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Antiepileptic Potential of *Justicia insularis* Leaf Extract in Swiss Mice

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Abstract

Epilepsy is a prevalent neurological condition characterized by repetitive seizures, necessitating the exploration of efficacious treatments. Justicia insularis, a plant with a historical use in treating convulsions and epilepsy, has garnered attention as a potential antiepileptic agent. This study aimed to investigate the antiepileptic properties of Justicia insularis leaf extract, focusing on generated seizure models in mice. The analysis of variance (ANOVA), specifically one-way ANOVA, was utilized to assess the statistical significance of the data. Seizure models were employed to evaluate the antiepileptic effects of Justicia insularis leaf extract. The p-values obtained from the one-way ANOVA were significant, with values of $ap<0.005$, $bp<0.01$, and $cp<0.001$. These results suggest the statistical significance of the observed effects in the seizure models. This study provides valuable insights into the therapeutic potential of Justicia insularis leaf extract as a treatment for epilepsy. The significant findings underscore the importance of further research into the use of natural medicines for managing epilepsy, potentially offering new avenues for treatment.

Keywords: *Justicia insularis*; antiepileptic; seizures; convulsion; GABAA receptor; flavonoids.

INTRODUCTION

Epilepsy is a persistent neurological condition characterised by the occurrence of repetitive seizures. This event can potentially influence individuals across various age groups and substantially influence their overall well-being. Despite various antiepileptic treatments, many persons diagnosed with epilepsy encounter insufficient seizure management and often endure unfavourable prescription side effects. his has increased interest in alternative therapies, including herbal remedies (Espinosa-Garcia et al., 2021).

Justicia insularis, sometimes called "African Water Willow", is classified under the Acanthaceae botanical family. It has been widely used in many traditional medicinal practices in diverse cultural contexts. *Justicia insularis* is often grown within the home gardens of West and Central Africa, with particular prevalence in countries such as Guinea, Sierra Leone, Ghana, Togo, Benin, Nigeria, Cameroon, and the Democratic Republic of Congo (Christian et al., 2020). *Justicia insularis* has been used as a traditional cure for convulsions, epilepsy, and associated ailments across several African groups. In Senegal, providing a leaf decoction derived from *J. insularis* to pregnant women during the last month of their pregnancy is common practice to alleviate the discomfort associated with labour (Christian et al., 2020). In the Western area of Cameroon, the use of it is seen in conjunction with the foliage of three other botanical species (*Aloe buettneri*, *Hibiscus macranthus*, and *Dicliptera verticillata*) to manage dysmenorrhoea and some instances of female infertility (Meguem et al., 2021). Additionally, in Togo and Northeast Ghana, the cooked leaves of *J. insularis* are administered to infants to facilitate bowel movement, and the leaves are also used topically to enhance the healing process of wounds (Irinmwinuwa et al., 2023). Nevertheless, it is well observed that traditional knowledge frequently lacks empirical substantiation via scientific methods. Hence, the primary objective of this investigation was to conduct a scientific assessment of the antiepileptic properties of *Justicia insularis* leaf extract via the use of pentylenetetrazole, aminophylline and isoniazid-induced mice.

MATERIALS AND METHODS

Experimental Animals

Swiss mice of either sex used for these experiments were obtained from the Faculty of Pharmacy Animal House, University of Uyo, Uyo. The animals were housed in standard cages and were maintained on standard pelleted feed (Guinea feed) and water *ad libitum.* Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee,

University of Uyo. All animal experiments complied with the National Institute of Health Guide for Care and Laboratory Animals (pub. No. 85-23, revised 1985).

Plant Collection and Identification

The plant material *Justicia insularis* (leaves) was collected from the University of Uyo premises, Akwa Ibom State, Nigeria, in March 2023. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

Preparation of Extract

The leaves were washed and shade-dried for two weeks. The dried plants' materials were chopped into small pieces and reduced to powder using an electric grinder. The powdered leaves material (1.5 kg) was soaked for 72 h in ethanol (50%). This was later filtered, and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract was stored in a refrigerator at -4°C until used for the proposed experiments.

