Journal of Medicinal Herbs
journal homepage:**www.jhd.iaushk.ac.ir**

Anticonvulsant activity of methanol leaf extract of *Combretum hypopilinum* Diels (Combretaceae) in laboratory animals

Alhassan Zakariyya[*1], Yau Jamilu[1], Hussaini Isah Marte[2]

[1]*Pharmacology and Therapeutics, Ahmadu Bello University Zaria;*
Email:elzaky02@gmail.com*

[2]*Pharmacology, university of maiduguri, Nigeria;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *January 22th2021*
Accepted *May 20th2021*

Key words:
- ✓ *Anticonvulsant*
- ✓ *Combretum hypopilinum*
- ✓ *Epilepsy*
- ✓ *Pentylenetetrazole*
- ✓ *Picrotoxin*

ABSTRACT

Background & Aim: The use of plants as medicine is an ancient practice common to all societies especially the African society and this practice continues to exist in developing nations. *Combretum hypopilinum* is harvested from the wild for local use of its gum, medicinal uses and timber, it is one of the eight most widely used plants for management of epilepsy in northern Nigeria. This study aimed at examining the anticonvulsant properties of methanol leaf extract of *Combretum hypopilinum* (MECH) in acute animal models as well as the possible mechanism involved in its anticonvulsant activities. .

Experimental: The LD_{50} of the extract was calculated using OECD 423 starting with the limited dose of 5000mg/kg while the anticonvulsant activity of the extract was examined using maximal electroshock (MES), pentylenetetrazole (PTZ), strychnine and picrotoxin induced seizure models. For investigating the possible mechanism of the extract in PTZ model, flumazenil, naloxone, L-Argenin and sildenafil were administered to interact with the extract.

Results: The LD_{50} of the extract was found to be greater than 2000mg/kg but less than 5000 mg/kg (oral). In maximal electroshock test, the extract protects only 30% of chicks against tonic hind limb extension (THLE) at the dose of 600 mg/kg. However, it significantly ($p<0.05$) and dose dependently decreased the mean recovery time of the convulsed chicks. In PTZ model, the extract at the dose of 600mg/kg, protected mice by 66.66% and significantly ($p<0.005$) delayed the mean onset of seizure. The extract significantly ($p<0.005$) delayed the mean onset of seizure and increased the mean latency of mortality for unprotected mice in both strychnine and picrotoxin models. Flumazenil, naloxone, L-argenine and sildenafil reversed the anticonvulsant activity of MECH.

Recommended applications/industries: The study suggested that MECH possessed bioactive component(s) responsible for its anticonvulsant effect, therefore, justify its use for the management of epilepsy amongst herbalists in Northern part of Nigeria.

1. Introduction

Epilepsy is a spectrum of brain disorder ranging from severe, life threatening and disabling, to ones that are much more benign. In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensation, emotion and behaviour or sometimes convulsion, muscle spasm and loss of consciousness (Okoh-Esene *et al.*, 2013).

It is considered as spectrum disorder because of its different causes, different seizure types, ability to vary in severity, range of co-existing conditions and impact from person to person (Venkateshwarlu *et al.*, 2013).

During an episode, some people may have convulsions (sudden onset of repetitive generalized contraction of muscles) and loss consciousness (Venkateshwarlu *et al*., 2013). In Nigeria, the prevalence of epilepsy, based on different communities, varies from 15-37/1000, however, one of the early publications on the prevalence of epilepsy in Nigeria reported a prevalence of between 8-13/1000 inhabitant in the urban communities of Lagos (Ogunri, 2006). This prevalence rate is lower than what was obtained in most rural African communities and similar to that of western countries. The age distribution among Nigerians appears to be similar to that described among the Caucasians in which 70-85%of patients with epilepsy have onset of seizure below 30 years of age. However, among adult Nigerians, a study reported a mean age of 21 years with dominance of partial seizure (53%) (Ogunri, 2006). It is thought that in most parts of Africa, males readily come to hospital for socio-economic reasons and hence, predominate in the hospital populations. The male sex predominance may also be due to occupational and social exposure to epileptogenic insult such as cranial trauma and alcohol (Ogunri, 2006).

In spite of several advancements in the field of synthetic chemistry, plants continue to be one of the major raw materials for drugs used in treating various ailments of humans. Plants remedies are effective and without many side effects provided they are selected properly (Panvan *et al.*, 2011).

Herbal medicines, rather than merely curing a particular disease, aim at returning the body back to its neutral state of health. The phytochemical components of medicinal plants often act additively and synergistically to improve health (Panvan *et al.*, 2011).

Combretum hypopilinum is a deciduous shrub or small tree growing from 4- 18 metres tall. The plant is harvested from the wild for local use of its gum, medicinal uses and timber. Sub-species geitoxophyllum is the most widely spread occurring in the savannah from Senegal to southern Nigeria. Sub-species binderiantum occurs from Ghana to Nigeria and is widely spread in East Africa and sub-species *hypopilinum* is in Guinea to southern Nigeria across to Sudan and Uganda.It is commonly known as Jar taramniya or jar ganyee in Hausa, buskidaneehi in Fulfulde, katankara in Kanuri and aro in Yoruba language of Nigeria (Burkill, 1985).

The bark yields a gum and it is used to cure toothache or to plug a carious tooth. In Guinea, a leaf-macerate is considered effective in treating diarrhea and as depurative and chalagogue. The leaves are used as a purgative and the roots are boiled and the decoction drunk warm as a treatment for dysentery and snakebites. In Ivory Coast, the leaves are held to be strongly diuretic and of benefit in cases of general oedema. A root decoction is taken for stomachache in the Gambian and the roots are said to have unspecified medicinal use in the Soudano-Guinean region of Africa (Burkill, 1985). It is one of the eight most widely used plants for the management of epilepsy in northern Nigeria (Muazu and Kaita, 2008).

