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**Abstract**

The research study is an approach to establish the possibility of the anticonvulsant activity of the ethanolic extract of the bark of *Bruguiera cylindrica* Blume. The mangrove species of plants are found along the coastlines of the seashores spreading across the delta regions and the estuarine creeks of the world in tropical regions. India is home to a large number of Mangrove species. The Mangroves occupy their specific locations depending upon the requirement of substrate and water replenishment. *Bruguiera* and *Rhizophora* spp. are found in the firm bottom of sandy regions of low tide. *Bruguiera cylindrica* Blume (F. Rhizophoraceae) is a woody shrub evergreen mangrove. All the parts of this plant have been used for disease remedies which is attributed to its diverse bioactive compounds. The traditional and folk ways predominantly used and still uses the extracts of various parts of *B. cylindrica* as a cure for different ailments. This review reveals the meticulous study done on ethanolic extract of the bark of *Bruguiera cylindrica* Blume which was found to be anticonvulsant activity and also proven safe in toxicity study. The extract (100- 400g/ kg) produced significant dose dependant delay in the onset of seizure in MES model on oral administration that is comparable to phenytoin (20 mg/ kg i.p.). This extract produced significant dose dependant reduction in intensity of seizures in the Lithium- Pilocarpine epilepsy model which was comparable to standard drug diazepam (10mg/ kg). The results obtained from this study and findings suggest for the use *Bruguiera cylindrica* for the better management of convulsions and justify traditional uses.

**Keywords:** *Bruguiera cylindrica*, Ethanolic extract; Anticonvulsant; MES; Lithium-Pilocarpine.
Introduction

Throughout ancient times until the modern age, Epilepsy has been recorded since and associated with spiritual or supernatural condition. [1] The word epilepsy is derived from late Latin which means to seize, to possess or afflict [2]. Epilepsy is identified as not a single disease but a group of neurological diseases. It occurs as a seizure when brain activity is not normal which causes unusual behaviour, sensations and at times there is no awareness of the surroundings. Some of the patients may develop epilepsy due to any infection in brain like meningitis, viral encephalitis, type of birth defects, due to stroke, injuries or tumours in brain and brain stem. [3] Male and females from any geographical place, race, or ethnic background could develop the symptoms of epilepsy. Epilepsy could be defined as the manifestation of paroxysmal and neuronal discharges in the brain that are disordered. Epilepsy is recently looked upon as a spectrum of clinical diseases and not as a single disease due to the occurrence of seizures those which displayed difference based on clinical representation, extent, location and mode of propagation of the paroxysmal discharge. There is a subtle difference between epilepsy and seizures. Seizures are said to be paroxysmal events that are found to be occurring due to abnormal hyper synchronous discharge from an aggregate of central nervous system or the (CNS) neurons.

To diagnose epilepsy, it should be unprovoked seizures at least for two and more times. Provoked seizures could be due to external injuries, other neurological diseases or any kind of brain trauma. Epilepsy is a disease of the brain that is non communicable one. Recurrent seizures characterize the epileptic attacks which are involuntary movements involving partial body or the entire body. It happens due to decreased resistance of excitatory neurons to fire and down regulation of inhibitory neurons. The event takes place at a particular region called ‘seizure focus’ and the excessive, abnormal neuronal firing results into a wave of depolarization that is termed as paroxysmal deploring shift. [4] Epilepsy is not curable though there are available modern drugs that are able to control the seizures to some extent. Again, there are several conditions such as non-responsive cases which need to go for surgery and also sometimes neurostimulation or lifestyle changes approaches are advised for treatment purpose. [5]

Organisation of WHO had estimated in the year 2013 that around 33 million people around the world was epileptic. It is studied that out of all the epileptic patients, 80% are residents of the developing countries. [6] It has been observed that developing as well as the underdeveloped countries like the South Asian and the African countries are the places most severely affected by epileptic and related disorders. Poverty stricken population in these countries might not even receive the needful treatment which is termed as ‘treatment gap’. Expensive drugs, non-availability of long- term treatment facility has made the scenario even worst. Nearabout 90% patients in Africa are untreated either due to the non- availability of the medicine or the treatment facility. [7]

There are antiepileptic drugs available to treat different types of seizures. Even though they are effective to reduce seizures but the side effects cannot be avoided for these drugs. On an ideal situation antiepileptic drug should suppress the seizures without causing any side effects. The unavailability medicines in the rural and poor regions the alternative way of remedies could be the medicines prepared from plant extracts or natural flora from the local region. The procedures call
for proper research and identification of the medicinal properties of the particular plant species. Scientific evaluation of such kind of bio-compounds in plants could provide with far better relief than the synthetic medicine if the efficacy is established. Hence phytotherapy used as traditional medicines might play a significant role in management of epileptic seizures and convulsions especially among the too low-income group of population.

