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Research paper

Anti-epileptic Potentials of the Partitioned Fractions of *Chamaecrista mimosoides*

Asinamai N. Medugu [1], *James Yakubu [2], Hussaini I. Marte [1], Timothy S. Yerima [1]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria*
[2] *Department of Pure and Applied Chemistry, Faculty of Science, University of Maiduguri, Borno State, Nigeria*
**Corresponding author E-mail: Jamesyakubu96@gmail.com*

Abstract

This research work aimed to establish scientific basis for the use of *Chamaecrista mimosoides*, in traditional medicine as anti-epileptic medication. The whole plant part of *Chamaecrista mimosoides* was extracted with ethanol and screened for phytochemicals. Acute toxicity study was carried out using Lorke's method and the antiepileptic activity was evaluated using maximal electroshock induced seizure test in day-old chicks, pentylenetetrazole (PTZ) and strychnine using mice. The phytochemical study revealed the presence of saponins, cardiac glycosides, tannins, flavonoids, terpenoids and cardenolides. Both the chloroform, ethylacetate and n-butanol portions at 100, 250, and 500mg/kg body weight did not protect the chicks against tonic hind limb extension (THLE) in maximal electro-shock test (MEST). The chloroform and n-butanol portions at doses of 250 and 500 mg/kg body weight protected 40% and 60% of mice against clonic spasm induced by pentylenetetrazole, while ethyl-acetate soluble portion did not protect the mice against clonic spasm induced by pentylene-tetrazole at all doses used when compared to Valproic acid (200 mg/kg) protected all the mice (100%) against clonic spasm induced by pentylenetetrazole. The chloroform soluble portion at the doses of 100, 250 and 500 mg/kg body weight protected 40%, 100%, 100% against death induced by strychnine, while ethylacetate and n-butanol portions did not protect the rats against death induced by strychnine but prolonged the onset of convulsion. In all the tests, phenobarbitone (20 mg/kg) was used as positive control and protected 80% of mice against convulsion induced by strychnine. The antiepileptic investigation suggests that the chloroform portion of *Chamaecrista mimosoides* has a promising antiepileptic activity.

Keywords: *Chamaecrista mimosoides Phytochemical Antiepileptic; Pentylenetetrazole (PTZ); Maximal Electroshock Induced Seizure (MEST); Strychnine.*

1. Introduction

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Newman and Cragg, 2001). Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. Scientific evidences report that the potential of plants in general and higher plants in particular as a source of new drugs has not been fully explored. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax quinones (Iwu et al., 1999; Asaolu, 2003).

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime (Sander and Shorvon, 1996). In Nigeria, the estimated prevalence of epilepsy is 8 per 1000 people indicating a substantial burden of the disease in the country (Owolabi et al., 2020).

Chamaecrista mimosoides is an annual or short-lived perennial herb, sometimes prostrate but more commonly growing as an erect sub shrub up to 1.2 metres. This widespread plant was once placed in the genus Cassia. It is a small perennial shrub up to about 60 centimetres high but sometimes much taller, has leaves with 20-60 pairs of leaflets A decoction of the leaves is used as a tea in Japan. The leaves are cooked and eaten as a vegetable. Medicinally, the roots are used in the treatment of dysentery and stomach pains (Gautam and Harisha, 2017). In tropical Africa, roots used for colic. Entire plant used as remedy for facial eruptions. In northwestern Tanzania, aerial parts of *C. mimosoides* are pounded and mixed with animal fat, applied topically or taken orally for fractures, cleaning of the uterus by pregnant women, as antibacterial. Also, aerial parts pounded with the leaves of Cassia polytricha and the paste tied around a fracture to promote healing. In Japan, raw material used as diuretic or antidote in folk remedy (Dave and Ledwani, 2012) In Uganda, decoction of fresh leaves drunk for pediatric cough (Namukobe et al., 2011). In southern Benin, used for the treatment of typhoid fever (Kakpo et al., 2019).

Currently available antiepileptic drugs (AED) are synthetic molecules that have serious adverse effects such as weight gain, hepatotoxicity, teratogenicity and withdrawal symptoms (McNamara, 2006). Pharmacotherapy of epilepsy with available AED is symptomatic as these drugs inhibit seizure and do not cure the underlying disease process in the brain (Schmidt, 2002).

