT assay.

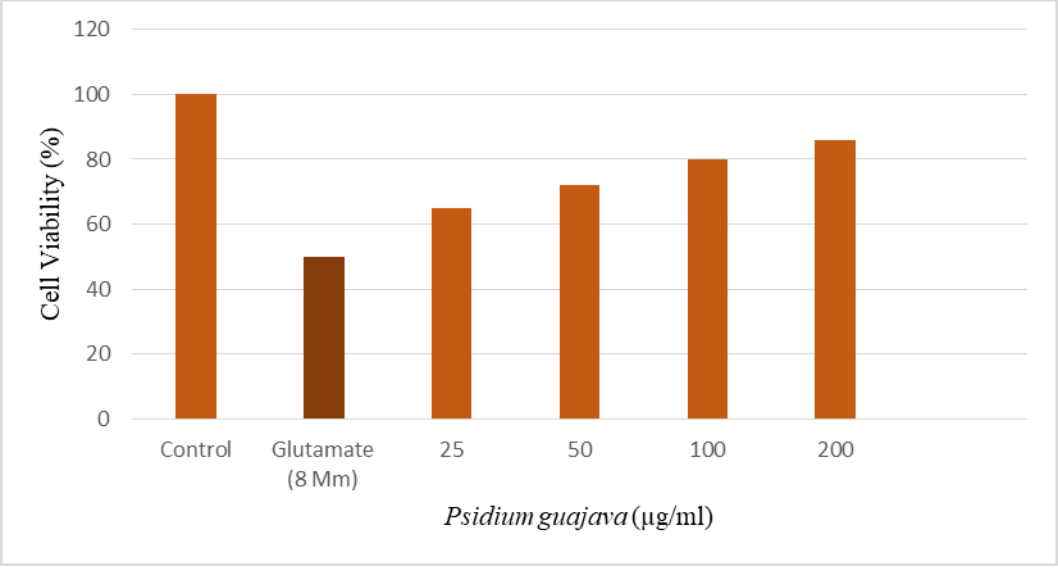

Fig 2: Effect of Psidium guajava on cell viability of glutamate-injured N2a cells. The cells were pretreated with different concentrations of the extract for 2 h and then exposed to glutamate (8 mM) for 24 h. The cell viability was quantitated by MTT assay.

DISCUSSION

The present study demonstrates that *Psidium guajava* leaf extract exhibits significant neuroprotective effects against glutamate-induced cytotoxicity in PC12 and N2a cells in a dose-dependent manner. Glutamate-mediated excitotoxicity is a well-established mechanism involved in neuronal damage associated with epilepsy and other neurodegenerative disorders.

PC12 and N2a cells are commonly used in vitro neuronal models for evaluating neurotoxicity and neuroprotection. These cells are sensitive to glutamate-induced damage, making them suitable for studying excitotoxic mechanisms relevant to epilepsy. Excess glutamate leads to neuronal cell death through receptor-mediated calcium influx and oxidative stress, which are key contributors to epileptic neuronal injury.

In the present study, exposure to glutamate significantly reduced cell viability, confirming excitotoxic neuronal damage. Treatment with *Psidium guajava* leaf extract significantly improved cell viability in a concentration-dependent manner, indicating its protective role against glutamate-induced neuronal injury. This protective effect may be attributed to the presence of bioactive phytoconstituents with antioxidant and neuroprotective properties.[13]

CONCLUSION

The present study concludes that *Psidium guajava* leaf extract possesses significant neuroprotective activity against glutamate-induced excitotoxicity in PC12 and N2a neuronal cell lines. Exposure to glutamate markedly reduced cell viability, confirming successful induction of neuronal damage. Treatment with *Psidium guajava* leaf extract significantly increased cell viability in a dose-dependent manner, as evidenced by the MTT assay. This indicates that the extract is effective in protecting neuronal cells from glutamate-induced toxicity. Similar protective effects observed in both PC12 and N2a cells suggest that the extract has consistent neuroprotective action in different neuronal models commonly used for epilepsy research. The protective effect observed may be attributed to the presence of bioactive phytoconstituents, particularly phenolic compounds and flavonoids, which are known for their antioxidant and neuroprotective properties. These compounds may help in reducing oxidative stress and preventing neuronal damage caused by excessive glutamate activity. Although the study is limited to in vitro evaluation, the findings provide scientific support for the potential role of *Psidium guajava* leaf extract as a neuroprotective and antiepileptic candidate. Further investigations involving mechanistic studies and in vivo epilepsy models are required to confirm and extend these findings.

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