



EVALUATION OF ANTICONVULSANT ACTIVITY OF ETHANOL LEAF EXTRACT OF *Pavetta crassipes* K. SCHUM (*RUBIACEAE*) ON 4-AMINOPYRIDINE AND PENTYLENETETRAZOLE INDUCED SEIZURES IN ALBINO MICE

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ABSTRACT

Epilepsy is one of the most common neurological disorders that is affecting more than 50 million people worldwide and it has several underlying causative factors. The chronic nature of epilepsy and the inability of the current drugs to have a long term seizure control in all patients has led to the need to investigate plants that are used in traditional medicine for the management of epilepsy. *Pavetta crassipes* (K. Schum) Rubiaceae is used in Northern Nigerian folk medicine to manage a range of diseases including epilepsy, cough and other diseases and its efficacy is well known in northern Nigeria. This study was carried out to determine the anticonvulsant effects of ethanol leaf extract of *P. crassipes* on mice using 4-aminopyridine and pentylenetetrazole (PTZ) induced seizure models at doses 250, 500 and 1000 mg/kg intraperitoneally. The intraperitoneal LD₅₀ in mice was estimated at 3807.88 mg/kg. The extract decreased the time for the onset of myoclonic jerk, the time for the onset of convulsion, the number of convulsion and the duration of convulsion at 250, 500 and 1000 mg/kg and also protected 80, 100 and 100% of the mice at 250, 500 and 1000 mg/kg doses respectively in the 4-aminopyridine-induced convulsion. The extract reduced the increased the latency of convulsion at 250, 500 and 1000 mg/kg. The extract reduced the number and duration of convulsion at 250, 500 and 1000 mg/kg and the extract protected 40%, 40% and 60% of the mice at 250, 500 and 1000 mg/kg against PTZ-induced seizures. The findings from the research suggest that ethanol leaf extract of *P. crassipes* has a potent anticonvulsant properties that are effective in the management of epilepsy and the maximum ameliorating effects observed in the 4-aminopyridine induced seizures suggests seizure control by the extract via interactions with potassium channels leading to decreasing neuronal, calcium channel blockade, leading to inhibition of the excessive influx of presynaptic Ca²⁺ caused by 4-AP and stabilization of neurons against the excessive glutamate concentrations generated by 4-AP. PTZ induced seizure showed ameliorating effects of the extract via enhancement of GABAergic neurotransmission in the brain by restoration of central GABAergic tone. The extract is also believed to counteract the mechanical channel antagonism imposed by PTZ, promoting a higher binding affinity for endogenous GABA molecules in the brain. This structural adjustment maximizes both the open-state frequency and duration of the integral Cl⁻ pore, reinstating inhibitory postsynaptic potentials (IPSPs) that blocks the propagation of seizure firing in the brain.

Key words: Epilepsy, PTZ, 4-aminopyridine, GABAergic tone, potassium channels, *Pavetta crassipes*

INTRODUCTION

Epilepsy is a neurological disorder that is characterized by repetitive and uncontrolled seizures. Seizures are events of brain dysfunction which occur as a result of recurrent firing of cerebral neurons (Fisher *et al.*, 2014). Epilepsy disorder characterized by an imbalance in neuronal electrical activity results to various recurrent and unpredictable seizures which affects the patient's negatively (Fisher *et al.*, 2014). Some epileptic syndromes have been related to progressive cortical thinning and brain volume loss, as well as to neuronal death in several brain regions (Galovic *et al.*, 2019; Whelan *et al.*, 2018). The chronic neurological disorder of epilepsy occur with recurrent episodic attacks, epileptic seizures, convulsion and their somatic and psychiatric consequences. Recent data suggest that it affects around 60-70 million people worldwide from all ages, races, social classes, and geographical locations (Perez-Malagon and Lopez-Gonzalez, 2021). Seizures have been classified into two broad groups: focal and generalized. Focal seizures involve only a portion of the brain, typically one of the cerebral hemispheres (Bradley, 2012). A complex partial seizure is a seizure that is associated with unilateral cerebral hemisphere involvement and causes impairment of awareness or responsiveness, that is, alteration of consciousness (Johnston *et al.*, 2000). Complex partial seizures are often preceded by an aura (Johnston, *et al.*, 2000). Complex partial seizures occur when excessive and synchronous electrical brain activity causes the impaired awareness and responsiveness (Patsalos *et al.*, 2008). The abnormal electrical activity might spread to the rest of the brain and cause a focal to bilateral seizure or a generalized tonic-clonic seizure (Johnson *et al.*, 2000).