2. Material and methods

2.1. Plant collection and identification

The whole plant (*Chamaecrista mimosoides*) was collected from Maiduguri in Jere local government area, Borno State. The collection was done between the months of June and August, 2017. The plant was identified and authenticated by Professor S.S. Sanusi; a Taxonomist with the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. A voucher specimen was deposited for future reference.

2.2. Preparation of the plant extracts

The whole plant (*Chamaecrista mimosoides*) was air dried at room temperature for 2 weeks and was size-reduced into coarse powder using pestle and mortar the powdered plant material (2 kg) will be defatted with petroleum ether (2.5L) for 24 hours using soxhlet extractor. The marc was air dried and macerated with (5L) of ethanol (99%$^{v}/_{v}$) for 4 days with occasional shaking. The filtrate was evaporated to dryness in *vacuo* at 40^{o}C and stored in a desiccator. The extract was subsequently referred to as *Chamaecrista mimosoides* extract (CME). A fresh aqueous suspension of the extract in 2% tween-80 was prepared for each study.

2.3. Fractionation of the plant extract

One hundred grammes (100g) of the crude ethanol extract was sequentially portioned using chloroform, ethylacetate and n-butanol in increasing order of polarity. The fractions obtained were concentrated to dryness in vacuo at 40^{o}C and kept ascetically until further use.\

2.4. Chemical studies

2.4.1. Preliminary qualitative phytochemical screening
The screening was done in accordance with the standard protocol as describe by Evans, 2009. The extract was screened for the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenoids, cardiac glycosides, and carbohydrate.

2.5. Pharmacological studies

2.5.1. Animal

Male and female Swiss albino mice (19-21 g) maintained at the animal house of the Department of Pharmacology and Toxicology, University of Maiduguri were used for the study. They were housed in a well-ventilated room, fed with commercially obtained feed (Vital feed, Jos). All experiments were conducted in accordance with the standard ethic described by ICLAS and CIOMS.

2.5.2. Drugs and drug solutions

Pentylenetetrazole was purchased from Sigma Chemical Co. (St. Louis. USA). Sodium valproate (Fawdon Manufacturing Centre, Newcastle-upon-Tyne, UK) and Phenytoin (Manfes Pharmaceutical limited, Nigeria). The drug solutions were prepared fresh for each day's experiment to maintain stability of the drugs used. The solutions were kept in air-tight, amber coloured containers and stored in the refrigerator ready for use.

2.5.3. Routes of drug administration

The extract, phenytoin and sodium valproate were administered intraperitoneally while pentylenetetrazole was administered subcutaneously.

2.5.4. Acute toxicity studies

The acute toxicity of the CME crude extract was investigated in mice using intra-peritoneal route. The method used was as described by Lorke (1983). The study was carried out in two phases, in Phase I, 3 groups of three mice each was treated with the extract of the plant at doses of 10, 100 and 1000 mg/kg body weight i.p and observed for signs of toxicity and death for 24 hours. In Phase II, 3 group each containing one mouse was injected with four more specific doses of the extract based on result obtained in phase I. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived. $LD_{50} = \sqrt{a\ x\ b}$, where a = lowest dose that killed animal and b = highest dose that did not kill any animal.

2.5.6. Pentylenetetrazole-induced seizure in mice

Twenty-five mice (18-21g) were divided into five groups each containing five mice. The first three groups received CME (three different doses) the fourth group (Valproic acid 200mg/kg) and the fifth group received 10mL normal saline per kg body weight intraperitoneally respectively. Thirty minutes later, mice in all the groups receive 60 mg/kg of pentylenetetrazole subcutaneously and observed over a period of 30 minutes. Absence of a clonic spasm of at least 5 seconds' duration indicated the compounds ability to abolish the effect of pentylenetetrazole on seizure threshold (Swinyard et al., 1989).

