



Toxicity Study and Anticonvulsant Effect of Ethanol Leaf Extract of *Piliostigma thonningii* Milne-Redhead (Fabaceae)

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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: *Piliostigma thonningii* (Schumach) Milne-Redhead [Fabaceae] is a plant widely used locally for the treatment and management of several ailments which include epilepsy in Northeastern Nigeria.

Objectives: This study aimed at evaluation of the toxicity and anticonvulsant effect of ethanol leaf extract of *Piliostigma thonningii* in rats and mice with a view to determining the efficacy of the plant as an anticonvulsant drug.

Methods: Fresh leaves of *Piliostigma thonningii* were air-dried, pulverized and extracted using soxhlet extraction apparatus. Acute toxicity study was carried out by Lorke's method and the anticonvulsant activity of the ethanol leaf extract was carried using pentylenetetrazole and strychnine-induced convulsion model on Wistar strain albino rats and mice respectively.

Result: The soxhlet extraction yielded 21.04% ^{w/w} of extract after being concentrated. The oral and intraperitoneal LD₅₀ were ≥ 5000 mg/kg implying that the extract is relatively safe according to literatures. Anticonvulsant effect of the ethanol leaf extract using pentylenetetrazole (PTZ), revealed the ability of the extract to confer protection on rats treated with doses of 200, 400 and 600 mg/Kg bd. wt. by exerting 60%, 80% and 80% protection on rat against PTZ induced convulsion respectively in a dose dependent manner as well as protected 20%, 60% and 80% of mice against death induced by strychnine when treated with 100, 200 and 400 mg/kg of ethanol extract.

Conclusion: The ethanol leaf extract of *Piliostigma thonningii* was able to provide anticonvulsant effect and is relatively safe for consumption as medicine.

Keywords: Phytochemicals; Acute Toxicity; Strychnine; Pentylenetetrazole; Anticonvulsant.

INTRODUCTION

Plant products have been part of phytomedicines since time immemorial. The use of medicinal plants in West Africa is probably as old as the duration of human settlement in the region (Abdulrahman *et al.*, 2010; Sodipo *et al.*, 2011). These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Newman and Cragg, 2001). The pharmacological properties of African medicinal plants are immense: remedies made from plants play an important role in the health of millions of people especially in the rural areas (Grabley and Thiricke, 1999). The availability, low cost and accessibility of these plants in Tropical and Sub-tropical Africa

coupled with the global crisis of drug resistance incidences, adverse effects amongst other appalling negative scientific reports of conventional drugs make it convenient for in-depth survey of medicinal plants from this part of the world (Usman *et al.*, 2009).

Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax quinones (Iwu, 1993; Asaolu, 2003).

Piliostigma thonningii (Schumach) Milne-Redhead [Fabaceae] is a plant used for medicinal purposes in many African countries. Different parts of the plant have been used traditionally for the treatment of various diseases in humans and animals (Djuma, 2003). For example, the roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections (Iwalewa *et al.*, 1990; Jimoh and Oladeji, 2005). The leaf is useful in the treatment of malaria fever (Akinniyi and Sultanbawa, 1983; Rabo and Sanusi, 2001). The leaves of *Piliostigma thonningii* are used to treat wounds, chronic ulcers, diarrhoea, toothache and gingivitis, cough, and bronchitis (Watt and Breyer-Brandwijk, 1962). The dry leaf powder has been reported to contain alkaloids, saponins, flavonoids and tannins (Stahl, 2005).

Toxicology is the science that deals with the study of the adverse effects caused by chemicals or physical agents in living organisms under specific conditions of exposure (Doull *et al.*, 2008). Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of the test item and to estimate safety margins (Robbinson *et al.*, 2009). Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which

may be due to over dosage or certain factors and these may lead to toxicity and death (Okigbo *et al.*, 2009). Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime (Sander and Shorvon, 1996). The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy (Mattson, 1995).

