Anticonvulsant effect of methanol leaf extract of *Tetrapleura tetraptera* (Mimosaceae) in mice.

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ABSTRACT

Background: The leaf of *Tetrapleura tetraptera* (Schumach & Thonn.) (Mimosaceae) is used in folklore medicine for treatment of various disease conditions such as; epilepsy and rheumatoid arthritis.

Objective: This study sought to evaluate anticonvulsant activity of methanol leaf extract of *Tetrapleura tetraptera* (MeTT).

Methods: Preliminary qualitative analysis of phytochemical components was carried out. MeTT (100-400 mg/kg, *p.o*.) or vehicle (distilled water; 10 ml/kg, *p.o*.) was administered to mice 1 h before picrotoxin (PIC) (10 mg/kg, *i.p*.) or pentylenetetrazol (PTZ) (90 mg/kg, *i.p*.). The latency and duration of seizure as well as percentage mortality were recorded.

Results: The percentage yield was 6.79$^{w}/_{w}$. Preliminary qualitative phytochemical screening revealed presence of tannins, cardiac glycosides, terpenoids, saponins, flavonoids and alkaloids. MeTT (200 mg/kg) significantly prolonged the onset and reduced the duration (60% protection) of picrotoxin-induced seizures when compared to vehicle-treated control. MeTT also produced significant prolongation of latency to seizure in PTZ-induced seizure. Moreover, the fractions produced 40, 40 and 20% protection, respectively, for aqueous, ethylacetate and n-hexane fractions in picrotoxin-induced seizures.

Conclusion: Findings from this study showed that the methanol leaf extract of *Tetrapleura tetraptera* possesses anticonvulsant activity. Hence, could be a potential phytotherapeutic agent in the management of seizures.

Keywords*:* Epilepsy, seizure, picrotoxin, pentylenetetrazol, flavonoid

Effet anticonvulsivant de l'extrait de feuilles de méthanol de *Tetrapleura tetraptera* (Mimosaceae) chez la souris.

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RESUME

Contexte : La feuille de *Tetrapleura tetraptera* (Schumach & Thonn.) (Mimosaceae) est utilisée dans la médecine traditionnelle pour traiter diverses maladies, telles que l'épilepsie et la polyarthrite rhumatoïde.

Objectif : Cette étude visait à évaluer l'activité anticonvulsive de l'extrait de feuilles de *Tetrapleura tetraptera* (MeTT).

Méthodes : Une analyse qualitative préliminaire des composants phytochimiques a été réalisée. Du MeTT (100-400 mg/kg, *p.o*.) ou du véhicule (eau distillée ; 10 ml/kg, *p.o*.) a été administré à la souris 1 heure avant la picrotoxine (PIC) (10 mg/kg, *i.p*.) ou le pentylènetétrazol (PTZ) (90 mg/kg, *i.p*.). La latence et la durée de la crise, ainsi que le pourcentage de mortalité ont été enregistrés.

Résultats : Le rendement en pourcentage était de 6,79% en poids. Le dépistage phytochimique qualitatif préliminaire a révélé la présence de tanins, de glycosides cardiaques, de terpénoïdes, de saponines, de flavonoïdes et d'alcaloïdes. Le MeTT (200 mg/kg) *a prolongé de manière significative l'apparition et réduit la durée (60% de protection) des crises induites par la picrotoxine par rapport au témoin traité avec le véhicule. Le meTT a également provoqué une prolongation significative de la latence des crises lors des crises induites par le PTZ. De plus, les fractions ont produit respectivement une protection de 40, 40 et 20% pour les fractions aqueuses, l'acétate d'éthyle et le n-hexane lors de crises induites par la picrotoxine*.

Conclusion : Les résultats de cette étude ont montré que l'extrait au méthanol de feuille de *Tetrapleura tetraptera* possède une activité anticonvulsive. Par conséquent, pourrait être un agent phytothérapeutique potentiel dans la gestion des crises.

