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Original Research Article

Comparative Anticonvulsant Activity of Leaf, Stem Bark and Root Bark Extracts of *Bombax costatum* Pellegr. and Vuillet in Acute Models of Epilepsy

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ABSTRACT

Bombax costatum is a tropical medicinal plant utilized conventionally for the treatment of epilepsy. The parts used are leaves, stem and roots. Nevertheless, there is paucity of scientific proof to support its use in epilepsy. This study evaluated the anticonvulsant properties of hydroalcoholic extracts of the three plant parts using animal models. Phytochemical testing and median lethal dose (LD_{50}) determination of the extracts were done. Graded doses of the leaves, stem bark and root bark extracts (125, 250 and 500 mg/kg) were administered orally in the anticonvulsant studies. Seizures were induced in chicks by administration of maximal electroshock; and in mice by the administration of pentylenetetrazole (PTZ), picrotoxin and strychnine. Oral LD_{50} of the three extracts was >5000 mg/kg in chicks and mice. The extracts did not yield considerable activity against the electroshock-induced seizures at all tested doses. In the PTZ test, all the extracts increased the onset of seizures. The increase was considerable (p<0.05) with the stem bark (125 and 250 mg/kg) and root (500 mg/kg) extracts. A similar increase in the onset of seizures was observed in the picrotoxin test. Also, the root extract (125, 250 and 500 mg/kg) produced a dose-dependent protection (33.33, 50 and 66.67%, respectively) against the picrotoxin-induced seizures. In the strychnine test, just the root extract significantly (p<0.05) raised the seizure threshold at doses of 250 and 500 mg/kg. In conclusion, the findings demonstrated that the leaf, stem bark and root bark extracts of *B. costatum* possess anticonvulsant activity in animal models of epilepsy.

Keywords: *Bombax costatum*, Anticonvulsant, Pentylenetetrazole, Picrotoxin, Strychnine.

Introduction

Epilepsy is a well-known chronic neurological illness that disturbs people of all ages and has a universal dissemination.[1,2] The fundamental indication of the disease is epileptic seizures; which is characterized by spontaneous and recurrent episodes of abnormal firing of a neuronal population and disturbances of consciousness.[3,4] As stated by the World Health Organization (WHO), about 50 million people have active epilepsy, making it one of the frequent neurological disorder that largely affects individuals in low and middle-income countries.[2] The high burden of epilepsy in these countries could be due to poor knowledge, inadequate diagnostic facilities and human resources, as well as high cost of effective drugs.[5-7] Thus, in order to lessen the distress associated with the disease and bridge the treatment gap, there is need for a multi-dimensional perspective incorporating the development of traditionally agreeable, available and cheaper drugs that can enhance therapeutic outcome especially in people of resource-poor communities.[7]

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In Nigeria and many other communities of developing countries, people naturally make use of readily available and well-known ethno-medicinal plants for the treatment of epilepsy.[7-9] Extracts from some of the plants employed in diverse forms of ethno-medicine against epilepsy have been investigated using animal models and novel bioassays.[10,11] Still, a lot of these plants employed in African ethno-medicine are yet to be investigated and thus, devoid of scientific proof to support their effectiveness. An example of such plants is *Bombax costatum* Pellegr. and Vuillet (Family: Bombacaceae).[12]

B. costatum is found largely in the Savanna areas of West Africa. It is commonly recognized in English as red-flowered silk cotton tree and in Nigerian languages as "*Gurjiiyaa"*, "*Joohi"* and *"Akpu"* (in Hausa, Fulfulde and Igbo, respectively).[13] Different parts of *B. costatum* are used in ethno-medicine against many ailments including epilepsy, oedema, hernia, headache, fever, insanity, skin diseases, yellow fever, diarrhoea and liver problems.[13-15] Basically, the powdered plant parts are macerated and eaten in a sauce or applied as a bath against epilepsy.[15] Scientific investigations have reported that *B. costatum* possesses antioxidant, anti-inflammatory and hepatoprotective activities.[12,16,17] To our knowledge, scientific reports on the anticonvulsant activity of *B. costatum* are scarce despite its well-commended efficacy and acceptability in the management of epilepsy. In this regard, this study was performed to explore the anticonvulsant potentials of the hydroalcoholic extracts of *B. costatum* leaves, stem bark and root bark using acute models of epilepsy.

