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Evaluation of *invitro* anti-oxidant and anti-epileptic activities of *Calliandra haematocephala* using zebra fish model

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Abstract

Background: *Calliandra haematocephala* Hassk commonly known as the red powder puff, is a small ornamental shrub belongs to Fabaceae. It is widely distributed in tropical and subtropical regions, including India, Southeast Asia, Africa, and Central America. Though popularly cultivated for its vibrant crimson flowers, the plant also holds considerable ethnomedicinal value. Traditionally, various parts of *Calliandra haematocephala* have been used in the treatment of inflammatory disorders, epilepsy, fever, bacterial infections, and as a natural blood purifier. Tribal and folk communities (Ogbomoso, Oyo State) in Nigeria have employed its extracts for their anti-inflammatory, anticonvulsant, immunomodulatory, and antibacterial properties. Ethnomedicinal review revealed that this *Calliandra haematocephala* used for China, Jinjiang, Yunnan people leaf, flower and bark used by tranquilizing effect. A study was undertaken to investigate Antiepileptic and *In vitro* Antioxidant activities of *Calliandra haematocephala.*

Materials and methods: Leaves were collected from the Alagarkoil foot Hills, Madurai district, Tamil Nadu in the month of March 2025.It was authenticated by Dr. Stephen, Professor of Botany, The American College, Madurai-625002. Collected leaves were washed with water, shade dried powdered and aqueous extract were prepared. The extract was concentrated and stored in airtight container for further use.

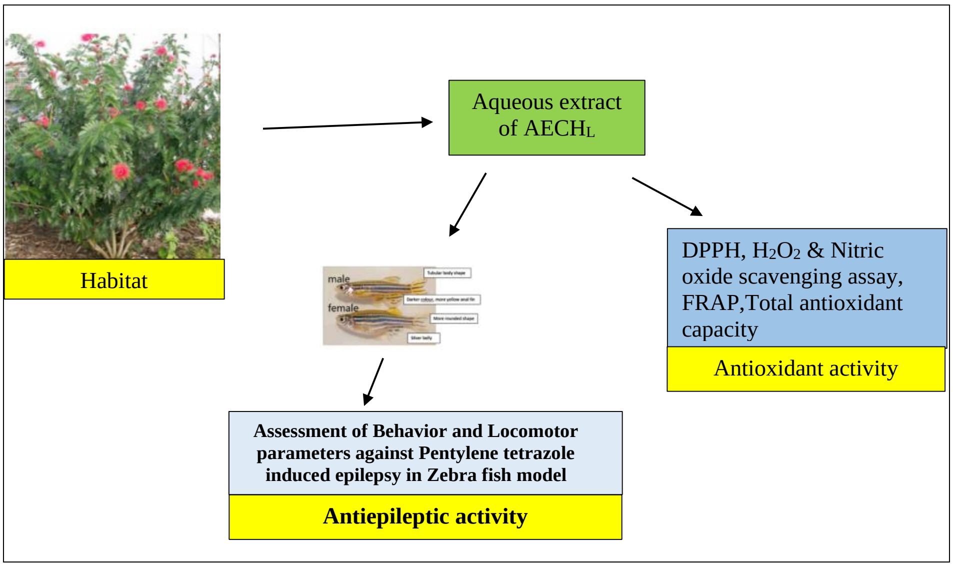
These compounds are believed to contribute to its therapeutic potential. In the present study, fresh leaves of *Calliandra haematocephala* were collected, cleaned and crushed to juice using blender, the resulting juice was used for further analysis.

Results and discussion: The present study was evaluated for the pharmacological studies of Antioxidant and Anti epileptic activities of *Calliandra haematocephala* leaves using Zebrafish Model in comparision with standard for two activities.

Keywords: *Calliandra haematocephala*, Fabaceae, Evaluation of anti-epileptic activity and *Invitro* Anti-oxidant Assay

Introduction

Graphical abstract



Calliandra haematocephala, belongs to Fabaceae, is an ornamental shrub commonly known as Red Powder Puff plant. It is native to Bolivia, but widely cultivated in India [1]. In Southern India the plant traditionally used to treat pain, inflammatory conditions, and neurological disorders [2], In Tamil Nadu (Kani, Paliyar) [3] leaf and flower preparations for used to treat skin infections, wounds, fever and inflammatory disorders. In some parts of northeastern

India (Irula, Kurumba) indigenous users rely on aqueous leaf extracts for gastrointestinal issues and as a general tonic [4]. In Central and South America, Nigeria, Colombia people used leaf decoction to treat diabetics and Yoruba, Nupe people used to treat malaria [5, 6]. In south western Nigeria Tude people used antioxidant and blood purifier [7]. In China, Jinjiang, Yunnan people leaf, flower and bark used by tranquilizing effect [8]. Phytochemical survey revealed the presence of flavanoids, terpenoids, saponins, phenolic compounds, glycosides and alkaloids [9]. It exhibited pharmacological activities such as, antimicrobial [10], antioxidant and anti-inflammatory [11], antidiabetic [12], hepatoprotective [13]. The objective of the study is to evaluate the pharmacognostical, physiochemical parameters, qualitative and quantitative estimation of *Calliandra haematocephala* leaves. An investigation was studied to identify the Antiepileptic activity of *Calliandra haematocephala* using Zebra fish model.

