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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTICONVULSANT ACTIVITY OF ETHANOL LEAF EXTRACT OF *ANTHOCLEISTA DJALONENSIS* (LOGANIACEAE) IN MICE

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ABSTRACT

Anthocleista djalonensis (Loganiaceae) is used traditionally for the treatment of convulsions, hypertension, stomach pains, hemorrhoids, syphilis, diabetes, and other conditions. The objective of this work was to access the phytochemicals (using standard methods) and anticonvulsant potential (using adult Swiss albino mice) of the ethanol leaf extract of *A. djalonensis* (EEAD). The acute toxicity profile of EEAD was investigated following standard guidelines established by the OECD 423. The study examined the anticonvulsant activity of EEAD at 200, 400 and 800 mg/kg in pentylenetetrazole- and strychnine-induced seizure tests. The phytochemical analysis of the extract was carried out and revealed the presence of phenols, tannins, terpenoids and alkaloids. When given orally to mice, the extract's median lethal dose (LD50) was more than 2000 mg/kg. Despite the fact that there was no significant difference between the control and 200 mg/kg doses, the ethanol leaf extract of *A. djalonensis* showed a significant ($P<0.05$) dose-dependent increase in the start of clonic and tonic seizures.. The 800 mg/kg dose of the extract produced an almost similar effect as standard drug (diazepam) in stopping the occurrence of clonic and tonic seizure. Also, the higher the dose of EEAD, the higher its protection of the mice against mortality in both strychnine and pentylenetetrazole-induced seizure models as 800 mg/kg dose produced complete protection against mortality similar to diazepam. These findings suggest that EEAD possess anticonvulsant activity which justifies the traditional utility of the plant in the management of convulsion.

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INTRODUCTION

Epilepsy is mostly among the prevailing long-term nervous disorders worldwide, in which its characteristics include irregular, unprovoked and repeated seizures. Whilst epilepsy can affect all age groups, young children (below age 2) and the aged (above 75 years of age) are the dominant population usually affected [1, 2]. The World Health Organization (WHO) states that around fifty million

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people globally have epilepsy and forty million of these people are living in less developed nations, including Nigeria [3].

Seizure is a health condition that is characterized by an interruption in the nerve cell activity of the brain, resulting in uncontrolled muscle contractility as well as abrupt, forceful and jerky body apparent motion. It is linked to a variety of other health conditions, such as inflammatory brain infection, severe fever and epilepsy [4].

The conventional treatment approach for convulsion consists primarily of anticonvulsant medications such as hydantoins and barbiturates that are dispensed chronically with the goal to cause prophylactic epileptic seizures in people at risk and to provide symptomatic relief from seizures by interacting with a variety of cellular targets [5]. Although these medications frequently control or reduce the frequency of convulsions in majority of patients, some patients show little or no improvement. Orthodox anticonvulsant drugs have been linked to some life threatening side effects such as hepatotoxicity, aplastic anemia, and neurotoxicity and often times the patients are at risk of severe adverse drug interactions [6]. The drawbacks necessitated the search for newer alternative of natural origins with proven efficacy and fewer adverse effects and by extension improving the patient compliance. Plants have long been identified as a source of life-saving drugs, and as a result, they are evaluated for the creation of more potent and secure anti-anticonvulsants [7].

Anthocleista djalonensis Afzel. Ex R.Br. (Gentianaceae) is common medicinal plant that is widely used in Nigeria. The seeds, bark and roots are particularly used among the Igbos as antipyretic, laxative and remedy for various stomach disorders. Aqueous extract of the leaves mixed with lemon juice is used by the Abros of Ghana to cure epilepsy while in Casamane, Senegal, it is used as a diuretic. Also, it has been noted that an aqueous extract of *A. djalonensis* can lower blood pressure in cats and can also tone down and amplify rabbit intestinal contractions. It is called "Uvuru" in Igbo language [8]. Among the Igbos, "Uvuru" is further used in the treatment of constipation, malaria fever, typhoid fever, hypertension, stomach aches, hemorrhoids, syphilis, and diabetes [9]. According to ethnomedical reports, the plant has also been used to treat epilepsy and its related morbidities, including depression, anxiety, and insomnia [10-12]. The stem bark extract was found to protect mice from pentylenetetrazole-induced epileptic convulsions in a previous study, potentially through enhancing antioxidant defense and GABAergic signaling. However, the leaf extract commonly used in Nigeria have not been evaluated [13]. The present study investigated the anticonvulsant activity of *A. djalonensis* leaf extract in two chemoconvulsant-induced seizures models with the aim of validating the ethnomedicinal utility of the plant in the management of convulsive disorders.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *A. djalonensis* were collected from Ibadan, Oyo state, Nigeria (in November, 2021) and authenticated at the herbarium section of the Department of Plant Biology by Mr. Boluwatife Ajayi. The voucher number (UILH/002/2022/107525) was issued.

