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PHYTOCHEMICAL INVESTIGATION AND ANTIEPELEPTIC ACTIVITY OF *ERYTHERINA SUBEROSA*

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Abstract:
This study presents the phytochemical investigation of leaf extract of the plant Erythrina suberosa and antiepileptic activity by using PTZ (pentylene tetrazole) model. Almost 50 million people around the world are suffering from chronic epileptic disorder with or without appearance of convulsions on the physique. Severe side effects results upon long term uses of synthetic antiepileptic drugs and addiction obligations as well. Thus, the researchers around the globe are searching for different natural resources. Erythrina suberosa is a traditional medicinal plant used to treat seizures. Phytochemical investigation by GCMS method showed the presence of alkaloids, triterpenoids, flavonoids, sterols, proteins and phenols. The present studies reveals the anti convulsant activity of methanolic leaf extract of Erythrina suberosa by using PTZ induced convulsions model in mice successively. However, the anticonvulsant activity of this specific specie of plant has not been studied in depth. In Pentylene tetrazole (PTZ) model test, parameters like onset of tonic convulsions, myoclonic convulsions and duration of tonic-clonic convulsions were observed in the different test groups. In conclusion, we presented that the methanolic extract of Erythrina suberosa has anticonvulsant effect in the model, suggesting their possible anticonvulsant action in the central nervous system gave significant protection (P<0.001) against PTZ induce convulsion.

Keywords: *Erythrina suberosa, antiepileptic, phytochemical, PTZ induce convulsions, methanolic leaf extract*

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INTRODUCTION:

Erythrina suberosa Roxb is a well-known medicinal plant commonly known as Gul-e-nishtar in urdu and coral tree in English. It is an ornamental tall tree that belongs to family fabiaceae, usually grown on plains and hilly areas of Pakistan, India, Nepal, and Barma and Vietnam (1). It is medium to large sized deciduous tree having prickles on the branches and grown as an ornamental due to presence of ribbon like beautiful red flowers in bunches. The leaflets are heart like and broad about 7 to 20 cm in size. The bright red flowers are auxiliary and terminal on the branches. The calyx is campanulate to become 2-labiate. The upper stamens are free from the lower and the pods are 12.5n15 cm long, terateand tapering at the trimmings. The ripe seeds are black in color (2). The genus *Erythrina* included about 110 species in the tropics, which having different phytochemical compounds like alkaloids, flavonoids, terpenes and lactins. Being the part of traditional folk medicine, different species of *Erythrina* have been used in cure of neurological disorders like anxiety, hypnotic, analgesic and hypotensive and anti convalescent. Different parts of *E. suberosa* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antibacterial. In the some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds (3). Stem bark has hypoglycemic, cytotoxic and antispasmodic properties. Seed oil and leaves of *Erythrina suberosa* has antifungal and antibacterial properties (4). The Coral plant "*Erythrina suberosa"* is highly potential medicinal plant of multiple uses commonly found all over the world (5). However, anti-epileptic study is still not discussed of this specific species of *Erythrina* so the objective of the present study was to investigate anti convulsant activity of methanolic leaf extract of *Erythrina suberosa* against the seizures induce by PTZ (phenthylene tetrazole*).*

MATERIAL AND METHOD:

Plant material and extraction

The leaves of *Erythrina suberosa* are collected in April 2018 from the near campus of Riphah International University, Islamabad where this plant is cultivated as an ornamental plant.

The shed dried healthy leaves will be pulverized separately using mechanical grinder followed by sieving of 40µm mesh size for further study. The leaves were shade dried until water evaporated completely and then grinded. The grinded powder (1 kg) was extracted for 10 days with 70% methanol with mixing at regular interval. The macerated plant material was filtered. The filtrate was concentrated on a rotary evaporator to obtain the thick semi-solid paste under reduced pressure i.e. *Erythrina suberosa* methanolic leaf extract *(E.sub.),*yielding 1.42 % w/w.

Chemicals

Distilled water, normal saline, pentylenetetrazole (PTZ), methanol, diazepam.

