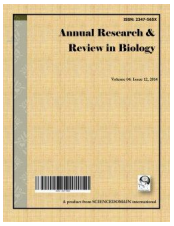

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Phytochemical and Antiepileptic Activity of the Ethanol Leaf Extracts of *Culcasia falcifolia* in Pentylenetetrazole Induced Seizure in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author AGP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DMM and AGMN managed the analyses of the study. Author PMW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the phytochemicals and the anticonvulsant activity of the ethanol leaf extract of *Culcasia falcifolia* used in the traditional medical treatment of epilepsy.
Methodology: The phytochemical screening was carried out using standard protocol while the anticonvulsant activity was studied using Pentylenetetrazole in mice.
Result: The preliminary phytochemical screening carried out on the ethanol extract of *Culcasia falcifolia* revealed the presence of alkaloids, flavonoids, saponins, tannins, polyphenols, and glycosides. In the anticonvulsant activity, there was a significant (*p< 0.05, **p<0.01) increase in the mean latency of tonic convulsion (243.72 ± 6.90*, 402.56 ± 5.52**) and significant (*p< 0.05,

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p<0.01) decrease in the mean duration of tonic convulsion (192.62 ± 7.72*, 158.99 ± 8.66) in a dose-dependent manner at the dose of 200 and 400 mg/kg body wt. respectively. The extract at 400 mg/kg body wt. showed 100% protection against mortality.
Conclusion: The results of this study suggest that the ethanol leaf extract of *Culcasia falcifolia* possesses anticonvulsant activity in PTZ induced seizure in mice.

Keywords: Culcasia falcifolia; phytochemicals; antiepileptic; pentylenetetrazole.

1. INTRODUCTION

Epilepsy is regarded as a multifactor and highly diversified neurological disorder characterised by recurrent unprovoked seizures. Physiological studies show that seizures reflect brief, abnormal and synchronous hyperactivity of a neuronal population in the brain. Such brain dysfunction can be accompanied by motor, sensory and autonomic disturbances depending on brain region involved in the origin and/or spread of seizures [1]. Epilepsy is a disorder that can occur in all mammalian species, probably more frequently as brains have become more complex. Epilepsy is also remarkably uniformly distributed around the world. There are no racial, geographical or social class boundaries. It occurs in both sexes, at all ages, especially in childhood, adolescence and increasingly in ageing populations [2]. Antiepileptic drugs are the drugs that are used for the treatment of epilepsy. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure. An effective anticonvulsant would prevent the spread of the seizure within the brain and offer protection against possible excitotoxic effects, which may result in brain damage [3]. Antiepileptic drugs are related to reactions, including teratogenicity, chronic toxicity and adverse effects, on cognition and behaviour interminable poisonous quality and adverse impacts, on cognition and behaviour [4]. Majority of the antiepileptic drugs are consumed lifelong, concomitant administration of other drugs predisposes to the risk of drug interaction [5]. Antiepileptic drugs are neither preventive nor curative and are employed solely as a means of controlling symptoms [6].

Plant extracts can be an important source of natural and safer drugs for the treatment of epilepsy [7]. Herbal medicines are widely used due to their wide applicability and therapeutic efficacy with low adverse effects. Several medicinal plants have been studied for their anticonvulsant activity in various animal models. *Culcasia falcifolia* is a medicinal plant which is traditionally used in Nandi County, Kenya in the treatment of epilepsy. The leaves of *Culcasia falcifolia* is used as ash (internal) for epilepsy, edema, dry cough. It is used as a tonic or ashes were taken with porridge [8]. *Culcasia falcifolia* is a perennial climber, epiphytic on trees and growing to several meters, stem with adventitious roots, penetrating bark with short clasping roots at the nodes. The leaves are oblong, leathery, dark glossy green. The plant is native to damp evergreen forest in the shade; riverine and swamp forests; marshy forest. It is found in Kenya, Ethiopia, Malawi, Tanzania, Uganda, Zambia, and Zimbabwe [9]. There is no scientific documentation of the antiepileptic activity of *Culcasia falcifolia* till date. In the wider frame of the ongoing research on the treatment of epilepsy, the present study aimed at evaluating the mechanism and neuroprotective effects of *Culcasia falcifolia* in the treatment of epilepsy.

