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Research Article

HARNESSING THE NEUROPROTECTIVE POTENTIAL OF *PUERARIA TUBEROSA* TUBER THROUGH OXIDATIVE STRESS MODULATION AND ANTICONVULSANT ACTIVITY

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ABSTRACT

Objectives: The study aimed to assess the phytochemical profile, anticonvulsant activity, and antioxidant potential of ethanol, butanol, and aqueous *Pueraria tuberosa* tuber extracts.

Methods: Preliminary phytochemical screening was performed using standard chemical tests. In male Swiss albino mice, anticonvulsant activity was assessed using two models: seizures caused by pentylenetetrazole (PTZ) and maximal electroshock seizures (MES). Male Wistar rats were administered the extracts (100 mg/kg, p.o.) for 14 days to evaluate their antioxidant levels. Catalase, superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) levels were assessed in brain tissue homogenates. One-way analysis of variance and Dunnett's test were used for statistical analysis.

Results: Phytochemical analysis confirmed the presence of steroids, saponins, flavonoids, alkaloids, and tannins in all extracts. In the MES model, the ethanol and butanol extracts significantly reduced extensor phase duration (6.54±1 s and 6.32±0.57 s, $p<0.001$, respectively) compared to the control, closely approaching the standard drug phenytoin (4.33±0.57 s, $p<0.001$). In the PTZ model, the butanol extract showed the highest anticonvulsant activity, with a delayed onset of clonus (138.33±0.57 s, $p<0.001$), prolonged clonus duration (304.23±1.52 s, $p<0.001$), and 83.3% protection. The ethanol and aqueous extracts provided 66.6% and 33.3% protection, respectively. According to antioxidant analysis, the butanol and ethanol extracts had substantial antioxidant effects, as evidenced by the considerable increases in catalase, SOD, and GSH levels and the decrease in MDA concentrations.

Conclusion: The extracts of *P. tuberosa*, particularly the butanol and ethanol fractions, exhibited strong anticonvulsant and antioxidant activities, indicating their therapeutic potential in the management of seizures and oxidative stress-associated neurological disorders.

Keywords: Epilepsy, Flavonoids, Indian Kudzu, Reactive nitrogen species, Reactive oxygen species.

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INTRODUCTION

One of the most prevalent long-term neurological conditions in the world, epilepsy is characterized by frequent, erratic, and mostly spontaneous convulsions. Affecting 1–2% of the global population, it significantly impacts various aspects of quality of life [1].

Oxidative stress (OS) and mitochondrial dysfunction are increasingly acknowledged as key contributors to the pathophysiology of neurological disorders, including epilepsy [2]. A major cause of many illnesses, including ischemia, atherosclerosis, neurological diseases, and aging, OS is an imbalance in the rate of oxidant generation and elimination [3]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are elevated in recurrent seizures, according to experiments, and natural antioxidant substances that scavenge ROS and/or RNS have been reported to reduce epileptic discharge episodes and seizure-induced pathologies [4]. High concentrations of ROS, which are extremely reactive chemicals, can poison macromolecules such as lipids, proteins, and DNA, impairing the structural integrity and functionality of cells [5].

Furthermore, at blood concentrations within the therapeutic range for epileptic seizures, the majority of anticonvulsant medications exhibit side effects such as ataxia, drowsiness, and cognitive impairment. Finding a novel anticonvulsant that is more effective and has a better safety profile is therefore crucial. Given this, several plants extract and product may help treat seizures or convulsions; as a result, natural products provide a prospective source of novel antiepileptic medications (AED) [6]. Unfortunately, more than 60% of people continue to experience seizures even after receiving proper therapy, and more than 30% of people are still resistant to AEDs despite advancements in treatment. Consequently, there is a continuing need to create new medicines [7].