There are many medicinal plants used in the management of epilepsy, some of which have been explored scientifically and their anticonvulsants activities have been established. Like many other medicinal plants, the anticonvulsant activity of Combretum hypopilnium is yet to be established. Hence, its traditional uses in epilepsy among herbalists in Northern Nigeria needs to be scientifically approved.

2. Materials and Methods

2.1. Chemicals/ reagents

Some of the chemicals/reagents that were used include the following: Pentylenetetrazole, Phenobarbitone (sigma Aldrich USA), Picrotoxin (Sigma chemicals India), strychnine (BDH chemicals ltd poole England), Diazepam (Valium$^{(R)}$Rouche Switzerland), Sodium Valproate (Epilim$^{(R)}$ Sanofi) Phenytoin (Sigma chemicals India), L-arginine, sildenafil (Viagra$^{(R)}$ Pfizer USA), normal saline, methanol (Sigma-Aldrich, St.Louis U.S.A.), Flumazenil, Naloxone((BIOMOL Research Lab PA. U.S.A).

2.2. Plant extraction

The leaves of *Combretum hypopilinum* plant were air dried under shade and were crushed into a coarse powder with the aid of a mortar and pestle. A portion (500g) of the powdered leaves was extracted with 1 Litre of absolute methanol for 8 hours using Soxhlet method of extraction. The filtrate was collected in a round bottom flask where it was decanted into an evaporating dish and evaporated to dryness over water bath maintained at about 50˚C. The dried methanol leaf

extract of *Combretum hypopilinum* was stored in an airtight container. The solutions of the extract were always freshly prepared for each study by dissolution of the appropriate amount required in deionized water under standard laboratory conditions.

2.3. Experimental animals

The pharmacological experiments were conducted using adult Swiss Albino mice of both sexes (20-26 g) obtained from the Animal House of Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were housed in well ventilated cages, fed with their normal feed and water *ad libitum*; and maintained under standard laboratory conditions in accordance with the protocols approved by the University Ethical Committee on use and care of experimental animals.

Day-old chicks were obtained immediately before the experiment and they were handled and maintained on a Standard Animal Feeds obtained from Vital Feeds (Kaduna, Nigeria) and fed with food and water *ad libitum* under standard laboratory conditions. All experiments performed on these animals were in accordance with the protocols approved by the University Ethical Committee on use and care of experimental animals with the approval number ABUCAUC/2021/049.

2.4. Preliminary phytochemical Studies

Preliminary phytochemical screening of the methanol leaf extract of *Combretum hypopilinum* was carried out according to the methods described by (Evans, 2002; Sofowora, 1993).

2.5. Elemental analysis

The dried powdered leaves of *Combretum hypopilinum* were placed in a weighing crucible and ashed at 500 ˚C in a hot spot furnace for three hours. The ash material was prepared for the determination of trace element. A portion of zero point (0.5 g) of the ashed sample was digested by heating for two minutes with a mixture of 10mL each of nitric acid (HNO_3), HCl and perchloric acid in a 500 mL flask. The aliquot obtained from this mixture by filtration was mixed with a 10 ml of 2M HNO_3 and 30 ml of distilled water in a 100mL volumetric flask. The volume was made up to 100ml mark with distilled water. Blank sample and standard solution for the various elements were similarly done. All samples were placed in a plastic container and stored in a refrigerator maintained at 4 ˚C prior to analysis. Flame emission spectrometer (Model FGA-330L; Gallenkamp, Weiss, UK) was used to determine the presence of essential element.

2.6. Acute toxicity studies

The median lethal dose (LD_{50}) was investigated in both mice and chicks using the OECD guideline 423 (1996), starting with a limited dose of 5000 mg/kg body weight (as recommended by the guideline) of test animals since previous literatures indicate that the plant is relatively non-toxic.

The study was divided into two phases; in the first phase, three mice and three chicks were randomly selected and were fasted for 3-4 hours. The extract (5000 mg/kg) was administered to each mouse and chick base on the fasted body weight, food was withheld for further 1-2 hours after administration of the extract. In the second phase, three mice and three chicks were also randomly selected and the extract was administered to each mouse and chick at the dose of 2000 mg/kg based on their fasted body weight, food was withheld for further 1-2 hours after administration of the extract. The animals (mice and chicks) were observed individually for signs of toxicity at least once during the first 30 minutes and periodically during the first 24 hours with special attention given during the first 4 hours for 14 days.

The median lethal dose (LD_{50}) was calculated according to the guideline as the percentage of the highest non-toxic dose (the dose that at least two animals survived) used in the experiment.

2.7. Maximal electroshock - induce seizure test (MEST)

The method of Swinyard and Kupferberg (1985) was adopted. Fifty chicks of both sexes were randomly divided into five groups (ten chicks each). Group I received distilled water (10 ml/kg i.p), group II, III and IV received 600 mg/kg, 300mg/kg and 150mg/kg of the extract respectively while group V received phenytoin (20 mg/kg i.p). Forty five minutes later, MES was delivered to each chick to induce seizure using electroshock machine by placing the corneal electrode to the upper eyelid of each chick. A current of 80mA, 100Hz pulse frequency, 0.6 ms pulse width and 0.6 s stimulus duration were set and maintained throughout the study to produce tonic seizures. The number of

animals protected from tonic hind limb extension seizure (i.e. abolition of tonic hind limb extension within 10 s after delivery of the electroshock was considered as protected mouse) and the recovery time in unprotected animals was recorded in each dose group.