2.5.7. Maximum electroshock induced seizure in chicks

Fifty (50) chicks of both sexes were used for this study. They were grouped into ten chicks per group. Group I received vehicular treatment; group II-IV received 100, 250 and 500 mg/kg b dwt. of CME was administered i.p the group 2 received phenytoin (20 mg/kg i.p) as a reference standard. Thirty minutes after pretreatment, maximal electroshock was administered to induce seizure in the chicks using Ugobasile Electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and pulse width were set and maintained at 90 mA, 0.80 second, 200 pulse/second

and 0.8 m/s respectively. The ability to prevent this feature or prolong the latency and/or onset of the tonic hindlimb extension was considered as an indication of an anticonvulsant activity (Sayyah et al., 2002).

2.5.8. 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 CME. 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 CME could prevent strychnine-induced seizures (Raza et al., 2001).

2.6. Statistical analysis

Data obtained from convulsive tests were analysed using Graphpad Prism version 8.0 for windows. The results were expressed as mean ± standard error of mean (Mean ± SEM) for time of onset of convulsion, as well as percentage of inhibition of convulsion (percentage protection) and or percentage mortality. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test.

3. Results

3.1. Plant extraction

The extractive value of the whole plant ethanol extract was found to be 152g representing a yield of 5.2%.

3.2. Partition portions of ethanol whole plant extract of *Chamaecrista mimosoides*

Successive partitioning of whole plant extract (100g) of *Chamaecrista mimosoides* yielded Ethylacetate portion (3.96g), Butanol portion (6.06g). chloroform portion (41.0g) and residual aqueous portion (21g) (Table 1).

Table 1: Yield of Partition Portions of Ethanol Extract of Whole Plant of *Chamaecrista Mimosoides*

Portion s	Yield (g)	Percent (%)
Butanol	6.06	6.06
Ethyl acetate	3.96	3.96
Chloroform	41	41
Residual aqueous	21	21

3.3. Photochemical constitutions of ethanol whole plant extract of *Chamaecrista mimosoides*

The preliminary phytochemical screening of the whole plant extract of *Chamaecrista mimosoides* using ethanol and the 3 soluble portions using chloroform, ethyl acetate and n-butanol as solvents revealed the presence of some phytochemicals such as flavonoids, terpenoids, cardiac glycosides, saponins and tannins. Ethanol extract had the highest number of phytochemicals while ethyl acetate portion had fewer phytochemicals, most notably is the absence of terpenoids in ethylacetate portions. Alkaloid, carbohydrates and anthraquinones were all absent in the ethanol extract and the three portions. The result of the phytochemical screening is shown in Table 2.

Table 2: Phytochemical Screening of Chloroform, Ethylacetate and N-Butanol Soluble Portions of the Whole Plant of *Chamaecrista Mimosoides*

S/No.	Test	CLP	EAP	BUP
1.	Test for carbohydrate	-	-	+
2.	Test for tannins	+	+	+
3.	Test for Anthraquinones		-	-
4.	Test for cardiac glycosides	+	+	+
5.	Test for terpenoids	+	-	+
6.	Test for saponins	+	+	+
7.	Test for flavonoids	+	+	+
8.	Test for alkaloids	-	-	-

Portion; BUP = n-Butanol Portion; CLP = Chloroform portion

3.4. Pharmacological studies

3.4.1. Acute toxicity studies

Signs and symptoms observed in test animals injected with crude extracts and partitioned portions included decreased locomotor activity, breathing difficulty and immobility (Table 4.3).

Table 3: LD_{50} Values of Crude and Partitioned Portions of *Chamaecrista mimosoides*

Test	Animal Species	Route	LD_{50} Value
Chloroform	Mice	i.p	3808
Ethly Acetate	Mice	i.p	3808
n-Butanol	Mice	i.p	>5000

Key: i.p. = intraperitoneal.

