

Original Research Article

Anticonvulsant potentials of *Hippocratea welwitschii* Oliv (Celastraceae) aqueous crude extract

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Abstract

Purpose: To propose a specific mode of action for the activity of the crude aqueous extract of *Hippocratea welwitschii* Oliv (Celastraceae) roots using specific models.

Methods: The extract was evaluated for anticonvulsant activity using picrotoxin, 4-amino pyridine, N-methyl-D-aspartate (NMDA) and the effects of flumazenil on the extract on seizures caused by pentylenetetrazole (PTZ) in adult Swiss mice of either sex, locally bred, and weighing 20 ± 2 g at doses of 125, 250, and 500 mg/kg. Five groups of ten mice each were used for all tests. Extracts were given orally to all mice, while the convulsing agents were administered intraperitoneally after administration of the extract.

Results: The extract at 125 and 250 mg/kg provided 100 and 80 % protection against mortality, respectively, while at a dose of 250 mg/kg, it afforded 60 % protection against NMDA-induced clonic spasm; this suggests the involvement of glutamatergic neurotransmission in its anticonvulsant activity. The extract did not produce any protection against death in the picrotoxin test, but it was able to prolong the time of death (14.72 ± 1.10) and onset of seizure (11.78 ± 1.21) at 125 mg/kg dose of the extract in the treated mice. Quite notably, the 500 mg/kg dose of the extract delayed seizure onset (16.49 ± 0.33) and time of death (18.36 ± 1.88) of the treated mice in 4-Aminopyridine induced convulsions.

Conclusion: The aqueous crude extract of *Hippocratea welwitschii* exhibits promising antiepileptic potential, which can be further harnessed through more thorough biological analyses and additional research on the chemical description of the anticonvulsant active principles and definite mechanism of anticonvulsant action.

Keywords: 4-Aminopyridine, Anticonvulsant, Convulsant, *Hippocratea welwitschii*, N-Methyl-D-aspartate

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INTRODUCTION

Hippocratea welwitschii Oliv (locally called 'Nya worouruambombo' in Efik, or 'Nya worouruambomb' in Ibibio) belongs to the Celastraceae family, which primarily consists of shrubs. However, certain species, like *Hippocratea*, have branchlets that resemble

climbers and coil around their supports [1]. A member of the bittersweet shrub family, *Hippocratea welwitschii* is a staff vine that is indigenous to Ghana, Nigeria, and Liberia. In addition to being a closed, primary, or mature secondary forest shrub, or in secondary shrub thickets from Guinea to western Cameroon, the plant is found throughout Africa, extending to

Angola, Uganda, and Tanganyika (Tanzania) [2]. According to Okwute *et al*, it is a large woody climber with glabrous leaves that are broadly oval and lack noticeable reticulation [3]. One of the plant's most significant uses is in the treatment of epilepsy in South-South Nigeria, where it has enormous medicinal significance among indigenous and traditional healers in Africa [1]. The phytochemicals present in the root extract of *Hippocratea welwitschii* have been reported to be terpenoids, phenols, saponins, tannins, flavonoids, oxalic acid, cyanide, glycosides and alkaloids [4] and these phytochemicals may largely be responsible for the medicinal properties observed in the plant [5]. Several biological activities of the plant have been assessed and recorded, including the broad-spectrum activity of the volatile oil extracted from the plant's roots against harmful bacteria and fungi, as well as the antibacterial activity of the methanol extract of the leaves [1,6].

Epilepsy is a chronic condition characterized by recurrent seizures [7]. It can have a variety of causes, including genetics, brain trauma, inflammation, and tumours, and can manifest in a variety of ways, ranging from a brief loss of consciousness to focal or widespread convulsions, depending on the part of the brain that has developed a propensity for neuronal hyperexcitability and hypersynchronization and its physiological function [8]. The first step treatment option for epilepsy is usually to use one of the available antiepileptic drugs (i.e., monotherapy) and this is often based on age of patient, co-morbidities, seizure type and other factors. Conventional treatment of epilepsy largely comprises anticonvulsant medications (examples are clobazam, felbamate and ethosuximide) [9]. Antiepileptic drugs may act mainly by one of three main mechanisms – they may accomplish seizure reduction by suppressing neuronal intrinsic (lacosamide and carbamazepine) or synaptic excitation (usually mediated by the neurotransmitter glutamate) for example phenytoin and perampanel and promoting synaptic inhibition (usually mediated by the neurotransmitter gamma amino – butyric acid or GABA) examples of these includes valproic acid and pregabalin [10]. While the mechanism of action of several antiepileptic medications is still unknown (such as that of gabapentin, Levetiracetam, Pregabalin, and Cannabidiol (CBD)), it has been demonstrated that some have multiple target systems (divalproate, for example, impacts Na^+ , Ca^{2+} and GABA) [11]. Unfortunately, instead of treating epileptogenesis, the long-term buildup of problems that contribute to seizures, all currently

available medications tackle ictogenesis, or the expression of seizures. This suggests that there is still no complete cure for epilepsy because all treatments are symptomatic rather than causative. In over 70 % of cases, this symptomatic therapy is successful [12].