The medicinal plant *Pueraria tuberosa* (Roxb. ex Willd.) DC., also referred to as Indian Kudzu or Vidarikand, is a member of the Fabaceae family. Native to India, Pakistan, and Nepal, it is a fast-growing perennial climber characterized by its large tuberous roots [8]. Traditional medicinal systems, especially Ayurveda, and ethnomedicine both make extensive use of the plant's tuber. Its anti-aging and health-promoting properties are demonstrated by its traditional clinical use in Ayurveda (referred to as "Rasayana drugs") [9]. Numerous phytoconstituents, including alkaloids, sugars, steroids, glycosides, tannins, terpenoids, flavonoids, coumarins, and anthocyanidins, are present in the tuber [10,11]. Specifically, flavonoids and isoflavones are known for their potential health benefits, particularly in reducing OS and providing neuroprotective effects [12]. Recent studies have highlighted the anticonvulsant properties of various natural compounds; however, the specific effects of *P. tuberosa* tuber extracts in modulating OS and preventing seizure activity have yet to be comprehensively studied.

This research aims to investigate the neuroprotective potential of *P. tuberosa* tuber extracts, specifically focusing on their ability to modulate OS markers and exhibit anticonvulsant activity. By examining both the antioxidant and seizure-suppressing effects of this plant, we hope to shed light on its therapeutic potential as a complementary treatment for epilepsy, providing an alternative or adjunct to conventional AEDs. This study will evaluate key OS markers, assess seizure threshold levels, and contribute to the understanding of *P. tuberosa* as a promising natural agent for neuroprotection in epilepsy management.

METHODS

Collection and authentication of plant material

In July, tubers of *P. tuberosa* (Roxb. ex Willd.) DC. (Fabaceae family) were gathered from Karad, Maharashtra, India's local market. The Department of Botany, Krishna Mahavidyalaya, Rethare-BK, Maharashtra, India, then identified and verified the plant material.

Extraction and fractionation of plant material

P. tuberosa tubers were air-dried under a shed and subsequently coarsely powdered using a mixer grinder. A 1000 g portion of the crushed material underwent continuous hot extraction with petroleum ether (60–80°C) (6500 mL) to defeat the drug material for 48–52 h. After complete defatting, the marc was separated, air-dried, and subjected to further extraction. The defatted crude drug was then extracted with ethanol using a Soxhlet apparatus and concentrated using a vacuum rotary evaporator to get the ethanol extract (16.0% w/w). The ethanol extract was further fractionated with 4:1 (water: ethanol) with n-butanol to get n-butanol extract (6.198 % w/w), and the remaining mark left after fractionated with n-butanol was aqueous extract (9.632 % w/w) [13,14].

Preliminary phytochemical screening

A number of chemical tests were performed on the extract to check for the presence of secondary metabolites [15].

Anticonvulsant activity of *P. tuberosa*

Preparation of test sample

Dose 50 mg of the aqueous extract, ethanol extract, and butanol extracts were suspended in 1% Tween-80 solution.

Animals

Groups of 6–8 male Albino Swiss mice weighing between 22 and 25 g were kept in cages with a 12:12 h light–dark cycle and controlled temperatures of 25±1°C and 45–55% relative humidity. They have unlimited access to nourishment and beverages. The Department of Pharmacology at Satara College of Pharmacy, Satara's Institutional Animal Ethical Committee authorized the experimental procedure (Registration No. 1314/PO/Re/S/2009/CCSEA).

Assessment of anticonvulsant activity

Maximum electroshock-induced seizures (MES)

Five groups of six mice each were randomly assigned to the animals. One milliliter of normal saline was given to Group I, 25 mg of phenytoin were given intraperitoneally to Group II, 50 mg of ethanol extract, 50 mg of butanol extract, and 50 mg of *P. tuberosa* aqueous extract were given orally to Group V.

Convulsions in both control and treated animals were induced using ear electrodes to deliver a maximum electroshock (Inco Electroconvulsiometer, model #100-3) of 150 mA for 0.2 s [16]. Multiple convulsion phases, including flexion, extension, clonus, and stupor, were caused by MES. The duration of hind limb tonic extension was taken as the endpoint, with a reduction or complete prevention of this extension regarded as a protective effect.