Despite of the use of *Piliostigma thonningii* in traditional medicine by the local people in the North-Eastern part of Nigeria in the management of epileptic conditions, only a few studies have been carried out to evaluate the convulsant activity and the safety of the leaves of *Piliostigma thonningii*, given its rich phytochemical composition. It is, therefore, pertinent to examine the phytochemicals responsible for the plant's medicinal uses in Tropical Africa particularly those found in the Arid and Semi-Arid region of Nigeria.

This study was carried out to evaluate the acute toxicity and anticonvulsant efficacy of ethanol extract of the leaf *Piliostigma thonningii*.

METHODOLOGY

Sample Collection, Identification and Preparation

Fresh leaves of the plant, *Piliostigma thonningii* were collect from Jumu'a, a village in Potiskum, Yobe State, Nigeria. The leaf was identified and authenticated to be *Piliostigma thonningii* by a Plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. It was given a voucher specimen number 548A and deposited at the Postgraduate Research Laboratory of the Department of Chemistry, Faculty of Science, University of Maiduguri.

The leaves were cleaned by hand picking foreign materials and air-dried under shade at room temperature for seven days and pulverized using mortar and pestle and then subjected to the following analysis.

Plant Extraction

Eight hundred grammes (800 g) of the powdered leaves was exhaustively soxhlet extracted using ethanol as solvent. The extract fraction was concentrated to dryness by slow evaporation process at room temperature. It was weighed, labelled and kept in a desiccator until required.

Experimental Animals

All the experiments performed on laboratory animals in this study followed the standard procedure for the treatment of animals. Ethical approval was obtained on 30th 03, 2015 from the University of Maiduguri Teaching Hospital by the Research and Ethical Clearance Committee. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals, CIOMS and ICLAS, (2012).

A total of forty-eight albino rats (100-180 g) and twenty-five (25) mice of both sexes were purchased from the animal house of the Faculty of Pharmacy, University of Maiduguri, Borno State. They were housed in clean plastic, well-ventilated cages with saw dust as beddings under 12 hours light/12 hours dark cycle conditions of normal room temperature and humidity in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for the analysis. They were fed with standard fed (ECWA, Jos) and allowed water *ad libitum*.

Extract Preparation

Ethanol leaf extract of *Piliostigma thonningii* (2 g) was dissolved in 10 ml distilled water, to give a stock solution of 200 mg/ml.

$$\text{Stock solution} = \frac{2000 \text{ mg}}{10 \text{ ml}} = 200 \text{ mg/ml}$$

$$\text{Volume to be administered} = \frac{\text{Dose} \times \text{Body Weight in kg}}{\text{Concentration of the Extract in mg}}$$

Acute Toxicity Evaluation (LD₅₀)

The acute toxicity (LD₅₀) of the crude leaf ethanol extract of *Piliostigma thonningii* was determined using standard conventional procedure as described by Lorke (1983). In this study, two different routes of administration were considered; the oral and intraperitoneal. In phase I, rats were divided into 3 groups of three rats each for each route (a total of nine rats) and then treated with the crude ethanol extract at doses of 10, 100 and 1000 mg/kg bd. wt. intraperitoneally and orally and observed for 24 hours for mortality. In the phase II, the animals of each group (for each route) were divided into three groups of one animal each and the ethanol extract was administered at doses that were determined after the phase I. The rats were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The LD₅₀ was then calculated using the formula:

$$\text{LD}_{50} = \sqrt{a \times b}$$

Where a = least dose that killed a rat
b = highest doses that did not kill a rat

Pentylentetrazole Induced-convulsant in Rats

The methods of Nwafor (1998) was adopted for this experiment with some modifications. Adult rats of both sexes were used for this experiment, food was withdrawn 12 hours before the experiment, but water was made available *ad libitum* until the start of the experiment. The rats were randomly divided into five groups of five rats each and treated as follows: group A rats were given a convulsive dose of pentylentetrazole (PTZ) (60 mg/kg bd. wt. *s.c.*) Groups B-D received doses of 200, 400 and 600 mg/kg

