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Phytochemical screening and anticonvulsant studies of ethyl acetate fraction of *Globimetula braunii* on laboratory animals

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the phytochemical properties and anticonvulsant activities of EAF of *G. braunii* on laboratory animals. The activity was assessed based on anticonvulsant activity using maximal electroshock test in chicks, as well as pentylenetetrazole and 4-AP-induced seizures in mice. The preliminary phytochemical screening carried out on the crude ethanol extract revealed the presence of saponins, carbohydrates, flavonoids, tannins, anthraquinones and steroids. Similarly, tannins, flavonoids and steroids/terpenes were found to be present in the EAF.

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ABSTRACT

Objective: To investigate the phytochemical properties and the anticonvulsant potential of the ethyl acetate soluble fraction of ethanol leaf extract of *Globimetula braunii*, a plant used in ethnomedicine for the treatment of epilepsy.

Methods: The phytochemical screening was carried out using standard protocol while the anticonvulsant activity was studied using maximal electroshock test in chicks, pentylenetetrazole and 4-aminopyridine-induced seizures in mice.

Results: The preliminary phytochemical screening carried out on the crude ethanol extract revealed the presence of saponins, carbohydrates, flavonoids, tannins, anthraquinones and steroids. Similarly, tannins, flavonoids and steroids/terpenes were found to be present in the ethyl acetate fraction. In the pharmacological screening, 150 mg/kg of the fraction protected 83.33% of animals against pentylenetetrazole-induced seizure in mice whereas sodium valproate a standard anti-epileptic drug offered 100% protection. In the 4-aminopyridine-induced seizure model, the fraction produced a significant ($P<0.05$) increase in the mean onset of seizure in unprotected animals. The fraction did not exhibit a significant activity against maximal electroshock convulsion. The median lethal dose of the fraction was found to be 1 261.91 mg/kg.

Conclusions: These results suggest that the ethyl acetate fraction of *Globimetula braunii* leaves extract possesses psychoactive compound that may be useful in the management of petit mal epilepsy and lend credence to the ethnomedical use of the plant in the management of epilepsy.

KEYWORDS

Epilepsy, *Globimetula braunii*, Seizure, Medicinal, Pentylenetetrazole

1. Introduction

Epilepsy is a common and diverse set of chronic neurological disorders characterized by seizures[1]. About 75%-80% of epileptic patients may be provided with adequate seizure control with the help of conventional antiepileptic drugs. However, over 30% of people with epilepsy do not have seizures control even with best available medications[2]. Currently available antiepileptic drugs do not affect epileptogenesis and are associated with serious side effects,

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including teratogenicity, chronic toxicity and adverse effects on cognition and behavior[3]. Almost all the currently available antiepileptic drugs are associated with drug interaction making it difficult to attain easy seizure control[4]. There is an urgent need for the development of newer antiepileptic agents with better safety and efficacy profile. There is a reawakening interest in traditional medicine in the management of epilepsy, especially in developing countries[5]. Researches are needed to validate the folkloric use of these medicinal plants in order to provide evidence of their safety and efficacy[6]. One of such medicinal plants used in the traditional management of epilepsy but with paucity of scientific verification literature is *Globimetula braunii* (*G. braunii*) (Mistletoe).

G. braunii (Engler) Van Tlegh is a member of the Loranthaceae family, a hemi-parasitic shrub that grows on dicotyledones trees and attached itself to the host by modified roots. The vernacular names in Nigeria include *"Kauchii"* (Hausa) and *"Afomoonishano"* (Yoruba). The leaves of *G. braunii* are used for the treatment of disease like cardiovascular diseases[7], hepatic illness[8,9] and malaria[10]. It also has common application in treating rheumatism, epilepsy, infertility, stomach problems, and as a laxative[11]. To the best of our search, there is no report of the anticonvulsant activity of *G. braunii* in the literature. In furtherance with an attempt to isolate the bioactive principles of the plant, this study therefore aimed at establishing the phytochemical constituent present and evaluating the anticonvulsant potential of the ethyl acetate fraction of *G. braunii*.

2. Materials and methods

2.1. Materials

2.1.1. Collection and identification of plant material

The leaves of *G. braunii* parasitic on *Piliostigma thonningii* were collected in October 2011, at the Botanical garden of medicinal plants of Sheda science and Technology complex (Shestco), Abuja, Nigeria. This was confirmed and authenticated at the herbarium, Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria, by comparing with existing specimens (No. 9016 for *G. braunii* and No. 7151 for *Piliostigma thonningii*).

