Methanol leaf extract of Cusonea aborea Hochst (Araliaceae) possesses anticonvulsant activity in laboratory animals

Adamu Bello Shuaibu*[1], Hassan Maharazu Yusuf[1], Abdullahi Balarabe Nazifi[1], Abdullahi Hamza Yaro[1], Lawal Alhassan Bichi[1]

[1]Department of Pharmacology and Therapeutics, Bayero University, Kano.
Submitted: 21st Dec., 2023; Accepted: 7th March, 2024; Published online: 30th April, 2024
DOI: https://doi.org/10.54117/jcbr.v4i2.2
**Corresponding Author: Adamu Bello Shuaibu; abshuaibu.pha@buk.edu.ng, adamushuaibu77@yahoo.com; 08030527090*

Abstract

Cusonea aborea is used in the treatment of both infectious and non-infectious diseases. The objective of this study was to investigate the anticonvulsant activity of methanol leaf extract of the plant in experimental animals. Phytochemical screening and acute toxicity studies were conducted. The anticonvulsant activity of the extract at doses of 75, 150, 300 and 600 mg/kg was evaluated in chicks (using maximal electroshock test) and mice (using pentylenetetrazole, 4-aminopyridine, strychnine, picrotoxin and isoniazid-induced) seizures models. Phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins, streroids, anthraquinones and triterpines. Intraperitoneal median lethal dose was estimated to be 2000 and 3800 mg/kg in mice and chicks respectively. The extract offered 40 % protection at 150, 300 and 600 mg/kg against maximal electroshock test. Protection of 50 and 33.33 % was offered at 75 and 150 mg/kg respectively against pentylenetetrazole-induced seizures. At 300 mg/kg, the extract confered 66.67 % protection and significantly (p<0.05) increased the onet of seizures in 4-aminopyridine test. Protection of 83.33 % and significant increase (p<0.05) in the onset of seizure was seen at 300 mg/kg in strychnine-induced seizures. There was significant (p<0.05) increase in the onset of seizures at 300 mg/kg against picritoxin-induced seizures. No protection was offered by the extract against isoniazid-induced seizures at all of the tested doses. The findings revealed that the methanol leaf extract of C*usonea aborea* possesses anticonvulsant activity and this may provide scientific basis for the use of the plant in the treatment of epilepsy.

Keywords: Anticonvulsant, *Cusonea aborea*, Epilepsy, Isoniazid, 4-aminopyridine, Pentylenetetrazole

Introduction

Epilepsy is a chronic neurological condition characterized by abnormal electrical activity in the brain causing recurrent seizures or unusual behavior, sensations and sometimes loss of awareness (WHO, 2023). It is the second most common disorder of the brain after stroke and has no age, racial social, sexual or geographical boundries (WHO, 2017). It affects 0.5-1 % of the world population out of which 80 % live in low and middle income countries (Mittal *et al.,* 2005; WHO, 2023). The etiology of seizure attack includes: brain injury, stroke, serious events before birth, brain tumor, infection (encephalitis, bacterial meningitis, cystercercosis), metabolic abnormalities, neurotoxicity, drug withdrawal and genetic factors (WHO, 2023). Epileptic pateints suffer in most cases from comorbidities such as

depression, anxiety, intellectual disabilities and physical injuries (WHO, 2023).

People with epilepsy and their families are stigmatized and discriminated in most parts of the world leading to human rights violations and social exclusion (Susmita, 2018; WHO, 2023). It poses a significant negative economic effect in terms of health care services costs and loss of working hours which affects productivity (WHO, 2017). Reducing the stigma is a key focus of action for epilepsy patient support organizations across the world; it is rather a more important issue than treating the clinical symptoms of the disease, even though the two are frequently inexorably linked (Fernandes *et al*., 2020).

Currrent treatment options for epilepsy include anti-epileptic drugs (AEDs) and brain surgery (Ahmad *et al.* 2020). However, approximately a quater of patients remain pharmacoresistant (drug-resistant epilepsy) (Bohosova *et al*., 2021). Factors like, side effects (neurotoxicity & dose related and chronic toxicity), teratogenic tendencies, drug interactions and duration of therapy significantly affect drug compliance and overcome therapeutic benefits (Pande *et al*., 2009; Mittal *et al*., 2015; Erkec & Arichan, 2015; WHO, 2023) Thus there is an urgent need to discover newer antiepileptic drugs with greater clinical efficacy, tolerability, minimal side effects and devoid of or limited unfavorable drug interactions. The drug should also be able to effectively tackle the actual epileptic events at molecular level (Ibrahim *et al*. 2008; Landmark and Johannessen, 2008; Alshabi *et al*., 2022). Herbal medicine is accepted worldwide and extensively used in epileptic treatment in many countries, but there is lack of robust evidence for its efficacy and safety (Liu *et al.,* 2017).

Cussonia arborea Hochst. ex A. Rich. is a medium-sized deciduous tree with rough and corky bark. It is widely distributed throughout Africa, from western to central and eastern regions of the continent (Oladele *et al.*, 2017). *Cusonea arborea* is reffered to as *Cabbage tree (English), Hannun kuturu/Gasaya or Takaddan Giwa (Hausa), Ako-sigo (Yoruba) and Burmarlahi* (*Fulfulde*). Tradomedical care has significantly recognized the plant for its use in the treatment of wounds, cancer, malaria, mental illness, seizures, conjunctivitis, sexually transmitted infections, painful menstruation, leprosy, as diuretics in oedema, yellow fever etc. (Burkill, 1985; Kogan *et al*., 2015 and Ken, 2021). *In-vivo* and *in-vitro* studies have discovered that the plant possesses antimicrobial, antibacterial, antihyperglycemic, antiplasmodial, anticancer and immunomodulatory effects (Oladele *et al.*, 2017; Katarzyna *et al.,* 2020). Based on preliminary investigation the folkloric claim on the use of the plant in management of epilepsy has not been validated and this provided the justification for the current study.

Materials and methods

Drugs, chemicals and equipments:

Methanol, strychinine, pentylenetetrazole and picrotoxin (Sigma chemical Co., St. Louis, USA) 4-amino pyridine (Merch-schuchardt, Germany), sodium valproate (Sanofi aventis,UK), phenobarbitone (Lab Renaudin, France), phenytoin (Parker-Davis and Co. Ltd), diazepam (Roche Ltd, France), Electroconvulsive machine (Orchid International EC01 India), analytical balance (Mettler Instrument Corporation, U.S.A.) .