3.4.2. Effects of partition portions of CME on maximal electroshock test (MEST) in chicks

The chloroform soluble portion at 100, 250, and 500mg/kg body weight did not protect the chicks against tonic hind limb extension (THLE) in maximal electro-shock test (MEST). It however significantly ($p<0.05$) decreased the mean recovery time from 11.7±0.73 min (normal saline group) by 38% and 63% minutes at doses 250 and 500 mg/kg body weight, respectively in a dose-dependent manner. (Table 4)
The ethyl-acetate soluble portion (100, 250, and 500mg/kg body weight) did not protect the chicks against tonic hind limb extension (THLE) in maximal electro-shock test (MEST). It however significantly ($p<0.05$) decreased the mean recovery time from 11.7±0.73 min (normal saline group) by 49%, 56% and 62% at the dose 100, 250 and 500 mg/kg body weight respectively in a dose dependent manner. (Table 5)
The n-butanol soluble portion (100, 250, and 500mg/kg body weight) did not protect the chicks against tonic hind limb extension (THLE) in maximal electro-shock test (MEST). It however significantly ($p<0.05$) decreased the mean recovery time from 11.7±0.73 min (normal saline group) by 68%, 74% and 95% minutes at doses 100, 250 and 500 mg/kg body weight respectively, in a dose-dependent manner. In all the tests, phenytoin (20 mg/kg body weight) was used as positive control and it produced 100% protection of the chicks and the mice. (Table 6)

Table 4: Effects of Chloroform Soluble Portion on Maximal Electroshock Test (MEST) in Chicks

Treatment (mg/kg)	Mean Recovery Time (min.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	11.7±0.73	0/10	0
100	9.8±0.51	0/10	0
250	7.3±0.37*	0/10	0
500	4.3±0.37*	0/10	0
Phenytoin (20mg/kg)	00*	10/10	100

Data presented as Mean ± SEM, n =10, CLP = Chloroform Portion, *represent $p<0.05$ by student t-test.

Table 5: Effects of Ethylacetate Soluble Portion on Maximal Electroshock Test (MEST) in Chicks

Treatment (mg/kg)	Mean Recovery Time (min.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	11.7±0.73	0/10	0
100	6.0±0.60*	0/10	0
250	5.1±0.45*	0/10	0
500	4.4±0.40*	0/10	0
Phenytoin (20mg/kg)	00*	10/10	100

Data presented as Mean ± SEM, n =10, EAP = Ethylacetate Portion, *represent $p<0.05$ by student t-test.

Table 6: Effects of Ethylacetate Soluble Portion on Maximal Electroshock Test (MEST) in Chicks

Treatment (mg/kg)	Mean Recovery Time (min.±SEM)	Quantal Protection	%Protection
N/Saline (10m//kg)	11.7±0.73	0/10	0
100	3.8±0.52*	0/10	0
250	3.0±0.35*	0/10	0
500	2.2±0.20*	0/10	0
Phenytoin (20mg/kg)	00*	10/10	100

Data presented as Mean ± SEM, n =10, BUP = n-Butanol Portion, *represent $p<0.05$ by student t-test.

3.4.3. Effects of partition portions of CME on pentylenetetrazole-induced convulsion in mice

The chloroform soluble portion at higher doses of 250 and 500 mg/kg body weight protected 40% of mice against clonic spasm induced by pentylenetetrazole. All the doses also significantly ($p<0.05$) increased the latency of convulsed animals in a dose-dependent manner from 3.6±0.40min in normal saline treated group by 67%, 169% and 214% at 100, 250 500 mg/kg body weight respectively. Valproic acid (200 mg/kg) protected all the mice (100%) against clonic spasm induced by pentylenetetrazole (Table 7).
The ethyl-acetate soluble portion did protect the mice against clonic spasm induced by pentylenetetrazole at all doses used. However, it significantly ($p<0.05$) increased the latency of convulsed animals in a dose-dependent manner from 3.6±0.40 in normal saline treated group by 150%, 200% and 211% at 100, 250 and 500 mg/kg body weight respectively. Valproic acid (200 mg/kg) protected all the mice (100%) against clonic spasm induced by pentylenetetrazole (Table 8).
The n-butanol soluble portion at higher doses of 250 and 500 mg/kg body weight protected 40% and 60% of the mice respectively against clonic spasm induced by pentylenetetrazole. Similarly, it significantly ($p<0.05$) increased the latency of convulsed animals in a dose-dependent manner by 83%, 114% and 205% at 100, 250 and 500 mg/kg body weight respectively. Valproic acid (200 mg/kg) protected all the mice (100%) against clonic spasm induced by pentylenetetrazole (Table 9).

