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Unraveling the Anti-Epileptic Potential of Pavonia Procumbens: Phytochemical Insights and Neuroprotective Potential

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Abstract

There remains a significant challenge when it comes to managing epilepsy despite the advancement of diagnostic techniques, so alternatives like traditional medicine are being explored. It is the purpose of this study to investigate the mechanisms underlying Pavonia's antiepileptic effect. Flavonoid was identified as a major component of Pavonia extracts by phytochemical analysis. A significant scavenging effect of EEPP was observed at 88.03 % and 88.26 % against superoxide and hydroxyl radicals, similar to standard drug rutin. This study found that EEPP inhibited 95.69% of the lipid peroxidation induced by Fe2+/ascorbate, which was comparable to the activity of rutin (87.77%). EEPP inhibits acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in a concentration-dependent manner. As a result of increasing concentrations (10-500 g/ml), the inhibitory ability of the drug increased from 13.36% to 88.53%, paralleling standard drug efficacy (33.44%-94.56%). Additionally, BuChE inhibition was also up from 7.05% to 79.36%, reflecting standard efficacy (22.36%-83.35%). There is no doubt that EEPP inhibits both enzymes more effectively than the standard, although it requires higher concentrations to be equally effective. Based on these findings, Pavonia may be effective at treating anxiety related to epilepsy. It has been suggested that cholinergic modulation may be involved in Pavonia's efficacy. It will be important to elucidate the neurobiological mechanisms behind EEPP, validate findings in animal models, identify bioactive compounds, as well as investigate broader neuroprotective effects in future research. In the context of neurological diseases, EEPP offers promise as an adjunctive therapy.

Keywords: Epilepsy, *Pavonia procumbens*, neuroprotective, traditional medicine, cholinesterase inhibition

Introduction

There is a significant portion of the world's population that suffers from epilepsy, a neurological condition marked by recurrent seizures due to abnormal electrical activity occurring in the brain [1,2]. It

is important to realize that seizures can manifest themselves in different ways, including muscle spasms, loss of consciousness, or strange sensations or behaviors [3]. There have been advances in diagnostic techniques such as CT scans and EEG over the past few decades, but managing epilepsy remains a challenging process [4]. A variety of triggers, such as anxiety, lack of sleep, and certain medications, can potentially exasperate seizures.

It has been reported that despite the fact that there are numerous antiepileptic medications available to help manage seizures, some patients still experience insufficient seizure control or significant side effects when taking these medications [5,6]. Consequently, there has been an increase in the use of alternative treatments, such as plant-based drugs, over the last few years [7-10]. There is an abundance of medicinal plants with potential therapeutic properties for epilepsy in India, due to its lush biodiversity and extensive history of herbal medicine.

Among these plants, the *Pavonia procumbens* has garnered a great deal of attention for its reported medicinal properties, including antidiabetic [11], antibacterial [12], and antioxidant properties [13]. However, despite the promising findings of *Pavonia procumbens*, the antiepileptic potential of the plant remains largely unexplored. In light of these findings, the purpose of this study is to investigate the efficacy of *Pavonia procumbens* in treating epilepsy, and also to investigate the preventive and therapeutic mechanisms behind the anti-epileptic activity of *Pavonia procumbens.*

Methodology

Collection of plant specimens

From the local area in Chennai, the fresh leaves of *Pavonia procumbens* were collected and it was authenticated by Dr. A. Balasubramanian, Former SIDDHA Research consultant (AYUSH) Ministry of Health and family welfare, New Delhi.

Plant extraction process

Approximately 1kg of dried powdered leaf material was sequentially extracted using chloroform, benzene, pet-ether, and ethanol in a Soxhlet apparatus. Then extracts were concentrated under reduced pressure, and residual solvents traces were removed. Subsequently, the extracts were stored in vacuum desiccators for further use. Additionally, aqueous extract was prepared by macerating the leaf powder with double-distilled water. Then this extract also concentrated in a water bath and stored in a desiccator.

Preliminary phytochemical screening [14,15]

As per the standard procedure, the chemical test to detect the secondary metabolites such as carbohydrates, alkaloids, flavonoids, glycosides, sterols, tannins, Saponins etc., were carried out.

