Evaluation of anticonvulsant activity of methanol leaf extract of *Opilia celtidifolia* diels (opiliaceae) in mice and chicks

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Abstract

Epilepsy affects 1% of the global population, with around 2.4 million new diagnoses annually. Approximately 80% of individuals with epilepsy reside in low- and middle-income countries, with a prevalence of 8.2 per 1000 in sub-Saharan Africa and 8 per 1000 in Nigeria. This study evaluated the anticonvulsant properties of methanol leaf extract of *Opilia celtidifolia* (MEC) in mice and chicks.

The median lethal dose (LD_{50}) of the extract was determined in mice and chicks. The acute anticonvulsant properties of MEC were evaluated using pentylene tetrazole (PTZ), maximal electroshock (MES), strychnine, and picrotoxin-induced convulsions, while the chronic anticonvulsant effect was assessed through PTZ-induced kindling. The effects of MEC on cognition, coordination, motor deficits, sedation, and locomotion were assessed using the Y Maze, Beam Walking, and Open Field tests.

The extract increased the mean percentage of survival and significantly delayed the mean onset of PTZ-induced seizure ($p<0.05$), strychnine-induced seizure ($p<0.01$), and picrotoxin-induced seizures ($p<0.05$) at a dose of 1000 mg/kg. However, the extract did not provide protection against MES-induced seizures. In the kindling model, the extract-treated mice exhibited a decrease in the seizure threshold, with an associated increase in oxidative stress. The extract treated mice showed significant ($p<0.05$) decrease in locomotor activity with apparent sedative effect but there was no obvious cognitive impairment in learning and memory, coordination or motor deficits.

MEC is apparently non-toxic and possesses anticonvulsant activity against some animal models of epilepsy. It also exhibited decreased locomotor activity with apparent sedative effect in mice.

Keywords: Anticonvulsant, *Opilia sp*, Epilepsy, Y Maze Test, Beam Walking Test, Open field Test

Introduction

Epilepsy is a chronic brain function disorder associated with unprovoked recurrent seizures that are generated from the brain's abnormal and excessive cortical neuronal activity (Roger *et al.*, 2017, Stephen, 2018). Approximately 1% of the world's population has epilepsy, making it the fourth most common neurological disease after migraine, stroke, and Alzheimer's disease (Roger *et al.*, 2017). It is estimated that 5 million people are diagnosed with epilepsy globally each year, and around 50 million people worldwide have epilepsy, with nearly 80% of that population

living in low- and middle-income countries (WHO, 2023). The estimated proportion of the population with active epilepsy is between 4 and 10 per 1000 people, while its prevalence in sub-Saharan Africa and Nigeria is 8.2 and 8 per 1000, respectively (WHO, 2023, Abigail *et al.,* 2012, Owolabi *et al.*, 2019).

About 70% of epileptic patients could live seizure-free if properly diagnosed and treated (WHO, 2023), however, none of the existing anti-seizure drugs can improve the epileptogenic process. In fact, about 25-30% of patients develop refractory seizures that are difficult to manage (Xia *et al.,* 2017) with the existing drugs. Additionally, anti-epileptic therapy is associated with various side effects, including phenytoin-induced gingival hyperplasia and valproate-associated alopecia and weight gain, dose-related toxicity, and teratogenic effects (Nivedha *et al.*, 2017). There is need for safer and more effective anti-epilepsy medicines.

Opilia celtidifolia (*Opilia amentacea*), known as "Rugargada" in Hausa (Liadi *et al*., 2016), "aga" in Igbo, and "àárà̩ṣá", "à̩ṣá" or "à̩ṣáràgbà" in Yoruba, has been used by traditional medical practitioners across West Africa for the treatment of various ailments including sleeping sickness, leprosy, headache (Burkill, 1997), jaundice (Amang *et al.,* 2020), malaria (Liadi *et al*., 2016), skin disorders (Gronhaug *et al.,* 2008), dental abscesses, fever (Gronhaug *et al.,* 2010). This study aimed to assess the anticonvulsant activity of methanol leaf extract of *Opilia celtidifolia* (MEC) using mice and chicks models. Its effects on cognition, coordination, motor deficits, sedation, and locomotion were also investigated.

