Original Research Article

ANTICONVULSANT POTENTIAL OF DICHLOROMETHANE EXTRACT OF *Aspilia africana* LEAF IN MICE

ABSTRACT

Aim: This study was carried out to determine the anticonvulsant potential of *A.africana* leaf extracts in its non-polar (dichloromethane) constituents. **Study Design:** The study was adapted from three methods of seizure stimulation of the *in vivo* animal model using the maximal electroshock (MES), pentylenetetrazole (PTZ), strychnine (STC) methods and in addition, motor coordination. **Place and Duration of Study:** The study was carried out in the respective laboratories of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria between the months of October to November, 2017. **Methodology:** The powdered leaf of *A. africana* (2000 g) was extracted cold in 10 liters of dichloromethane (100%). The extract was made using rotary evaporator at a maximum temperature 45°C for both extracts respectively. The total dried extract obtained from the 2000 g powdered leaves of *A. africana* was 51.9 g given 2.6% w/w yield (dichloromethane). The dose was established based on preliminary data that proof ratio 1/33 of the LD50 (6.6 g/kg) safe in mice using intraperitoneal route; hence dose 200, 100, 50 and 25 mg/kg applied according to the study design. **Results:** Computer software Graph pad PRISM® version 5.00 was used for data analysis. The marked delayed onset of seizure indicated anti-seizure potential dichloromethane extract of *A.africana* leaf with the maximal delayed onset of seizure, largely recorded in the least dose, 25 mg/kg significantly $P = .05$ compared with the normal control in the applied methods. The study also indicated reduced motor planning potential of same

A.africana leaf significantly $P = .05$ compared with normal control. **Conclusion:** this study suggests antiseizure potential of *A.africana* leaf extracts.

Keywords: *A.africana*, Antiseizure, Pentylenetetrazole (PTZ), Strychnine (STC), Maximal electroshock (MES).

INTRODUCTION

Convulsion is a chronic neurological condition with history across all ages, race and gender with prevalence variance across countries, it is characterized by uncontrolled muscle contraction and relaxation of the affected body part resulting to bladder and bowel incontinent (Krumholz *et al.*, 2015). Because convulsion is often a symptom of an epileptic seizure, the term convulsion is often used as a synonym for seizure. However, not all epileptic seizures lead to convulsions, and not all convulsions are caused by epileptic seizures (Fisher *et al.*, 2005; 2014).

It is the third most common chronic neurological disorders characterized by seizures after stroke and Alzheimer's disease (Paul, 2013; Aliyu *et al.*, 2014). According to the WHO, 2017 fact sheet report about 50 million people suffers epilepsy worldwide especially 75-80% of the population live in developing countries with little or no access to medical services or treatment (Nazifi *et al.*, 2017; WHO, 2017). The prevalence of epilepsy in Nigeria is about 6.2 to 20.8 per 1000 (Banerjee *et al.*, 2009; Osakwe and Alo, 2014). However, it has been reported not less than 30% of epileptic seizure associated individuals do not have seizures control even with the best available medications (Yemitan and Adeyemi, 2013; Aliyu *et al.*, 2014; NINDS, 2016; Nazifi *et al.*, 2017). Furthermore, undesirable side effects such as psychosis, agitations, aggression, allergies, sedation, blood dyscrasias, teratogenesis, changes in mood, memory problems and intolerance of anticonvulsant drugs used clinically often render treatment difficult; thus, search

for new, safer and affordable antiepileptic drugs is geared towards naturally-occurring compounds, which is thought to belong to new structural, chemical classes or other mechanism of actions especially from herbal sources.

It is acknowledged by several authorities that natural products remained the keystone in drug discovery and maintains important role in antiepileptic seizure even in the future (Malami *et al.*, 2016). Various medicinal plants are known for their anticonvulsant value and their extracts is an important source of chemicals for the development of better and safer drugs for the treatment of epilepsy as earlier stated. Similarly, the use of herbal medicine in the management of epilepsy is widely accepted among rural dwellers in most Nigerian communities and their efficacies are well acclaimed. Some of these medicinal plants remain unexplored for their value as sources of antiepileptic drugs and therefore, research is encouraged to validate the folkloric claims of these medicinal plants so as to provide scientific evidence of their safety and efficacy (Nazifi *et al.*, 2017).