The mainstay of epilepsy management is the use of antiepileptic drugs which have proved to be effective in reducing the onset and severity of neuronal attacks. Therapy is symptomatic, in that available drugs inhibit seizure but neither effective prophylaxis nor cure is available (Puranam and McNamara, 2001). The current available synthetic antiepileptic drugs provide seizure control in up to 70% patients with 30% of patients not responding to treatment. Moreover these drugs are associated with many side effects like teratogenicity, sedation, fatigue and behavioral disturbances (Avanzini and Franchetti, 2003). Nearly 80% of epilepsy cases live in low- and middle-income countries (LMIC) and as such, antiepileptic drugs are not readily available and affordable to the large number of epilepsy patients in developing countries (Rincon *et al.*, 2021; Beghi, 2020). In developing countries like Nigeria, the World health Organization (WHO) has recommended the initiation of programs designed to incorporate the use of medicinal plants for the management of diseases like epilepsy, cancer and diabetes (WHO, 1978). Plants are believed to be important as a source of new lead compounds with potential therapeutic benefits for the management of this debilitating neurological disorders and they are believed to have fewer side effects (Eisner, 1990). One of such plants is *Pavetta crassipes*. *P. crassipes* is reported to be used medicinally in the management of respiratory infections and abdominal disorders. The leaves are used in Tanzania in the treatment of gonorrhea. In Central Africa and Northwest Nigeria, the leaves are taken as a cough remedy (Watt and Breyer-Brandwijk, 1962). The plant is also used in Northern Nigeria for the treatment of epilepsy. However, there is no

comprehensive data to support the use of this plant in the treatment of epilepsy using different chemical models of epilepsy. Therefore, this study was carried out to evaluate the effects of *P. crassipes* in 4-aminopyridine and pentylenetetrazole induced seizures in albino mice.

MATERIALS AND METHODS

Materials

Pavetta crassipes leaves, pestle and mortar, n-Hexane, ethanol, filter paper, funnel, weighing balance, analytical balance, syringe (5ml and 1ml), measuring cylinder, stop watch, beakers, conical flasks, glass rod, spatula, hand gloves, permanent marker, masking tape, distilled water and rotary evaporator.

Reagents

Valproic acid powder (Sigma-Aldrich, USA), Pentylenetetrazole powder (Sigma-Aldrich, USA), 4-Aminopyridine powder (Sigma-Aldrich, USA), normal saline (Advacare Pharma) and distilled water.

Plant Material Source, Collection and Identification

Pavetta crassipes (K. Schum) Rubiaceae leaves were collected in the month of January 2022, from the outskirts of Kaduna, along Kaduna-Zaria Highway, in Kaduna State, Nigeria. The plant sample was identified and authenticated by Prof. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Borno State. A voucher number UM/FPH/18c/001/001 was obtained in the November, 2020 and deposited at the Department of Pharmacognosy Repository, Faculty of Pharmacy, University of Maiduguri.

METHODOLOGY

Handling and Extraction Process

The leaves of the *Pavetta crassipes* were air-dried at room temperature under the

shade until a constant weight was obtained. The dried leaves were pounded into powdered form using a wooden pestle and mortar. The weight of the powdered leaves was determined using a weighing balance. The powdered sample weighing 1,220 gram was soaked in 5 liters of n-Hexane in a ratio of 1:4 and allowed to stand for 72 hours with occasional shaking to extract the fat in the powdered leaves. The resulting mixture was filtered using a Whatman filter paper, a funnel, retort stand and a conical flask. The process of fat extraction from the powdered leaves was repeated three times until a clear solvent was seen. Rotary evaporator was used to recover the solvent at 40°C. The filtrate was collected in a glass container and allowed to evaporate to dryness in a laboratory oven. After drying, the dried extract was weighed, collected in a container and stored in a refrigerator for further use. The extraction yield was calculated by dividing the weight of the extract by the weight of the powdered sample and then multiplying the result by 100.