Mots-clés *:* épilepsie, convulsion, picrotoxine, pentylènetétrazole, flavonoïde

INTRODUCTION

Seizure is a paroxysmal alteration of neurologic function caused by excessive, hyper-synchronous discharge of neurons in the brain while epilepsy is a heterogeneous clinical condition characterized by recurrent unprovoked seizures. [1]Epilepsy is one of the most common neurologic conditions affecting 1% of the population and about one-third of patients have refractory epilepsy. [2] Some seizures do respond to antiepileptic drugs (AEDs) but not without debilitating adverse effects, [3] hence, the need for safer and more effective drugs from medicinal plants. The leaves of *Tetrapleura tetraptera* (TT) are used in traditional African medicine in the management of convulsion, leprosy, inflammation, type-2 diabetes mellitus and rheumatoid arthritis. [4, 5] Interestingly, the anticonvulsant effect of the volatile oil extract from fresh fruits of *Tetrapleura tetraptera* showed potent effect against leptazol-induced convulsions. [6,7]

Hence, this study sought to evaluate the protective effects of *Tetrapleura tetraptera* (TT) leaf extract and fractions on chemical-induced seizures in mice.

METHODS

Experimental animals

This study was carried out using male mice (20-25g) obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were housed in plastic cages with wood shavings as beddings, at room temperature and under standard environmental conditions (12 hours light/dark cycle). They were placed on standard rodent diet (Livestock feed Plc, Lagos, Nigeria) and drinking water *ad libitum*. The experimental procedures adopted in this study was approved by Health Research Ethics Committee of the College of Medicine, University of Lagos, with reference number CMUL/HREC/04/17/123 and in accordance with the United States National Institutes of Health Guidelines for Care and use of laboratory Animals in Biomedical Research (2011). Animals used in this study were acclimatized for 2 weeks before the commencement of the experiment.

Plant collection

The leaves of *Tetrapleura Tetraptera* ("Aridan" -Yoruba, Southwest Nigeria; "Uyayak"- Annang/Ibibio, Middlebelt Nigeria; "Yanayan"- Urhobo [8], Middlebelt Nigeria; "Uhiokrihio"- Igbo, Eastern Nigeria; "Abogolo"- Igala, North Central Nigeria; "Dawo"- Hausa, Northern Nigeria; "Prekese"- Twi, Ghana) were harvested at the National Horticultural Research Institute (NIHORT), Jericho reservation area, Ibadan, Oyo State, Nigeria . The leaves (voucher number: NH 07), were identified and authenticated by Mr Fariyike T. A. at the Vegetable Program of the National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria. Dried, pulverized TT leaves (2200 g) were preliminarily processed by cold maceration in methanol (7.5 L). The extract was filtered, after which the filtrate was concentrated using a rotatory evaporator at 40^{0}C. The dry extract was then weighed, stored in labelled samples bottles and preserved in the refrigerator.

Tannin, alkaloid, saponin, steroid, flavonoid, anthraquinones, cardiac glycosides and terpenoid content were qualitatively determined in the methanol extract by standard methods as reported in the manual of food quality control. [9]

Fractionation

Approximately 50 g of the crude methanol extract was weighed, dissolved in 250 ml distilled water followed by fractionation using a total of 750 ml each of four solvents in order of their increasing polarity (fig. 1). Aqueous fraction was left over after butanol fraction had separated out. The fractions were concentrated using a rotary evaporator at 40ºC. The fractions were weighed and transferred into labelled sample bottles then stored in the refrigerator pending analysis.