Total phenolic content determination [16]

The total phenolic content of the plant was estimated based on the literature and preliminary phytochemical screening results. Colorimetric measurements were performed using Rutin and FC (Folin Ciocault's) reagents.

Standard solution

Several dilutions of 1, 2, 4, 6, 8, and 10µg/ml of rutin were made by weighing and dissolving 1 mg of rutin in 100ml of distilled water. Mix 1.25ml of FC reagent with a volume from above aliquots. It was left for 5 mins. Following that, 2.5ml of 20% sodium carbonate was added and allowed to react for 30 minutes before being diluted to 10ml. At 765 nm, the absorbance was measured. Using the absorbance and concentrations, a calibration curve was plotted.

Sample solution

An extract of 0.5 grams was weighed and dissolved in 100 milliliters of water. 0.1 ml of this solution was filled into a 10ml standard flask along with 1.25ml of FC reagent and allowed to react for five minutes, then 2.5ml of 20% sodium carbonate was added and the volume was brought up to 10ml. For the reaction to be complete, it was left for 30 minutes. At 765 nm, the absorbance was measured. Rutin equivalents were determined from the total phenolic content calculated from the calibration curve.

Chromatography study

HPTLC Fingerprinting [17]

Preparation of sample

Approximately 1 gram of powdered plant parts was extracted with ethanol, subsequently evaporated to dryness, and dissolved in 1 ml of ethanol separately by using a sonicator before filtration. A description of the chromatographic conditions can be found in (Table 1). In Table 2, solvent systems are listed according to sample proportions.

Table: 1 Optimized HPLTC Chromatographic Conditions

Application Mode	CAMAG - Linomat IV Applicator
Application Syringe	Hamilton – Bonaduz 695.0014 CA
Filtering System	Advance Micro-devices Pvt. Ltd. PTFE 0.2µm
Stationary Phase	MERCK-TLC/HPTLC Silica gel 60 F254 on Aluminum sheets (10X4 cm)
Applying distance	10mm
Band Width	8mm
Band spacing	6mm
Volume of sample	10µL
Separation Mode	CAMAG-Twin Through Chamber
Development Distance	80 mm from plate base
Plate drying	5min with cold air (hair dryer)
Spraying Mode	Diptank of 500 mL capacity
Spraying time	The plate is immersed in the spray reagent for 0.5 to 1.0 min
Drying Mode, Temperature & time	CAMAG-TLC Plate Heater Preheated at 100± 5^0C for 5 min

Table:2 Solvent System for HPTLC Fingerprinting

S.No	Sample	Solvent System	Visualization mode/Spray Reagent
1	Ethanol extract of *Pavonia procumbens* (EEPP)	ethyl acetate: formic acid: water (6:1:1)	Anisaldehyde+H_2SO_4
2	Standard Flavonoid (Rutin)	ethyl acetate: formic acid: water (6:1:1)	Anisaldehyde+H_2SO_4

***In-vitro* anti-oxidant assay**

SOD assay

Nitro blue tetrazolium (NBT) reduction was used to determine superoxide radical scavenging activity. This assay generates superoxide radicals through the nonenzymatic PMS/NADH system, which reduces NBT into a purple color formazan. There were 0.5 mL of phosphate buffer of 7.4pH, NADH 1ml, NBT 1ml, PMS 0.1ml, also EEPP and standard drug (Rutin) 3ml of various concentrations (10-50 µg/mL, in 90% ethanol). The mixture was measured for absorbance at 560 nm at 25ºC for one hour after incubation to determine the amount of formazan formed. [18,19]

Hydroxyl radical scavenging assay

Fe3+/ascorbate/EDTA/H2O2 systems were used to generate hydroxyl radicals. The system produces hydroxyl radicals that attack deoxyribose, resulting in thiobarbituric acid (TBA) and TBARS. As a reaction mixture, 0.1 mL 2-deoxy-2-ribose (10mM) was added, 0.33 mL phosphate buffer was added, 0.1 mL $FeCl_3$ was added, 0.1 mL EDTA was added, 0.1 mL H_2O_2 was added, 0.1 mL ascorbic acid was added, and 1.0 mL concentrations of EEPP and standard (Rutin) were added. When the reaction mixture was incubated for 45 mins at 37ºC, 1.0 mL of 2.8% (v/v) TCA was added, along with 1.0 mL of TBA, 0.5% (v/v) in 0.025 mol/L sodium hydroxide having 0.2% BHA, and the pink chromogen was developed by incubating it for 15 minutes at 95ºC. In comparison with a blank solution, the absorbance was measured at 532 nm after cooling. [19]