Materials and Methods

Drugs and chemicals

The standard drugs used were diazepam (Valium®, Roche, UK), phenobarbitone (Lab Renaudin, France), sodium valproate (Sanofi-Aventis, UK) and phenytoin sodium (Parker-Davis and Co. Ltd. Detroit). Chemo-convulsants used were pentylenetetrazole, strychnine and picrotoxin (Sigma-Aldrich Chemical Co., USA), while the reagents include ethanol (Sigma Chemical Co., USA), ferric chloride and sulphuric acid (BDH Ltd Poole, England).

Experimental animals

Albino mice (18-22 g) of either sex were acquired from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Day-old ranger cockerels (30-40 g) were acquired from the National Animal Production Research Institute, Shika, Zaria. The animals were maintained under standard laboratory setting and given access to feed and water *ad libitum*. The studies were carried out with the authorization of the Ahmadu Bello University Committee on Animal Use and Care, with the ethical approval number: ABUCAUC/2020/50.

Collection, identification and extraction of plant material

The leaves, stem bark and root bark of *B. costatum* were gotten from Basawa, Sabon Gari Local Government of Kaduna State in August, 2019. The verification of the plant was done in the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria by Sanusi Namadi. The plant was issued a voucher specimen number (No. 1211) after comparing with an existing reference voucher specimen. The plant parts were air-dried under shade and then pulverized. The hydroalcoholic extracts of the three plant parts were prepared by subjecting 100 g of each powdered material to cold maceration with 2 L of 70% v/v aqueous ethanol (70% absolute ethanol and 30% water) and occasional shaking for 1 week. The mixtures were filtered and concentrated to dryness over a water bath kept at 45ºC. The extracts obtained were weighed, transferred to clean airtight containers and then kept in a desiccator till required for further studies.

Phytochemical test

The three extracts of *B. costatum* were subjected to preliminary phytochemical tests using standard procedures.[18]

Acute toxicity study

The up-and-down procedure as explained by the Organization for Economic Co-operation and Development (OECD) guideline 425 was adopted[19] to ascertain the acute oral toxicity profile of *B. costatum* extracts. In this study, the limit test at 5,000 mg/kg was carried out for each extract using five mice. The mice were dosed orally and sequentially with 5000 mg/kg of the extracts after they were fasted for four hours. Food was further withheld for 2 hours and they were then observed twice during the first 30 minutes after dosing, then periodically during the first 24 hours for signs of toxicity such as changes in fur and skin, eyes and mucous membranes, salivation, tremors, diarrhoea, lethargy, sleep, convulsions, coma and death and then daily for two weeks. The median lethal dose (LD_{50}) for each extract was subsequently estimated. This experiment was repeated using chicks.

Experimental design for anticonvulsant studies

In each experiment, eleven groups of animals were used. Group I mice were negative control (administered distilled water, 10 mL/kg) while group II mice were designated as positive control (pretreated with standard drugs). The other groups were test groups. Groups III-V received the leaf extract (125, 250 and 500 mg/kg), groups VI-VIII received the stem bark extract (125, 250 and 500 mg/kg), and lastly, groups IX-XI received the root bark extract (125, 250 and 500 mg/kg). All pretreatments were by oral gavage. The selected doses for the extracts (125, 250 and 500 mg/kg) were 2.5, 5.0 and 10% of their oral LD_{50} values respectively.

Maximal electroshock-induced convulsion test

Maximal electroshock test (MEST) was performed on day-old chicks[20] using eleven groups of ten chicks each. The chicks were treated as described above with phenytoin (20 mg/kg) as the positive control. An hour after pretreatment, tonic-clonic convulsion was induced to the chicks by passing alternating current from an electroconvulsive machine (Model 7801, Ugo Basile, Italy) through corneal electrodes which were placed on their upper eyelids. The current, frequency, shock duration and pulse width which produced tonic hind limb extension (THLE) were maintained at 80 mA, 100 pulse/s. 0.8 sec and 0.6 ms, respectively. The chicks were observed for THLE and recovery from the generalized seizures.

