Original Research Article

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

ABSTRACT

Aim: The study was carried out to determine the anticonvulsant potential of dichloromethane leaf extract of *Aspilia africana*. **Study Design:** Three seizure models namely: pentylenetetrazole (PTZ), strychnine (STC) and maximal electroshock (MES) were used, motor coordination behavior of the animals was also assessed. **Place and Duration of Study:** The study was carried out between the months of October and November, 2017 at Pharmacology laboratory, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria. **Methodology:** The powdered leaves of *A. africana* (2000 g) were extracted with 10 liters of 100% dichloromethane. Doses of 25, 50, 100 and 200 mg/kg were administered to the test animals. **Results:** The chemical models, STC & PTZ showed 50% & 100% survival respectively. The delayed onset of seizure suggested anti-seizure potential of dichloromethane extract of *A. africana* leaf with the maximal latency period of seizure recorded in the 25 mg/kg dose in these chemical models, this was significant, $P = .05$ compared to the normal control. The MES method showed that at doses of 25 & 50, 100 mg/kg, the animals were 66.7% & 50% free of hind limb tonic extension (HLTE). Furthermore, there was reduced motor co-ordination potential at 25 mg/kg by 68.5%, this was significant, $P = .05$, compared to normal control. **Conclusion:** From the foregoing, it can be seen that the dichloromethane leaf extract of *A. africana* has antiseizure potential

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 [1].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 [2, 3].

It is the third most common chronic neurological disorders characterized by seizures after stroke and Alzheimer's disease [4, 5]. 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 [6, 7]. The prevalence of epilepsy in Nigeria is about 6.2 to 20.8 per 1000 [8, 9]. 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 [10, 5, 11, 6]. 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 [12]. Various medicinal plants are known for their anticonvulsant value and their extracts are 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 [6].

The plant *Aspilia africana* (wild sunflower), also called iodine or haemorrhage plant because of its characteristics, is said to be an *Asteraceae* species and was evaluated for its potential chemoprotective bioactivities among others.

Justification to the Study

No available literature indicating antiseizure potential as well as other related behavioural changes evaluation of non-polar dichloromethane extract of *A.africana*leaf exists as at the time of this study. Thus, its polar aprotic property suggests flavonoids, and tannins pull as revealed in literatures. The study was established following the antiepileptic local claim of *A.africana* leaves among the Joinkrama People of the Engenni ethnic nationality of Ahoada West Local Government Area of Rivers State, Nigeria.

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 were prepared and deposited at the Herbarium Unit of the same department, NDUP 098. Fresh leaves of *A.africana* were collected, air-dried for 14 days and made powder using industrial grinder. The powdered leaf of *A. africana* (2000 g) was extracted cold in 10 liters dichloromethane (100%). The mixture was filtered and the filtrate concentrated using a rotary evaporator at maximum temperature of 45°C. 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 dichloromethane extract of *A. africana* leaf was stored in the refrigerator at 4°C till needed for use. The study dose was established based on preliminary data that proof ratio 1/33 of the LD50 (6.6 g/kg) safe in mice [13, 14] using intraperitoneal route (i.p); hence 200, 100, 50 and 25 mg/kg applied in the study. The semi-solid paste of the dichloromethane extract was dissolved in 20% of tween80 and as such, the subjects in control group received 20% tween80 normal saline.

Experimental animals

One hundred and eight (108) 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 [15, 16] were followed in this study. The

research study approval was subjected to the Postgraduate Research Ethical Committee, University of Port Harcourt, Nigeria.

Administration of extract

The extract was administered to the male mice respectively through the i.p route. The volume of extract 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 as it bypasses enteral route metabolic effects (first pass effect). 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 as well as maintain uniform route of administration for both extract and the chemical substances [17].The dichloromethane extract as non-polar extract was made i.p applicable via dissolution of the semi-solid extract in 20% tween80 including 20%tween80 normal saline for control group.

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*.,[18].

Maximum electroshock induced convulsion in mice

The method of Swinyard and Kufferberg [19] and Browning [20] was employed. Thirty six (36) male mice were randomly allotted into six groups of six mice each (n=6). The first group receivedi.p 10 mL per body weight 20% tween80 normal saline and the second group received i.p60 mg/kg phenobarbitone (analytical grade) while the third, fourth, fifth and sixth group 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 protection from electroshock [21].