Experimental Design

Table 1 illustrates the experimental design used for the three seizure models. The design of the models was the same across all models, with the exception of the administration of different standard drugs for each kind of convulsion. Specifically, phenobarbital (40 mg/kg) was used as the standard drug for pentylenetetrazole and aminophylline-induced convulsions, while diazepam (5 mg/kg) was employed as the standard drug for isoniazid-induced convulsions. The different doses of the extract and standard drugs were administered to the mice an hour before induction of convulsion.

Table 1. Experimental design of experimentally induced convulsion in Swiss mice.

GROUPS	AGENT	DOSAGE (mg/kg/day)
I	Normal Saline	10 mL/kg
II	Low dose of the ethanol leaf extract of *Justicia insularis*	150
III	Middle dose of the ethanol leaf extract of *Justicia insularis*	300
IV	High dose of the ethanol leaf extract of *Justicia insularis*	450
V	Phenobarbital	40

Source: Field data (2023)

Statistical Analysis and Data Evaluation

The results were analysed using the statistical software package for social sciences (SPSS) version 16.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and all results were presented as Mean ± SEM.

RESULTS AND DISCUSSION

Results

PTZ –induced convulsion

Administration of leaf extract of *Justicia insularis* (150-450 mg/kg) provided a considerable degree of protection for the mice against seizure induced by pentylenetetrazole. The leaf extract prolonged the onset of myoclonic convulsion in a dose-dependent fashion, but the effect was insignificant ($p>0.05$) compared to the control. Similarly, the leaf extract exerted a significant ($p<0.05$) prolongation of time for the onset of tonic convulsion in a dose-dependent manner, which was significant ($p<0.05$) at the higher doses (300 and 450 mg/kg) (Table 2). The time of death of treated animals was dose-dependently and insignificantly ($p>0.05$) prolonged compared with the control. The standard drug, phenobarbital, offered 33.33% protection to the treated animals (Table 2).

Table 2. Effect of ethanol leaf extract of *Justicia insularis* on Pentylenetetrazole-induced convulsion.

TREATMENT	DOSE (mg/kg)	Onset of myoclonic (Min)	Onset of tonic (Min)	Time of death (Min)	No. of death
Control (normal saline)	-	0.32 ±0.04	1.35 ± 0.06	1.68±0.37	6/6
Phenobarb	40	1.23 ±0.09[c]	18.28±1.82[c]	30.09±0.01[c]	2/6
leaf extract	150	0.33 ±0.01	1.32± 0.14	3.89±0.32	6/6
	300	0.34 ±0.06	2.06± 0.25[a]	2.99±0.38	6/6
	450	0.46± 0.04	2.57±0.02[a]	3.64±0.63	6/6

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.005$; [b]$p<0.01$; [c]$p < 0.001$, compared to control. (n=6).

Aminophylline-induced convulsion

Administration of the leaf extract of *J. insularis* (150-450 mg/kg) caused a significant ($p<0.01$-0.001) delay in the onset of seizure induced by aminophylline in a dose-dependent fashion. The delay was significant ($p<0.01$-0.001) in both myoclonic and tonic convulsions,

especially at higher doses (300 and 450 mg/kg) of the extract (Table 3). The time of death of the treated animals was dose-dependently and significantly ($p<0.05$-0.01) prolonged at higher doses (300 and 450 mg/kg) of the extract when compared with the control. The standard drug, Phenobarbital, offered 100% protection to the treated mice.

Table 3. Effect of ethanol leaf extract of *Justicia insularis* on Aminophylline-induced convulsion.