The residue of the defatted powdered leaves was allowed to dry. The dried powdered leaves weighing 1,150g was then macerated in 5 liters of ethanol in a ratio of 1:4 and left to stand for 72 hours with occasional shaking at room temperature. The resulting mixture was filtered using a Whatman filter paper, a funnel, retort stand and a conical flask. The solvent was repeatedly recovered using a rotary evaporator at 40°C. The extraction process was repeated three times in order to obtain maximum yield. The filtrate was collected in a glass container and allowed to evaporate to dryness in a laboratory oven. After drying, the dried powdered extract was weighed, collected in an airtight container and stored in a refrigerator for subsequent use. The extraction yield was calculated by dividing the weight of the extract by the weight of the powdered sample and then multiplying the result by 100.

Experimental Animals

Fifty male Swiss Albino mice weighing between 25-30 g and aged 3-4 months were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, Bayero University, Kano, Kano State. The animals were acclimatized at the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri. The animals were maintained at ambient room temperature, 12 hour light/dark cycle and standard laboratory conditions. The animals were fed with standard animal feed (Vital Feed Plc, Bukuru Jos) and water was provided *ad libitum*. Ethical approval for the research was requested from the Faculty of Pharmacy Research and Ethics Committee in the month of December, 2020. Ethical approval number; FP/03/20/PGA-18-08-01-08800 was obtained in January, 2021. Animals were grouped into five groups of five (a minimum of 5 mice per group), with group 1 serving as negative control, group 2 serving as positive control and groups 3, 4 and 5 serving as test group. Group 3 was pretreated with low dose, group 4 was pretreated with medium dose while group 5 was pretreated with high dose of the extract.

Experimental Site

The experiments were conducted in the Department of Pharmacology and the Department of Pharmacognosy laboratories of the Faculty of Pharmacy, University of Maiduguri, Borno State.

Phytochemical Screening

Phytochemical screening was carried out on the ethanol leaf extract for the qualitative determination of major chemical constituents such as alkaloids, glycosides, flavonoids, phenol, tannins, saponins and steroids using the methods described by Sofowora, 2008, Evans, 2009 and Mohammed, 2014.

Test for Tannins

A small quantity (0.5 g) of the extract was boiled with water and filtered. Two drops of ferric chloride were added to the filtrate. Formation of a blue-black, or dark green precipitate was taken as evidence for the presence of tannins (Evans, 2009; Mohammed, 2014).

Tests for Flavonoids

The extract was dissolved in 2 ml distilled water. 2 ml of the mixture was dissolved in 10% aqueous sodium hydroxide solution. The solution was observed for the presence of a yellow colour, a change in colour from yellow to colourless on addition of dilute hydrochloric acid indicates the presence of flavonoids (Evans, 2009; Mohammed, 2014).

Test for Cardiac Glycosides

The extracts (0.5 g) were respectively dissolved in 5 ml glacial acetic acid containing traces of ferric chloride in a test tube was held at an angle of 45° and 1 ml of concentrated sulphuric acid was added carefully down the side, a purple ring colour indicates its presences (Evans, 2009; Mohammed, 2014).

Test for Steroids

A small quantity of the extract was dissolved in 1.0 ml chloroform and to it 1.0 ml of concentrated sulphuric acid was added down the test tube. Formation of red or yellow coloration was taken as an indication for the presence of steroids (Sofowora, 2008; Mohammed, 2014).

Test for Alkaloids

The extract 0.5 g was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3.0 ml of the filtrate was divided into three. To the first 1.0 ml few drops of freshly prepared Dragendorff reagent was added and observed for formation of orange to brownish precipitate. To the second, one drop of

Meyer reagent was added observed for formation of white to yellowish or cream colour precipitate. To the third, 1.0 ml of Wagner reagent was added to give a brown or reddish or reddish-brown precipitate (Evans, 2009; Mohammed, 2014).

