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Phytochemical, antibacterial and anticonvulsant activity of the stem bark of *Lannea kerstingii* Engl. & K. Krause (Anacadiaceae)

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Abstract
The stem bark of *Lannea kerstingii* Engl. & K. Krause was investigated for its phytochemistry, acute toxicity, antibacterial and anticonvulsant activities. Standard methods were used to evaluate phytochemistry while antibacterial activity was determined using agar diffusion and broth dilution methods on *Staphylococcus aureus, Salmonella typhii, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Escherichia coli* and *Bacillus subtilis*. Maximal electroshock-induced seizures test in chicks and pentylenetetrazole-induced seizures test in mice were used to determine the anticonvulsant activity. Phytochemical studies revealed the presence of flavonoids, tannins, carbohydrates steroids and triterpenes. Ethyl acetate and methanol fractions of the stem bark were found to be active against *S. aureus, S. typhi, P. aeruginosa, K. pneumoniae, Proteus sp, E. coli, Bacillus subtilis* with zone of inhibition ranging from 20-27.5mm and MIC ranging from 6.25mg/mL to 100mg/mL and MBC from 50mg/mL and above. LD_{50} was found to be 2154.066 mg/kg. The crude methanol extract of the stem bark afforded dose (150, 300 and 600mg/kg) dependent protection to the laboratory animals against the hind limb tonic extension though not statistically significant ($P<0.05$) showing the inability of the extract to inhibit seizure discharge within the brainstem seizure substrate. Meanwhile the extract at doses of 300 and 600mg/kg significantly ($P<0.05$) prolonged the onset of seizure in pentylenetetrazole (PTZ) test showing the potential of this plant in raising seizure threshold in the brain therefore making it beneficial in the treatment of myoclonic and absence seizures. This justifies the use of the plant in treating convulsion.

Keywords: *Lannea kerstingii;* Anticonvulsant; Phytochemical; Antibacterial; Phytochemistry

INTRODUCTION

Epilepsy is one of the most common brain disorders. It is regarded as a symptom of pathological excitatory processes in the brain, rather than a disease in itself, and is commonly defined as a group of chronic neurological disorders characterized by recurrent and unprovoked seizures [1]. Throughout history, persons suffering from epilepsy have been regarded as being possessed by evil spirits or punishment from gods [2]. Every year approximately 250,000

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new cases are added to this figure. It is roughly estimated that 28-30% of patients are resistant to the available medical therapies [3]. In developed countries, the prevalence of epilepsy is estimated to be 5 % while in Africa; it is estimated to be 10 % [4]. About 75-80 % of the world's population uses medicinal plants for the treatment of epilepsy due to lesser side effects and better compatibility with the human body [5].

The active principles of many drugs found in plants are secondary metabolites [6] thus basic phytochemical investigation of plant extracts (known to have biological activity) for the major phytoconstituents is very vital as well as determining the particular constituent responsible for such activity. This will go a long way to propose a template or lead that can be used for designing of other drugs. Thus, medicinal plants represent a rich source from which anticonvulsant agents may be obtained.

An example of a plant with great medicinal properties is *Lannea kerstingii,* a tree with a height of 12m and 40cm in diameter, with a wide-spreading and relatively dense crown. The bark is smooth to slightly fissured, fissures spiral around the trunk (spiral grain), pale grey with pinkish, white-striped slash. The stem is brown, densely pubescent (mainly at juvenile stage) [7]. *L. kerstingii* is widely utilized in traditional medicine by various cultures worldwide and their applications vary from region to region. In Sudan, a decoction of the back is used to treat swellings [8]. The stem bark and roots are consumed by natives from Northern Côte d'Ivoire, as traditional remedies for the treatment of diarrhoea, gastritis, rheumatic, sterility, intestinal helminthiasis [7,9]. Traditional healers in Zaria report that the plant is used to treat convulsion, diarrhoea [10,11] and epilepsy. Thus, this study is aimed at assessing the phytochemical properties, acute toxicity, antibacterial, and anti-convulsant activity of the methanol and ethyl acetate stem-bark extracts of *Lannea. kerstingii.*

EXPERIMENTAL

Chemicals. All solvents used in the experiments were from Merck (Darmstadt, Germany). Pentylenetetrazole (Sigma, UK), Phenytoin (Sigma, UK) and valproic acid (Sigma, UK).