Materials and Methods

Chemicals and drugs

The chemicals and drugs used in this study include Picrotoxin; Phenytoin; Pentylenetetrazole; Methanol; Phenobarbitone (Sigma Aldrich St. Louis USA), Strychnine (BDH Chemicals Ltd Poole England), Diazepam (Valium$^{(R)}$ Roche Switzerland), Sodium Valproate (Epilim$^{(R)}$ Sanofi), Vinpocetine (Tyonex) and Distilled water. All drug preparations have NAFDAC registration and batch numbers.

Equipments

Ugo Basile Electroconvulsiometer (Model no. 7801) (Gemonio, Italy) and High Precision Weighing balance (A123 Digital scale) (Mumbai, India) were used.

Experimental animals

Adult Swiss albino mice of both sexes were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Day-old chicks were obtained from Chi Hatchery, off Lagos-Ibadan Express way. The animals were housed and maintained according to standard laboratory conditions and allowed to acclimatize. They were maintained on a natural light/dark cycle and housed in laboratory polypropylene cages at room temperature with access to food and 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 (2011). Ethical clearance for the study was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2023/016).

Collection and Identification of Plant

The sample consisting of leaves, flowers, fruits and stem of the plant *Opilia celtidifolia* was collected from Galadimawa, Zaria Local Government Area of Kaduna State. The sample was then taken to the Herbarium Unit, Department of Botany, Ahmadu Bello University Zaria, where it was identified by

the taxonomist Namadi Sunusi (voucher number of ABU0901534).

Plant Extraction and Preparation

The leaves were washed with clean water, air-dried under shade at room temperature until constant weight was acquired. Leaves were then ground into a powdered form using a pestle and mortar. The powder was extracted with 100% absolute methanol using Soxhlet extraction method. The filtrate obtained was then collected and decanted into an evaporating dish, then evaporated on a water bath at 40°C to dryness. The dried extract was then weighed and stored in a desiccator.

Preliminary Phytochemical Screening

The methanol leaf extract of *Opilia celtidifolia* (MEC) was subjected to preliminary phytochemical screening according to Sofowora (1982), Trease *et al.*, (1996) and Silva *et al.* (1998).

Acute Toxicity Studies

The median lethal dose (LD_{50}) of MEC was determined orally in both mice and chicks using the Organisation for Economic Co-operation and Development (OECD) guideline 425 (2001). A dose of 5,000 mg/kg was administered to a mouse and a chick. The animals were then observed for signs of toxicity at least once during the first 30 minutes and periodically during the first 24 hours. Subsequently, two more mice and chicks were administered with 5,000 mg/kg of the extract and were also observed for signs of toxicity or death for 14 days.

Pentylene tetrazole (PTZ)-induced convulsion in mice

The Swinyard *et al.*, (1989) model for PTZ-induced convulsions was adopted. Thirty mice were divided into five groups, with six mice in each group. Group one received 10 ml/kg of distilled water *po*, groups two, three, and four received MEC at doses of 250, 500, and 1000 mg/kg, respectively while group five received 200 mg/kg of sodium valproate *po*. One hour later, all the mice were injected with 85 mg/kg of PTZ subcutaneously. Observation for the absence of clonic spasms (loss of righting reflex) lasting for at least five seconds was made over 30 minutes period.

Maximal electroshock-induced convulsion in chicks

The Swinyard *et al*., model for maximal electroshock-induced convulsions in chicks, as modified by Sayyah *et al*., (2002), was adopted. Fifty-day-old chicks were randomly divided into five groups, with ten in each group. Group one received 10 ml/kg of distilled water *po*, while groups two, three, and four received MEC at doses of 250, 500, and 1000 mg/kg *po*, respectively. Group five, the positive control group, received 20 mg/kg of phenytoin *po*. One hour later, an electroconvulsiometer was connected to a stabilizer, and corneal electrodes, dipped in normal saline, were placed on the upper eyelids of the chicks to induce seizures. The maximal electroshock (MES) parameters used throughout the study were a frequency of 100 pulse/sec, current of 80 mA, pulse width of 0.6 ms, and shock duration of 0.6 sec. The chicks were observed for the absence of hind limb tonic extension (HLTE) and/or prolongation of its latency or onset were recorded over a 30-minute period.

Strychnine-induced seizure in mice

The Porter *et al*., (1984) model for strychnine-induced convulsions was adopted. Thirty mice were divided into five groups, with six mice in each group. Group one received 10 ml/kg of distilled water *po*, while groups two, three, and four received MEC at doses of 250, 500, and 1000 mg/kg *po*, respectively. Group five received 30 mg/kg of phenobarbital *po*. Sixty minutes later, all animals were injected subcutaneously (S.C.) with 1.5 mg/kg of strychnine. The absence of tonic extension of the hind limbs after strychnine administration

was recorded over a 30-minute observation period.