Animals

Swiss albino mice (18-25g) of either sex were obtained from Animal Facility, Department of Pharmacology and Therapeutics, Bayero University, Kano. Day old Ranger cockerels (30-40 g) were obtained from Yammfy chicks Illemona, Kwara State, Nigeria. The animals were kept in a well-ventilated condition at ambient temperature and were fed with a

standard animal feed with adequate access to water *ad libitum*. The experimental animals used were handled in accordance with the Bayero University guidelines for the care and use of laboratory animals (Ref No: BUK/CHS/REC/117).

Collection and identification of plant material

Fresh leaves of *Cusonoea aborea* were collected from Kiru Local Government area, Kano state, Nigeria. It was identified and authenticated by Baha'uddeen Said Adam, a taxonomist with the Department of Botany, Faculty of Life Sciences, Bayero University, Kano, Nigeria by comparing with an already deposited voucher specimen number (BUKHAN 0543) as reference at the Herbarium Section of the department.

Plant preparation and extraction

The leaves of the plant were shade dried at room temperature for two weeks until constant weight was attained. The dried leaves were then grinded into fine powdered form using pestle and mortar. The powdered leaf (2300 g) was extracted with 10 L of 70% v/v methanol (70 % Methanol: 30 % water) for 1 week using cold maceration extraction method with occasional shaking. The extract obtained was then evaporated in a thermostat oven at 50 ºC. The dried extract was weighed and then stored in a desiccator until when needed.

Phytochemical screening

Preliminary phytochemical screening (Sofowora, 1993; Evans, 2009) was conducted on the methanol leaf extract of *Cusonoea aborea* in oder to screen for the presence of secondary metabolites such as alkaloids, cardiac glycosides, saponins, tannins, streroids and anthraquinones.

Acute toxicity study

Median lethal dose (LD_{50}) of methanol leaf extract of *Cusonoea aborea* was determined via intraperitoneal route (*i.p*) in mice using the method described by Lorke (1983). The study was carried out in two phases. In the first phase, nine chicks/mice of either sex were randomly selected and divided into three groups of three chicks/mice per group, which were treated with 10, 100, 1000 mg/kg of the methanol leaf extract of *Cusonoea aborea*. The treated animals were then observed for signs of toxicity including death over a period of 24 hours. In the second phase, three chicks/mice were treated with more specific doses of the extract *i.p* (based on the outcome of first phase study) and also observed for signs of toxicity and death over 24 hours. The LD_{50} was calculated as the geometric mean of the lowest dose that caused death and the highest dosage for which the animal survived.

Anticonvulsant studies

Maximal electroshock (MEST)-induced convulsion test in chicks

Maximal electroshock (MEST) induced convulsion test was conducted using the method described by Swinyard and Kupferberg (1985). Fifty day old cockerels were randomly divided into five groups each containing ten chicks. The first group received distilled water (10 mL/kg) *i.p* and served as a negative control. Groups 2 - 4 received graded doses of *Cusonoea aborea* extract (150, 300 and 600 mg/kg *i.p* respectively) while the fifth group received phenytoin (20 mg/kg, *i.p*) and served as positive control. Thirty minutes later, maximum electroshock was administered to induce seizure in the chicks using Orchid international (EC01) electro convulsive machine connected to a corneal electrodes, placed on the upper eyelids of the chicks after dipping them in normal saline. The current, shock duration, frequency and pulse width were set and maintained at 150 mA, 0.2 s, 50 Hz and 0.6 ms^{-1} respectively throughout the study. Tonic hind limb extension was considered as convulsion. Anticonvulsant

activity was considered as the ability of the extract to prevent features of tonic hind limb extension.

Pentylenetetrazole-induced convulsion test in mice

The method described by Swinyard *et al*., (1989) was employed. Thirty mice were randomly divided into five groups of six mice each. The first group received distilled water (10 mL/kg *i.p.*); groups 2 – 4 were treated with 150, 300 and 600 mg/kg (*i.p*) of *Cusonoea aborea* extract respectively and the fifth group received 200 mg/kg of sodium valproate. Thirty minutes post treatment, mice in all the groups were administered 80 mg/kg body weight of freshly prepared pentylenetetrazole (PTZ) subcutaneously (*s.c.*). Thirty minutes later each mouse was observed for for onset of seizures. Episodes of clonic spasm (loss of righting reflex) was considered as convulsions. The absence of loss of righting reflex during the 30 minutes of observation was regarded as protection against PTZ-induced convulsions.

Strychnine-induced convulsion test in mice

The study was conducted using method described by (Lehmann *et al*., 1988).Thirty mice were divided into five groups of six mice each, the group 1 received distilled water (10 mL/kg body weight *i.p.*); groups 2 – 4 were treated with 150, 300 and 600 mg/kg (*i.p*) of *Cusonoea aborea* extract respectively. Group 5 received 30 mg/kg of phenobarbitone *i.p.* Thirty minutes later, mice in all the groups were treated with 1 mg/kg (*s.c.*) of freshly prepared strychnine. Abolition of tonic extensor jerks of the hind limbs and/or latency of death was considered as protection against strychnine-induced convulsion.

4-aminopyridine-induced convulsion test in mice

The study was performed as described by Yamaguchi and Rogawski (1992). Thirty (30) mice were divided into five groups each containing six mice each. Group 1 served as negative control and was pretreated with normal saline 10 mL/kg (*i.p.*) body weight. Groups 2 – 4 were treated with 150, 300 and 600 mg/kg (*i.p*) of *Cusonoea aborea* extract respectively while Group 5 were pretreated with 30 mg/kg body weight phenobarbitone (*i.p*). Thirty minutes after pretreatment; 4-aminopyridine was freshly prepared and administered at a dose of 14 mg/kg body weight (*s.c.*) to each mouse in all the groups. The mice were observed for 30 minutes for presence or absence of tonic extension as well as onset of seizures.

Picrotoxin-induced convulsion test in mice

The method described by Ogbonnia *et al.* (2003) was employed. Thirty mice were randomly divided into five groups containing six mice in each group. The first group served as control and was pretreated with normal saline 10mL/kg body weight *i.p.* Groups 2 – 4 were pretreated were treated with 150, 300 and 600 mg/kg (*i.p*) of *Cusonoea aborea extract* respectively while group 5 were pretreated with phenobarbitone 30 mg/kg (*i.p.*). Thirty minutes after pretreatments; mice in all groups were treated with freshly prepared picrotoxin (1.2 mg/kg, *s.c*). The mice were then observed for presence or absence of convulsion for 30 minutes.

