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Pharmacological justification for the ethnomedical use of *Clausena anisata* root-bark extract in the management of epilepsy

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ABSTRACT

Various morphological parts of the tropical plant, *Clausena anisata* (Wild) Hook [family: Rutaceae], have ethnomedical claim for use in the management of epilepsy. This study examined the antiepileptic activity of *Clausena anisata* root bark, stem bark and leaf ethanolic extracts (i.e. CARE, CASE and CALE respectively) against pentylenetetrazole (PTZ) induced seizures in mice. Phytochemical and acute toxicity tests were performed on the extracts followed by oral administration of graded doses of CASE (500, 750 and 1000 mg/kg), CARE and CALE (400, 600 and 800 mg/kg) to the mice, thirty minutes before the administration of PTZ (90 mg/kg i.p.). The anticonvulsant effect of the extracts and diazepam (4 mg/kg) were compared. CALE was found to possess large amount of saponins, CARE large amounts of tannins and saponins, CASE large amounts of flavonoids, alkaloids and tannins. While CARE at the dose level of 800 mg/kg significantly ($p<0.05$) delayed the onset of convulsions and afforded 33.33 % protection, neither CALE nor CASE could exert any significant protective effect on PTZ induced convulsions, whereas diazepam totally abolished the episodes of convulsions. This study suggests that the ethanolic root bark extract of *Clausena anisata* contains bioactive constituents that may be beneficial in petit mal epilepsy and lend pharmacological credence to the ethnomedical claim for the use of the plant in the management of epilepsy.

***Abbreviations*:** NMDA= N-methyl-D-aspartate, SCMC= sodium carboxy methyl cellulose, CARE= *Clausena anisata* root bark ethanolic extract, CASE= *Clausena anisata* stem bark ethanolic extract, CALE= *Clausena anisata* leaf ethanolic extract, PTZ= pentylenetetrazole.

INTRODUCTION

Convulsion, a common chronic neurological condition due to sudden excessive disorderly discharge from the cerebral neurons (Kabir et al., 2005) and characterized by recurrent unprovoked epileptic seizures, has now become the most serious disorder, which accounts for about 1% of the world's burden of diseases (Hema et al., 2009) affecting approximately 40-50 million people worldwide (Fisher et al., 2005; Njamnshi et al., 2010). All the currently available anticonvulsants (drugs which have the ability to suppress or mo dify seizures without loss of consciousness or anesthesia) in the market are synthetic molecules. However, the side effects and the drug interactions are the major restrictions in its clinical utility. Herbal medicines are widely used due to their therapeutic efficacy coupled with least side effects, which initiate the scientific research regarding the anticonvulsant activity. Medicinal plants used for the therapy of epilepsy, in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening (Hema et al., 2009; Njamnshi et al., 2010; Maiha et al., 2009; Salih and Mustafa, 2008; Ojewole, 2005; Adeyemi, 2010; Ezugwu and Odoh, 2003; Ngo Bum et al., 2001; Ngo Bum et al., 2009a; Ngo Bum et al., 2009b). *Clausena anisata* (Fam. Rutaceae) plant is a tropical shrub o r tree up to 10 meters