Picrotoxin-induced convulsion test in mice

The model described by Yagamuchi *et al*., (1992) for picrotoxin-induced convulsions was adopted. Thirty mice were divided into five groups, each containing six mice. The first group was administered 10 mL/kg distilled water orally, the second, third, and fourth groups were given MEC orally at 250, 500, and 1000 mg/kg, respectively, while the fifth group was given diazepam at 10 mg/kg orally. An hour later, all mice were given a subcutaneous injection of 4 mg/kg picrotoxin. The absence of tonic extension or the latency of tonic hind limb over a 30-minutes observation period was recorded.

PTZ-Induced Kindling

The model described by Rocha *et al*., as modified by Tadayuki *et al*., (2018), was used to establish the neuroprotective effect of the extract. Sixty Swiss albino mice were used, and they were randomly divided into five groups of 12 mice each. The animals were habituated after measuring their body weight. A sub-convulsive dose of PTZ (35 mg/kg) was injected subcutaneously every other day for a total of ten (10) injections.

Group one (negative control) was administered 10 mL/kg distilled water orally 60 minutes before injection of the sub-convulsive dose (35 mg/kg) of PTZ throughout the kindling days.

Group two was administered MEC orally at 1000 mg/kg 60 minutes before injection of sub-convulsive dose (35 mg/kg) of PTZ throughout the kindling days.

Group three was administered MEC orally at 1000 mg/kg 60 minutes before injection of sub-convulsive dose of PTZ during the first to fifth kindling days, then distilled water at 10 mL/kg and sub-convulsive dose (35 mg/kg) of PTZ throughout the remaining days.

Group four was administered distilled water at 10 mL/kg and sub-convulsive dose (35 mg/kg) of PTZ during the first to fifth kindling days, then MEC at 1000 mg/kg 60 minutes before injection of sub-convulsive dose (35 mg/kg) of PTZ during the remaining kindling days.

Group five (positive control) was administered sodium valproate at 200 mg/kg 60 minutes before injection of sub-convulsive dose of PTZ throughout the kindling days.

Table 1 PTZ-Induced Kindling

DAYS / GROUPS	**DAY 1**	**DAY 3**	**DAY 5**	**DAY 7**	**DAY 9**	**DAY 11**	**DAY 13**	**DAY 15**	**DAY 17**	**DAY 19**
GRP I	**PTZ ONLY**									
GRP II	**MEC + PTZ**									
GRP III	**MEC + PTZ**					**PTZ ONLY**				
GRP IV	**PTZ ONLY**					**MEC + PTZ**				
GRP V	**VALPROATE + PTZ**									

All the animals were observed for at least 30 minutes and the epileptic behaviours classified and scored according to the modified Racine scale:
0: normal behaviour, no abnormality
1: immobilization, lying on belly
2: head nodding, facial, forelimb, or hind limb myoclonus
3: continuous whole-body myoclonus, myoclonic jerks, tail held up stiffly
4: rearing, tonic seizure, falling down on its side
5: tonic-clonic seizure, falling down on its back, wild rushing and jumping
6: death

Behavioural Studies

Y Maze Test

The method described by Dellu *et al*., 1992 was used to assess cognitive impairment (learning and memory). Thirty experimental mice were randomly divided into five groups of six mice each. The mice were allowed to acclimatize to the testing area in their cage for about ten minutes. Group one received 10 ml/kg distilled water orally, groups two, three, and four received MEC *po* at 250, 500, and 1000 mg/kg respectively while group five received vinpocetine at 10 mg/kg. During an eight-minute session, each mouse was placed at the end of an arm of the Y-maze and allowed to move freely through it. Entry into an arm was recorded when the hind paws of the mice completely entered an arm of the maze. The series of arm alternation, defined as successive entries into three arms on overlapping triplet sets (ABC, BCA, or CAB), but not CAC, BAB, or ABA, which were regarded as errors, were recorded. The Y-maze apparatus was cleaned with 70% ethanol to prevent any cues.

$$Percentage\ of\ Spontaneous\ alternation = \frac{spontaneous\ alternation}{total\ number\ of\ arm\ entries - 2} \times 100$$

Beam Walking Test

The protocol described by Feeney *et al*., (1982) was used for the beam walking test to determine coordination and motor deficits. The test was conducted over two days, which involved the training and testing days. Five experimental groups of six mice each were used. The mice were allowed to acclimatize to the testing area in their cage for about 10 minutes prior to the test.