Pentylenetetrazole (PTZ)-induced seizures

Five sets of animals were formed, each consisting of six mice. Group I administered 1 ml of normal saline, Group II administered 80 mg/kg of PTZ, and 4 mg/kg Diazepam, group III- administered 50 mg/kg each of ethanol, butanol, and aqueous extract of *P. tuberosa*.

Between 9:00 and 10:00 am, an intraperitoneal dose of PTZ (50 mg/kg B.W.) caused convulsions. Following the injection, each experimental animal was housed in a separate cage and monitored continuously for behavioral alterations that, in accordance with Ito *et al*.'s criteria, define tonic–clonic convulsions (1977a). This study only included mice that exhibited typical tonic–clonic convulsions (duration of convulsions: 60–70 s) within 3–5 min following PTZ injection. The percentage of animals in each trial that experienced ineffective PTZ-induced convulsions was estimated to be between 0 and 5%. Physiological saline (0.9% NaCl) was given orally to the control group.

After 45 min of normal saline, standard medication, and *P. tuberosa* extracts, PTZ (80 mg/kg, intraperitoneal) was given. After receiving a PTZ injection, the animals were monitored for 30 min. In this model, the capacity of various *P. tuberosa* extracts to postpone the start of myoclonic spasms and clonic convulsions was used to evaluate their anticonvulsant properties [17-19].

Biochemical estimation of OS markers

Animal and treatment schedule

Male Wistar rats weighing 200–250 g were used in the experiments. For 14 days in a row, the rats were given 100 mg/kg of different *P. tuberosa* extracts every day. The animals' brains were promptly removed, cleaned with ice–cold saline, and preserved at −80°C when they were put to sleep on the 14 day by spinal cord dislocation.

Tissue preparation

Samples of brain tissue were thawed and mixed in 0.1 M phosphate buffer, which is extremely cold (pH 7.4). After centrifuging the homogenates for 60 min at 15,000 rpm, the supernatant was gathered. Glutathione (GSH) levels, lipid peroxidation, and protein content were measured in aliquots taken from the right hemisphere. The catalyze activity was measured immediately after the sample was generated, and the superoxide dismutase (SOD) was evaluated 24 h later. Using the Lowry *et al*. (1951) method, protein content was measured using purified bovine serum albumin as a reference [20,21].

Estimation of malondialdehyde (MDA)

MDA serves as a biomarker for lipid peroxidation. Through the use of spectrophotometry, it can combine with thiobarbituric acid (TBA) to produce a complex. The findings are given as nmol/g of the tissue's moist weight. One milliliter of 0.6% TBA solution, 3 mL of 1% phosphoric acid, and 0.5 mL of brain or liver homogenate were combined for the assay. After 45 min of incubation in a boiling water bath, the mixture was cooled and mixed with 4 mL of n-butanol. The absorbance of the supernatant was measured at 535 and 520 nm after the butanol phase was separated by centrifugation. The amount of MDA in liver and brain tissues was measured [22].

Estimation of GSH

The method of Rotruck *et al*. (1972), which measures reduced GSH at 412 nm, was used to measure GSH peroxidase (GPx) activity. GPx activity was measured in units/mg protein, or μmoles of GSH used every minute per milligram of protein [23,24].

Estimation of catalase

To measure catalase activity, 0.1 mL of supernatant was added to a cuvette that contained 1.0 mL of freshly made 30 mM H_2O_2 and 1.9 mL of 50 mM phosphate buffer (pH 7.0). Catalase activity was quantified in units per milligram of protein, and the rate at which $H2O_2$ decomposed was measured using spectrophotometry at 240 nm [25,26].

Estimation of SOD

SOD activity was estimated following the method of Kakkar *et al.* (1984). The assay mixture contained 0.2 mL of nicotinamide adenine dinucleotide reduced disodium salt (NADH) (750 μM), 0.3 mL of nitroblue tetrazolium (300 μM), 0.1 mL of phenazine methosulfate (186 μM), and 1.2 mL of sodium pyrophosphate buffer (0.052 M, pH 8.3). NADH was added to start the reaction, which was then left to run for 90 s at 30°C. Then, with vigorous mixing, 0.1 mL of glacial acetic acid and 4.0 mL of n-butanol were added to halt it. Centrifugation was used to separate the butanol layer after the mixture had stood for 10 min. SOD activity was quantified as units per milligram of protein, and the chromogen's intensity in the butanol layer was assessed using spectrophotometry at 560 nm [27,28].