**Phytotherapy**

The plant extracts could be used for therapies for epilepsy which could improve the situation of scarcity of antiepileptic drugs or the side effects offered by them. This is a traditional way of treating epileptic patients and is an age-old practice. Medicine history traces that phytotherapy or plant extract uses were random in the oldest civilisations like in China, Egypt, Iran and India. Ayurveda in India is a study of medicine that mainly relates to the herbal or the plant extract medicines. Indian peninsula is a treasure trove for medicinal plants given to the varied geographical distribution throughout the country. There is this need for the proper identification of the medicinal properties and their scientific investigation for the efficacy of the drug to be prepared. There is still the need of robust evidence of efficacy and the toxicity of the plant parts to be used for drug development.

Here we would like to focus on orange mangrove and review the investigation done of the anticonvulsant activity of the ethanolic extract of the bark of *Bruguiera cylindrica* blume. (F. Rhizophoraceae), a tropical plant found in the coastal habitat. *Bruguiera cylindrica* is a mangrove which is evergreen tree which is a rare mangrove found mostly in the western coast of India and the swamps in Bangladesh, Australia.\[8\] *Bruguiera cylindrica* blume is a perennial columnal tree and attains about 25m height in its lifetime. It is woody, deliquescent and evergreen type of plant. There are various names given to this plant in local languages- Kandel, Kanil, Lahan zumbar, Sona Champa, Thusia, Kakandam, Vurudu.

Ancient and recent literature as well have revealed that various scientific researches have been carries out on this plan to explore its stupendous medicinal properties. True to say that all of the parts of the plant more or less have some or the other medicinal property that has been used as traditional remedy to particular ailments. Leaves, fruits, bark and roots have all been used traditionally as antiviral, cytotoxic, antibacterial, antimalarial and against diarrhoea or fever.

Medicinal properties of the bark of this plant have been studied for its antibacterial and antimicrobial properties. \[9\] The leaves of this plants are found to be antioxidant. Also, ant plasmodial, cytotoxic, insecticidal and antifeedant in nature. \[10\] The stems are reported to have antihyperglycemic properties. \[11\] There are reports about that brugine alkaloid is contained in the stem and bark of plant. The stems and twigs of the plant are found to be anti-inflammatory materials \[12\] and is considered as a protectant against or retrovirus diseases like HIV \[13\]. The wood of B. cylindrica species has a prominent activity against Cancer with anti-tumour propagating action. \[14\] This plant also contains complex protective agents like monoterpenoids, diterpenoids, triterpenoids along with flavonoids, tannins, alkaloids and steroids.
Having the insight that the plant *Bruguiera cylindrica* is equipped with the important bio-
compounds, we aim to evaluate and ascertain the antiepileptic properties of the bark of this plant
and to rationalise the uses on the basis of scientific findings.

**Materials and Methods**

**Plant Material**

The bark of *Bruguiera cylindrica* Blume (Rhizophoraceae) was collected from the water of
estuarine ecosystem at Gorai creek, Borivali, Mumbai. It was identified and authenticated by A.
Bennjamin, Botanical Survey of India, Pune. Voucher Specimen number - PPB- 1.

**Experimental animals**

Swiss albino mice of either sex weighing 18- 25gm and Wistar rats of either sex weighing 180-
200gm were used for the research study. The animals were housed in Dr. D.Y. Patil Institute of
Pharmaceutical Sciences and research, Pimpri, Pune. All of the animals were kept in a well-
lit and cross- ventilated room at 27± 2, light and dark cycles of 12 hours respectively for a week and
during the experiments being carried on. All the animals of either sex were housed in groups of
six under the standard conditions of laboratory with abundant access to standard pellet of diet and
water. Experiments mentioned in the study were all conducted in accordance with the guidelines
of the local animal ethical committee. The clearance to carry out the work had been obtained from
the Institutional animal ethical committee bearing number DYPIPSR/IAEC/15-16/P-05.

**Preparation of extract**

The stem barks of *B. cylindrica* has been collected and washed under tap water and then dried in
shades. Dried up bark was then powdered and passed through sieve of mesh size number 85. Next,
500 gm. of coarse powder was taken to be defat with petroleum ether 80. The powder was then
placed in thimbles that are made up of cellulosic filter paper extracted with ethanol solvent in
Soxhlet apparatus at a temperature within 60 degree C for 72 hours. The extractive value of
ethanolic bark was obtained to be 10.20 % w/w. The extract was concentrated under reduced
pressure in rotary evaporator to produce a syrupy viscous mass. The viscous mass thus yielded
was allowed to dry in porcelain dishes. After drying the dark brown solid mass was weighed and
stored for pharmacological investigations to be done later.