During the training day, the mice were set to cross a beam of 12 mm three times each. The mice were allowed to rest for 10 minutes in their home cages in between the training, and the balance beam apparatus was cleaned with 70% ethanol. Pushing or poking of the mice was done if they performed any form of stalling or sniffing to encourage them to continue moving forward.

On the test day, group one was administered 10 ml/kg of distilled water, groups two, three, and four were administered MEC orally at 250, 500, and 1000 mg/kg, respectively, while group five was administered 10 mg/kg of diazepam orally. They were then made to perform the test done during training. Foot slips, falls, and the time utilized to traverse to the escape box were recorded.

Open field Test

Sedation and locomotion were assessed using the open field test, as described by Gupta *et*

al., (1971). The apparatus used consisted of a wooden field arena divided systematically into 16 organized squares (measuring 15 cm × 15 cm each), alternatively painted in black and white. The mice were divided into five groups of six mice each. Each mouse was placed in the middle of the arena, and the number of squares visited was counted while it was allowed to move freely for three minutes. Group one was orally administered with 10 mL/kg distilled water, while the second, third, and fourth groups were administered MEC *po* at 250, 500, and 1000 mg/kg respectively whereas group five was administered 10 mg/kg diazepam. They were then subjected to the open field test 60 minutes post-treatment. A unit of locomotion was counted manually when the mouse entered into a different square with its four limbs. The total number and frequency were determined after a period of six minutes. In between the tests for each mouse, the apparatus was cleaned with 70% ethanol to remove odors that could have influenced the behavior of the next mouse to be tested.

Oxidative Stress Biomarkers

At the end of the pentylene tetrazole induced kindling, the animals were anesthetized then euthanized. Their brain samples were removed and homogenized with 10 times ($^{w}/_{v}$) ice-cold 0.1M phosphate buffer (pH 7.4) to produce 10% $^{w}/_{v}$ homogenates. The homogenate was then centrifuged at 10,000 rpm for 15 minutes, and the resultant supernatant was used to assay for oxidative stress biomarkers, including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) using spectrophotometric assay kits.

Statistical Analysis

Data was analyzed using SPSS version 23 and *p-values* ≤0.05 was considered statistically significant. Results were presented as tables, lines, charts and graph as appropriate and expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was carried out for acute models of anticonvulsant studies, Y maze test, beam walking test and open field test, while Bonferoni post hoc test was used in the analysis of oxidative stress biomarkers. Mixed ANOVA followed by Bonferoni post hoc test was carried out in the analysis of chronic anticonvulsant study model.

RESULTS

Phytochemical screening

The phytochemical screening of methanol leaf extract of *Opilia celtidifolia* (MEC) revealed the presence of various secondary metabolites, including alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids (Table 2).

Table 2: Phytochemical constituents

Phytoconstituents	Inferences
Alkaloids	+
Cardiac Glycosides	+
Saponins	+
Phenolic compounds	+
Tannins	+
Steroids	+
Carbohydrates	+
Flavonoids	+
Terpenoids	+
Anthraquinones	-

Keys= + Present, - Absent

Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was found to be greater than 5,000 mg/kg in both mice and chicks, indicating that it is relatively safe and non-toxic.

Pentylene tetrazole-induced convulsion in mice

The MEC exhibited a significant ($p \leq 0.05$) delay in the mean onset of PTZ-induced seizure at doses of 500 mg/kg (12.56 ± 04.08) and 1000 mg/kg (13.21 ± 02.30) compared to the negative control (04.08 ± 00.38). Furthermore, the extract demonstrated 66.67% mortality prevention at a dose of 1000 mg/kg while the negative and positive controls provided 50% and 100% protection, respectively (Table 3).

