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Examining the Ethanol Leaf Extract of *Celosia Leptostachya* in Mice for its Sedative and Anticonvulsant Properties

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Implication for health policy/practice/research/medical education

Celosia leptostachya leaves contain anticonvulsant metabolites coupled with sedative substance. Hence, it might be beneficial in the treatment of convulsion and insomnia.

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Abstract

Background: The goal of this research was to find out if Celosia leptostachyahas anticonvulsant properties using Pentylenetetrazole (PTZ), Brucine (BRC), and Maximal electroshock (MES) induced seizures in mice.

Materials andMethods: PTZ, BRC, and MES induced seizures in mice were used. Group1 received normal saline, Goup5 received standard drugs while Groups 2, 3 and 4, were respectively treated with 100, 150 and 200 mg/kg of the extract. The sedative-hypnotic behaviour was assayed using diazepam-induced sleep.

Results: In MES-induced seizures, ethanol extract of Celosia leptostachya(EECL) at 100 mg/kg, 150 mg/kg, and 200 mg/kg protected the animals against hind limb tonic extension and also, in convulsed mice significantly ($P< 0.05$) decreased mean recovery time suggesting inhibition of Na+ gated channels. In BR-induced seizures, EECL at 150 and 200 mg/kg offered protection against mortality but did not significantly ($P>0.05$) affect both the mean onset time and mean duration of convulsion. In PTZ-induced seizures, EECL at 200 mg/kg offered protection against mortality and significantly ($P<0.05$) attenuated both mean onset time and mean duration of convulsion suggesting activity on GABAA receptors. The EECL 100, 150, and 200 mg/kg doses significantly ($P < 0.05$) prolonged the total duration of Diazepam-induced sleeping time in mice without affecting the mean onset of sleep, indicating sedative action of the extract.

Conclusion: The data may provide the pharmacological basis for the use of the plant alone or in combination with other plant (s) in the management of epileptic seizures and insomnia

Keywords: Anticonvulsant, brucine, seizures, insomnia, Celosia leptostacya

Introduction

Epilepsy is one of the major frequent distresses of man [1]. At least, 50 million people in the world are affected by epilepsy. This account for about 1% of the total world sickness [2]. Also, it is a persistent neurological chaos that disturbs equally males and females [3]. Neurologists

reported that it is the second most regular disease of neurons worldwide [4]. Epilepsy however, is a common chronic disorder of neurons known by repeated unprovoked seizures (or convulsion). It repeatedly interrupts normal brain function unpredictably. This is known as epileptic seizures. This arises because of unexpected, excess and rapid release of cerebral neurons mainly in the grey matter of the brain [5].

This affects not only the victim of epilepsy but also the family and the entire community [6].Hence, epilepsy is still well known as one of the most stigmatized brain diseases. This stigmatization is common among developing nations possibly due to low levels of education, lack of exposure, and poor information concerning the real nature of the disease. The disgrace and trepidation linked with epilepsy put off lots of affected persons from looking for healing. Consequent upon this, their epileptic condition usually run out of hand and hence limits their chances of being educated, employed, and socially relevant [7].

Treatment of diseases with medicinal plants began before human civilization [8]. Medicinal plants from folk therapies have impacted extensively in the finding of new drugs and are now well thought-out as an alternative source for the discovery of drugs that have antiepileptic effects with researchable novel and high therapeutic index[9]. Many natural products and their derivatives expounded synthetically have been effectively developed for clinical use to heal human diseases in almost all treatable facets [10]. The African continent is well known from time immemorial for the use of plants as therapeutic agents of which phytotherapy is still in use in the management of diseases in most low-income African countries [11].

Regardless of the flourishing growth of diverse new antiepileptic drugs in latest decades, the hunt for new therapies with improved efficacy and tolerability are very important area of interest[12]. Existing treatment with antiepileptic drugs only suppresses seizures but do not correct the fundamental *epileptogenic* process [13]. Since, prevention of epilepsy in patients at risk is not clinically successful; hence, there is increasing concern that the efficacy of drug management of epilepsy has not yielded better outcomes despite the development of new drugs for epilepsy.

Celosialeptostachya (*C. leptostachya*) plant belongs to the family of *amarantheceae*. There are no hairs on its stem but very slim and looks like an angle tip in nature with decumbent support. This plant can grow up to 30cm in height and can get to 60cm high when lifted from the stalk. It

does have ovate leaves but the leaves are smooth and very conspicuous. *C. leptostachya* is well distributed in Abia State, South East of Nigeria. The plant's leaves are eatable and serve as cooking vegetables for soup. *C. leptostachya* possesses numerous medicinal values, which traditional medicine practitioners exploit especially in the treatment of diseases such as boils, fever, snake bites, scorpion stings, eye infections, wounds and pain, and most notably, convulsions. Based on personal interaction with the traditional healer, when an epileptic victim is brought to him, the fresh leaves of the plant would be fetched and squeezed manually to get at least 10ml of the liquid content and administered orally and through the eyes. The victims usually recover between 5-10 minutes after treatment. Hence, this work was designed to investigate its anticonvulsant activity using an animal model of epilepsy.

1. Materials and Methods

Plant material collection and authentication

The fresh leaves of *C. leptostachya* were collected within the vicinity of UmuhuNvosi in Isiala-Ngwa South Local Government Area of Abia State, Nigeria on 8th May 2022. The plant was identified and authenticated by Mr. Ibe K. Ndukwe of the Herbarium unit, Department of the Forestry MichealOkpara University of Agriculture Umudike, Nigeria with the Herbarium number "FHI3081".