Lipid per-oxidation assay

Ascorbic acid, $FeCl_3$, and Tris-HCl buffer 7.0pH were added to a reaction mixture of rat liver homogenate (0.1 mL, 25% w/v) at 37ºC in the presence or absence of EEPP at various concentrations (50-250µg/mL) for 1 h at 37ºC. TBARS measurements were used to measure lipid peroxide formation. Distilled water was then added, and the mixture was kept at 100°C for 1 hour. A mixture of n-butanol and pyridine (10:1 v/v) was added to the reaction mixture after cooling, was shaken vigorously, and centrifuged for 10 mins at 4000rotation speed. TBARS were measured by measuring the absorbance at 532 nm of the butanol-pyridine layer [20]

In-vitro anti-inflammatory assay (Protein denaturation method)

A weighed amount of EEPP was dissolved in distilled water to make a concentration of 1 mg/ml. As a result, various concentrations of 500, 250, 100, 50 and 25 g/ml were obtained by serially diluting this solution. A standard drug, Acetyl Salicylic Acid, was used to prepare solutions with similar concentrations. In vitro anti-inflammatory activity was assessed using the procedure with minor modifications [21]. The test drug and the standard drug were diluted in phosphate buffered saline solution (PBS) by 0.5ml per concentration. This was then incubated at 27°C with 2ml of albumin from egg. Following 15 minutes of reaction, the tubes were transferred to a 70°C water bath and allowed to stand for 10 minutes. In a UV-Vis measurement at 660nm, the reaction mixture was cooled and the absorbance was measured in distilled water as blank solution. The reaction mixtures without drugs were considered as control solutions.

$$\% \text{ of inhibition} = \frac{ABS\ test - ABS\ control}{ABS\ control} X\ 100$$

Acetyl-cholinesterase (AChE) and Butyryl-cholinesterase (BChE) Inhibition assay (Ellman's method) [22,23]

Based on a colorimetric method, acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates for the AChE and BChE activity assays. 10 µL of EEPP solution in 0.2% DMSO, 79 µL of 20 mM sodium phosphate buffer (pH 7.6), and 1 µL enzyme preparation (with final concentrations: 0.087 unit/mL for AChE, or 0.035 unit/mL for BChE, and final concentrations: 1 to 500/1000 µM for

compounds tested) were mixed and preincubated for 15 min. 10 µL of substrate solution were added to the mixture and incubated for 30 minutes (final concentrations of 1.5 mM or 4 mM for acetylthiocholine iodide and butyrylthiocholine iodide). DTNB-phosphate-ethanol reagent was used to stop the reaction. A microplate reader was used to measure the absorption immediately at 412 nm. AChE or BChE inhibition dose response curves were calculated with galanthamine as standard to calculate the IC50.

Statistics

In accordance with procedure, values are considered as triplicates and presented as Mean and Standard error. To find the difference between the samples, the data was subjected to Analysis of variance (one-way analysis). Dunnet's test was used to evaluate significant differences between groups and P>0.001 was considered

Results

Preliminary phytochemical study

Formulating pharmacopoeial standards relies on phytochemical analysis. Some of the most important phytochemical found in Pavonia extracts were listed in below table 3

Table: 3 Preliminary phytochemical screening

S.No	Constituents	Solvents				
		Pet-ether	Benzene	Chloroform	Ethanol	Water
1	Carbohydrates	_	_	_	+	+
2	Alkaloids	_	+	_	+	+
3	Tannins	_	_	_	+	+
4	Glycosides	_	_	_	+	+
5	Steroids	+	+	+	_	_
6	Flavonoids	_	_	_	+	+
7	Triterpenoids	+	+	+	+	_
8	Saponins	_	_	_	+	+
9	Polyphenols	_	_	_	+	+
10	Gum and mucilages	_	_	_	_	+