Materials and methods

Preparation of aqueous extract of *Calliandra haematocephala* (AECH$_L$)

The fresh, matured leaves of *Callindra haemtocephala* were collected, carefully washed with water, and shade-dried. The dried leaves were then ground into a fine powder. Approximately 20 g of the powdered material was boiled with 200 ml of water for 2 hours. The extract was subsequently filtered and concentrated to dryness. Separate batches were prepared as required for the study.

Preparation of fresh juice of *Calliandra haematocephala* leaves (*C. haematocephalafrj*)

Fresh leaves of 20g were weighed, washed with water and 20ml of water is added to make a 20 ml of *Calliandra haematocephala* fresh juice.

Evaluation of Antiepieptic activities of *Calliandra haematocephala* Leaves

Experimental Animal

Adult zebrafish (*Danio rerio*) of both sexes, measuring approximately 3–4 cm in length and weighing 0.5–1.0 g, were obtained from a certified aquaculture supplier. The fish were housed in 10-liter glass aquaria with continuous aeration and maintained at a temperature of 27 ± 1°C under a controlled 14-hour light and 10-hour dark photoperiod. They were fed a commercial zebrafish diet twice daily. Before experimentation, the fish were acclimatized to laboratory conditions for seven days to minimize environmental stress and to ensure physiological stabilization (Lawrence, 2007) [14].

1. Acute toxicity study

Procedure

In the acute toxicity study, adult zebrafish were exposed to the aqueous extract of *Calliandra haematocephala* at concentrations of 50 mg/L and 100 mg/L for 24 hours in accordance with OECD (2013) guidelines [15] Each group consisted of 10 males and 10 females (total 20 fish per group), and a separate control group was maintained without treatment. After 24 hours of observation, no mortality was recorded in either the control group or the treated groups. All fish appeared normal, with no alterations in swimming behavior, loss of equilibrium, or respiratory distress. No external lesions or abnormal mucus secretion were observed. Since no mortality occurred even at the highest concentration tested (100 mg/L), the median lethal concentration (LC_{50}) could not be determined, and the 24-hour LC_{50} value was therefore considered to be greater than 100 mg/L under the present test conditions. The results were obtained in Fig.1 and Table.1.

Sex determination in zebrafish (Danio rerio). Image of male and female zebrafish and characteristics used to determine sex. Males have a longer and thinner tubular body shape and are darker in colour with a more yellow anal fin. Females have a rounder body shape and a silver belly.

Experimental design

The zebrafish were randomly divided into fourteen groups, each consisting of five animals (n = 5). The dose range was selected based on preliminary dose-finding and pilot studies as well as previously reported literature on zebrafish models of epilepsy (Stewart *et al*., 2014) [16].

Fig 1: Acute toxicity study of Zebra fish

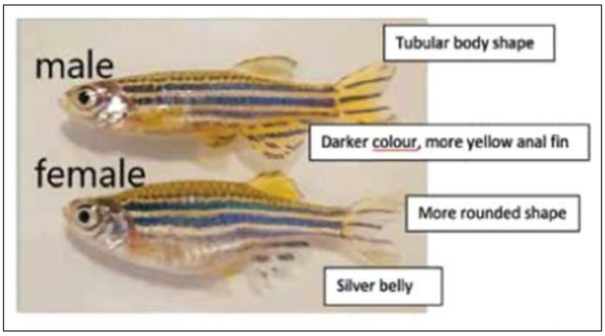


Fig 2: Male and Female Zebra fish

Fig 3: Inducing group of Zebra fish

Fig 2: After treatment of male and female Zebra fish

Drugs used
Inducing agent: Pentylenetetrazole (PTZ),
Standard drug: Phenobarbitone-4mg/ml

Test sample
Aqueous extract of *Calliandra haematocephala* leaves ($AECH_L$)

Induction of Seizures
Seizures were chemically induced using pentylenetetrazole (PTZ), a GABA-A receptor antagonist commonly used to model epileptic seizures in zebrafish.
A 15 mM solution of PTZ was freshly prepared in aquarium water and used as an immersion medium.
After treatment with the test extract or standard drug for 30 minutes, zebrafish were transferred to the pentylenetetrazole -containing tank for 20 minutes to induce seizures. This method has been widely validated for simulating epileptic phenotypes in zebrafish and allows for non-invasive, reproducible seizure induction (Baraban *et al*., 2005) [17].

Treatment Protocol Using Bath Immersion
The test groups were treated using the bath immersion method, a standard route of drug administration in aquatic models. Zebrafish were immersed individually in tanks containing the respective concentrations of plant extract or phenobarbitone for 30 minutes. This was followed by immediate transfer to the pentylenetetrazole immersion tank. Control animals were immersed in the vehicle solution only. The bath immersion approach ensures systemic absorption of the compound via gills and skin, providing effective systemic exposure without invasive procedures (Afrikanova *et al*., 2013) [18]. The results were obtained in Table 2 & Fig.4,5,6.

Evaluation of Parameters
Behavioral Seizure Assessment
Post pentylenetetrazole exposure, each fish was individually observed for 20 minutes to assess seizure-like behaviour. Seizure activity was scored using a previously validated behavioural scoring scale

- 0 – no response
- 1 – short swim burst
- 2 – rapid swim with whirlpool behaviour
- 3 – clonic seizure with abnormal posture
- 4 – tonic seizure with loss of posture.

