



RESEARCH ARTICLE

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Evaluation of anticonvulsant activity of *Bixa Orellana* Linn. seed

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ARTICLE INFO ABSTRACT



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Introduction: Epilepsy is a neurological disorder with recurrent seizures, often inadequately controlled by existing medications. Despite the availability of antiepileptic drugs, many patients experience inadequate seizure control and adverse effects, prompting the exploration of alternative treatments. *Bixa orellana* Linn., known as Annatto or Achiote, has traditional use for seizures.

Objective: This study explored the anticonvulsant properties of *Bixa orellana* Linn. seed extract using animal models.

Method: The extract was then tested in animal models, including the maximal electroshock seizure (MES) and Pentylenetetrazole (PTZ) models.

Results and Discussion: The extract exhibited significant anticonvulsant activity in both the maximal electroshock seizure (MES) and Pentylenetetrazole (PTZ) models. It delayed clonus onset, reduced the duration of the tonic extensor phase, and provided 100% protection against convulsions at medium and high doses in the MES model. In the PTZ model, it increased convulsion latency and offered high protection. *Bixa orellana* Linn. seeds show promise as a natural antiepileptic agent, warranting further research for mechanistic understanding and identification of bioactive compounds.

Conclusion: Developing new antiepileptic drugs from natural sources like *Bixa orellana* Linn. could offer alternative treatment options for epilepsy.

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Introduction

Epilepsy, a chronic neurological disorder characterized by recurrent seizures, affects millions of people worldwide (Sirven, 2015). Despite the availability of several antiepileptic drugs, a significant number of patients continue to experience inadequate seizure control and adverse effects. This has prompted researchers to explore alternative treatment options from natural sources, including medicinal plants (Manchishi et al., 2017). Anticonvulsants are substances or medications that suppress or control seizures and are commonly used in the management of epilepsy (Goldenberg, 2010).

Bixa orellana Linn., commonly known as Annatto or Achiote, is a tropical plant native to Central and South America. It is widely cultivated for its seeds, which are rich in natural pigments used as a food colorant and spice. Besides its culinary applications, *Bixa orellana* Linn. has a long history of use in traditional medicine for various health conditions, including seizures and convulsions. *Bixa orellana* Linn. has gained attention in recent years due to its potential therapeutic properties, including anticonvulsant activity. The seeds of *Bixa orellana* Linn. are known to contain various bioactive compounds, such as alkaloids, flavonoids, phenols, and terpenes, which are



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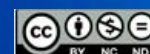
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believed to contribute to its pharmacological effects (Raddatz-Mota et al., 2017).

The evaluation of anticonvulsant activity involves assessing the ability of *Bixa orellana* Linn. seeds extracts or its constituents to prevent or reduce seizures induced by various experimental methods. These methods often include chemically-induced convulsions, such as maximal electroshock seizures (MES) and Pentylenetetrazole (PTZ) models in animals. By observing the effects of *Bixa orellana* Linn. seeds on seizure parameters, such as seizure severity, duration, and latency, researchers can determine its potential as an anticonvulsant agent. Understanding the anticonvulsant activity of *Bixa orellana* Linn. seeds may not only provide insights into its traditional use but also open avenues for the development of new antiepileptic drugs or complementary therapies. Additionally, the exploration of natural products for their anticonvulsant properties may contribute to the discovery of novel compounds with improved safety profiles and fewer side effects.

In this study, we aim to evaluate the anticonvulsant activity of *Bixa orellana* Linn. seeds by conducting comprehensive experiments using animal models of seizures. The findings of this research may provide valuable evidence regarding the potential of *Bixa orellana* Linn. seeds as a natural antiepileptic agent, thereby contributing to the development of alternative treatment options for epilepsy and related conditions.

Material and Methods

Plant material

The seeds of *Bixa orellana* Linn. was collected from local village of Mangalore and authenticated by Botanist, Pilikula Nisargadhama, Mangalore. The seeds were dried in shadow and were subjected to size reduction by using mixy, to coarse powder.

Plant extract

The air-dried *Bixa orellana* Linn. seeds powder weighed amount (200 g) was then subjected to continuous hot extraction with ethanol in a Soxhlet apparatus. The extract was filtered through a cotton plug, followed by Whatman filter paper (No. 1), and dried at 40-50 °C to get a reddish-brown material for final usage. The extract was stored in the refrigerator at 4 – 80 °C (Shiriki et al., 2019).