TREATMENT	DOSE (mg/kg)	Onset of myoclonic (Min)	Onset of tonic (Min)	Time of death (Min)	No. of death
Control (normal saline)	-	10.42 ± 0.36	12.31 ± 4.27	15.30 ± 5.45	6/6
Phenobarb	40	12.96 ± 0.76[c]	0.00 ± 0.00[c]	0.00 ± 0.00[c]	6/6
leaf extract	150	10.94 ± 1.82	12.37 ± 2.41	16.79 ± 5.21	6/6
	300	14.62 ± 2.66	22.82 ± 1.39[a]	26.65 ± 1.91[a]	6/6
	450	22.77 ± 3.19[c]	26.49 ±3.66[a]	30.22 ± 4.36[b]	6/6

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.005$; [b]$p<0.01$; [c]$p < 0.001$, compared to control. (n=6).

Isoniazid-induced convulsion

Administration of the leaf extract of *Justicia insularis* (150-450 mg/kg) caused a significant ($p<0.01$-0.001) delay in the onset of seizure induced by isoniazid in a non-dose-dependent fashion. The delay was significant ($p<0.01$-0.001) in both myoclonic and tonic convulsions, especially at the lower doses (150 and 300 mg/kg) (Table 4). The time of death of the treated animals was prolonged significantly ($p<0.05$) at the lowest dose (150 mg/kg) of the extract when compared with the control, while the standard drug, diazepam, also offered a more significant ($p<0.01$) prolongation of the time of death to the mice treated with it (Table 4).

Table 4. Effect of ethanol leaf extract of *Justicia insularis* on Aminophylline-induced convulsion.

TREATMENT	DOSE (mg/kg)	Onset of myoclonic (Min)	Onset of tonic (Min)	Time of death (Min)	No. of death
Control (normal saline)	-	13.73±3.90	29.08± 1.23	34.95±1.64	6/6
Diazepam	5	41.17±0.10[b]	26.19± 0.53[b]	84.09±4.05[b]	6/6
leaf extract	150	41.81±1.23[b]	55.77± 1.72[b]	56.39±1.86[a]	6/6
	300	26.96±0.59[a]	40.73±3.05[a]	47.64±6.25	6/6
	450	13.73±3.90	29.08±1.23	35.95±1.44	6/6

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.005$; [b]$p<0.01$; [c]$p < 0.001$, compared to control. (n=6).

Discussion

The efficacy of the leaf extract derived from *Justicia insularis*, a botanical species often used in traditional medicine for managing convulsions and epilepsy, was assessed in relation to its potential to mitigate experimentally induced convulsions. The leaf extract was found to possess significant activity against seizures induced by pentelyenetetrazole, aminophylline and isoniazid, offering considerable protection in some cases, with lower doses being more potent. The precise mechanisms of aminophylline-induced seizures seem multifaceted, including various intricate pathways that remain uncertain. The available evidence indicates that seizures caused by aminophylline may be attributed to two mechanisms: adenosine receptor antagonism and suppression of cerebral nucleotidase activity (Tescarollo et al., 2020). These mechanisms decrease adenosine levels in the brain, ultimately leading to disinhibition. However, according to a paper by Sharma and Sandhir (2010), it was shown that diphenylhydantoin, which is a potent inhibitor of adenosine absorption, did not show efficacy in avoiding these seizures. In addition to its non-specific inhibition of adenosine receptors (Maleki-Sadeghi et al., 2022), aminophylline is believed to have inhibitory effects on adenosine production. Nevertheless, a study by Ray et al. (2005) has shown that seizures triggered by aminophylline may be attributed to oxidative stress resulting from the production of free radicals and reactive oxygen species. The leaf extract has demonstrated notable antioxidant capabilities (Adeyemi & Babatunde, 2014). This activity may be attributed to the ability of its phytochemical ingredients to scavenge free radicals. The leaf extract may have also facilitated adenosine production, contributing to its anticonvulsant properties.

