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EVALUATION OF ANTICONVULSANT PROPERTIES OF LEUCAS ASPERA AERIAL PARTS ON EXPERIMENTAL RODENTS

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Abstract: Epilepsy is the most common disorder the Brain or Neurological symptom, affecting more than 50 million people worldwide, caused by sudden abnormal and recurrent electric discharge from the affected brain cells, the present investigation has been undertaken to study the anticonvulsant property of 95% Ethanolic extract of aerial part of *Leucas aspera.* The Phyto constituents are extracted by using continuous extraction method in soxhlet Extractor with solvents like petroleum Ether & 95% Ethanol. By executing the Qualitative & Quantitative Analysis we found and conformed that the Phytochemicals like flavonoids, terpinoids, saponins, carbohydrates, proteins etc are present, The anticonvulsant activity of the *Leucas aspera* was estimated by PTZ (Pentylenetetrazole) induced convulsion and Maximal electro shock induced convulsion models in experimental rodents, From the studies it is concluded that 95% ethanolic extract of aerial parts of *Leucas aspera* shows anticonvulsant property, by reducing the convulsive phase and mortality.

Keywords: Anticonvulsant, *Leucas aspera*, indigenous system, soxhlet Extraction, Phytochemicals, medicinal plants

Introduction*:* Epilepsy is a common neurological disorder, it is a group of syndromes that involves spontaneous, intermittent, abnormal electrical activity in the brain. There is spontaneous occurrence of brief episodes associated with disturbance in consciousness and excessive EEG spikes, The overall incidence of epilepsy in developed countries has been found to be around 50 cases per 100,000 persons per year and rises steeply in older age. Round about 50 million people were affected by this worldwide.[1] Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness.[2]

Epilepsy accounts for a significant proportion of the world's disease burden, affecting around 50 million people worldwide. The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. Globally, an estimated 5 million people are diagnosed with epilepsy each year. In high-income countries, there are estimated to be 49 per 10,0000 people diagnosed with epilepsy each year. In low- and middle-income countries, this figure can be as high as 139 per 100 000[3] All anti-epileptic drugs currently available in the market such as phenobarbital, benzodiazepines, phenytoin, sodium valproate, ethosuximide, trimethadione, carbamazepine etc., are unable to control seizures efficiently. Furthermore the dose-related neurotoxicity and other side effects associated with AED limit their clinical use.[4]

Traditional medicines are widely used in Developing and under developed countries, with up to 80% of the population relying on them or folk treatments for their primary health care. Medicinal plants are thought to be a major source of novel chemical compounds with medicinal properties. Several plants used for the treatment of epilepsy in various systems of traditional medicine have shown action when tested in contemporary bioassays for anticonvulsant activity, and many more are still being studied scientifically.[5] *Leucas aspera is* a perennial herb found commonly in tropical regions. It is an herbaceous aromatic weed belonging to the family Lamiaceae (Labiatae), Traditionally it is

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known as "Thumbai in Tamil and "Dronapushpi" in Sanskrit, in folklore practices it is used as an antipyretic and insecticide, Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue.[6] Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites and literature survey reveles that it has many secondary metabolites responsible for its pharmacological actions so the present work has been taken to screen the anticonvulsant properties of the aerial parts of *Leucas aspera* plant.

Material and Methods: Selection of Plant Material : The aerial parts of *Leucas aspera* were collected from local area (Uchangidurga) around Harapanahalli, Vijayanagara (D) Karnataka, *Leucas aspera* plant material was authenticated by Prof. K Prabhu Sir, Dept. of Pharmacognosy, S.C.S College of Pharmacy Harapanahalli. Herbarium (SCSCOP. Ph. Col. Herb. No. 09/2022-2023) is deposited in the department of Pharmacognosy.

Preparation of *Leucas aspera* Extract [7, 8.]: Whole plant of *Leucas aspera* were dried under shade, mixed together & then made in to coarse powder with mechanical grinder. The dried powder material was deflated with Petroleum ether (60˚-80˚) to remove waxy substance and Chlorophyll, which usually interfere in the isolation of Phyto-constituents. It is done by using Soxhlet extractor for 12hrs, The marc after defatted with Petroleum Ether was dried and again extracted with Ethanol (95% v\v) in a Soxhlet extractor for 72hrs. The percentage yield was calculated for the extracts with reference to the crude plant material taken using the reference formula given . The percentage yield of the each extract is tabulated in Table No.1

Preliminary phytochemical analysis [9,10,11.]: The obtained extract will be subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K.Kokate and R.K.Khandelwal results obtained are coreleated with the data obtained in review of literature.