**Acute Toxicity Study**

Acute toxicity studies of the extract were performed according to OECD guideline number 423.
*Rattus norvigicus* (females) 8 and 12 weeks old that are non- pregnant nulliparous were housed in
a clean room at 22° C. Animals were fasted before administering the dose and were held overnight
with water ad libitum. Dose of extracts were prepared in a certain way so that the volume wouldn’t exceed 1 ml/ 100 gm of the body weight. The groups were formed with three animals in each group and each of the group received a single dose. At a concentration of 5, 50, 300, 2000 mg/ kg body weight respectively were administered in increasing order in five fixed level doses.
Anticonvulsant Activity

Anticonvulsant activity was carried out using two different models, Maximal Electric Shock (MES) and Lithium-pilocarpine induced convulsion in rats.

Maximal Electroshock Model

Comprising of five animals in each group, animals were divided into five groups where all were kept on fast overnight with water ad libitum. The extract was suspended in 0.6% sodium carboxy cellulose 60 min prior to administration and induction of MES. Administration with plane vehicle 2ml/100 body weight to the first group and was considered as control. Second group was administered with standard drug phenytoin (20mg/kg body weight), intraperitoneally. This group was taken considered as standard. Remaining three groups of the animals were administered with BC extract that was designated as Test I to III the dose was increasing in order as BC-100 mg/kg body weight; BC-200 mg/kg body weight and BC-400 mg/kg body weight respectively. The animals were applied with supramaximal electrical stimulus of 150 mA for 0.2 seconds through the corneal electrodes on cornea. The animals were then observed through various phases of MES seizures occurring as tonic hind limb flexion, tonic hind limb extensor and tonic-clinic phase. Abolition or decrease in the duration of extensor phase was taken as the index of antiepileptic activity of the extracts. The data was recorded in tabular form with the mean ± SEM and analysed by applying the one-way analysis of variance (ANOVA). ANOVA is a collection of statistical models and their associated estimation procedures which is used to analyse the differences in the means. The procedure was developed by the statistician Ronald Fisher.

Lithium Pilocarpine induced convulsions in rats

Wistar rats of either of the sexes was divided into five groups. Each of which contained five animals with first group as control and the second as standard. The first group was administered with vehicle only and the second group administered with diazepam (10mg/kg). Pilocarpine was administered (30mg/kg, i.p.) after the administration of lithium sulphate (m Eq/kg/ip) which was responsible for the induced convulsions. BC extracts were administered in the increasing order in the dose which was mentioned earlier. The effect of the BC extract at the dose of 100, 200 and 400 mg/kg/ip was observed on the severity of the seizures. The severity of the status epilepticus was observed every 15 minutes till the time period of 90 minutes. After that it was observed every 30 minutes till 180 minutes. Seizure activities like the fictive scratching, tremors, nodding of head and forelimb clonus were all observed and recorded in tabular form and entire data were analysed using the one-way analysis of variance or ANOVA.

Results

1. Acute Toxicity Study
The acute toxicity studies of BC extracts gave the LD$_{50}$ as 2.12 g/ kg and 3.12 mg/ kg for oral and intraperitoneal routes respectively.

2. Anticonvulsant Activity

Table NO. 1. Effect of Ethanolic extract of bark of BC on Maximal electroshock induced convulsions in rats

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Groups</th>
<th>Flexion</th>
<th>Extension</th>
<th>Clonus</th>
<th>Stupor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>11.60±0.27</td>
<td>15.02±0.10</td>
<td>11.04±0.45</td>
<td>10.08±0.40</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>02.98±0.19**</td>
<td>04.00±0.07**</td>
<td>03.58±0.19**</td>
<td>03.10±0.07**</td>
</tr>
<tr>
<td>3</td>
<td>Test-1</td>
<td>08.26±0.22**</td>
<td>12.42±0.19**</td>
<td>09.42±0.20**</td>
<td>08.08±0.08**</td>
</tr>
<tr>
<td>4</td>
<td>Test-2</td>
<td>05.04±0.18**</td>
<td>06.72±0.21**</td>
<td>05.80±0.14**</td>
<td>05.04±0.10**</td>
</tr>
<tr>
<td>5</td>
<td>Test-3</td>
<td>03.24±0.17**</td>
<td>04.46±0.15**</td>
<td>03.38±0.11**</td>
<td>03.52±0.06**</td>
</tr>
</tbody>
</table>

Table No. 2. Effect of Ethanolic extract of BC extract on Lithium pilocarpine induced status epilepticus in rats.