Table 3 Effect of methanol leaf extract of *Opilia celtidifolia* on pentylene tetrazole-induced convulsion in mice

Treatment (mg/kg)	Mean Onset of seizure (min)	(%) Seizure protection	(%) Mortality protection	Mean Onset of Mortality (min)
DW 10 mL/kg	04.08 ± 00.38	0	50.00	19.17 ± 04.48
MEC 250	05.34 ± 02.13	0	33.33	18.52 ± 03.35
MEC 500	12.56 ± 04.08*	16.67	50.00	19.51 ± 04.32
MEC 1000	13.21 ±02.30*	0	66.67	26.25 ± 02.28
SV 200	>30.00**	100	100.00	>30.00

Data presented as Mean ± SEM, n = 6; * = $p < 0.05$; ** = $p < 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test), Distilled water = DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; SV =Sodium Valproate

Strychnine-induced seizure in mice

The MEC exhibited a dose-dependent delay in the onset of strychnine-induced seizures. The delay was significant ($p \leq 0.01$) at a dose of 1000 mg/kg (26.47 ± 03.12) when compared to the negative control (09.35 ± 01.01). At a dose of 1000 mg/kg, the extract provided 83.33% protection against seizures, while the negative and positive controls provided 0% and 100% protection, respectively. Moreover, the extract showed 100%, 83.33%, and 83.33% mortality prevention at doses of 250, 500, and 1000 mg/kg, respectively, while the negative and positive controls protected 50% and 100%, respectively (Table 4).

Table 4 Effect of methanol leaf extract of *Opilia celtidifolia* on strychnine-induced convulsion in mice

Treatment (mg/kg)	Mean Onset of seizure (min)	(%) Seizure protection	(%) Mortality protection	Mean Onset of Mortality (min)
DW 10 mL/kg	09.35 ± 01.01	0	50.00	21.57 ± 03.42
MEC 250	13.30 ± 03.25	16.67	100.00	>30.00

MEC 500	17.44 ± 03.56	33.33	83.33	26.45 ± 03.14
MEC 1000	26.47 ± 03.12**	83.33	83.33	26.52 ± 03.08
PHE 30	>30.00**	100	100.00	>30.00

Data presented as Mean ± SEM, n = 6; ** = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test) Distilled water= DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; PHE =Phenobarbital

Picrotoxin-induced seizure in mice

The MEC induced a statistically significant ($p \leq 0.05$) delay in the mean onset of picrotoxin-induced seizure at a dose of 1000 mg/kg (20.33 ± 03.27) in comparison to the negative control (12.32 ± 01.35). Furthermore, at the highest dose of 1000 mg/kg, it prevented 33% mortality, whereas the negative control had 0% protection and the positive control provided complete protection (Table 5).

Table 5 Effect of methanol leaf extract of *Opilia celtidifolia* on picrotoxin-induced convulsion in mice

Treatment (mg/kg)	Mean Onset of seizure (min)	(%) Seizure protection	(%) Mortality protection	Mean Onset of Mortality (min)
DW mL/kg	12.32 ± 01.35	0	0.00	18.09 ± 01.52
MEC 250	15.41 ± 01.37	0	16.67	23.31 ± 01.37
MEC 500	11.14 ± 00.51	0	16.67	21.09 ± 02.12
MEC 1000	20.33 ± 03.27*	33.33	33.33	22.49 ± 02.24
DZP10	>30.00**	100	100.00	>30.00**

Data presented as Mean ± SEM, n = 6; * = $p \leq 0.05$; ** = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test) Distilled water=DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; DZP =Diazepam;

Maximal electroshock (MES)-induced convulsion in chicks

The MEC did not exhibit significant ($p \leq 0.05$) protection against MES-induced seizure, and it did not significantly delay the mean recovery time compared to the negative control. The positive control, on the other hand, provided complete protection against MES-induced seizure (Table 6).

Table 6 Effect of methanol leaf extract of *Opilia celtidifolia* on MES-induced convulsion in chicks

Treatment (mg/kg)	(%) Seizure protection	(%) Mortality protection	Mean Recovery time (min)
DW mL/kg	0	100.00	09.09 ± 01.19
MEC 250	0	100.00	09.31 ± 01.08
MEC 500	0	100.00	10.56 ± 01.10
MEC 1000	10	100.00	08.13 ± 01.23
PHY 20	100	100.00	00.00**

Data presented as Mean ± SEM, n = 10; = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test) Distilled water = DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; PHY =Phenytoin**

PTZ-Induced Kindling

In the PTZ-induced kindling model, the groups treated with MEC, namely group two (MEC + PTZ), group three (MEC + PTZ; PTZ), and group four (PTZ; MEC + PTZ), had marginal means of the modified Racine scale of 1.97, 1.88, and 1.93, respectively. These values were statistically insignificant ($p>0.05$) when compared to the negative control group (group one, PTZ only, mean = 1.475) and the positive control group (group five, sodium valproate + PTZ, mean = 1.183) (see Figure 1).