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high and grows in and on the margins of evergreen forests (Hamza et al., 2006; Moshi et al., 2005). The leaves are pinnate, compound with 10 - 17, alternate, and with a terminal one, densely dotted with glands and have a strong scent; the branched inflorescences originate with an axillary spray; the small white flowers have orange-yellow stamens (Uwaifo, 1984). Previous phytochemical studies carried out on the extracts of various parts of the plant showed that *Clausena anisata* contains a diverse group of chemical compounds (Ekundayo, 1986; Chakraborty et al., 1995; Ito et al., 2000; Mester et al., 1977; Okunade, 1987). The traditional medicinal value of various morphological parts of *Clausena anisata* have been reported to be useful as effective remedies against parasitic infections, especially flatworm infestations, such as taeniasis and schistosomiasis, as well as in eye complaints; influenza and other respiratory ailments; heart disorders and hypertension; abdominal cramps, constipation and gastroenteritis; hepatic diseases causing bad breath; malaria; diabetes; fevers and pyrexia; boils, rheumatism, arthritis and other inflammatory conditions; headaches, body pains, toothaches and swollen gums; convulsions and some mental disorders; impotence and sterility; blood tonic; and dysentery in cattle (Hamza et al., 2006; Moshi et al., 2005, Uwaifo, 1984; Ekundayo, 1986; Chakraborty et al., 1995; Ito et al., 2000; Mester et al., 1977; Okunade, 1987; Ojewole, 2002). A review of the literature has not revealed any study on its anticonvulsant activity. This study, therefore, aims at evaluating the anticonvulsant activity of the ethanolic extract of the leaf, root bark and stem bark of *Clausena anisata* against pentylenetetrazole (PTZ) induced seizures in young albino mice (18 – 30g). The selection of the plant's parts for the present study relied on two sources viz, the folk literature and their present use in Temeke district (Daressalam, Tanzania) by traditional healers, who employ *C. anisata* against epilepsy and as an anticonvulsant (Moshi et al., 2005). This study will hopefully expose new frontiers by improving the current applications of this plant and provide a scientific basis for the traditional claims of this ethnic medicinal plant. In future the development of formulation by this plant constituents will give good anti-convulsion drug at lower cost.

MATERIALS AND METHODS

Preparation of plant material and extracts

Fresh stem bark, root bark and leaves of *Clausena anisata* were collected from Obukpa village in Nsukka L.G.A, Enugu state, Nigeria and authenticated by Mr. A. O. Ozioko of the Bioresources Development and Conservation Programme Centre (BDCP), Nsukka, Enugu State, Nigeria and a voucher specimen is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka. The materials were washed, shade dried, powdered, passed through sieve no. 60 and stored in air tight containers for experimental work. A weighed quantity (500 g) of each of the air-dried powdered drug was taken and extracted with ethanol (90 %) in a Soxhlet extractor. The extracts were concentrated in a rotary evaporator (yield 32.21, 40.75 and 29.86 % w/w for the stem bark, leaf and root bark extracts, respectively on dry weight basis). The ethanol extracts were dissolved in distilled water containing 2 % w/v Sodium carboxy methyl cellulose (as a suspending agent).

Experimental animals

Swiss albino mice (18-30 g) of either sex were used for the study. The animals were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka two weeks prior to the study, so that they could adapt to the new environment. The mice, maintained on standard rodent feed and water *ad libitum*, were housed in polypropylene cages at room temperature throughout the study. This study was conducted in accordance with Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of University of Nigeria, Nsukka, following the 18th WMA General Assembly Helsinki, June 1964 and updated by the 59th WMA General Assembly, Seoul, October 2008.

Acute toxicity studies: LD_{50}

Oral median lethal doses of CASE, CALE and CARE were determined using the method of Lorke (1983). Briefly, the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the ethanolic root bark, stem bark and leaves extracts of the plant at doses of 10,100 and 1000mg/kg body weight (p.o.), and were observed for signs of toxicity and death for 24 hours. In the second phase, 2 groups each containing one mouse were administered with 900 and 1200 mg/kg of ethanolic leaves extract; whereas 3 groups each containing one mouse were administered with 400, 900 and 1200 mg/kg of ethanolic root bark, and 1600, 2900 and 5000 mg/kg of ethanolic stem bark extract. The LD_{50} value for each extract was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

Phytochemical tests of the plant extracts

Various phytochemical tests were carried out on CASE, CALE and CARE to determine the presence of saponins, flavonoids, alkaloids and tannins following the method outlined by Wagner et al (1984).

Test for saponins

5ml of extract was vigorously shaken with 10ml of water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins.

Test for flavonoids

Extracts plus small quantity of Magnesium chips plus drops of concentrated hydrochloric acid down the side of test tube. Reddish coloration was taken as evidence for the presence of flavanoids.

Test for alkaloids

2 ml of the extract plus picric acid. An orange colouration was taken as evidence for the presence of alkaloids.

Test for tannins

Extract plus 4ml of water and drops of ferric chloride. Immediate green precipitate was taken as evidence for the presence of tannins.

