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Anticonvulsant Activity of *Lophira alata* (Ochnaceae) Stem Bark Extract in Mice

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Abstract
Lophira alata, a tree found in many countries in the tropics has diverse ethnobotanical uses including the management of pain, inflammation and convulsion. This study evaluated the anticonvulsant activity of ethanol extract of L. alata stem bark. Phytochemical screening and the mean lethal dose (LD_{50}) of the extract were determined. Anticonvulsant activity was evaluated in murine models of convulsion using the pentylenetetrazole and maximal electroshock induced tests at dose levels of 100 to 800 mg/kg. The results of phytochemical screening indicated the presence of tannins, saponins, steroids, glycosides, carbohydrates alkaloids and flavonoids, and the LD_{50} of L. alata was determined to be greater than 5000 mg/kg. L. alata afforded significant protection in both models at the higher doses used in this study. L. alata possesses anticonvulsant activity at high doses and this may account for its use in ethnomedicine

Keywords: *Lophira alata,* seizure, convulsion, epilepsy

INTRODUCTION
Lophira alata is found in the tropical forests of West, Central and East Africa in countries such as Nigeria, Cameroun, Democratic Republic of Congo, Uganda and Sudan. Common names for *L. alata* include iron red wood or azobe; *namijin kadai* (Hausa), *aba* or *akufo* (Igbo), *Ekki* (Yoruba), *Iku luo* or *luo* (Lugbara and Oteng tribes, Uganda) and *bongosi* [Cameroun] (Persinos and Quimby, 1968; Tih *et al.,* 1992b; Abderamane *et al.,* 2011; Ajiboye *et al.,* 2014. World Agroforestry, 2022).

L. alata is used locally for the treatment of systemic disorders such as epilepsy, pain and inflammation, and as an antiseptic and antimicrobial agent (Sanberg and Cronlund, 1982; Abderamane *et al.,* 2011; Balde *et al.,* 2015; Wahab, 2015).

The stem bark extract of *Lophira alata* is used for the relief of pain, inflammation, pyrexia and convulsions, while the roots, leaves and stem barks are used for their cognitive enhancing and anti-aging properties (Sanberg and Cronlund, 1982; Abderamane *et al.,* 2011; Cyril-Olutayo *et al.,* 2012; Balde *et al.,* 2015; Wahab, 2015; Babawale *et al.,* 2016).

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In pre-clinical studies, aqueous extract of the stem bark was shown to possess antidepressant, anxiolytic and anticonvulsant activity (Iniaghe *et al.,* 2015), while the methanol extract demonstrated antimutagenic, antioxidant, anticancer and insecticidal properties (Ajiboye *et al.*, 2014; Kuete *et al.,* 2017; Remi-Esan and Bankole, 2020). The leaves were evaluated for biological activity and the aqueous, methanol and ethanol extracts were found to have antibacterial and antiplasmodial activity (Falade *et al.,* 2014; Falade *et al.,* 2018; Mouafo *et al.,* 2021) while the root extract inhibited growth of cancerous cells in *in vitro* studies (Ezenyi *et al.*, 2021).

Epilepsy, a non-communicable disease which affects both children and adults requires long term pharmacotherapy. It is a common neurological disease with a high index of discrimination, social stigma and premature death (Baskind and Birbeck, 2005; Fiest *et al.,* 2014; WHO, 2019). Drugs currently used for the management of epilepsy though effective in preventing seizures possess adverse effects which often render them *user unfriendly* and can lead to poor adherence (Golderberg, 2010; Wahab, 2010). The development of anti-epileptic medications with minimal adverse effects is the focus of many laboratories in our world today (WHO, 2019).

Though studies have been carried out on the aqueous and methanol extract of *Lophira alata* stem bark and leaves, studies on the anticonvulsant potential of ethanol extract of *L. alata* stem bark to the best of our knowledge are not documented in existing Literature. In this study, we seek therefore, to evaluate the effects of the ethanol extract of *Lophira alata* stem bark in murine models of both chemical and electrically induced convulsion.