2. MATERIALS AND METHODS

2.1 Collection of Material

The leaves of *Culcasia falcifolia* were collected along the river Kiŋ́wal in Kaptildil, Nandi County, Rift Valley, Kenya. The leaves were identified and authenticated from the National Herbarium, Kenya (PS 22/05). The leaves were washed with tap water to remove dust and other unwanted materials accumulated on the leaves from their natural environment. The dust-free leaves were allowed to dry under shade in the laboratory for two weeks. The dried leaves were powdered using laboratory electric blender to obtain a powder. The powdered leaf was further passed through a sieve to obtain finer particles. The leaf sample was stored in a clean, dry labelled glass beaker until needed for extract preparation and analysis.

2.2 Extract Preparation

The dried plant material was mixed and macerated with absolute ethanol at a 1:20 ratio (100 g in 1 L solvent) for 7 days. The extract was then filtrated through Whatman No 1 filter paper and then followed by rotor evaporated the

supernatant by using the BUCHI Switzerland rotary evaporator to remove the ethanol and to obtain concentrated, oily extract. The crude extract was then stored in a sterile universal glass bottle until further use.

2.3 Experimental Animals

Male Swiss albino mice weighing between 20 to 25 g were used for the study. The animals were placed in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºC and relative humidity of 30 to 70%. A 12:12 light: day cycle was followed. All animals were supplied water and food ad libidum. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA.

2.4 Chemicals Used

Wagner's reagent; Dragendorff's reagent; magnesium metal; concentrate Hydrochloric acid; 1.8% sodium chloride solution; 10% lead acetate solution; glacial acetic acid, 5% FeCl3 and conc. sulphuric acid; Molisch's reagent; chloroform; acetic anhydride; ninhydrin solution; 4% NaOH and 1% copper sulphate solution; ferric chloride solution; Sodium acetate buffer iodine solution; Acetic acid; Na_2SO_3 solution; trichloroacetic acid; copper tartrate reagent. All the chemicals used in this study were purchased from Sigma Aldrich.

2.5 Preliminary Phytochemical Screening

The extract obtained was subjected to following phytochemical tests for identification of various phyto-constituents.

2.5.1 Test for alkaloids

i) **Wagner's test*:*** To 2 ml ethanol leaf extract of *C. falcifolia* 1 ml of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids [10].

ii) **Dragendorff's tests:** To 2 ml ethanol leaf extract of *C. falcifolia* 1 ml of Dragendorff's reagent were added in a test tube. Formation of orange-brown precipitate indicates the presence of alkaloids [10].

2.5.2 Test for flavonoids

i) **Shinoda tests:** To 2 ml ethanol leaf extract of *C. falcifolia* 0.5 grams of magnesium fragments were added in a test tube, followed by addition of 5 drops (drop-wise) concentrate hydrochloric acid. The appearance of orange, red colour indicates the presence of flavonoids [10].

ii) **NaOH tests:** To 2 ml of ethanol leaf extract of *C. falcifolia*, 2 ml of 10% sodium hydroxide solution was added in a test tube. Formation of an intense yellow colour indicates the presence of flavonoids [11].

2.5.3 Test for saponins

i) **Foam test:** 2 ml of ethanol leaf extract of *C. falcifolia* was diluted with 20 ml of distilled water, and it was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins [10].

2.5.4 Test for tannins

i) **Gelatin test:** To the ethanol leaf extract of *C. falcifolia* aqueous solution of gelatin and sodium chloride are added. Formation of white precipitate indicates the presence of tannins [12].

ii) **Lead acetate test:** To 5 ml of ethanol leaf extract of *C. falcifolia* a few drops of 10% lead acetate solution were added in a test tube. Formation of yellow precipitate indicates the presence of tannins [12].