Table 7: Effects of Chloroform Portion on Pentylenetetrazole-Induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of Convulsion (mm.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	3.6±0.40	0/5	0
100	6.0±0.32*	0/5	0
250	9.7±1.45*	2/5	40
500	11.3±0.88*	2/5	40
Sodium Valproate (200mg/kg)	00*	5/5	100

Data presented as Mean ± SEM, n =5, CLP = Chloroform portion, *represent $p<0.05$ by student t-test.

Table 8: Effects of Ethylacetate Portion on Pentylenetetrazole-Induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of Convulsion (min.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	3.6±0.40	0/5	0
100	9.0±1.42*	0/5	0
250	11.0±0.60*	0/5	00
500	11.2±0.58*	0/5	00
Sodium Valproate (200mg/kg)	00*	5/5	100

Data presented as Mean ± SEM, n =5, EAP = Ethylacetate portion, *represent p<0.05 by student t-test.

Table 9: Effects of n-Butanol Portion on Pentylenetetrazole-Induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of Convulsion (min.±SEM)	Quantal Protection	%Protection
N/Saline (10ml/kg)	3.6±0.40	0/5	0
100	6.6±0.51*	0/5	0
250	7.7±0.33*	2/5	40
500	11.0±1.00*	3/5	60
Sodium Valproate (200mg/kg)	00	5/5	100

Data presented as Mean ± SEM, n =5, CLP = n-Butanol portion, *represent p<0.05 by student t-test.

3.4.4. Effects of partition portions of CME on strychnine-induced convulsion in mice

The chloroform soluble portion at the doses of 100, 250 and 500 mg/kg body weight protected 40%, 100% and 100% of the mice respectively against death induced by strychnine. At a higher dose of 500 mg/kg body weight, it completely abolished strychnine-induced convulsion in mice. Similarly, on the time of death, the chloroform portion significantly (P<0.05) prolonged the death of convulsed mice from 11.8±0.37mm. in normal saline treated group to 16.0±00mm. at the dose of 100 mg/kg body weight (Table 10).

The ethyl-acetate portion significantly (P<0.05) prolonged the onset of convulsion in a dose-dependent manner from 10.2±0.37mm. in normal saline treated group by 109% at doses of 250 and 500 mg/kg body weight respectively. Similarly, in the time of death, the EAP significantly (P<0.05) prolonged the death of convulsed mice from 11.8±0.37mm. in normal saline treated group by 66%, 83% and 103% at doses of 100, 250 and 500 mg/kg body weight, respectively and did not protect the mice from strychnine-induced death at all the doses used. (Table 11).

The n-butanol soluble portion did not significantly (p>0.05) prolong the onset of convulsion at all the doses used (100, 250 and 500 mg/kg body weight) compared to normal saline treated group. Similarly, in the time of death, the ethylacetate portion significantly (P<0.05) prolonged the death of convulsed mice at all doses used.

In all the tests, phenobarbitone (20 mg/kg) was used as positive control and protected 80% of mice against convulsion induced by strychnine (Table 12).

Table 10: Effects of Chloroform Portion 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	11.7±0.67	16.0±.00*	2/5	40
250	13.2±0.86	00*	5/5	100
500	00*	00*	5/5	100
Phenobarbitone (20mg/kg)	24.0±1.84	24.00±00*	4/5	80

Data presented as Mean ± SEM, n =5, CLP = Chloroform portion, *represent p<0.05 by student t-test.

Table 11: Effects of Ethylacetate Portion 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	15.0±1.30	19.6±2.01*	0/5	0
250	21.4±1.03*	21.6±1.44*	0/5	0
500	21.4±1.94*	22.6±1.72*	0/5	0
Phenobarbitone (20mg/kg)	24.0±1.84*	24.00±00*	4/5	80

Data presented as Mean ± SEM, n =5, EAP = Ethyl-acetate portion, *represent p<0.05 by student t-test.

Table 12: Effects of n-Butanol Portion 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	9.8±0.83	14.0±0.71	0/5	0
250	11.2±0.73	12.2±0.73	0/5	0
500	11.2±0.73	15.8±0.73	0/5	0
Phenobarbitone (20mg/kg)	24.0±1.84*	24.00±00	4/5	80

Data presented as Mean ± SEM, n =5, EAP = n-Butanol portion, *represent p<0.05 by student t-test.

