Pharmacological Screening for Antiepileptic Activity of *Alstonia Venenata* R.Br Stem Bark Extracts *In-vivo* Methods

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**A B S T R A C T**

The ethanolic extract of *Alstonia venenata* R.Br stem bark was subjected for phytochemical investigation and LD₅₀ studies. It was found that ethanolic extract contained reducing sugars, proteins, Aminoacids, flavonoids, phenolic compounds and tannins. Phenolic compounds and flavonoids are responsible for antiepileptic activities. Significantly (P< 0.05 - 0.01) produce antiepileptic effect in mice at 100mg/kg comparing to 50mg/kg by oral administration, it is concluded that, the ethanolic extract of stem bark of *Alstonia venenata* R.Br. Possess antiepileptic activites. It is not possible to elucidate the action mechanism through which *Alstonia venenata* R.Br. exerts its effects. Further studies are necessary to confirm and extend the results.

**Keywords:** *Alstonia venenata* R.Br stem bark, soxhlet apparatus, Mice, PTZ induced convulsions.

**A R T I C L E  I N F O**

**CONTENTS**

1. Introduction .......................................................... 733
2. Materials and Methods ............................................. 734
3. Results and discussion ............................................. 736
4. Conclusion ............................................................ 737
5. References ............................................................ 737

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1. **Introduction**

**Epilepsy:** Epilepsy is a common term used to describe “Seizures”. It is defined as a chronic disorder which causes disturbances in the electrical signaling and its transmission in the brain. It is characterized by recurrent and sudden occurrence of seizure [2].
Seizures: Seizures are defined as brief disturbances in the electrical activity and physicochemical functioning of the brain. These have transient signs caused due to neuronal excitation which leads to the generation of action potential causing abnormal, excessive, repeated neuronal discharges.

Convulsions: These are defined as involuntary, violent contractions of the skeletal muscles which produce contortion (twisting or bending) of body and limbs.

Classification of Antiepileptic Agents:

Barbiturate: phenobarbitone
Deoxybarbiturate: primidone
Hydantoin: phenytoin, fosphenytoin
Iminostilbene: carbamazepine, oxcarbazepine
Succinimide: ethosuximide
Aliphatic carboxylic acid: valproic acid.
Benzodiazepines: clonazepam, diazepam,
Newer drugs: vigabatrin, topiramate, tiagabine, Zonisamide

General mechanism of action:
Barbiturates act primarily on the β-subunit of GABA-A receptor and increases the duration of opening of the GABA-gated chloride channels. This leads to influx of more chloride ions and thus prolonged hyperpolarization of the postsynaptic cell. This in turn causes the inhibition of neurotransmission. Hence, barbiturates act by enhancing the inhibitory effects of GABA. At higher concentrations, they exhibit a GABA mimetic action by directly opening the chloride channel and increasing the chloride conductance. They have been also found to inhibit voltage-dependent sodium and potassium channels [4]. All the above mentioned actions of barbiturates at multiple sites account for their CNS depressant effects.

Pharmacological actions: [1, 2]

a. On central nervous system
b. On respiratory system
c. On cardiovascular system
d. On liver
e. On smooth muscle
f. On skeletal muscle
g. On renal system
h. On gastrointestinal tract

2. Materials and Methods

Plant Name: Alstonia venenta R.Br.

Family: Apocynaceae

The plant material was collected from Tirupati.

Chemical constituents: Plant is a rich source of indole alkaloids. Major alkaloids in the stem bark are alstovenine, 3-dehydroalstovine, resperine (0.003-0.3%), venoxidine and kopsinine.

Therapeutic uses: Analivegam is a medicinal plant commonly used for snake bites by the traditional and tribal physicians of India. The root of Analivegam is used for the treatment of skin diseases, leprosy and other venomous bites.

Preparation of extract:
Alstonia venenta R.Br stem bark. was shade dried and made into coarse powdered which was passed through a# 40 mesh sieve to get uniform particle size and was extracted using Petroleum ether, and ethanol by continuous hot percolation process using soxhlet apparatus.