Animals

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house Srinivas College of Pharmacy, Mangalore at least 2 weeks prior to the study, so that they could adapt to the new environment. Animal house was

well maintained under standard hygienic conditions, at 22 ± 2 °C, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water ad libitum. The animals are now placed in separate cages. Cleaning and sanitation were done on alternate days. Paddy husk was provided as bedding material which was cleaned every day. The cages were maintained clean. The study was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee of Srinivas College of Pharmacy, Mangalore. Ref no: SCP/IAEC/F150/P114/2021 dated 13/01/2021.

Acute toxicity studies

Following the guidelines provided by the Organization for Economic Cooperation and Development (OECD) on testing the acute toxicity, an investigation was carried out. During the 24 to 72 hour observation periods, no adverse effects or death were seen in albino rats exposed to ethanolic extract of *Bixa orellana* Linn. Seeds (EEBOS) at doses up to 2 g/kg, p.o. The rats were continuously examined for 5 hours throughout this time to check for any obvious behavioral, neurological, or autonomic toxic effects. After 24 to 72 hours, the animals were sacrificed (Chinedu et al., 2013).

Assessment of Anticonvulsant activity

Maximal Electroshock seizure (MES) model

The acclimatized animals are randomly divided and into 5 groups of 6 animals each (n=6).

- Group I – Received 1% v/v Tween 80 orally.
- Group II – Received Phenytoin sodium 25mg/kg intraperitoneally.
- Group III – Received 100 mg/Kg EEBOS orally.
- Group IV – Received 200 mg/Kg EEBOS orally.
- Group V – Received 400 mg/Kg EEBOS orally.

Group I was maintained as normal control which was given with 3 % Tween 80 only (10 mL/Kg p.o.) only once daily for 7 days. Group II was injected with phenytoin (25 mg/Kg, i.p.) alone on 1st day and after 30 min a 60-mA current was delivered transauricularly for 0.2 sec on mice via small alligator clips attached to cornea by using electroconvulsive meter (ECM) and different phases of convulsions were recorded. Groups III, IV and V were treated with different doses of EEBOS (100, 200, and 400 mg/Kg p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of 3 % Tween 80 and EEBOS a 60-mA current was delivered transauricularly for 0.2 sec in mice via small alligator clips attached to cornea by using ECM and different phases of convulsion were recorded. Anticonvulsant activity was expressed as the percentage of inhibition of

convulsion compared with control animals. All precautions were taken to minimize animals suffering (Castel-Branco et al., 2009).

PTZ-induced seizures

The acclimatized animals are randomly divided and into 4 groups of 6 animals each (n=6).

- Group I – Received 1% v/v Tween 80 orally.
- Group II – Received Diazepam 5mg/kg intraperitoneally.
- Group IV – Received 100 mg/kg EEBOS orally.
- Group IV – Received 200 mg/kg EEBOS orally.
- Group V – Received 400 mg/kg EEBOS orally.

Group I was maintained as control which was given with 3 % Tween 80 (10 mL/Kg p.o.) once daily for 7 days. Group II was administered with diazepam (5 mg/Kg i.p.) alone on 1st day only after 30 min treatment anticonvulsant activity was recorded. Groups III, IV and V were treated with different doses of EEBOS (100, 200, and 400 mg/Kg p.o.) respectively once daily for 7 days on 7th day 30 min after diazepam and 60 min after extract administration PTZ (80 mg/Kg i.p.) was

administered. The following parameters were recorded during test session of initial, 30min and up to 24 h (Shimada et al., 2018).

Statistical analysis

By using the outcome parameters Mean values and Standard Deviation, the impacts of EEBOS were estimated. The analysis of variance (ANOVA) method was used to compare the outcomes of the investigated medicines. ANOVA was used to analyse the data, and Dunnett's test was then performed. The significance level was set at *p<0.05. Graph-pad Prism software was used for all of the statistical analysis.

Results

Effect of extract on Maximal Electroshock seizure (MES) induced seizures in mice

A standard drug phenytoin had exhibited significant anticonvulsant effect by abolishing the tonic extension phase and offered 100 % animals protection. Medium and high doses of EEBOS but not lower dose (100 mg/Kg) had delayed the onset of clonus, decreased the duration of tonic extensor phase and offered a good percentage protection of the animals (100 %) when compared to control group. (Table 1)

Table 1: Effect of EEBOS on MES induced convulsion in mice.