RESULTS

Extraction Profile of *Piliostigma thonningii* Leaf

The extraction of the leaf of *Piliostigma thonningii* using ethanol produced extract with 21.04% weight

b. wt.) of the ethanol extract (*i.p.*), pentylentetrazole (100 mg/kg) was injected subcutaneously (*s.c.*) on the back of the neck of the rats after 30 minutes while group E were treated with 200 mg/kg of Sodium Valproate. Seizures manifested as tonic convulsions (tonic hind limb extension). The ability to prevent this feature or prolong the latency or onset of the tonic hind-limb extension over a 24-hour period was taken as indication of anticonvulsant activity. The onset of tonic convulsions and number of mice presenting convulsions per minute and the duration of convulsions was recorded. Rats that did not show tonic hind limb extension during the period of observation were considered as not having convulsed. The rats were also monitored for instances of death up to 24 hours after the experiment.

$$\% \text{ Survival} = \frac{\text{Number of survived animals}}{\text{Total Number of Animals}} \times 100$$

Strychnine-induced Convulsion in Mice

The method used is as described by Lehmann *et al.* (1988). In brief, strychnine convulsions followed by death was induced in mice by the subcutaneous injection of 1mg/kg of strychnine nitrate. Thirty minutes prior to administration of strychnine three groups of 5 mice each were intraperitoneally pretreated with ethanol extract of *Piliostigma thonningii*. The fourth group was treated with phenobarbitone sodium (20mg/kg *i.p.*) which served as the positive control while the fifth group received normal saline 10ml/kg as the negative control. Mice were observed for tonic extensor jerks of the hind limbs followed by death in 30 minutes. Abolition of tonic extensor jerks of the hind limb was considered an indicator that *Piliostigma thonningii* could prevent strychnine-induced seizures (Raza *et al.*, 2001).

Statistical Analysis

Results of pharmacological study was analysed using GraphPad Prism version 8.0 2016 Model for windows One-way Analyses of Variance (ANOVA) test followed by Tukey-Kramer's Multiple Comparison test was used to analyse and compare the results at 95 % confidence level. Values of $p < 0.05$ was considered significant. Results were expressed as mean \pm standard error of mean.

which was brownish in colour and gummy in texture. The result of the extraction profile is shown on Table 1.

Table 1. The extraction profile of air-dried powdered leaf of *Piliostigma thonningii*

S/N	Fraction	Mass	%Yield (w/w)	Colour	Texture
	Ethanol	148.29	21.04	Brown	gummy mass

Acute Toxicity (LD₅₀)

Table 2 represents the result of acute toxicity of *P. thonningii* on rats. No death was recorded on administration of up to 5000 mg/kg dose of the extract via both the oral and intraperitoneal routes. Though behavioural signs of toxicity were observed in rat when 5000 mg/kg of the extract was administered via intraperitoneal route which included; paw licking,

stretching and reduced activity but it revived 5 hrs after the exhibition of clinical signs. Thus, LD₅₀ of the crude ethanol leaf extract of *Piliostigma thonningii* in rats administered via both oral and intraperitoneal route were calculated as ≥ 5000 mg/kg bd. wt. which made it impossible to estimate the LD₅₀ via Lorke's method.

Table 2. Acute toxicity effect of ethanol leaf extract of *Piliostigma thonningii* on rats

Phase	Dose (mg/kg)		No. of rat	Mortality rate	
	Oral route	IP route			
I		10	3	0/3	0/3
		100	3	0/3	0/3
		1000	3	0/3	0/3
II		1600	1	0/1	0/1
		2900	1	0/1	0/1
		5000	1	0/1	0/1

Effect of Ethanol Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice

The ethanol extract of *P. thonningii* at doses of 200, 400 and 600 mg/kg bd. wt. exerted 60 %, 80 % and 80 % protection to rats against PTZ induced convulsion respectively. It was observed that the mean number of spasms of group B and D were significantly ($p < 0.05$) different from the control group. Meanwhile, the mean time of onset of convulsion increased with increasing doses of extract from 18.00 \pm 0.71 min to 29.60 \pm 0.51 min for 200 mg/kg bd. wt. to 600 mg/kg bd. wt. of the extract when compared to the negative control that had 4.00 \pm 0.32 min. There was no significance difference of the effect extract administered to animals of groups B and C. However, the mean onset of death was also dose dependent for the rats treated with 200, 400 and 600 mg/Kg of the ethanol extract. The mean onset of deaths were significantly different from the control group at $p < 0.05$. Valproic acid (200mg/kg) protected all the rats (100%) against clonic spasm induced by pentylenetetrazole as presented in Table 3.