METHODOLOGY

Plant Material

Stem bark, leaves and flowers of *Lophira alata* collected in a forest in Okeigbo, Ondo State, South-West Nigeria in February 2019 were taken to the Forest Research Institute of Nigeria, Ibadan for identification. Identification and authentication were undertaken by Mr. K. Adeniji and a voucher specimen (FHI 112824) was prepared and deposited for reference purposes. Stem barks were cleaned, pulverized and extracted with 98% v/v ethanol for 72 hours. The extract was filtered, evaporated and dried in an oven at 40^{o}C and final product -dark brown crystals- was stored in a refrigerator at 4^{o}C.

Following the procedures laid down by Trease and Evans (1983), the extract was screened for phytochemical constituents. The frothing test was used to detect the presence of saponins while the sodium hydroxide, lead ethanoate and Shinoda's tests were used to test for the presence of flavonoids. The presence of alkaloids was determined using Mayer and Dragendoff's tests; Keller-Kiliani test was used to show the presence of glycosides; Liebermann-Burchard's and ferric chloride tests were used respectively to evaluate the presence of steroids and tannins; Molisch and Fehling's tests were used to demonstrate the presence of carbohydrate in the ethanol extract of *Lophira alata* stem bark.

Animal Study

Male and female Swiss albino mice (20-28 g) procured from the Animal House Unit, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin were used for both toxicity studies and anticonvulsant screening. Male and female mice were separately housed in polypropylene cages and fed with standard animal feed (Topfeed^{R}) and water *ad libitum*. The cages were cleaned daily and animals were humanely handled according to the guidelines of the National Institutes of Health (2015). Ethical approval (EC/FP/019/09)

was obtained from the Institutional Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria before the experimental studies with animals were undertaken.

Acute Toxicity

Following the method described by Lorke (1983), with some modifications, 18 mice were used for the acute toxicity tests. In Phase I, 9 mice in groups of 3 per group were administered 10, 100 and 1000 mg/kg of *L. alata* and keenly monitored for 24 hours. Unusual behaviours and mortality were noted. Thereafter, 9 mice in groups of 3 per group, were treated with 1600, 2900 and 5000 mg/kg *L. alata* and observed for 24 hours.

Chemically induced Convulsions

The method of Swinyard and Kupferberg, (1985) was used to evaluate pentylenetetrazole (PTZ) induced convulsions in mice. Thirty-six mice randomly placed in 6 groups of 6 mice each were treated with distilled water (group 1), 100, 200, 400 and 800 mg/kg *L. alata* orally (groups 2-5) or diazepam 2 mg/kg intraperitoneally (i/p) - group 6. Thirty minutes post treatment, 70 mg/kg PTZ was administered i/p to each mouse. Mice were then placed in individual cages and observed for 1 hour for signs of seizure, onset, type and duration of seizure, death or recovery from seizure.

Electrically induced Convulsions

Mice in 6 groups of 6 animals each received either distilled water, 100-800 mg/kg *L. alata* orally or 30 mg/kg phenobarbitone i/p prior to exposure to electroshock of 50 mA current delivered with the aid of electrodes clipped to the ears. Protection from convulsion or tonic extension of hind limb and death were recorded by observers unaware of drug treatment (Swinyard *et al.*, 1952).

RESULTS

Results of phytochemical analysis and LD_{50} studies

The percentage yield of *L. alata extract* was 16.5% and phytochemicals identified from the extract are tannins, saponins, steroids, flavonoids, glycosides, carbohydrate and alkaloids. As no death was recorded in both phases of the acute toxicity study, the oral LD_{50} of *L. alata* was determined to be more than 5,000 mg/kg.

Effect of *L. alata* on Chemically and Electrically induced Convulsions

The highest dose (800 mg/kg) gave lowest mortality rates in both models. Results are presented in Tables 1 and 2

Table 1: Effect of *L. alata* in the PTZ induced convulsion

Treatment	Onset of convulsion (s)	Quantal Protection	(%) Mortality
Distilled Water	6.8 ± 2.10*	0/6*	100.00
LA 100 mg/kg	24.51 ± 4.65*	2/6*	66.67
LA 200 mg/kg	48.8 ± 6.20	4/6	33.33
LA 400 mg/kg	27.50 ± 8.10*	3/6*	50.00
LA 800 mg/kg	63.25 ± 5.9	5/6	16.67
Diazepam 2 mg/kg	None	6/6	0

LA *Lophira alata.* * Significantly different from Diazepam

Table 2: Effect of *L. alata* in the Maximal Electroshock induced convulsion

Treatment	Quantal Protection	(%) Mortality
Distilled Water	0/6*	100.00
LA 100 mg/kg	1/6*	83.33
LA 200 mg/kg	3/6*	50.00
LA 400 mg/kg	3/6*	50.00
LA 800 mg/kg	4/6	33.33
Phenobarbitone 30 mg/kg	6/6	0

LA *Lophira alata.* * Significantly different from Diazepam

DISCUSSION

This study evaluated the effects of the ethanol extract of *Lophira alata* on the chemical and electrically induced convulsions in mice.