Statistical analysis

GraphPad Prism version 5 was used to analyze the data, and the results were displayed as mean±standard deviation. A one-way analysis of variance and Dunnett's *post hoc* test were used to evaluate group differences; $p<0.05$ was deemed statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extract

The ethanol, aqueous, and butanol extracts were found to contain steroids, saponins, flavonoids, alkaloids, and tannins.

MES test

The study evaluated the anticonvulsant potential of different extracts of *P. tuberosa* tuber in MES-induced convulsions in mice, focusing on the time spent in different phases of convulsion: flexion, extensor, clonus, and stupor (Figs.1-4).

In the control group, the mice displayed longer convulsive phases, especially in the extensor phase (9.66±0.57 s); with a prolonged stupor phase of 40.37±0.57 s. This result reflects the typical convulsive response in untreated MES-induced rats.

The standard drug, phenytoin at 25 mg/kg, effectively reduced the duration of the extensor phase to (4.33±0.57 s, ***p<0.001), indicating a significant anticonvulsant effect by minimizing the most critical phase associated with convulsive severity. The clonus and stupor times remained consistent with the control group, with all animals recovering post-convulsion.

Among the *P. tuberosa* extracts, butanol extracts at 50 mg/kg showed promising anticonvulsant activity. The ethanol extract shortened the

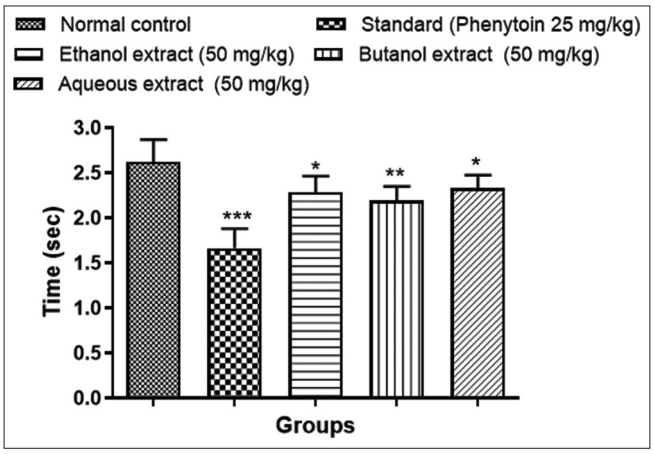

Fig. 1: Effect of *Pueraria tuberosa* extracts on flexion period. All values are expressed as mean±standard deviation (n=6); One-way analysis of variance followed by Dunnett's test; *p<0.05, **p<0.01 and ***p<0.001 when compared with normal control

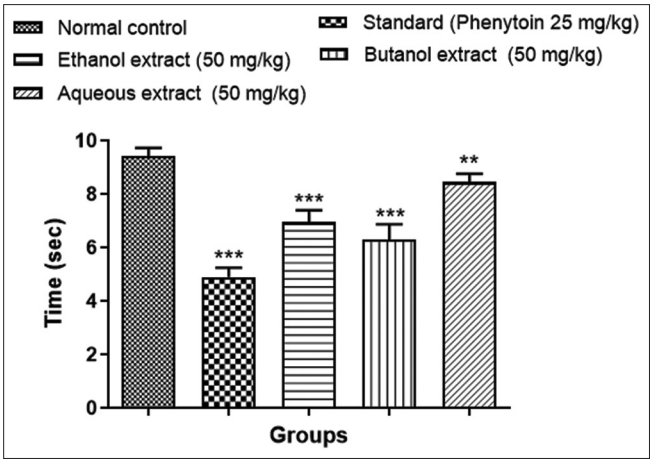

Fig. 2: Effect of *Pueraria tuberosa* extracts on extensor period. All values are expressed as mean±standard deviation (n=6); One-way analysis of variance followed by Dunnett's test; *p<0.05, **p<0.01 and ***p<0.001 when compared with normal control