Additionally, the onset latency (time from pentylenetetrazol e exposure to the first seizure), seizure duration, and frequency were recorded manually by a trained observer in a blinded manner. This behavioral scoring system has been widely employed to quantify seizure severity in zebrafish models (Kalueff *et al*., 2014) [19].

Procedure
Adult zebrafish (*Danio rerio*), comprising equal numbers of males and females, were acclimatized in laboratory conditions for seven days prior to the experiment. Healthy and active fish of uniform size and weight were selected and maintained in aerated aquaria with controlled water quality parameters (temperature 26 ± 2 °C, pH 6.8–7.5, dissolved oxygen > 5 mg/L, photo period 14 h light/10 h dark). Fish

were not fed 24 hours before experimentation to avoid interference with drug absorption and metabolism.

The antiepileptic activity of the aqueous extract of *Calliandra haematocephala* leaves was evaluated using phenobarbitone sodium 0.04µg/ml (PBN) as the standard reference drug.

The test was performed according to OECD (2013) guidelines with minor modifications for zebra fish. Groups of fish (10 males and 10 females per group) were exposed to different concentrations of the aqueous extract (50 mg/L to 1.5 g/L), while one group received PBN (0.04µg/L) as standard and another group served as control.

Table 1: Anti-epileptic activity of Zebra fish experimental design

Group	Treatment	Dose	Route of Administration	Number of Animals (n)	
				Male	Female
Group I	Pentylene tetrazole (PTZ)	15mM	Immersion	5	5
Group II	Standard (Phenobarbitone) + 15 mM PTZ	0.04µg/L	Immersion	5	5
Group III	$AECH_L$ + 15 mM PTZ	50mg/L	Immersion	5	5
Group IV	$AECH_L$+ 15 mM PTZ	100mg/L	Immersion	5	5
Group V	$AECH_L$ + 15 mM PTZ	200mg/L	Immersion	5	5
Group VI	$AECH_L$+ 15 mM PTZ	400mg/L	Immersion	5	5
Group VII	$AECH_L$+ 15 mM PTZ	500mg/L	Immersion	5	5
Group VIII	$AECH_L$+ 15mM PTZ	600mg/l	Immersion	5	5
Group IX	$AECH_L$+ 15 mM PTZ	700mg/L	Immersion	5	5
Group X	$AECH_L$+ 15 mM PTZ	800mg/L	Immersion	5	5
Group XI	$AECH_L$+ 15 mM PTZ	900mg/L	Immersion	5	5
Group XII	$AECH_L$+ 15 mM PTZ	1g/L	Immersion	5	5
Group XIII	$AECH_L$ + 15 mM PTZ	1.25g/L	Immersion	5	5
Group XIV	$AECH_L$ + 15 mM PTZ	1.5g/L	Immersion	5	5

Each group of fish was transferred into glass aquarium containing the prepared test solution. After exposure, the onset of loss of equilibrium and immobility was monitored, and the duration of sedation was recorded. Fish were then placed in fresh water to recover, and the recovery time (defined as the interval between transfer to fresh water and regaining of normal swimming behaviour with equilibrium) was noted separately for males and females. Observations were made for all concentrations up to 1.5 g/L.

The behavioral parameters were expressed as recovery time (in minutes) for each group. A comparison was made between the PBN standard group and the plant extract groups to assess the concentration-dependent effect of the extract on zebra fish behaviour.

Locomotor data

Adult zebra fish (Danio rerio), aged 3–6 months, of both sexes (five males and five females in each group, n = 10) were procured and acclimatized in laboratory aquaria under standard husbandry conditions (26–28 °C, pH 7.0 ± 0.2, conductivity 400–450 µS, with a 14:10 h light–dark cycle). Fish were fed twice daily with commercial feed, and water was renewed on alternate days. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), following CPCSEA guidelines. Ethical committee clearance certifies that the project proposal no:11/2025 for 50 numbers of Zebra fish.

Experimental Procedure

Each fish was individually transferred to a transparent experimental tank (37x19cm), filled with 3–4 cm depth system water) and allowed to acclimate for 5 min. Baseline locomotor activity was recorded for 1 minute using an overhead digital video were analyzed using ToxTrac Software. Fish in the treatment groups were pre-exposed by immersion in the assigned concentration of the aqueous extract for 30 min. Following pretreatment, the fish were transferred to the test tank containing 15 mM PTZ solution, and locomotor activity was recorded for 1 minute. Parameters measured included Average velocity (cm/sec), Average total distance moved (cm). Data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test was applied using Graph pad prism.

***In vitro* Antioxidant Assay**

Determination of DPPH free radical scavenging assay

The effect of *C. haematocephalafrj* on DPPH radical was estimated using the method of LiyanaPathiranan *et al.* (2005) [20]. About 0.1 ml of DPPH-methanol solution (0.135 mM) was mixed with 1.0 ml of different concentrations of sample. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Vitamin C was used as standard drug. The results are obtained in Tab 5 & Fig 7. The percentage of free radical scavenging was calculated according to the following equation

$$\%\text{scavenging} = 100 - \frac{(\text{Abs sample} - \text{Abs blank})}{\text{Abs Control}} \times 100.$$

Determination of Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay was determined according to the method of (Rana MG 1996) [21] Various concentrations are prepared. 1ml of *C. haematocephalafrj* were added to 2 ml of hydrogen peroxide solution (10 mM) in phosphate buffer (50 mM, pH 7.4). The extract was replaced by methanol for control. Reaction mixture was incubated at room temperature for 30 min. The unreacted hydrogen peroxide was determined by measuring the absorbance of the reaction mixture at 230 nm with respect to the blank (methanol) using UV/visible spectrophotometer.