Isoniazid-induced convulsion test in mice

Isoniazid-induced convulsion test was assessed using the method described by Corda *et al.,* 1982. The mice were divided into 6 different treatment groups (n=6). Group 1 were given distilled water (control, 10 mL/kg), groups 2, 3 and 4 were given graded doses of *Cusonoea aborea extract* (150, 300, 600 mg/kg *ip*) respectively. Group 5 recieved diazepam (5 mg/kg), while group 6 were administered pyridoxine (300 mg/kg). Thirty minutes later, the mice were given isoniazid

(300 mg/kg, *i.p*) and were observed for 1 hour for latency to convulsion and death.

Data analysis

Data obtained were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test using Statistical Package for Social Sciences (SPSS) version 22 software. Values of $p<0.05$ were considered statistically significant. The data were then presented as mean ± the standard error of the mean (S.E.M.).

Results

The preliminary phytochemical screening of the methanol leaf extract of *Cusonea aborea*revealed the presence of alkaloids, cardiac glycosides, saponins, triterpenes, tannins and flavanoids (Table 1).

Table 1: Phytochemical constituents present in the methanol leaf extract of *Cusonea arborea*

Constituents	Inference
Cardiac glycosides	+
Saponins	+
Triterpenes	+
Tannins	+
Steroids	+
Flavonoid	+
Alkaloid	+
Anthraqiunones	+

Key: Present= (+)

Acute toxicity study on methanol leaf extract of *Cusonea aborea*

The intraperitoneal LD_{50} of the methanol leaf extract of *Cusonea aborea* in mice was found to be 2000 mg/kg and 3800mg/kg in chicks.

Effect of methanol leaf extract of *Cusonea aborea* on maximal electroshock test in chicks

The methanol leaf extract of *Cusonea aborea* at all the tested doses (150, 300 and 600 mg/kg body weight) provided 40 % protection against hind limb tonic extension (HLTE) in the maximal electroshock induced convulsion test. While the standard drug phenytoin (20 mg/kg) provided 100 % protection against HLTE. There was significant ($p< 0.01$) delay in the mean recovery period at 150 mg/kg (Figure:1).

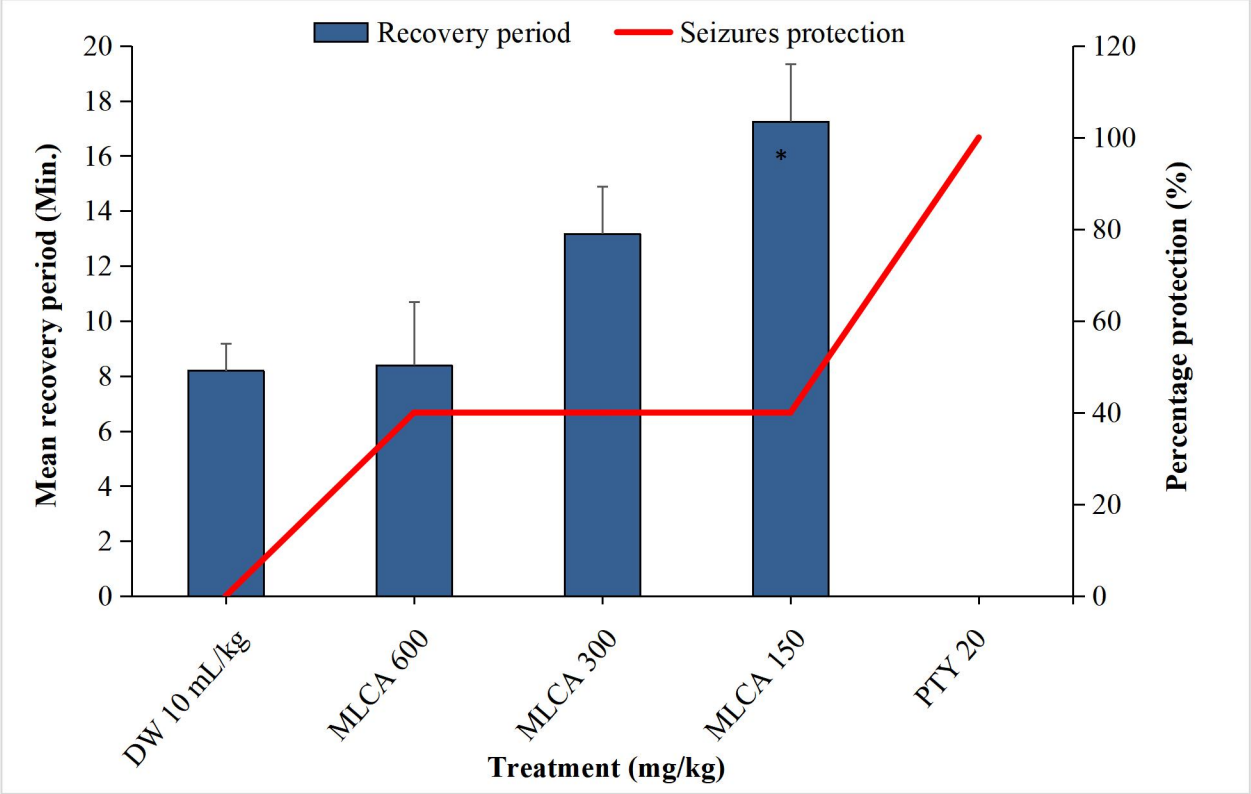

FIG. 1: Effect of methanol leaf extract of *Cusonea aborea* on maximal electroshock test in chicks .

Values are presented as Mean ± SEM, *p<0.01 compared to distilled water control group - One way ANOVA, followed by Dunnet's *post hoc* test, n=10, DW = Distilled water, MLCA = Methanol leaf extract of *Cusonea aborea*, PTY = Phenytoin

Effect of methanol leaf extract of *Cusonea aborea* on pentylenetetrazole-induced seizures in mice

In the PTZ test methanol leaf extract of *Cusonea aborea* offered 33.33 and 50 % protection against tonic clonic seizures at 150 and 75 mg/kg respectively, while 100 % protection was offered in the standard drug (Sodium valproate, 200 mg/kg). The extract (75 mg/kg) significantly (p<0.01) decreased the mean onset of seizure compared to negative control group (Table 2).