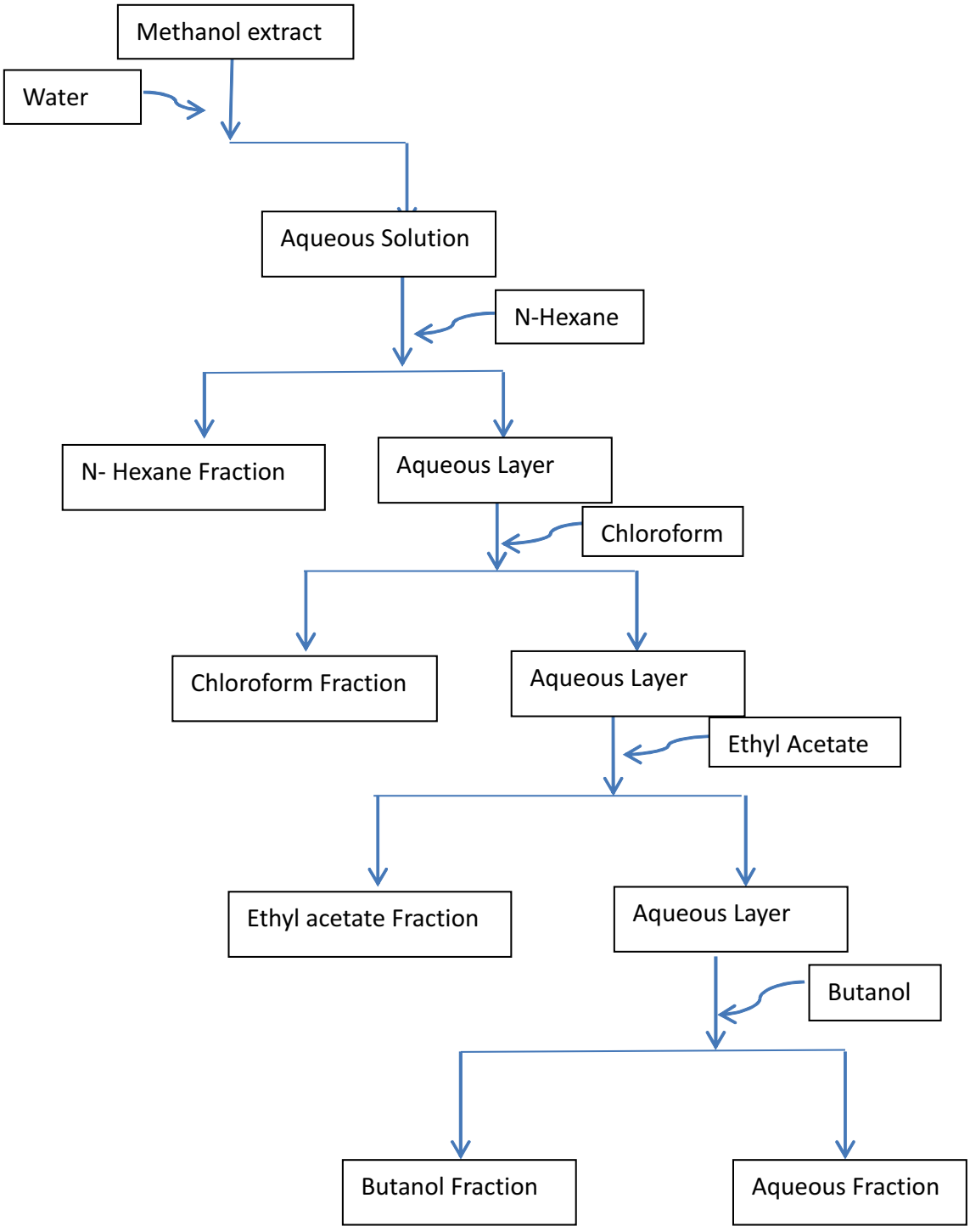

Fig. 1 Organogram showing the stepwise fractionation procedures using solvents in order of their increasing polarity

Acute toxicity testing

The protocol of the Organization of Economic Cooperation and Development (OECD) TG 420 [10] was used to determine the acute toxicity effect of the extract at a maximum dose of 4000 mg/kg (p.o.). The animals were then observed during the next 2 h for behavioural/physical changes and over the next 14 days for mortality.

Pharmacological evaluation

Anticonvulsant activity test on Picrotoxin-induced convulsions:

Twenty-five mice were randomly divided into 5 groups (n= 5) as follows: Group 1: distilled water (10 ml/kg, p.o., control), group 2: diazepam (5 mg/kg, p.o., as a reference standard) and group 3-5: MeTT (100, 200 and 400 mg/kg, p.o. respectively). One hour after treatment, the mice were given picrotoxin (10 mg/kg, i. p.). The onset and duration of convulsions as well as number of deaths were recorded.

Anticonvulsant activity test on Pentylenetetrazol-induced convulsions

Twenty-five mice were randomly divided into 5 groups (n= 5) as follows: Group 1: distilled water (10 ml/kg, p.o., control), group 2: diazepam (5 mg/kg, p.o., as a reference standard) and group 3-5: MeTT (100, 200 and 400 mg/kg, p.o. respectively). One hour after treatment, the mice were given pentylenetetrazol (90 mg/kg, i. p.). The onset and duration of convulsions as well as number of deaths were recorded.