Effect of Ethanol Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice

The crude extract of *Piliostigma thonningii* at doses of 100, 200 and 400mg/kg body weight protected 20%, 60% and 80% of mice respectively against death induced by strychnine. However, crude extract of *Piliostigma thonningii* significantly ($P < 0.05$) prolonged the onset of convulsion in a dose-dependent manner from 10.2 \pm 0.37min. In normal saline treated group by 60%, 111% and 127% at doses of 100, 200 and 400 mg/kg body weight respectively. Similarly, in the time of death, the crude extract of *Piliostigma thonningii* significantly ($P < 0.05$) prolonged the time of death of convulsed mice from 11.8 \pm 0.37min. in normal saline treated group by 52%, 103% and 129% at doses of 100, 200 and 400 mg/kg body weight respectively. Result of the study is shown in Table 4.

Table 3: Effect of ethanol leaf extract of *Piliostigma thonningii* on pentylenetetrazole induced convulsion

Extract pretreatment (mg/kg)	Mean±SEM onset of spasm (min)	Mean±SEM onset of convulsion (min)	Mean±SEM of onset of death (min)	Quantal death	Survival (%)
*control + 60 mg/kg of PTZ	9.60±1.14	4.00±0.71	13.00±2.92	1/5	20
200 mg/kg + 60 mg/kg of PTZ	18.20±5.89 ^a	18.00±1.14 ^a	44.40±1.40 ^a	3/5	60
400 mg/kg + 60 mg/kg of PTZ	28.40±2.07 ^b	24.80±2.35 ^b	50.00±0.00 ^b	4/5	80
600 mg/kg + 60 mg/kg of PTZ	28.40±2.07 ^{bc}	29.60±1.60 ^c	60.00±0.00 ^c	4/5	80
Sodium Valproate (200mg/kg)	00	0.0 0.00	0.00.00	5/5	100

*Control= Rats administered distilled water,

Values across column with different alphabets as superscript are statistically ($p > 0.05$) significant

Data presented as Mean \pm SEM, $n = 5$; *represent $p < 0.05$ by student t -test.

Table 4: Effects of Crude Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice

Treatment (mg/kg)	Mean Onset of Convulsion (min.±SEM)	Mean Time of Death (min.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	10.2±0.37	11.8±0.37	0/5	0
100	16.3±1.20*	18.0±1.53*	1/5	20
200	21.5±0.50*	24.0±1.00*	3/5	60
400	23.2±0.86*	27.0±0.00*	4/5	80
Phenobarbitone (20mg/kg)	24.0±0.00*	24.00±0.00*	4/5	80

Data presented as Mean \pm SEM, $n = 5$; *represent $p < 0.05$ by student t -test.

DISCUSSION

The observation of present study indicates that ethanol extract of *Piliostigma thonningii* possesses anticonvulsant activity in rats. There was no mortality at all dose levels of *Piliostigma thonningii* administered to the laboratory rats. The intraperitoneal and oral ≥ 5000 mg/kg b. wt. obtained in this study is about 25 times greater than the minimum effective dose of 200 mg/Kg. Earlier reports have shown that if the LD₅₀ of a test substance is three times more than the minimum effective dose; the substance is considered a good candidate for further studies (Madara et al., 2010) reported that oral administration is about 100 times less toxic than the intraperitoneal. Clarke and Clarke (1977) were of the opinion that compounds with LD₅₀ of 1500 mg/kg and above have low toxicity. The extract is therefore safe and this could explain the safe use of the plant by the local people who have been using it in traditional management of depressive illnesses in North-Eastern Nigeria (Idris et al., 2014).