Preparation of plants and extraction procedure

The fresh leaves of *C. leptostachya*collected were washed under running water to remove any possible dirt and thereafter dried up at room temperature. The dried leaves were ground into the powdered form using a mortar and pestle. A portion of the resulting powdered leaves (800g) was subjected to extraction using ethanol (absolute) for 72 hours using the maceration method. The extract was collected from the separating funnel by filtration using Whatman filter No. 25. The solvent was evaporated from the resulting filtrate on a water bath set at a low temperature (40^oC).

Phytochemical screening

The preliminary phytochemistry of *C. leptostachya*ethanol leaf extract was carried out to determine different secondary metabolites and these include the following test; for tannins, some quantities of *C. leptostachya* extract about 0.5g by approximation was dissolved in 1ml of

distilled water, stirred and filtered. Some drops of ferric Chloride reagent was introduced into the filtered solution. The presence of blue-black, green or blue green precipitate indicates the presence of tannins [14] For alkaloids, 0.5g of the *C. leptostachya* extract was turned in 5ml of 1% diluted HCl_{aq}on heated water bath. Thereafter, 1ml of the resulting solution was added with some few drops of Mayer's reagent, Dragendort's reagent, and *picic* acid solution. The presence of precipitates was seen an indication of the presence of alkaloids in the extract [15].For saponins, approximately 0.5g of *C. leptostachya* extract was dissolved in water and thoroughly shaken in a test tube. The continuous appearance of frothing upon heating was seen as indication of the presence of saponin[15]. For steroids, about 0.5g of the *C. leptostachya* was collected and liquefied in water and filtered thereafter. 1ml of the resulting solution was introduced to 2ml of H_2SO_4 in a test tube. Steroid was taken to be present so long as reddish brown ring is seen within the interface [15].

For terpenoids, some portion of *C. leptostachya* extract was dissolved in water and 5ml of the portion received 2ml of chloroform and subjected to evaporation by means of water bath. Thereafter, the resulting portion was boiled in 3ml of concentrated H_2SO_4. The appearance of grey colouration was taken to indicate the availability of terpenoid.

For flavonoids, lead *sub acetate* test was used. 100mg of the extract of *C. leptostachya* was liquefy in 5ml of water and filtered thereafter. Lead *sub acetate* of about two to three drops was introduced. Precipitation of yellow colouration suggests the availability of flavonoids. For anthraquinones, some quantities of *C. leptostachya* was collected into a conical flask containing 10ml of benzene and was allowed to thoroughly mix for 10minutes. It was filtered and 10ml of solution of 10% ammonia was introduced to it and shaken very strongly within 30 seconds. Any appearance of pink, violet and or red colour suggest the presence of anthraquinones[16].

Thin layer chromatography with retention factor (TLC)

In preparation of TLC, 25ml of water, 75g of silica gel, 100ml of methanol and 50ml of chloroform were used. Formation and activation of TLC plate was done with an oven (110^0C)under 1hour [17].To separate TLC, 3g of the ethanol extract of *C. leptostachya*was dissolved in its solvent. These solvents were made from hexane and methanol in the ratio of 4:1. Spotting of the solution of the sample was done with the help of capillary tube on silica gel TLC

plate placed 1cm from the plate's edge while the drop was allowed to dry[17]. The action of capillary tube helps the plate positioned in the TLC chromo tank to ascend the TLC pate. The solvent front was marked and given time to dry after the plate had been removed. In order to detect the spot, iodine solution was used to make it more visual and clearer. The distance moved by solvent and spot from the retention factor of different spots were measured by the aid of meter rule[18]. This is calculated as;

$$R_f = \frac{\text{Distance move by compound}}{\text{Distance move by solvent front}}$$ [19]

Experimental animals

Adult mice of both sexes weighing between 18-25 g were sourced from Animal House of the Department of Pharmacology and Therapeutics. The animals were housed in propylene cages situated in well-ventilated rooms at the Pharmacology Animal House and had free access to standard pellets (Guinea Feeds, Plc Nigeria) and water ad libitum. All experiments performed on laboratory animals were in accordance with the Ebonyi State University Animal Research Ethical Committee. The animals were handled according to the International Guidelines for Care and Handling of Experimental Animals (NIH).

Acute toxicity tests

The LD_{50} of the plant extract was tested to determine the safety of the agents using Lork's (1983) method. The study was carried out in two phases on adult mice. The animals were grouped into three in the first phase, each group having three mice. The mice were administered *C. leptostachya* leaf extract at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/ kg using an orogastric cannula and observed for signs of toxicity and mortality for 24 hours. In the second phase of the study, mice were divided into three groups, with each having one mouse, and orally administered 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg of the leaf extract respectively. These animals were first observed for 4 hours and later for 24 hours. Toxicity signs and mortality were recorded for each group after the observation period. The experimental mice were monitored further for 72 hours for delayed toxicity.

Anticonvulsant studies

Pentylenetetrazole–induced seizure in mice (PTZ)

The method previously described by Swinyard, (1989) and modified by Shimada and Yamagata (2018) was employed [20]. Thirty mice were randomly divided into five groups of six mice each. Group one representing the negative control group was given 10ml per kg normal saline via the intraperitoneal route, while groups two, three, and four were pre-treated with the ethanol extract of *C. leptostachya* at doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg body weight via the intraperitoneal route. The fifth group of mice (positive control group) was treated with 10mg/kg Diazepam intraperitoneally. Following 30 minutes of EECL pre-treatment, PTZ (90 mg/kg) was given to the animals through the subcutaneous route. The animals were kept under watch for any sign of absence or threshold seizures within 30 minutes of PTZ inducement.