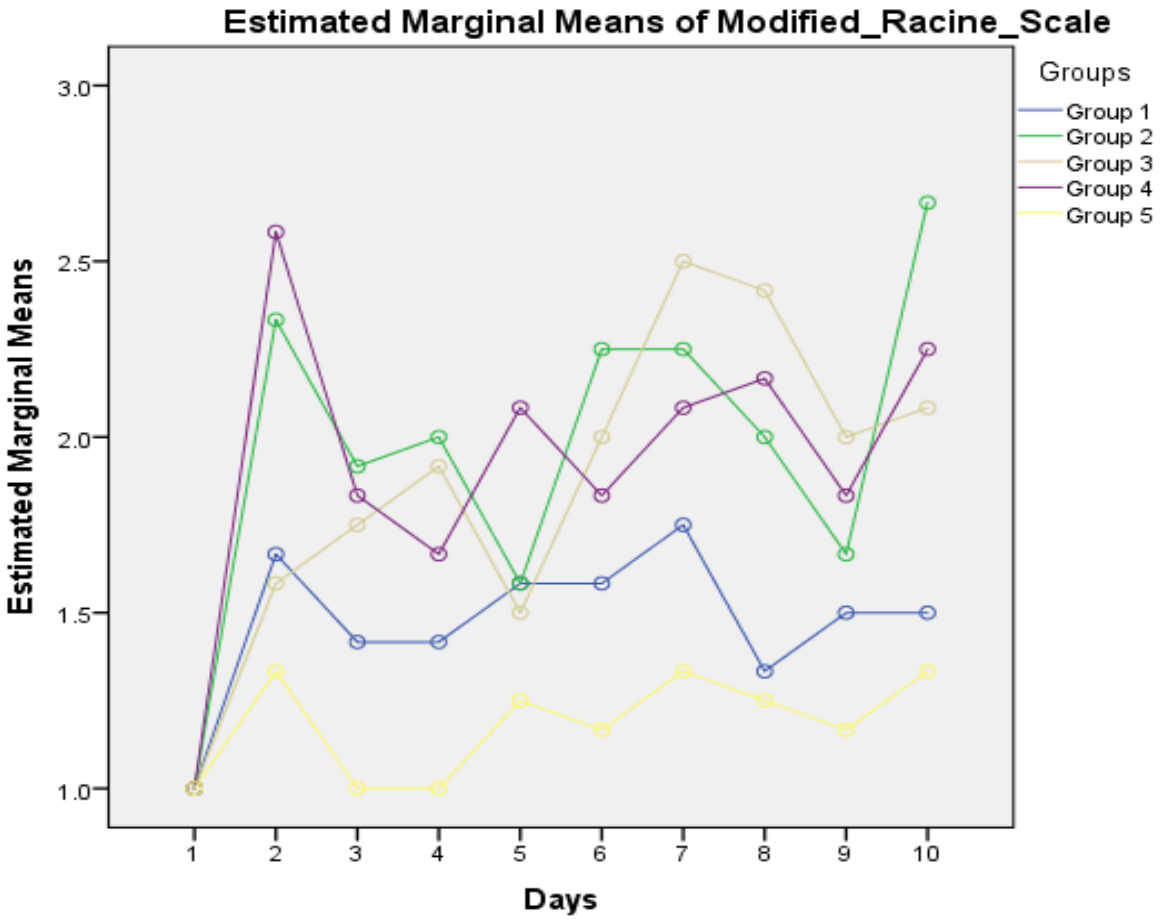


Figure 1: Estimated marginal means of modified racine scale during PTZ-induced kindling

Oxidative Stress Biomarkers

The PTZ-induced kindling model in mice showed a significant ($p \leq 0.05$) increase in the levels of superoxide dismutase in group four mice, which were administered PTZ then MEC+PTZ, when compared to group five mice, which were administered valproate then PTZ. Additionally, there was a significant ($p \leq 0.05$) increase in the levels of catalase in group four (PTZ; MEC+PTZ) when compared to group one (PTZ only) (Table 7).