Test for Saponins

About 2.0ml of the test solution was placed in a test tube and shaken vigorously for few minutes. Frothing which persists on warming was taken as an evidence for the presence of saponins (Sofowora, 2008; Mohammed, 2014).

Test for phenols

A small quantity of the extract was added to a test tube. 2 ml of distilled water was added to the test tube and shaken gently. 3 drops of 10% aqueous Ferric Chloride (FeCl_3) was added to the mixture. Formation of a blue-black, or dark green precipitate was taken as evidence for the presence of phenols (Sofowora, 2008; Mohammed, 2014).

Determination of Median Lethal Dose (LD_{50}).

The Intraperitoneal median lethal dose (LD_{50}) of the ethanol leaf extract was determined using modified Lorke's method (1983). Twelve adult male mice (weighing between 25-30 grams) were randomly picked for determination of the LD_{50} . The method has two phases. In the first phase, three groups of mice, two per group were treated with crude ethanol extract at doses of 10, 100 and 1000 mg/kg and observed for signs of toxicity and death for 24 hours. Based on the outcome, no death was observed at the first phase. Three more groups of mice, two per group were treated with the crude ethanol extract at 1600, 2900 and 5000 mg/kg and observed for signs of toxicity for 24 hours. The LD_{50} value was determined by calculating the geometric

mean of the lowest dose that caused death and the highest dose that the animal survived.

ACUTE ANTICONVULSANT SCREENING

4-Aminopyridine (4-AP)-Induced Convulsion in Mice

Twenty five adult male mice (weighing between 25-30 grams) were randomly picked (simple randomization) and divided into 5 groups each containing five mice. The first group served as control and was pretreated with normal saline 10 ml/kg intraperitoneally. The second, third and fourth groups were pretreated with 250, 500 and 1000 mg/kg of the ethanol extract respectively, while the fifth group was pretreated with 200 mg/kg sodium valproate, all through the intraperitoneal route. Thirty minutes post treatment 4-AP was administered at a dose of 9 mg/kg subcutaneously to each mouse. The mice were observed for 30 minutes for signs, such as intermittent forelimb extension, tonic seizures and death. Ability of the extract to protect the mice from convulsion and lethality within the 30 minutes observation period was considered as an indication of anticonvulsant activity (Yamagachi and Rogawski, 1992).

Pentylentetrazole (PTZ)-Induced Convulsion in Mice

The method of Swinyard *et al.* (1989) was employed to induce convulsion in mice using PTZ. Twenty five mice were randomly picked (simple randomization) and divided into five groups of five. The first group received normal saline 10 ml/kg body weight, the second, third, and fourth groups received 250, 500, and 1000 mg/kg intraperitoneally (I.P) of the extract, while the fifth group was injected with sodium valproate at 200 mg/kg. Thirty minutes after pretreatment, the animals received PTZ, 65 mg/kg via intraperitoneal route I.P. Mice were observed over a period of 30 minutes. The absence of convulsion for 30 minutes duration indicates the extract's

ability to abolish the effect of PTZ-induced convulsion threshold (Swinyard *et al.*, 1989).

Data Analysis

Results are presented as mean $\pm$ standard error of mean (SEM) and “n” represents the number of animals per group. Inferential statistical analysis was done using one-way ANOVA. Turkey post-hoc test were used. Turkey’s multiple comparison analysis method compared each experimental group to the each control group and homogeneity of variance were tested in the test groups. Differences between compared data in the groups were considered statistically significant at p value $p < 0.05$.

RESULTS

Extract Percentage Yield

Weight of the powdered *Pavetta crassipes* leaves is 1.22 kg (1,220 g) and the extractive value of the n-Hexane extract is 31.61 g with a percentage yield of 2.59%. The extractive value of the ethanol extract is 143.39 g with percentage yield of 12.45% (Table 1).