2.8. Pentylenetetrazole (PTZ)-induce seizure

The method of Swinyard*et al* (1989) was adopted in this study. Thirty mice of both sexes were randomly divided into five groups (six mice each). Group I received distilled water (10 ml/kg i.p), group II, III, and IV received 600 mg/kg, 300 mg/kg and 150mg/kgof the extract, respectively, while group V received sodium valproate (200 mg/kg i.p). Forty five minutes later, each group was treated with a convulsive dose of PTZ (70 mg/kg i.p) and was observed for thirty minutes. The absence of clonic spasm indicates the extract's ability to protect the mice from the effect of PTZ-induce seizure.

2.9. Strychnine-induce seizure

The method of Porter et *al*. (1984) was adopted. Thirty mice of both sexes were divided into five groups (six mice each). Group I received distilled water (10ml/kg i.p), group II, III and IV received 600mg/kg, 300mg/kg and 150mg/kgof the extract respectively while group V received phenobarbitone (20mg/kg i.p). Forty five minutes later, the mice in each group were treated with strychnine (1mg/kg i.p). Abolition of tonic extensor jerks of hind limbs was considered as protection from strychnine-induced seizure.

2.10. Picrotoxin-induced seizure

The method of Yagamuchi and Rogawaski (1992) was adopted. Thirty mice were randomly divided into five groups of six mice each. Group I served as negative control and received distilled water (10 ml/kg i.p). Group II, III, and IV received 600 mg/kg, 300 mg/kg and 150 mg/kg of the extract, respectively, while group V received phenobarbitone (20 mg/kg i.p). Forty five min later, each group received a solution of picrotoxin (4 mg/kg i.p) and was observed for the presence or absence of tonic hind limb extension within 30 minutes period. Prolongation of the latency of tonic hind limb extension was also considered as indication of anticonvulsant activity.

2.11. Interaction studies

2.11.1. Effect of flumazenil on anticonvulsant activity of methanol leaf extract of Combretum hypopilinum in PTZ induced seizure.

In this study, five groups of six mice were randomly selected. Group I were treated with distilled water (10ml/kg i.p), group II and V received the extract (600 mg/kg p.o) and Diazepam (0.5mg/kg) respectively. Group III and IV received flumazenil (5 mg/kg) 15 min before the administration of the extract (600 mg/kg p.o) and Diazepam (0.5 mg/kg), respectively. Forty fiveminutes later, PTZ (70mg/kg i.p) was administered to each group and were assessed for onset and latency of clonic convulsion.

2.11.2. Effect of naloxone on anticonvulsant activity of methanol leaf extract of Combretum hypopilinum in PTZ induced seizure

In this study, five groups of six mice each were randomly selected. Group I were treated with distilled water (10ml/kg i.p), group II and V received the extract (600 mg/kg p.o) and sodium valproate (200mg/kg), respectively. Group III and IV received naloxone (0.3 mg/kg) 15min before the administration of the extract (600 mg/kg p.o) and sodium valproate (200 mg/kg) respectively. Forty five minutes later, PTZ (70 mg/kg i.p) was administered to each group and were assessed for onset and latency of clonic convulsion.

2.11.3. Effect of methanol leaf extract of Combretum hypopilinum in NO-cGMP pathways in PTZ induced seizure

In this studies, seven groups of six mice each were randomly selected. Group I were treated with distilled water (10 ml/kg i.p), group II and III received the extract (600 mg/kg p.o) and sodium valproate (200mg/kg), respectively. Group IV and V received L-argenine (NO precursor) with the concentration of 50 mg/kg, 15 min before the administration of the extract (600mg/kg) and sodium valproate (200mg/kg), respectively. Group VI and VII received sildenafil (phosphodiesterase-5 inhibitor) with the concentration of 5 mg/kg, 15 min before the administration of the extract (600 mg/kg) and sodium valproate (200 mg/kg), respectively. Forty five minutes later, PTZ (70 mg/kg i.p) was administered to each group and were assessed for onset and latency of clonic convulsion.

2.12. Data presentation and analysis

Results were expressed as the mean ± standard error of mean (SEM) and percentages in the form of tables and charts. Statistical analysis was carried out using one way analysis of variance (ANOVA) which was followed by Bonferoni post hoc test where the result is statistically significant. Significant differences between means were assessed at 95% level of significance i.e. P-value less than or equal to 0.05 ($p \leq 0.05$) was considered significant.

3. Results and discussion

The extraction of the powdered leaf of *Combretum hypopilinum* with 90% methanol afforded a yield of 8.4% w/w.

Preliminary phytochemical screening of the methanol leaf extract of *Comcbretum hypopilinum* revealed the presence of alkaloids, flavonoids, saponins, steroids/ terpenoids, and tannins (Table 1).

Preliminary elemental analysis of the methanol leaf extract of *Comcbretum hypopilinum* revealed the presence of sodium, magnesium, aluminum, chlorine and calcium. Some of which are implicated in seizure (Table 2).

Table 1. *Phytochemical* constituents of the methanol leaf extract of *Comcbretum hypopilinum*

Constituents	Inference
Alkaloids	+
Anthraquinones	-
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Cardiac glycosides	+
Triterpenes	+

Table 2. Elemental composition of the methanol leaf extract of *Comcbretum hypopilinum*.