The percentage inhibition was calculated according to the following equation. The results are obtained in Tab 6 & Fig 8.

$$\text{Percentage inhibition (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

IC_{50} values were estimated graphically using a non- linear regression algorithm

Determination of nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and its scavenging effect was determined as per Green *et al*., 1982; Morocci *et al*., 1994 [22] Different concentration of *C. haematocephalafrj* in phosphate buffer was incubated with sodium nitroprusside for 5 hours at 25°C. Control experiments were performed with equal amount of buffer instead of solution. After 5 hours of incubation, 0.5ml of supernatant liquid was removed and 0.5ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization with sulphanilamide and its subsequent coupling was read at 546nm. Vitamin C was used as standard and the nitric oxide scavenging was expressed in terms of ascorbic acid equivalents [23] and its results are presented in Tab 7 & Fig 9

Determination of ferric reducing antioxidant power (FRAP) activity

Ferric reducing antioxidant power activity was determined according to the method of Maruthamuthu Vijayalakshmi (2016) [24] Different concentrations of *C. haematocephalafrj* and its various fractions was added to 2.5ml of 0.2M sodium phosphate buffer (PH 6.6) and 2.5ml of 1% potassium ferricyanide solution. The reaction mixture was vortexed well and then incubated at 50°C for 20min using vortex shaker. At the end of the incubation, 2.5ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000rpm for 10 min. The supernatant (2.5ml) was mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. The colored solution was read a700nm against the blank with reference to standard using UV-Spectrophotometer. Vitamin C was used as standard. The results are presented in Tab 8 & Fig 10

Determination of total antioxidant capacity content

Total antioxidant capacity was determined according to the method of Prieto P (1999) [25]. *C.haematocephala*frj in different concentrations were prepared individually containing 3ml of distilled water and 1ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695nm. Mean values from three independent samples were calculated for each *C. haematocephalafrj*. Vitamin C was used as standard. The results were displayed in Tab 9&Fig 11

Results and Discussion

Behavior Assessment

These findings demonstrate that the aqueous extract exerts a concentration-dependent effect on behavioral recovery, with a significant reduction in recovery time observed at concentrations above 400 mg/L. The results suggest possible central nervous system (CNS) activity of the extract, which may be attributed to its phytoconstituents such as flavonoids, Pyrolidine alkaloids, saponins. Flavonoids in particular are known to interact with the GABAergic system and modulate sedative and anticonvulsant activity. Flavonoids in particular are known to interact with the GABAergic system and modulate sedative and anticonvulsant activity, Pyrolidine alkaloids of Succinamide acts as, a Neuroprotective potential while antioxidant compounds may enhance neuronal stability and recovery. Interestingly, female zebrafish generally recovered faster than males at higher concentrations, suggesting potential sex-related differences in metabolism or sensitivity to the bioactive compounds.

When compared to phenobarbitone(0.04µg/ml), which produced stable recovery times, the extract at higher concentrations demonstrated enhanced recovery, implying either potentiation of neuronal excitability or an independent mechanism distinct from phenobarbitone's sedative action.

Locomotor activity

The present study demonstrated that the aqueous leaf extract of *Calliandra haematocephala* exerted significant protective effects against PTZ-induced locomotor hyperactivity in zebrafish. PTZ, a GABA-A receptor antagonist, is well known to induce seizure-like phenotypes in zebrafish, manifested as increased velocity and total distance moved (Baraban *et al*., 2005). In the current assay, PTZ produced a robust increase in locomotor activity, consistent with seizure induction.

Pretreatment with the plant extract attenuated the PTZ-induced hyperactivity in a dose-dependent manner. At higher concentrations ($\geq$ 900 mg/L), locomotor activity was significantly reduced, approaching baseline control values. This indicates that the extract may possess anticonvulsant activity by counteracting PTZ-induced excitability.

The protective effect could be attributed to the phytoconstituents of *Calliandra haematocephala*, including flavonoids, saponins, and alkaloids, which have been reported to modulate the GABAergic system and exhibit antioxidant properties (Joseph and Mathew, 2021; Kumar *et al*., 2019). Antioxidant mechanisms are particularly relevant, as oxidative stress is implicated in seizure pathophysiology.

At lower concentrations (50–200 mg/L), the extract failed to significantly suppress PTZ-induced hyperlocomotion, suggesting that a threshold concentration is required to elicit anticonvulsant effects. This dose-dependent trend highlights the pharmacological potential of the plant extract and supports its traditional ethnomedicinal use in neurological disorders.