Brucine-induced seizure in mice

This test was conducted on EECL of the plant to investigate the effect of brucine and its anticonvulsant activity. The method described by Fan et al (2014) was adopted [20]. Thirty mice were grouped into five; each consisting of six mice. The first group received normal saline (10 ml/kg). The mice in the second, third and fourth groups received graded doses (100mg/kg, 150 mg/kg, and 200 mg/kg) of EECL intraperitoneally. Mice in the fifth group received 20mg/kg of the standard drug, phenobarbital. Thirty (30) minutes post-treatment, 110 mg/kg brucine was administered to each mouse via the intraperitoneal route. The mice were subsequently observed for hind limb tonic seizures for thirty minutes. The nonappearance of tonic hind limb extension or persistence of the latency of tonic hind limb extension was considered an indication of anticonvulsant activity.

Maximal electroshock-induced seizure test in mice

The method described by Swinyard and Kupferberg (1985), modified by Tutka, et al (2018) was employed in this study [20]. Thirty (30) mice were indiscriminately selected and shared into five groups with each housing 6 mice. The first group of mice received normal saline (10 ml/kg). The second, third, and fourth groups received intraperitoneally 100 mg/kg, 150 mg/kg, and 200 mg/kg of EECL respectively. The fifth group was given 20mg/kg of phenytoin. Thirty minutes later, an alternating current of 50Hz and 35mA was delivered to the animals in each group

through ear-electrodes for 0.2sec. Electrodes were moistened with saline before fastening the ear of the mouse to enhance electrical contact. Seizures were manifested as hind limb tonic extension (HLTE). Prevention of MES-induced hind limb tonic extension (HLTE) by the extract was considered as protection against MES-induced seizure. The percentage protection against seizure, onset seizure, mortality, and recovering time were noted [21].

Behavioural study on the ethanol leaf extract ofCelosia leptostachya

Diazepam-induced sleep in mice

The method described by Beretzet al., (1978) and modified by Okomoloet al., (2011) was adopted in this study[22]. Mice were randomly divided into four groups containing 5 mice each. The first group received normal saline (10 ml/kg) intraperitoneally. The second, third, and fourth groups received 100, 150, and 200 mg/kg of ethanol leaf extract of *C .leptostachya* per kg body weight via the same route. After 30 minutes of administration of normal saline and plant extract, all the mice in each group received diazepam at 20 mg/kg i.p. The mice were placed individually in propylene cages for observation. The onset and duration of sleep were determined for each animal. The time interval between the administrations of diazepam to the loss of righting reflex was recorded as the onset of sleep while the interval between the loss and the recovery of righting reflex was regarded as the duration of sleep.

Statistical analysis

Results were expressed as Mean ± Standard Error of Mean and analysed with a statistical package for social sciences (SPSS version 20) by using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A difference in the mean $P < 0.05$ was considered significant.

2. Results

Phytochemical analysis

Preliminary phytochemistry of extract of *C. leptostachya* leaf using different solvents: ethanol, and water revealed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, steroids, saponins, balsam, and resins, while anthraquinone, cardiac glycoside, and tannins were found to be absent both in water and ethanol extract. However, saponins and flavonoids were shown to be abundant in ethanol and less in water (Table 1).

The fingerprint of TLC with retention factor

The fingerprint of TLC revealed that saponins (Rf 0.04) and flavonoids (Rf 0.05) have the least retention factor showing that the plant is rich in these secondary metabolites. Also, carbohydrates (Rf 0.80), alkaloids (Rf 0.8), balsam (Rf 0.70), and Resins (Rf 0.65) have high retention factors. This is because they have less affinity towards the stationary face of the TLC plate, hence traveling more distance compared with saponins and flavonoids. This is why they were more in abundance during preliminary quantitative phytochemical screening (Table 2 and Figure 1).

Acute toxicity tests

The ethanol leaf extract of *C. leptostachya* did not produce any lethality or significant toxicity signs in mice up to 5000 mg/kg body weight for 24 and 72 hours post-treatment.

PTZ – induced seizures in mice

In PTZ-induced seizures, EECL 100 mg/kg, did not offer any protection against mortality and also did not significantly ($P>0.05$) affect both mean onset time and mean duration of convulsion compared with mice that received normal saline. This study further indicated that EECL at 150 mg/kg offered 66.7% protection against mortality but did not significantly ($P>0.05$) affect both mean onset time and mean duration of convulsion. However, EECL at 200 mg/kg offered 100% protection against mortality and significantly ($P<0.05$) decreased both mean onset time and mean duration of convulsion in convulsed animals similar to the effects elicited by the standard drug; Diazepam (Table 2).

Brucine–induced seizures in mice

The observation from this study brucine-induced seizures indicated that EECL at 100 mg/kg, did not offer any protection against seizure, or mortality and did not significantly ($P>0.05$) affect both onset time and recovering time in convulsed mice. However, EECL at 150 mg/kg offered 66.67%protection against mortality but did not significantly ($P>0.05$) affect both the mean onset time and mean duration of convulsion in convulsed animals while EECL at 200 mg/kg offered 100% protection against mortality similar to standard drug Phenobarbital but did not significantly ($P>0.005$) affect both onset and duration of convulsion in animals. (Table 3 and Figure 3).

Maximal electroshock-induced seizures in mice

This study also revealed that EECL at 100, 150 and 200 mg/kg respectively protected 16.67%, 50%, and 66.67% of mice against hind limb tonic extension induced by maximal electroshock and in convulsed mice, a significantly ($P < 0.05$) decreased in mean recovery time was noted for each dose (Table 4 and Figure 4).