The plant *Aspilia africana* (wild sunflower), also called iodine or haemorrhage plant because of its characteristics, it is an *Asteraceae* species, was evaluated for its potential chemoprotective bioactivities among others. No ethnomedicinal information is available on its antiseizure/anticonvulsant activities as at the time of this study.

MATERIALS AND METHODS

Plant material and preparation of *Aspilia africana* extraction

Fresh leaves of *Aspilia africana* were sourced from Wilberforce Island rain forest around the Niger Delta University, Bayelsa State, Nigeria. On the 17th of February, 2017, and were authenticated by DR. Gideon Alade of the Department of Pharmacognosy and Herbal medicine,

Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The voucher specimen of the plant leaves was prepared and deposited at the Herbarium Unit of the Department of Pharmacognosy and Herbal medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State. Fresh leaves of *A. africana* were collected, air-dried for 14 days and milled into powder with the aid of industrial grinder. The powdered leaf of *A. africana* (2000 g) was extracted cold in 10 liters dichloromethane (100%) with daily gentle shaking for 96 hours. The mixture was filtered and the filtrate concentrated using a rotary evaporator at a maximum temperature of 45°C to obtain the crude extract of dichloromethane of *A. africana* leaf. Further drying of the extracts was carried out using the freeze-dryer to obtain powder extracts. The total dried extract obtained from the 2000 g powdered leaves of *A. africana* was 51.9 g given 2.6% w/w yield of dichloromethane. The semi-solid paste of the both extracts of the *A. africana* leaf was then respectively stored in the refrigerator at 4°C till needed for use. The dose was established based on preliminary data that proof ratio 1/33 of the LD50 (6.6 g/kg) (Oko and Agiang, 2011; Etoa *et al.*, 2007) safe in mice using intraperitoneal route (i.p); hence 200, 100, 50 and 25 mg/kg applied in the study.

Experimental animals

Twenty four (24) male mice weighed between 23 g and 30 g were obtained from the animal house of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria and was acclimatized for two weeks at the Animal Breeding and Research Unit, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria. The animals were kept under conducive laboratory conditions and fed with standard animal feed (Grower's pelletized), and water ad libitum. The principle of laboratory animal care as prescribed by the National Institute of Health-

NIH (publication No. 85-23) guidelines and procedures (Michael and Altman, 2002) were followed in this study. The research study was subjected to the Postgraduate Research Ethical Committee, University of Port Harcourt, Nigeria, for approval.

Administration of extracts

The extract was administered to the male mice respectively through the i.p route. The volume of extracts administered intraperitoneally was 10 mL/kg in all cases. The i.p route was used in this study because it is considered to be faster, more consistent in its outcome and is readily reproducible. This route is preferred in central nervous system related studies because of the possibility of interference of metabolic processes with the test agents given through the oral route (De Carvalho *et al.,* 2011).

Anti seizure Study Design

The study design was adapted from three methods of seizure induction of the *in vivo* animal model as described by Kasthuri *et al., (*2013).

Maximum electroshock induced convulsion in mice

The method of Swinyard and Kufferberg (1985) and Browning (1992) was employed. Twenty four (24) male mice were randomly allotted into six groups of four mice each. The first group received i.p 10 mL per kg body weight normal saline and the second group received i.p 60 mg/kg phenobarbitone (analytical grade) while the third, fourth, fifth and sixth groups received 25, 50, 100 and 200 mg of the dichloromethane extract per kg body weight i.p respectively. Thirty minutes later, maximum electroshock was administered to induce seizure in the mice using Ugo Basile electroconvulsive machine (Model 18182 v 220) with an electrode clipped to each ear of

the mice. The current, shock duration, frequency and pulse width set and maintained at 60 mA, 2.0 s, 100 pulse per second and 2.0 ms respectively. Abolition of hind limb tonic extension (HLTE) was considered as protection from electroshock (Imoru *et al.,* 2015).