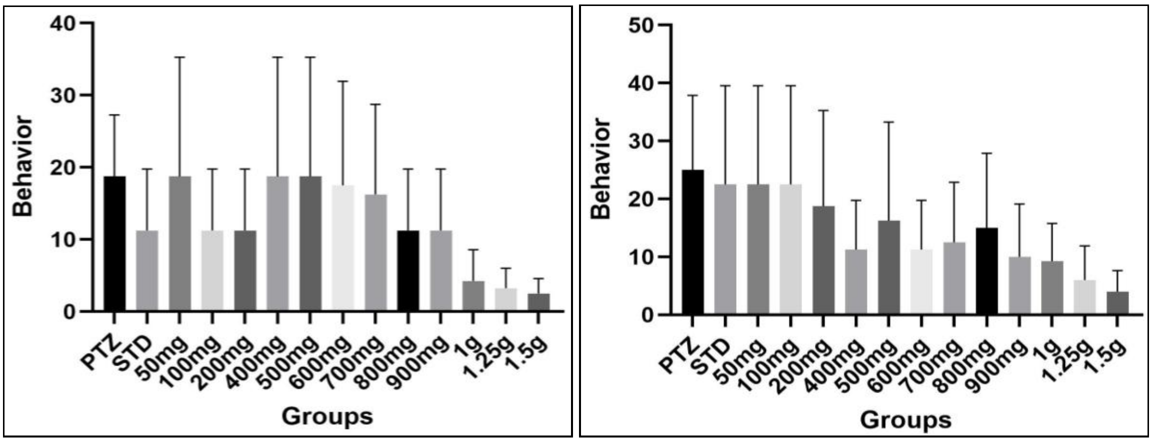
Table 2: Assessment of Behavior and Locomotor parameters against Pentylene tetrazole induced epilepsy in Zebra fish model

S.No.	Group	Behavior		Velocity (cm/sec)		Total distance moved (cm/sec)	
		Male	Female	Male	Female	Male	Female
1	PTZ	-	-	0.92±0.032	0.88±0.0418	55.2±1.972	53±2.5111
2	PBN	30±4.41	20±8.81	0.72±0.0311*	0.71±0.0233	43.2±2.391**	42.4±1.390
3	50mg/L	45±4.41	45±8.81	0.81±0.081	0.81±0.0806	54±2.415	60±3.726
4	100mg/L	45±4.41	45±8.81	0.81±0.081	0.81±0.0806	55.4±3.844	58±2.0206
5	200mg/L	40±6.00	40±4.40	0.8±0.062	0.83±0.0878	48±3.726	50±5.270
6	400mg/L	30±4.40	30±4.40	0.83±0.0121	0.82±0.0254	49.8±0.726	48.8±1.527
7	500mg/L	30±7.21	25±6.23	0.74±0.052	0.78±0.0499	47.4±3.162	46.6±2.995
8	600mg/L	25±4.41	20±6	0.85±0.0549	0.77±0.0447	51±3.543	46.4±2.688
9	700mg/L	25±6	30±4.41	0.85±0.371	0.74±0.0253	50.8±2.229	44.6±1.518
10	800mg/L	30±5.77	20±4.40	0.83±0.0227	0.75±0.0396	49.6±1.364	45.2±2.380
11	900mg/L	20±4.40	20±4.40	0.74±0.0394	0.7±0.0240*	44.6±2.374*	42±1.433*
12	1g/L	15±3.71	10±1.45	0.69±0.400**	0.67±0.0121**	41.8±2.403**	40±0.726**
13	1.25g/L	12±3.05	6±1.45	0.63±0.0346***	0.66±0.0198**	37.8±2.081****	39.6±1.190**
14	1.5g/L	8±1.76	5±0.88	0.61±0.044****	0.64±0.0371**	36.6±2.645****	38.4±2.229***

In the PTZ-induced zebrafish epilepsy model, the aqueous leaf extract of *Calliandra haematocephala* exhibited a clear dose-dependent anticonvulsant effect. Behavioural scores in both male and female zebrafish showed no significant differences between the disease, treatment, and standard groups ($p > 0.05$), indicating that the extract did not alter behavioural seizure scoring under these conditions.

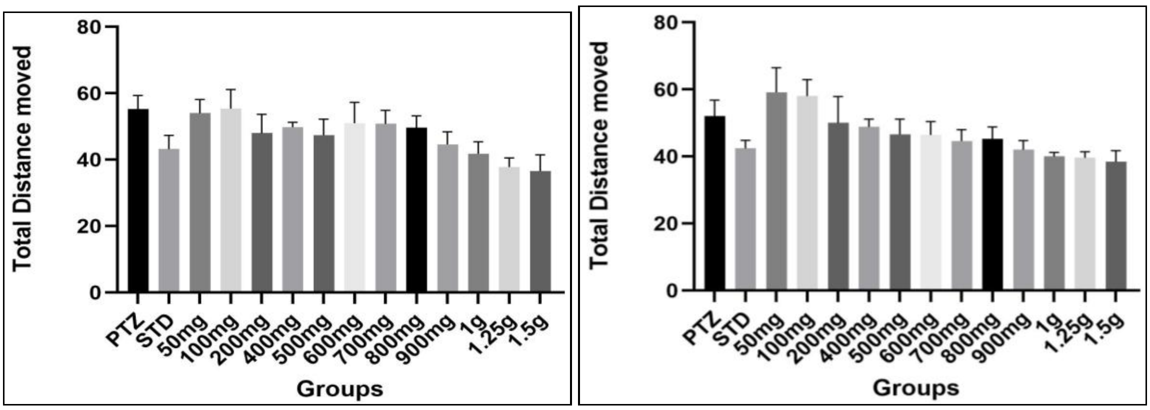
In contrast, locomotor parameters demonstrated marked improvements. In male zebrafish, velocity was significantly reduced at 1 g ($p = 0.0036$**), 1.25 g ($p = 0.0002$***), and 1.5 g ($p < 0.0001$****), while total distance moved decreased significantly at 900 mg ($p = 0.0329$*), 1 g ($p = 0.0011$**), 1.25 g ($p < 0.0001$****), and 1.5 g ($p < 0.0001$****). The standard drug also showed significant protection versus disease ($p = 0.0414$* for velocity; $p = 0.0063$** for distance).