Total Phenolic content estimation

Table: 4 Content of chemical constituents in the various extract of *Pavonia procumbens*

	Extract				
	Pet-ether	Benzene	Chloroform	Ethanol	Water
Percentage yield	4.29	3.44	9.37	25.38	18.33
Total phenol content	32.29±5.32	20.22±2.38	23.84±3.33	321.35±29.37	297.52±21.29

Chromatography study
HPTLC fingerprinting study

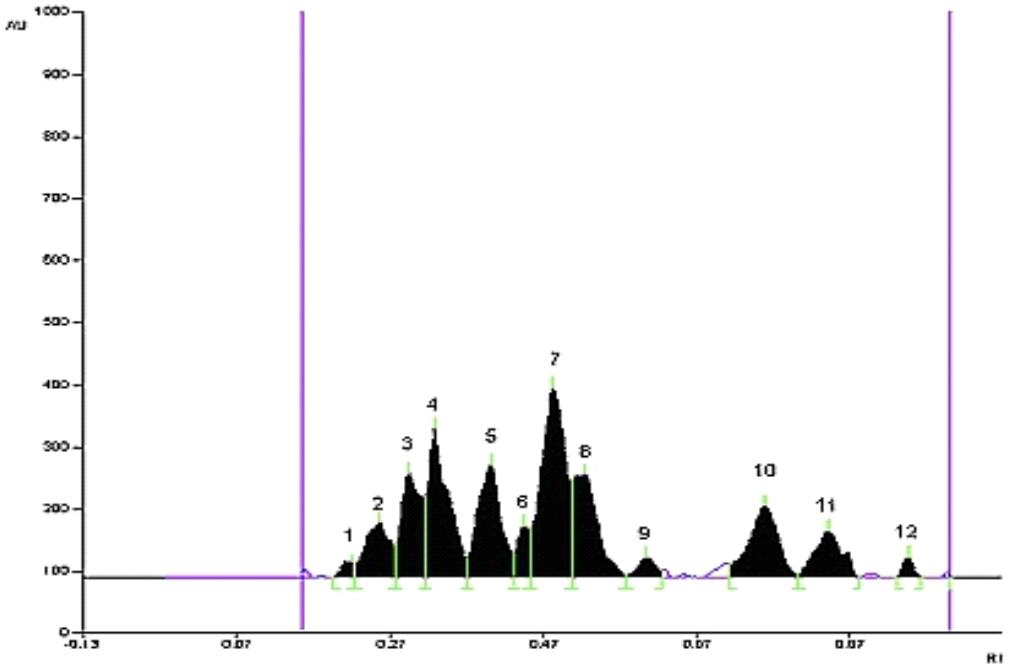

Figure:1 HPTLC chromatogram of Ethanol extract of leaves of Pavonia obtained at 320