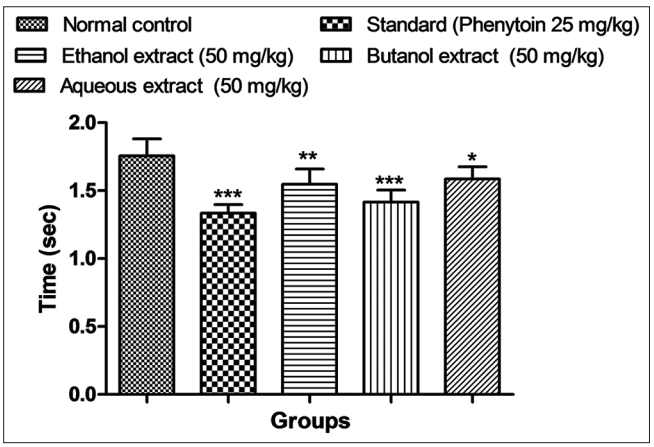

Fig. 3: Effect of *Pueraria tuberosa* extracts on clonus period. All values are expressed as mean±standard deviation (n=6); One-way analysis of variance followed by Dunnett's test; *p<0.05, **p<0.01 and ***p<0.001 when compared with normal control

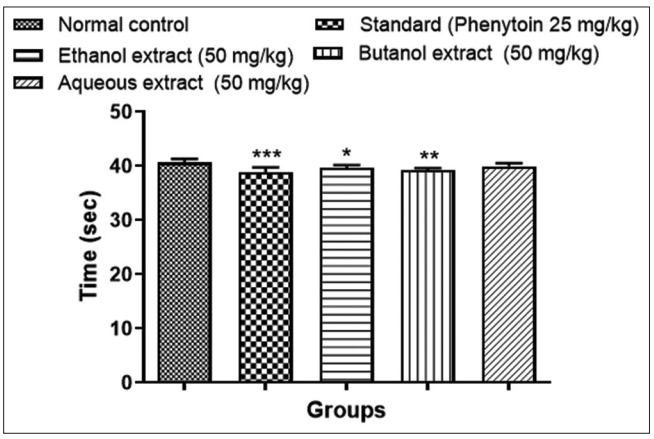

Fig. 4: Effect of *Pueraria tuberosa* extracts on stupor period. All values are expressed as mean±standard deviation (n=6); One-way analysis of variance followed by Dunnett's test; *p<0.05, **p<0.01 and ***p<0.001 when compared with normal control

stupor phase to 39.46±1 s and drastically decreased the extensor phase duration to 6.54±1 s, p<0.001, which was comparable to the action of phenytoin. Similarly, the butanol extract decreased the extensor phase even further to (6.32±0.57 s, ***p<0.001) and the stupor phase to (39.06±1.73 s, *p<0.01), suggesting that this extract may have the most potent anticonvulsant activity among the tested extracts.

The aqueous extract, although less effective than the ethanol and butanol extracts, still showed a reduction in the extensor phase (8.33±1.52 s, *p<0.01) and a shorter stupor phase (39.78±1.15 s, compared to the control.

Overall, the findings suggest that the ethanol and butanol extracts of *P. tuberosa* tuber exhibited significant anticonvulsant activity, particularly in reducing the extensor phase, similar to the standard drug phenytoin. The effectiveness of these extracts in shortening the duration of convulsive phases points to their potential therapeutic value in managing seizures.

PTZ -induced seizures

This study evaluated the anticonvulsant properties of *P. tuberosa* tuber extracts against mice's seizures caused by PTZ, looking at metrics such as clonus onset, duration, and % protection.

The effects of the tested extracts on the onset of clonus, duration of clonus, and percentage protection against convulsions are summarized in Table 1.

The control group, treated with normal saline, exhibited an onset of clonus at 81.33±1.57 s and a clonus duration of 76.28±0.57 s, with no protection against seizures (% protection=0). In contrast, diazepam (4 mg/kg, i.p.), used as the standard anticonvulsant, significantly delayed the onset of clonus (140.66±1.15 s, ***p<0.001) and markedly prolonged the clonus duration (698.25±2.51 s, ***p<0.001), achieving 100% protection.