Element	Inference	Mean concentration (µg/g)
Magnesium	+	920.33 ± 1.24
Alminium	+	101.33 ± 1.31
Phosphorus	+	204.00± 1.51
Sulphur	+	378.00± 0.81
Chlorine	+	447.33± 1.00
Iron	+	37.62± 1.42
Calcium	+	131.62±0.28
Copper	_	0.00± 0.00
Zinc	_	0.00± 0.00
Sodium	+	23.88± 1.40

Data presented as Mean ± SD, + = Presence - = Absence

The curative purposes of medicinal plants are often attributed to their secondary metabolites such as alkaloids, glycosides, essential oil, flavonoids and tannins etc. (Hameed *et al.*, 2015). The preliminary phytochemical screening of the methanol leaf extract of *Combretum hypopilinum* revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, carbohydrates, and cardiac glycosides which are known to be responsible for various pharmacologic activity. However, triterpenic steroids and saponins have been reported to possess anticonvulsant activity in experimental seizure models such as MEST and PTZ (Kasture *et al.*, 2002).

Essential elements (Fe, Cu, Zn etc) and non-essential elements (Pb, Ni, Cd, etc) influence biochemical processes (metabolism) in the human body. The elemental analysis of the extract revealed the presence of Mg, Al, Ca, Cl, Na, Fe, Pb, Cd and Chromium. It is been reported that hypocalcemia and hypomagnesemia lead almost exclusively to CNS irritability clinically manifesting with seizures, whereas disorders of potassium rarely produce symptoms in the CNS, with muscle weakness being their major clinical manifestation (Nardone *et al.*, 2016). The presence of these trace elements (Mg and Ca) might contribute to the anticonvulsant action of the extract.

3.1. Acute toxicity studies

Determination of mean lethal dose of plant extract using acute toxicity studies provides information regarding the margin of safety of the extract in a biological system which is very important for traditional medicine practitioners. The oral mean lethal dose (LD_{50}) value of methanol leaf extract of *Combretum hypopilinum* in both mice and chickswas found to be less than 5000 mg/kg but greater than 2000 mg/kg according to OECD guideline 423. This suggests that the plant is relatively non-toxic and safe at given doses.

3.2. Effect of methanol leaf extract of Combretum hypopilinum (MECH) on maximal electroshock induced seizure in chicks

At the doses of 300 mg/kg and 150 mg/kg, MECH did not offer significant protection against tonic hind limb extension induced by maximal electroshock in all animals (Table 3). Only 30% of the chicks were protected by the highest dose of MECH (600 mg/kg). In contrast to MECH, phenytoin at a dose of 20 mg/kg, completely protected the chicks from tonic hind limb extensions. However, MECH produced a significant

(P<0.05) and dose dependent decrease in the mean recovery time in all treated groups as compared to the control group with the highest dose (600mg/kg) having the least recovery time (3.7 ±0.35).

Table 3. Effect of methanol leaf extract of *Combretum hypopilinum* on maximal electroshock induced seizure in chicks.

Treatment (mg/kg)	Mean recovery time (min)	Quantal protection	Percentage protection (%)
Control	9.7 ± 0.83	0/10	0
MECH (600)	3.7 ± 0.35**	3/10	30
MECH (300)	6.1 ± 0.20*	0/10	0
MECH (150)	6.6 ± 0.30*	0/10	0
PHY (20)	---	10/10	100

Data presented as Mean ± SEM, n = 10; * p ≤ 0.05; ** p ≤ 0.001 compared to control; (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison), Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum*; PHY = Phenytoin.

Maximal electroshock is a model of acute seizure and one of the gold standards in early stage of antiepileptic drug screening and probably the best validated preclinical test to predict effectiveness of drugs against Grand mal seizure (Sarma and Bhattacharyya, 2014). The activity of methanol leaf extract of *Combretum hypopilinum* against MES was demonstrated by the extract's ability to significantly and dose dependently decrease the mean recovery time of seizure and also protect some of the chicks against seizure. Like phenytoin which is the standard treatment in this experiment that offered 100% protection, the extract is said to have a compound that interacts with voltage gated sodium channel thereby stabilizing neuronal membranes by preventing depolarization. Therefore, it is likely to have effect against generalized tonic-clonic seizures as well as preventing seizure spread.

3.3. Effect of methanol leaf extract of Combretum hypopilinum on pentylenetetrazole induced seizure in mice

The methanol leaf extract of *Combretum hypopilinum* offered protection against seizure induced by pentylenetetrazole (70mg/kg). At the dose of 600 mg/kg and 150 mg/kg the percentage protection against seizure was found to be 66.66% and 16.66%, respectively, while no protection was offered at the dose of 300mg/kg. The extract at the dose of 600 mg/kg showed a significant increase (p<0.001) in the mean onset of seizure and the mean latency of mortality compared to the control group. However, at the dose of 300 mg/kg and 150 mg/kg, MECH did not produce significant increase in the mean onset of seizure and mean latency of mortality when compared with the control group. Valproate, which is the standard drug used, protected all animals against pentylenetetrazole induced seizure (Table 4).

Table 4. Effect of methanol leaf extract of *Combretum hypopilinum* on pentylenetetrazole induced-seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Mean latency of mortality (min)
Control	4.66 ± 0.21	16.66	6.00 ± 0.44
MECH (600)	8.16 ± 0.30*	66.66	9.50 ± 2.50
MECH (300)	5.66 ± 0.61	0.00	7.50 ± 0.67
MECH (150)	4.66 ± 0.33	16.66	7.00 ± 0.70
VAL (200)	---------	100	---------

Data presented as Mean ± SEM, n = 10; * p≤ 0.05; ** p ≤ 0.001 compared to control; (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum*; Val = Valproate;

Pentylenetetrazol (PTZ) is often used experimentally in search of newer antiepileptic drugs (AEDs) to induce seizures in animals. It is a noncompetitive antagonist that blocks GABA-mediated Cl^- influx through an allosteric interaction in the Cl^- channel, thus leading to neuronal membrane depolarization, propagation and maintenance of seizure activity (Ziyaurrahman and Jayvadan, 2012). Defects in GABA neurotransmission are linked to epilepsy in both experimental animal models and human syndromes (Ambavade *et al.*, 2009). Drugs such as barbiturates, benzodiazepines, felbamate and valproate that enhance $GABA_A$ receptor-mediated inhibitory neurotransmission can also block PTZ-induced clonic seizures. This study revealed that administration of MECH and valproate has increased the onset of myoclonus and clonic seizures in mice.

