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Research Article
Sedative and Anti-convulsant Effects of the Stem Bark Extract of *Combretum hypopilinum* in Laboratory Animals

[1]Abubakar Kabiru, [2]Usman Aminu, [1]Yerima Musa, [1]Muhammad Abubakaar Amali, [3]Samaila Hassan, [1]Abdullahi Suleiman, [1]Rabiu Tijani Giaze and [2]Abdulhamid Imam Tanko

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Pharmacology and Therapeutics, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Pharmacy, Sir Yahaya Memorial Hospital, Birnin Kebbi, Kebbi State, Nigeria

Abstract

Background and Objectives: Limited efficacy and negative property of currently available antiepileptic drugs limit their use and cause difficulties in patient management. The aim of this study was to evaluate the sedative and anti-convulsant effects of *Combretum hypopilinum*. *Combretum hypopilinum* stem bark was extracted using 95% methanol. Preliminary phytochemical screening of the crude methanol stem bark extract was carried out using standard procedures. **Materials and Methods:** Diazepam induced sleep was employed for the sedative effect. The anti-convulsant effect was evaluated by using MEST, PTZ and strychnine induced seizure models. Motor coordination deficit was also evaluated by using the beam walk assay method. **Results:** The result of the phytochemical screening revealed the presence of secondary metabolites such as; steroids, flavonoids and alkaloids. Acute toxicity studies were carried out and oral LD_{50} were found to be 3807.89 and >5000 mg kg^{-1} b.wt., respectively. The extract showed a dose dependent and statistically significant decrease in the onset of sleep. The extract was active in maximal electroshock seizure test at doses of 200 and 400 mg kg^{-1}. The extract also exerted significant activity in Pentylenetetrazole (PTZ) seizure test at a dose of 400 mg kg^{-1}. The extract had no effect on Strychnine (STC) induced seizure test. In the beam walk assay test, the extract revealed no significant motor deficit in all tested doses. **Conclusion:** From the results of the present work, it may be concluded that the *Combretum hypopilinum* extract contains bioactive principles with central nervous system depressant effect, this may also support the traditional use of the plant in treatment of epilepsy and other mental illnesses.

Key words: *Combretum hypopilinum*, epilepsy, sedative, anti-convulsant, phytochemical screening

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Corresponding Author: Abubakar Kabiru, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria Tel: +2348035863780

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are about 3.3 billion people in developing world who depend on plant-based traditional medicine for their healthcare needs. Almost 87% of the world's inhabitants rely mainly on traditional medicine for their primary health care[1]. Epilepsy is a neurological disorder of the brain that affects people worldwide. It is identified as recurrent seizures, which are brief involuntary movements which may involve a body part or entire body. Some instances this is followed by loss of consciousness and control of bowel or bladder function[2]. It is the fourth most common neurological disorder in the United States after migraine, stroke and Alzheimer's disease[3].

Limited efficacy and negative property of currently available antiepileptic drugs limit their use and cause difficulties in patient management. Unwanted events, withdrawal symptoms, harmful interactions with other drugs and economic consequences, especially in developing countries limit the long term use of antiepileptic drugs[4]. Studies on economic consequences of epilepsy management have shown that in Northern Nigeria, an annual cost per patient was about $285.2 and in Southern Nigeria the cost was around $395 per individual patient[5].

Several drugs sold today are simple synthetic modifications or copies of the naturally obtained substances. For example, salicin from *Salix alba* Linn. (white willow) and emetine[6]. More than 355 species of medicinal plants have been evaluated and found to have anti-seizure activity using *in vivo*/*in vitro* models[7]. A lot of plants are now being used traditionally for the management of epilepsy and seizures, but till date only a handful have been documented[8]. *Combretum hypopilium* has been used by traditional medicine practitioners in Zuru, Kebbi state of Nigeria, for the treatment of epilepsy. Previous studies have shown that the plant possesses antimicrobial and antineoplastic properties[9]. Furthermore, the currently available antiepileptic drugs can provide only symptomatic relief as these drugs suppress seizures and have no effect on epileptogenesis[10]. These claims and the need for continued search and development of new drugs that are cheap, safe and effective for management of epilepsy from plants and other sources were part of the justification for this study

MATERIALS AND METHODS

Study area: The study was carried out at the Pharmacology and Toxicology Laboratory of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto from January-June, 2019.