Pentylenetetrazole-induced convulsion test

The pentylenetetrazole-induced convulsion test was conducted on eleven groups of six mice. The mice were treated as described above with sodium valproate (200 mg/kg) as the positive control. An hour after pretreatment, each mouse received 85 mg/kg body weight of newly prepared pentylenetetrazole (PTZ) subcutaneously (s.c). The mice were then monitored for 30 minutes for the absence or presence of a clonic spasm.[21]

Picrotoxin-induced convulsion test

In this test, mice were treated as described above with diazepam (5 mg/kg) as the positive control. An hour after pretreatment, each mouse received freshly prepared picrotoxin (5 mg/kg, s.c.) and then they were monitored for 30 min for the presence of clonic or tonic seizures. The absence of tonic-clonic seizures or prolongation of the onset of seizures within the observation period was regarded as a sign of anticonvulsant activity.[22]

Strychnine-induced convulsion test

The method depicted by Porter *et al*.[23] was adapted. Eleven groups of mice were treated as described above with phenobarbitone (20 mg/kg) as the positive control. An hour later, all the mice were injected with strychnine (1 mg/kg body weight, s.c). The onset of tonic-clonic convulsion and the proportion of mice presenting with convulsion were recorded. The absence of tonic extension jerks of the hind limbs within 30 min of the observation period was regarded as a sign of anticonvulsant action.

Statistical analysis

The statistical analysis of data was done using SPSS software (Version 20, IBM, USA). The disparity between means was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results were presented as Mean ± S.E.M. and values of $p < 0.05$ were interpreted as significant.

Results and Discussion

The leaf, stem bark and roots of *B. costatum* have been utilized in African Traditional Medicine in the remedy of epilepsy. This research evaluated the anticonvulsant potentials of the three different parts of the plant using maximal electroshock and chemically-induced seizure tests. The outcome of the studies revealed that the hydroalcoholic extracts of *B. costatum* possess anticonvulsant activities in chemically-induced models of epilepsy.

The extraction of 100 g each of the powdered leaves, stem bark and root bark of *B. costatum* yielded 9.4, 5.7 and 5.7% $^{w}/_{w}$ of extracts respectively. Phytochemical screening of the extracts showed the occurrence of secondary metabolites including flavonoids, alkaloids, tannins, saponins, anthraquinones, cardiac glycosides, steroids and triterpenes (Table 1). Some of these phyto-constituents have been reported to possess anticonvulsant activities in established models of

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epilepsy.[8,24] Alkaloids have been described to exhibit anticonvulsant activity through inhibition of sodium channels, modulation of gamma-amino butyric acid (GABA), serotonin and norepinephrine,[25] while flavonoids were reported to exert anticonvulsant activity through modulation of $GABA_A$ benzodiazepine receptor.[26] Similarly, anthraquinone derivatives have been shown to inhibit generalized myoclonic convulsions.[27] The phyto-constituents in the three different plant parts were not the same. Flavonoids, tannins, cardiac glycosides, steroids and triterpenes were common to all the extracts, while the root extract in addition retained alkaloids and anthraquinones. This variation could be responsible for the higher anticonvulsant activity associated with the root bark extract.

Acute toxicity study is performed to assess the adverse effects of a test substance due to acute accidental or deliberate exposure. It is also useful in the determination of the range of doses that could be used for pharmacological studies.[28] In this research, acute toxicity test in mice and chicks revealed neither observable signs of toxicity nor mortality after a single administration of 5000 mg/kg of the leaf, stem bark and root bark extracts of *B. costatum* during the 14 days observation period. The oral LD_{50} of each extract was thus assessed to be > 5000 mg/kg in mice and chicks. This suggests that the extracts are practically non-toxic in both species when administered orally.[29] In this study, the doses selected for the pharmacological studies were less than 20% of the LD_{50}, and doses of this level have been shown to be well tolerated by experimental animals.[30]