Shrivastava et al. (2022) explain that the anticonvulsant action of pentylenetetrazole (PTZ) may be attributed to its ability to block the activity of gamma-aminobutyric acid (GABA) at GABAA receptors. Gamma-aminobutyric acid (GABA) is a prominent inhibitory neurotransmitter involved in the pathophysiology of epilepsy. The modulation of GABA neurotransmission may reduce or intensify convulsions (Akyuz et al., 2021). Phenobarbitone and diazepam, commonly used medications for epilepsy, have been shown to exert their antiepileptic properties by

augmenting GABA-mediated inhibition inside the brain (Okokon et al., 2021). The efficacy of phenytoin in preventing PTZ-induced seizures was limited, perhaps due to its mechanism of action, including the inhibition of sodium ion influx into brain cells, hence impeding the production of repetitive action potentials (Okokon et al., 2021). The observed delay in PTZ-induced convulsion and protective effects on animals resulting from the administration of *J. insularis* leaf extract provide evidence supporting its central nervous system depressive properties and its capacity to increase GABA-mediated inhibition inside the brain.

Isoniazid, a pharmaceutical agent used to treat tuberculosis, has been found to induce status epilepticus, a prolonged seizure activity, by reducing Gamma-Aminobutyric Acid (GABA) levels in the brain. GABA is a crucial inhibitory neurotransmitter in the mammalian brain. This effect is achieved by inhibiting pyridoxal-5-phosphate-dependent Glutamic Acid Decarboxylase (GAD), an enzyme involved in GABA synthesis. Pyridoxal-5-phosphate is the biologically active form of pyridoxine, functioning as a cofactor for glutamic acid decarboxylase (GAD), an essential enzyme involved in the manufacture of gamma-aminobutyric acid (Ghatge et al., 2021). Recurrent seizures characteristic of status epilepticus result from reduced GABA levels (Rho and Boison, 2022). Despite the limited efficacy of existing anticonvulsant medications in treating seizures generated by isoniazid, intravenous diazepam continues to be utilised for seizure management in the absence of pyridoxine (Okokon et al., 2021). The administration of *Justicia insularis* leaf extract demonstrated a substantial protective effect against isoniazid (INH)-induced convulsions in the treated mice. The observed behaviour might be attributed to the extract's capacity to augment GABA production in the brain through the actions of its phytoconstituents.

The antiepileptic effect of flavonoids, which are secondary metabolites found in plants, has been extensively documented in many studies. These compounds have been shown to modulate the GABAA-Cl-channel complex, similar to benzodiazepines, indicating their potential therapeutic use in treating epilepsy (Choudhary et al., 2011). The antiepileptic effect of flavonoids is attributed to their ability to modulate the GABAA-Cl-channel complex, facilitated by their structural resemblance to benzodiazepines (Choudhary et al., 2011). Additionally, the compound apigenin, which belongs to the class of flavonoids, has been identified as a ligand that acts on the central nervous system, like benzodiazepines. It has also shown effectiveness in mitigating convulsions generated by picrotoxin (Jäger et al., 2009). Extracts derived from *Justicia insularis* leaves have a high concentration of flavonoids and other phenolic compounds known for their potent antioxidant properties, and these metabolites function by scavenging free radicals and regulating the GABAA-Cl-channel complex inside the central nervous system (CNS), demonstrating their anticonvulsant properties (Oyomah et al., 2019).

The results of this study have confirmed the anticonvulsant potential of leaf extract of *Justicia insularis* and justify its use traditionally in the treatment of convulsion.

CONCLUSION

The findings presented in this study provide scientific support for the traditional use of *Justicia insularis* as an antiepileptic remedy. The leaf extract demonstrated significant anticonvulsant effects across various induced seizure models, indicating its potential as a natural therapy for epilepsy management. Interactions with GABAA receptors and antioxidant mechanisms likely mediate the observed effects. Further research is warranted to elucidate the specific compounds responsible for these effects and to explore their mechanisms of action in more detail. The study contributes to the growing knowledge surrounding herbal remedies for epilepsy and underscores the importance of integrating traditional knowledge with modern scientific approaches.

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***Competing Interests*:** The authors declare that there are no conflicts of interest concerning this manuscript.

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