Evaluation of anticonvulsant activity of fractions on Picrotoxin-induced convulsions

Due to observed anticonvulsant activity using picrotoxin as chemoconvulsant, the extract fractions were further tested on picrotoxin-induced convulsions. Thirty five mice were used (n= 5). Group 1: vehicle (10 ml/kg, p.o., control), group 2: diazepam (5 mg/kg, p.o., as a reference standard) and group 3-7: were each given (100 mg/kg p.o.) n-hexane (HETT), ethyl acetate (EATT), chloroform (CHTT), butanol (BUTT) and aqueous (AQTT) fraction, respectively. One hour after treatment, the mice were given picrotoxin (10 mg/kg i.p.). The presence or absence, onset and duration of clonic convulsions were noted for 30 mins following administration of convulsants, latency and duration of seizure as well as number of deaths were recorded.

RESULTS

Extraction and phytochemical screening

Approximately 149.4652 g (6.79%) crude extract was obtained from 2200 g of dried, pulverized MeTT leaf. The results of the qualitative phytochemical screening showed the presence of tannins, cardiac glycosides, terpenoids, saponins, flavonoids and alkaloids while anthraquinone glycosides and steroids were not detected.

Fractionation

Starting from the least polar; n-Hexane (11.66 g), chloroform (4.26 g), ethyl acetate (2.37 g), butanol (12.43 g) and aqueous (8.23 g) fractions were obtained by batch extraction (Table 1).

Table 1: Percentage yield of fractions obtained from *Tetrapleura tetraptera* methanol leaf extracts

Solvent fraction	Weight of fraction (g)	Percentage yield (%)
N-hexane	11.65	11.66%
Chloroform	4.26	4.26%
Ethyl acetate	2.37	2.37%
N-butanol	12.43	12.43%
Aqueous	8.23	8.23%

Acute toxicity

No significant behavioral changes and toxic symptoms were observed within the first 24 h and no mortality occurred over the next 14 days.

Effect of MeTT on Picrotoxin-induced seizure

MeTT (100, 200, 400 mg/kg) significantly ($p< 0.05$) delayed the onset of seizure in comparison to vehicle control group. Moreover, the pre-treatment of mice with diazepam prevented the occurrence of seizures (Table 2). MeTT (200mg/kg) also reduced the duration of seizures (34.4±8.86 s; 60% protection) compared with 912.4±22.4 s in vehicle-treated control.

Table 2: Effect of *Tetrapleura tetraptera* methanol leaf extract on picrotoxin-induced seizure

Treatment	Dose (mg/kg)	Onset (s)	Duration (s)	% protection	% mortality
Distilled water	10ml/kg	180.3±18.88	912.4±22.40	-	100
MeTT 100	100	447.0±45.79 [a]	33.8±8.49 [a]	-	100
MeTT 200	200	499.0±29.64 [a]	34.4±8.86 [a]	60	40
MeTT 400	400	469.0±32.60 [a]	39.6±9.68 [a]	-	100
Diazepam	5	442.4±180.75 [a]	11.8±4.58 [a]	60	40

[a] $p <0.05$ statistically significant compared with control (one-way ANOVA followed by Tukey's post hoc multiple comparison test.

Effect of MeTT on Pentylenetetrazol-induced seizure

MeTT (200 mg/kg) significantly ($p< 0.05$) delayed onset of seizure when compared with control group (Table 3). However, there was no significant change in the duration of seizures when compared with control.

Table 3: *Effect of MeTT on Pentylenetetrazol-induced seizure*

Treatment	Dose (mg/kg)	Onset (s)	Duration (s)	% protection	% mortality
Distilled water	10ml/kg	113.20±15.78	11.6±1.72	-	100
$MeTT_{100}$	100	153.00±10.59	15.0±4.56	-	100
$MeTT_{200}$	200	195.80±52.36[b]	13.8±4.33	20	80
$MeTT_{400}$	400	135.00±18.64	20.4±6.88	20	80
Diazepam	5	-	-	100	-

MeTT: methanol extract of *Tetrapleura tetraptera*

[b] $p <0.05$ statistically significant when compared to control.
One-way ANOVA followed by Tukey's post hoc
multiple comparison test

Effect of fractions on Picrotoxin-induced seizure

Investigation of anticonvulsant activity of the fraction against picrotoxin-induced seizures showed that the aqueous (AQTT) and ethylacetate (EATT) fractions each produced 40% protection from seizures while the n-hexane fraction (HETT) gave 20% protection from seizures (Table 4).