The MEST is a validated method for pre-clinical evaluation of drugs useful against generalized tonic-clonic and partial seizures.[10,31-33] It also allows assessment of the capacity of a compound to avert seizure spread via neural tissues.[7,34] Standard drugs like carbamazepine, sodium valproate, phenytoin, oxcarbazepine and lamotrigine have been reported to suppress THLE induced by maximal electroshock and are also effective against generalized seizures.[35,36] In this study, the administration of electrical shock produced THLE in all chicks in the negative control. The administration of hydroalcoholic extracts of *B. costatum* leaves, stem bark and root bark did not produce a significant reduction ($F_{10, 76} = 1.907$, $p > 0.05$) in the duration of THLE when compared to the negative control. Also, the extract did not offer substantial protection against the maximal electroshock-induced seizures as only 20% protection was produced each by the leaf and root extracts at doses of 125 and 250 mg/kg, respectively. However, the standard drug (Phenytoin, 20 mg/kg) granted 90% protection against seizures (Table 2). Thus, the results obtained suggest that *B. costatum* extracts may not be beneficial in generalized tonic-clonic seizures. Chemically-induced seizure models have been utilized generally in the preclinical testing of anticonvulsant activity of new drugs. For example, the PTZ test denotes a valid model for human generalized myoclonic seizures, while picrotoxin and strychnine are models for primary generalized seizures.[37,38] The results obtained from the three chemically-induced seizure models suggest that *B. costatum* extracts contain important compounds that may be beneficial against myoclonic and tonic-clonic seizures.

In the PTZ test, subcutaneous administration of 85 mg/kg of PTZ produced myoclonic jerks in all the negative control mice. In contrast, the standard drug (Sodium valproate, 200 mg/kg) provided 83.33% protection against PTZ-induced seizures. Considering the hydroalcoholic extracts of *B. costatum*, oral administration of all the extracts increased the onset of PTZ-induced myoclonic jerks. The increase was significant ($F_{10, 32} = 1.492$, $p < 0.05$) with the stem bark (125 and 250 mg/kg) and root (500 mg/kg) extracts (Figure 1A). The leaf extract (250 and 500 mg/kg) and the root extract (125 and 250 mg/kg) protected 33.33% of the mice against seizures, while the stem bark extract rendered 50% protection at 500 mg/kg (Figure 1B). The PTZ test is one of the gold standard models for human absence seizures and is widely employed in screening the clinical activity of investigational drugs with the ability to raise seizure threshold.[32,39] PTZ induces seizures by preventing the key inhibitory pathways mediated by GABA in the central nervous system. Consequently, standard anticonvulsants like sodium valproate, phenobarbitone, ethosuximide and gabapentin inhibit PTZ-induced seizures by facilitating GABA mediated neurotransmission and they have all proven useful in the treatment of absence seizures.[40,41] Therefore, the anticonvulsant actions exhibited by the extracts of *B. costatum* in PTZ test suggests that they can increase the seizure threshold in the brain and may be effective against absence seizures.

Picrotoxin is a natural toxin with a well-defined binding site in the chloride channel of $GABA_A$ receptor.[38] When administered in rodents, it blocks $GABA_A$ receptor/chloride channel which prevents chloride conductance into the brain and consequently, inhibiting GABAergic neurotransmission and hence causing excitation.[38,42,43] In this research, oral administration of *B. costatum* extracts increased the mean latency of picrotoxin-induced seizures. The increase was significant ($F_{9, 32} = 3.511$, $p < 0.05$) with the leaf extract at 250 mg/kg and the stem bark extract at 125 and 250 mg/kg when compared to the negative control (Figure 2A). Amongst the three extracts, the root bark extract was more efficacious as it provided a dose-dependent protection of 33.33, 50 and 66.67% against seizures at doses of 125, 250 and 500 mg/kg, respectively. The standard drug used (Diazepam, 5 mg/kg) rendered 100% protection against picrotoxin-induced seizures (Figure 2B). The actions of *B. costatum* extracts in this model further suggest they may contain compounds that interrelate with GABAergic pathway and may also be beneficial in the treatment of tonic-clonic seizures.