Table 2: Effect of methanol leaf extract of C*usonea aborea* on pentylenetetrazole-induced seizure in mice

Treatment Mg/Kg	Mean Onset Of Seizures (Min)	Quantal Protection	% Protection	% Mortality
DW 10 mL/kg	11.20±0.66	0/6	0.00	33.33
MLCA 300	11.75±1.25	1/6	16.67	16.67
MLCA 150	8.33±0.67	2/6	33.33	16.67
MLCA 75	5.50±0.50*	3/6	50.00	0.00

SVP 200	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, * = p<0.01 compared to distilled water control group – One-way ANOVA followed by Dunnet's *post hoc* test, n=6, DW = Distilled water, MLCA = Methanol leaf extract of *Cusonea aborea*, SVP = Sodium valproate

Effect of methanol leaf extract *Cusonea aborea* on 4-aminopyridine-induced seizure in mice

The leaf extract of *Cusonea aborea* (300 mg/kg) protected 66.67 % of the mice against 4-aminopyridine induced seizures. While only 16.67 % of the animals were protected at the doses of 75 and 150 mg/kg. There was significant (p< 0.05) increase in the mean onset of seizures at 75, 150 and 300 mg/kg respectively when compared to distilled water control group (Table 3).

Table 3: Effect of methanol leaf extract of *Cusonea aborea* on 4-Aminopyridine-induced seizure in mice

Treatment (mg/kg)	Mean Onset of Seizures (min)	Quantal Protection	% Protection	% Mortality
D/W10 mL/kg	13.16±0.40	0/6	0.00	100.00
MLCA 300	25.00±1.00*	4/6	66.67	33.33
MLCA 150	18.00±1.41*	1/6	16.67	66.67
MLCA 75	18.50±1.65*	1/6	16.67	83.33
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, * p = <0.05, compared to distilled water control group – One-way ANOVA followed by Dunnet *post hoc* test, n=6, DW – Distilled water, MLCA = Methanol leaf extract of *Cusonea aborea*, PHB = Phenobarbitone

Effect of methanol leaf extract of *Cusonea aborea* on strychnine-induced seizures in mice

The extract offered protection of 66.67 and 83.33 % against strychnine-induced seizures at 150 and 300 mg/kg respectively. There was also significant (p<0.05) increase in the mean onset of seizures at the same doses of 150 and 300 mg/kg when compared to distilled water control group (Table 4).

Table 4: Effect of methanol leaf extract of *Cusonea aborea* on strychnine-induced seizure in mice

Treatment (mg/kg)	Mean Onset of Seizures (min)	Quantal Protection	% Protection	% Mortality
D/W10 mL/kg	8.25±0.25	2/6	33.33	16.67
MLCA 300	11.00±0.00*	5/6	83.33	0.00
MLCA 150	20.00±0.00*	4/6	66.67	0.00
MLCA 75	1.17±0.14	0/6	0.00	100.00
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, * = p<0.05 compared to distilled water control group – One-way ANOVA followed by Dunnet's *post hoc* test, n=6, DW= Distilled water, MLCA= Methanol leaf extract of *Cusonea aborea*, PHB = Phenobarbitone

Effect of methanol leaf extract of *Cusonea aborea* on picrotoxin-induced seizures in mice

Cusonea aborea extract at 75 mg/kg offered 16.67 % and 50 % protection against picrotoxin-induced seizures and mortality respectively. There was significant (p<0.05) increase in the mean onset of seizures at dose of 300 mg/kg compared to negative control group (Table 5).

Table 5: Effect of methanol leaf extract of *Cusonea aborea* on picritoxin-induced seizure in mice

Treatment (mg/kg)	Mean Onset Of Seizures (min)	Quantal Protection	% Protection	% Mortality
D/W10 mL/kg	13.40±0.51	0/6	0.00	0.00
MLCA 300	17.80±1.16*	0/6	0.00	50.00
MLCA 150	12.83±0.60	0/6	0.00	33.33
MLCA 75	12.00±1.10	1/6	16.67	16.67
PHB 30	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM, * p = <0.05 compared to distilled water control group – One-way ANOVA followed by Dunnet's *post hoc* test, n=6, DW – Distilled water, MLCA= Methanol leaf extract of *Cusonea aborea*, PHB = Phenobarbitone

Effect of methanol leaf extract of *Cusonea aborea* on Isoniazid-induced seizure in mice

Administration of the extract at all tested doses did not offer protection against both tonic-clonic seizures and mortality While diazeparm 5 mg/kg produced significant (p<0.05) increase in the latency to seizures when compared to negative control group and together with pyridoxine 300 mg/kg provided 83.33 % protection each against isoniazid-induced seizures (Table: 6).

Table 6: Effect of methanol leaf extract of C*usonea aborea* on isoniazid-induced seizure in mice

Treatment (mg/kg)	Mean Onset of Seizures (min)	Quantal Protection	% Protection	Latency To Death (min)	% Mortality
D/W10 mL/kg	27.17±1.64	0/6	0.00	34.67±1.89	100.00
MLCA 300	31.33±2.51	0/6	0.00	40.83±3.18	100.00
MLCA 150	36.33±2.26	0/6	0.00	36.33±2.26	100.00
MLCA 75	29.67±1.48	0/6	0.00	38.67±2.94	100.00
DZP 5	60.00±0.00*	5/6	83.33	-	0.00
PDZ 300	39.00±0.00	5/6	83.33	-	0.00

Onset of seizures presented as Mean ± SEM, * p = <0.05, compared to distilled water control group – One-way ANOVA followed by Dunnet's *post hoc* test, n=6, DW – Distilled water, MLCA = Methanol leaf extract of *Cusonea aborea*, DZP = Diazeparm, PDZ= Pyridoxine

Discussion

There is an extensive ongoing search for medicinal plants for the treatment of epilepsy due to the unwanted side effects, high cost, and less efficacy of synthetic drugs. This is because plants are considered as cheaper, accessible and devoid of side effects; and thus mostly used by people in the developing countries (Manchishi, 2018). Many medicinal plants prescribed for epilepsy treatment by traditional herbalists have demonstrated an encouraging anticonvulsant activity against stimuli-induced in *vitro* and *in vivo* seizure models (Sucher *et al*., 2015; Kaur *et al*. 2021). The main aim of the present study was to validate the folkloric claim of the use of the leaves of *Cusonea aborea* in the treatment of epilepsy.