Strychnine-induced convulsion in mice

The method of Porter *et al.*,[22] was employed. As stated in the MES method earlier, the first group received i.p 10 mL per body weight 20% tween80 normal saline and the second, third, fourth, and fifth group receive i.p 25, 50, 100 and 200 mg per body weightand the sixth group received i.p1.0 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 anticonvulsant indicator in strychnine-induced convulsions.

Pentylenetetrazole (PTZ) induced convulsion in mice

The method of Swinyard *et al.*, [23] was employed. As treated in the STC method, thirty minutes later, male 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 the extract's ability to abolish the pentylenetetrazole seizure stimulation.

Motor coordination evaluation

Test for motor coordination and motor planning potential of *A.africana* leaf extract of dichloromethane in thirty six (36) male mice was adopted from Swinyard and Kufferberg [19]

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 extract 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

Survival rate

The chemical methods of seizure stimulation revealed survival rate of the subjects from seizure exposure for at least 24 h. The PTZ seizure survival rate suggests 100% protection in lowest dose, 25mg/kg of *A.africana* leaf extract of dichloromethane as seen in the figure 1 below showing survival rate in dose descending order. The STC method of seizure stimulation revealed a unique pattern in the survival rating of the subjects exposure to seizure unlike the PTZ method, survival was observed in the highest dose of the plant extract at 25% protection over 24 h with 200 mg/kg while 25, 50 mg/kg rating 50% survival along the standard group as seen in figure 2.

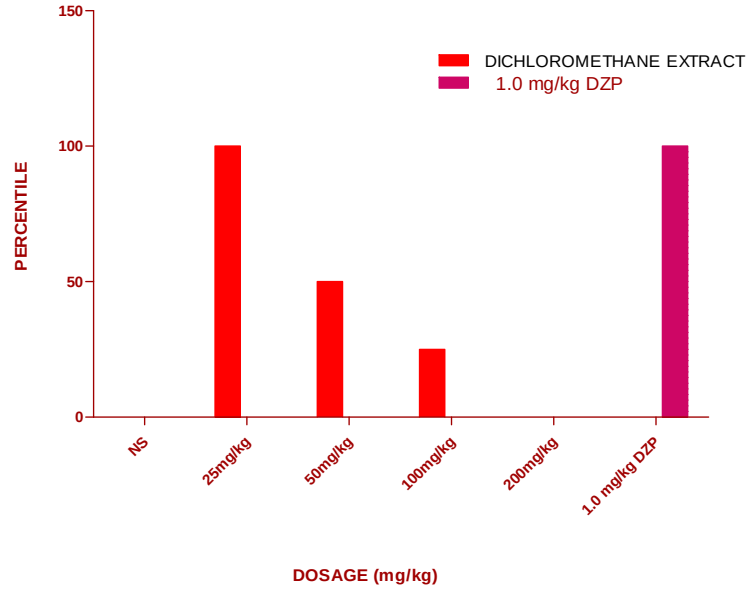


Figure 1: Survival rate after PTZ induced convulsions at different doses of dichloromethane extract of *A.africana* leaf , NS = 20% tween80 Normal saline, DZP = Diazepam.