Diazepam-induced sleep

There was no significant difference in the mean onset of sleep in the mice, which received 100 mg/kg, 150 mg/kg, and 200 mg/kg when compared with the control (normal saline). However, a significant difference in the mean duration of sleep was observed when mice that received 100 mg/kg, 150 mg/kg, and 200 mg/kg of ethanol leaf extract of *C. leptostrachya* were compared with the mice that received the normal saline (Table 5).

Table 1:Phytochemical screening of *Celosia leptostachaya* using different solvents

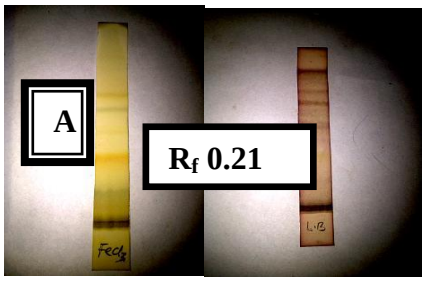
Experiment	Aqueous extract	Ethanol
Alkaloids	+	+
Saponins	+	+++
Tannins	-	-
Flavonoids	++	+++
Carbohydrates	+	+
Steroids	+	+++
Terpenoids	+	++
Anthraquinone	-	-
Balsam	+	+
Resins	+	+
Cardiacglycoside	-	-

Key
+ = Present
++= Very present
+++= Abundant

- = Absent

Table 2:TLC of the ethanol extract of *C. leptostachya with its* retention factor

TLC plate	Secondary metabolite	Retention factor (R_f)
A	Steroids	0.21
B	Carbohydrates	0.80
C	Saponins	0.04
D	Terpenoids	0.25
E	Flavonoids	0.05
F	Balsam	0.70
G	Alkaloids	0.85
H	Resins	0.65

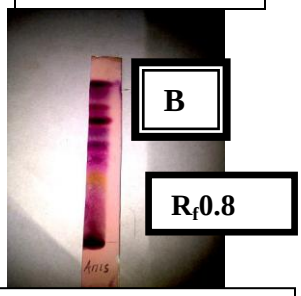


Steroids EECL

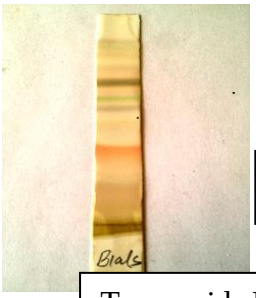
C

R_f0.04

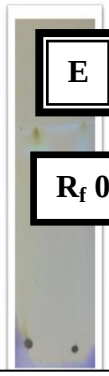
Saponins EECL



Carbohydrates EECL

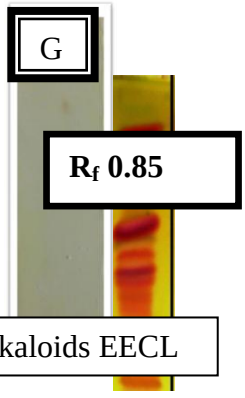


Terpenoids EECL



Flavonoids EECL

Balsam EECL



Alkaloids EECL

H

R_f 0.65

Resins EECL

Figure 1: Finger print of thin layer chrromatograhy of ethanol extract of *Celosia Leptostachya*

Table 2: The effect of ethanol leaf extract of *Celosia leptostachya* on pentylenetetrazole induced seizures in mice after 30 minutes of administration

*[#]Data presented as Mean ± SEM, * = P < 0.05; [#]= P> 0.05 n = 6; EECL = Ethanol Extract of *Celosia leptostachya*;

Treatment mg/kg	Mean±SD onset of convulsion (mins)	Mean±SD duration of convulsion (mins)	Quantile protection against seizure	Quantile protection against mortality	Percentage protected against mortality (%)
Normal saline	0.15±0.4	0.92±0.10	0/6	0/6	0
EECL (100)	0.25±0.23[#]	1.22±0.23[#]	0/6	0/6	0
EECL (150)	1.05±0[#]	1.35±0.22[#]	0/6	4/6	66.7
EECL (200)	1.210.51[*]	0.05±0.28[*]	4/6	6/6	100
Diazepam (10)	0.00±0.00[#]	0.00±0.00[#]	6/6	6/6	100