2.5.5 Test for cardiac glycoside

i) **Keller kiliani test:** To 2 ml of the ethanol leaf extract of *C. falcifolia* 1 ml of glacial acetic acid, 3 drops 5% $FeCl_3$ and 0.5 ml conc. H_2SO_4 were added by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of glycosides [10].

2.5.6 Test for terpenoids

i) **Liebermann-burchard test:** To 2 ml of the ethanol leaf extract of *C. falcifolia*, 10 drops of acetic anhydride was added and mixed well. To this 5 ml of concentrated sulphuric acid was added from the sides of the test tube, the appearance of greenish blue colour indicates the presence of triterpenoids [10].

2.5.7 Test for amino acid/ protein

i) **Biuret's test:** To 3 ml of ethanol leaf extract of *C. falcifolia* 1 ml of 4% w/v sodium hydroxide and 1ml of 1% w/v

copper sulphate were added. The change in colour of the solution to violet or pink indicates the presence of proteins [10].

ii) **Millon's test:** To 3 ml ethanol leaf extract of *C. falcifolia* 5 ml of Millon's reagent was added and heated the appearance of a white precipitate which changed to brick red on heating indicates the presence of proteins [10].

2.5.8 Tests for phenols

i) **Ferric chloride test:** To 3 ml ethanol leaf extract of *C. falcifolia,* 3 ml of 5% ferric acid solution was added was added and observed for the formation of green or blue colour which may indicate the presence of phenols [10].

2.5.9 Tests for oils and resins

The 2 ml of ethanol leaf extract of *C. falcifolia* was applied on filter paper. Development of a transparent appearance on the filter paper indicates that the presence of oils and resins [13].

2.6 Acute oral Toxicity Study of *Culcasia falcifolia*

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines. Swiss Albino mice were selected by random sampling technique for this study. The animals fasted for 4 hours with free access to water. Following the period of fasting, the animals were weighed and the extract was administered. The dose level to be used as a starting dose was selected from the four fixed-dose levels 5, 50, 300 and 2000 mg/kg b. wt. Initially 5 mg/kg b. wt. of the extract was orally administered for three mice and was frequently observed for mortality and signs of toxicity for the first 30 minutes, 24 hours (first 4 hours with special attention) and daily thereafter for 14 days. If mortality was observed in two out of three animals, then the dose administered was considered as a toxic dose. However, if mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of the plant extracts were employed for further toxicity studies. Three animals were used for each step. The following general behaviours were observed during the study: sedation; hypnotics; convulsion; ptosis; pain distress; stupor reaction; salivation; somatomotor activity; muscle relaxation; piloerection; change in skin colour; lacrimal secretion; lethargy; diarrhea [14].

2.7 Assessment of Anticonvulsant Activity of the *Culcasia falcifolia* Leaves

Animals were randomly allotted into four groups of six mice each. Mice fasted overnight before the test, but the water was supplied *ad libitum.*

- Group I – Control mice (received 0.1% carboxy methyl cellulose)
- Group II - standard (diazepam 5 mg/kg body weight)
- Group III – ethanol extract of *Culcasia falcifolia* 200 mg/kg body weight
- Group IV – ethanol extract of *Culcasia falcifolia* 400 mg/kg body weight

The first group served as control which received 0.1% carboxy methyl cellulose. The second group served as a positive control which received standard drug diazepam 5 mg/kg only once, the third and fourth group received 200 and 400 mg/kg of the ethanol extract of *Culcasia falcifolia* respectively for twenty-one days. On the 21st day, pentylenetetrazole (PTZ) (60 mg/kg body weight, i. p) was administered to all the groups to induce clonic convulsions. PTZ was administered 60 minutes after the administration of the two doses (200 and 400 mg/kg b. wt.) ethanol extract of *Culcasia falcifolia* to group III and IV. And 30 minutes after the administration of diazepam to group II. Following the administration of PTZ, mice were placed in separate cages and were observed for the occurrence of seizures, initially for 30 minutes and later up to 24 hours. The following parameters were observed: Latency of convulsions (the time before the onset of tonic convulsions), duration of tonic convulsions, and mortality protection (percentage of deaths in 24 hours) were recorded [15].