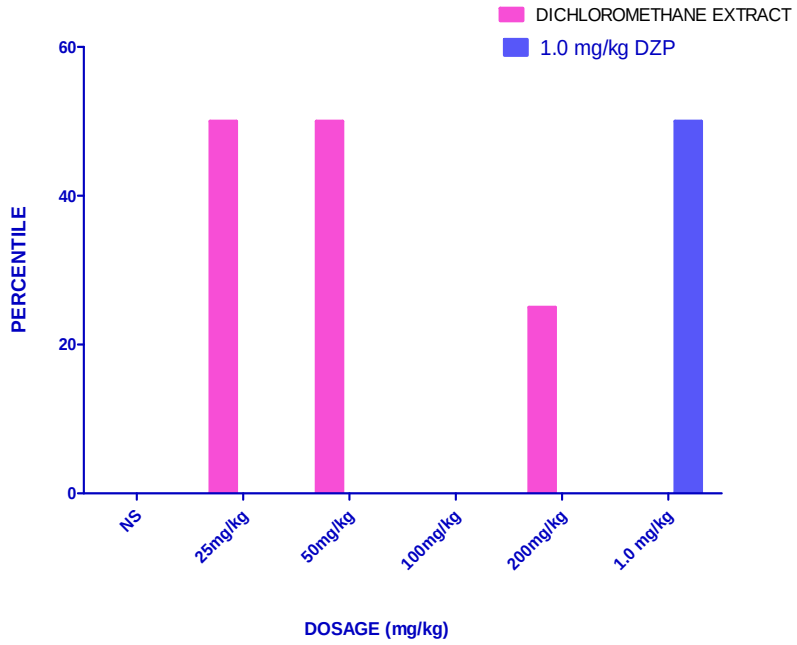


Figure 2: Survival rate after STC induced convulsions at different doses of dichloromethane extract of *A.africana* leaf. NS = 20% tween80 Normal saline, DZP = Diazepam.

Maximal Electro-Shock

The plant extract was evaluated using the incidence of HLTE seizure revealing 66.7% protection of seizure in the lowest dose, 25 mg/kg of dichloromethane extract of *A.africana* leaf with the mid doses,50, 100 mg/kg suggesting 50% protection against HLTE seizure, see table 1 below.

Table 1. MES evaluation of dichloromethane extract of *A.africana* leaf.

Treatment	Dose	Incidence of HLTE seizure (%)
NS	10 mL/kg	100
PHB	60 mg/kg	0*
DEAA1	25 mg/kg	33.3*
DEAA2	50 mg/kg	50
DEAA3	100 mg/kg	50
DEAA4	200 mg/kg	66.7

N=6, * denotes significant *P* =.05 compared to normal control value, NS= 20 % tween80Normal saline, PHB = Phenobarbitone, DEAA = Dichloromethane extract of *A.africana.*

Seizure latency period

The dichloromethane extract of *A.africana* leaf was evaluated of latency period using the chemical method of seizure stimulation. The STC method showed unique pattern in figure 3 below similar to figure 2 above. The figure 3 revealed decreased latency periods in dose

depended pattern except at the high dose, 200 mg/kg. The test group, 25 mg/kg and standard group responded equally linked to the glycine receptor inhibitory mechanism of STC in addition of the fact that the test substance is un-purified and doesn't suggest glycine/acetylcholine substrate. Figure 4 showed dose independence pattern with the highest latency period of over 300 s seen at the lowest dose yet.

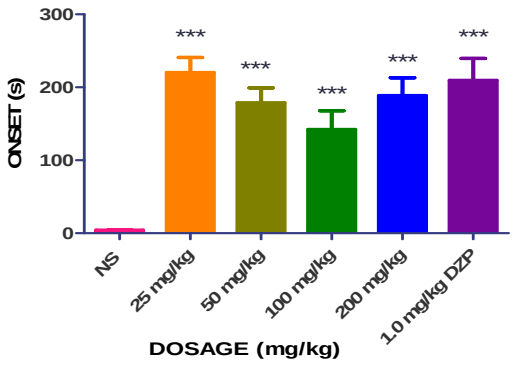
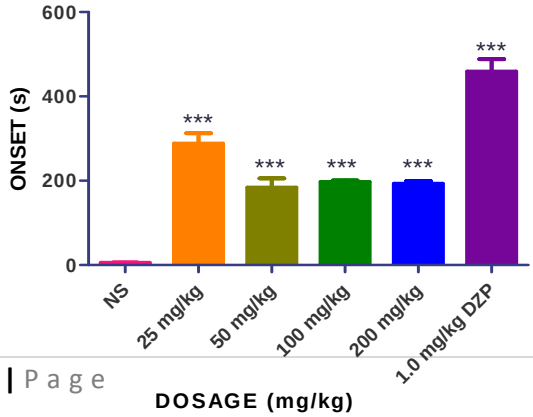


Figure 3: Effects Effects of dichloromethane extract of *A.africana* leaf on STC induced seizure onset. Data analyzed using Newman-Keuls Multiple Comparison Test showed,*** denotes statistically significant (*P*=.01). S = Seconds, NS = 20% tween80 Normal saline, DZP = Diazepam.