Since Valproate exact its anticonvulsant activity by inhibiting neuronal hyperexcitability via enhancement of GABA mediated inhibition, this blocking effect of MECH suggest that its anticonvulsant action may be due to its interference with GABAergic system and might be a potential agent in the management of petit mal (absence) seizures (Rang *et al.*, 2005).

3.4. Effect of methanol leaf extract of Combretum hypopilinum on strychnine induced seizure in mice

The methanol leaf extract of *Combretum hypopilinum* and the standard drug (phenobarbitone) offered protection against seizure induced by strychnine (1mg/kg). At the dose of 600 mg/kg and 300 mg/kg the percentage protection against seizure was found to be 66.66% and 16.66%, respectively, while no protection was offered at the dose of 150 mg/kg. The highest dose of the extract (600 mg/kg) and phenobarbitone (20 mg/kg) showed a significant increase (P<0.001) in the mean onset of seizure and the mean latency of mortality compared with the control group. While at the dose of 300 mg/kg and 150 mg/kg, the extract increased the mean onset of seizure and mean latency of mortality which was not statistically significant ($P>0.05$) compared with the control (Table 5).

Table 5. Effect of methanol leaf extract of *Combretum hypopilinum* on strichynine induced-seizure in mice.

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Quantal protection	Mean latency of mortality (min)
Control	3.00 ± 0.00	0	0/6	3.00 ± 0.00
MECH (600)	6.83 ± 0.70*	33.33	2/6	8.83 ± 1.27*
MECH (300)	4.83 ± 0.60	16.66	1/6	4.83 ± 0.60
MECH (150)	4.50 ± 0.42	0	0/6	5.83 ± 0.47
PHB (20)	12.83 ± 0.65**	50	3/6	17.16 ± 0.60**

Data presented as Mean ± SEM, n = 6; * P ≤ 0.05; ** P < 0.001 compared to control; (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum;* PHB = Phenobarbitone.

Strychnine is an alkaloid with a strong convulsant activity that excites the CNS by specifically antagonizing the inhibitory neurotransmitter amino acid (glycine) at postsynaptic receptors. Inhibitory glycine receptors are abundant in the spinal cord and brain stem where they are mainly involved in regulation of motor functions (Krieger, 2001). This study revealed that the extract significantly increase the onset of seizure and latency of mortality which suggest the possible involvement of glycinergic pathway in the mechanism of anticonvulsant action of MECH.

3.5. Effect of methanol leaf extract of Combretum hypopilinum on picrotoxin induced seizure in mice

One-way ANOVA which was followed by Bonferroni post hoc test showed that the extract at the dose of 600 mg/kg and 300 mg/kg produced a significant increase (P<0.001) in the mean onset of seizure and mean latency of mortality as compared with the control group (Table 6). However, the extract at the dose of 150 mg/kg increased the mean onset of seizure and mean latency of mortality which was not statistically significant (P<0.05) when compared with the control group. The extract did not offer any significant percentage protection against mortality as compared with phenobarbitone group which offered 100% protection.

Table 6. Effect of methanol leaf extract of *Combretum hypopilinum* on picrotoxin-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Quantal protection	Mean latency of mortality (min)
CONTROL	7.66 ± 0.21	0	0/6	18.66 ± 0.42
MECH (600)	17.66 ± 2.06**	16.67	1/6	25.00 ± 0.83*
MECH (300)	14.66 ± 3.32*	0	0/6	24.50 ± 3.33*
MECH (150)	10.4 ± 3.50	0	0/6	19.80 ± 1.52
PHB (20)	0.00 ± 0.00	100	6/6	0.00 ± 0.00

Data presented as Mean ± SEM, n = 6; * P ≤ 0.05; ** P < 0.001 compared to control; (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum;* PHB = Phenobarbitone;

In mammals, it has been shown that picrotoxin blocks GABA presynaptic inhibition and strychnine-resistant postsynaptic inhibition in the central nervous system (Kumar and Singh, 2016). Picrotoxin

selectively antagonizes the effects of the predominant inhibitory transmitter, gamma-aminobutyric acid (GABA), at all levels of the central nervous system (Kumar and Singh, 2016). The convulsant action of picrotoxin is via blockade of $GABA_A$ receptor-linked chloride ion channel, which normally opens to allow increased chloride ion conductance following the activation of $GABA_A$ receptors by GABA (Nicoll, 2001; Velíšek, 2006). Data from this study showed that methanol leaf extract of *Combretum htpopilinum* exhibited anticonvulsant activity against picrotoxin induced seizure by significantly and dose dependently increasing the mean onset of myoclonic jerk and seizure as well as the mean latency of mortality.

3.6. Effect of flumazenil on anticonvulsant activity of methanol leaf extract of Combretum hypopilinum on PTZ-induced seizure in mice

Flumazenil at a dose of 5mg/kg produced a significant reduction ($P<0.001$) in the mean onset of seizure when compared with the groups that were treated with extract (MECH 600mg/kg) andDiazepam (0.5mg/kg) only. It also abolished the protective effect of diazepam against mortality. There was no significant decrease ($P>0.05$) in the mean latency of mortality when compared with the group that received the extract only (Table 7).