In an earlier study carried out, n-hexane, ethyl acetate, methanolic and aqueous crude extracts of the root of *Hippocratea welwitschii* were subjected to anticonvulsant evaluation using the maximal electroshock test (MEST) in chicks, subcutaneous pentylenetetrazole (scPTZ), beam walking assay and strychnine-induced seizures test in mice [4]. From the results of the tests, the aqueous crude extract was seen to show very good anticonvulsant activity, especially in the subcutaneous Pentylenetetrazole (PTZ) – induced Seizure where the crude extract at 125 mg/kg showed 80 % protection against death in the mice used. In this paper, we present further exploration into the anticonvulsant activity of the aqueous crude extract of *Hippocratea welwitschii* roots using specific models.

EXPERIMENTAL

Collection and identification of plant materials

Fresh roots of *Hippocratea welwitschii* were collected from Ukanafum in Akwa Ibom state, which is located in the South-South region of Nigeria (with coordinates Latitude 4.8965° North and 7.5969° East) sometimes around August 2021. The plant was thereafter identified and authenticated at the University of Nigeria, Nsukka (UNN), Department of Pharmacognosy and Environmental Medicine, by plant taxonomists and assigned a Voucher reference number PCG/UNN/0305. After that, a voucher specimen was deposited in the herbarium section of UNN for future reference. The plant material was cleaned to get rid of debris and cut into smaller pieces before drying (under shade) for three weeks. The plant sample was then milled into powder with the aid of an electric hammer mill (model TRAPP TRF 80 Hammer mill foliage) and then stored in a moisture-free environment until required for further use.

Preparation of the crude extract

The powdered sample, 150 g, was boiled in 1 L of distilled water for six hours to produce the plant material's crude extract. After filtering and cooling, the filtrate was freeze-dried to produce a crunchy, dark brown powder. After that, the dried extract was stored in a firmly sealed vial at 4 °C

in the refrigerator until it was needed for further studies.

Animals

Adult Swiss mice of either sex, locally bred and weighing 20 ± 2 g, were acquired from the Animal House Facility in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. Throughout the research, the mice were kept in polypropylene cages with normal environmental conditions, fed a regular mouse diet, and given unlimited access to water. The Ahmadu Bello University committee on animal use carefully authorized the protocol (Reg no. ABUCAUC 2017/018).

Chemicals and Reagents

All the drugs used for the studies were produced by Sigma Aldrich and supplied by Adel Crystal Lake, Lagos, Nigeria. All other general chemicals, including analytical-grade solvents, were purchased from Femimat Chemicals.

Picrotoxin-induced seizure in mice

The method of Muhammad *et al* was used as previously described [13]. Five groups of ten mice each were randomly selected from among fifty (50) animals. Distilled water was given to the first group (10 ml/kg). The crude extract was administered at three graded doses (125, 250, and 500 mg/kg) to the second, third, and fourth groups, respectively. Every treatment was administered orally. The fifth group of mice was given intraperitoneal injections of 10 mg diazepam/kg body weight. Each mouse received a convulsive dose of picrotoxin (10 mg/kg per body weight) 60 minutes after extract administration, and 30 minutes for the diazepam treatment group. After that, they were all monitored for thirty minutes for hind limb tonic seizures. Anticonvulsant activity was considered to be indicated by either the absence of tonic hind limb extension or the prolongation of the latency of the hind limb tonic extension [13].

4-Aminopyridine-induced seizure in mice

The approach used was similar to that previously described [13]. A total of fifty (50) mice were split into five groups, each with ten mice, at random. Distilled water (10 ml/kg) was given to the first group. Three graded doses of the crude extract (125, 250, and 500 mg/kg) were given to the second, third, and fourth groups, respectively. All of the treatments were administered orally. 30 mg of phenobarbitone per kilogram of body weight was administered intraperitoneally to the

fifth group. 4-Aminopyridine was given to each mouse at a convulsive dose of 15 mg/kg per kg body weight 30 minutes after treatment (for all oral pre-treatment) and 15 minutes after phenobarbitone. For thirty minutes, the mice were monitored for typical behavioural symptoms, including trembling, hyperactivity, intermittent clonus of the forelimb and hindlimb followed by hindlimb extension, tonic seizures, opisthotonus, and death. An indication of anti-convulsant activity was the extract's capacity to prevent the mice from dying within a 30-minute observation period [13].