Preparation of plant materials: A sample of the plant was collected in Zuru local government area, Kebbi state, Northern Nigeria. It was authenticated at the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo, Sokoto, by Dr H. E. Mshelia. A voucher specimen number (PCG/D/US/Comb/0001) was deposited at the herbarium for future reference. Subsequently the stem bark of the plant was collected, washed with distilled water, shade-dried for one week and pulverized by using a pestle and mortar.

The powdered plant material (500 g) was continuously extracted with 2000 mL of 95% methanol by maceration for 3 days, extract obtained was filtered and concentrated in a rotary evaporator (Büchi Labortechnik AG, Switzerland) at 45°C. A brown product called methanol stem bark extract of *Combretum hypopilinum* was obtained. The extract was dried in an oven at a temperature of 40°C to remove any remaining moisture and stored at room temperature in an air tight container.

Phytochemical screening: Phytochemical screening was carried out on the plant by using standard procedures[7].

Experimental animals: Five to six weeks old, male and female Swiss albino mice weighing (20-33 g), two day old ranger cockerels weighing 20-35 g were obtained from animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and Ojuanu Agricultural Enterprises, Sokoto, respectively. The animals were housed at the animal facility of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. They were allowed free access to water and standard animal feed *ad libitum*. All procedures were performed according to the guidelines of care and use of laboratory animals as approved by the Animals Ethics Committee of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto, Nigeria.

Experimental design

Acute toxicity study: The method described by Lorke[11] was used, three groups of three mice or chicks were treated with the methanol stem bark extract of *Combretum hypopilinum* at doses of 10, 100 and 1000 mg kg^{-1} b.wt., orally and observed for signs of toxicity and death for 24 h. During second phase, the mice or chicks were divided into three groups of one mouse each. Group one, two and three received 1600, 2900 and 5000 mg kg^{-1} b.wt., respectively. Signs of toxicity and death were observed. The LD_{50} values were calculated using the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

where, D_0 is the highest dose that animal survived and D_{100} is the lowest dose that killed the animal.

Diazepam-induced sleep test in mice: Methanol stem bark extract of *Combretum hypopilinum* (CHME) was investigated for its Central Nervous System (CNS) effect using the diazepam-induced sleep test. Mice were divided into 4 groups of 6 each. Group (I-III) was treated with 100, 200 and 400 mg kg^{-1} doses of *Combretum hypopilinum* methanol stem bark extract. Group IV (control group) was treated with normal saline (10 mL kg^{-1}, p.o). After 1 h, receiving of CHME and normal saline, each animal was injected with Diazepam 5 mg kg^{-1} i.p. The criterion for sleep was the loss of righting reflex in which the mice cannot roll back when turned over[12]. The sleeping time was noted by recording the interval between the loss and regain of righting reflex[13].

Maximal Electroshock Seizure (MES) test in chicks: The method was employed, two day old ranger cockerels were divided into 8 chicks per group. The test groups (I, II and III) were treated with 100, 200 and 400 mg kg^{-1} doses of the extract. Group IV was administered Phenytoin (20 mg kg^{-1}, i.p), while group V received normal saline (10 mL kg^{-1}). Thirty minutes later, MES were induced in the chicks using the Ugo Basile Electro Convulsive Therapy (ECT) machine (Model 57800-001) with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and the pulse width were set and maintained at 90 mA, 0.80 sec, 200 pulse sec^{-1} and 0.8 msec, respectively[14,15]. Seizure was manifested as Tonic Hind Limb Extension (THLE)[16]. Ability to prevent this feature or decrease the recovery time post seizure was an indication of anti-convulsant activity[15].