Pentylenetetrazole-induced Seizure in mice

The method of Vogel *et al.* (1997) was employed. The animals were divided into eleven (11) groups of 6 animals each. Group I, II and III were treated with *Clausena anisata* ethanolic stem bark extract (CASE) (500, 750 and 1000 mg/kg body weight respectively) orally. Group IV, V and VI were treated with *Clausena anisata* ethanolic leaves extract (CALE) (400, 600 and 800 mg/kg body weight respectively). Group VII, VIII and IX were treated with *Clausena anisata* ethanolic root bark extract (CARE) (400, 600 and 800 mg/kg body weight respectively). Group X served as the standard and was treated with Diazepam (4 mg/kg body weight). Group XI served as control and received 1% Sodium carboxy methyl cellulose (10 ml/kg, orally). Thirty minutes post treatment, mice in all the groups received 90 mg pentylenetetrazole per kg intraperitoneally (i.p). The animals were then individually placed in trays and observed over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold. The time taken before onset of clonic convulsions and percentage mortality was also recorded.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test (SPSS-15). Differences between groups were considered significant at $p < 0.05$ levels. All values were expressed as Mean ± SEM.

RESULTS AND DISCUSSION

Acute toxicity studies were carried out to evaluate the drug's toxicity and to determine the minimum lethal dose of the drug extracts, using swiss albino mice. The dose levels chosen for the anticonvulsant screening of the extracts were based on the results of the acute toxicity test (LD_{50}) which were 948.68 mg/kg for both the ethanolic leaves and root bark extracts (i.e. CALE and CARE) and 1265 mg/kg for the ethanolic stem bark extract (i.e. CASE). The results obtained from the phytochemical tests carried out on CALE, CASE and CARE are shown in Table 1. CALE was found to possess trace amount of alkaloids and large amount of saponins but no flavonoids and tannins; CASE contains large amounts of saponins, flavonoids, alkaloids and tannins; whereas CARE was found to contain moderate amounts of tannins and saponins, but large amounts of flavonoids and alkaloids, consistent with earlier reports (Mester *et al.,* 1977; Ojewole, 2002).

Table. 1: Qualitative chemical tests for phyto constituents in *Clausena anisata* ethanolic extracts.

Test	CASE	CALE	CARE
Saponins	+++	+++	++
Flavonoids	++	---	++
Alkaloids	++	+	++
Tannins	++	---	+

CASE: *Clausena anisata* stem bark extract; CALE: *Clausena anisata* leaves extract; CARE: *Clausena anisata* root bark extract; +.... low concentration; ++.... moderate concentration; +++.... high concentration; --- .... absent.

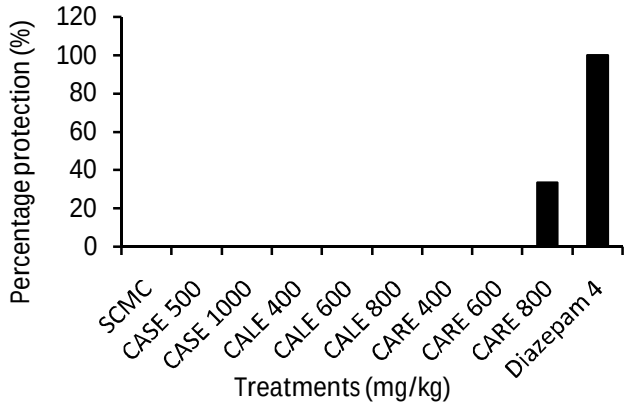

Fig. 1: Effect of ethanolic extracts of *C. anisata* on pentylenetetrazol-induced seizure in mice (n=6 per dose).

Legend: CASE: *Clausena anisata* stem bark extract; CALE: *Clausena anisata* leaves extract; CARE: *Clausena anisata* root bark extract; SCMC: Sodium carboxy methyl cellulose. Histogram represents the percentage of protected animals. The percentage of protection of SCMC was compared to the percentage of protection of the groups treated with the extracts (i.e. CARE, CASE and CALE) and Diazepam. [CASE 750 was not shown].