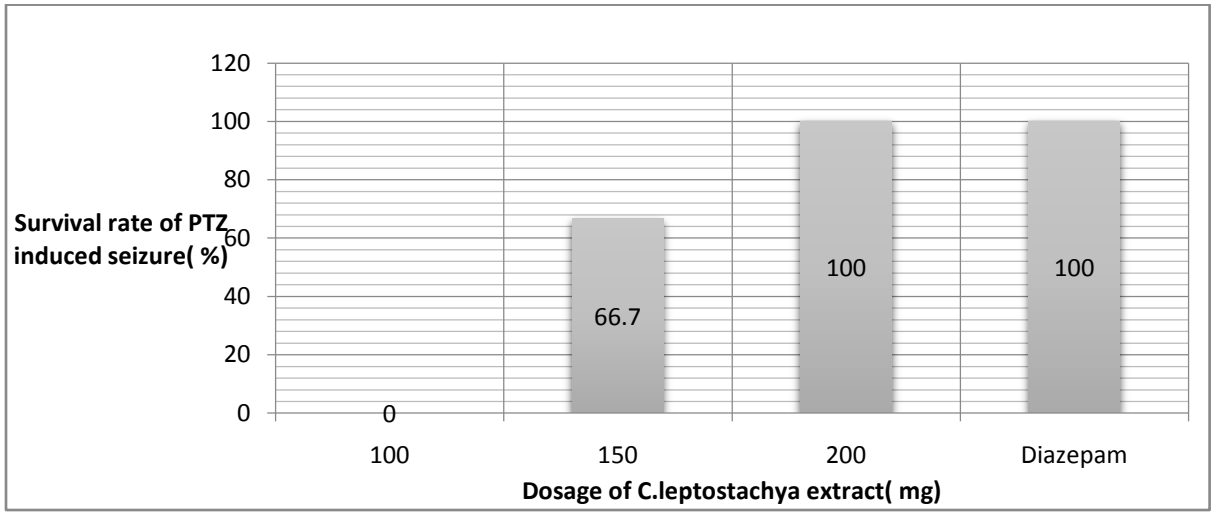


Figure 2. Percentage survival of mice against PTZ induced seizure

Table 3: The effect of ethanol leaf extract of *Celosia leptostachya* on brucine induced seizures in mice after 30 minutes of administration

Treatment mg/kg	Mean ±SD Onset of convulsion	Mean±SD Duration of convulsion (mins)	Quantile protection against seizure	Quantile protection against mortality	Percentage protected against mortality (%)
Normal saline	0.43±0.11	1.25±0.11	0/6	0/6	0
EECL (100)	0.95±0.34$^{\#}$	2.74±0.49$^{\#}$	0/6	0/6	0
EECL (150)	4.34±0.73$^{\#}$	2.47±1.06$^{\#}$	0/6	4/6	66.7
EECL (200)	14.46±22.	15.02±3.32*	2/6	6/6	100
Phenobarbital(20)	0.00±0.00$^{\#}$	0.00±0.00$^{\#}$	6/6	6/6	100

* $^{\#}$Data presented as Mean ± SEM, * = P < 0.05; $^{\#}$= P> 0.05 n = 6; EECL = Ethanol Extract of *Celosia leptostachya*;

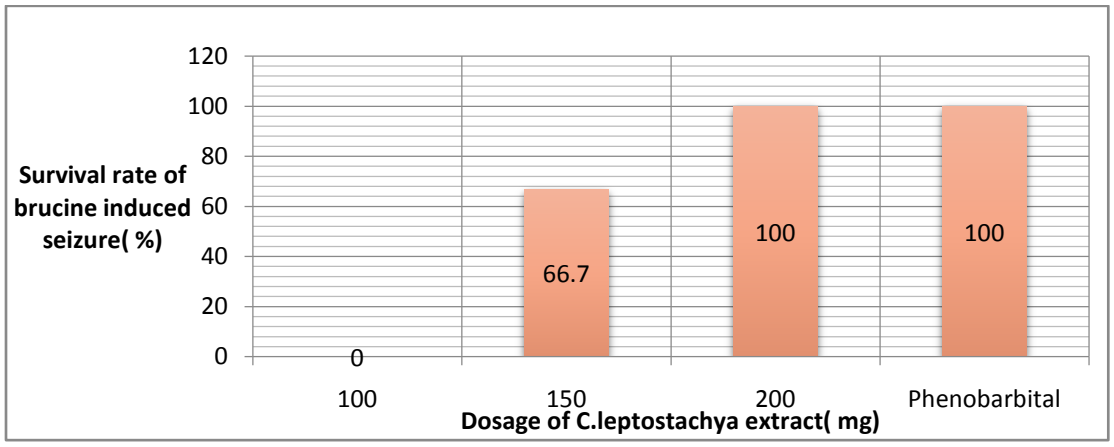


Figure 3. Percentage survival of mice against brucine induced seizure

Table 4: The effect of ethanol leaf extract of *Celosia leptostachya* on maximal electroshock induced seizures in mice after 30 minutes of administration

Treatment (mg/kg)	Mean onset of convulsion (min)	Mean duration of convulsion (min)	No of mice protected against convulsion	% Protection against convulsion
Normal saline	2.28±0.08	1.24±0.05	0/6	0
EECL(100)	3.51±0.81$^{\#}$	0.32±0.06*	1/6	16.67
EECL(150)	2.61±1.17$^{\#}$	0.17±0.08*	3/6	50
EECL(200)	2.72±1.72$^{\#}$	0.11±0.07*	4/6	66.67
Phenytoin (20)	0.00±0.00$^{\#}$	0.00±0.00*	6/6	100

* $^{\#}$Data presented as Mean ± SEM, * = P < 0.05; $^{\#}$= P> 0.05 n = 6; EECL = Ethanol Extract of *Celosia leptostachya*;