Table 1 Percentage Yield of Extract

Weight of powdered leaf sample (g)	Solvent added	Weight of extract (g)	Percentage yield (%)
1,220	n- Hexane	31.61	2.59
1,150	Ethanol	143.39	12.45

Percentage yield of n-Hexane extract is given as weight of n-Hexane extract/weight of dried leaf powder x 100
Percentage yield of ethanol extract is given as weight of ethanol extract/weight of dried leaf powder x 100

Median Lethal Dose Values (LD₅₀)

The intraperitoneal LD₅₀ in mice was estimated at 3807.88 mg/kg (Table 2). Death was observed at 5000 mg/kg. LD₅₀ was calculated as square root of (2900 x 5000).

Table 2: Median Lethal Dose (LD₅₀) in Mice

Phase	Dose (mg/kg)	Number of mice	Mortality
Phase one	10	2	0/2
	100	2	0/2
	1000	2	0/2
Phase two	1600	2	0/2
	2900	2	0/2
	5000	2	2/2

LD₅₀ = 3807.88 mg/kg

Phytochemical Constituents of the Ethanol Leaf Extract of *Pavetta crassipes*

The preliminary phytochemical screening of the ethanol leaf extract of *Pavetta crassipes* revealed that cardiac glycosides, tannins, phenols, alkaloids, saponins, flavonoids are present in the plant extract while and steroids are absent in the extract.

Table 3: Phytochemical Constituents of Ethanol Leaf Extract of *Pavetta crassipes*

Phytochemical constituents	Results
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Cardiac glucosides	+
Tannins	+
Phenols	+
Alkaloids	+
Saponins	+
Steroids	-
Flavonoids	+

+ denotes present, - denotes absent

Effects of Ethanol Leaf Extract of *Pavetta crassipes* on 4-Aminopyridine-Induced Seizures in Mice

Pavetta crassipes leaf extract at 250, 500 and 1000 mg/kg significantly ($p < 0.05$) reduced the time for the onset of myoclonic jerk in mice in the 4-aminopyridine induced seizure compared to the negative control group. The extract also significantly increased the latency of convulsion ($p < 0.05$) at 250, 500 and 1000 mg/kg in a dose dependent manner compared to the

negative control group (Table 4). There is statistically significant difference ($p < 0.05$) in the number of convulsion between the negative control group and the test groups at 250, 500 and 1000 mg/kg. The extract at 250, 500 and 1000 mg/kg significantly produced a dose dependent reduction in the duration of convulsion compared to the negative control group (Table 4). The extract at 250, 500 and 1000 mg/kg protected 80%, 100% and 100% of mice respectively against 4-aminopyridine-induced seizure (Table 4).

Table 4: Effects of Ethanol Leaf Extract of *Pavetta crassipes* on 4-Aminopyridine-Induced Seizures in Mice

	Negative control (10mg/kg NS)	Positive control (200mg/kg SV)	250mg/kg EEPC	500mg/kg EEPC	1000mg/kg EEPC
Onset of myoclonic jerk (minutes)	10.60±0.51 [#]	30±0.00*	20.60±2.79*	27.20±2.80*	27.00±3.00*
Onset of convulsion (minutes)	12.20±0.58 [#]	30±0.00*	24.33±3.56*	27.33±2.67*	30±0.00*
Number of convulsion	3.20±0.58	0±0.00*	0.60±0.24*	0.40±0.04*	0±0.00*
Duration of convulsion (minutes)	0.19±0.04 [#]	0±0.00*	0.04±0.02*	0.03±0.02*	0±0.00*

Quantal protection (%)	0	100	80	100	100
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Values are expressed as Mean $\pm$ S.E.M., n=5, * denotes significant difference with negative control group, # denotes significant difference with positive control group, p< 0.05 - One way ANOVA, NS= Normal Saline, EEPC= Ethanol leaf extract of *Pavetta crassipes*, SV= Sodium valproate, Ethanol leaf extract of *Pavetta crassipes* and sodium valproate were administered 30 minutes intraperitoneally before the injection of 4-aminopyridine (9 mg/kg) intraperitoneally

Effects of Ethanol Leaf Extract of *Pavetta crassipes* on Pentylentetrazole (PTZ)-Induced Seizures in Mice

There is statistically significant difference (p< 0.05) in the latency of convulsion between the test group at 250, 500 and 1000 mg/kg and the negative control group (Table 5). There is also statistically significant difference (p< 0.05) in the

number of convulsion between the test groups at 250, 500 and 1000 mg/kg compared to the negative control group. The extract at 250, 500 and 1000 mg/kg significantly (p<0.05) reduced the duration of convulsion compared to the negative control group (Table 5). The extract at 250, 500 and 1000 mg/kg protected 40%, 40% and 60% of mice respectively against PTZ-induced seizure (Table 5).