Preparation of *Anthocleista djalonensis* Extract

Fresh leaves of *A. djalonensis* were air dried under shade at room temperature (32 ˚C) and pulverized into coarse powder. The powder (786 g) was extracted into 70% ethanol by cold maceration and the extract was concentrated *in vacuo*. The dried extract was weighed, labeled as EEAD and refrigerated until needed for analysis. The yield of the extract was computed in percentage using the formula below:

$$\%\ \text{yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of powdered plant material}} \times 100\%$$

Experimental Animals

Adult Swiss albino mice were obtained from the animal house facility of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria, The animals were maintained under standard laboratory conditions of temperature (23.0±2.0°C), humidity, and light. All animals had access to water and food *ad libitum*. The animals were kept in polypropylene cages throughout the study. The experiment was performed in consonance with the University of Ilorin Research policy, ethics and regulations governing the care and use of experimental animals as contained in "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985). Approval for the conduct of the study was obtained from the Animal Use and Ethics Committee of the University of Ilorin. The approval number for the study is (UERC/ASN/2022/2346).

Acute Oral Toxicity Test
Acute toxicity testing of the plant extract was performed using the OECD method. Two groups of overnight fasted mice (n=3) were given an ethanolic extract of the plant at an oral dose of 2000 mg/kg. The animals were thereafter continuously observed for signs of autonomous and central nervous system dysfunction and changes in behavioural pattern for a period of 2 hours in the first instance, then after 24 hours, 72 hours and up to 2 weeks for evidence of toxicity or mortality [14].

Phytochemical Screening
The presence or absence of phytochemicals (tannins, carbohydrate, terpenoids, steroids, glycosides and saponins) in the leaf extract of the plant was determined using standard methods. [15-17].

Anticonvulsant Study
Experimental design
Animals were divided into five groups (n = 5 per group). The groups include the negative control group (intraperitoneal administration of 10 ml/kg of 0.9% normal saline), standard or positive control group (intraperitoneal administration of 1 mg/kg of diazepam), EEAD I (oral administration of 200 mg/kg of EEAD), EEAD II (oral administration of 400 mg/kg of EEAD) and EEAD III (oral administration of 800 mg/kg of EEAD).

Strychnine-Induced Seizure
The method as described by Quintans-Júnior and co-workers [18] was used. Animals in first group received normal saline (vehicle) orally, animals in the second group received a 1 mg/kg dose of diazepam intraperitoneally (*i.p*) route, while animals in group 3, 4 and 5 were administered with EEAD as doses of 200, 400 and 800 mg/kg, respectively. Thirty minutes later, each mouse received 2 mg/kg strychnine intraperitoneally. Immediately after strychnine administration, animal was individually placed in a cage and observed for about an hour for the onset of convulsion, duration of convulsions, time to death of animal after convulsions and percentage of mortality rate.

Pentylenetetrazole- Induced Seizure
The method as described by Mante with his co-workers [19] was used. Animals in group 1 were administered orally with normal saline (vehicle), animals in group 2 were administered with diazepam, at 1 mg/kg dose by *i.p* route, while animals in group 3, 4 and 5 were administered with EEAD as doses of 200, 400 and 800 mg/kg, respectively. Sixty minutes later after convulsion was induced with *i.p.* injection of pentylenetetrazole (PTZ) at dose of 85 mg/kg, and the animals were observed for the onset of convulsions for a period of 30 min post-PTZ injection.
Motility Protection offered by the extract and standard drug was calculated using the formula below:

$$\%\ \text{Mortality Protection} = \frac{\text{A} - \text{B}}{\text{A}} \times 100\%$$

Where;
A = Total number of animal in group.
B = Total number of dead animal in group

Statistical Analysis
The data obtained were analyzed using Graphpad prism computer program. Results were presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by post-hoc Dunnett's test was used to determine the differences between the different group employed in this study. The one-way ANOVA test was performed using a 95% confidence interval.