Among the tested extracts, the butanol extract at 50 mg/kg demonstrated the highest efficacy. It significantly delayed the onset of clonus to (138.33±0.57 s ***p<0.001) and extended the clonus duration to 304.23±1.52 s (***p<0.001), with a protection rate of 83.3%. The ethanol extract also exhibited substantial anticonvulsant activity, with an onset of clonus at 132.66±0.57 s (***p<0.001) and a clonus duration of 286.53±2.51 s (***p<0.001), providing 66.6% protection.

The aqueous extract, while less effective than the ethanol and butanol extracts, still showed significant anticonvulsant activity compared to the control. It delayed the onset of clonus to 123.33±0.57 s (*p<0.05) and increased the clonus duration to 204.37±2.51 s (*p<0.05), with a protection rate of 33.3%.

The findings demonstrated that all tested extracts exhibited anticonvulsant activity, with the butanol extract showing the highest potency, followed by the ethanol and then the aqueous extract. This activity is likely attributable to the presence of bioactive phytochemicals, including alkaloids, flavonoids, and saponins, which are known to mediate neuroprotection and seizure suppression through mechanisms such as modulation of GABAergic transmission, inhibition of voltage-gated sodium and calcium channels, and antioxidant actions [29-32].

The greater efficacy of the butanol and ethanol extracts may be linked to their enriched content of lipophilic compounds, which possess superior blood–brain barrier permeability and thus engage central nervous system targets more effectively [33]. Notably, the anticonvulsant profile of the butanol extract, approaching that of diazepam, suggests possible interaction with GABA-A receptors at the benzodiazepine-binding site, alongside additional membrane-stabilizing and antioxidant effects [34,35].

Although the aqueous extract displayed comparatively weaker activity, it still produced a statistically significant anticonvulsant effect, highlighting the importance of employing multiple extraction approaches to maximize the recovery of active constituents.

The primary limitation of the present study is the absence of a comprehensive dose–response evaluation. Future research will incorporate at least two additional doses (e.g., 25 mg/kg and 100 mg/kg) to establish a proper dose–response curve and will also focus on isolating and characterizing the specific phytoconstituents responsible for the observed effects, with particular emphasis on delineating whether their anticonvulsant action arises primarily from potentiation of GABAergic inhibition, blockade of excitatory ion channels (Na^+, Ca^{2+}), activation of antioxidant defenses, neuroprotective pathways, or a synergistic combination of these mechanisms.

OS markers

This study evaluated the impact of various *P. tuberosa* tuber extracts on OS markers in rats, specifically measuring catalase, SOD, GSH, and MDA levels (Table 2).

These markers are essential indicators of antioxidant activity, where elevated catalase, SOD, and GSH levels suggest antioxidant potential, while lower MDA levels indicate reduced lipid peroxidation and OS.

Table 1: Effect of *Pueraria tuberosa* extracts on PTZ-induced convulsions

Treatment	Onset of clonus (in secondsQ)	Duration of clonus (in seconds)	% protection
Control (normal saline)	81.33±1.57	76.28±0.57	0
Diazepam, (4 mg/kg)	140.66±1.15***	698.25±2.51***	100
Ethanol extract (50 mg/kg)	132.66±0.57***	286.53±2.51***	66.6
Butanol extract (50 mg/kg)	138.33±0.57***	304.23±1.52***	83.3
Aqueous extract (50 mg/kg)	123.33±0.57*	204.37±2.51*	33.3

PTZ: Pentylenetetrazole. All values are expressed as mean±standard deviation (n=6; One-way analysis of variance followed by Dunnett's test; *p<0.05, **p<0.01, and ***p<0.001 compared with normal control

Table 2: Biochemical analysis of antioxidant and oxidative stress markers following treatment with different extracts of *Pueraria tuberosa*