Table 5: Effects of Ethanol Leaf Extract of *Pavetta crassipes* on Pentylentetrazole (PTZ)-Induced Seizures in Mice

	Negative control (10 mg/kg NS)	Positive control (200 mg/kg SV)	250 mg/kg EEPC	500 mg/kg EEPC	1000 mg/Kg EEPC
Onset of myoclonic jerk (minutes)	2.50 $\pm$ 0.86 [#]	30 $\pm$ 0.00*	5.00 $\pm$ 0.15	3.00 $\pm$ 0.32	8.00 $\pm$ 0.50
Onset of convulsion (minutes)	4.20 $\pm$ 1.02 [#]	30 $\pm$ 00*	15.00 $\pm$ 0.15*	13.50 $\pm$ 0.15*	19.00 $\pm$ 0.32*
Number of convulsion	4.40 $\pm$ 0.51 [#]	0 $\pm$ 00*	1.00 $\pm$ 0.06*	1.00 $\pm$ 0.32*	0.80 $\pm$ 0.20*
Duration of convulsion (minutes)	0.40 $\pm$ 0.05 [#]	0 $\pm$ 00*	0.07 $\pm$ 0.02*	0.05 $\pm$ 0.01*	0.02 $\pm$ 0.01*
Quantal protection (in %)	0	100	40	40	60

Values are expressed as Mean $\pm$ S.E.M., n=5, * denotes significant difference with negative control group, # denotes significant difference with positive control group, p< 0.05 - One way ANOVA, NS= Normal Saline, EEPC= Ethanol leaf extract of *Pavetta crassipes*, SV= Sodium valproate, Ethanol leaf extract of *Pavetta crassipes*

and sodium valproate were administered 30 minutes intraperitoneally before the injection of PTZ (65 mg/kg) intraperitoneally

DISCUSSION

The findings of this study suggests that ethanol leaf extract of *Pavetta crassipes* (K. Schum) Rubiaceae possesses anticonvulsant activity. The percentage yield of ethanol leaf extract of *Pavetta crassipes* was found to be 12.45%. The acute toxicity studies suggest that the ethanol leaf extract of *Pavetta crassipes* is not toxic in mice, following intraperitoneal administration according to classification by Corbett *et al.*, (1984). The doses of the extract used in this study were lower than 1142 mg/kg, which is lower than 30% of the LD₅₀. These doses are relatively safe for ethnopharmacological research (Vongtau *et al.*, 2004).

4-aminopyridine is a known potassium channel blocker that affects all forms of brains neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release. The 4-aminopyridine (4-AP) chemical model provides a practical way for determining a drug's and extract's capacity to reduce the propagation of highly refractory or localized focal epileptic seizures (Wickenden, 2002). At the molecular level, 4-AP acts as a highly selective antagonist of voltage-gated potassium (K⁺) channels, specifically targeting Kv1 dendrotoxin-sensitive subfamilies found on presynaptic terminals. Under healthy conditions, the outward movement (efflux) of K⁺ ions down their electrochemical gradient is required to repolarize the axonal membrane immediately following an action potential. When 4-AP inhibits these channels, the repolarization phase is massively delayed, which results to a prolonged presynaptic depolarization and a massive, voltage-dependent opening of presynaptic L-type and N-type calcium (Ca²⁺) channels. The resulting rush of intracellular Ca²⁺ triggers a spontaneous, uncontrolled release of

excitatory neurotransmitters—predominantly glutamate—into the synaptic cleft, overwhelming postsynaptic receptors and causing severe, lethal tonic seizures (Yamaguchi & Rogawski, 1992).