The leaf extract was also observed to have an effect on pentylenetetrazole (PTZ) induced convulsion on rats. The extract appeared to reduce the convulsant effect in a dose dependent manner. It depressed the central nervous system stimulation from convulsing which agrees with the reports of Abdulrahman et al., (2005). This finding also agrees with the reports in rats using different plant extracts (Sandabe et al., 2003; Abdulrahman et al., 2005; 2007; Usman et al., 2008, Abdulrahman et al., 2012). The PTZ is a known convulsant and anticonvulsant activity in (PTZ) (s.c) test identifies compounds that can raise the seizure threshold in the brain (White et al., 1998; Raza et al., 2001). It acts by stimulation of the medulla (Franz, 1975). The ability of the plant's ethanol extract to protect rats stimulated with PTZ may be an indication of its depressant effects on both the spinal cord and brain stem. Triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as Maximal electroshock (MES) and PTZ (Chauhan et al., 1988; Kasture et al., 2002).

Some flavonoids are also reported to have protective effects against PTZ, picrotoxin and N-methyl-D, L-aspartic acid (NMDLA)-induced convulsions (Johnston and Beart, 2004).

In the strychnine-induced seizure study, it is known that strychnine directly antagonizes the inhibitory spinal reflexes of glycine (Sayin *et al.*, 1993). The ethanol extract at the dose of 200mg/kg and 400mg/kg protected 20%, 60% and 80% of the mice against strychnine-induced death. The convulsing action of strychnine is due to the interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter of the motor neurons and interneurons in the spinal cord. Strychnine sensitive

postsynaptic inhibition in higher centers of the CNS is also mediated by glycine. Strychnine acts as a selective, competitive antagonist at all glycine receptors (Rajendra *et al.*, 1997). The ability of the ethanol extract to prevent the strychnine-induced seizures demonstrate additional anticonvulsant effects mediated via glycine receptors (Ogbonnia *et al.*, 2003).

The depressant activity of the ethanol leaf extract of *Piliostigma thonningii* may be attributed to the presence of phytochemicals reported by Yakubu *et al.* (2016) which may have singly or acted synergistically with one another and might be responsible for the anticonvulsant effect.

CONCLUSION

The ethanol leaf extract of *Piliostigma thonningii* exhibited anticonvulsant activity in experimental animal models. The leaf extract had no observable

toxic effect on rats. The results of this study provide support for the traditional use of *P. thonningii* as an anticonvulsant drug.

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REFERENCES

- Abdulrahman, F.I., Akan, J.C., Sodipo, O.A. and Onyeyili, P.A. (2010). Effect of aqueous root-bark extract of *Vitex doniana* sweet on hematological parameters in rats, *J. Am. Sci.*, 6: 8-12.
- Abdulrahman, F.I., Onyeyili, P.A., Sandabe, U.K. and Ogugbuaja, V.O. (2007). Evaluation of the effects of the aqueous extract of *Vitex doniana* root-bark on the peripheral and central nervous system of laboratory animals, *J. Appl. Sci.* 7(10): 1397-1403.
- Abdulrahman, F.I., Sandabe, U.K. and Iguniwei, P.B. (2005). Anticonvulsant, antinociceptive and antipyretic effects of aqueous extract of *Terminalia avicennoides* stem bark on rats, *Sahel J. Vet. Sci.* 4(1): 43-47.
- Abdulrahman, F.I., Tijjani, M.A., Khan, I.Z. and Sandabe, U.K. (2012). Anti-inflammatory, anticonvulsant and antipyretic properties of ethanolic extract of *Vitex doniana* sweet stem bark, *Int. J. Pharm.* 3(4): 288-292.
- Akinniyi, J. A. and Sultanbawa, M.U.S. (1983). A glossary of Kanuri names of plants with botanical names, distribution and uses, *Annal Borno*, 1: 85-98.
- Asaolu, M.F. (2003). Chemical composition and phytochemical screening of the seeds of *Garcinia kola*. *Pakistan J. Sci. Ind. Res.*, 46: 145-147.
- Chauhan, A.K., Dobhal, M.P. and Joshio, B.C. (1988). A review of medicinal plant showing anticonvulsant activity, *J. Ethnopharmacol.* 22: 11-23.
- CIOMS and ICLAS (2012): Principles find medical sciences and the International Council for Laboratory Animal Science. Guiding Principles for Biomedical Research Involving Animals. find pdf. <http://idas.Org/wp-content/uploads/2013/03/CIOMS-ICLAS>. Access Date: 22/4/2015.
- Clarke, E.G.C. and Clarke, M.L. (1977). *Veterinary Toxicology*, 2nd ed. Bailliere Tindall, New York. p. 10.
- Djuma, (2003). Djuma Game Reserve Copyright(C). 1998–2003.
- Doull, J., Klaassen, D.C. and Amdur, M.D. (2008). Casarett and Doull's Toxicology. The Basic Science of Poisons. 7th ed. McGraw-Hill companies Inc. New York. pp. 45-583.
- Franz, D.N. (1975). Central nervous system stimulants. In: *The Pharmacological Basis of Therapeutics* (Goodman, L.S. and Gilman, A. editors) Macmillan Publishing Company, Philadelphia USA. pp. 1317-1342.
- Idris, M., Abdulrahman, F.I., Tijjani, M.A. and Sandabe, U.K. (2014). Effects of ethanol leaf extract of *Terminalia avicennoides* Guill and Perr. on the central and peripheral nervous system, *Int. J. Phytopharm Res.* 5(4): 178-183.

