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Evaluation of Anticonvulsant Properties of Methanol Aerial Extract of *Bryophyllum Pinnatum* (Lam.) Oken (Crassulaceae) In Mice and Chicks

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Bryophyllum pinnatum* is an environmental weed from the Crassulaceae family that is often used to treat convulsion, hypertension, diarrhea, insect bites, asthma and other ailments.

Objectives: To evaluate the anticonvulsant potential of methanol aerial extract of *Bryophyllum pinnatum* plant in mice and chicks.

Materials and Methods: The anticonvulsant potential was studied using Maximal electroshock Test (MEST) and pentylenetetrazol (PTZ) test in one day old chicks and mice at 250, 500 and 1000 mg/kg body weight of the extract *i.p* respectively. Positive control drugs used were sodium valproate (200 mg/kg) and phenytoin (20 mg/kg) in PTZ and MEST respectively while distilled water (10 ml/kg) *i.p* was used as negative control in all experiments.

Results: The intraperitoneal LD_{50} of the extract was found to be greater than 5000 mg/kg body weight with an indication that the extract is relatively safe. Significant ($p \leq 0.05$) prolongation of the mean onset of seizures was recorded with the extract at 1000 mg/kg body weight compared with normal saline treated group in PTZ induced seizures. *Bryopyllum pinnatum* methanol aerial extract also significantly reduced the mean recovery time of seizures at doses 500 and 1000 mg/kg induced by MEST when compared with normal saline treated group. Conversely, a significant ($p \leq 0.05$) delay in the mean onset of seizures was recorded with standard drugs, sodium valproate (200 mg/kg) and phenytoin (20 mg/kg) in PTZ and MEST respectively.

Conclusion: Methanol extract of the aerial part of *Bryophyllum pinnatum* possesses anticonvulsant activities.

Keywords: *Bryophyllum pinnatum*, Anticonvulsant, Epilepsy, Maximal electroshock, Pentylenetetrazol.

INTRODUCTION

Epilepsy is a chronic neurological disorder with a severe morbidity (Fisher *et al.*, 2005). Epilepsy is a disease that affects about 50 million people across the globe and 85% of this population resides in developing countries, it is second commonest neurological disorder. It is estimated that 0.5-1% of world population are affected by this disorder, about 85% of this population are residing in developing countries (Pedley and Kale, 1996; Sridharan, 2002). (The prevalence of epilepsy in Nigeria is 3.7- 4.1% (Banerjee *et al*., 2009). Conventional antiseizure drugs like phenobarbitone, phenytoin, sodium valproate, clonazepam among others, have been in use for a quite period of time. Even with the introduction of these antiepileptic drugs (AED), there is no known cure for epilepsy and relapse is still high (Loscher, 2002). It is therefore imperative, to search for ideal antiseizure drugs with high specificity, efficacy and tolerable side effects. The plant kingdom has become an important target in the search of lead compounds in the treatment of many neurological disorders including epilepsy (Chindo *et al.*, 2014).

Bryophyllum pinnatum (Lam.) Oken, belongs to the family Crassulaceae commonly known as air plant, miracle leaf, life plant is a perennial herb growing

widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America (Yemitan *et al.,* 2005). It usually grows 30-120 cm tall, but can sometimes reach up to 2 m in height reproduces through seeds and also vegetatively from leaf bulbils. In Nigeria, it is locally known as 'Sutura' by the Hausas in northern Nigeria, 'Abamoda' by the Yoruba's in western Nigeria and Odaa Opue by the Igbo's in eastern part of Nigeria. The leaves and stembark are bitter tonic, astringent to bowels, analgesic, carminative, and are useful in diarrhea and vomiting. Antimicrobial, antifungal, anti-ulcer, anti-inflammatory and analgesic activities of leaf extract were reported (Quazi *et al.,* 2011). The juice from fresh leaves is used to treat smallpox, otitis, cough, asthma, palpitation, headache and convulsion (Jain *et al.*, 2010). It is largely used in folk medicines for the treatment of hypertension and kidney stone (Lans, 2006); pulmonary infections and rheumatoid arthritis (Majaz *et al.,* 2011). Hitherto, there has been no scientific claim on the anticonvulsant potential of aerial part of the plant. The aim of the present study is therefore to screen the aerial part extract of the plant for anticonvulsant activity.

METHODOLOGY

Plant collection and authentication

The plant sample comprising the leaves and stem bark (aerial part) were collected from University of Ibadan botanical garden, Ibadan in Oyo State, Nigeria in March 2019 and was authenticated by Mr. Namadi Sanusi of the Herbarium Section of the Department of Botany, Ahmadu Bello University, Zaria, through comparison with herbarium reference, voucher specimen (Number 01838) was obtained.