Estimation of Total Phenolic Content [12]: The quantitative estimation Phenolic content of the plant extract was carried out by determining the total phenolic content of *Leucas aspera* herb extract followed by Folin-ciocalteu assay method described by Kavithachandran et al,. in which 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-ciocalteu's phenol reagent. After 5 minutes,10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionised distilled water and mixed properly, volume was made up to 25ml and The mixture was kept in the dark for 90 minutes at 23^0C, after which the absorbance was read at 650nm. In the reaction tube as a result of complex redox reaction between phenols and phosphomolybdic acid in Folin-ciocaltau reagent in alkaline medium, blue color develops due to the formation of molybdenum blue. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

Animals: Albino mice (weighing 20-25 g) of either sex were used in this study. All experimental animals were procured from Sri Venkateshwara Enterprises, Banglore, Karnataka. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C under 12 hrs dark/light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water ad libitum was provided. The husk in the cages was renewed thrice a week to ensure hygienity and maximum comfort for animals. Ethical clearance for usage of the experimental animals was obtained from the Institutional animal ethical committee, Certificate reference no: TMAES/IAEC/18th/05/2022-23 dated: 05-08-2023 prior to the beginning of the research work.

Acute oral toxicity study: Acute toxicity study for the aerial parts of *Leucas aspera* was done according to the OECD guidelines No: 423 and low and high dose was selected for treatment. The Alcoholic extract (95 %) of *Leucas aspera* was administered orally in the escalating dosages, up to 2000 mg/kg to different groups of Mice (n=3, in each). The animals were observed for behavioral and physiological variations initially continuously for 4 h, followed by 4th hourly for 12 h and there after once daily for fourteen days. If toxic signs or lethality is not observed, then 1/5th and 1/10th part of the limit test dose were considered as test doses for the present investigation.

Determination of Anti-convulsant activity

A. Pentylenetetrazole (PTZ) induced convulsion [12,13] : Animals were divided into IV groups, (n=6) mice of either sex in one group.

Group I received respective vehicle (p.o); for 7 days

Group II was treated with standard drug (diazepam 5 mg/kg i.p).

Group III was treated with low dose of test extract (200 mg/kg p.o); for 7 days.
Group IV was treated with high dose of test extract (400mg/kg p.o); for 7 days.
All test and std. drug treated groups were statistically compared with vehicle groups. Vehicles, extracts are administered by oral route and standard drugs were administered by intra-peritoneal (i.p.) route. Test group were treated with plant extracts for seven days and on the experimental day, PTZ 65 mg/kg was injected intra-peritoneal to mice 45 min after vehicle or extracts and 30 min after the standard drug. Immediately after PTZ administration mice were observed for.
(1) Onset of convulsions (elapsed time from PTZ injection until convulsion occurred),
(2) Duration of convulsion (number of mice showing convulsions) and
(3) Mortality for the duration of 15 minutes (Swinyard *et al.*, 1952)

B. Maximum electro shock (MES) induced convulsions [14,15.] **:** The electroshock was applied via ear-clip electrodes separately to each mouse. The stimulus duration was 0.2 s and the current frequency 45 mA (60 Hz). Six mice of either sex in one group with a weight of 25±5 g,
Group I received respective vehicles (p.o); for 7 days
Group II was treated with standard drug (Phenytoin 25 mg /kg i.p); for 7 days.
Group III was treated with low dose of test extract (200mg/kg p.o); for 7 days.
Group IV was treated with high dose of test extract (400mg/kg p.o); for 7 days.
Mice were treated with extracts for seven days and on the experimental day test was started 60 min after administration of extracts and 30 min after standard drug (phenytoin 25 mg/kg i.p.).
The duration of hind limb tonic extension, total recovery time and the protection against mortality was recorded by using video recorder. and then the percentage protection was calculated as:

$$\% \text{ Reduction in THLE} = \frac{\text{Mean Duration of THLE in control - Mean duration of THLE in test}}{\text{Mean Duration of THLE in control}} \times 100$$

Note: Prevention or decrease in hind limb tonic extension was considered as protective action.

Results:

I. Preparation of extract and calculation of percentage yield

The percentage yield of the respective extract was given in the following table.

Table: 1 Percentage and Color of the Extract:

Sl. No	Solvent type	Wt. of the sample before extraction (gm)	Wt. of the extract (gm)	Percentage of yield (%)	Color of the extract	Consistency
01.	Petroleum Ether extract	260gm	4.61gm	1.77%	Dark Green	Gummy and highly Viscous
02	Ethanolic extract	250gm	26.5gm	10.60%	Blackish Green	Sticky and Viscous

II. Phyto-chemical analysis:

The prepared extract were tested for various chemical constituent according to standard procedure and results revealed the presence of alkaloids, proteins, flavonoids, phytosterol, saponin and Tannins etc in 95% ethanolic extract based on these findings 95% ethanolic extract is further subject for analysis.

III. Quantitative determination of Total polyphenolic content:

The total phenolic content of aerial part of the plant extract was 1.08 mg/G expressed as equivalent to Gallic acid.