Table 1: Phytochemical constituents of hydroalcoholic extracts of *Bombax costatum* leaf, stem bark and root bark
Key: + (Present); - (Absent)

Constituents	*Bombax costatum* extracts		
	Leaf	Stem bark	Root bark
Alkaloids	–	+	+
Flavonoids	+	+	+
Saponins	–	+	+
Tannins	+	+	+
Anthraquinones	–	–	+
Cardiac glycosides	+	+	+
Steroids and triterpenes	+	+	+

Table 2: Effect of hydroalcoholic extracts of *Bombax costatum* on maximal electroshock-Induced seizures in mice

Treatment (mg/kg)	Recovery period (Min.)	Quantal protection	Percentage protection (%)
D/W 10 mL/kg	8.22 ± 0.88	0/10	0.00
PHT 20	9.00 ± 0.00	9/10	90.00
BCLE 125	7.57 ± 0.92	2/10	20.00
BCLE 250	8.75 ± 1.25	1/10	10.00
BCLE 500	7.90 ± 0.66	0/10	0.00
BCSE 125	12.25 ± 2.11*	1/10	10.00
BCSE 250	8.22 ± 0.97	0/10	0.00
BCSE 500	12.00 ± 1.66*	0/10	0.00
BCRE 125	8.33 ± 1.05	0/10	0.00
BCRE 250	7.43 ± 1.09	2/10	20.00
BCRE 500	10.11 ± 1.01	0/10	0.00

Data are presented as Mean ± S.E.M., *=p<0.05, as compared to D/W group – One way ANOVA followed by Tukey's post hoc test, n = 10, D/W = Distilled water, PHT = Phenytoin, BCLE = *Bombax costatum* leaf extract, BCSE = *Bombax costatum* stem bark extract, BCRE = *Bombax costatum* root bark extract.

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Strychnine causes convulsions by blocking the postsynaptic inhibitory effect of glycine in the brainstem and spinal cord.[7,44] In this study, the administration of strychnine induced tonic seizures in all the negative control mice. The hydroalcoholic extracts of *B. costatum* did not produce a considerable increase in the mean onset of seizures except the root extract (250 and 500 mg/kg) where significant ($F_{10, 49}$ = 2.602, p<0.05) increase in the onset was observed when compared to the negative control (Figure 3A). Nevertheless, just the stem bark extract offered 33.33% protection at the dose of 125 mg/kg. Meanwhile the standard drug (Phenobarbitone, 20 mg/kg) protected 83.33% of mice against the strychnine-induced convulsions. (Figure 3B). From the foregoing, it can be inferred that the anticonvulsant effect of *B. costatum* extracts may also involve glycine-mediated inhibitory pathways.