In female zebrafish, velocity was significantly reduced at 900 mg ($p = 0.0364$*), 1 g ($p = 0.0078$**), 1.25 g ($p = 0.004$**), and 1.5 g ($p = 0.0012$**), while total distance moved was significantly reduced at 900 mg ($p = 0.0375$*), 1 g ($p = 0.003$**), 1.25 g ($p = 0.0018$**), and 1.5 g ($p = 0.0004$***). Comparisons of the standard drug with lower doses (50 mg, 100 mg) also revealed highly significant differences ($p < 0.0001$****).



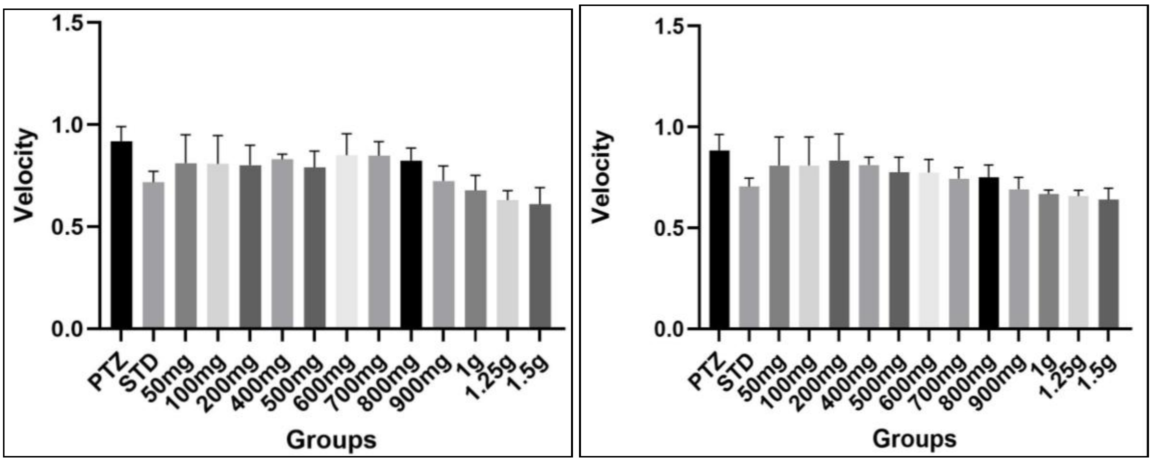
[A] [B]

Fig 4: Behavior assessment of male[A] and female [B] Zebra fish



[C] [D]

Fig 5: Total Distance moved of male [C] and female [D]



[E] [F]

[A] **Fig 6:** Velocity of male [E] and female [F] [A]

In vitro anti-oxidant assay

In vitro anti-oxidant activity of *C. haematocephalafrj* were determined by five methods and the results are depicted below

DPPH free radical scavenging assay

Various concentrations (0 to 10 µg/ml) of *C. haematocephal a*$_{\mathrm{frj}}$ were treated with DPPH and the absorbance was obsereved and displayed in Tab 3&Fig 7

Table 3: Determination of DPPH free radical scavenging assay of *C. haematocephalaafrj*

S.No.	Concentration (µg/ml)	Percentage of inhibition	
		C.haematocephala$_{\mathrm{frj}}$	**Ascorbicacid**
1	0	0	0
2	2	17.57	29.92
3	4	34.14	37
4	6	39.46	43.83
5	8	43.2	50.09
6	10	49.79	55.28
	IC_{50}	9.081	7.846

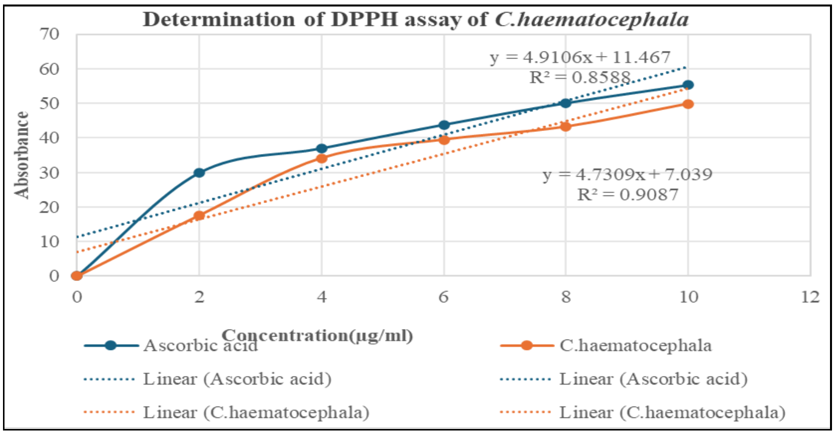


Fig 7: Graphical data of DPPH free radical scavenging assay of *C. haematocephala*$_{\mathrm{frj}}$

The inhibitory concentration IC_{50} value of *C. haemtocephal afrj* was found to be 9.08µg/ml in comparison with ascorbic acid as standard 7.85 µg/ml

Hydrogen peroxide scavenging assay

Various concentrations (0 to10 µg/ml) of *C. haaematocepha la*$_{\mathrm{frj}}$ were treated with hydrogen peroxide and the absorbance was obsereved and displayed in Tab 6 & Fig 8.