Table 7 Mean levels of oxidative stress biomarkers in PTZ-induced kindled mice

Treatment (mg/kg)	**SOD (IU/MG PROTEIN)**	**GSH (UG/MG PROTEIN)**	**MDA (UMO/MG PROTEIN)**	**CAT (U/MG PROTEIN)**

PTZ only	11.85 ± 0.83	10.73 ± 1.17	225.78 ± 9.16	6.80 ± 0.55
MEC + PTZ	12.71 ± 1.27	10.65 ± 1.01	226.63 ± 17.11	9.03 ± 0.65
MEC + PTZ; PTZ	11.96 ± 0.79	11.40 ± 0.84	238.17 ± 22.20	7.16 ± 0.81
PTZ; MEC + PTZ	13.89 ± 1.07*	10.15 ± 0.67	218.97 ± 11.49	10.04 ± 0.70*
Sodium valproate + PTZ	9.69 ± 0.53*	11.43 ± 0.79	180.68 ± 17.75	8.16 ± 0.83

Data presented as Mean ± SEM; n = 12; * = $p \leq 0.05$ significant difference as compared to the DW group; (One Way Anova followed by Bonferroni Post hoc test for Multiple comparism), Distilled water = DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; reduced glutathione=GSH; superoxide dismutase=SOD; malondialdehyde MDA; Catalase=CAT

Behavioural Studies

Y Maze Test

In mice treated with the MEC, there was no significant difference in the mean percentage of spontaneous alternation compared to the negative control group. However, a statistically significant decrease ($p \leq 0.01$) was observed in the mean number of arm entries at the highest dose of MEC, compared to the negative control group (Table 8).

Table 8 Effect of MEC on cognition in mice using Y maze test

Treatment (mg/kg)	Mean Percentage of spontaneous alternation	Mean Number of arm entries	Mean Number of actual alternation
DW mL/kg	23.06 ± 2.63	24.83 ± 1.92	5.17 ± 0.54
MEC 250	22.05 ± 3.19	26.67 ± 1.75	5.50 ± 0.92
MEC 500	18.75 ± 2.24	28.50 ± 3.38	5.17 ± 1.08
MEC 1000	14.87 ± 5.51	12.50 ± 3.14**	2.33 ± 0.99

VPC 10	20.41 ± 2.47	27.50 ± 2.59	5.33 ± 0.88

Data presented as Mean ± SEM, n = 6; ** = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test) Distilled water =DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; Vinpocetine=VPC;

Open Field Test (OFT)

The methanol leaf extract of *Opilia celtidifolia* significantly ($p \leq 0.01$) reduced the mean number of distinct squares explored at a dose of 1000 mg/kg (9.33 ± 3.69) compared to the negative control (62.50 ± 6.87). Similarly, the positive control (Diazepam; 38.50 ± 7.82) also exhibited a significant ($p \leq 0.05$) decrease in the mean number of different squares visited as shown in Table 9.

Table 9 Assessment of The Locomotor and Sedative Effect of Methanol Leaf Extract of *Opilia celtidifolia* in Mice

Treatment (mg/kg)	Mean number of squares visited	Mean Frequency of squares visited (min)
DW mL/kg	62.50 ± 6.87	20.83 ± 2.29
MEC 250	80.83 ± 5.85	26.94 ± 1.95
MEC 500	55.17 ± 7.34	18.39 ± 2.45
MEC 1000	9.33 ± 3.69**	3.11 ± 1.23
DZP 10	38.50 ± 7.82*	12.83 ± 2.61

Data presented as Mean ± SEM, n = 6; * = $p \leq 0.05$; ** = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test for Multiple comparism) Distilled water =DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; DZP =Diazepam;

Beam Walking Test

There was no significant difference in the mean number of foot slip or mean time utilized to traverse to the escape box nor was there fall in the extract treated mice when compared to the negative control (Table 10).

Table 10 Effect of methanol leaf extract on coordination and motor activities in mice using beam walking test

Treatment (mg/kg)	Number of Foot slips	Number of Falls	Time utilized to traverse to the escape box
DW mL/kg	0.67 ± 0.33	0	00.13 ± 00.01
MEC 250	0.00	0	00.20 ± 00.03
MEC 500	0.17 ± 0.17	0	00.19 ± 00.06
MEC 1000	0.50 ± 0.50	0	00.19 ± 00.04
DZP 10	0.67 ± 0.33	0	00.17 ± 00.04

Data presented as Mean ± SEM, n = 6; * = $p \leq 0.05$; ** = $p \leq 0.01$ significant difference as compared to the DW group; (One Way Anova followed by Dunnett Post hoc test) Distilled water =DW; MEC = Methanol leaf extract of *Opilia celtidifolia*; DZP=Diazepam;

Discussion

The phytochemical analysis of methanol leaf extract of *Opilia celtidifolia* (MEC) revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids.