Effect of naloxone on anticonvulsant activity of methanol leaf extract of Combretum hypopilinum on PTZ-induced seizure in mice

The extract at the dose of 600mg/kg produced a significant increase ($P<0.05$) in the mean onset of seizure when compared with the control group. Pre-treatment with naloxone significantly ($P<0.05$) reversed this anticonvulsant effect of the extract which was observed as a decrease in the mean onset of seizure ($p<0.001$) by comparing the two groups (Table 8).

3.7. Effect of L-argenine and sildenafil on anticonvulsant activity of methanol leaf extract of Combretum hypopilinum on PTZ-induced seizure in mice

Pre-treatment with L-Arginine (50mg/kg) and Sildenafil (5 mg/kg) produced a significant decrease in the mean onset of seizure and the mean latency of mortality when compared with the group receiving the extract (600 mg/kg). Valproate (standard drug) at the dose of 200 mg/kg protected all the animals against PTZ-induced seizure but pre-treatment with L-Arginine (50 mg/kg) and Sildenafil (5 mg/kg) reduced the effect of valproate by increasing the susceptibility of brain tissue to PTZ-induced seizure causing animals to convulse leading to mortality (Table 9).

Table 7. Effect of flumazenil on anticonvulsant activity of methanol leaf extract of *Combretum hypopilinum* on PTZ-induced seizure in mice.

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Quantal protection	Mean latency of mortality (min)
Control	5.00 ± 0.37	0	0/6	8.00 ± 0.57
MECH (600)	11.60 ± 0.74*	50	3/6	17.80 ± 0.5*
FLU + MECH (600)	6.20 ± 0.58a	33.33	2/6	11.20 ± 1.00
DIA (0.5)	16.20 ± 0.80**	83.33	5/6	--------
FLU + DIA (0.5)	11.80 ± 0.58 b	66.66	4/6	15.60 ± 0.00*

Data presented as Mean ± SEM, n = 6; * $p \leq 0.05$; ** $p \leq 0.001$ compared to control, a : $p \leq 0.01$ compared to MECH, b : $p \leq 0.05$ compared to DIA (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum*; DIA = Diazepam; FLU= Flumazenil

Table 8. Effect of naloxone on anticonvulsant activity of methanol leaf extract of *Combretum hypopilinum* on PTZ-induced seizure in mice.

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Quantal protection (%)	Mean latency of mortality (%)
Control	3.20 ± 0.2	0	0/6	3.80 ± 0.37
MECH (600)	8.20 ± 0.37*	66.66	4/6	5.20 ± 2.57
NAL + MECH (600)	5.00 ± 0.44a	16.66	1/6	5.60 ± 1.53
VAL (200)	——	100	6/6	0.00 ± 0.00
NAL + VAL(200)	9.60 ± 0.50	66.66	4/6	14.73 ± 0.56

Data presented as Mean ± SEM, n = 6; * $p \leq 0.05$ compared to control, a : $p \leq 0.05$ compared to MECH (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum*; VAL = Valproate; NAL= Naloxone.

Table 9. Effect of L-argenine and sildenafil on anticonvulsant activity of methanol leaf extract of *Combretum hypopilinum* on PTZ-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Percentage protection (%)	Quantal protection	Mean latency of mortality (%)
CONTROL	3.20 ± 0.2	0	0/6	3.80 ± 0.37
MECH (600)	10.60 ± 0.24*	66.66	4/6	14.20 ± 3.76
VAL (200)	——	100	6/6	0.00 ± 0.00
LARG + MECH (600)	5.80 ± 0.20a	50	3/6	11.00 ± 1.00*
LARG + VAL(200)	13.60 ± 0.50	83.33	5/6	16.30 ± 0.40
SIL + MECH (600)	6.40 ± 0.24 a	50	3/6	5.60 ± 1.43
SIL + VAL(200)	12.20 ± 0.20	83.33	5/6	14.00 ± 0.26

Data presented as Mean ± SEM, n = 6; * p≤ 0.05 compared to control, a: p ≤ 0.05 compared to MECH (One way ANOVA followed by Bonferroni Post hoc test for multiple comparison) Control = Distilled water; MECH = Methanol extract of *Combretum hypopilinum*; VAL = Valproate; NAL= Naloxone; LARG= L-Arginine; SIL= Sildenafil

Pre-treatment with flumazenil antagonized the anticonvulsant effect of MECH and diazepam by decreasing the onset of myoclonic jerk and clonic seizures induced by PTZ, suggesting further the involvement of the GABAA-BDZ receptor complex in the anticonvulsant activity of the extract. This result is similar to some previous findings (Rashidian *et al.*, 2017, Nassiri and Schwann, 2007, Ya'u *et al.*, 2014) that reported flumazenil blocked the anticonvulsant activity of plant extract known to have potential anticonvulsant properties.

It is well established that central opioidergic system possesses seizure-modulating properties. Several investigations have suggested that low doses of morphine (μ-opioid receptor agonist) have an anticonvulsive effect, while in high doses, it increase the seizure susceptibility induced by chemoconvulsant drugs such as picrotoxin and PTZ (Nejad *et al*., 2017). Also anticonvulsant activity of Kappa receptor agonists has been well established in previous studies that revealed their effectiveness against PTZ, bicuculline and MES induced seizures. They also attenuate the kindling effect produced by repeated administration of PTZ. To investigate the possible involvement of opioidergic system in the anticonvulsant action of MECH, naloxone (an opioid antagonist) was used and it was found that naloxone significantly decreased the onset of myoclonus and clonic seizures. This finding is in line with other reports that revealed the ability of naloxone to decrease the anticonvulsants effect of other compounds (Ramin *et al.*, 2015, Rashidian *et al*., 2017, Nassiri and Schwann, 2007). Therefore, this study may confirm the possible involvement of opioidergic system in the mechanism of anticonvulsant action of MECH.