4. Discussions

Preliminary phytochemical screening of the whole plant extract revealed the presence of cardenolides, flavonoids, saponins, tannins and terpenoids. The butanol soluble portion and the chloroform soluble portion of the whole plant extract retained all the phytochemicals. However, the ethyl acetate soluble portion of the whole plant extract retained all but terpenoids the phytochemicals already identified in the crude ethanol extract of the whole plant. Therapeutic benefits of traditional remedies might depend upon a combination of constituents (Amos et al., 2001), such as those identified in this plant.

Medicinal plants are a rich source of bioactive phytochemicals. Studies carried out during the past 2-3 decades have shown that these phytochemicals have an important role in preventing chronic diseases in human (Saxena et al., 2013). The most abundant phytochemical detected in the present work is terpenoids. They are also considered the most structurally diverse group; they function as phyto-alexins in plant direct defense responses which involves herbivores and their natural enemies (McCaskill and Croteau, 1998). They are reported to have neuro-pharmacological activity including anticonvulsant activity (Saxena et al., 2013). Flavonoids have also gained recent attention because of their broad biological and pharmacological activities but the best described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Tapas et al., 2008). Flavonoids have been found to inhibit almost all the mechanisms involved in seizures generation in epilepsy. They have been found

to modulate neuronal Na^+ channels (Nicholson et al., 2010), Ca^{2+} channels (Cogolludo et al., 2007), GABAergic pathway glutamatergic pathway and opioid pathway (Engelborghs et al., 2000).

Intraperitoneal acute toxicity studies conducted in mice showed the butanol soluble fraction have LD_{50} value of >5000mg/kg b. wt. while the chloroform and ethylacetate have the value of 3808mg/kg b. wt. Clarke and Clarke (1977) reported that compounds with LD_{50} of 1500 mg/kg and above are considered to be of low toxicity.

The maximal electroshock test (MEST) is a non-mechanistic seizures model that has clearly defined end points such as inhibition of HLTE (Stables and Kupferberg, 1997). There is no false negative in the MES test and the currently available AEDs that are clinically effective in the management of generalized tonic–clonic and partial seizures such as carbamazepine, phenytoin, primidone, phenobarbital, valproate, and lamotrigine all suppress HLTE in MES test (Browning, 1992; Rho and Sankar, 1999).

Protection against hind limb tonic extension (HLTE) in the maximal electroshock test (MEST) predicts anticonvulsant activity of antiepileptic drugs (AED) that prevent the spread of the epileptic seizure discharges from an epileptic focus during seizures. The three portions in MEST did not protect the chicks against seizures induced by maximal electroshock suggesting non-activity against generalized tonic clonic and partial seizures. They in fact, reduced onset of seizures in the animals.

Pentylenetetrazole (PTZ) is a known convulsant and anticonvulsant activity in subcutaneous pentylenetetrazole test identifies compounds that can raise the seizure threshold in the brain (White et al., 1998). Antiepileptic drugs (AEDs) effective in the therapy of generalized seizures of (absence or myoclonic) petit mal type such as Ethosuximide (ETX), Valproic acid (VPA), Phenobarbitone (PHB), and Benzodiazepine (BDZ) exhibit dose-dependent suppression of various seizure patterns induced by PTZ (Loscher et al., 1991). At cellular level, one of the basic mechanisms of actions of AEDs such as ETX and VPA is the suppression of T-type calcium currents in thalamic neurons (Rho and Sankar, 1999; Meldrum, 1996). Besides increasing GABA levels, VPA may also have antiepileptic activity by reducing the high-frequency firing of neurons by blocking voltage-gated sodium, potassium, and calcium channels (Johannessen and Johannessen, 2003).