Animals

Swiss albino mice (25-35g) of either twelve will be obtained from the animal house of Department of Pharmacology, Riphah Institute of Pharmaceutical Sciences, Islamabad. The animals will be kept under standard environmental conditions (25±2°C) in 12 hrs light and dark cycle each. Water and food will be made available at libitum.

Phytochemical investigation

Phytochemical investigation of active fraction will be carried out by using gas chromatography-mass spectroscopy (GC-MS).

Antiepileptic activity

Male swiss albino mice were divided into 5 groups (four animals in each group) and injected intra-parental with normal saline (10ml/kg), test compound which is *E.suberosa* (600mg/kg) and diazepam (1mg/kg). After 30 minutes, an intra-parental dose of PTZ (90mg/kg) was given to all animals and observed for onset time of myoclonic jerks, tonic-clonic seizures and the duration of tonic-clonic seizures for 30 minutes via digital video camera. Drugs that delayed onset of these seizures and/or shortened duration of tonic-clonic seizures considered to exhibit anticonvulsant effect (6). The animals were observed for mortality (1 % mortality = number of mice dead after convulsion/total number of mice used × 100).

Acute toxicity test

Mice in two groups (each having four mice) were administered with *E.suberosa* high doses of 800mg and900mg/kg via i.p then kept under observation for 24 hours and observed for toxicity symptoms or death.