- Iwalewa, E.O., Lege-Oguntoye, L., Rai, P.P., Iyaniwura, T.T. and Etkin, N.L. (1990). *In-vitro* antimalarial activities of leaf extract of *Cassia occidentals* and *Guiera senegalensis* in *Plasmodium yoelli nigeriensis*, West Afr. J. Pharmacol. Drug Res. 9: 19-21.
- Iwu, M.M., Duncan, A.R. and Okunji, C.O. (1999). New Antimicrobials of plant origin. In Janick, J. (ed) Perspectives in New crops and New uses. ASHS Press, Alexandria, V.A, 1999, pp. 457- 462.
- Jimoh, F.O. and Oladiji, A.T. (2005). Preliminary study on *Piliostigma thonningii* seeds: preliminary analysis, mineral composition and phytochemical screening, Afr J. Biotech, 4: 1439-1442.
- Johnston, G.A.R. and Beart, P.M. (2004). Flavonoids: Some of the wisdom of sage? Brit. J. Pharmacol., 142: 809-810
- Kasture, V.S., Kasture, S.B. and Chopde, C.T. (2002). Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals, Pharmacol. Biochem. Behav, 72: 965-972.
- Lorke, D. (1983). Approach to acute toxicity test, Arch. Toxicol. 54: 275 – 287.
- Madara, A.A., Ajayi, J.A., Salawu, O.A. and Tijani, A.Y. (2010). Anti-malarial activity of ethanolic leaf extract of *Piliostigma thonningii* Schum. (Cesalpiniaceae) in mice infected with *Plasmodium berghei-berghei*. Afr. J. Biotech, 9(23): 3475-3480.
- Mattsaon, H.R. (1995). Antiepileptic drug monitoring: A reappraisal, *Epilepsia*. 36(Suppl. S): S22-S29.
- Newman, D.J. and Cragg, G.M. (2007). Natural products as sources of new drugs over the last 25 years, J. Nat. Prod. 70: 461–477.
- Nwafor, P.A. (1998). Anticoceptive and other pharmacological effects of *Asparagus pubescence* bark root and *Cassia nigricans* leaves. PhD Thesis, (unpublished) University of Jos, Nigeria.
- Okigbo, R.N., Anuagasi, C.L. and Amadi, J.E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa, J. Med. Plant Res. 3(2): 086 -095.
- Rabo, E.T. and Sanusi, S.S. (2001). An Inventory of Medicinal Plants of the Nigerian Savannah. Leviathan books, Lagos, Nigeria. pp. 21- 24.
- Raza, M.F., Shaheen, M.I., Choudhary, A., Suria, A.U., Raham, S.S. and delorenzo, R.J. (2001). Anticonvulsant activities of the FS-1 sub-fraction isolated from root of *Delphinium denudatum*. *Phytother. Res.*, 15: 426-430.
- Robinson, S., Chapman, K., Hudson, S., Sparrow, S., Spencer-Briggs, D., Danks, A., Hill, R., Everett, D., Mulier, B., Old, S. and Bruce, C. (2009). Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals. National center for the Replacement, Refinement and Reduction of Animals in Research.
- Sandabe, U.K., Onyeyili, P.A. and Chibuzo, G.A. (2003). Neuropharmacological effects of *Ficus sycomorus* stem bark in rats, Vet. Arh. 73(2): 103–110.