N-Methyl-D-aspartate (NMDA)-induced seizures in mice

For this study, the methods previously outlined by Muhammad *et al* were used. Five groups of ten mice each were randomly selected from among fifty (50) animals. Distilled water was given to the first group (10 ml/kg). The crude extract was administered at three graded doses (125, 250, and 500 mg/kg) to the second, third, and fourth groups, respectively. Every treatment was administered orally. Diazepam (10 mg/kg body weight) was administered intraperitoneally to the fifth group. Each mouse received a subcutaneous convulsant dosage of NMDA (100 mg/kg) 60 minutes after extract administration and 30 minutes after diazepam therapy. Animals were deemed protected if they did not exhibit turning behaviour within 30 minutes [16,17]. An animal exhibits turning behaviour when it completes two consecutive 360° rounds [13].

Effects of flumazenil on anticonvulsant effects of extracts on pentylenetetrazole (PTZ)-induced seizures

The mice were grouped into five ($n=10$) at random. Group 1 was given 10 ml/kg of distilled water. Normal saline (10 ml/kg, orally), Minimum effective dose (MED) of the crude extract (90 mg/kg, orally), and Diazepam (10 mg/kg, intraperitoneally) were given to groups two through four, respectively. Diazepam (10 mg/kg) and MED of crude extract were given to groups five and six, respectively. Fifteen minutes before treatment, flumazenil (2 mg/kg) was administered subcutaneously to groups two through four. The convulsive dose of PTZ (85 mg/kg) was administered to all groups two through six. Within 30 minutes following the administration of PTZ, the observations were recorded [13].

Statistical analysis

Values were expressed as Mean $\pm$ standard error of the mean (SEM). Data were analyzed

using One-Way ANOVA followed by Dunnett post hoc t-test for multiple comparisons. A p -value < 0.05 was considered significant.

RESULTS

With the aid of specific models, viz., Picrotoxin-induced seizure, 4-Aminopyridine-induced seizure, *N*-Methyl-D-aspartate (NMDA)-induced seizure and the effects of flumazenil on the extracts on PTZ-induced seizures in mice, the anticonvulsant activity of aqueous crude extract of *Hippocratea welwitschii* root was determined. Tables 1 through 3 show the findings from the various studies.

DISCUSSION

Evaluation of the anticonvulsant activity of the aqueous crude extract of roots of *Hippocratea welwitschii* using the models listed above showed some potential, as seen in the results above and provides scientific evidence for anticonvulsant use of the plant. The root aqueous extract at all doses could not offer any

protection against seizure and mortality in the picrotoxin induced seizure (Table 1); it showed an extended time for the onset of seizure and mortality better than the methanol extract of the sample plant (*Hippocratea welwitschii*) as presented in an earlier study [13]. The 4-Aminopyridine induced seizure on the animals showed delayed onset of seizure comparable to the standard agent (phenobarbitone) using the root aqueous extract at a dose of 500 mg/kg, but no protection against death, which is also in tandem with the results obtained for the methanol extract of the same plant [13]. In the NMDA-induced clonic seizure, the root aqueous extract showed very promising activity as it gave 100 % protection against death at a dose of 125 mg/kg, and between 60 – 80 % protection against death at other doses tested. It equally significantly extended onset of seizure to 10.92, 13.46 and 10.22 mins at doses of 125, 250 and 500 mg/kg, respectively, which were all better than methanol fraction and standard drug (diazepam), with mean onset of seizure time of 7.15 mins [13].

Table 1: Anticonvulsant activity of *Hippocratea welwitschii* roots aqueous extract on picrotoxin-induced seizures

Sample	Treatment (mg/kg)	Mean onset of seizure (min)	QP protection against seizures	Mean time of death (min)	QP protection against mortality
Control	0	10.5±0.54	0/10	14.24±1.50	0/10
Crude extract	125	11.78 ± 1.21	0/10	14.72 ± 1.10	0/10
Crude extract	250	10.11 ± 1.71	0/10	11.24 ± 1.49	0/10
Crude extract	500	7.64 ± 0.74	0/10	9.38 ± 1.02	0/10
Diazepam	10	25.18±0.00	9/10	25.41±0.00**	9/10