2.1.2. Preparation of extract

The plant leaves were washed, air dried under the shade until constant weight was obtained, size was reduced using laboratory blender and subsequently referred to as powdered plant material. The powdered plant material (250 g) was subjected to extraction with ethanol in a Soxhlet apparatus for 48 h. The Solvent was removed in vacuo to yield a residue (80 g) referred to as *G. braunii* ethanolic extract. The ethanol extract (70 g) was suspended in distilled water and partitioned successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to obtain *n*-hexane fraction, chloroform fraction, ethyl acetate fraction (EAF), *n*-butanol fraction and the aqueous fraction respectively.

2.1.3. Animals

Locally bred adult Swiss albino mice of either sex weighing (20±2) g were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. Day old ranger cockerels (30 ±5) g were obtained from Zarm farms Kwara state, Nigeria. The mice, maintained on standard rodent faced and water *ad libitum*, were housed in polypropylene cages at room temperature throughout the study.

2.2. Methodology

2.2.1. Phytochemical screening

Portion of the ethanol extract and that of EAF of *G. braunii* were subjected to preliminary phytochemical screening using standard methods[12].

2.2.2. Acute toxicity studies in mice

The method used was described by Lorke[13]. All mice were injected intra peritoneally (*i.p*). The study was divided into two phase. In the first phase, nine mice of either sex were divided into groups of three mice each. Group one received 10 mg/kg extract while groups two and three received 100 mg/kg and 1 000 mg/kg respectively. The animals were observed for signs and symptoms of toxicity including mortality for forty eight hours after treatment. In the second phase, four mice of either sex were divided into four groups of one mouse each. The first mouse received extract at a dose of 600 mg/kg while the second, third and fourth received the extract at doses of 1 000 mg/kg, 1 600 mg/kg and 2 900 mg/kg respectively, based on the outcome of the first phase. The mice were also observed for twenty four hours. The final LD_{50} was estimated as the square root of the products of the lowest lethal dose and the highest non-lethal dose *i.e.* geometric mean of consecutive doses for which 0% and 100% survival rates were recorded.

2.2.3. Maximal electroshock seizures test in chicks

Day old ranger cockerels (fifty in number) were randomly divided into five groups of ten chicks each. The first group received normal saline (10 mL/kg) *i.p*, second, third and fourth groups were administered 75 mg/kg, 150 mg/kg and 300 mg/kg *i.p* of EAF respectively, and the fifth group was administered 20 mg/kg of phenytoin. Thirty minutes after treatment, maximal electroshock was administered to induce seizures in the chicks using Ugo Basile electroconvulsive machine (Model 7801) with comeal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.8 seconds, 100 pulses/seconds and 0.8 m/s respectively. A current of 70 mA which produced tonic seizures in 80% of the control

Table 1
Results of phytochemical analysis of ethyl acetate fraction of *G. braunii* leaves.

Constituents	Test	Observation	Inferences
Steroids/Terpenes	a. Lieberman burchard	Greenish upper and brown ring at interphases was observed.	+
	b. Salkwski	Red ring with at the interphases	
Flavonoids	a. Sodium hydroxide	Yellow colouration was observed.	+
	b. Shinoda test	Red colouration was observed.	
	c. Ferric chloride test	Green precipitate was observed.	
Tannins	a. Ferric chloride test	Green precipitate was observed.	+
	b. Strong lead sub-acetate test	Green precipitate was observed.	
Saponins	Frothing	Froth which persisted for up to 15 minutes was observed.	-
Alkaloids	a.Wagner's test	No change was observed.	-
	b.Meyer's test	No change was observed.	
	c.Dragendroff's test	No change was observed.	
Anthraquinones	a.Bontrager's test	Bright pink colour was observed in the upper layers.	-
Carbohydrates	a. Molisch's test	A reddish coloured ring was observed at interphases.	-
	b. Fehling's test	A brick red precipitate was observed.	

+: present, -: not present.

chicks, were used throughout the study. An episode of tonic extension of the hind limbs was regarded as full convulsion, while lack of tonic extension of the hind limbs was regarded as protection. In unprotected animals, the recovery time was recorded[14].