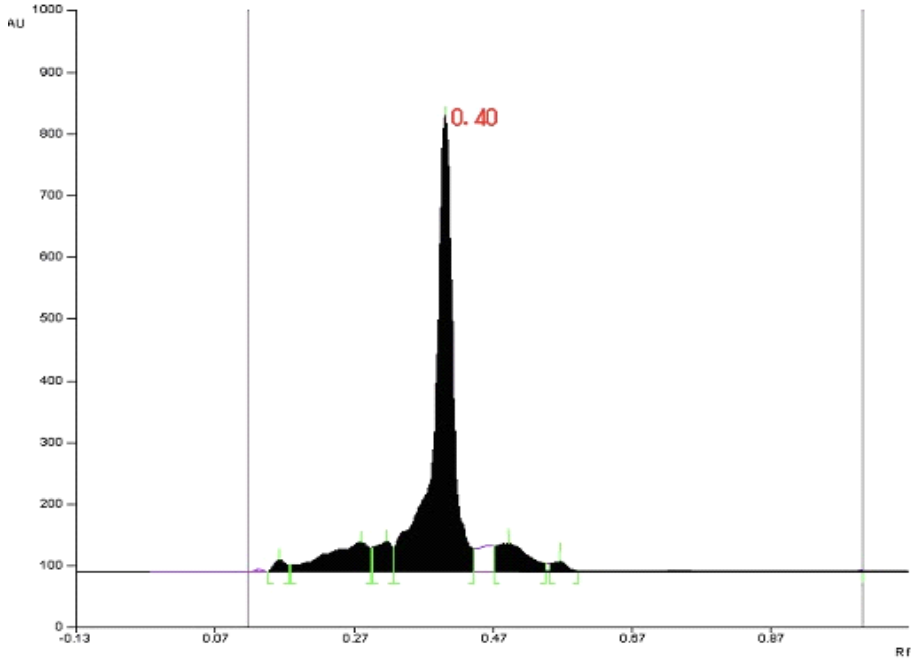

Figure: 2 Standard Rutin (Flavonoid) Chromatogram

A Rf value of 0.40 was determined for the standard Rutin and it was close to the peak 5 of the extract chromatogram, thus identifying it as rutin. Upon quantification, 0.118mg/mg w/w of rutin was found in the extract.

Anti-oxidant assay

SOD Assay

There was a concentration dependent scavenging effect of EEPP on superoxide radicals. At the concentration of 50µg/ml, EEPP exhibited 88.03±2.23% of scavenging activity, while rutin showed 86.846±2.436% of scavenging activity at the same concentration. (Figure 3)

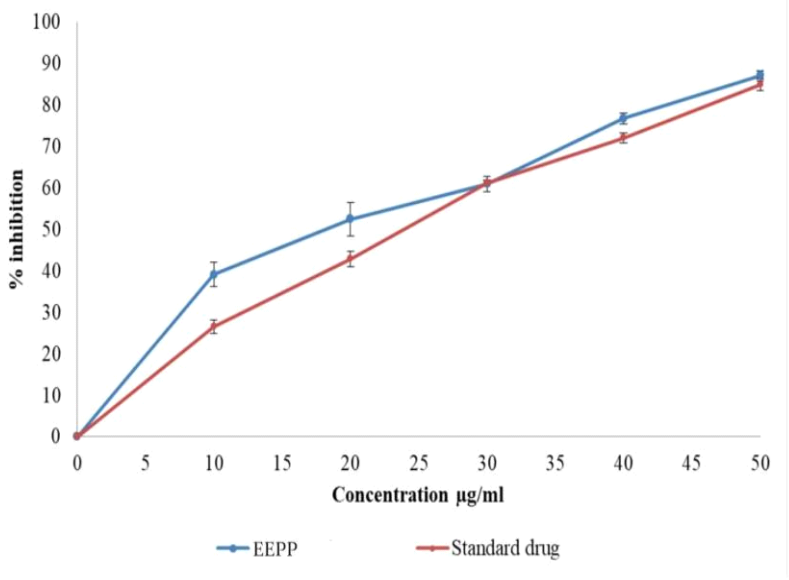

Figure: 3 Superoxide radical scavenging activity

Hydroxyl radical scavenging activity

The percentage inhibition of hydroxyl radicals was 88.262±1.566% for EEPP at the concentration of 50µg/ml. Inhibitory activity of isolated molecule was compared to that of standard drug rutin (89.428±1.564%) at the same concentration. In comparison with rutin, EEPP had similar percentage scavenging activity. (Figure 4)

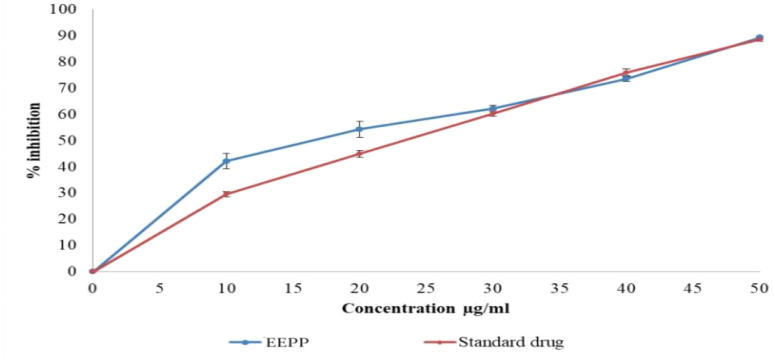

Figure: 4 Hydroxyl radical scavenging activity

Lipid peroxidation scavenging assay

The lipid peroxidation induced by Fe2+/ascorbate was inhibited by EEPP in a concentration dependent manner. A significant inhibitory effect on lipid peroxidation was observed, with a percentage inhibition of 93.686±2.324. Rutin used as the standard drug, exhibited inhibition of 87.77±2.377 at the concentration of 50µg/ml. These findings indicated that the EEPP extract possess a comparable inhibitory activity to the standard drug rutin. (Figure 5)