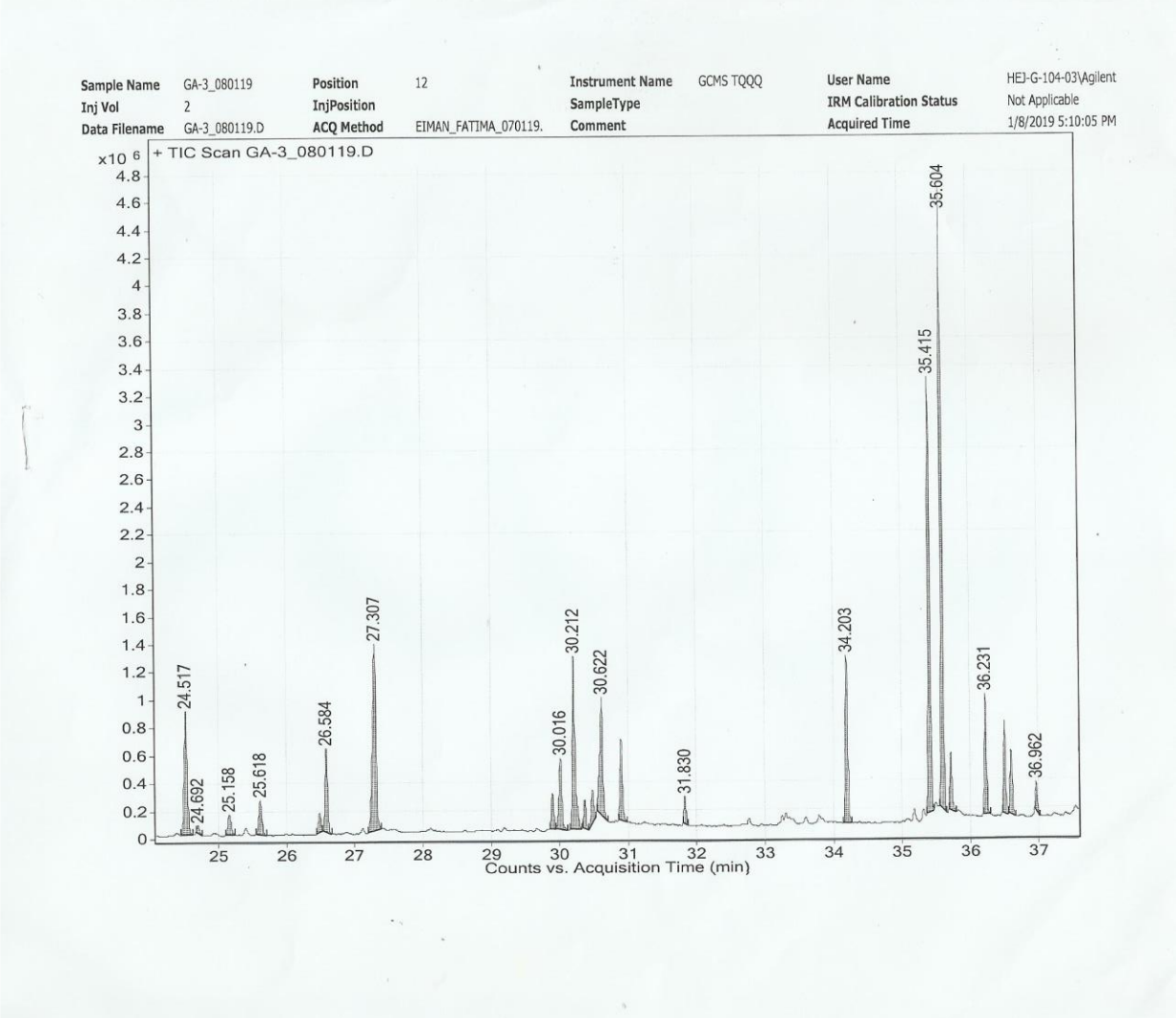
Statistical analysis

Data expressed are mean ± standard error of mean (SEM, n = number of experiments).The 1-statistical parameter applied is one-way analysis of variance (ANOVA) with Tukey post-hoc test. P <0.05 was noted as significantly different. The bar-graphs were analyzed using Graph Pad program (GraphPAD, San Diego, CA, USA).

RESULTS:

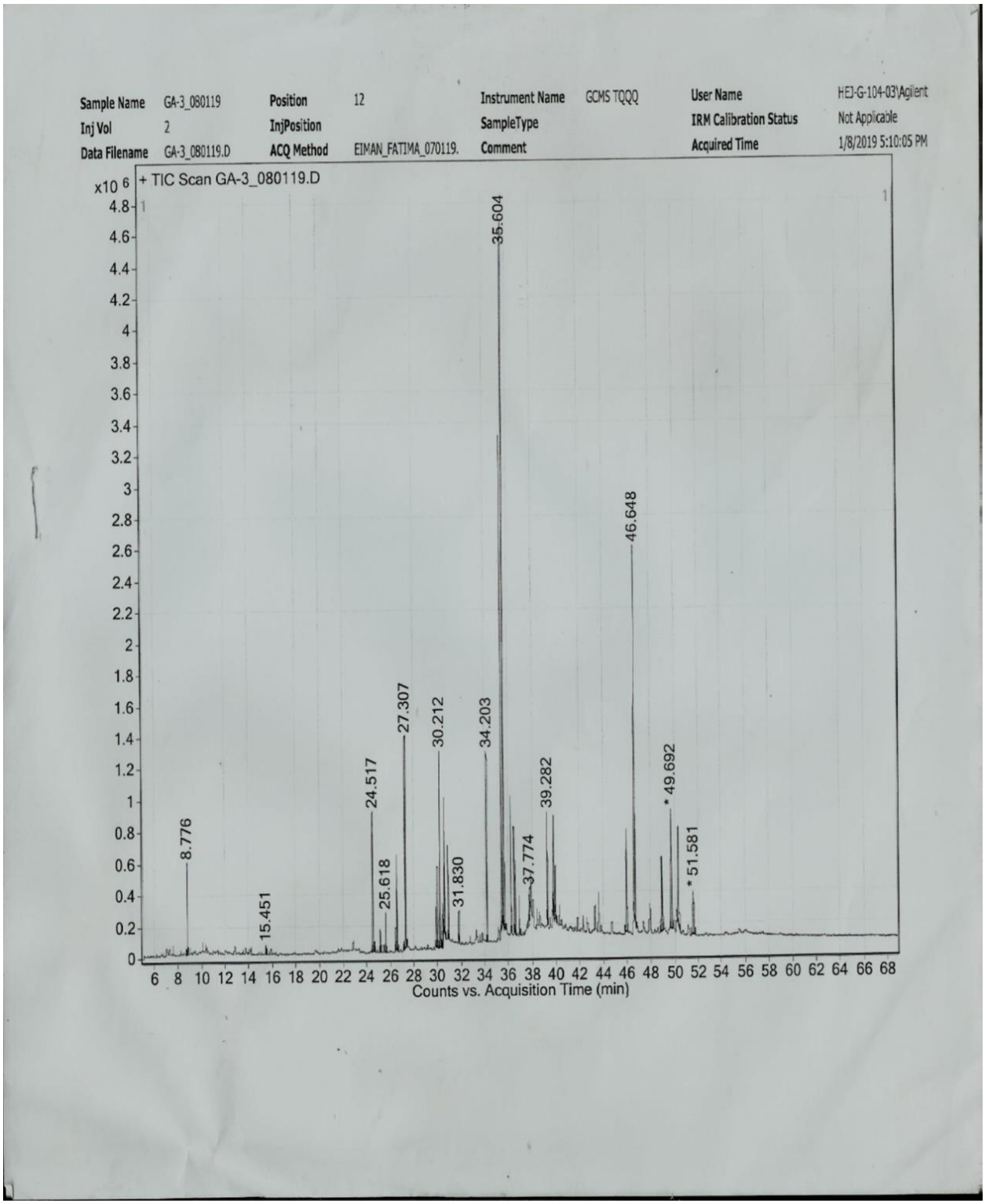
Phytochemical analysis

E.suberosa was tested positive for the presence of tannins, triterpenoids, protein and amino acids, lactins and carbohydrates. Methanolic leaves extract analysis by GCMS



alkaloids, flavonoids, saponins, steroids, esters,

results 36 different peaks which contains 1-Butanol; 3-methyl-, formate ,2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(R)- ,3,7,11,15-Tetramethyl-2-hexadecan-1-ol. Octadecane, 1-(ethenyloxy)- ,3,7,11,15-Tetramethyl-2-hexadecen-1-ol ,7-Hexadecenoic acid ,methyl ester, (z)-,Hexadecanoic acid, methyl ester, n-Hexadecanoic acid ,9,12-Octadecadienoic acid, methyl ester,(E,E)-,9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-, Phytol, Octadecanoic acid, methyl ester, 9,12-octadecadienoic acid(Z,Z)-,3,4-Dihydroisoquinoline,1-[1-phenethyl]-6,7-dimethoxy-, Morphinan, 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-17 methyl-,(5α,6α)- , Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Behenic alcohol , 4-[3-Hydroxy-4-methoxybenzyl]-7-hydroxy-6-methoxyisoquinoline , 1-Hexadecanol, 2-methyl- , Squalene , 10-Hydroxy-5,7-dimethoxy-2,3-dimethyl-1,4-anthracenedione , 1-Hexadecanol, 2- methyl- , vitamin E, Campesterol , stigmasterol ,9,19-Cycloergost-24(28)-en-3-ol, 4, 14-dimethyl-, acetate, (3β, 4α,5α)- ,Lupeol , Lup-20(29)-en-3-one, 1-Heptatriacotanol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15.451
24.517
25.618
27.307
30.212
31.830
34.203
35.604
37.774
39.282
46.648
* 49.692
* 51.581
Counts vs. Acquisition Time (min)