Phytochemicals are secondary metabolic compounds present in plants that serve as defensive mechanism against predation by many microorganisms, insects and herbivorous (Vaghasiya, 2011). Various phytoconstituents present in medicinal plants belong to the class of alkaloids, terpenes, triterpenoids, lipids, flavonoids, glycosides, coumarins etc. and they have been documented to possess anticonvulsant activities as reported from previous studies (Jangra *et al*., 2022). These compounds have been reported to be involved in the amelioration of convulsions or act as modulators of the activities of the central nervous system as confirmed by different animal models (He *et al.,* 2021). Their actions are directed on different targets such as synapses, receptors, associated neuronal pathways, ion channels, immune system, inflammatory mediators, and glial cells that are implicated in the occurrence and progression of epilepsy (Mohammed *et al.,* 2017 ; He *et al.,* 2021). Examples of medicinal plants with documented antiepileptic activities include: *Dorema ammoniacum* gum (Motevalian *et al.,* 2017) *Albizia amara* (Sedahmed *et al*., 2021) and *Strychnos spinosa* (Shuaibu *et al.,* 2023).

Acute toxicity study of plant extracts is performed to assess the potential inherent toxicity that may be displayed in a short period of time upon a single dose exposure. It is mostly conducted via the oral route as it is considered as a viable route for accidental human exposure for hazardous substances and it allows for hazard classification of test substances (Ng'uni *et al*., 2018). Evaluation of the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part for assessment of safety of the extract (Ping *et al*. 2013). The results from toxicity studies provides accurate information on potentially relevant adverse effects of the substance (extract) being evaluated (Jordan *et al*., 2010). According to Loomis and Hayes (1996), toxicity of a chemical compound has been categorized on the basis of the quantity necessary to cause harm. These ranges from extremely toxic (1mg/kg or less), highly toxic (1 to 50 mg/kg), moderately toxic (50 to 500 mg/kg), slightly toxic (0.5 to 5 g/kg), practically nontoxic (5 to 15 g/kg) to relatively harmless (more than 15 g/kg). In the present study, the intraperitoneal LD_{50} of the methanol leaf extract of *Cusonea aborea* in mice and chicks was found to be slightly toxic.

Maximal electroshock-induced seizure in laboratory animals represents the grand mal type of convulsion. It is used for pre-clinical evaluation of compounds effective against generalized seizures of the tonic-clonic and partial in nature (Mares and Kubova; 2006; Malami *et al*., 2017; Velisek, 2017; Ahmad *et al*. 2019). The tonic extensor stage of convulsion is selectively eliminated by the compounds that are effective against the aforementioned types of seizure. Hind limb tonic clonic seizures (HLTE) induced by MEST can be antagonized by compounds that block the voltage-depended Na^+ channels

(Ambavadea *et al*., 2009 ; Solliman *et al*. 2016) such as carbamazepine, phenytoin, oxcarbazepine and lamotrigine (Banach and Borowicz, 2015; Yuen and Troconiz, 2015). Therefore the protection provided by methanol leaf extract of *Cusonea aborea* against MEST-induced seizure in chicks in this study indicates that the plant possess anticonvulsant activity.

Pentylenetetrazole is a proconvulsant that exerts its effect by inhibiting or attenuating the activity of gamma amino butyric acid (GABA) at the receptor site (GABA-A) (Bum *et al*., 2010). PTZ-induced clonic seizure is a form of forebrain-controlled seizure in which there is increase in the activity of the main epileptogenic areas (Loscher and Czuczwar, 1985; Swinyard and Kupferberg, 1985 and Manavi *et al*., 2022). The mechanism behind the PTZ-induced seizures involves decrease in the level of gamma-aminobutyric acid (GABA) in the brain cortex. GABA is the major inhibitory neurotransmitter in the mammalian central nervous system and decrease in its activity has been implicated in a the etiopathogenesis of convulsion. This is because GABA mediates the inhibition of neuronal responsiveness and activity by increasing chloride ion conductance through the opening of the chloride ion channels (Corda *et al.,* 1990; Riazi *et al.,* 2004 and Motevalean *et al*., 2017). The model is useful for studying petit mal epilepsy (Nejad *et al.,* 2017). Treatment of this type of epilepsy involves the use of anticonvulsant drugs such as diazeparm, phenobarbitone, valproate, felbamate, gabapentin and clonazepam. These drugs inhibit PTZ-induced seizure by enhancing the action of GABA-receptors, thus facilitating the GABA-mediated opening of chloride channels (Czuczwar and Patsalos, 2001; Greenfield Jr., 2013; Ahmed *et al.,* 2019). The decrease in latency to seizures observed in half of the mice that convulsed at the lower test dose was likely due to factors that have significant pharmacological influences on seizure susceptibility in acute PTZ model such as age, sex and physiologic stress (Yuskaitis *et al*., 2021). The protection offered by the methanol leaf extract of *Cussonea aborea* against PTZ-induced seizures suggests that the plant possesses anticonvulsant activity which is likely via enhancement of GABAergic neurotransmission.. Thus the plant could thus be effective in the pharmacotherapy of petit mal (absence) or myoclonic seizures.

Four (4)-aminopyridine, a non-selective potassium channel antagonist is a powerful pro-convulsant in both man and animals (Kobayashi *et al.,* 2008; Heuzeroth *et al*., 2019). It induces tonic-clonic type of seizures via antagonistic action on potassium channels (Yamaguchi and Rogawski, 1992). Substantial genetic, molecular, physiological and pharmacological studies have provided evidence that supports the role of K^+ channels in the control of neuronal excitability and epileptogenesis (Kobayashi *et al.,* 2008). The main physiological roles of potassium channels are to repolarize membrane potentials that have previously been depolarized by sodium and calcium channel activation and consequently regulate neurotransmitter release (Kobayashi *et al.,* 2008). Potassium channel activators would enhance potassium currents and reduce neuronal excitability; therefore, K^+-channel openers may have potential as antiepileptic drugs (Rogawski and Loscher, 2004). The ability of the methanol leaf extract of *Cussonea aborea* to protect the animals and significantly increase the mean onset of seizures induced by 4-aminopyridine suggest its likely interractions with potssium channels. Therefore modification of K^+ channels, in particular their activation, might be a potential therapeutic target for epileptic seizures.