IV.Acute oral toxicity study (OECD 423): Acute oral toxicity studies were carried out according to OECD guideline No 423, 2000 mg/kg of extract was administered as per OECD guidelines per orally to 3 animals of single sex. Toxic effects were observed or monitored for 72 hours. No change in normal behavior or any abnormality in behavior was observed and no mortality was seen. Thus it was concluded that plant extract was nontoxic up to 2000 mg/kg doses, so $1/5^{th}$ (400mg/kg High dose) and $1/10^{th}$(200mg/kg low dose) of the administered dose were selected for future studies as per OECD guidelines.

Table No 03: Effect alcoholic extracts of *Lecus aepera* on the pentylenetetrazole-induced convulsion in mice.

Experimental group	Dose mg/kg b.w	Onset of clonic convulsion (min.)	Duration of convulsion (min)	Mortality/used (%)
Control	------	1.077 ± 0.11	5.25 ± 0.21	4/6 (66%)
Std	5 mg/kg	0.00 ±0. 00***	0.00±0.00***	0/6 (0%)
Low Dose	200 mg/kg	1.763 ±0.12	2.786±0.140*	3/6 (50%)
High Dse	400 mg/ kg	3.63±0.13**	2.42±0.13*	1/6 (16%)

(All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Table No 04: Anticonvulsant effect of alcoholic extracts of *Lecus aepera* on the MES-induced convulsion in mice.

Experimental group	Dose mg/kg b.w	Mean duration of THLE in sec.	% Reduction in THLE	Mortality/used (%)
Control	--------	27.83±1.5	-------	4/6 (66%)
Std	5 mg/kg	5.33±1.57 ***	80.84%***	0/6 (0%)
Low Dose	200 mg/kg	24.50±1.23	12%	3/6 (50%)
High Dse	400 mg/ kg	19.16±1.87*	32%*	0/6 (0%)

(All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001)

Discussion and Summary: Epilepsy is a chronic neurological disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge from the cerebral neurons. It is the second most common Brain disorder with an annual incidence of 50 cases/100000 per year. Overall it accounts for 1% of the world's burden of diseases, and reported 2% prevalence rate, *Leucas aspera* is a perennial herb found commonly in tropical regions, It is an herbaceous aromatic weed belonging to the family Lamiaceae (Labiatae),Traditionally it is known as "Thumbai" in Tamil and "Dronapushpi" in Sanskrit traditionally it is used as an antipyretic and insecticide, Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites and literature survey reveles that it has many secondary metabolites responsible for its pharmacological actions so the present project work has been taken to screen the anticonvulsant properties of the aerial parts of *Leucas aspera plant.*

The results of the present study demonstrate that alcoholic extract of aerial parts of *Leucas aspera* shows the anti-convulsant property in experimentally induced convulsive animals like mice, the PTZ induced anti-convulsant model all extracts are compared with control group and results revels that High dose (400 mg/kg) of the plant extract significantly delayed the onset of convulsion and reduced the duration of convulsion and also protected the experimental animals from the mortality, where as std drug Diazepam (5 mg/kg) completely blocked the colonic convulsion and mortality in mice against PTZ induced convulsion.

In MES induced convulsion model High dose (400 mg/kg) of plant extract significantly reduces the duration of HLTE and mortality when compared to control where as STD drug Phenytoin (25 mg/kg) significantly reduces MES induced HLTE and

completely prevented various phases of convulsion and death of the experimental animal.

From the studies it is found that ethanolic extract of *Leucas aspera* posses anti convusulnt property may be due various phytoconstituents and literature survey reveals the same.

In our study the shade dried plant powder was subjected to series of soxhlet extraction process with different solvents like pet ether and 95% ethanol, from our studies we found that many phytoconstituents are present in ethanolic extract and it is was confirmed by percentage yield and phytoconstituent analysis.Further the extract was subjected to quantification studies like Total phenolic content and total phenolic content found to be 1.08 mg/g of Gallic acid.

GABA is the major inhibitory neurotransmitter in Brain and inhibition of its neurotransmission has been one of the under laying factor epilepsy, it has been reported that STD drug like Diazepam and Phenobarbitone exert their anti-convulsant activity by enhancing the GABA mediated inhibition so plant might have acted up on the GABAnergic neurotransmission. The MES induced convulsion in animals represents grandma type of epilepsy in animals the plant extract High dose significantly decrease the duration of HLTE induced by MES in treated and reduces the mortality when compared to control group, this property of the plant extract might be due to various secondary metabolites present in the extract like phenols, Flavonoids,Terpenoids etc but further extensive work has to be carried out to co-relate the anti-epileptic property at receptor or cellular level.

Conclusion: From the present studies it is concluded that ethanolic extract of aerial parts of ethanolic extract of *Leucas aspera* shows anti-convulsant property and reduces the duration of convulsive phases and mortility in experimental models, However further studies must be carried out at cellular level and further clinical studies to be carried out to use this aromatic weed plant as anti-convulsant.

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