Preparation of Extract

The aerial part of the plant was shade dried at room temperature and pulverized manually using mortar and pestle. 1.5 kg of the pulverized aerial part was macerated with methanol for 72 hours and concentrated in vacuo using rotary evaporator at 40^{o}C to afford the crude extract. The dried extract was weighed and stored for further experiments.

Experimental animals

Swiss albino mice (24 ± 6 g) of either sex were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. One-day old Ranger cockerels (32 ± 7 g) were obtained from Chi Farms Ltd along Ibadan-Lagos express way, Ibadan, Oyo state. The animals were kept in a well-ventilated condition at ambient temperature and fed with a standard animal feed with adequate access to water *ad libitum*. The experimental animals used were handled in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No.80-23) revised in 1996.

Drugs, chemicals and equipments

Methanol, pentylenetetrazol (*Sigma chemical Co., St. Louis, USA*), sodium valproate, phenytoin (*Parker-Davis and Co. Ltd*), Electroconvulsive machine (*Ugo Basile, Model 7801, Italy*), analytical balance (*Mettler Instrument Corporation, U.S.A*.) and Soxhlet apparatus.

Acute toxicity studies

The intraperitoneal LD_{50} was determined in mice and chicks respectively. The method of Lorke (1983) was adopted for the study. This method was carried out in two phases. In the first phase, 9 mice and chicks were divided randomly into 3 groups of 3 mice and chicks each. Varying doses of the extract (10, 100 and 1000 mg/kg body weight) were administered intraperitonially (*i.p.)* to groups 1, 2 and 3 respectively and observed for 24 hours for any sign of toxicity and mortality. In the second phase, three groups with one mouse each were treated with doses of 1600, 2900 and 5000 mg/kg of the extract intraperitonially (*i.p.)* based on the result of the first phase and observed for signs of toxicity and death. The median lethal dose was estimated as a geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

Anticonvulsant studies

Maximum electroshock test in chicks

The methods of Swinyard and Kupferberg (1985) was employed. 50 Day-old chicks weighing between 24 g and 39 g were randomly divided into five groups of ten chick each. The first group was treated with normal saline 10 ml/kg *i.p*. The second, third and fourth groups were administered with 250 mg/kg, 500 mg/kg and 1000 mg/kg doses of the extract *i.p* respectively, while the fifth group was treated with phenytoin 20 mg/kg *i.p*. as positive control. Thirty (30) minutes post treatment with phenytoin and 60 minutes post-treatment with the extract, electroshock was administered to each animal via the corneal electrode to induce convulsion. The current, shock duration, frequency and pulse width were maintained at 80 mA 0.8 sec, 100 pulse per second and 0.6 ms respectively. The durations of tonic hind-limb extension (THLE) were noted and recorded. Chicks

that failed to produce THLE were considered protected.

Pentylenetetrazol-induced convulsion test in mice

The method of Swinyard *et al*., (1989) was employed. Thirty (30) mice of both sexes weighing between 17g and 30 g were randomly divided into five groups of six (6) mice each. Mice in group I were treated with normal saline 10 ml/kg *i.p*. The second, third and fourth groups were treated with 250, 500 and 1000 mg/kg doses of the extract respectively, while the fifth group was treated with sodium valproate (200 mg/kg, *i.p*). Thirty minutes post treatment, mice in all groups received 90 mg/kg body weight of freshly prepared PTZ subcutaneously. Each mouse was observed for 30 minutes for onset of seizures. Episodes of clonic spasm for at least 5 seconds was considered as convulsions. The absence of clonic spasm during the 30 minutes of observation was regarded as protection against PTZ induced convulsions.

Statistical analysis

Data were analyzed using SPSS version 20. Results were expressed as mean ± standard error of mean (SEM). Differences between the means were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparison. *p values* of ≤ 0.05 were considered significant. SPSS version 20 was used for the analysis.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the crude methanol aerial extract of *Bryophyllum pinnatum* revealed the presence of alkaloids, steroids, triterpenes, flavonoids, tannins, saponins, glycosides and flavonoids (Table I).

TABLE I: Phytochemical constituents of methanol aerial extracts of *Bryophyllum pinnatum*

CONSTITUENTS	INFERENCE
FLAVONOIDS	
a. Sodium Hydroxide test	+
b. Shinoda test	+
ALKALOIDS	
a. Dragendorff's test	+
GLYCOSIDES	
a. Keller-Kelliani's test	+
STEROIDS	
a. Salkowski test	+
TRITERPENOID	
a. Lieberman Burchard	+
SAPONINS	
a. Frothing	+
TANNINS	
a. Ferric chloride	+

Acute toxicity study

The intraperitoneal LD_{50} of the methanol extract was found to be above 5000 mg/kg body weight.