Plant collection and extraction. The plant was collected in May 2011 in Zaria, Kaduna State, Nigeria. It was then taken to the herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria for identification. It was identified by comparison with a herbarium specimen (voucher specimen 1832). After identification, the stem bark was removed and dried under shade. The size was reduced using mortar and pestle, filtered for homogeneity and kept away from light until further use. The stem bark (580g) was extracted exhaustively with petroleum-ether using maceration method. The marc was divided into two. One was extracted with methanol and the other extracted with sequentially with ethyl acetate and then methanol using maceration method with intermittent shaking and solvents changed every 3 hours. The maceration process was then repeated several times for exhaustive extraction. The extracts were kept in a clean, corked container until when needed.

Phytochemical screening. Basic phytochemical screening to detect the presence or absence of plant chemical constituents such as alkaloids, tannins, saponins, anthraquinones, flavonoids, carbohydrates, cardiac glycoside, anthraquinones, steroids and triterpenes were carried out using standard procedures [12-14] on the petroleum ether and crude methanol extracts of the stem bark of *L. kerstingii*.

Antibacterial activity. The test microorganisms selected were based on their clinical and pharmacological importance. The

bacterial strains were obtained from the Department of Clinical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. The clinical bacteria used were: *Stapylococcus aureus, Salmonella typhii, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Escherichia coli* and *Bacillus subtilis*. The bacterial strains were grown in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C. Agar-well diffusion method was used to determine the antibacterial activity of both extracts. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 cfuml^{-1}). Two hundred microliters of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 0.1mL of the crude extract at 10 mgmL^{-1} were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extracts. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of cefuroxime and sparfloxacin at a concentration of 1 mg/mL and 10 µg/mL respectively [15]. The activity index was also calculated using the formula:

Activity index = mean zone of inhibition of extract/ zone of inhibition obtained by standard antibiotic

Minimum inhibitory concentration (MIC). A 2.0 mL aliquots of different concentrations of each of the extract solution were added to 18 mL of pre-sterilized molten nutrient agar and SDA at 40°C to give final concentration regimes of 2.5 mg/mL and 40.0 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial and fungal cultures. The plates were later incubated at 37°C for 24 h, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism [16].

Minimum bactericidal concentration (MBC). Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates and SDA plates, and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extracts that did not show any growth on a new set of agar plates [17].

Animals. Swiss albino mice weighing 18 to 30g maintained at the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria were used for the experiments. The mice were housed in transparent plastic cages padded with wood shavings, under standard conditions of temperature, relative humidity and light/dark cycles (12/12 h). The animals were fed with the standard laboratory feeds and water *ad libitum*. This research was carried out according to the rules governing the use of laboratory animals as acceptable internationally. The mice were approved for use by the AFC committee after reviewing the protocol. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. All efforts were made to minimize the number of rats used and their suffering.

Acute toxicity study. Lorke's method [18] was used which involved the use of 13 animals and divided into two phases. At the first phase, a total of 9 mice of average weight of 28.3 g were used. The animals were divided into 3 groups of 3 animals each. The dried methanolic extracts of stem bark of *L. kerstingii* was re-dissolved in water and administered intraperitoneally (IP) to the mice at doses of 10mg/kg, 100mg/kg and 1000mg/kg. All animals had unrestricted access to water and animal feed and were then

observed in their cages for 24 h. The animals were observed for possible signs of toxicity (anorexia, drowsiness, apnoea, immobility, twitches and irritation) and, or death. All dead animals were immediately removed from the cage as soon as possible once death was observed, counted and recorded. The second stage followed the end of the first stage and 4 new mice were selected and placed 1 per cage. They were separately given 400mg/kg, 800mg/kg, 1600mg/kg and 2900mg/kg and were further observed for the next 24 h. LD_{50} was then calculated using the formula