Some of the compounds in the plant have demonstrated hepatoprotective effects, potentially attributable to tannins and saponins (Amang *et al.*, 2020). Additionally, saponins have been identified as contributors to the plant's immunostimulatory, antipyretic, antiparasitic, and antispasmodic activities (Gronhaug *et al.*, 2008, Shihata *et al*., 1977). Furthermore, polysaccharides extracted from the plant have been found to possess complement-fixing and macrophage-stimulating activities (Togola *et al.*, 2005), which may be responsible for its wound-healing properties.

The MEC showed a significant delay in the mean onset of picrotoxin-induced seizures at the highest dose, along with an increased percentage of survival. This implies that the extract may have an effect on the $GABA_A$ receptor chloride channels, as picrotoxin is a non-competitive antagonist of these channels in different regions of the central nervous system, leading to inhibition of GABA activity and resulting in death in most cases, usually secondary to generalized tonic-clonic seizures (Abdul- Ghani *et al*., 1980, Ya'u *et al.*, 2015).

Compounds that have shown effectiveness against maximal electroshock (MES)-induced seizure models are known to block sodium channels (Stephen, 2018). In this study, the

MEC did not provide significant protection against maximal electroshock (MES)-induced seizures, nor did it significantly delay the mean recovery time. This further suggests that the extract may be acting mainly through the $GABA_A$ chloride channel

The MEC exhibited sedative characteristics and suppressed locomotor activity in mice, as evidenced by the decreased number of squares visited in the open field test and the reduced number of arm entries in the Y maze test. This further supports the observation that the extract has GABAmimetic properties. However, the extract does not appear to affect the cognitive abilities of the mice, as indicated by the absence of significant differences in spontaneous alternation in the Y maze test.

In the PTZ-induced kindling model, all groups treated with the extract demonstrated higher marginal mean of the modified Racine scale compared to both the negative control (PTZ only) and positive control (sodium valproate + PTZ). These suggestions suggest that extended co-administration of the extract with sub-convulsive doses of PTZ may decrease the seizure threshold, thereby increasing susceptibility to seizures and potentially exacerbating the condition. They also indicate that the extract exhibited no therapeutic efficacy in the PTZ-induced kindling model, and instead, it potentially exacerbated seizure severity.

In the PTZ-induced kindled mice, group four (PTZ; MEC+PTZ) showed a significant increase in brain superoxide dismutase levels compared to group five (Valproate + PTZ) and a significant increase in catalase levels compared to group one (PTZ only). These findings suggest increased oxidative stress in the brains of mice treated with the extract during PTZ-induced kindling.

During mitochondrial respiration and energy generation, there is a continuous production of oxygen-derived free radicals, also known as reactive oxygen species (ROS), in the cell. However, their degradation and removal by intracellular ROS scavengers prevent cellular injury associated with free radical production. Oxidative stress occurs when there is an imbalance between the increased production and decreased scavenging of ROS, leading to excessive free radicals (Scott, 2021). Some of the scavengers that prevent cellular injury include catalase, which decomposes H_2O_2 ($2H_2O_2 \rightarrow O_2 + 2H_2O$), superoxide dismutases (SODs), which converts O_2• to H_2O_2 ($2O_2$• + $2H \rightarrow H_2O_2 + O_2$), and glutathione peroxidase, which catalyzes free radical breakdown (H_2O_2 + 2GSH → GSSG [glutathione homodimer] + $2H_2O$, or 2˙OH + 2GSH → GSSG + 2H2O) (Scott, 2021). An increase in free radicals causes overproduction of malondialdehyde because it is one of the final products of polyunsaturated fatty acid peroxidation in the cells (Stefan *et al.,* 2004).

The administration of the extract did not lead to any notable motor deficits or coordination impairment, as evidenced by the lack of significant difference in the mean number of foot slips or mean time to reach the escape box between the extract-treated mice and the negative control during the beam walking test. Additionally, there was no significant difference in the mean number of falls observed during the same test.

Conclusion

In conclusion, the methanol leaf extract of *Opilia celtidifolia* has anticonvulsant properties which it exerts through its effects on GABA chloride channel. Additionally, the extract also increased oxidative stress and the mean racine score when administered during PTZ-induced kindling, suggesting it may have a pro-convulsant effect in this model.

Furthermore, the extract had a sedative effect and reduced locomotor activity, but did not affect cognitive abilities such as learning and memory. Overall, the extract may be a useful source of anticonvulsant remedy.

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