Group	Treatment	Dose (per kg)	Latency (on set of clonus) (sec/30 min) Mean ± SEM	Duration of tonic flexion (sec/ 30 min) Mean ± SEM	Duration of tonic extensor phase (sec/30min) Mean ± SEM	% Protection
I	Control	1% v/v Tween 80	3 ± 0.57	3 ± 0.57	15 ± 0.73	33
II	Standard	Phenytoin sodium 25 mg/kg	10.16 ± 0.30**	---**	----**	100
III	Test 1	EEBOS 100 mg/kg	3 ± 0.36ns	3.83 ± 0.65ns	12.16 ± 0.33**	100
IV	Test 2	EEBOS 200 mg/kg	8.16 ± 0.30**	---**	3.16 ± 0.47**	100
V	Test 3	EEBOS 400 mg/kg	8.16 ± 0.47**	0.33 ± 0.33**	5.33 ± 0.61**	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

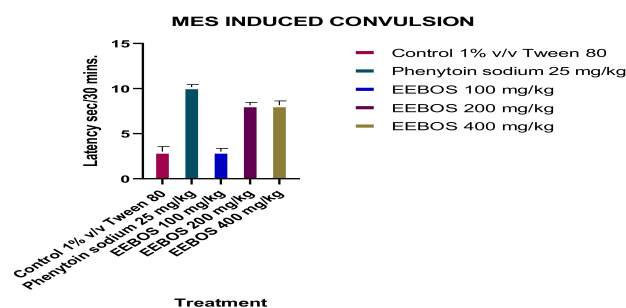


Figure 1: Duration of Latency (onset of clonus)

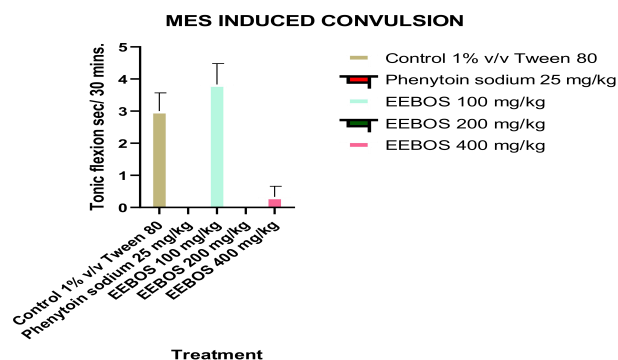


Figure 2: Duration of Tonic Flexion.

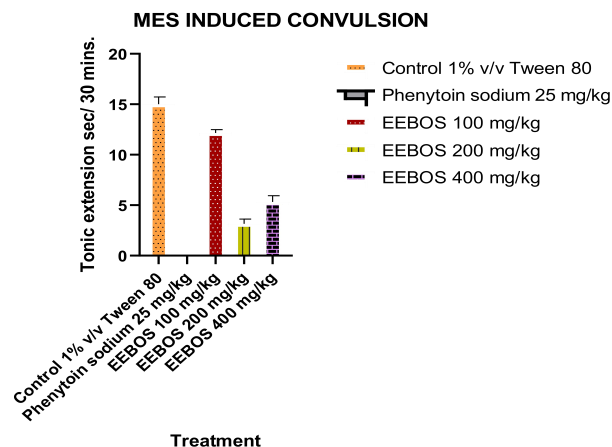


Figure 3: Duration of Tonic Extension.

Effect of extract on PTZ induced convulsion in mice

A standard drug diazepam treated group showed no convulsion after PTZ treatment it protects 100 % of the animal. In all the doses of EEBOS significantly increased latency, on set of tonic convulsions and protects up to 83 %, 100 % and 100 % respectively of mice. (Table 2).

Table 2: Effect of EEBOS on PTZ induced convulsion in mice.

Group	Treatment	Dose (per kg)	Latency (onset of clonus) (sec/30 min) Mean $\pm$ SEM	Onset of tonic convulsion (sec/30 min) Mean $\pm$ SEM	Status of animal (30min in) (No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
I	Control	1% v/v Tween 80	46 $\pm$ 0.577	210.5 $\pm$ 13.69	3	2	33
II	Standard	Diazepam 5 mg/kg	792.66 $\pm$ 46.37**	----**	ALL	ALL	100
III	Test 1	EEBOS 100 mg/kg	267.16 $\pm$ 14.96**	398.16 $\pm$ 20.26**	ALL	5	83
IV	Test 2	EEBOS 200 mg/kg	409.16 $\pm$ 49.23**	457.5 $\pm$ 23.85**	ALL	ALL	100
V	Test 3	EEBOS 400 mg/kg	475.66 $\pm$ 32.06**	651.5 $\pm$ 34.00**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