Effect of methanol aerial extract of *B. pinnatum* on maximal electroshock test in chicks

The methanol aerial extract of *Bryopyllum pinnatum* reduced the mean recovery time of seizures significantly ($p \leq 0.05$) at doses 500 and 1000 mg/kg induced by MEST when compared with normal saline treated group. The standard phenytoin provided 100% protection against tonic hind limb extension (THLE) induced by maximal electroshock seizure (Table 2).

TABLE 2: Effect of methanol aerial extract of *B. pinnatum* on maximal electroshock test in chicks

Treatment (mg/kg)	Mean Recovery Period (Min)	Quantal Protection	% Protection
NS 10 ml/kg	9.8 ± 0.77	0/10	0.00
MAE 250	8.2 ± 0.61	0/10	0.00
MAE 500	6.8 ± 0.71*	5/10	50.00
MAE 1000	5.2 ± 0.49*	8/10	80.00
PHT 20	----------	10/10	100

Data were analyzed using one-way ANOVA. Values are expressed as Mean ± SEM, n=10, NS= Normal Saline, MEA = Methanol aerial extract of *Bryopyllum pinnatum*, PHT = Phenytoin, SEM= Standard Error of Mean, * = $p \leq 0.05$ compared with Normal Saline.

Effect of methanol aerial extract of *B. pinnatum* on pentylenetetrazol-induced seizures in mice

Bryopyllum pinnatum extract produced a significant ($p \leq 0.05$) increase in mean onset of seizures at 1000 mg/kg. Similarly, sodium valproate showed 100% protection against PTZ-induced convulsion (Table 3).

TABLE 3: Effect of methanol aerial extract of *B. pinnatum* on Pentylenetetrazol-induced seizures in mice

Treatment (Mg/Kg)	Mean Onset Of Seizures (Min)	Quantal Protection	% Protection
NS 10 ml/kg	10.50 ± 2.17	0/6	0.00
MAE 250	11.67 ± 3.27	0/6	0.00
MAE 500	12.33 ± 4.89	1/6	16.67
MAE 1000	20.67 ± 3.20*	3/6	50.00
SV 200	25.50 ± 0.71*	6/6	100

Data were analyzed using one-way ANOVA. Values are expressed as Mean ± SEM, n=6, NS = Normal Saline, MEA = Methanol aerial extract of *Bryopyllum pinnatum*, SV = Sodium Valproate, SEM= Standard Error of Mean, * = $p \leq 0.05$ versus N/Saline

DISCUSSION

Phytochemical screening of methanol aerial extract of *B. Pinnatum* revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and triterpenoid which might be responsible for the anticonvulsant activities observed because these phytochemicals from other plants have been reported to have anticonvulsant property in animal models of epilepsy like PTZ and MES (Mishra *et al.*, 2011). The intraperitoneal LD_{50} value of the aerial extracts of *B. Pinnatum* was found to be greater than 5000 mg/kg body weight with an indication that the extract is practically safe (Lorke, 1983). Furthermore, the doses of the extract used in this study were lower than 30% of the LD_{50}. These doses are relatively safe for ethnopharmacological research (Vongtau *et al*, 2004). The maximal electroshock (MEST) model identifies drugs that are likely to be effective in the management of generalized tonic clonic seizures (Magaji *et al*, 2013). Agents that are active against the tonic hind limb extension (THLE) induced by MEST act by limiting the spread of seizures (Porter and Meldrum, 2018). Drugs such as phenytoin, lamotrigine and carbamazepine have been shown to abolish tonic hind limb extension in MEST test primarily by prolonging the inactive state of Na^+, consequently, preventing the repetitive firing of the neurons. Ability of the extract to significantly reduce mean recovery time of seizures and protect at least 50% of the chicks against MEST suggests that it might possess compounds with ability to abolish seizure spread. This further suggests that it may be of value in the treatment of generalized tonic clonic and partial seizures.

On the other hand, the PTZ seizure model screens agents with activity against petit mal epilepsy. Antiseizure drugs such as phenobarbitone, benzodiazepines, ethosuximide, and sodium valproate are active against seizures induced by PTZ. Drugs that abolish petit mal epilepsy act by enhancing $GABA_A$ inhibitory action and block T-type Ca^{2+} current (Malawska, 2005). The methanol aerial extract of *B. Pinnatum* showed a dose-dependent increase in the mean onset of seizures in the PTZ model an indication of the extract's ability to

increase seizure threshold and therefore, may be effective in the therapy of absence seizures.

CONCLUSION

The result obtained above clearly showed that the methanol aerial extract of *Bryophyllum pinnatum* contained bioactive substances that are useful in the treatment of absence seizures and further provides scientific justification for its use in ethnomedicine against convulsions.

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