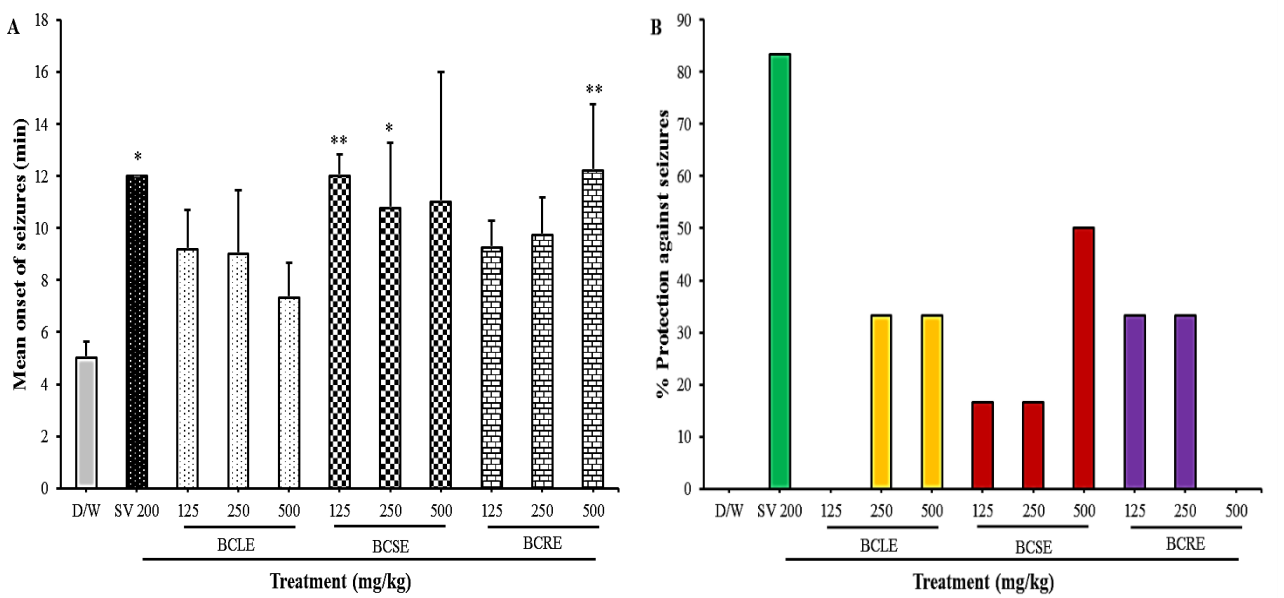

Figure 1: Effect of hydroalcoholic extracts of *Bombax costatum* on pentylenetetrazole-induced seizures in mice
Data are presented as Mean ± S.E.M., * = p < 0.05, ** = p < 0.01 as compared to D/W group – One way ANOVA followed by Tukey's post hoc test, n = 6, D/W = Distilled water, SV = Sodium valproate, BCLE = *Bombax costatum* leaf extract, BCSE = *Bombax costatum* stem bark extract, BCRE = *Bombax costatum* root bark extract

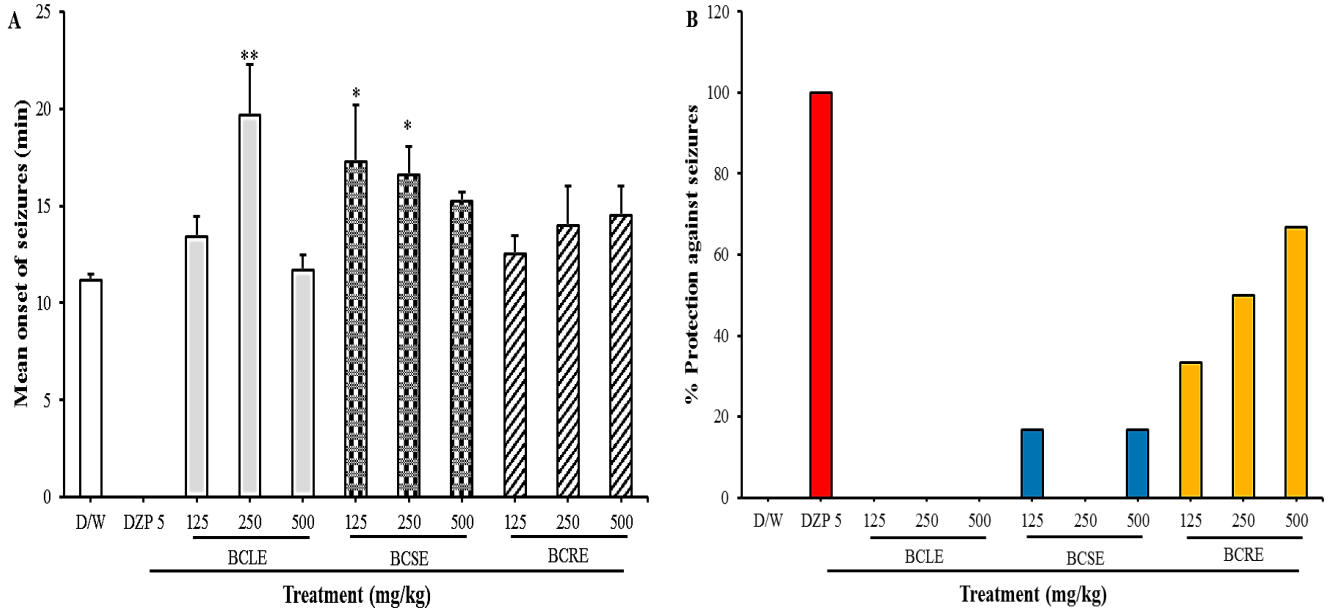

Figure 2: Effect of hydroalcoholic extracts of *Bombax costatum* on picrotoxin-induced seizures in mice
Data are presented as Mean ± S.E.M., * = p < 0.05, ** = p < 0.01 as compared to D/W group – One way ANOVA followed by Tukey's post hoc test, n=6, D/W = Distilled water, DZP = Diazepam, BCLE = *Bombax costatum* leaf extract, BCSE = *Bombax costatum* stem bark extract, BCRE = *Bombax costatum* root bark extract