Table 4: Effect of MeTT fractions on picrotoxin induced seizures in mice

Treatment	Dose	Onset (s)	Duration (s)	% protection	% mortality
Distilled water	10ml/kg	180.30±18.88	912.40±22.4	-	100
AQTT	100mg/kg	700.00±143.97[a]	11.80±1.46[a]	40	60
BUTT	100mg/kg	566.20±53.69[a]	23.20±6.57[a]	-	100
EATT	100mg/kg	561.40±104.68[a]	23.80±8.33[a]	40	60
HETT	100mg/kg	513.80±61.70[a]	15.60±2.42[a]	20	80
CHTT	100mg/kg	467.00±48.22[a]	17.80±6.45[a]	-	100
Diazepam	5 mg/kg	442.40±180.75[a]	11.80±4.58[a]	60	40

AQTT= aqueous fraction, BUTT= butanol fraction, EATT= ethyl acetate fraction, HETT = n-hexane fraction,

CHTT=chloroform fraction

[a] $p < 0.05$ statistically significant when compared to control. (one-way ANOVA followed by Tukey's post hoc multiple comparison test).

DISCUSSION

In this study, the methanol extract and fractions of the leaves of TT were investigated for their acute toxicity levels and anticonvulsant properties in order to establish a scientific basis and validation for its folkloric use in the treatment of epilepsy. The most effective dose and fraction was also investigated.

In pursuit of the discovery of an effective remedy for epilepsy, it is important that the chemoconvulsants have statistically significant activity against convulsions by delaying the onset, and also reducing the duration of seizures. At present, no single epilepsy therapy screening program (ETSP) can solely predict the clinical potential of an investigational compound since each one has a different mechanism of action in different parts of the brain. Thus, in this study, picrotoxin and pentylenetetrazol were used. Excitatory and inhibitory neurotransmission plays a critical role in mediating normal neuronal signaling, and an imbalance between these two pathways can contribute to the onset of seizures and ultimately epileptogenesis. Picrotoxin is a GABA (A) chloride (Cl) channel blocker which causes an increase in neuronal nitric oxide synthase (nNOS) activity which leads to the onset of seizures in various regions of the brain. It acts on GABA (A) receptors through a non-competitive antagonism of the chloride channel, stabilizing the GABA (A) receptor-coupled Cl-channel in a non-conducting state and thus reducing GABAergic inhibitory tone. [11] In this study, pretreatment of mice with MeTT prolonged the latency to seizure while reducing the duration of seizure in the picrotoxin-induced seizures model indicative of its ability to enhance GABAergic activity.

Pentylenetetrazol, at high doses has been discovered to cause convulsions. Pentylenetetrazol binds to GABA (A) receptors and produces an opposite effect to anticonvulsant sedatives like phenobarbital or diazepam. [12] Though its mechanism of action is not yet well understood, a study found that pentylenetetrazol increases calcium and sodium influx, both of which depolarize the neuron. These effects are antagonized by calcium channel blockers and it was concluded that PTZ acts at calcium channels causing them to loose selectivity and conduct sodium ions as well. [13] in this study, the pretreatment of mice with MeTT prolonged the onset of seizure but no significant effect on duration

of seizure.

Findings from this study showed that the methanol leaf extract of TT possess anticonvulsant effects. The significant action shown by TT in the prolongation of seizure latency and reduction in the duration of seizure, as well as protection from convulsant-induced mortality corroborates the use of TT by traditional healers the management of convulsion.[14]

Findings from this study showed that the aqueous and ethylacetate fractions contain the active principles responsible for the observed pharmacologic activity.

It is recommended that further analysis using other solvents can be focused on for comparative studies. Also, characterization and isolation of the specific compound(s) responsible for observed anticonvulsant activity in the leaves be investigated which can lead to further structural activity relationship investigations and modifications until a possible cure for epilepsy can be discovered.

CONCLUSION

These results suggest methanol leaf extract of *Tetrapleura tetraptera* possesses anticonvulsant activity and thus, lend credence to the claimed benefit by traditional medicine practitioners in the treatment of epilepsy. Hence, further study is required to isolate and characterize the phytochemical constituent responsible for the anticonvulsant effect.

ACKNOWLEDGEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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