Pentylenetetrazole-induced seizure (PTZ) test in mice: Animals were divided into five groups of five mice each. Groups I, II and III received CHME 100, 200 and 400 mg kg^{-1}, p.o, respectively. Groups IV and V received diazepam (5 mg kg^{-1}) and 0.9% w/v of normal saline (10 mL kg^{-1}) i.p, respectively. Seizure was induced by administering 90 mg kg^{-1} of sc PTZ[14] as modified by Vellucci and Webster[17]. CHME was administered orally, 1 h before the PTZ administration. Absence of an episode of clonic spasm of at least 5 sec duration, hind limb extension or death indicated the extract's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Strychnine (STN) induced seizure test in mice: Twenty five mice were divided into 5 groups (n = 5). Groups I, II and III received CHME 100, 200 and 400 mg kg^{-1} p.o, respectively. Group IV received diazepam (5 mg kg^{-1}) i.p, as positive control, while Group V served as negative control received 0.9% w/v of normal saline (10 mL kg^{-1}). The extract was administered orally, 60 min prior to the administration of strychnine nitrate (2.5 mg kg^{-1}) i.p. The animals were observed for 30 min by placing them in separate cages. The onset of seizures (tonic-clonic convulsions) and time of death were recorded[18].

Beam walking assay: Mice were trained to walk from a start platform along a ruler (80 cm long and 3 cm wide) elevated 30 cm above the bench by a metal support to a goal box. Three trials were performed for each mouse. Only the mouse that showed no neurological deficit and walked successfully along the ruler was admitted into the study and grouped into 5 groups of 6 mice each[19]. Mice in the first group were given normal saline (p.o), second, third and fourth groups received graded doses of the extract and the fifth group was given diazepam (0.5 mg kg^{-1}) (p.o). One hour later, each mouse was placed at one end of a beam (60 cm long, 8 mm in diameter and elevated 30 cm above the bench by a metal support) and allowed to walk to the goal box at the other end. The number of foot slips, which is a sensitive measure of motor coordination deficit[19] was recorded for each mouse by using a tally counter.

RESULTS

Phytochemical constituents of methanol stem bark extract of *Combretum hypopilinum*: The methanol stem bark revealed the presence of steroids, glycosides, flavonoids, tannins, alkaloids, saponins, carbohydrates, reducing sugars and proteins (Table 1).

Table 1: Phytochemical screening of methanol stem bark extract of *Combretum hypopilinum*

Phytochemicals	Observation
Steroids	+
Glycosides	+
Flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Carbohydrates	+
Anthraquinones	-
Proteins	+

+: Present, -: Absent

Acute toxicity studies (LD_{50}): The median lethal dose (LD_{50}) in mice was calculated to be 3807.89 mg kg^{-1} b.wt., and in chicks it was found to be >5000 mg kg^{-1} b.wt., orally as shown in Table 2.

Effects of *Combretum hypopilinum* methanol stem bark extract in the diazepam induced sleep test in mice: The result of diazepam induced sleep revealed a dose dependent decrease in onset of sleep in all treated groups. The decrease in sleep onset was statistically significant ($p<0.05$) compared with control (Table 3).

Anti-convulsant effect of *Combretum hypopilinum* on Maximal Electroshock-induced Seizure (MES) in chicks: The result of MEST showed dose dependent reduction in recovery time after Hind Limb Tonic Extension (HLTE) in chicks. The reduction was statistically significant ($p<0.05$) at doses of 200 and 400 mg kg^{-1} b.wt., (Table 4). At highest dose, the extract offered 25% protection against the MEST. Phenytoin at 20 mg kg^{-1}, a known anti-convulsant agent showed significant ($p<0.05$) reduction in the recovery time and provided 75% protection against MEST.

Anti-convulsant effect of *Combretum hypopilinum* on pentylenetetrazole-induced seizures in mice: In the PTZ test *Combretum hypopilinum* showed dose dependent increase in onset of seizure. The increase was significant ($p<0.05$) at the highest dose (400 mg kg^{-1} b.wt.,) of the extract. At the same dose, extract offered 20% protection against PTZ induced test. Diazepam (5 mg kg^{-1}) group offered 100% protection against PTZ induced seizure (Table 5).

Anti-convulsant effect of *Combretum hypopilinum* on strychnine-induced seizures in mice: The result of the STC test showed that the extract did not significantly increase the mean onset of seizure nor provide protection in STC induced seizure. Diazepam (5 mg kg^{-1}) significantly ($p<0.05$) increased seizure mean onset of the seizure and protected all the mice from death (Table 6).