Table 4: Determination of Hydrogen peroxide scavenging assay of *C. haematocepalafrj*

S.No.	Concentration (µg/ml)	Percentage of inhibition	
		C.haemtocephla$_{\mathrm{frj}}$	**Ascorbicacid**
1	0	0	0
2	2	22.1	29.93
3	4	30.46	55.03
4	6	48.47	62.23
5	8	51.95	72.09
6	10	76.73	95.26
	IC_{50}	6.669	4.721

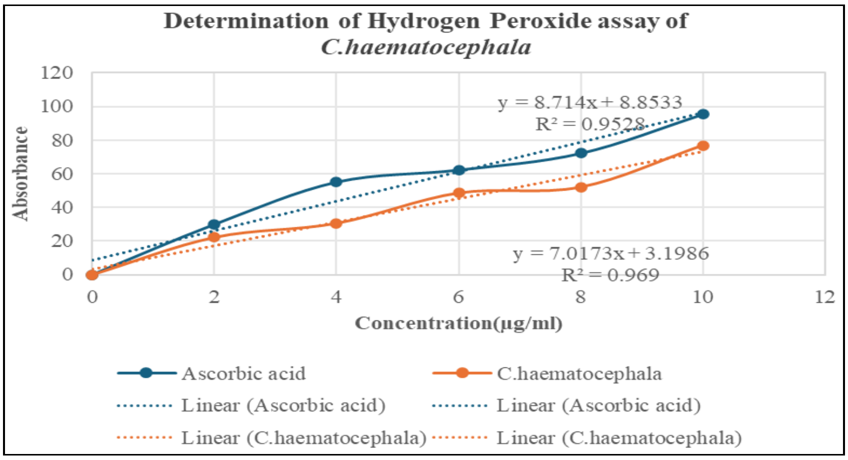


Fig 8: Graphical data of Hydrogen peroxide scavenging assay of *C. haematocephalafrj*

The inhibitory concentration IC_{50} value of *C. haematocephala*$_{frj}$ was found to be 6.67µg/ml in comparison with ascorbic acid as standard 4.72µg/ml

Nitric oxide scavenging assay

Various concentrations (0 to 25 µg/ml) of *C. haematocephala*$_{frj}$ were treated with griess reagent and the absorbance was obsereved and displayed in Tab 5 & Fig 9.

Table 5: Determination of Nitric oxide scavenging assay of *C. haematocephalafrj*

S.No.	Concentration (µg/ml)	Percentage of inhibition	
		C.haematocephala$_{frj}$	**Ascorbicacid**
1	0	0	0
2	5	18.67	27.25
3	10	27.28	32.03
4	15	32.07	38.4
5	20	32.64	44.24
6	25	33.24	50.08
	IC_{50}	13.55	9.09

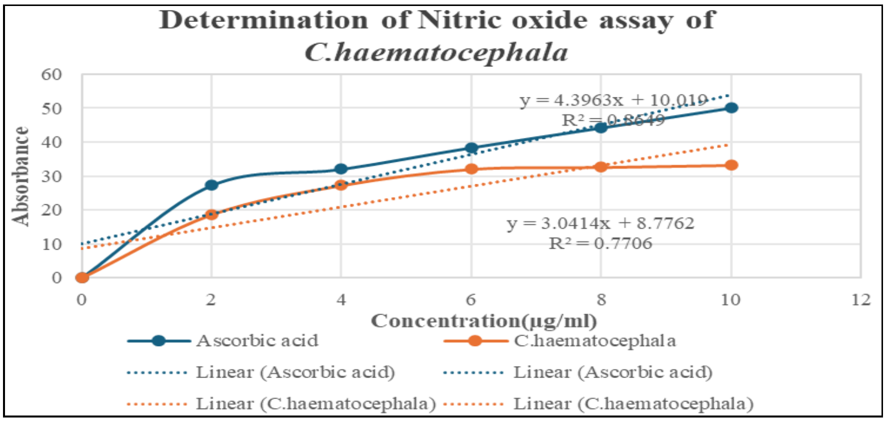


Fig 9: Graphical data of Nitric oxide scavenging assay of *C. haematocephalafrj*

The inhibitory concentration IC_{50} value of *C. haematocephalafrj* was found to be 13.55µg/ml in comparison with ascorbic acid as standard 9.09µg/ml

Ferric reducing anti-oxidant power (FRAP) activity

Various concentrations (0 to10 µg/ml) of *C. haematocephal afrj* were treated with potassium ferricyanide and the absorbance was obsereved and displayed in Tab 6 & Fig 10.