Strychnine-induced convulsion in mice

The method of Porter et al. (1984) was employed. As stated in the MES method earlier, the first group received i.p 10 mL per kg body weight normal saline and the second, third, fourth, and fifth groups receive i.p 25, 50, 100 and 200 mg per kg body weight and the sixth group received i.p 1.0 mg/kg of diazepam (Swipha Nig. Ltd.). Thirty minutes later, mice in all the groups received i.p 2.0 mg/kg of strychnine (Sigma chemicals Co. St Luis U.S.A). Abolition of tonic extensor jerks of the hind limbs was considered an indicator that the testing materials could prevent strychnine-induced convulsions.

Pentylenetetrazole (PTZ) induced convulsion in mice

The method of Swinyard *et al.,* (1989) was employed. As treated in the STC method, thirty minutes later, mice in all the groups receive i.p 80 mg/kg pentylenetetrazole (Sigma chemicals Co. St Luis U.S.A). 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.

Motor coordination evaluation

Test for motor coordination and motor planning potential of *A.africana* leaf in twenty four male mice was adopted from Swinyard and Kufferberg (1985) using Ugo Basile, Italy Rota Rod (for mice 47600 V04). The rota rod is set at 6 revolutions per minute (rpm) for 120 s with baseline record established by placing each of the mice on the rota rod for at least 120 s constant grip

were selected, weighed and divided into the normal control group, phenobarbitone group and test groups of 25, 50,100 and 200 mg/kg respectively. Thirty minutes after the controls and test substances have been administered. The falling time from the rotating rod was recorded for each mouse per group.

Method of Data Analysis

The laboratory data was expressed as mean, standard error of mean (M ± SEM). Statistical difference for parametric data was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test, SNK). Difference considered statistically significant with P =.05 for all comparisons. Computer software Graph pad PRISM® version 5.00 was used for the analysis.

RESULTS AND DISCUSSION

Table 1. Dichloromethane extract of *A.africana* on antiseizure evaluation in MES

Treatment	Dose	Incidence of HLTE seizure (%)
NSt	10 mL/kg	100
PHB	60 mg/kg	0*
DEAA1	25 mg/kg	50
DEAA2	50 mg/kg	50
DEAA3	100 mg/kg	75
DEAA4	200 mg/kg	75

N = 4, * denotes significant *P* =.05 compared to control value, NSt= normal saline + 20 % tween 80 control, PHB=Phenobarbitone, DEAA= Dichloro methane extract of *A.africana.*

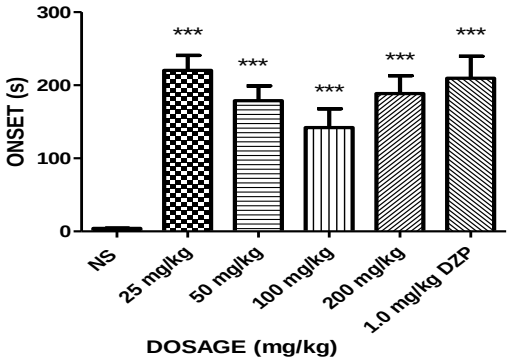

Figure. 1: Dichloromethane extraction of *A.africana* of STC showing graph of delayed onset animal seizure. Data analyzed using Newman-Keuls Multiple Comparison Test showed,*** denotes statistically significant (*P* =.01).NS = Normal control, DZP = Diazepam.

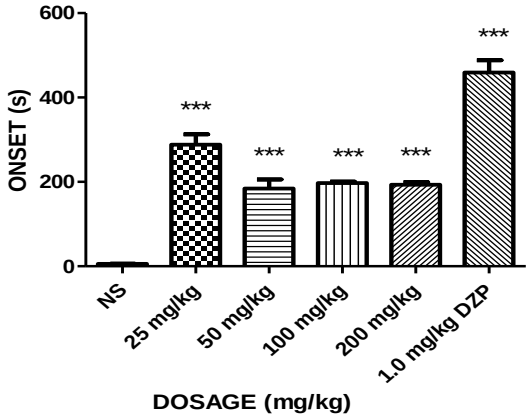

Figure. 2: Dichloromethane extract of *A.africana* of PTZ method graph showing delayed onset of animal seizure. Data analyzed using Newman-Keuls Multiple Comparison Test showed, *** denotes statistically significant (*P* =.01). NS = Normal control, DZP = Diazepam.