2.2.4. Pentylenetetrazole induced seizure in mice (PTZ)

Thirty six mice were randomly divided into six groups of six mice each. Group one (negative control group) was given 10 mL/kg *i.p* of normal saline, group two, three and four were given 75 mg/kg, 150 mg/kg and 300 mg/kg *i.p* of EAF respectively. Fifth group (positive control) received 200 mg/kg *i.p* sodium valproate. Thirty minutes after treatment, 90 mg/kg of freshly prepared solution of PTZ (CD_{97}) was administered to each mouse, subcutaneously. The mice were observed for thirty minutes for presence or absence of threshold seizures (*i.e.* an episode of clonic spasm of at least five seconds duration)[15].

2.2.5. 4-Aminopyridine-induced seizures test (4-AP)

The method previously described by Yamaguchi and Rogawski was adopted[16]. Thirty six albino mice were randomly divided into six groups of six mice each. Group one (negative control) was administered with 10 mL/kg of normal saline while groups two, three and four received EAF (75, 150 and 300 mg/kg), *i.p*. The fifth group received phenobarbitone (20 mg/kg). Thirty minutes after treatment, 13.5 mg/kg of freshly prepared 4-AP was administered subcutaneously to each mouse. Ability of the extract to protect the mice from lethality within a thirty minutes observation period was considered an indication of anticonvulsant activity[17].

3. Results

The preliminary phytochemical screening revealed the presence of saponins, carbohydrates flavonoids, cardiac glycoside, anthraquinones and steroids in the crude ethanol extract while the ethyl acetate fraction was found to contain tannins, flavonoids and steroids/terpenes (Table 1).

All the control animals exhibited seizures after delivery of electroshock. The extract did not protect the animals against tonic limbs extension at all the tested doses (Table 2).

Table 2
Effect of EAF against maximal electroshock in chicks.

Treatments	Percentage protection %	Mean recovery time (min)
Normal saline 10 mL/kg	0.00	6.20±0.32
EAF 75 mg/kg	0.00	8.30±0.87
EAF 150 mg/kg	0.00	6.20±0.49
EAF 300 mg/kg	0.00	12.30±3.12
Phenytoin 20 mg/kg	80.00	5.00±2.00

Protection against seizure expressed as percentages. n=10.

The entire control animal exhibited myoclonic jerk and some exhibited threshold seizures and loss of righting reflex with tonic forelimbs extension. The extract exhibited anticonvulsant effect on seizure included by subcutaneous PTZ. It offered maximum protection against threshold seizure with 88.33% at a dose of 150 mg/kg (Table 3).

Table 3
Effect of EAF against pentylenetrazole induced seizure.

Treatment	Mean Onset of Jerking (min)	Quantal Protection of Jerking	Mean Onset of clonic Spasm (min)	Quantal Protection of Seizure	Mean Time of Death (min)	Quantal Protection of Death
Normal saline 10 mL/kg	3.67±0.55	0/6	4.80±0.58	1/6	13.60±2.54	1/6
EAF 75 mg/kg	4.17±0.48	0/6	7.40±1.91	1/6	9.50±1.66	2/6
EAF 150 mg/kg	3.60±0.24	1/6	8.00	5/6	8.00	5/6
EAF 300 mg/kg	5.30±0.93*	0/6	10.33±2.33	3/6	12.00	4/6
VPA 200 mg/kg	7.00±0.70	5/6	0.00	6/6	0.00	6/6

Protection against seizure and mortality expressed as percentages; Mean onset of seizure is expressed as mean±SEM. *P<0.05 (compared with normal saline treated control); VPA: sodium valproate. n=6.

The extract produced a reasonable protection against 4-AP induced seizure compared to the control. However, the extract produced an increase in the mean onset of seizure in unprotected animals (Table 4).

Table 4
Effect of EAF against subcutaneous 4-AP induced seizure.

Treatment	Quantal protection of seizure	Mean onset of seizure in unprotected animals (min)
Normal saline 10 mL/kg	0/6	15.00±2.66
EAF 75 mg/kg	0/6	14.67±1.52
EAF 150 mg/kg	0/6	15.33±2.04
EAF 300 mg/kg	0/6	19.67±1.26
Phenobarbitone 20 mg/kg	5/6	24.60

Protection against seizure and mortality expressed as percentages; Mean onset of seizure expressed as mean±SEM (n=6).

4. Discussion

The result of preliminary phytochemical screening carried out on the crude ethanol extract revealed the presence of saponins, carbohydrates flavonoids, cardiac glycoside, anthraquinones and steroids, while that of the ethyl acetate fraction revealed the presence of tannins, flavonoids and steroids/terpenes. These phytochemical constituents have been reported to be associated with different pharmacological activities of plants[18]. Triterpenes and steroids, among other phytochemicals have been reported to possess anticonvulsant activity[19].