<table>
<thead>
<tr>
<th>Time after Pilocarpine (min)</th>
<th>Control</th>
<th>Standard</th>
<th>Test-1</th>
<th>Test-2</th>
<th>Test-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>02±0.25</td>
<td>0.66±0.33*</td>
<td>1.33±0.21</td>
<td>1.5±0.35</td>
<td>1±0.36</td>
</tr>
<tr>
<td>30 min</td>
<td>2.33±0.45</td>
<td>0.83±0.30**</td>
<td>1.50±0.22</td>
<td>1.5±0.22</td>
<td>1.16±0.30*</td>
</tr>
<tr>
<td>45 min</td>
<td>2.83±0.30</td>
<td>1.16±0.30**</td>
<td>2.00±0.36</td>
<td>1.66±0.21</td>
<td>1.5±0.22*</td>
</tr>
<tr>
<td>60 min</td>
<td>3.50±0.22</td>
<td>1.16±0.30**</td>
<td>2.00±0.36*</td>
<td>1.8±0.3*</td>
<td>1.6±0.42**</td>
</tr>
</tbody>
</table>
2.a. Maximal Electroshock Model

The *Bruguiera cylindrica* [BC] extract (100- 400 mg/ kg) produced a dose dependant delay in seizure onset induced by MES method. The effect of BC extract at dose 400 mg/ kg was found to be similar to that of standard drug phenytoin. (Table 1).

2.b. Lithium- pilocarpine induced convulsions in rats

Rats treated with lithium- pilocarpine (LP) displayed stage- 4 Status Epilepticus in all animals. The BC extract reduced the intensity of seizures and checked the intensity comparable to diazepam at a dose of 400 mg/ kg at 150 min (Table 2). BC dose of 100 mg/ kg and 200 mg/kg were found to control LP induced status epilepticus in rats within 180 minutes.

![Fig. 1: Effect of Ethanolic extract of Bark of *Bruguiera cylindrica* Blume on Maximal electroshock induced convulsion in rats.](image)

Values are expressed as Mean ± S.E.M., * p<0.05, ** p<0.01
Where,
Control: Normal saline + MES (Inducer)
Standard: Phenytoin at 20mg/kg orally.
BC-100: Ethanolic extract of bark of Bruguiera cylindrica Blume at 100mg/kg orally.
BC-200: Ethanolic extract of bark of Bruguiera cylindrica Blume at 200mg/kg orally.
BC-400: Ethanolic extract of bark of Bruguiera cylindrica Blume at 400mg/kg orally.

![Effect of Ethanolic extract of Bark of Bruguiera cylindrica Blume on Lithium Pilocarpine induced status epilepticus in rats.](image)

**Fig. 2** Effect of Ethanolic extract of Bark of *Bruguiera cylindrica* Blume on Lithium Pilocarpine induced status epilepticus in rats.

Values are expressed as Mean ± S.E.M., *p<0.05, **p<0.01

Where,
Control: Normal Saline + lithium pilocarpine
Standard : Diazepam 1mg/kg i.p
BC-100 : Ethanolic extract of bark of *Bruguiera cylindrica* at 100mg/kg orally.
BC-200 : Ethanolic extract of bark of *Bruguiera cylindrica* at 200mg/kg orally.
BC-400 : Ethanolic extract of bark of *Bruguiera cylindrica* at 400mg/kg orally.

Discussion

The BC extract was found to have significant broad dose range as is evident in the acute toxicity study. It showed the oral LD$_{50}$ of 2.12 g/ kg that is quite high as compared to phenytoin with oral LD$_{50}$ of 150 mg/ kg. The extract of BC in the dose range of 100- 400 mg/ kg produced a significant dose dependant (P< 0.1) reduction in convulsions in the MES model and displayed the efficacy in flexion, extension, clonus, and stupor (Figure 1). The extract 400mg/ kg also exhibited the anticonvulsant effect as compared to phenytoin at 20 mg/ kg. All of these observations suggest that the BC extract exerts significant glycinergic and GABAergic initiating mechanism. The two act as inhibitory neurotransmitters in the nervous system and are associated with convulsions. The **BC extract** might be inducing the release of those neurotransmitters and that way inhibiting the convulsions. The extract of BC had shown significant dose dependant decrease (p< 0.01) in the symptoms of status epilepticus in rats. This was found similar to the standard drug **diazepam** at the dose of 10 mg/ kg (Figure 2). It is quite evident from the table 2 that the relief from LT induced status epilepticus was observed in the span of 45 minutes of administration in the Test 3 group with the dose of 400 mg/ kg of extract. Then an observation of 45 minutes to 150 minutes showed a dose dependent decrease in convulsions. Hence, it is evident that the extract of BC is potentially effective against the LT induced status epilepticus.

Conclusion

The extensive research done and the result thus obtained from this particular study is hopeful for the treatment of convulsion and epileptic attacks. This is evident from the observations which have shown that the extract of *Bruguiera cylindrica* Blume has potent anticonvulsant activity. The fact also justifies the use of traditional and vernacular medicines for the treatment of CNS related disorders like epilepsy.
Acknowledgements
The authors are grateful to Godrej Ecological Centre, Mumbai for the contribution in plant collection and the Principal, Dr. S. S. Chitlange for providing with all the facilities needed.

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