$LD_{50} = \sqrt{}$ (lowest dose that killed an animal x highest dose that did not kill an animal)

Maximal electroshock-induced seizures test in chicks. Day old white cockerels (50) were randomly divided into five groups each containing 10 chicks. The first group received normal saline 10 mL/Kg body weight IP, second , third and fourth groups were treated with 150, 300, 600 mg/kg of the methanolic extract of the stem bark of *Lannea kerstingii* (IP), and the fifth group was injected with 20 mg/Kg of Phenytoin IP. Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (model 7801). The shock duration, frequency, current and pulse width were set and maintained at 0.6s, 150 pulse/s, 80 mA and 0.8 ms, respectively. A current of about 80 mA, which produced tonic seizures in about 90% of the negative control chicks, was used throughout the study. Seizures were manifested as tonic hind limb extension (THLE) [19]. The ability to prevent this feature or prolong the latency and/ or onset of the THLE was considered as an indication of anticonvulsant activity [19,20].

Pentylenetetrazole-induced seizures test in mice. The method of Swinyard's team [19] was employed. Thirty (30) mice were divided into five groups each containing six (6) mice. The first group received normal saline 10 mL/Kg body weight IP, second, third, and fourth groups received 150, 300, 600 mg/kg body weight IP of the methanol extract fraction of the stem bark of *L. kerstingii*, while the fifth group was injected with sodium valproic acid 200 mg/Kg body weight IP. Thirty minutes after treatment, mice in all the groups received Pentylenetetrazole (PTZ) 85 mg/Kg subcutaneously. Mice were observed over a period of thirty minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated the extract's ability to abolish the effect of Pentylenetetrazole on seizure threshold or the ability to prolong mean onset of seizure is an indication of the compound's ability to reverse the effect of PTZ [21]. Injections were administered in volumes not higher than 10 mL/kg of body weight of animals.

Statistical analysis. The data will be presented in tables as mean SEM. The anticonvulsant activity was analysed for statistical significance using analysis of variance (one-way ANOVA) and chi square using Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

Extraction and yield. Successive extraction of the stem-bark of *L. kerstingii* with petroleum ether and methanol using cold maceration leads to 3.75 and 67.5g of petroleum ether and crude methanol extract respectively from the stem bark of this plant (Table 1). The Ethyl acetate and the methanol fractions show yields of 1.84 and 10.92 % respectively as shown in Table 1.

Phytochemical constituents. The crude methanol extract of the stem-bark as well as the ethyl acetate and the methanol fractions all contained tannins, flavonoids, steroids and triterpenes as shown in Table 2.

Antibacterial activity. The ethyl acetate and methanol fractions of the stem bark were found to be active against both Gram positive and Gram-negative bacteria. They inhibited the growth of *S. aureus, S. typhi, P.*

aeruginosa, K. pneumoniae, Proteus vulgaris, E. coli, B. subtilis with zones of inhibition ranging from 16.5 – 27.0 as shown in Tables 3 and 4. The activity index of the extracts against standard drug (gentamicin) ranged from 0.7 to 1.35 at concentrations of 25 and 50mg/ml for the ethyl acetate fraction and 0.65 to 1.28 for the methanol fraction of the stem-bark of *L. kerstingii* (Tables 3 and 4). The MIC ranged from 6.25mg/mL to 100mg/mL with the least inhibitory effect on *K. pneumonia* and *B. subtilis* while the MBC ranged from /mL 12.50 mg/mL to 100 mg/mL with the least inhibitory effect on *B. subtilis* as shown in Table 5. The methanol extract of the stem bark of *L. kerstingii* has been reported to have antibacterial effects against *E. faecalis, P. mirabilis, P. aeruginosa, S. aureus, MRSA,* and *S. epidermidis* and inactive against *E. coli* [22]. The result of the antimicrobial sensitivity test against the different bacteria species shown in Table 4 is in line with that of literature except that there was activity of this plant on *E. coli.*