Picrotoxin is a non-competitive $GABA_A$-receptor antagonist which produces seizures by blocking the chloride ion channels linked to $GABA_A$ receptors, as a result of which prevents the entry of chloride ions into neuronal cells. This leads to decreased GABA transmission and activity in the brain (Lason *et al.,* 2013; Ahmad *et al*., 2019). The GABAergic ionotropic receptors can mediate both pre-and postsynaptic inhibition. Presynaptic inhibition mediated by GABA often leads to inhibition of neurotransmitter release from the excitatory arm (Macdermot, *et al*., 1999). Standard anticonvulsant drugs such as phenobarbitone, sodium valproate, benzodiazepines and the newer antiepileptics such as tiagabine and gabapentin are known to enhance GABAergic neurotransmission by facilitating chloride ion influx through the chloride channels of $GABA_A$-receptors, thus effectively suppressing seizures induced by picrotoxin (Porter *et al*., 1984; Taylor, 1995 ;Waller and Sampson, 2018; Wapa *et al*., 2018; Ahmad *et al*., 2019 and Shuaibu *et al*., 2023). Since the methanol leaf extract of *Cusonea aborea* has been able to increase the latency to seizures and also offer protection in the picrotoxin-induced seizure test, points to an action of the extract is likely to be on GABA-mediated neurotransmission.

Isoniazid (INH) is an antitubercular drug that is commonly associated with recurrent seizures especially at toxic levels (Gokhale *et al*., 2009; Minns *et al.,* 2010). Seizures occur because of pyridoxine (vitamin B6) -induced deficiency which leads to decrease in inhibitory neurotransmitter Gamma Amino Butyric Acid (GABA) resulting into the decrease in threshold for convulsions (Okutur *et al*., 2006). Pyridoxal 5-phosphate is an active form of pyridoxine and it is a co-factor for glutamic acid decarboxylase which is required for GABA synthesis. INH inhibits glutamic acid decarboxylase by binding to pyridoxal 5-phosphate and reduces synthesis of GABA (Tsubouchi *et al*., 2014; Sridhar *et al*., 2012 ; Okutur *et al*., 2006; Sinan *et al*., 2013; Vasu and Saluja, 2006). Deficiency of GABA can therefore manifest itself in form of seizures especialy in acute toxicity (Kukuia *et al*., 2016). The inability of the methanol leaf exttact of *Cusonea aborea* to prolong the latency to seizures and offer protection against isoniazid-induced seizures and mortality suggests that its anti-convulsant action may not likely involve the synthesis of GABA.

Conclusion

The findings from this study revealed that the methanol leaf extract of *Cusonea aborea* possesses anticonvulsant activity in maximal electroshock test, pentylenetetrazole,4-aminopyridine, strychnine and picrotoxin-induced seizure models. This provides scientific evidence for the ethnomedicinal use of the plant in the management of different forms of epilepsy.

Conflict of interest

The authors declare no conflict of interest

Acknowledgement

The authors appreciate the technical assisstance of Mal. Abdussabur Imam and Mal. Abba of the Department of Pharmacology and Therapeutics and Mal. Aminu Department of Pharmacognosy Bayero University Kano.

References

Ahmed AD, Maiha BB, Danjuma NM, Nazifi AB (2019). Methanol leaf extract of *Albizia chevalieri* Harms possesses anticonvulsantactivity in acute and chronic models of epilepsy *Journal of Herbal Drugs*. 10 (1): 1-9,

Ahmad SK, Sani S (2020). Surgical Treatments of Epilepsy. *Semin. Neurol*. 40, 696–707.

Alshabi AM, Shaikh IA, Asdaq SMB (2022).The antiepileptic potential of Vateria indica Linn in experimental animal models: Effect on brain GABA levels and molecular mechanisms. *Saudi Journal of Biological Sciences*, https://doi.org/10.1016/j.sjbs.02.059

Asehinde S, Ajayi A, Bakre A, Omorogbe O, Adebesin A, Umukoro S (2018). Research Paper: Effects of Jobelyn® on Isoniazid-Induced Seizures, Biomarkers of Oxidative Stress and Glutamate Decarboxylase Activity in Mice. *BCN.9*(6), 389-396.

Banach M, Borowicz KK (2015). Effects of chronic lamotrigine administration on maximal electroshock- induced seizures in mice. *CNS and Neurological Disorders - Drug Targets*.14(7):855-862.

Bohosova J, Vajcner J, Jabandziev P, Oslejskova H, Slaby O, Stefania AS (2021). MicroRNAs in the development of resistance toantiseizure drugs and their potential as biomarkers in pharmacoresistant epilepsy. *Epilepsia.* 00:1–16. DOI: 10.1111/epi.17063

Bum EN, Nkantchoua GN, Njikam N, Taiwe GS, Ngoupaye GT, Pelanken MM (2010). Anticonvulsant and sedative activity of leaves of *Senna spectabilis* in mice. *Int. J. Pharmacol.* **6**: 123- 128.

Burkill HM (1985). The useful plant of West Tropical Africa. Vol. 1, Royal Botanic Gardens, Kew.

Corda MG, Costa E, Guidotti A (1982). Specific proconvulsant action of an imidazobenzodiazepine (Ro 15-1788) on isoniazid convulsions. *Neuropharmacology.21*(1), 91-4. [DOI:10.1016/0028- 3908(82)90217-9]

Corda MG, Longoni B, Orlandi M, Biggio G (1990). Decrease in the function of the gamma-aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylenetetrazol to rats. *J Neurochem*. 55(4):1216-1221.

Czuczwar SJ, Patsalos PN (2001). The new generation of GABA enhancers potential in the treatment of epilepsy. *CNS Drugs*. 15(5): 339-350.

Evans WC (2009). Trease and Evans Pharmacognosy.16th ed. Elsevier Health Sciences, ondon, U.K.p. 135-144.

Fernandes LCB, Camara CCC, Soto-Blanco B (2012). Anticonvulsant Activity of Extracts of Plectranthus barbatus Leaves in Mice Evidence-Based Complementary and Alternative Medicine, Article ID 860153, 4 pages

Gokhale YA, Vaidya MS, Mehta AD, Rathod NN (2009). Isoniazid toxicity presenting as
status epilepticus and severe metabolic acidosis. *J Assoc Physicians India*. 57:70–1.

Greenfield Jr LJ (2013). Molecular mechanisms of antiseizure drug activity at GABAA receptors. *Seizure*. 22: 589-600.

He LY, Hu MB, Li RL, Zhao R, Fan LH, He L, Lu, F, Ye X, Huang Yl, Wu CJ (2021). Natural medicines for the treatment of epilepsy: bioactive components. Pharmacology and Mechanism. *Front Pharmacol*. 12(28):604040.