All the experimental animals in the negative control, pretreated with distilled water, were not protected from the PTZ-induced chemoconvulsion and did not survive it. The PTZ-induced convulsion is like symptoms observed in absence seizures. On the other hand, drugs used in the treatment of absence seizures suppress PTZ-induced seizures (McNamara, 2006). Consequently, pharmacologically active chemical substances that can suppress or prevent PTZ-induced convulsion are often speculated to have activity against absence seizures. PTZ is an antagonist of Gamma amino butyric acid (GABA) at $GABA_A$ receptor which has been widely implicated in epilepsy (Rang et al., 1996). In addition, drugs which protect animals against the generalized clonic seizure induced by PTZ are effective in protection and management of petit mal epilepsy (Alhwegy and Ahmad, 1993). Drug that are effective against petit mal seizure reduce T-type calcium current and these types of seizure can also be prevented by drugs that enhance GABA. In the positive control, sodium valproate (200mg/kg), a standard antiepileptic drug was used as control and it yielded 100% protection against the PTZ-induced seizures. The standard drug protected all the mice from death due to PTZ. Consequently, it can be inferred that sodium valproate not only suppresses seizures but also has the capability of lowering the chances of mortality. It is believed to act by altering the function of the neurotransmitter GABA (as GABA transaminase inhibitor) in the human brain. Its principal mechanism of action is believed to be the inhibition of the transamination of GABA (by inhibiting GABA transaminase, then GABA would increase in concentration). However, several other mechanisms of action in neuropsychiatric disorders have been proposed for valproate in recent years. Sodium valproate also blocks the voltage-gated sodium channels and T-type calcium channel. These mechanisms make it a broad-spectrum anticonvulsant drug. The crude extract and the three portions at the tested doses demonstrated dose-graded protection against PTZ-induced seizures. The chloroform and n-butanol soluble portion at higher doses of 250 and 500mg/kg body weight protected 40% and 60% of mice respectively, against clonic spasm induced by pentylenetetrazole. However, the ethylacetate soluble portion offered no protection to the mice at all doses tested. There was also delays in the onset of convulsion at all tested doses of the three soluble portions and these delays were statistically significant ($p<0.05$) when compared with the onset in the distilled water group.

Protection of the animals against PTZ-induced seizures predicts anticonvulsant activity and the delayed onset of seizure indicates that it can raise seizure threshold (White et al., 1995). This also indicates its probable effectiveness against absence seizures (McNamara, 2016) which could be speculated to involve the enhancement of GABAnergic neurotransmission and/or action in the brain and possible glutamate receptor antagonist's action. However, since the crude extract and the three soluble portions did not protect the animals against seizures evoked by electroshock, it could be theorized that it does not have glutamate receptor antagonism.

Protection of the mice against PTZ-induced seizure predicts anticonvulsant activity and the delayed onset of seizure indicates that it can raise seizure threshold (White et al., 1995). This also indicates its probable effectiveness against absence seizure (McNamara, 2006) which could be speculated to involve the enhancement. The order of relative potencies is crude ethanol extract, n-butanol portion, chloroform portion and ethyl acetate portion.

In the strychnine-induced seizure model, it is known that strychnine directly antagonizes the inhibitory spinal reflexes of glycine (Sayin et al., 1993). The chloroform portion of the whole plant of *Chamaecrista mimosoides* at the dose of 250mg/kg and 500mg/kg protected 100% of the mice against strychnine-induced death. While both the ethyl acetate and n-butanol portion did not protect the mice against strychnine-induced death, they however, the ethyl acetate portion at the dose of 250mg/kg and 500mg/kg, significantly increased the time of onset of convulsion and time of 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 (Larson, 1969; Rajendra et al., 1997). The ability of the chloroform portion of the whole plant of *Chamaecrista mimosoides* to prevent the strychnine-induced seizures demonstrate additional anticonvulsant effects mediated via glycine receptors (Ogbonnia et al., 2003). The order of relative potencies is chloroform portion, n-butanol portion and ethylacetate portion.

5. Conclusion

The phytochemical study revealed the presence of saponins, cardiac glycosides, tannins, flavonoids, terpenoids and cardenolides. The three portions had no observable toxic effect on mice within the duration of time evaluated. The portions have no activity against generalized tonic-clonic seizures when maximal electroshock was used. The anticonvulsant effect of the whole plant ethanol extract and the chloroform portion in this study contains active ingredients which may have act synergistically, since the plant contains flavonoids, glycosides and saponins of reported anticonvulsant activity. These findings justify the traditional use of this plant in the control and /or treatment of convulsions and epilepsy.

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