Soxhlet extraction:
It is an automatic, continuous process. The extraction time is less than 24 hours for a 500 gm sample. The disadvantage is that some compounds like carotenoids may decompose due to high temperature during heating.

Ethanol extraction process:
Drug: Menstrum ratio : 150gms: 700ml.
Number of cycles : 13 cycles.
Percentage yield : 7.6.

Drug preparation: The drug was prepared by dissolving the extract in 2% W/V Tween 80.

Test for reducing sugars:

Fehling's test: 1ml Fehling’s A and 1ml Fehling’s B solutions was mixed and boiled for one minute. Add equal volume of test solution. Heated in boiling water bath for
5 minute. First yellow, then brick red precipitate was observed, indicates presence of reducing sugars.

**Benedict’s test:**

Equal volume of Benedict’s reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 minutes. A red color solution was observed, indicates presence of reducing sugars.

**Test for proteins:**

**Biuret test (General test):**

To 3 ml test solution add 4% NaOH and few drops of 1% CuSO₄ solution. Slightly violet color was observed, indicates slightly presence of proteins.

**Test for amino acids:**

**a) Test for ninhydrin:**

Heat 3 ml of test solution and 3 drops of 5% ninhydrin solution in boiling water bath for 10 min. Purple color was observed.

**b) Test for Cysteine:**

To 5 ml test solution add few drops of 40% NaOH and 10% lead acetate solution. Boil the solution black ppt of lead sulphate is not formed.

**Test for cardiac glycosides:**

**Legal’s test (test for cardenoloides):**

To extract, add 1 ml pyridine and 1 ml of sodium nitroprusside. Red color was not observed, indicates absence of cardiac glycosides.

**Test for flavonoids:**

**Lead acetate test:** 2 ml extract were treated with few drops of 5% lead acetate solution. A yellow precipitate was not observed, indicates absence of flavonoids.

Heat test solution with zinc and HCL, pink to red colour was not observed.

**Shinoda test:**

To dry powder or extract, add 5 ml of 95% ethanol, few drops of conc. HCL and 0.5 gms of magnesium turnings. Orange colour was observed, indicates presence of flavonoids.

**Test for tannins and phenols:**

a. To 2 ml of extract were treated with few drops 5% FeCl₃ solution.

b. Deep blue – black color was observed, indicates presence of phenols.

c. To 2 ml of extract were treated with few drops lead acetate solution. A white precipitate was observed, indicates presence of tannins.

d. To 2 ml of extract were treated with few drops dilute iodine solution. Transient red colour was observed, indicates presence of tannins.

**Anti Epileptic Activity:**

**Pentylenetetrazole (PTZ)-induced epileptic seizure model:**

**Grouping of animals:**

Swiss albino mice were divided into five groups, either of sex.

- Control group [6].
- Standard group [6].
- Test group 1 [6].
- Test group 2 [6].

Total number of animals required 24.

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**Weight of the animals between 20 to 35 Gms.**

**Doses for each group:**

- **Control group:** 2%W/V Tween80 10 ml/kg body weight. p. o. route.
- **Standard group:** Phenobarbitone 30 mg/kg body weight. p. o. Route

**Test group 1:** Ethanolic extract of *Alstonia venenata* R.Br. Stem bark 50 mg/kg body weight. p. o. route.

**Test group 2:** Ethanolic extract of *Alstonia venenata* R.Br. Stem bark 100 mg/kg body weight .p.o. route.
Group 1-4 received convulsive dose of 80 mg/kg i.p of Pentylenetetrazole.

**Procedure:**

Animals were weighed and marked. Animals were divided into four groups consisting of six animals each. Group 1 served as epileptic control receiving tween 80 2%W/V (10ml/kg, p.o.) and the animals of group II and III received ethanolic extract of AV orally at a dose of 50,100 mg/kg respectively. Group V received standard drug phenol barbitone (30mg/kg, p.o). Group I-IV received convulsive dose of 80 mg/kg, i.p of PTZ. The animals were observed for onset and duration of myclonic seizure along with their percentage inhibition.