Table 6: Determination of ferric reducing anti-oxidant power (FRAP) activity of *C. haematocephalaafrj*

S.No.	Concentration (µg/ml)	Percentage of inhibition	
		C.haematocephala$_{frj}$	**Ascorbicacid**
1	0	0	0
2	2	19.56	31.1
3	4	22.5	37.29
4	6	25.49	43.32
5	8	28.04	47.23
6	10	31.43	53.42
	IC_{50}	15.87	8.18

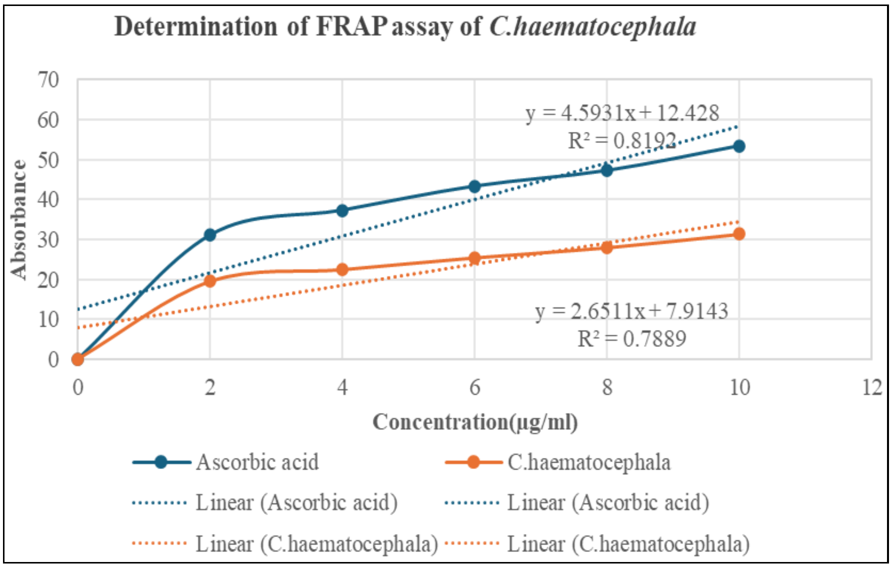


Fig 10: Graphical data of ferric reducing antioxidant power (FRAP) activity of *C. haematocephalafrj*

The inhibitory concentration IC_{50} value of *C. haematocephalafrj* was found to be 15.87 µg/ml in comparison with ascorbic acid as standard 8.18 µg/ml

Total antioxidant capacity

Various concentrations (0 to10 µg/ml) of *C. haematocephalaafrj* were treated with molybdate reagent and the absorbance was obsereved and displayed in Tab7 & Fig 11.

Table 7: Determination of total anti-oxidant capacity of *C. haematocephalafrj*

S.No.	Concentration (µg/ml)	Percentage of inhibition	
		C.haemtocephala **frj**	**Ascorbicacid**
1	0	0	0
2	2	24.57	28.23
3	4	28.54	36.47
4	6	34.75	53.23
5	8	38.36	73.67
6	10	42.12	91.17
	IC_{50}	10.94	5.33

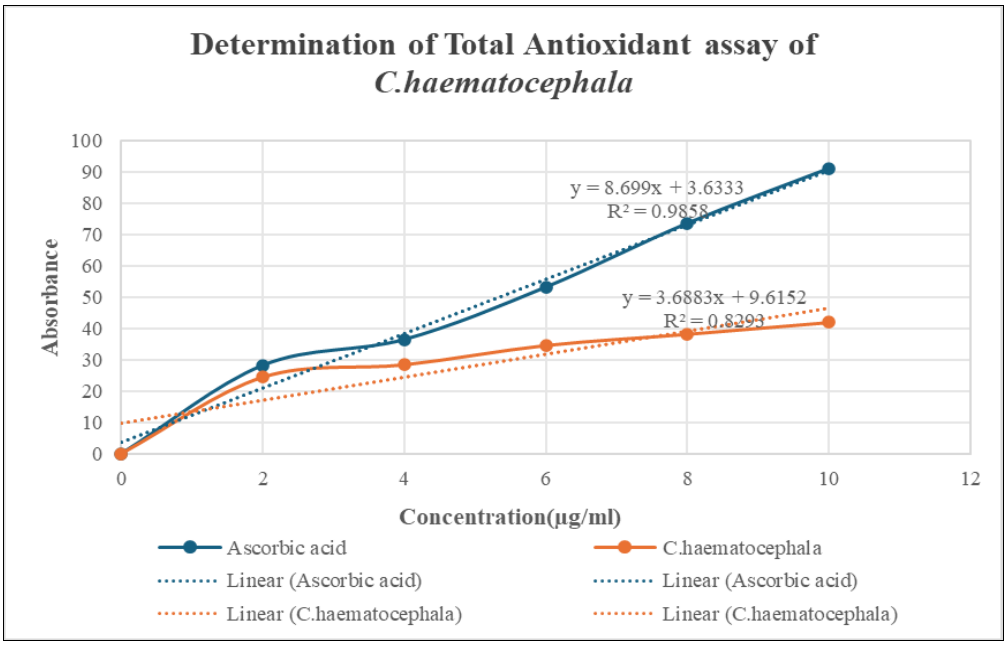


Fig 11: Graphical data of total antioxidant capacity of *C. haematocephalafrj*

The inhibitory concentration IC_{50} value of *C. haematocephalaafrj* was found to be 10.94 µg/ml in comparison with ascorbic acid as standard 5.33 µg/ml

Conclusion
The present investigation demonstrates that the leaf extract of *Calliandra haematocephala* possesses significant antiepileptic activity in zebra fish model. These results provide preliminary evidence supporting the ethnopharmacological claims of *Calliandra haematocephala* in neurological disorders, particularly in relation to its antiepileptic and neuroprotective potential. However, further studies involving biochemical assays, receptor binding, and histopathological analysis are necessary to establish the precise mechanism of action and confirm the safety and efficacy of the extract.

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