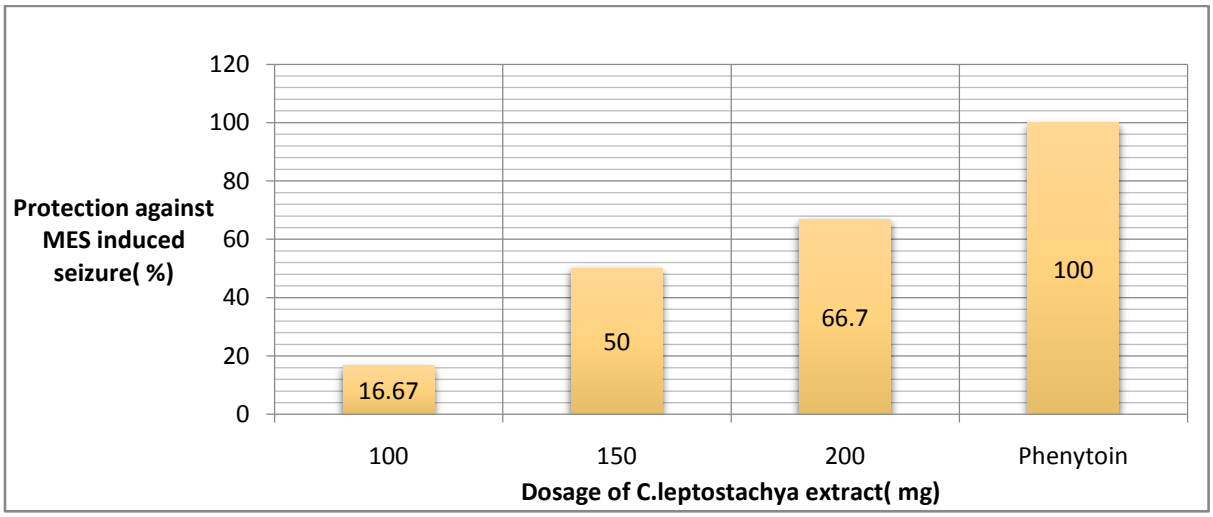


Figure 4. Percentage protection against MES induced seizure in mice

Table 5: The effect of ethanol leaf extract of *Celosia leptostachya* on diazepam induced sleep

Treatment (mg/kg)	Mean± SD onset of sleep (min)	Mean± SD duration of sleep (min)
Normal saline	3.15±0.02	107.76±0.47
EECL 100	3.19±0.04$^{\#}$	254.10±24.68*
EECL 150	3.18±0.08$^{\#}$	306.64±1.61*
EECL 200	3.69±0.25$^{\#}$	243.10±1.71*

$^{\#}$represents P > 0.05; * represents P < 0.05; EECL = Ethanol Extract of *Celosia leptostachya*;

3. Discussion

Phytochemical screening offers basic information regarding the diverse classes of secondary metabolites present in a plant and the medicinal significance of such plant extract[23]. Based on the results obtained from the phytochemical screening, it is not likely to credit with certainty the observed anticonvulsant effect of *C. leptostachya*to one or several active principles amongst those detected in the phytochemical screening. It has been reported in some animal model of seizure that certain plant secondary metabolites such as steroids along with saponins and trterpenic have antiseizure effects against MES and PTZ induced seizures[24].

For MES-induced convulsion, the ability of this extract to significantly decrease the mean recovering time of convulsed mice compared to normal saline-group post exposure to maximal

electroshock stimulus, showed that these extracts probably antagonized the electrically induced seizures by inhibiting voltage gated Na^+ channels or by antagonism of glutamatergic excitation mediated by N-methyl-D- aspartate- receptor complex. Inhibition of Na^+ ion channels would always stabilize neuronal membranes protecting mice against convulsion induced by maximal electroshock stimulus. Maximal electroshock test for screening potentially active anticonvulsant agents (medicinal plants) is believed to identify anticonvulsant agents that are protective against partial seizures and generalized seizures. The MES test model for anticonvulsant screening has a clearly defined (consistent) end point (inhibition of the tonic hind limb extension phase) and is highly reproducible[25]. MES is one of the best validated preclinical tests that predict drugs effective against generalized (Tonic – clonic seizures). Phenytoin and Carbamazepine have been shown to protect animals against hind limb tonic extension (HLTE) induced by maximal electroshock stimulus. [26] The behavioural and electrographic seizures generated in this model are consistent with the human disorder[27]. This model: MES essentially identifies those compounds which prevent seizure spread through neural tissue. Hence, the ability of the ethanol extract to inhibit seizures induced by electroshock stimulus and also shorten the recovery time of convulsed mice imply that it is likely to exhibit activity against generalized tonic-clonic seizures[28]. *PTZ* which is a derivative of *tetrazole* is the model agent in the class of "systemic" convulsants[29]. PTZparenteral administration has consistent convulsant actions in mice, rats, cats and primates. PTZ originally produces myoclonic jerks which subsequently become generalized which could lead to a generalized tonic-clonic seizure. Research has shown that PTZ reduces *GABAergic* tone[30]. Gamma amino butyric acid is the chief inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The enhancement of GABA neurotransmission is said to antagonize seizures while its inhibition promotes seizure[31]. Clonic seizures provoked by PTZ are blocked by drugs that lessen T-type calcium currents and drugs that enhance the inhibitory neurotransmission by GABA receptors. Compounds which are able to suppress PTZ-induced seizures are presumed to be effective in the treatment of absence seizures[32].

In table 2, the extract demonstrated dose dependent activity on PTZ-induced seizures by decreasing recovering time in convulsed animals compared with the negative control. However, it also offered 100% protection against mortality at 200mg similar to the standard drug:

diazepam. The extract also prevented convulsion in four (4) animals which is better when compared with negative control which offered no protection against convulsion.

This suggest that the ethanol extract of *C. leptostachya*has effects on GABAergic neurotransmission. Dopamine has been shown to reduce seizure threshold in the brain and specific antagonists of dopamine have been shown to protect experimental animals against PTZ-induced seizures[33]. A study in 2013, founded that PTZ increases calcium influx and sodium influx, both of which depolarized the neuron[34]. Because these effects were antagonized by calcium channel blockers, it was concluded that PTZ acts at calcium channels, and it causes calcium channels to lose selectivity and conduct sodium ions as well[35]. The ability of the ethanol extract to protect mice against PTZ- induced seizures suggests it may probably act by modulating GABA receptor mediated inhibitory neurotransmission and hence may be applicable as a potential antiabsence seizure therapy for the management of *petit mal* (absence) epilepsy.