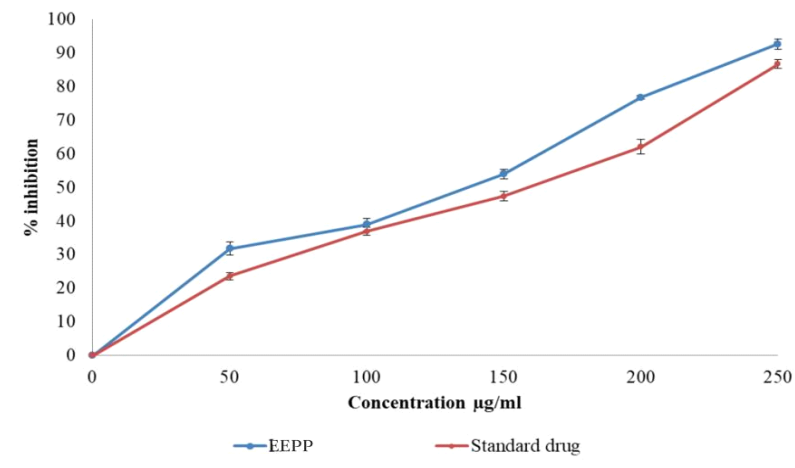

Figure: 5 Lipid peroxidation scavenging activity

***In-vitro* acetyl-cholinesterase and Butyryl-cholinesterase inhibition of EEPP**

The concentration dependent effects of plant extract on acetyl-cholinesterase and Butyryl-cholinesterase were described in figure 6 and 7. The results reveal a clear dose-response relation between EEPP concentration and the inhibition of both acetyl-cholinesterase and Butyryl-cholinesterase activities. In terms of AChE inhibition, the standard compound displayed a progressive increase in inhibition percentage, ranging from 33.44% at 10µg/ml to a robust 94.56% at 500 µg/ml. Similarly, EEPP demonstrated a rising inhibition trend, with percentages ranging from 13.36 to 88.53% across the same concentration range. Remarkable, at higher concentration (250 and 500 µg/ml), EEPP approached the inhibitory efficacy of the standard compound. It was also observed that butyrylcholinesterase inhibition followed a similar trend. EEPP, on the other hand, showed a gradual increase in inhibition percentages, from 7.05% to 79.36%, while the standard compound showed an increase from 22.36% to 83.35%. In contrast, EEPP inhibited both acetylcholinesterase and butyrylcholinesterase more potently than the standard, indicating a lower potency. According to these results, EEPP inhibits both acetylcholinesterase and butyrylcholinesterase at high concentrations, although higher concentrations are required to achieve comparable efficacy. Potential therapeutic applications targeting cholinesterase-related conditions may benefit from further research into the specific bioactive components within EEPP.