No	Name of Compound	Molecular formula	Molecular weight	Retention index (iu)
1	1-Butanol,3-methyl-,formate	$C_6H_{12}O_2$	116	818
2	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R) -	$C_{11}H_{16}O_2$	180	1426
3	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	1890
4	7-Hexadecanoic acid, methyl ester,(Z)-	$C_{17}H_{32}O_2$	268	1886
5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1878
6	9,12,15-Octadecatrienoic acid,(Z,Z,Z),	$C_{18}H_{30}O_2$	278	2191
7	9,12-Octadecadienoic acid(Z,Z)-	$C_{18}H_{32}O_2$	280	2183
8	Octadecanoic acid	$C_{18}H_{36}O_2$	284	
9	9,12,15-Octadecatrienoic acid,methyl ester,(Z,Z,Z)-	$C_{19}H_{32}O_2$	292	2101
10	9,12-Octadecadienoic acid,methyl ester,(E,E)-	$C_{19}H_{34}O_2$	294	2093
11	3,4-dihydroisoquinoline, 1[1-phenethyl]-6,7-dimethoxy-	$C_{19}H_{21}NO_2$	295	2363
12	Phytol	$C_{20}H_{40}O$	296	2045
13	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	2077
14	4-[3-Hydroxy-4-methoxybenzyl]-7-	$C_{18}H_{17}NO_4$	311	2831
15	hydroxy-6-methoxyisoquinoline	$C_{18}H_{16}O_5$	312	2820
16	Morphinan, 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-17-methyl-(5α,6α)-	$C_{19}H_{23}NO_3$	313	
17	Behenic alcohol	$C_{22}H_{46}O$	326	2451
18	Hexadecanoic acid,-2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	2498
19	1,2-Benzenedicarboxalic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2704
20	Campesterol	$C_{28}H_{48}O$	400	2632
21	Squalene	$C_{30}H_5O$	410	2914
22	Stigmasterol	$C_{29}H_{48}O$	412	2739
23	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	2831
24	Lupeol	$C_{30}H_{50}O$	426	2848
25	Vitamin E	$C_{29}H_{50}O_2$	430	
26	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl,acetate,(3β,4α,5α)	$C_{32}H_{52}O_2$	468	2900
27	1-Heptatriacotanol	$C_{37}H_{76}O$	536	3942

Effect of *E.sub* on PTZ-induced seizures

Dose-dependent (600mg, 800mg, 900mg/kg) effect produces by *E. suberosa*. It delayed onset time of PTZ(90mg/kg)mediated both myoclonic jerks and tonic-clonic seizures, while decreased time duration of tonic-clonic seizures. In control saline groups, onset time of myoclonic jerks,tonic-clonic seizures and duration of tonic-clonic seizures were 34.12±6.4,43.6 and 45±2.74 sec. respectively. In *E.sub*.(600mg/kg)treated group, average onset times of myoclonic jerks and tonic-clonic seizures increased to 39.635±6.0and52.55±75.1 respectively, while duration time of tonic-clonic seizures reduced to 15.775sec.In diazepam(1mg/kg)treated group, onset times of

myoclonic jerks and tonic-clonic seizures increased to 90.5±2.53 and 130±1.86sec.respectively,while duration time of tonic-clonic seizures reduced to12.5±2.33sec.($p<0.001$ vs saline group). The saline treated group animals showed 100% mortality and immediately died..*E.sub.* at 600mg/kg, reduces PTZ- induced seizures mortality rate to 75%($P<0.05$ vs. saline group) and ($P<0.001$ vs. saline group).Diazepam at test dose of 1.0mg/kg, reduced mortality to 0% ($P<0.001$ vs. saline group) as shown in Table 1.

Table 1: Effect of *E.sub*. Leaf extract and diazepam on pentylenetetrazole (PTZ)-induced seizures mortality in mice.

GROUPS	% MORTALITY
Normal Saline(10mL/kg)+PTZ(90mg/kg)	100.00
E.sub.(600mg/kg)+PTZ(90mg/kg)	25.00
Diazepam(1mg/kg)+PTZ(90mg/kg)	0.00