Administration of L-arginine enhanced seizure susceptibility through excessive release of NO in chemical seizure models induced by GABA antagonists. NO is a gaseous free radical, which is synthesized from the amino-acid L-arginine by nitric oxide synthase (NOS) and function as a neuronal messenger and as a modulator of neurotransmitters in the brain. It is a potent stimulator of guanylyl cyclase, resulting in an increased levels of cGMP.This effect is likely a consequence of hyperexcitability caused by NO-induced cGMP synthesis (Riazi *et al.*, 2006). Several lines of evidence suggested NO as a modulator of seizure activity with diverse proconvulsant effects based on the type of seizure, source of NO and other neurotransmitter systems involved (Hosseini *et al.* 2006).

Pre-treatment with L-arginine at the dose of 50mg/kg decreased the anticonvulsant activity of MECH and Valproate. This is consistent with various reports where L-arginine (NO donor) decreased the antiepileptic effect of compounds in PTZ model of epilepsy (Adongo, 2015). This further confirms the possible involvement of the nitric oxide pathway in the anticonvulsant effect of MECH.

The important role of NO in modulation of seizure threshold raises the hypothesis that sildenafil may affect seizure susceptibility through NO-dependent mechanisms according to some literatures (Adongo, 2015). Sildenafil is a selective phosphodiesterase 5 (PDE5) inhibitor used in the treatment of erectile dysfunction. Intra-cellular cGMP concentrations are also regulated not only by soluble guanylate cyclase (sGC), but also by PDE5 (Adongo, 2015). PDE5 is connected with the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway and catalyzes hydrolysis of both cGMP and cAMP to GMP and AMP respectively. Its inhibition causes an increase in intracellular cGMP level, which leads to smooth muscle relaxation and improves blood flow to the penis (Uthayathas *et al.*, 2007). It is also found in other

tissues, including lungs, all types of smooth muscle, and several brain structures. The ability of sildenafil to cross the blood–brain barrier andthe presence of PDE5 in different brain areas suggest that this drug may exert somecentral actions (Nieoczym *et al.*, 2010). This study revealed that Sildenafil decrease anticonvulsant effect of MECH and valproate by decreasing the mean onset of myoclonus and the mean onset of clonic seizure. This may also confirm the possible involvement of NO-cGMP pathway in the mechanism of anticonvulsant action of MECH. Other reports showed Sildenafil reduces the threshold of PTZ and bicuculline- induced clonic seizures of some extract and other classical AEDs also support this study (Riazi *et al.*, 2006; Nieoczym *et al.*, 2010).

4. Conclusion

The results of the present study showed that the methanol leaf extract of *Combretum hypopilinum* possessed anticonvulsant property which might be the rationale for its common use among traditional medicine practitioners in northern Nigeria for the treatment of epilepsy. It was further shown that the anticonvulsant activity of methanol leaf extract of *Combretum hypopilinum* may be due to involvement of GABAergic, opioidergic and NO-cGMP pathways.

5. References

Adongo, D., Mante, P. and Woode, E. 2017. Anticonvulsant effect of antiaristoxicaria aqueous extract: Investigation using animal models of temporal lobe epilepsy. *BMC Research Notes.* 10(1) 22-33 .

Ambavade, S.D., Mhetre, N.A., Muthal, A.P. and Bodhankar, S.L. 2009. Pharmacological evaluation of anticonvulsant activity of root extract of Saussurea lappa in mice. *European Journal of Integrative Medicine*, 1:131-137.

Burkill, H.M. 2000. The useful plants of west tropical Africa. Vol 15. Royal Botanical Garden Kew. UK.

Dhir, A., Naida, P.S. and Kulkarni, S.K. 2007. Neuroprotective effect of nimesulide, a preferencial COX-2 inhibitor against pentylenetetrazole PTZ-induced chemical parameters in mice. *African Journal of Biotechnology*, 16: 691-697 .

Hameed, U.R., Rehman, R., Ullah, T.A., Farhan, Z. M., Asad A.M., Tahir, I. and Salman K. 2015. Elemental analysis and evaluation of phytochemical and biological properties of Orbancheagyptica. *Global Journal of Pharmacology*, 9(1): 51-55.

Hosseini, M., Hamid R.S., Soodabeh, S., Hassan, A. and Ali, G. 2009. The effect of L-argenine and L-NAME on PTZ-induced seizures in Ovariectomized rats, an in vivo study. Seizure, 18:695-698.

Kasture, V.S., Kasture, S.B., Joshua, A.J., Damodaran, A. and Amit, A. 2002. Ootropic activity of BacoMind™, an enriched phytochemical composition from Bacopa monnieri. *Journal of Natural Remedies*, 7: 166-173.

Krieger, R. 2001. Handbook of pesticide toxicology. 2nd Ed. Vol 1. Academic Press, San Diego, California. pp. 156

Kumar, S. and Singh, G. 2016. Pathophysiology of epilepsy: an updated review. *International Journal of Medical and Health Research*, 2(10): 32-36 .

Muazu, J. and Kaita, A.H. 2008. A review of traditional plants used in the treatment of epilepsy amongst the Hausa/Fulani Tribes of Northern Nigeria. African Journal of Traditional Complementary and Alternative Medicines, 5(4): 387-390.

Nardone, R., Francesco, B. and Eugen, T. 2016. Acute symptomatic seizures caused by electrolyte disturbances. *Journal of Clinical Neurology*, 12(1): 21-33.