Table 2. Dichloromethane extract of *A.africana* effect on motor coordination

Treatment	Dose	Rota rod performance (s)	% inhibition of motor coordination
NSt	10 mL/kg	120 .0 ± 0.0	0
PHB	60 mg/kg	85.0 ± 24.0	29.2
DEAA1	25 mg/kg	37.8 ± 7.4	68.5*
DEAA2	50 mg/kg	53.8 ± 10.3	55.2
DEAA3	100 mg/kg	59.8 ± 18.0	50.2
DEAA4	200 mg/kg	63.3 ±22.7	47.3

Values are presented as the Mean ± SEM (n = 4).NSc= normal salinec ontrol, PHB = phenobarbitone, DEAA = dichloromethane extract of *Aspilia africana*; * denotes Significant *P* =.05 compared with the normal saline control group (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test).

DISCUSSION

Dichloromethane extract of *A.africana* leaf prove potent against seizure at 50 % protection in both 25 mg/kg and 50 mg/kg of as shown in table 1 above. This study outcome contrast an evaluation of anticonvulsant activity study by Kar *et al.*, (2014) as well as Gollapalle *et al.*, (2016) and similar to (Nazifi *et al.*, 2017). The anticonvulsant indication can be said to be due to its phytoconstituents inclusion, (Oko and Agiang, 2011) with flavonoids well known

scientifically for its GABAergic activity. The dose independent pattern seen in this study may be attributed to the fact that the test substance is a crude drug.

The strychnine method also show delayed onset of seizure with least delayed onset period of 180 s with 25 mg/kg as the highest delayed onset period of over 200 s as shown in figure 1 above with statistical significant (P = .05) when compared with the normal control group. From the figure 1 above, the standard group and the highest response of the test group, 25 mg/kg rated almost same because of the suggestive mechanism of action of strychnine, implicating glycine mediation rather GABA potentiation as mostly seen in some other chemical agents used in seizure stimulation. This study outcome appears similar to anticonvulsant evaluation study by (Nazifi *et al.,* 2017).

The pattern of anti-seizure response in the PTZ method, doses as seen in figure 2 above is a suggestion that the anti-seizure potential in a dose-independent order as seen in other methods' outcomes as such may be very difficult for the locals to detect its anti-seizure potency especially with an un-accessible solvent like dichloromethane. However, this also border on the insight of the likely mechanism of action even as a crude drug.

The crude drug, *A.africana* leaf extract tested positive to reduced motor coordination using rota rod means of evaluation complimenting the various models of seizure evaluation. The mean value of the pretreated groups with *A.africana* leaf extracts showed calmness among the doses with 25 mg/kg with statistically significant (P = .05) compared to the normal control (see table 2 above). Further expression was made by estimating percentage of motor coordination inhibition which reflects similar to the mean score. This is a clear pointer or indication of anxiolytic, sedative and antiseizure potentials with the earlier not scientifically measured in this study but

apparent. It is also interesting to note that the crude drug reduced muscular tone was more pronounce in the test substances (dichloromethane extract of *A.africana*) than the standard drug, phenobarbitone see table 2 above. The mean value of the motor evaluation with the rota rod also shows dose independence. This can be attributed to the fact that the test substance are not pure compounds corresponding with antiseizure models used in this study and several other plant extracts studies especially similar to that of the Nazifi *et al.,* (2017) including other studies with similar pattern of outcome compared to an evaluation of central nervous system effect study by Lidianne *et al.,* (2010) as well as *in vivo* sedative and muscle relaxants activity study by Rauf *et al.,* (2015).

CONCLUSIONS

The study has indicated scientific evidence of the presence of antiseizure potentials in dichloromethane extract of *A.africana* leaf as well reduced motor coordination pointing to muscular relaxant potential. The study has also expressed *A.africana* leaf extract to be sensitive to seizure control at the lowest dose 25 mg/kg in the electrical and chemical methods as well as reduced motor coordination.

COMPETING INTERESTS

The authors declare no conflict of interest.

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