Values are expressed as Mean ± SEM. N = 10. ** $p < 0.01$ vs N/saline (Control). QP – Quantal protection. Control group received only distilled water

Table 2: Anticonvulsant activity of *Hippocratea welwitschii* roots aqueous extract on 4 – amino pyridine induced seizures

Sample	Treatment (mg/kg)	Onset of seizure (min)	QP protection against seizures	Mean time of Death (min)
Control	0	11.84±0.12	0/10	15.68±1.24
Crude extract	125	13.55±0.42	0/10	15.05 ± 1.03
Crude extract	250	12.69±0.65	0/10	13.84 ± 1.32
Crude extract	500	16.49±0.33	1/10	18.36 ± 1.88
Phenobarbitone	30	16.87±0.45*	10/10	

Values are expressed as Mean ± SEM, * $P < 0.05$ vs control. N = 9 – 10. Control group received only distilled water

Table 3: Anticonvulsant activity of *Hippocratea welwitschii* roots aqueous extract on nmda-induced seizures

Sample	Treatment (mg/kg)	Mean onset of seizure (min)	QP protection against seizures	Mean time of Death (min)	QP protection against mortality
Control	0	5.12±0.43	2/10	11.15±2.68	6/10
Crude extract	125	10.92 ± 5.50	4/10	-	10/10
Crude extract	250	13.46 ± 4.04	6/10	12.32 ± 0.33	8/10
Crude extract	500	10.22 ± 4.04	4/10	14.35 ± 2.46	7/10
Diazepam	10	7.15±2.66*	7/10	11.24±0.00	9/10

Values are expressed as Mean ± SEM. N = 10. * $P < 0.05$ vs N/saline. Control group received only distilled water

In comparison to conventional agent (diazepam), which provided 90 % protection against death, the aqueous root extract of *Hippocratea welwitschii* at a dose of 250 mg/kg provided 60 % protection against NMDA-induced clonic spasm and 100 % protection against mortality at a dose of 125 mg/kg. The NMDA (N-methyl-D-aspartate)-induced mouse seizure model is a widely used experimental model for investigating neurotoxicity linked to excessive NMDA receptor activation, testing anticonvulsant medications, and studying seizure mechanisms. The NMDA receptor, a subtype of the glutamate receptor involved in excitatory neurotransmission, is synthetically agonistic. Excitotoxicity from overactivation of NMDA receptors causes seizures and, in extreme situations, neurodegeneration [14]. Extract's capacity to shield mice from seizures brought on by NMA points to the role glutamatergic neurotransmission plays in its anticonvulsant properties.

Picrotoxin-induced seizure paradigm may be used to clarify the likely mechanism of action of an anticonvulsant medication on the GABA receptor. Picrotoxin is a non-competitive antagonist of the GABA A receptor that selectively blocks chloride channels. Barbiturates, benzodiazepines, valproic acid, vigabatrin, gabapentin, tiagabine, and other medications that stop picrotoxin-induced seizures may improve GABA-mediated neurotransmission by disrupting the GABAergic pathway [15,16]. The extracts' inability to protect mice against picrotoxin-induced seizures suggests that they may not interact with picrotoxin sites in the GABAA-chloride ion channel complex to produce their anti-convulsant effects. The K⁺ channels have a major role in controlling all facets of neuronal excitability, such as resting membrane potential, sensitivity to synaptic inputs, frequency adaptation, and neurotransmitter release [17]. Drugs like phenytoin that prevent the spread of seizures are effective antagonists of seizures brought on by K⁺ channel blockade, whereas medications that have specific actions on other cellular targets may be weak or inactive, most likely because they are unable to reduce the spread of intense (non-NMDA receptor-mediated) excitation evoked by 4-Aminopyridine [15,18]. This extract's incapacity to generate significant effects against 4-Amino Pyridine-induced seizures suggests that it may not be interacting with K⁺ channels to achieve its anticonvulsant properties. The effects of flumazenil on the anticonvulsant effects of the extracts on PTZ-induced seizures failed the predictive validity.

CONCLUSION

Hippocratea welwitschii aqueous root extract shows potential antiepileptic properties. The potential anticonvulsant property of the extract suggests its suitability for medical intervention in seizures. *Hippocratea welwitschii* roots can thus be considered a potential source for new anti-epileptic drug for pharmaco-resistant epilepsy. While this study has shown promising results in reducing seizure frequency and severity in animal models, additional pharmacological investigations using isolated active ingredients are necessary to confirm efficacy and mechanism of action.

DECLARATIONS

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None.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Ethical approval

The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee with approval no. ABUCAUC 2017/018.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

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