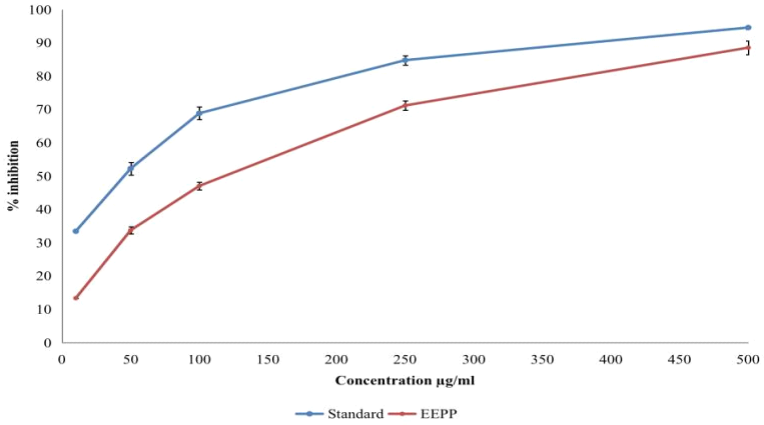

Figure: 6 Acetyl-cholinesterase inhibition of EEPP in protein denaturation assay

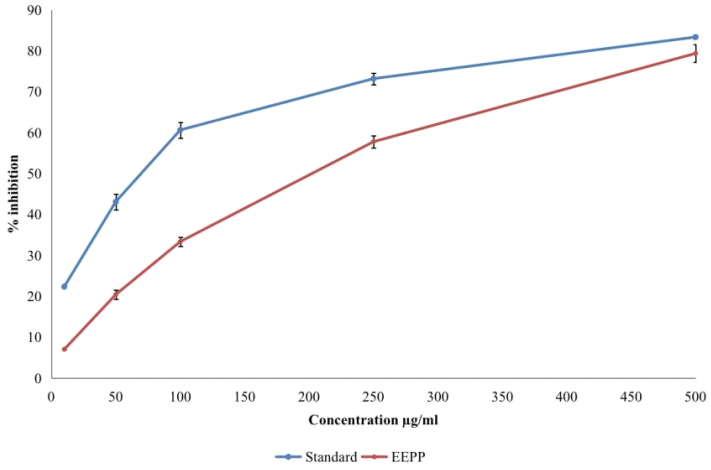

Figure: 7 Butyryl-cholinesterase inhibition of EEPP in Ellman's method

Discussion

In various studies, research has demonstrated a connection between epilepsy and behavioral disorders like anxiety and depression. Emotional disorders are more prevalent in epilepsy patients than in patients with other chronic diseases [24,25]. It remains unclear, however, how anxiety develops in epilepsy. The dysfunction of cholinergic receptors has been implicated in epilepsy. It has been shown that anxiety and depression are affected by cholinergic transmission [26].

Through the hydrolysis of acetylcholine (ACh) in the brain, acetylcholinesterase (AChE) terminates cholinergic transmission. Therefore, various therapeutic contexts have investigated AChE inhibition [27,28].

Pavonia leaf ethanol extract (EEPP) is shown to be potentially effective for inhibiting AChE in this study. Cholinergic dysfunction may contribute to epilepsy-related anxiety, as indicated by the concentration-dependent inhibition of AChE observed in the study. Additionally, Pavonia species contain rutin, a substance that has neurobiological significance, as reported in the literature. Butyrylcholinesterase, a potential therapeutic target for epilepsy, also hydrolyzes ACh, although less efficiently. Increasing acetylcholine levels in the brain may improve cognition by inhibiting butyrylcholinesterase [29].

In neurological conditions, drugs such as galantamine and rivastigmine demonstrate dual inhibition of both AChE and butyrylcholinesterase [30,31].

These findings suggest that EEPP inhibits both AChE and butyrylcholinesterase concentration-dependently, as demonstrated in the study. It has been suggested that epilepsy-related anxiety is related to modulation of cholinergic transmission. According to the study, EEPP inhibits cholinesterase activity and cholinergic dysfunction has been associated with epilepsy-related anxiety, suggesting that further investigation is warranted. It may be feasible to develop therapeutic interventions in epilepsy-associated anxiety based on an understanding of the exact mechanisms underlying the extract's effects on cholinesterases.

Conclusion

Based on this study, Pavonia leaf ethanol extract (EEPP) demonstrates therapeutic potential to address epilepsy-related anxiety, likely through its concentration-dependent inhibition of acetylcholinesterase and butyrylcholinesterase. These findings are consistent with previous findings that cholinergic dysfunction plays a role in epilepsy and anxiety. As a modulator of neurotransmitter dynamics, EEPP's inhibition of key enzymes may offer insight into the role of acetylcholine in epilepsy-associated anxiety. EEPP future research should focus on revealing the specific neurobiological mechanisms underlying its effects, validating findings in animal models, identifying bioactive compounds, and exploring its broader neuroprotective properties. The potential for novel adjunctive therapies for neurological diseases is suggested by EEPP as a source of bioactive compounds.

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