Table 1. % yield of the different extracts obtained from the extraction of the stem-bark of *Lannea kerstingii.*

Solvent	Colour	Weight (g)	% Yield
Petroleum ether	Pale yellow	3.75	0.74
Methanol	Dark brown	67.5	13.5
Ethyl acetate fraction	Greenish brown	10.72	1.84
Methanol fraction	Dark brown	63.32	10.92

Table 2. Phytochemical constituents in petroleum ether and methanol extracts of the stem-bark of *Lannea kerstingii*

Constituent	Tests	Fractionated extract		Crude methanol (after defatting)
		Ethyl acetate fraction	Methanol fraction	
Tannins	Pb subacetate	+	+	+
	$FeCl_3$	+	+	+
Flavonoids	Shinoda	+	+	+
	NaOH	+	+	+
Alkaloids	Meyer's	+	+	+
	Wagner's	+	+	+
	Dragendorff	+	+	+
Cardiac glycoside	Keller-Killiani	-	-	-
Saponins	Frothing	-	-	-
Unsaturated steroids and Triterpenes	Liebermann-Burchard's	+	-	+
Anthraquinones	Borntrager	-	-	-
	Modified Borntrager	-	-	-

+ = present and - = absent

Table 3. Zone of inhibition the ethyl acetate extract of the stem bark of *Lannea kerstingii* against some microbes

Test organism	Zone of inhibition (mm) / activity index						Standard drugs		
	Ethyl acetate fraction (mg/mL)						*Cipro*	Amox	Gen
	50.00	25.00	12.50	6.26	3.13	1.53			
S. aureus	25.00/1.19	24.00/1.14	21.00/1.00	9.50/0.45	-	-	40.00	-	21.00
S. typhi	25.00/1.00	20.00/0.80	14.5/0.58	-	-	-	40.00	25.00	25.00
P. aeruginosa	20.00/0.77	19.50/0.73	12.50/0.47	9.50/0.35	-	-	40.00	-	26.50
K. pneumoniae	20.00/0.66	20.00/0.66	13.00/0.43	10.00/0.33	-	-	29.00	-	30.00
P. vulgaris	20.00/0.80	17.50/0.70	12.50/0.50	10.50/0.42	-	-	33.00	-	25.00
E. coli	20.00/0.84	19.00/0.80	17.50/0.74		-	-	-	-	23.50
B. subtilis	27.00/1.35	22.50/1.13	19.50/0.96	13.50/0.67	9.00	-	24.50	22.50	20.00

- = no activity, Cipro = Ciprofloxacin, Amox = Amoxicillin, Gen = Gentamicin

Table 4. Zone of inhibition of the methanol extract of *Lannea kerstingii* against some clinical isolates

Test organism	Zone of inhibition (mm) /activity index						Standard drugs		
	Methanolic fraction (mg/mL)						*Cipro*	Amox	Gen
	50.00	25.00	12.50	6.26	3.13	1.53			
S. aureus	27.00/1.28	22.50/1.07	19.00/0.90	12.50/0.60	-	-	40.00	-	21.00
S. typhi	25.00/1.00	19.50/0.78	16.50/0.66	8.50/0.34	-	-	40.00	25.00	25.00
P. aeruginosa	22.50/0.85	20.00/0.75	15.00/0.57	14.00/0.53	9.50	-	40.00	-	26.50
K. pneumonia	20.00/0.66	19.50/0.65	16.50/0.55	-	-	-	29.00	-	30.00
P. vulgaris	20.50/0.82	20.00/0.80	17.50/0.70	12.50/0.50	-	-	33.00	-	25.00
E. coli	20.00/0.85	19.00/0.80	17.50/0.74	-	-	-	-	-	23.50
B. subtilis	23.50/1.18	19.50/0.97	19.50/0.97	18.50/0.93	-	-	24.50	22.50	20.00