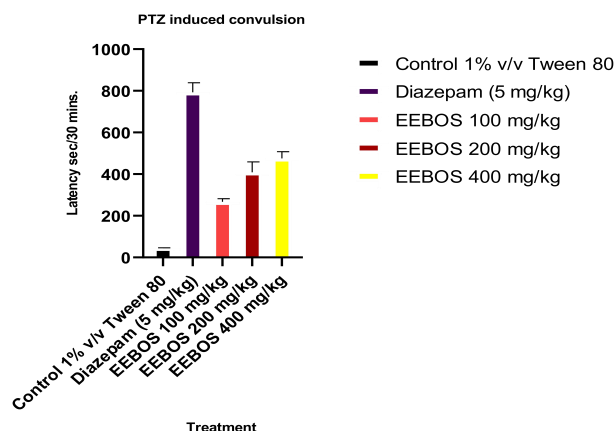


Figure 4: Duration of Latency.

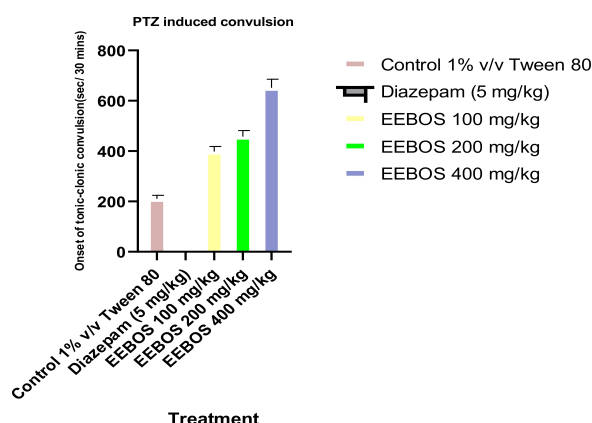


Figure 5: Duration of onset of tonic clonic convulsion.

Discussion

Epilepsy is a neurological disorder characterized by recurrent seizures, affecting millions globally (Anwar et al., 2020). However, current anticonvulsant drugs have limited efficacy, with only about 50 % of patients experiencing seizure control and 25 % showing some improvement (Wahab, 2010). Undesirable side effects also complicate treatment, necessitating the search for new antiepileptic drugs (Hanaya et al., 2016). One promising approach is investigating naturally occurring compounds, such as Bixa Orellana leaves, which may offer unique structures and mechanisms of action.

In the MES-induced convulsion animal model (representing grand mal epilepsy), antiepileptic drugs that block the tonic extension phase inhibit seizure spread. They can target voltage-dependent Na⁺ channels (e.g., phenytoin, valproate, felbamate, lamotrigine) or NMDA receptors (e.g., felbamate) (Loscher et al., 2021). EEBOS has demonstrated anticonvulsant activity by abolishing the tonic extension phase, possibly by blocking Na⁺

channels or acting as an NMDA antagonist. The PTZ-induced convulsion animal model mimics petit mal epilepsy and helps assess potential anticonvulsant drugs. PTZ acts as a GABA A receptor antagonist, affecting inhibitory GABAergic signaling and excitatory glutamate and aspartate systems. Notably, EEBOS has shown anticonvulsant activity against PTZ-induced convulsions, possibly acting as a GABA A agonist (Rogawski, 2006).

EEBOS has displayed anticonvulsant effects in both the MES and PTZ models, indicating its versatility in targeting different aspects of epileptic seizures. Its dual mechanisms of action, inhibiting Na⁺ channels or acting as an NMDA antagonist in the MES model and functioning as a GABA A agonist in the PTZ model, make it an attractive candidate for novel antiepileptic drug development. While promising, further research is essential to fully understand EEBOS's pharmacological properties, safety, and efficacy in treating epilepsy in humans. Rigorous clinical trials are necessary to validate its potential as an antiepileptic medication, ensuring better seizure control and improved quality of life for epilepsy patients. The quest for effective antiepileptic drugs remains critical, and naturally occurring compounds like EEBOS offer valuable avenues for exploration.

Conclusion

Current anticonvulsant drugs have limited efficacy and undesirable side effects, necessitating the search for new treatments. Naturally occurring compounds, like EEBOS, offer promise as potential antiepileptic drugs. EEBOS demonstrated anticonvulsant activity in both MES and PTZ models, potentially through Na⁺ channel inhibition, NMDA receptor antagonism, and GABA A agonism. These findings highlight EEBOS as a candidate for developing novel, effective antiepileptic medications.

Conflict of interest: None

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