RESULTS

Effect of EEAD on Acute Oral Toxicity Testing
At the dose of 2000 mg/kg administered to a group of animals (*n* = 6), no mortality or toxic effect of the extract was observed 24 h following the administration. Also, throughout the two-week observation period of the experiment, there was no change in all the parameters observed**.**

Phytochemical Constituents of EEAD:
The results of the phytochemical assay indicate the presence of a number of phytochemicals such as alkaloids, tannins, terpenoids and phenols. A comprehensive list of phytochemicals present in the leaves of *Anthocleista djalonensis* is presented in Table 1.

Strychnine-Induced Convulsion
The onset of clonic and tonic seizures was significantly delayed by EEAD at 400 mg/kg dose when compared to 200 mg/kg dose and control (normal saline). Administration of diazepam and 800 mg/kg dose of EEAD completely stopped the occurrence of clonic and tonic seizures. The

standard drug (diazepam) and EEAD at 400 mg/kg and 800 mg/kg doses were statistically significant ($p<0.05$) in delaying the onset of clonic and tonic seizures when compared to control (Table 2).

Table 1: Phytochemical Constituents of the Ethanol Leaf Extract of *A. djalonensis*

Phytochemicals	Remark
Phenols	Present
Tannins	Present
Flavonoids	Absent
Terpenoids	Present
Saponins	Absent
Glycosides	Absent
Steroids	Absent
Carbohydrates	Absent
Alkaloids	Present

Table 2: Effect of *A. djalonensis* Leaf Extract on Strychnine-Induced Seizures in Mice

Treatment and route	Seizure onset + S.E.M. (min.)		Mortality protection (%)
	Clonic	Tonic	
Control-NS (10 mL/kg, i.p.)	3.564±0.5053	4.768±0.5094	0
EEAD (200 mg/kg, p.o.)	4.554±0.2721	6.362±0.1777	0
EEAD (400 mg/kg, p.o.)	12.13±0.5736*	15.43±0.3638*	80
EEAD (800 mg/kg, p.o.)	0.000±0.000*	0.000±0.000*	100
Diazepam (1mg/kg, i.p.)	0.000±0.000*	0.000±0.000*	100

Values are mean ± SEM (n=5), * significantly ($p<0.05$) different from control (one way ANOVA followed by post-hoc Dunnett's multiple comparison test), NS (Normal Saline).

Effect of EEAD on Pentylenetetrazole-Induced Convulsion

The onset of clonic and tonic seizures was significantly delayed by EEAD at 400 mg/kg dose when compared to 200 mg/kg dose. Administration of diazepam completely stopped the occurrence of clonic and tonic seizures while 800mg/kg dose significantly delayed the onset of clonic seizures and completely stopped the occurrence of tonic seizures. The standard drug (diazepam) and EEAD at 400 mg/kg and 800 mg/kg doses were statistically significant ($p<0.05$) in delaying the onset of clonic and tonic seizures when compared to control (Table 3).

DISCUSSION

After stroke, epilepsy is the second leading neurological disorder with 50 million people on various anticonvulsant treatments worldwide. Most commonly used anticonvulsants result into undesired effects such as cognitive impairment, dose-related neurotoxicity, and a spectrum of systemic side effects [20]. The aim of the present study was to evaluate the anticonvulsant effect of the leaf extract of *A. djalonensis* using two antiepileptic test models.

The yield of the extract following aqueous extraction was 2.57%w/w and was sufficient for the conduct of the study. In spite of many herbal uses and chemical structure of plants, it can bring about deleterious and toxicological effects of such plants [21]. In the present study, EEAD was evaluated for acute oral toxicity testing; no neurobehavioural changes and mortality was observed at maximum dose (2000 mg/kg) after 24 h of observation. On the basis of results, optimal doses of EEAD 200, 400 and 800 mg/kg were selected for anticonvulsant evaluation.