The data presented in this study provide scientific evidence that EAF obtained from the crude ethanol extract of *G. braunii* leaves may contain psychoactive principles that are relevant to the management of convulsive disorder. The intraperitoneal median lethal dose of EAF found to be 1 261.91 mg/kg in mice suggests that it is relatively toxic[20]. However, it is relatively safe at the dose employed in this study.

Maximal electroshock seizure can be prevented by sodium channel blockers such as phenytoin, valproate, felbamate and lamotrigine[21] or agents that block glutamatergic neurotransmission mediated by n-methyl-d-aspartate (NMDA) receptors[22]. Agents which protect animal against MEST have been shown to be beneficial in the management of generalized tonic clonic seizure. Therefore, the absence of anticonvulsant ability in MEST suggests that EAF may not be useful in the treatment of generalized tonic clonic and partial seizures.

EAF protected mice against pentylenetetrazole and significantly delayed the onset of myoclonus jerks and tonic seizures. It is widely accepted that PTZ causes seizures by blocking the major GABAergic inhibitory pathways[23]. Standard antiepileptic drugs such as diazepam and phenobarbitone are thought to produce their effects by enhancing GABA mediated inhibition in the brain[24]. Seizures induced by PTZ can also be blocked by drugs such as ethosuximide by reducing T-type Ca^{2+} currents[25].

Activation of the NMDA receptor system is also involved in the initiation and propagation of PTZ-induced seizures[26]. In this regard, drugs such as felbamate that reduces glutamate release by blocking presynaptic NMDA receptors in the entorhinal cortex have demonstrated anticonvulsant activity against PTZ induced seizures[27]. It is therefore possible that the anticonvulsant effect shown in this study by EAF against seizures produced by PTZ might be due to enhancement of GABAergic neurotransmission, inhibition of T-type Ca^{2+} current or blockade of glutamateergic neuro transmission mediated by NMDA receptor, which are not tested in this study.

K^+ channels play a significant role in controlling all aspect of neuronal excitability[28]. Sodium channel blockers, such as phenytoin which prevent seizure spread effectively antagonize seizures induced by K^+ channel blocker such as 4-AP while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-AP. EAF failed to abolish the spontaneous discharges induced by 4-AP in mice. The inability of the fractions to produce significant activities against 4-AP induced seizure suggests that they may likely not be interacting with K^+ channel in producing their anticonvulsant activities.

The findings of this study suggest that the EAF of *G. braunii* leaves extract possesses psychoactive principles that may be useful in the management of petit mal epilepsy and lend credence to the ethnomedical use of the plant in the management of epilepsy.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The ethyl acetate soluble fraction of ethanol leaf extract of *G. braunii* used in ethnomedicine for the treatment of epilepsy and diabetes among other ailments, was subjected to phytochemical and anticonvulsant screening.

Research frontiers

The present research work presents a report of the anticonvulsant activity of *G. braunii*, as no information could be found in the literature. In furtherance with an attempt to isolate the bioactive principles of the plant, this study aimed at establishing the phytochemical constituent present, and evaluating the anticonvulsant potential of the EAF of *G. braunii*.

Related reports

The leaves of *G. braunii* are used for the treatment of ailments like cardiovascular diseases (Ouedraogo *et al*.,

2004), hepatic illness (Phillipson and Wright, 1991; Olagunju *et al*., 1999; Al-Ghaithi *et al*., 2004) and malaria (Traore *et al*., 2000).

Innovations and breakthroughs

These results suggest that the EAF extract of *G. braunii* leaves possesses psychoactive compounds.

Applications

These results may be useful in the management of petit mal epilepsy and lend credence to the ethnomedical use of the plant in the management of epilepsy.

Peer review

This is a valuable research work in which authors have demonstrated the phytochemical properties and anticonvulsant activities of EAF of *G. braunii* on laboratory animals. The activity was assessed based on anticonvulsant activity using maximal electroshock test in chicks, as well as pentylenetetrazole and 4-AP-induced seizures in mice. The preliminary phytochemical screening carried out on the crude ethanol extract revealed the presence of saponins, carbohydrates, flavonoids, tannins, anthraquinones and steroids. Similarly, tannins, flavonoids and steroids/terpenes were found to be present in the EAF.

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