**6. Statistical analysis:** [16,17,18]

Statistical analysis was carried out using Prism graph pad 6 software. All results were expressed as Mean ± S.E.M. The statistical analysis of all the results was done using one way analysis of variance (ANOVA) followed by Dunnett’s test. Coating of F11 Tablets (F11A – F11E); *F11 E is Optimized Batch.

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### 3. Results and Discussion

**Preliminary phytochemical analysis of Stem bark of Alstonia venenata R.Br extract:**

The revealed results of the preliminary phyto chemical analysis of Stem bark of *Alstonia venenata* R.Br extract results were shown below.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Test</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reducing agents</td>
<td>Fehling's &amp; Benedict</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Test for proteins</td>
<td>Biuret</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Test for Amino acids</td>
<td>Ninhydrin</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Test for Amino acids</td>
<td>Cysteine</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Test for Cardiac glycosides</td>
<td>Legal</td>
<td>_</td>
</tr>
<tr>
<td>6</td>
<td>Test for flavanoids</td>
<td>Shinoda</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Test for flavanoids</td>
<td>Lead acetate</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Test for Tannins</td>
<td>Lead acetate &amp; 5% FeCl₃</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Test for Starch</td>
<td>Tannic acid</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Test for Alkaloids</td>
<td>Dragendorf’s &amp; Hager’s &amp; Wagner’s</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Test for Saponin Glycosides</td>
<td>Foam</td>
<td>_</td>
</tr>
<tr>
<td>12</td>
<td>Test for Steroids</td>
<td>Salkowski</td>
<td>_</td>
</tr>
</tbody>
</table>

**Antiepileptic Activity**

**A. PTZ Induced Convulsions:** Ethanolic extract of stem bark of *Alstonia venenata* R.Br significantly (P<0.05, P<0.01) decrease the immobility time as compared to standard group.
Table 2: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on PTZ Induced convulsion

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Drug treatment</th>
<th>Onset of myoclonic epileptic seizure(sec)</th>
<th>Duration myoclonic epileptic seizure(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control, p.o</td>
<td>118.83±4.46</td>
<td>99.5±0.885</td>
</tr>
<tr>
<td>II</td>
<td>Test(50mg/kg) p.o</td>
<td>169.83±5 ***</td>
<td>69±4.74 ***</td>
</tr>
<tr>
<td>III</td>
<td>Test(100mg/kg) p.o</td>
<td>186.5±1.9 ***</td>
<td>47.33±2.3 ***</td>
</tr>
<tr>
<td>IV</td>
<td>Standard(30mg/kg) p.o</td>
<td>202.33±2 ***</td>
<td>36.5±2.01 ***</td>
</tr>
</tbody>
</table>

n=6. The observations are mean ± SEM. * P<0.05, *** P<0.0001, as compare to control. (ANOVA followed by Dunnett’s test). Test = ethanolic extract of stem bark of *Alstonia venenata* R.Br.

Figure 3: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on PTZ Induced convulsions

n=6. The observations are mean ± SEM. * P<0.05, *** P<0.0001, as compare to control. (ANOVA followed by Dunnett’s test). Test = ethanolic extract of stem bark of *Alstonia venenata* R.Br.

4. Conclusion

The ethanolic extract of *Alstonia venenata* R.Br stem bark was subjected for phytochemical investigation and LD<sub>50</sub> studies. It was found that ethanolic extract contained reducing sugars, proteins. Aminoacids, flavonoids, phenolic compounds and tannins. Phenolic compounds and flavonoids are responsible for anti-epileptic activities. Significantly (P< 0.05- 0.01) produce antiepileptic effect in mice at 100mg/kg comparing to 50mg/kg by oral administration. It is concluded that, the ethanolic extract of stem bark of *Alstonia venenata* R.Br. Possess antiepileptic activities. It is not possible to elucidate the action mechanism through which *Alstonia venenata* R.Br.exerts its effects. Further studies are necessary to confirm and extend the results. Epilepsy has become the most common problem in many people. Therefore the present work proposes the use of herbal formulation to overcome this condition, which also helps

5. References

3. Lippincott Williams and Wilkins. Review of Pharmacology. 3<sup>rd</sup> Edition page no. 158 to 168 and 323 to 329


