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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 LD_{50} 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

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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.

Fits: It is a common term used to describe epileptic seizures. Person of any age group can suffer from epilepsy, but, it is more prevalent in children between 1-10 years of age and in geriatrics above 60 years of age.
Epilepsy is mainly two types (i) Generalised Seizures, (ii) Partial Seizures.

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[2] of more chloride ions and thus prolonged hyperpolarization of the postsynaptic cell. This inturn 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

Figure 1: *Alstonia venenta*

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, venenatine, 3-dehydroalstovenine, 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 venenata 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 24hours for a 500gm sample. The disadvantage is that some compounds like carotenoids may decompose due to high temperature during heating.

Petroleum ether extraction process:

Drug: Menstrum ratio	: 150gms: 700ml
Number of cycles	: 13 cycles
Percentage yield	: 7.6

Ethanolic extraction process:

Drug: Menstrum ratio	: 150gms:700ml.
Number of cycles	: 14 cycles.
Percentage yield	: 6.5

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

5minute. 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 3ml test solution add 4%NaOH and few drops of 1% $CuS0_4$ solution. Slightly violet color was observed, indicates slightly presence of proteins.

Test for amino acids:

a) Test for ninhydrin:

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

b) Test for Cysteine:

To 5ml 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 1ml pyridine and 1ml of sodium nitroprusside.Red color was not observed, indicates absence of cardiac glycosides.

Test for flavonoids:

Lead acetate test: 2ml 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 5ml of 95%ethanol, few drops of conc.HCL and 0.5gms of magnesium turnings. Orange colour was observed, indicates presence of flavnoids.

Test for tannins and phenols:

a. To 2ml of extract were treated with few drops 5%$Fecl_3$ solution.
b. Deep blue – black color was observed, indicates presence of phenols.
c. To 2ml of extract were treated with few drops lead acetate solution. A white precipitate was observed, indicates presence of tannins.
d. To 2ml of extract were treated with few drops dilute iodine solution transient red colour was observed, indicates presence of tannins.
e. To 2ml of extract were treated with few drops potassium dichromate solution red colour ppt was observed, indicates presence of tannins.

Test for starch:

Tannic acid test: Mix 3ml test solution and few drops of tannic acid solution gives buff colour ppt, indicates presences of starch.

Test for alkaloids:

The extract were treated with dilute HCL and filtered. The filtrate was treated with various alkaloidal agents.

Dragendorff's test: 2-3ml filtrate was treated with Dragendorff's reagent, orange brown precipitate was observed, indicates presence of alkaloids.

Hager's test: 2-3ml filtrate was treated with Hager's reagent, yellow precipitate was observed, indicates presence of alkaloids.

Wagner's test: 2-3ml filtrate was treated with Wagner's reagent, reddish brown precipitate was observed, indicates presence of alkaloids.

Test for saponin glycosides.

Foam test: Shake the drug extract vigorously with water. Foam was not observed, indicates absence of saponin glycosides.

Test for steroid:

Salkowski reaction: To 2ml of extract, added 2ml chloroform and 2ml con.H2So4.Shake well. Chloroform layer was not observed indicates absence of steroid.

Pharmcological Screening (*In-Vivo* Methods):

Acute toxicity study of *Alstonia venenata R.Br* stem bark extract:

Acute toxicity study was performed for ethanolic extract of roots of *Alstonia venenata* R.Br. According to Blind Screening (Irwin 1959) Female mice selected by random sampling technique were employed in this study. Female mice were used because literature surveys of convential LD_{50} tests show that females are generally slightly more sensitive for the study. The animals were fasted prior to dosing. Ethanolic extract of stem bark of *Alstonia venenata* R.Br. was administered orally to different groups at the dose levels of 5, 50, 300, and 1000 mg/kg body weight. The animals were observed the behavioural, neurological and autonomic effects studies 24hrs for mortality with special attention during first 3hr and intermittently for 14 days. During acute toxicity study, no mortality was observed in animals treated with ethanolic extract of stem bark of *Alstonia venenata* R.Br. up to a high dose of 1,000mg/kg body weight.

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 groups -1 [6].
Test group-2 [6].
Total number of animals required 24.

Weight of the animals between 20 to 35 Gms.

Doses for each group:

Control group: 2%W/V Tween80 10ml/kg body weight. p. o.route.