Similarly, brucine is an alkaloid which is an inhibitor of glycine (inhibitory neurotransmitter) synthesis [36]. It is an antagonist to excitatory monotransmitter like AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (N-methyl-D-aspartate) and it also opposes Ca^{2+} induced excitotoxicity in the neurons. These factors are pro *epileptogenic* and precipitate seizures. Hence, inhibition of glycine leads to clonic and tonic seizures in laboratory animals. Thus, it is used as a research tool to induce seizures in the animals.

However, EECL 200 mg/kg prevented 100% mortality but failed to significantly affect both onset and recovering time of convulsion. This further demonstrates dose dependency of this drug. Brucine, a potent convulsant, selectively blocks inhibitory inputs by glycine receptors, predominantly at the spinal cord, to induce excitatory responses in the CNS (32), a mechanism that might also define the action of *C. leptostachya*.

Potentiating the total sleeping time by the ethanol extract of *C. leptostachya*signifies the presence of sedative compounds. Sedation comes from the activation of GABA receptors in the $GABA_A$ receptor complex thereby potentiating GABA mediated inhibitory action. By potentiating diazepam-induced sleep, the extract of *C. leptostchya*possesses sleep-inducing properties [37].The ability of the extract to potentiate the sedative property of diazepam hints that it may possibly act by interacting with GABA-mediated synaptic transmission.

4. Conclusion

From the results of this study, it can be observed that the ethanol leaf extract of *C. leptostachya* demonstrated anticonvulsant activities on the three animal model of epilepsy used in this study. However, its anticonvulsant activity was more effective on MES-induced seizure compare with brucine and PTZ induced seizures. Furthermore, the results of the present study can therefore be concluded that *Celosia leptostachya* leavescontain anticonvulsant metabolites coupled with sedative substance in mice which could account for its use in the treatment of febrile convulsion and insomnia. There is need to study more on this plant especially on isolation, purification and molecular docking because this plant may be the answer to our quest for a new antiepileptic agent that carry curative measures.

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Conflict of interest:

The authors declare absence of conflict interest in carrying out this research work.

Ethical clearance

This current research work was done after a permission to go ahead was granted from the ethical committee of the Ebonyi State University, Abakaliki Nigeria were the work was done with reference number **EBSU/DRIC/UREC/VOL.04/098.**

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5. References

1. Lukong KE, Ogunbolude Y, Kamdem JP. Breast cancer in Africa: prevalence, treatment options, herbal medicines, and socioeconomic determinants. Breast Cancer Res Treat. 2017;166(2):351-65.
2. Shi Y, Wang S, Ying J, Zhang M, Liu P, Zhang H, et al. Correlates of perceived stigma for people living with epilepsy: A meta-analysis. Epilepsy & Behavior. 2017;70:198-203.

3. Panebianco M, Al-Bachari S, Hutton JL, Marson AG. Gabapentin add-on treatment for drug-resistant focal epilepsy. The Cochrane database of systematic reviews. 2021;1(1):Cd001415.
4. Kirmani BF, Shapiro LA, Shetty AK. Neurological and Neurodegenerative Disorders: Novel Concepts and Treatment. Aging and disease. 2021;12(4):950-3.
5. Khawaja AM, Pati S, Ng Y-T. Management of Epilepsy Due to Hypothalamic Hamartomas. Pediatric neurology. 2017;75:29-42.
6. von Gaudecker JR, Taylor AG, Keeling AW, Buelow JM, Benjamin S. Living in the epilepsy treatment gap in rural South India: A focused ethnography of women and problems associated with stigma. Health care for women international. 2017;38(7):753-64.
7. Keikelame MJ, Swartz L. "I wonder if I did not mess up….": Shame and resistance among women with epilepsy in Cape Town, South Africa. Seizure. 2018;61:50-6.
8. Cavalcanti LP, Mota LA, Lustosa GP, Fortes MC, Mota DA, Lima AA, et al. Evaluation of the WHO classification of dengue disease severity during an epidemic in 2011 in the state of Ceará, Brazil. Memorias do Instituto Oswaldo Cruz. 2014;109(1):93-8.
9. Auditeau E, Chassagne F, Bourdy G, Bounlu M, Jost J, Luna J, et al. Herbal medicine for epilepsy seizures in Asia, Africa and Latin America: A systematic review. J Ethnopharmacol. 2019;234:119-53.
10. Łukawski K, Gryta P, Łuszczki J, Czuczwar SJ. Exploring the latest avenues for antiepileptic drug discovery and development. Expert opinion on drug discovery. 2016;11(4):369-82.
11. Mahomoodally MF. Traditional medicines in Africa: an appraisal of ten potent african medicinal plants. Evidence-based complementary and alternative medicine : eCAM. 2013;2013:617459.
12. Balestrini S, Sisodiya SM. Pharmacogenomics in epilepsy. Neuroscience letters. 2018;667:27-39.
13. Schulze-Bonhage A. A 2017 review of pharmacotherapy for treating focal epilepsy: where are we now and how will treatment develop? Expert opinion on pharmacotherapy2017;18(17):1845-53.
14. Nakaziba R, Lubega A. Phytochemical Analysis, Acute Toxicity, as well as Antihyperglycemic and Antidiabetic Activities of Corchorus olitorius L. Leaf Extracts. 2022;2022:1376817.
15. Thotathil V, Sidiq N. Phytochemical Analysis of Anastatica hierochuntica and Aerva javanica Grown in Qatar: Their Biological Activities and Identification of Some Active Ingredients. 2023;28(8).
16. Afzal T, Bibi Y, Ishaque M, Masood S, Qayyum A, Nisa S, et al. Pharmacological properties and preliminary phytochemical analysis of Pseudocaryopteris foetida (D.Don) P.D. Cantino leaves. Saudi journal of biological sciences. 2022;29(2):1185-90.
17. Deranieh RM, Joshi AS, Greenberg ML. Thin-layer chromatography of phospholipids. Methods in molecular biology (Clifton, NJ). 2013;1033:21-7.
18. Myher JJ, Kuksis A. General strategies in chromatographic analysis of lipids. Journal of chromatography B, Biomedical applications. 1995;671(1-2):3-33.
19. Karthika K, Paulsamy S. TLC and HPTLC Fingerprints of Various Secondary Metabolites in the Stem of the Traditional Medicinal Climber, Solena amplexicaulis. Indian journal of pharmaceutical sciences. 2015;77(1):111-6.
20. Shimada T, Yamagata K. Pentylenetetrazole-Induced Kindling Mouse Model. Journal of visualized experiments : JoVE. 2018(136).