The ability of *Pavetta crassipes* leaf extract to increase the latency of 4-aminopyridine-induced myoclonic jerk, its ability to significantly increase the latency of seizures in 4-aminopyridine-induced seizure at 1000 mg/kg and the ability of the extract to reduce the duration of convulsion in the mice against 4-aminopyridine-induced seizure suggest anticonvulsant activity of the extract. The significant anticonvulsant activity of the extract suggest its interaction with potassium and sodium channels in the brain and increases GABA levels in the brain to result in its anticonvulsant activity. The ethanol leaf extract of *Pavetta crassipes* blocks 4-AP-induced convulsions through complementary pathways downstream of the potassium channel block (Bariweni and Ozolua, 2017). Isolated tissue studies demonstrates that *Pavetta crassipes* contains bioactive compounds that inhibit calcium-dependent smooth muscle contractions, indicating clear voltage-gated calcium channel (VGCC) blocking properties (Vongtau *et al.*, 1998). When applied to the central nervous system of albino mice, calcium channel blockade inhibits the excessive influx of presynaptic Ca²⁺ caused by 4-AP. By antagonizing intracellular calcium accumulation, the extract directly reduces and weakens the hyper-synchronous, exocitotic release of vesicular glutamate into the synaptic gap, preventing the onset of excitotoxic cascades in the brain.

Furthermore, components within the extract help mitigate post-synaptic hyperexcitotoxicity. The phytochemical constituents interfere with ionotropic

glutamate receptors, specifically blocking N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor pathways (Johnston et al., 2000). By minimizing post-synaptic NMDA-mediated calcium surges and AMPA-mediated sodium (Na^+) influx, *Pavetta crassipes* acts as a functional buffer. It stabilizes neurons against the excessive glutamate concentrations generated by 4-AP, effectively dampening seizure propagation and preventing downstream excitotoxic cell death. Drugs like sodium valproate antagonize voltage-gated Na^+ channels and increase brain level of GABA, thereby reducing excitation triggered by 4-aminopyridine (Yamaguchi and Rogawski, 1992; Larson et al., 2021).

The ethanol leaf extract of *Pavetta crassipes* protected the mice against PTZ-induced seizures. PTZ, a well-known convulsing agent that has been shown to diminish the GABAergic tone (Macdonald and Baker, 1997; Alachkar et al., 2020) by the inhibition of benzodiazepine (BDZ) site of the GABA receptor channel complex (De Deyn et al., 1992). Gamma amino butyric acid (GABA) is a major inhibitory neurotransmitter in the brain, and the inhibition GABAergic neurotransmission has been thought to be one of the underlying factors responsible for epilepsy (Amabeoku et al., 1998; Alachkar et al., 2020). The enhancement of the GABAergic neurotransmission is reported to antagonize seizures in the brain (Leonard, 2003). Therefore, inhibition of PTZ-induced seizures by the extract suggests effects on GABAergic neurotransmission in the brain (Chen et al., 2020).

PTZ serves as a typical well known standard model for identifying therapeutic drugs and plant extracts that are effective against generalized myoclonic and absence seizures in human patients (Alachkar et al., 2020). From a cellular point of view and available scientific facts, PTZ induces paroxysmal depolarizing shifts and

sustained electrical bursts by functioning as a highly potent, non-competitive blocker directly at the gamma-aminobutyric acid type A (GABA_A) receptor-ionophore complex (Macdonald and Barker, 1977). By binding selectively to the t-butylbicyclophosphorothionate (TBPS) or picrotoxinin pore portal within the chloride channel matrix, PTZ directly inhibits the inward flux of chloride ions (Cl^-) evoked by endogenous GABA. Consequently, postsynaptic neurons are deprived of the hyperpolarizing currents required to blocks excessive neuronal firing, leading to immediate membrane depolarization, widespread loss of synaptic inhibition, and swift progression into tonic-clonic convulsions (De Deyn et al., 1992).