Key: - = no activity, Cipro= Ciprofloxacin, Amox = Amoxicillin, Gen = Gentamicin

Table 5: MIC and MBC of the ethyl acetate and methanol fractions of the stem bark of *L. kerstingii*

Test organism	Ethyl acetate fraction of stem-bark (mg/mL)		Methanolic fraction of stem-bark (mg/mL)	
	MIC	MBC	MIC	MBC
S. aureus	100.00	>100.00	100.00	>100.00
S. typhi	100.00	>100.00	100.00	>100.00
P. aeruginosa	100.00	>100.00	100.00	>100.00
K. pneumonia	6.25	50.00	25.00	50.00
P. vulgaris	50.00	>100.00	100.00	>100.00
E. coli	100.00	>100.00	100.00	>100.00
B. subtilis	6.25	12.50	6.25	25.00

Table 6. Phase I and II of Acute toxicity Test

	Group	Weight of mice (g)	Dose (mg/kg)	Stock (mg/mL)	Death observed
PHASE I	1	28.00	1000.00	100.00	2/3
		30.00	1000.00	100.00	
		31.00	1000.00	100.00	
	2	27.00	100.00	10.00	0/3
		26.00	100.00	10.00	
		19.00	100.00	10.00	
	3	34.00	10.00	1.00	0/3
		30.00	10.00	1.00	
		30.00	10.00	1.00	
PHASE II	1	30.00	2900.00	290.00	1/1
	2	27.00	1600.00	160.00	0/1
	3	27.00	800.00	80.00	0/1
	4	26.00	400.00	40.00	0/1

Table 7. Effect of methanol extract of the stem bark of *Lannea kerstingii* on maximal electroshock-induced seizures in a day old chick.

Group (n=10)	Treatment	Dose (mg/kg)	Mean onset (s) + SEM	Quantal Protection	Mean recovery time (min) + SEM	% protection
I	Normal saline	10mL/kg	1.72±0.06	0/10	7.81 ± 0.84	0.00
II	Extract	150.00	2.06±0.14	0/10	7.02 ± 0.60	0.00
III	Extract	300.00	2.53±0.09	0/10	6.22 ± 1.56	0.00
IV	Extract	600.00	2.82±0.13	0/10	5.51 ± 0.52	0.00
V	Phenytoin	20.00	*7.10±0.73	9/10	**4.2 ± 0.40	90.00

*SEM = Standard error of mean; Secs = Seconds; Min = Minutes, *=onset of single animal that showed seizures, **= mean recovery time of single animal that showed seizures*

Table 8. Effect of crude Methanol Extract of the stem bark of *Lannea kerstingii* on pentylenetetrazole-induced seizures in mice

Group n = 6	Treatment	Dose (mg/kg)	Mean onset of seizure ± SEM (s)	Quantal Protection	% Protection	% Mortality
I	Normal saline	10mL/kg	3.89 ± 0.30	0/6	0.00	100.00
II	Extract	150	6.30 ± 0.70	2/6	33.33	66.67
III	Extract	300	7.69 ± 0.39*	6/6	100.00	0.00
IV	Extract	600	8.79 ±1.11**	6/6	100.00	0.00
V	Valproic acid	200	9.99 ±1.14***	6/6	100.00	0.00

SEM = Standard Error of Mean, * = Significant at $p< 0.05$, ** = Significant at $p< 0.002$, *** = Significant at $p< 0.001$ as compared to negative control group.

This might be due to the difference in secondary metabolites as a result of geographical location. The ethyl acetate extract of the stem bark of *L. kerstingii* was also found to be effective against both Gram-positive and Gram-negative bacteria as shown in table 3. The activity index of the extracts against standard antibiotic shows that the plant extracts at concentration used were as effective against the test organisms as the standard drug. This work shows that the stem bark of the plant contains antimicrobial properties against a wide range of bacteria. Some species of the genus *Lannea* also exhibit antibacterial activity. An example can be seen with *Lannea acida*, which possesses antibacterial activity against a wide range of bacteria including methicillin resistant *Staphyloccocus aureus* [23]. Flavonoids have been shown to have anti-viral, anti-inflammatory, antiulcer, antioxidant, antidiabetic and antimicrobial activities [24, 25]. There is the possibility of *Lannea kerstingii* having these activities since it contains flavonoids in the ethyl acetate and methanol fractions of the stem-bark. .