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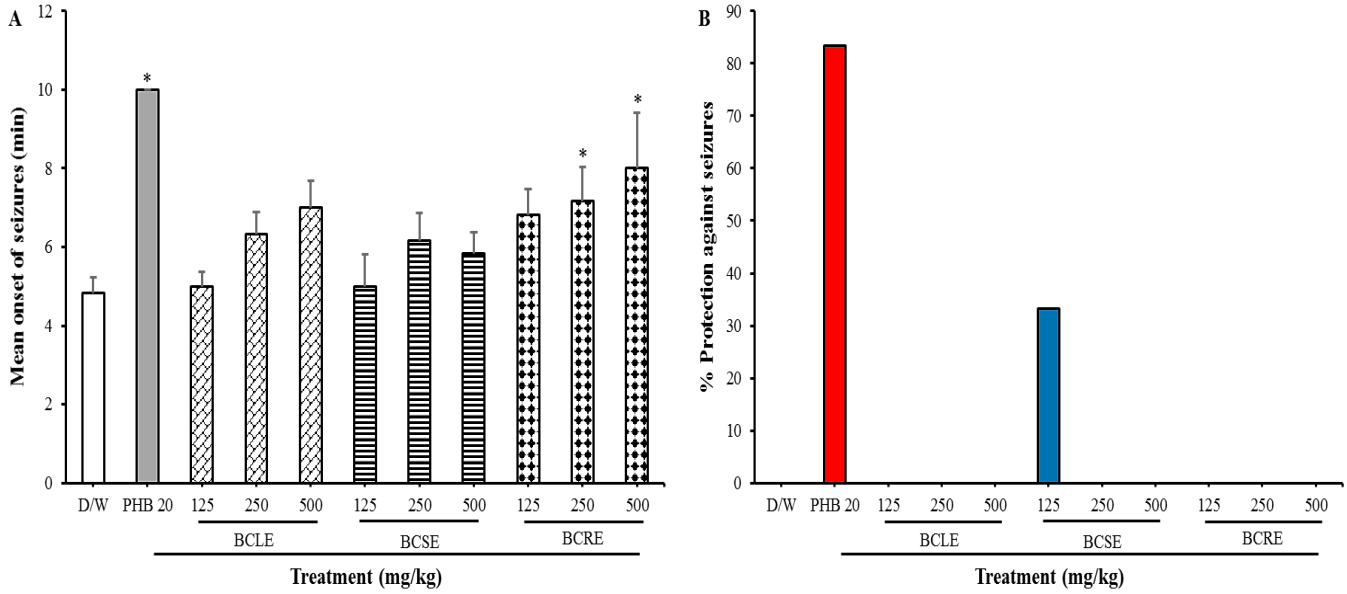

Figure 3: Effect of hydroalcoholic extracts of *Bombax costatum* on strychnine-induced seizures in mice
Data are presented as Mean ± S.E.M., * = $p < 0.05$, as compared to D/W group – One way ANOVA followed by Tukey's post hoc test, n = 6, D/W = Distilled water, PHB = Phenobarbitone, BCLE = *Bombax costatum* leaf extract, BCSE = *Bombax costatum* stem bark extract, BCRE = *Bombax costatum* root bark extract

Conclusion

The findings of this research revealed that the hydroalcoholic extracts of *Bombax costatum* leaves, stem bark and root bark possess anticonvulsant properties. The root bark was found to be the most effective part of the plant followed by the stem bark. This study provides credence for the ethno-medical use of the plant in the management of epilepsy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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