Treatment 100 mg/kg	Catalase units/ mg protein	SOD units/mg protein	GSH nmol/mg protein/mg protein	MDA nmol/mg protein/mg protein
Control vehicle	20.27±1.4606	10.51±1.33	660±36	9.60±2.00
Butanol extract	42.056±0.2052	16.59±1.76	710±60	1.10±0.45
Ethanol extract	40.41±1.7081	14.71±2.74	860±60	6.90±0.42
Aqueous extract	32.906±1.2677	14.08±1.76	700±115	7.40±0.40

SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde

Catalase activity

The control group exhibited a catalase activity level of 20.27±1.46 units/mg protein. In comparison, all *P. tuberosa* extracts significantly increased catalase activity. The butanol extract demonstrated the highest catalase level (42.06±0.21 units/mg protein), followed by the ethanol extract (40.41±1.71 units/mg protein) and the aqueous extract (32.91±1.27 units/mg protein). This result suggests that the butanol and ethanol extracts, in particular, enhance catalase activity, contributing to stronger antioxidant defenses against OS.

SOD activity

SOD levels in the control group were 10.51±1.33 units/mg protein. All extracts significantly elevated SOD activity, with the butanol extract showing the highest SOD levels (16.59±1.76 units/mg protein), followed by the ethanol (14.71±2.74 units/mg protein) and aqueous extracts (14.08±1.76 units/mg protein). Enhanced SOD activity from *P. tuberosa* extracts suggests a strong ability to neutralize superoxide radicals, reducing cellular OS.

GSH levels

The control group's GSH level was measured at 660±36 nmol/mg protein. All extracts maintained or slightly increased GSH levels, with the ethanol extract showing the highest concentration (860±60 nmol/mg protein), followed by the butanol (710±60 nmol/mg protein) and aqueous extracts (700±115 nmol/mg protein). Elevated GSH levels indicate an enhanced cellular capacity to counteract oxidative damage, reinforcing the role of *P. tuberosa* in promoting antioxidant defense.

MDA levels

The control group had the highest levels of MDA, a marker of OS and lipid peroxidation, at 9.60±2.00 nmol/mg protein. All extracts significantly lowered MDA levels, with the butanol extract showing the greatest reduction (1.10±0.45 nmol/mg protein), indicating a substantial decrease in lipid peroxidation. The ethanol (6.90±0.42 nmol/mg protein) and aqueous extracts (7.40±0.40 nmol/mg protein) also effectively reduced MDA levels, although to a lesser extent than the butanol extract.

CONCLUSION

The comprehensive evaluation of *P. tuberosa* tuber extracts demonstrated significant anticonvulsant and antioxidant activities, with the ethanol and butanol extracts showing the most pronounced effects across multiple experimental models. The detection of bioactive constituents, including steroids, saponins, flavonoids, alkaloids, and tannins, underscores the therapeutic potential of the extracts and likely accounts for their observed pharmacological activities. In the MES test and PTZ-induced seizure model, the butanol extract displayed the highest anticonvulsant potential. These results suggest the extracts' ability to modulate seizure pathways, possibly through neuroprotective mechanisms attributed to their phytochemical profile.

The butanol and ethanol extracts significantly enhanced levels of catalase, SOD, and GSH, which are crucial for combating OS. Concurrently, they reduced MDA levels, indicating their role in mitigating lipid peroxidation. The butanol extract consistently exhibited the most potent antioxidant activity, highlighting its potential for therapeutic use in conditions associated with OS.

Overall, the findings highlight the potential of *P. tuberosa* tuber extracts, particularly the butanol and ethanol extracts, as promising candidates for managing seizures and OS. Further studies focusing on the isolation and characterization of the active compounds, as well as their mechanisms of action, will be essential for the development of novel therapeutic agents.

AUTHOR'S CONTRIBUTIONS

Dr. Trupti Durgawale conceptualized, designed the study, and handled the data collection. Dr. Jyotiram Sawale conducted data analysis and prepared the initial draft of the article. Dr. Pratik Durgawale and Dr. Suhas Padmane supervised the study, contributed to data analysis and interpretation, and provided essential revisions. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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