Nassiri-Asl, M. and Schwann, S. 2007. Anticonvulsant effect of Passiflora incarnate extract in mice: Involvement of benzodiazepines and opioid receptors. *BMC Complementary and Alternative Medicine*, 7:26 .

Nejad, S.R., Motevalian, M., Fatemi, I. and Shojaii, A. 2017. Anticonvulsant effect of the hydroalcoholic extract of Alpinia officinarum rhizomes in mice: Involvement of benzodiazepine and opioid receptors. *Journal of Epilepsy Research*, 7 (1): 33-38.

Nicoll, R.A. 2001. Introduction to the pharmacology of CNS drugs. In: Katzung, B.G. (Ed.), Basic and clinical pharmacology. 8th Ed. New York, Lange Medical Books/Mcgraw Hill.

Nieoczym, D., Jarogniew, J.Ł., Stanisław, J.C. and Piotr, W. 2010. Effect of sildenafilon the anticonvulsant action of classical and second-generation antiepileptic drugs in maximal

electroshock-induced seizures in mice. *Epilepsia*, 51(8):1552- 1559.

Ogunri, A.O. 2006. Epilepsy in Nigeria: A review of etiology, epidemiology and management. Benin *Journal of Postgraduate Medicine*, 8 (1): 27-51.

Okoh-Esene, R.U., Okogun, J.I., Okwute, S.K. and Thomas, S.A. 2013. An overview of the facts, myths and treatment of the disease condition known as "Epilepsy". *Journal of Dental and Medical Sciences*, 6(2): 7-19 .

Panvan, K.K., Vidyasagar, G., Ramakrishna, D., Madhusudhana, I.R. and Gupta, A. 2011. Antiepileptic activity for methanolic extract of Vitexnegundo leaf against different animal models. Journal of Chemistry and Pharmaceutical Research, 3(4): 159-165.

Porter, R.J., Cereghino, J.J. and Gladding, G.D. 1984. Antiepileptic drug development program. *Cleve Clinical*, 51:293-305 .

Preux, P.M. and Druet-Cabanac, M. 2005. Epidemiology and aetiology in sub-Saharan Africa. *Lancet Neurol*, 4(1): 21-31.

Ramin, A., Nafiseh, N. and Majid, J.S. 2015. Interaction between Naloxone and Tramdolin kindling model of seizure in rat. *American Multidisciplinary International Research Journal*, 2(4):13-17.

Rang, H.P, Dale, M.M., Ritter, J.M. and Moore, P.K. 2005. Rang and Dales Pharmacology, 5th Ed. Churchill Livingstone. India. Pp. 456-473.

Rashidian, A., Kazemi, F., Saeed, M., Ahmad, R.D., Shahram, E.M. and Seyed, M.R. 2017. Anticonvulsant effects of aerial parts of Verbena officinalis extract in mice: Involvement of benzodiazepine and opioid receptors. *Journal of Evidence-Based Complementary and Alternative Medicine,* 22(4): 632-636.

Riazi, K., Roshanpour, M., Rafiei-Tabatabaei, N., Homayoun, H., Ebrahimi, F. and Dehpour, A.R. 2006. The proconvulsant effect of Sildenafil in mice: Role of nitric oxide Cgmp pathway. *British Journal of Pharmacology*, 147: 935-943.

Sarma, P. and Bhattacharyya, A. 2014. Models of epilepsy used in antiepileptic drug discovery: A review. International *Journal of Pharmacy and Pharmaceutical Sciences*, 7-1; (11) 6.

Sofowora, A. 1993. Medicinal plants and traditional medicine in Africa. 2nd Ed. Spectrum Books Ltd., Ibadan. Nigeria. pp. 289.

Swinyard, E.A., and Kupberg, H.J. 1985. Antiepileptic drugs: Detection, quantification and evaluation. Federation Proceedings, 44:39-43 .

Swinyard, E.A., Woodhead, J.H., White, H.S. and Franklin, M.R. 1989. General principles: Experimental selection, quantification and evaluation of anticonvulsants. In: Levy, H., Mattson, R.H., Meldrum B., Penry, J.K. and Dreifuss, F.E. (Eds.): Antiepileptic Drugs, 3ed Ed, New York, Raven Press, pp. 85- 102 .

Yemitan, K.O. and Adeyemi, O.O. 2013. Antiepileptogenic and anticonvulsant actions of Dalbergia saxatilis (Hook, F.) in sub-toxic chemical kindling and toxic convulsant models. *European Journal of Medicinal Plants*, 3(2): 288-296 .

Uthayathas, S., Karuppagounder, S.S., Thrash, B.M., Parameshwaran, K., Suppiramaniam, V. and Dhanasekaran, M. 2007. Versatile effects of sildenafil: *recent pharmacological applications. Pharmacological Reports*, 59:150–163.

Venkateshwarlu, E., Umasankar, K., Sharvanabhava, B.S. and Venkateshwar, R.J. 2013. Screening of the anticonvulsant activity of some isatin derivatives in experimental seizure model and its effect on brain GABA levels in mice. *American Journal of Pharmaceutical Science,* (3)1; 42-46.

Ya'u, J., Yaro, A.H., Musa, A.M., Abubakar, A., Yahaya, S.M., Chindo, B.A., Anuka, J.A. and Hussaini, I.M. 2014. Mechanisms of anticonvulsant Action of residual aqueous fraction (RAF) of the ethanol root bark extract of Carissa edulis. *Journal of Natural Sciences Research*, 4(22): 1329-1338.

Ziyaurrahman, A.R. and Jayvadan, P. 2012. Anticonvulsant effect of Boswellia serrata by modulation of endogenous biomarkers. *Der Pharmacia Lettre*, 4(4): 1308-1326.