Heuzeroth H, Wawra M, Fidzinski P, Dag R, Holtkamp M (2019). The 4-aminopyridine modelof acute seizures *in vitro* elucidates efficacy of new antiepileptic drugs. *Frontiers in Neuroscience*. 13: 677.

Ibrahim G, Abdulmumini S, Musa KY, Yaro AH (2008). Anticonvulsant activities of crude flavonoid fraction of the stem bark of Ficus sycomorus (moraceae). *Journal of pharmacology and toxicology*. 3(5):351-356

Jangra S, Manjusha A, Budhwar V (2022). Ethno medicinal plants with anticonvulsant activity through GABAergic mechanism-(A review) *Indian Journal of Natural Products and Resources.* 13(3), pp. 274-286.

Jordan SA, Cunningham DG, Marles RJ (2010). Assessment of herbal medicinal products:challenges, and opportunities to increase the knowledge base for safety assessment, *Toxicol. Appl. Pharmacol*. 243 (2) 198–216.

Katarzyna S, Urszula D, Piotr W (2020). Salvinorin A Does Not Affect Seizure Threshold in Mice Department of Animal Physiology and Pharmacology, Maria Curie-Skłodowska University, Institute of Biological Sciences. *Molecules*, 25, 1204; doi:10.3390/molecules25051204

Kaur J, Famta P, Famta M, Mehta M, Satija S, Sharma N, Vyas M, Khatik GL, Chellappan DK., Dua K (2021). Potential anti-epileptic phytoconstituents: an updated review. *J Ethnopharmacol*. 268:113565.

Ken F (2023). Tropical Plant Database. Tropical.theferns.info. <tropical.thefern.info/viewtropical.php?id=Cussonia+aborea>

Kobayashi K, Nishizawa Y, Sawada K, Ogura H, Miyabe M (2008). K^+-Channel openers suppress epileptiform activities induced by 4-aminopyridine in cultured rat hippocampal neurons. *Journal of Pharmacological Sciences*.108: 517-528.

Kogan HJ, Adam K, Gross RE, Rick S, Qian C, Carrie L, Megumi A, Amy L, Noah MW, Sosuke M, Shinich M, Katsunori T, Hiroyuki I, Steven J, Siegel B, Mitsuyuki M (2015). *Journal of Neuroscience*, 35 (49) 16282-16294; DOI:

Kukuia KKE, Ameyaw EO, Woode E, Mante PK, Adongo DW (2016). " Enhancement of inhibitory neurotransmission and inhibition of excitatory mechanisms underlie the anticonvulsant effects of Mallotus oppositifolius," *Journal of Pharmacy & Bioallied Sciences*. 8(3), pp. 253–261.

Landmark CJ, Johannessen SI (2008). Modifications of antiepileptic drugs for improved toleravility and efficacy. Persp Med Chem. 2:21-39

Lason W, Chlebicka M, Rejdak K (2013). Research advances in basic mechanisms of seizures and antiepileptic drug action. *Pharmacology Reports*. 65(4): 787-801.

Lehmann J, Hutchison A, McPherson SE, Mondadori C, Schmutz M, Sinton CM, Tsai C, Murphy DE, Wood PL (1988). A selective and competitive N-methyl-D- aspartate-type excitatory amino acid receptor antagonist. *Journal of Pharmacology and Experimental Therapeutics*; 246: 65-75.

Liu WT, Ge ZP, Yashu L, Jiayin L, Bingjin L (2017). The effects of herbal medicine on epilepsy. *Oncotarget*. 8 (29) pp: 48385-48397.

Loomis TA, Hayes AW (1996). Loomis's Essentials of Toxicology, Academic Press, CA.

Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*. **54:** 275-287.

L€oscher W, Czuczwar SJ (1985). Evaluation of the 5-hydroxytryptamine receptor agonist

8-hydroxy-2-(di-n-propylamino)tetralin in different rodent models of epilepsy, *Neurosci. Lett.* 60 (2) 201–206.

Macdermott AB, Role LW, Siegelbaum SA (1999). "Presynaptic ionotropic receptors and the control of transmitter release," *Annual Review of Neuroscience.* 22 (1), pp. 443–485.

Malami S, Kyari H, Danjuma NM, Ya'u J, Hussaini M (2016). Anticonvulsant properties of methanol leaf extract of *Laggera aurita* Linn. F. (Asteraceae) in laboratory animals. *Journal of Ethnopharmacology.* 191:301-306.

Manchishi MS (2018). Recent advances in antiepileptic herbal medicine, *Curr europharmacol*. **16**, 79-83.

Mares P, Kubova H (2006). Electrical stimulation induced model of seizures and Epilepsy. In Pitkanen A, Schwarzkroin PA, Moshe SL, (Eds). Elsevier Academic Press, USA. pp. 153-159.

Minns Ab, Ghafouri N, Clark RF (2010). Isoniazid-induced status epilepticus in a pediatric
patient after inadequate pyridoxine therapy. *Pediatr Emerg Care*. 26:380–1. https://doi.org/10.1097/PEC.0b013e3181db24b6.

Mittal S, Dixit PV, Chauhan B (2015). Screening model for Antiepileptic and various herbal Sources beneficial an Epilepsy: A review.*ejpmr.* 2(4) p.1-13

Motevalian M, Mehrzadi S, Ahadi S, Shojaii A (2017). Anticonvulsant activity of *Dorema ammoniacum* gum: evidence for the involvement of benzodiazepines and opioid receptors. *Research in Pharmaceutical Sciences*. 12(1): 53-59

Muhammad K, Magaji MG, Danjuma NM, Zezi AU, Gyang SS (2017). Methanol Leaf Extract of *Diospyros mespiliformis* Hochst. offers Protection against Some Chemoconvulsants. *Trop J Nat Prod Res.* 1(3):113-117

Ng'uni T, Klaasen JA, Fielding BC (2018). Acute toxicity studies of the South African medicinal plant. *Galenia africana*. Toxicol Rep.5:813–8.

Ogbonnia SO, Jager AK, Van Staden J, Coker HAB (2 0 0 3). Anticonvulsant Activity of *Schumanniophyton magnificum* Roots extract in mice. West *Afr. J. Pharmacol. Drug Res.* 19 (182): 33-36

Okutur S, Borlu F, Yazici Ç, Paksoy F (2006). Acute Isoniazid Intoxication: Convulsion, Rhabdomyolysis and Metabolic Acidosis. *Turk J Med Sci* 36 (6): 397-9.