Table 2: Acute toxicity studies of methanol stem bark extract of *Combretum hypopilinum*

Phase	Doses (mg kg^{-1})	Death/treated mice	Death/treated chicks
First	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Second	1600	0/1	0/1
	2900	0/1	0/1
	5000	1/1	0/1

Table 3: Effects of *Combretum hypopilinum* methanol stem bark extract and fractions in the diazepam induced sleep test in mice

Groups	Treatments	Doses (mg kg^{-1})	Sleep onset (min)	Duration of sleep (min)
1	CHMSE	100	4.50±0.50*	70.00±7.30
2	CHMSE	200	3.30±0.30*	77.70±3.40
3	CHMSE	400	2.50±0.40*	84.20±3.00
4	N/S	10 mL kg^{-1}	6.10±0.20	44.60±2.80

CHMSE: *Combrettium hypopilinium* crude methanol stem bark extract, N/S: Normal saline, data was presented as mean±SEM, (n = 6), *$p<0.05$ compared to control, one way ANOVA followed by Dunnett's *post hoc* test

Table 4: Anti-convulsant activity of methanol stem bark extract of *Combretum hypopilinum* in Maximal Electroshock induced Seizure Test (MEST) in chicks

Treatments	Doses (mg kg^{-1})	Recovery time (sec)	Quantal protection	Protected (%)	Mortality (%)
CHMSE	100	362.80±53.50	0/8	0	100
CHMSE	200	227.00±31.70*	0/8	0	100
CHMSE	400	160.80±42.80*	2/8	25	75
Phenytoin	20	38.00±25.50*	6/8	75*	25
N/S	10 mL kg^{-1}	488.00±73.80	0/8	0	100

CHMSE: *Combrettium hypopilinium* methanol stem bark extract, values are expressed as Mean±SEM, n = 8, *$p<0.05$ statistically significant as compared with the negative control, one way ANOVA followed by Dunnett's *post hoc* test

Table 5: Anti-convulsant effect of *Combretum hypopilinum* on pentylenetetrazole-induced seizure in mice

Treatments	Doses (mg kg^{-1})	Mean onset (sec)	Protected (%)	Quantal protection
CHMSE	100	144.60±24.80	0	0/5
CHMSE	200	188.60±23.20	0	0/5
CHMSE	400	278.40±27.90*	20	1/5
Diazepam	5	0.00±0.00*	100	5/5
N/S	10 mL kg^{-1}	138.60±32.20	0	0/5

Data presented as mean±SEM, N/S: Normal saline, CHMSE: *Combretum hypopilinium* crude methanol stem bark extract, (n = 5), *$p<0.05$, compared to control, One way ANOVA followed by Dunnetts' *post hoc* test

Table 6: Anti-convulsant effect of *Combretum hypopilinum* on strychnine-induced seizure in mice (n = 5)

Treatments	Doses (mg kg^{-1})	Mean onset (sec)	Protected (%)	Mortality rate (%)
CHMSE	100	353.60±23.50	0	100
CHMSE	200	367.40±22.18	0	80
CHMSE	400	383.40±32.40	0	20
Diazepam	5	627.00±56.71*	0	0
N/S	10 mL kg^{-1}	253.60±27.53	0	100

Data presented as mean±SEM, N/S: Normal saline, CHMSE: *Combrettium hypopilinium* crude methanol stem bark extract, (n = 5), *p<0.05, compared to control, One way ANOVA followed by Dunnett's *post hoc* test

Table 7: Effect of the methanol extract of *Combretum hypopilinum* on beam walking assay for motor coordination in mice

Treatments (mg kg^{-1})	Number of foot slip
CHMSE 100	1.00±0.40
CHMSE 200	1.30±0.30
CHMSE 400	1.30±0.60
Diazepam 5	3.50±0.30*
N/S 10	0.30±0.30

CHMSE: *Combretum hypopilinium* crude methanol stem bark extract, N/S: Normal saline, data presented as mean±SEM, (n = 5), *p<0.05, compared to control, One way ANOVA followed by Dunnett's *post hoc* test

Effect of the methanol extract of *Combretum hypopilinum* on beam walking assay for motor coordination in mice: In the beam walk assay test, the extract revealed no significant motor deficit in all doses tested (Table 7).