Table 3: Effect of *A. djalonensis* Leaf Extract on Pentylenetetrazole-Induced Seizure in Mice

Treatment and route	Seizure onset + S.E.M. (min.)		Mortality protection (%)
	Clonic	Tonic	
Control-NS (10 ml/kg, i.p.)	2.346±0.1347	4.170±0.2891	0
EEAD (200 mg/kg, p.o.)	3.288±0.2238	3.526±0.2263	0
EEAD (400 mg/kg, p.o.)	15.28±0.2202*	17.89±0.1027*	40
EEAD (800 mg/kg, p.o.)	5.050±0.550*	0.000±0.000*	100
Diazepam (10 mg/kg, i.p.)	> 24 h	0.000±0.000*	100

Interestingly, EEAD was observed to be rich in different types of phytochemicals such as phenolic and tannins which have been documented to relieve oxidative stress. Epileptic seizures are sometimes reported to result from a homeostatic imbalance between antioxidants and reactive oxygen species (ROS) and ROS-induced mitochondrial dysfunction is often ascertained accompanying seizures throughout epileptogenesis [22]. Similarly, many alkaloids and terpenoids have been reported to exert anticonvulsant activity by modulating GABA receptors which may be responsible for the anticonvulsant activity of EEAD [22]. The extract was observed to be neither non-mortal nor toxic to the experiential animals with a median lethal dose (LD_{50}) greater than 2000mg/kg. This may imply that the extract is safe with no toxic effects or at most minimal unobservable toxic effects in albino mice used to run the experiment as compared with some of the other anticonvulsant drugs used in the management of convulsion which have a plethora of toxic side effects [20].

The mechanism underlying strychnine-induced seizures involves direct antagonism of strychnine-sensitive glycine receptors, not only in the higher brain areas but also in the spinal cord and brainstem. This results in the loss of spinal reflexes and causes motor disturbance, increased muscle tone, hyperactivity of sensory, visual, and acoustic perception, tonic convulsions, and death through respiratory or spinal paralysis or by cardiac arrest. [4]. In strychnine induced convulsion, the onset of clonic and tonic seizures was significantly delayed by EEAD at 400 mg/kg dose when compared to 200 mg/kg dose and control (normal saline). Administration of diazepam and 800mg/kg dose of EEAD completely stopped the occurrence of clonic and tonic seizures. The standard drug (diazepam) and EEAD at 400 mg/kg and 800 mg/kg doses were statistically significant ($p<0.05$) in delaying the onset of clonic and tonic seizures when compared to control (Table 2) for over 24 hours. The results demonstrated that administration of *A. djalonensis* ethanol leaf extract at 400 mg/kg dose significantly increased the onset of clonic and tonic seizures in strychnine-induced convulsions when compared to 200 mg/kg dose and control. At 800 mg/kg dose, the occurrence of clonic and tonic seizures was completely stopped thereby suggesting the possible involvement of the extract on glycinergic neurotransmission [23].

Pentylenetetrazole (PTZ) induces seizure by interacting with the GABA neurotransmission (inhibition) and enhancing excitatory effects in the brain [4]. The onset of clonic and tonic seizures was significantly delayed by EEAD at 400 mg/kg dose when compared to 200 mg/kg dose. Administration of diazepam completely stopped the occurrence of clonic and tonic seizures while 800 mg/kg dose significantly delayed the onset of clonic seizures and completely stopped the occurrence of tonic seizures. The standard drug (diazepam) and EEAD at 400mg/kg and 800 mg/kg doses were statistically significant ($p<0.05$) in delaying the onset of clonic and tonic seizures when compared to control (Table 3). The result demonstrated that *A. djalonensis* ethanol leaf extract at 400 mg/kg and 800 mg/kg doses significantly delayed the onset of clonic seizures in PTZ-induced convulsion as compared to 200 mg/kg dose and control for more than 24 hours. The onset of tonic seizures was also delayed at 400 mg/kg dose while the occurrence was completely stopped at 800 mg/kg dose, inferring that the extract may act through GABA receptor in the prevention of convulsion.

The higher the dose of the extract, the higher its protection of the mice against mortality in both strychnine and pentylenetetrazole-induced seizure

models as 800 mg/kg dose produced complete protection against mortality similar to diazepam. The standard drug (diazepam) exerts its effects by increasing GABA-mediated suppression in the brain [24]. As evaluated in the experiment, it completely stopped the occurrence of clonic and tonic seizures in both strychnine and PTZ induced models as compared to the control group.

CONCLUSION

Ethanol leaf extract of *A. djalonensis* (EEAD) commonly used in traditional medicine for the management of epileptic seizure demonstrated anticonvulsant activity by significantly delaying seizure onset at 400 and 800 mg/kg doses in both pentylenetetrazole and strychnine-induced seizure models indicating that the extract is capable of interacting with the cerebral glycine and GABA receptors, respectively. Hence, the extract may be considered for the identification and isolation of novel anticonvulsant compounds and development of new anticonvulsant drugs.

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