The PTZ chemical model identifies compounds that can raise seizures threshold in the brain (Stables and Kupferberg, 1997; White et al., 1998) consistent with the human condition (Swinyard et al., 1989). The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs such as valproate, diazepam, and ethosuximide, which are useful in the treatment of the absence seizures suppresses PTZ-induced seizures (McNamara, 2006). It has been found empirically that drugs which inhibit PTZ-induced seizures and raise the threshold for production of electrically induced seizures are generally effective against absence seizures (Rang et al., 2003; Chen et al., 2020). Although the extract was not able to increase latency of PTZ-Induced myoclonic jerk, the ability of the extract to reduce the number and duration of convulsion and the extract ability to mildly protect mice against PTZ-induced seizures suggests anticonvulsant activity. Thus, the extract can be useful in the management of epilepsy as reported in previous study by Bariweni and Ozolua, 2017.

The ethanol leaf extract of *Pavetta crassipes* possesses the capacity to increase seizure latency, suppress clonic-tonic firing, and protect albino mice from PTZ-

induced lethality (Sanusi et al., 2023). This protective property of the extract suggest a fundamental restoration of central GABAergic tone. Phytochemical analyses reveal that *Pavetta crassipes* contains flavonoids and polyphenols (Bariweni and Ozolua, 2017). Phytochemicals contained in plant extracts such as flavonoids and polyphenols compounds have been reported to act as ligands at GABA_A receptors in the CNS (Jager and Saaby 2011). These compounds are known to act as positive allosteric modulators at the benzodiazepine binding domain of the heteromeric GABA_A macromolecule (Jäger and Saaby, 2011). By altering the conformational structure of the receptor, the extract counteracts the mechanical channel antagonism imposed by PTZ, promoting a higher binding affinity for endogenous GABA molecules. This structural adjustment maximizes both the open-state frequency and duration of the integral Cl⁻ pore, reinstating inhibitory postsynaptic potentials (IPSPs) that blocks the propagation of seizure firing.

In addition to allosteric modulation, the extract may also preserve GABA baseline levels at the brain by regulating its metabolic pathway. Secondary metabolites found in the extract can also stimulate glutamic acid decarboxylase (GAD), the primary enzyme responsible for synthesizing GABA from glutamate, while simultaneously suppressing GABA transaminase (GABA-T), the chief mitochondrial matrix enzyme responsible for GABA degradation (Alachkar et al., 2020). By balancing this enzymatic cycle, *Pavetta crassipes* extract ensures that adequate levels of GABA remain available in the synaptic space, preventing the catastrophic collapse of central nervous system inhibition evoked by PTZ injection.

The broad-spectrum anticonvulsant activity of *Pavetta crassipes* is results from the synergistic interactions of its secondary

metabolites compounds, rather than an isolated single compound (Sofowora, 2008). Quantitative and qualitative assays confirm the presence of alkaloids, saponins, tannins, anthraquinones, and flavonoids within the crude leaf extract (Bariweni and Ozolua, 2017). While flavonoids primarily target the GABA_A receptor complex to attenuate PTZ toxicity, the alkaloid found in the extract counteracts voltage-gated sodium and calcium currents, stabilizing excitable axonal membranes against 4-AP channel blocks. Concurrently, saponins and hydrophilic tannins contribute to generalized central nervous system depression, raising the threshold required to trigger paroxysmal bursts (Quintans-Júnior et al., 2008). This multi-component mechanism reduces the likelihood of metabolic resistance, making *Pavetta crassipes* natural alternative for further drug discovery.

CONCLUSION

Findings of this study suggest that ethanol leaf extract of *Pavetta crassipes* contains compounds with potential anticonvulsant properties that are effective in the management of epilepsy, and further provide scientific validity for the use of the leaf in the treatment of convulsion, thus supporting its ethnomedicinal use in the management of epilepsy in traditional medicine. The multiplicity of putative mechanisms of action as reflected by the broad spectrum of the extract's activity observed in this study can be attributed to the presence of a combination of different biologically active compounds in the extract that can be used for the treatment of epilepsy.

The leaf extract of *Pavetta crassipes* shows maximum or best ameliorating effects in preventing convulsions, myoclonic jerks and it gave a 100 percent seizure protection in 4-aminopyridine-induced seizures.

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