21. Tutka P, Mroz K, Mroz T, Buszewicz G, Aebisher D, Bartusik-Aebisher D, et al. Effects of androsterone on the protective action of various antiepileptic drugs against maximal electroshock-induced seizures in mice. Psychoneuroendocrinology. 2019;101:27-34.
22. Okomolo FC, Mbafor JT, Bum EN, Kouemou N, Kandeda AK, Talla E, et al. Evaluation of the sedative and anticonvulsant properties of three Cameroonian plants. African journal of traditional, complementary, and alternative medicines : AJTCAM. 2011;8(5 Suppl):181-90.
23. Danciu C, Muntean D, Alexa E, Farcas C, Oprean C, Zupko I. Phytochemical Characterization and Evaluation of the Antimicrobial, Antiproliferative and Pro-Apoptotic Potential of Ephedra alata Decne. Hydroalcoholic Extract against the MCF-7 Breast Cancer Cell Line. 2018;24(1).
24. Malami S, Kyari H, Danjuma NM, Ya'u J, Hussaini IM. Anticonvulsant properties of methanol leaf extract of Laggera Aurita Linn. F. (Asteraceae) in laboratory animals. Journal of ethnopharmacology. 2016;191:301-6.
25. Borowicz-Reutt KK, Poplawska M, Banach M, Wroblewska D. Influence of propafenone on the anticonvulsant activity of various novel antiepileptic drugs in the mouse maximal electroshock model. Pharmacological reports : PR. 2018;70(3):481-7.
26. Joshi R, Reeta KH, Sharma SK, Tripathi M, Gupta YK. Pharmacodynamic and pharmacokinetic interaction of Panchagavya Ghrita with phenytoin and carbamazepine in maximal electroshock induced seizures in rats. Ayu. 2015;36(2):196-202.
27. Baker EM, Thompson CH, Hawkins NA, Wagnon JL, Wengert ER, Patel MK, et al. The novel sodium channel modulator GS-458967 (GS967) is an effective treatment in a mouse model of SCN8A encephalopathy. Epilepsia. 2018;59(6):1166-76.
28. Tutka P, Mróz K, Mróz T, Buszewicz G, Aebisher D, Bartusik-Aebisher D, et al. Effects of androsterone on the protective action of various antiepileptic drugs against maximal electroshock-induced seizures in mice. Psychoneuroendocrinology. 2019;101:27-34.
29. Zhang B, Wong M. Pentylenetetrazole-induced seizures cause acute, but not chronic, mTOR pathway activation in rat. Epilepsia. 2012;53(3):506-11.
30. Bae MH, Bissonette GB, Mars WM, Michalopoulos GK, Achim CL, Depireux DA, et al. Hepatocyte growth factor (HGF) modulates GABAergic inhibition and seizure susceptibility. Experimental neurology. 2010;221(1):129-35.
31. Atherton JF, Menard A, Urbain N, Bevan MD. Short-term depression of external globus pallidus-subthalamic nucleus synaptic transmission and implications for patterning subthalamic activity. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(17):7130-44.
32. Karami R, Hosseini M, Mohammadpour T, Ghorbani A, Sadeghnia HR, Rakhshandeh H, et al. Effects of hydroalcoholic extract of Coriandrum sativum on oxidative damage in pentylenetetrazole-induced seizures in rats. Iranian journal of neurology. 2015;14(2):59-66.
33. Drexel M, Romanov RA, Wood J, Weger S, Heilbronn R, Wulff P, et al. Selective Silencing of Hippocampal Parvalbumin Interneurons Induces Development of Recurrent Spontaneous Limbic Seizures in Mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2017;37(34):8166-79.
34. N'Gouemo P. Probing the role of the sodium/calcium exchanger in pentylenetetrazole-induced generalized seizures in rats. Brain research bulletin. 2013;90:52-7.
35. Nieoczym D, Socała K, Łuszczki JJ, Czuczwar SJ, Wlaz P. Influence of sildenafil on the anticonvulsant action of selected antiepileptic drugs against pentylenetetrazole-induced clonic seizures in mice. Journal of neural transmission (Vienna, Austria : 1996). 2012;119(8):923-31.

36. Li S, Wang X-P. In vitro and in vivo evaluation of novel NGR-modified liposomes containing brucine. International journal of nanomedicine. 2017;12:5797-804.
37. Ayanwuyi LO, Kwanashie HO, Hussaini IM, Yaro AH. Preliminary Studies on the Behavioural Effects of the Methanol Extract of Leonotis nepetifolia Linn Stem in Mice. African journal of traditional, complementary, and alternative medicines : AJTCAM. 2016;13(4):15-21.