Figures 4: Effects of dichloromethane extract of *A.africana* leaf on PTZ induced seizure onset. Data analyzed using Newman-Keuls Multiple Comparison Test showed, *** denotes statistically significant (*P* =.01). S = Seconds, NS = 20% tween80 Normal saline, DZP = Diazepam.

Table 2. Motor coordination evaluation of dichloromethane extract of *A.africana* leaf.

Treatment	Dose	Rota rod performance (s)	% inhibition of motor coordination
NS	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

N = 6. Values are presented as the Mean ± SEM, NS = 20% tween80 normal saline, 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 100% (PTZ method) and 50% (STC method) survival rate within 24 h of exposure remarkable in the lowest dose. 25 mg/kg as shown in figure 1 & 2 above. The MES method suggests protection against HLTE seizure at66.7% observed in the lowest dose, 25 mg/kgand 50% inboth mid doses50 mg/kg and 100 mg/kg, see table 1. This study outcome contrast an evaluation of anticonvulsant activity

study by Kar *et al.,* [24] as well as Gollapalle *et al.,* [25] and similar to Nazifi *et al.,*[6].The latency period was further used to evaluate the anti-seizure potential of *A.africana* leaf extract of dichloromethane using the strychnine and pentylenetetrazole methods also show delayed onset (increased latency period) of seizure with the least delayed onset period of 180 s observed in the mid dose,100 mg/kg. The lowest dose, 25 mg/kg revealed the highest delayed onset period of over 200 s as shown in figure 3 above with statistical significant (P = .05) when compared with the normal control group. The pattern of response observed in figure 3 & 2 above, showed a unique resemblance of "U" shape pointing to increase latency period response in descending dose order with sudden increase latency period of seizure in the highest dose, 200 mg/kg with 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 receptor inhibition rather than GABA receptor inhibition as mostly known of some other chemical agents used in seizure stimulation. The preliminary study and survival/lethal evaluation of the plant extract indicated gross mortality at higher doses above 200mg/kg and survival at low dose less than 200 mg/kg (see figure 2) which also suggests body weight of the animal subject (mice) is incapable of accommodating high dose of the dichloromethane extract of *A.africana* leaf in the STC method contrary to recent study by Kemelayefa and Kagbo [26] but the figure 3 response is similar to a PTZ method study by Nazifi *et al.*,[6].Furthermore, the pattern of anticonvulsant potential can be said to be due to *A.africana* leaf phytoconstituents, [13] 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 crude drug, unpurified as such is subject to biochemical compromises. This study outcome appears similar to anticonvulsant evaluation study by Nazifi *et al.*,[6];Kemelayefa and Kagbo [26].The crude drug, *A.africana* leaf extract of

dichloromethane tested positive to reduced motor coordination using rota rod means of evaluation complimenting the various methods of seizure evaluationin this study. The mean value of the treated groups with *A.africana* leaf extract of dichloromethaneshowed calmness among the doses with 25 mg/kg 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 appears similar to the mean score. This is a clear pointer 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 reduced muscular tone was more pronounce in the test substance (dichloromethane extract of *A.africana* leaf) than the standard drug, phenobarbitone see table 2 above. The mean value of the motor evaluation also showed dose independence. This can be attributed to the fact that the test substance are not pure compounds corresponding with antiseizure group of drugs recommended per seizure model used in this study and several other plant extracts studies especially similar to that of the Nazifi *et al.*, [6];Kemelayefa and Kagbo [26] including other studies with similar pattern of outcome compared to an evaluation of central nervous system effect study by Lidianne *et al.*,[27] as well as *in vivo* sedative and muscle relaxants activity study by Rauf *et al.*,[28].

CONCLUSIONS

The study suggests antiseizure potential of *A.africana* leaf extract of dichloromethane as well as reduced motor coordination pointing to muscular relaxant potential as evidenced scientifically. 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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