- Sander, J.W.A.S. and Shorvon, S.D. (1987) Incidence and prevalence studies in epilepsy and their methodological problems: a review, J. Neuro. Neurosurg. Psych; 50: 829-39.
- Sodipo, O.A., Abdulrahman, F.I., Sandabe, U.K and Akinniyi JA (2011). Effects of the aqueous fruit extract of *Solanum macrocarpum* Linn. on Haematological parameters of triton-induced hyperlipidemic rats, Afr. J. Pharm. Pharmacol. 5(5): 632-639.
- Usman, H., Abdulrahman, F.I., Kaita, H.A. and Khan, I.Z. (2013). Antibacterial effects of cyanogenic glucoside isolated from the stem bark of *Bauhinia rufescens* Lam Int. J. Biol. Chem. Sci. 7(5): 2139-2150.
- Usman, H., Yaro, A.H. and Garba, M.M. (2008). Phytochemical screening and anticonvulsant screening of the ethanolic flower extract of *Newbouldia laevis* (Bignoniaceae) in mice, J. Pharmacol. Toxicol. 3(2): 127-133
- Watt, J. M. and Breyer-Brandwijk, M.G. (1962). Medicinal plants and Poisonous of Southern and Eastern Africa, E and S. Livingstone, London, p. 640.
- White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The nutritional institute of health anticonvulsant drug development program: Screening for efficacy. In: *Antiepileptic Drug Development; Advances in Neurology*, French, J., Leppik, I. E. and dichter, M. A. Editors. Lippincott-Raven Publishers; Philadelphia, 76: pp. 29-39.
- Yakubu, J., Usman, Y.B, Balami, M.V., Jonathan, N., Semiu, Y. and Teri, D.M. (2016). Phytochemical and Elemental Analysis of some Organic Solvent Leaf Extracts of *Piliostigma thonningii* (Schumach) Milne-Redhead, Ewemen J. Folk. Med. 2(2): 49-57.
- Lehmann, J., Hutchison, A., Mc Pherson, S.E., Mondadari, C., Schmutz, M., Sinton, C.M., Williams, M., Cheney, D.L. and Wood, P.L. (1988) A selective and competitive N-methyl-D-aspartate- type excitatory amino acid receptor antagonist, J. Pharmacol. Exp. Therapeut. p.01103.

- Raza, M., Shaheen, F., Choudhary, M.I., Sombati, S., Rafiq, A., Suria, A., Rahman, A. and Delorenzo, R.J. (2001). Anticonvulsant activities of ethanolic extract and aqueous fraction isolation from *Delphinium denudatum*, J. Ethnopharm. 78(1): 73-78
- Sayin, U., Cengiz, S. and Altug, T. (1993). Vigabatin as an anticonvulsant against pentyletetrazole seizures, Pharmacol. Res. 28: 325-31.
- Rajendra, S., Lynch, L.J. and P.R. Schofield, (1997). The glycine receptor. Pharmacol. Ther. 73: 121-146.
- Ogbonnia, S., VanStaden, J., Jager, A. K. and Coker, H. A. (2003). Anticonvulsant effect of *Glyphaea brevis* (Spreng) Moraches leaf extract in mice and preliminary phytochemical tests. Nig. Q. J. Hosp. Med. 13(3-4): 62-64.

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