3. RESULTS

The phytochemical analysis of the ethanol extract of *Culcasia falcifolia* revealed the presence of alkaloids, flavonoids, saponins, tannins, polyphenols and glycosides as shown in Table 1.

The acute oral toxicity test of ethanol extract of *Culcasia falcifolia* did not show mortality at the doses of 5, 50, 300 and 2000 mg/kg b. wt. in the mice used during the period (72 hours) of

observation. The extract did not show any signs of changes in all the activities generally observed such as diarrhea, hypnosis, convulsions, stupor reaction, piloerection, changes in skin colour, lacrimation, allergic reactions, and mortality in mice at the dose of 5, 50, 300 mg/kg b. wt. However, at the higher dose 2000 mg/kg b. wt. the extract showed signs of sedation and ptosis in mice. 1/10th (i.e. 200 mg/kg body weight) and 1/5th (i.e. 400 mg/kg body weight) of the 2000 mg/kg body wt. were selected for further studies. The mean latency of tonic convulsion were 125.37 ± 5.66, 492.59 ± 7.32, 243.72 ± 6.90, 402.56 ± 5.52 in groups I, II, III and IV respectively. The mean duration of tonic convulsions were 392.98 ± 6.43, 135.77 ± 6.78, 192.62 ± 7.72, 158.99 ± 8.66 in groups I, II, III and IV respectively. The group treated with diazepam and the group treated with 400 mg/kg body weight of an extract of *Culcasia falcifolia* showed 100% protection against mortality. Analysis of variance followed by Dunnett's t-test showed that latency of tonic convulsion and duration of tonic convulsion was statistically significant ($p<0.05$*, $p<0.01$**) in group II, group III and group IV when compared with control group I. The result is shown in Table 2.

Table 1. Qualitative phytochemical analysis of 50% aqueous-alcoholic leaves extract of *Culcasia falcifolia*

S.No	Plant constituents	Analysis
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Steroids	-
6	Terpenoids	-
7	Phenols	+
8	Phytosterols	+
9	Cardiac glycosides	+
10	Triterpenoids	-
11	Fixed oils and resins	-

Note: "+" = present; "-"= absent

4. DISCUSSION

In the present study, the effect of the ethanol extract of *Culcasia falcifolia* on seizure induced by PTZ in mice was evaluated. The results demonstrated that the extract is able to produce potent anticonvulsant activity on PTZ induced seizures in mice. The treatment of ethanol extract of *Culcasia falcifolia* on pentylenetetrazole-induced mice significantly reduced the duration of convulsion and delayed the onset of clonic convulsion in a dose-dependent manner. The dosage of the extract that showed lowest or no mortality and reduced the duration of myoclonic seizures was considered as the most effective dose for the protection against pentylenetetrazole-induced seizures. In the present study, the group treated with the higher dose, that is, 400 mg/kg body weight of ethanol extract of *Culcasia falcifolia* showed 100% protection against mortality, whereas, the group treated with the lower dose 200 mg/kg body weight of ethanol extract of *C. falcifolia,* showed 33.33% protection against mortality. Therefore, 400 mg of the extract of *C. falcifolia* is taken as the effective dosage. The standard drug, Diazepam (5 mg/kg body weight) was used in this study as a reference anticonvulsant agent, which showed significant activity by delaying the onset of myoclonic jerks, tonic convulsions and decreasing the frequency and duration of tonic convulsions and offered 100% protection. Diazepam acts through the activation of $GABA_A$ receptors and facilitates the GABA-mediated opening of Chloride channel. The protection of mice against PTZ-induced seizures by the standard anticonvulsant drugs, diazepam is expected as shown by various researchers that the anticonvulsant activity is enhanced by GABA-mediated inhibition [16]. Likewise, the ethanol extract of *C. falcifolia* at the dose of 400 mg/kg body weight showed significant activity by delaying the onset of myoclonic jerks, tonic convulsions and decreasing duration of tonic convulsions and offered 100% protection.