Standard group: Phenobarbitone 30mg/kg body weight. p.o. Route

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

Test group 2: Ethanolic extract of *Alstonia venenata* R.Br. Stem bark 100mg/kg body weight .p.o. route.

Group 1-4 received convulsive dose of 80 mg/kg i.p of Pentylenetetrazole.

Procedure: 19

Animals were weighed and marked. Animals were divided into four groups consisting of six animals each. Group 1 served as epileptic control receving 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

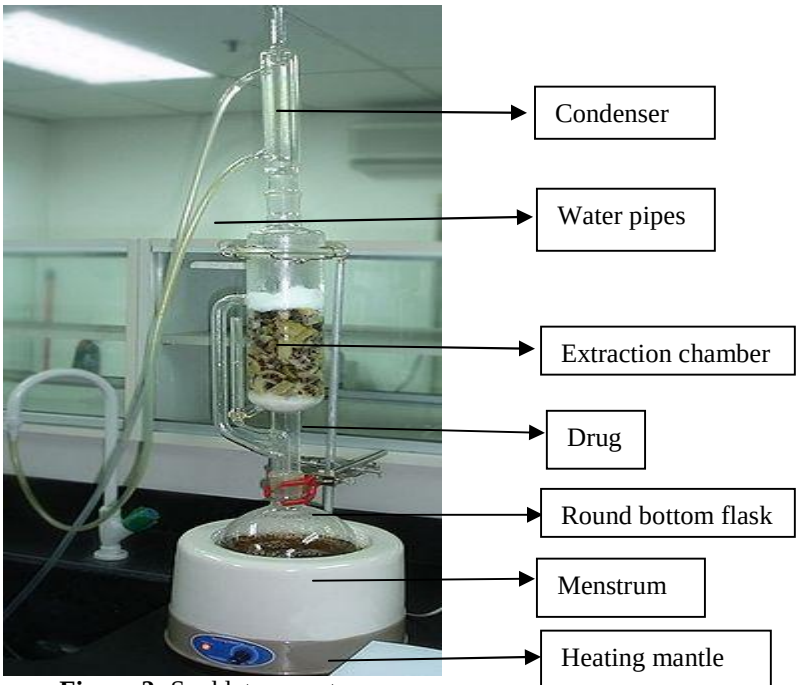

Figure 2: Soxhlet apparatus

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 1: Preliminary phytochemical analysis

S. No	Compound	Test	Ethanolic extract
1	Reducing agents	Fehling's & Benedicts	+
2	Test for proteins	Biuret	+
3	Test for Amino acids	Ninhydrin	+
4	Test for Amino acids	Cysteine	_
5	Test for Cardiac glycosides	Legal	_
6	Test for flavanoids	Shinoda	+
7	Test for flavanoids	Lead acetate	+
8	Test for Tannins	Lead acetate & 5% FeCl3	+
9	Test for Starch	Tannic acid	+
10	Test for Alkaloids	Dragendorff's & Hager's & Wagner's	+
11	Test for Saponin Glycosides	Foam	_
12	Test for Steroids	Salkowski	_

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

Group No.	Drug treatment	Onset of myoclonic epileptic seizure(sec)	Duration myoclonic epileptic seizure(sec)
I	Control, p.o	118.83±4.46	99.5±0.8851
II	Test(50mg/kg) p.o	169.83±5 ***	69±4.74 ***
III	Test(100mg/kg) p.o	186.5±1.9 ***	47.33±2.3 ***
IV	Standard(30mg/kg) p.o	202.33±2 ***	36.5±2.01 ***

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.

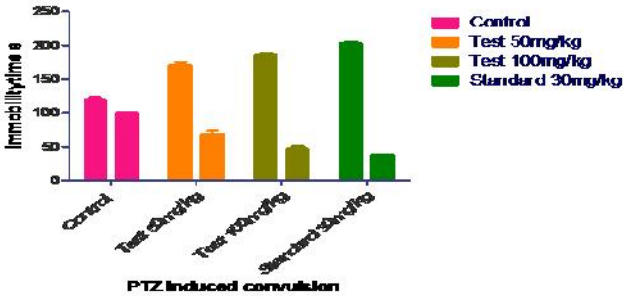

Figure 3: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on PTZ Induced convulsions

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_{50} 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 in reduced side effects. 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. 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

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