The results of the pentylenetetrazole-induced seizures test are depicted in Fig. 1 and Table 2. The administration of *Clausena anisata* ethanolic root bark extract (CARE) at a dose of 800 mg/kg body weight 30 min prior to the injection of PTZ, significantly ($p<0.05$) delayed the onset of convulsions (Table 2) and afforded 33.33% protection (Fig. 1). However, at lower doses employed in the study (400 and 600 mg/kg body weight), CARE could not exert any significant protective effect on PTZ induced convulsions. Neither CALE at doses of 400, 600 and 800 mg/kg body weight nor CASE at doses of 500, 750 and 1000 mg/kg body weight could exert any significant protective effect on PTZ induced convulsions. Diazepam (a known anticonvulsant) at a dose of 4 mg/kg totally abolished the episodes of convulsions and afforded 100% protection. Compared with SCMC (control), all the extracts at the doses used in the study possessed greater mean onset (time) of seizures, an indication that they are more sedating than the control.

The PTZ model is widely believed to be predictive of activity in common form of human epilepsy (Wickenden, 2002) and PTZ induced seizure is analogous to petit mal type of seizures and human generalized seizures (Loscher and Schmid, 1988). Compounds effective against this experimentally induced seizure models are effective against petit mal type of epilepsy (Vida, 1995). The effect of CARE (800 mg/kg) in the PTZ test could, therefore, suggest anticonvulsant efficacy against the above mentioned seizure types in man. The inability of CASE and CALE

as well as low doses of CARE (600 and 400 mg/kg) to protect the mice against PTZ-induced seizure suggests they may not be effective in the therapy of these seizure types in man.

Table. 2: Effect of ethanolic extracts of *C. anisata* on pentylenetetrazole-induced seizure in mice.

Treatment	Dose (mg/kg)	Quantal protection	Mean onset time (min)
CASE	500	0/6	3.467 ± 0.22
CASE	750	0/6	2.920 ± 0.78
CASE	1000	0/6	3.233 ± 1.93
CALE	400	0/6	3.075 ± 0.64
CALE	600	0/6	3.183 ± 1.27
CALE	800	0/6	2.714 ± 0.83
CARE	400	0/6	3.967 ± 0.55
CARE	600	0/6	3.503 ± 0.20
CARE	800	2/6	*9.133 ± 1.20
Diazepam	4	6/6	-
SCMC	10ml/kg	0/6	1.650 ± 0.20

CASE: *Clausena anisata* stem bark extract; CALE: *Clausena anisata* leaves extract; CARE: *Clausena anisata* root bark extract; SCMC: Sodium carboxy methyl cellulose.
* = P<0.05.

The oral administration of CARE (800 mg/kg), 30 min before the injection of PTZ, delayed the onset of seizures (Table 2). By implication, CARE contains bioactive constituents which possess anticonvulsant activity that may be beneficial in the management of petit mal epilepsy and this lend credence to the use of the plant in the management of epilepsy in traditional medicine (Moshi et al.,2005). Drugs that are effective against petit mal seizures reduce T-type calcium currents and these types of seizures can also be prevented by drugs that enhance $GABA_A$ – BZD (GABA- ergic- benzodiazepine) receptor mediated neurotransmission such as benzodiazepines (e.g. diazepam) and phenobarbitone (Mcdonald and Kelly, 1995). Studies have shown that activation of N-methylD-aspartate (NMDA) receptor are also involved in the initiation and generalization of PTZ induced seizures (Nevis and Amolde, 1989; Velisek et al., 1990). Drugs that block glutametargic excitation mediated by NMDA receptors, such as felbamate, have anticonvulsant property against PTZ induced seizures (Mcdonald and Kelly, 1995). Although the results of this study cannot specifically be used at this level to establish the mechanism of anticonvulsant action of CARE, the latter may be attributed to either one or more of the above mechanisms.

CONCLUSION

This study has shown that the ethanolic root bark extract of *Clausena anisata* (CARE) at a dose of 800 mg/kg body weight exhibited anticonvulsant activity on PTZ model of convulsion in mice. The results of the study, therefore, suggests that CARE contains bioactive constituents which possess anticonvulsant activity that may be beneficial in the management of petit mal epilepsy and lend credence to the use of the plant in the management of epilepsy in traditional medicine. The phytochemical tests revealed the predominance of saponins in CALE, tannins and saponins in CARE, flavonoids, alkaloids and tannins in CASE. Further phytopharmacological investigations is ongoing in our laboratory to determine the active molecule(s) responsible for the observed effect of CARE.

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