DISCUSSION

The preliminary phytochemical screening of the crude methanol stem bark extract of *Combretum hypopilinum* revealed the presence of secondary metabolites which have been previously reported to have various neuropharmacological effects[8,20]. Flavonoids and steroids have been indicated in various pharmacological actions on central nervous system including anti-convulsant and anxiolytic activity[21]. Flavonoids have been implicated in central inhibitory and neuromodulatory effects[22].

The LD_{50} values of crude methanol stem bark extract of *Combretum hypopilinum* in mice and chicks revealed that the plant is relatively safe[23]. Doses used in this work were selected to be less than one-quarter of the LD_{50} result for pharmacological safety[24].

The extract of *C. hypopilinum* significantly reduced the onset of sleep and increased the duration of sleep in this study. This activity might be achieved through the inhibition of diazepam metabolism or an action through the central mechanisms involved in the regulation of sleep[25,26]. Studies have shown that the activation of $GABA_A$ receptors in the central nervous system enhances sleep[27]. Hypnotics have more depression effect on the CNS than sedatives and this effect can be achieved through increasing the dose of sedative-hypnotic drugs and this leads to reduction in sleep onset and increase sleep duration[25].

In preliminary screening test for potential anti-convulsant agents, PTZ is among the most frequently used chemicals. PTZ is considered to act as an antagonist at $GABA_A$ receptor complex. Drugs that inhibit PTZ induced seizures are believed to be effective in controlling myoclonic and absence seizures in man[28].

Result from this study indicated that the tonic convulsion produced by PTZ was attenuated by *Combretum hypopilinum*. Studies have shown that diazepam exerts its antiepileptic effects by enhancing GABA-mediated inhibition in the brain[21]. It is possible that diazepam and *Combretum hypopililnum* inhibit PTZ induced convulsion enhancing GABA neurotransmission. Since, the *Combretum hypopililnum* delay the onset of PTZ induced convulsion, it might be possible that the extract may be interfering with GABA aminergic mechanism to exert its anti-convulsant effect. This work is corroborated the findings of Rasilingam *et al.*[21] and Tanko *et al.*[29].

Strychnine has been known to exert its convulsing effect through antagonizing the inhibitory spinal cord and brain stem reflexes via glycine and thereby increasing the spinal reflexes[30]. Inability of the extract to inhibit strychnine induced seizure showed its lack of effect on the glycine receptors in the spinal cord.

The maximal electroshock test is use to characterize agents with activity against generalized tonic clonic seizures with reference to clinically established antiepileptic drugs. Seizure produced by MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na^+ channels, such as; phenytoin, valproate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor, such as; felbamate[28]. The anti-convulsant activity indicated by the extract of *Combretum hypopilinum* in the MES model reveals that it might have blocked the seizure spread by inhibiting Na^+ channels and/or glutamatergic excitation through NMDA receptor.

Studies have shown that the number of foot slips made by mice in the motor coordination test is a sensitive indicator for evaluating benzodiazepine induced motor coordination deficit and it is a good indicator of doses providing clinical sedation[19]. The methanol stem bark extract of

Combretum hypopilinum showed no observable effects on motor coordination when compared with control implying that the inhibition effect observed in diazepam induced test might be initiated centrally not through peripheral neuromuscular blockade[31]. Thus, the sedative action of the extract observed was produced centrally. This work is similar to that of Nazifi *et al.*[32].

CONCLUSION

The stem bark of *Combretum hypopilinum* demonstrated sedative and anti-convulsant activities in the models used, this study has provided a scientific backing for the traditional use of the plant in the treatment of epilepsy and other mental disorders.

SIGNIFICANCE STATEMENT

This study was able to demonstrate that the extract contains active constituents with anti-convulsant effects possibly mediated via GABAergic pathways (as observed in both the PTZ and maximum electroshock induced tests). The extract could not protect the animals against strychnine induced seizure and did not protect the animals against lethality induced by strychnine. This study will also provide a lead for researchers who may want to investigate the mechanism of its anti-convulsant action.

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