Epilepsy affects about 50 million people worldwide and patients can achieve seizure free lives with the use of medications (WHO, 2019). Animal models are invaluable in the search for new antiepileptic medications and the need for development of effective antiepileptic medications with minimal adverse effects cannot be overemphasized (White, 2003; Bialer *et al*., 2007; Perrucca *et al*., 2007). The PTZ induced model of pharmacologic induction of convulsion is advantageous as it is simple, inexpensive and gives the researcher the opportunity to control timing/onset of seizures. PTZ is a central nervous system stimulant which is a non-competitive antagonist of gamma-aminobutyric acid-A receptor (Hansen *et al*., 2004; Shimata and Yamagata, 2018). PTZ which suppresses inhibitory synapses, causing an increase in neuronal activity and subsequent seizures has also been demonstrated to induce four behavioural phenomena in rodents vis freezing, myoclonic twitches, clonic seizures and tonic clonic seizures thus, it is useful for evaluating compounds with potential anti-epileptogenic properties. Treatment with effective doses of anti-epileptic drugs, such as phenytoin, phenobarbitone, and diazepam pre or post PTZ administration has been demonstrated to ameliorate seizure onset, severity and mortality. Thus, agents which can attenuate severity of seizures or prevent mortality are potential anti-epileptic agents (Tourov *et al*., 1996; White, 2003; Zhao and Holmes, 2006; Loscher, 2017: Shimata and Yamagata, 2018).

The maximal electroshock (MES) model, a model of generalized-tonic clonic seizures is a useful and valid tool for evaluating potential anti-seizure compounds compared to the focal or partial seizure model such as the 6 Hz psychomotor seizure model (Mares and Kubova, 2006). It has remained one of the most effective and validated tests for early screening of potential anti-epileptic compounds. The MES evaluates the ability of test compounds to prevent propagation of seizures in neural tissues (Rogawski, 2006; Castel-Branco *et al.,* 2009). The MES is advantageous as an epileptogenic agent as it is easy to conduct, standardize, requires minimal technical skill and acts only when the electrical current is applied. However, it has the demerit of being able to stimulate both neuronal and non-neuronal cells (Mares and Kubova, 2006; Castel-Branco *et al.,* 2009). Standard antiepileptic drugs are effective in abolishing MES induced seizures and drugs which abolish MES induced seizures in rodents have been shown to be effective in abating generalized tonic-clonic seizures in humans (Schmidt and Rogawski, 2002; White, 2003; Borowicz *et al.,* 2007).

Lophira alata delayed the onset of seizures and protected animals from death in this study suggestive of potential anti-epileptic properties. Protection from PTZ induced seizures and mortality could be indicative of GABAergic activity as compounds which ameliorate this type of convulsion have been shown to be agonists of GABA (White, 2003; Loscher, 2017: Shimata and Yamagata, 2018). The ability to reduce mortality in the MES suggests protective activity from generalized seizures as compounds which attenuate MES induced convulsion are able to protect from tonic clonic seizures (Schmidt and Rogawski, 2002; White, 2003; Borowicz *et al*., 2007). Maximum protection was seen at highest doses, while the middle and lowest doses afforded modest and minimal protection respectively. Mechanistic and biomolecular studies to determine exact mechanism of action and levels of neurotransmitters can be the target of future studies on extracts and isolates of *Lophira alata* stem bark. Taken together, these results lend credence to the ethnomedicinal use of *L. alata* in the management of epilepsy.

CONCLUSION

The ethanol extract of *Lophira alata* stem bark possesses anticonvulsant activity and can be explored as a potential candidate in the search for novel anticonvulsant medications.

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