GABA is a major inhibitory neurotransmitter in the brain, and the inhibition of its neurotransmission has been thought to be the underlying factor in epilepsy [17]. The enhancement of the GABAergic neurotransmission is reported to antagonise seizures, while the inhibition of the GABAergic neurotransmission promotes seizures [18]. PTZ is an antagonist of gamma-aminobutyric acid (GABA) at $GABA_A$ receptor which has been widely implicated in epilepsy [19]. The $GABA_A$ receptors are ligand-gated ion channels, which mediate the most common inhibitory transmission in synapses. The $GABA_A$ receptor function not only prevents the development of epilepsy but also inhibits the development of convulsive activity throughout the cerebral cortex tissues [20]. Studies have shown that drugs which protect animals against the seizure induced by PTZ reduce the T-type of Ca^{++} currents or drugs that inhibit GABA-mediated

Table 2. Effect of ethanol extract of *Culcasia falcifolia* on pentylentetrazole induced seizure in mice

Group	Drug treatment	Latency of tonic convulsion (sec)	Duration of tonic convulsion (sec)	(no. of animals alive after 30 min)	(no. of animals alive after 24 hours)	% protection
Group I	Control 0.1 % CMC	125.37±5.66	392.98±6.43	0	0	0
Group II	Diazepam 5 mg/kg	492.59±7.32***	135.77±6.78***	6	6	100
Group III	EECF 200 mg/kg	243.72±6.90**	192.62±7.72**	6	2	33.33
Group IV	EECF 400 mg/kg	402.56±5.52***	158.99±8.66***	6	6	100

*Note: EECF: Ethanol extract of Culcasia falcifolia; CMC: carboxy methyl cellulose; Values are in mean ± SEM (n=6); *p< 0.05, **p<0.01, ***p<0.001 vs. Control.*

neurotransmission, act by elevating the seizure threshold and are effective in myoclonic and absence seizures [19].

Prevention of PTZ-induced seizures in laboratory animals is the most commonly used preliminary screening test for characterising potential anti-convulsive drugs. The test is assumed to identify anticonvulsant drugs effective against generalised clonic seizures as PTZ produces clonic and tonic convulsions. It has been demonstrated that a neural pathway of PTZ convulsions is located in the forebrain while the brain stem is involved in the network of tonic PTZ induced convulsions. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsive action is by acting as an antagonist at the $GABA_A$ receptor complex. Drugs that offer protections against tonic-clonic seizures induced by PTZ in rodents are considered to be useful to control myoclonic and absence seizures in humans [20], [21]. Although the direct mechanism of PTZ is not known in detail, literature records reveal that it causes alterations in GABAergic systems, Glutamergic systems and antioxidant defense systems [22]. The observed anticonvulsant property of *Culcasia falcifolia* may be attributed to the presence of beneficial phytochemicals such as alkaloids, flavonoids, saponins, tannins and phenols.

5. CONCLUSIONS

Therefore, the findings of the present study, suggests that ethanol extract of *Culcasia falcifolia* leaves has anticonvulsant activity against PTZ-induced seizures in mice by modulating GABA receptor-mediated inhibitory neurotransmission, reducing the T-type of Ca^{++} currents, activating $GABA_A$ receptors and facilitating the GABA-mediated opening of Chloride channel. Based on the folkloric claims, we infer that the decoction of the leaves of *Culcasia falcifolia* could be integrated into health care system for the management of epilepsy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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