Acute toxicity test on the crude methanolic extract of the stem bark of *L. kerstingii*. At stage one, two animals died at dose of 1000 mg/kg. In stage two however, no animal died at doses of 400mg/kg to 1600mg/kg but the dose of 1900mg/kg killed the animal (Table 6). The LD_{50} of the methanolic crude extract of the stem bark of *L. kerstingii* was found to be 2154.066 mg/kg, which shows that the extract is relatively safe.

The methanol extract of the stem bark of *L. kerstingii* exhibited 0% protection against the maximal electroshock test (MEST) at all the dose level (Table 7) though there was slight increase in mean onset of seizure as the dose increased from 150mg/kg to 500mg/kg. There was also a decrease in the mean recovery time as the dose increases showing that the extract is dose dependent though not as comparable to that of the standard drug phenytoin

The extract showed a dose dependent protection against pentylenetetrazole induced seizures. At 150 mg/mL, the % protection was found to be 33.33 while with an increase in concentration to 300 mg/kg and 600 mg/kg, the extract showed 100% protection, which compares with the standard drug valproic acid (Table 8). The hind limb tonic extension (HLTE) produced electrically with the maximum electroshock test (MEST), is a common feature in many animal species including humans. The response of the brains of the animals to anticonvulsant is similar to that of humans. The crude methanolic extract of the stem-bark of *L. kerstingii* afforded dose dependent protection to the laboratory animals against the HLTE (Table 7) though not statistically significant ($P<0.05$) [showing the inability of the extract to inhibit or prevent seizure discharge within the brainstem seizure substrate [26]. The inability of the extract to significantly increase the mean onset of seizure in MEST experiment (Table 7) suggests that the extract is not effective in the control of generalized tonic-clonic and partial

seizures. The mean onset of seizure though not statistically significant increases with increase in dose.

The chemically-induced seizure using PTZ usually identifies drugs that raise seizure threshold in the brain [27]. The standard convulsant PTZ has also been shown to interact with γ-amino butyric acid (GABA) – a neurotransmitter – and the GABA receptor complex [28]. Drugs that inhibit the PTZ activity such as diazepam and valproic acid exert their effect by enhancing GABA mediated inhibition in the brain [29]. Maximum (100%) protection against PTZ induced seizures was observed T from 150mg/kg of the methanolic stem bark extract to 600mg/kg respectively. PTZ has been shown to interact with GABA neurotransmitters and the γ-amino butyric acid GABA receptor complex [30]. Antagonism of PTZ-induced seizures suggests effects on GABA-ergic neurotransmission. The crude methanol extract of the stem bark of *L. kerstingii* significantly ($P<0.001$) prolonged the onset of seizure in PTZ test (Table 8). The significant activity of the extract against pentylenetetrazole induced seizure ($P<0.001$) as shown in Table 8 shows that it has compounds with the potential of raising seizure threshold in the brain [31]. This activity may be due to the presence of tannins, flavonoids, steroids and triterpenes in this plant. Thus, this extract can be beneficial in the treatment of myoclonic and absence seizures [32].

Conclusion. The LD_{50} was found to be 2154.1mg/kg body weight and a maximum tolerated dose of 646.22mg/kg showing the relative safety of the extract. The crude methanol extract of the stem-bark of *L. kerstingii* contains tannins, flavonoids, steroids and triterpenes with broad antimicrobial activity. The extract afforded dose dependent protection to the laboratory animals against the HLTE though not statistically significant meanwhile the extract significantly ($P<0.05$) prolonged the onset of seizure in PTZ test showing the potential this plant in raising seizure threshold in the brain thus making it beneficial in the treatment of myoclonic and absence seizures.

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