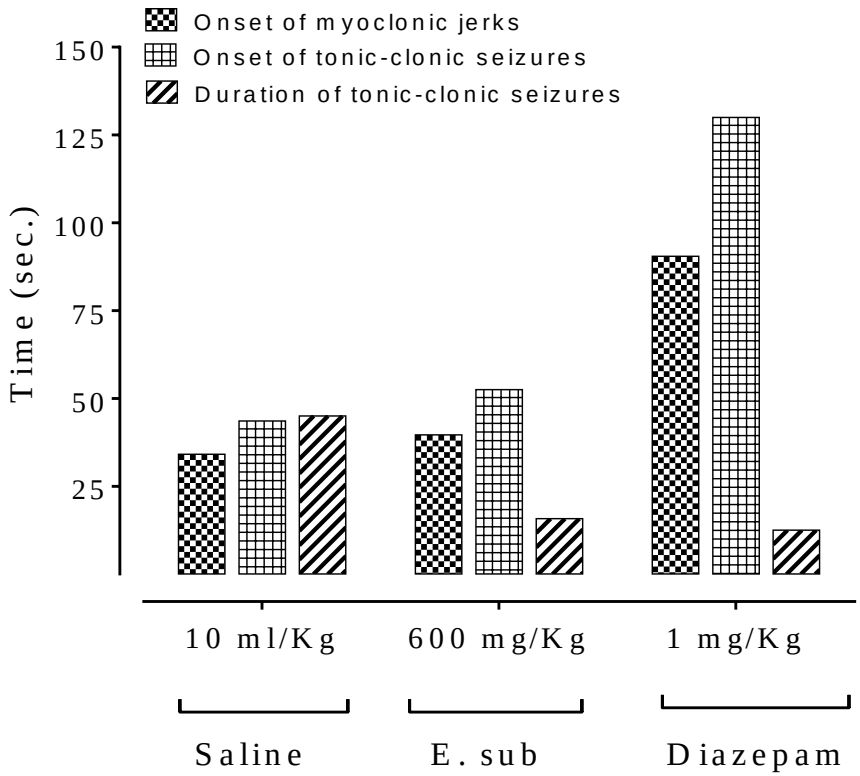
% mortality= (number of mice dead after convulsion/total number of mice used)×100,n=4.*P<0.05, vs.**.P<0.001 Vs. saline group, one-way ANOVA with post-hoc Tukey test.

Table 2: Effect of *Erythrina Suberosa* vs. Normal saline and control drug (diazepam).

GROUP	Onset of myoclonic seizure	Onset of tonic-clonic seizures	Du ration of tonic –clonic seizure	Status of animal alive after 30 minutes.
Normal saline(10ml/kg)	34.12±6.4	43.6±5.34	45±2.74 sec.	No
E.sub.(600mg)	39.635	52.5525	15.8	Yes
Diazepam(1mg/kg)	90.5±2.5	130±1.87	12.5±2.33sec.	Yes

Acute toxicity of *E.suberosa*

The high doses (800mg/kg and 900mg/kg) of *E.suberosa* caused mortality of mice .



DISCUSSION:

This study was conducted to evaluate phytochemical investigation by using GCMS analysis and antiepileptic activity of *Erythrina suberosa* by using PTZ induce seizures model in male swiss albino mice. PTZ-induced seizures is commonly used animal model for screening of anti-seizure drugs((Löscher and Schmidt, 1988) *E.suberosa* caused marked delay in onset time of both myoclonic jerks and tonic-clonic seizures, as well as decreased duration of tonic-clonic seizures and animals mortality demonstrating its anti-epileptic effect.PTZ evokes convulsions via inhibition of GABA (gamma-aminobutyric acid) neurotransmission by interfering with GABA A receptor stimulation((Ramanjaneyulu and Ticku, 1984).

CONCLUSION:

Methanolic leaf extract of *Erythrina suberosa* has strong anti convalescent activity as tested by PTZ induced convulsions model in mice. Natural plant originated drugs are safer and inexpensive as compare to synthetic antiepileptic drugs so, this activity indicate that methanolic leaf extract of plant *Erythrina suberosa* which has mortality rate 25% as compared to standard drug (diazepam) which has mortality rate 0%, but studied plant extract has positive anti epileptic effect and less toxic than synthetic drug diazepam. Phytochemical analysis by GCMS method determines the presence of important classes of phytoconstituents like alkaloids, flavonoids, sterols, triterpenoids, tannins, amino acids and protein. This indicates that the leaves can be useful for preventing different types of diseases because the therapeutic activity of a plant is due to availability of particular compounds which are biologically active. The present research can serve as a useful guide in establishment of quality parameters of formulations of the methanolic leaf extract of *Erythrina suberosa* and can also serve as an important guide for further investigation of phytoconstituents with different pharmacological aspects.

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