Oladele AO, Ibrahim AO, Almas J, Aisha F, Mohammed AM, Muhammad SA (2017). Immunomodulatory activities of isolated compounds from the root-bark of Cussoniaarborea, *Pharmaceutical Biology*. 55(1): 2240-2247,

Philippe G, Angenot L, Tits M, Friederich M (2004). "About the toxicity of some *Strychnos* species and their alkaloids," *Toxicon*. 44(4): pp. 405–416.

Ping KY, Dara I, Chen Y, Sreeramanan S, Sasidharan S (2013). Acute and Subchronic Toxicity Study of Euphorbia hirta L. Methanol Extract in Rats. *BioMed Research International.* Pp 1-14. http://dx.doi.org/10.1155/2013/182064

Rajendra S, Lynch JW, Schofield PR (1997). The glycine receptor. *Pharmacol. Ther*. (73)121–146

Ramesh CG, Michelle AL, Robin BD, Dejan M(2014). Chapter 17 Skeletal muscle toxicity biomarkers. In Biomarkers in Toxicology;

Elsevier: Amsterdam Academic press; pp 291-308

Raza ML, Zeeshan M, Ahmad M, Shaheen F, Simjee SU (2010). Anticonvulsant activity of DNS II fraction in the acute seizure models. *J Ethnopharmacol*.128(3):600-605.

Riazi K, Honar H, Homayoun H, Rashidi N, Dehghani M, Sadeghipour H (2004). Sex and estrus cycle differences in the modulatory effects of morphine on seizure susceptibility in mice. *Epilepsia.* 45(9):1035-1042

Rogawsk MA, Loscher W (2004). The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*. 5:553–564.

Sedahmed AA, Al-Nour MY, Mirghani MH, Abu-Algasim HE, Eltiab FA, Ali AA, Elhadi E, Arbab AH (2021). Phytochemical, in vivo, and in silico Anticonvulsant Activity Screening of *Albizia amara* Leaves ethanolic Extract. *Hacettepe University Journal 0f the Faculty of pharmacy.* 41(1) Pp 9-22.

Shuaibu AB, Nazifi AB, Yaro AH, Bichi LA (2023). Anticonvulsant Activity Of Methanol Leaf Extract Of *Strychnos Spinosa* (Lam.) In Mice And Chicks. *FUW Trends in Science & Technology Journal.* 8 (1) pp. 268 – 276

Sinan U, Tümay UY, Mehmet T, Alparslan K, Aytül T, Gülşen B (2013). Acute isoniazid intoxication: an uncommon cause of convulsion, coma and acidosis. *Tuberk Toraks*. 61(1): 50-53.

Sofowora A (1998). Medicinal plants and traditional medicine in Africa. 2th ed., Spectrum books limited, Ibadan, Nigeria, pp. 101-107 and 150-153.

Soliman GA, Yusufoglu H, Tatli-Çankaya I, Rehab F. Abdel-Rahman RF, Aarabaci Anul SA, Galip AG (2016). The potential anticonvulsant activity of the ethanolic extracts of *Achillea nobilis* and *Momordica charantia* in rats. *Journal of Pharmacy & Pharmacognosy Research*. 4 (3), 107-114

Sridhar A, Sandeep Y, Krishnakishore C, Sriramnaveen P, Manjusha Y, Sivakumar V (2012). Fatal Poisoning by Isoniazid andRifampicin. *Ind J Nephrol.* 22(5): 385-7.

Sucher NJ, Carles MC (2015). A pharmacological basis of herbal medicines for epilepsy. *Epilepsy Behav*. 52:308–18**.**

Susmita S, (2018). Medicinal uses of Plants for Nervous Disorders. *Adv Complement Alt Med*. 3(1).Pp1-6)

Swinyard EA, Kupferberg HJ (1985). Antiepileptic drugs: detection, quantification and evaluation. *Federal Proceedings*. 44: 39-43.

Swinyard EA, Woodhead JH, White HS, Franklin MR (1989). General Principles: Experimentalselection, quantification, and evaluation of anticonvulsants. In:Antiepileptic Drugs, Eds.Levy RH, Mattson B, Melrum Jkand Dreifuss FE 3rd edition (Raven Press. New York), p. 85-103.

Tsubouchi K, Ikematsu Y, Hashisako M, Harada E, Fujisawa HMN (2014). Convulsive Seizures with a Therapeutic Dose of Isoniazid*. Intern Med*. 53: 239-42.

Vaghasiya Y, Dave R, Chanda S (2011). Phytochemical analysis of some medicinal plants from western region of India, *Res J Med Plant*. **5**(5): 567-576.

Velısek L (2017). Models of Generalized Seizures in Freely Moving Animals. New York Medical College, Valhalla, New York. pp. 1-8.

Vasu T, Saluja J (2006). INH Induced Status Epilepticus: Response toPyridoxine. *Indian J.Chest Dis Allied Sci.* 48: 205-6.

Waller DG, Sampson AP (2018). Medical Pharmacology and Therapeutics (5thed). Elsevier, U.K. pp. 311-323.

Wapa LP, Nazifi AB, Malami S (2018). Evaluation of Anticonvulsant Activity of Methanol Leaf Extract of Peristrophe bicalyculata (Acanthaceae) in Experimental Animals *NJPBR*. 3(2):89-95

World Health Organisation and International Bureau for Epilepsy (2017). ILAE/IBE/WHO Global Campaign Against Epilepsy,. Epilepsy A manual for Medical and Clinical Officers in Africa, International LeagueAgainst Epilepsy, 8(5) p123.

World Health Organization (2023). Epilepsy: a public health imperative. Geneva: (https://www. who.int/mental_health/neurology/epilepsy/report_2023/en/, accessed 19 January, 2023).

Yamaguchi SI, Rogawski MA (1992). Effects of anticonvulsant drugs on 4-minopyridine induced seizures in mice. *Epilepsy Research*. 11: 9-16.

Yuen ESM, Troconiz IF (2015). Can pentylenetetrazole and maximal electroshock rodent seizure models quantitatively predict antiepileptic efficacy in humans? *Seizure*. 24:21-27.

Yuskaitis CJ, Rossitto LA, Groff KJ, Dhamne SC, Zhang B, Lalani LK, Singh AK, Rotenberg A, Sahin M (2021). Factors influencing the acute pentylenetetrazole-induced seizure paradigm and a literature review.(RESEARCH ARTICLE) *Annals of Clinical and Translational Neurology. 8(7): 1388–1397*