

**NEUROLOGICAL AND MOLECULAR INTERACTIONS OF PHYTOCONSTITUENTS
FROM *RICINUS COMMUNIS* ROOT EXTRACT- A PHARMACOLOGICAL AND
COMPUTATIONAL APPROACH**

ABSTRACT:

Most of the epileptic patients need polytherapy of conventional anticonvulsants and still not 100% cured. The major drawback due to these agents is their chronic side effects and drug interactions which restrict its use. On the other hand, nature has provided us plants to be used as natural remedy for diseases with least side effects and insignificant drug interactions, has motivated the researchers towards herbal remedy for anticonvulsant activity. The methanolic root extract of *Ricinus communis* was screened for its Antiepileptic activity in rodent models. Molecular docking studies using Schrodinger software was employed to study interactions with active site using PDB ID: 4MS4 (GABA agonist's), 3WFG (glutamate antagonist), 5HCV (aspartate antagonist), 6J8G (sodium channel antagonist). MERC showed a significant reduction in various phases of convulsions at 30 and 60 minutes and afforded significant protection against MES model. In PTZ, MERC extract treated groups at a dose (200 mg/kg and 400 mg/kg) showed significant antiepileptic activity with percentage Protection of 24 % and 33% respectively against seizures and significantly (* = $p < 0.0001$) increased the latency of the seizures. Molecular docking studies confirmed the GABA agonist's effects, and the results revealed that quercetin, gallic acid, luteolic acid, gentixic acid, catechin, vanillic acid, ricinoilc acid and standard phenytoin, clonazepam has highest Glide scores against all the selected proteins which indicates a stronger receptor -ligand binding affinity. From *in vivo* and molecular docking results it is clear that methanolic root extract of *Ricinus communis* possessed significant antiepileptic activity.

Keywords: *Ricinus communis*, Anti-epileptic, GABA, docking, glide score.

1. INTRODUCTION

Epilepsy is the commonest neuropsychiatric disorder characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbance of consciousness with or without characteristic body moments (convulsions) with sensory or psychiatric phenomena causing significant medical and social mobility. Epilepsy is a chronic neurological disorder that is manifested in the form of recurrent, spontaneous seizure episodes or convulsions, the latter reflecting sudden, stereotyped episodes with accompanying changes in motor activity, sensation, and behavior [1]. The incidence of epilepsy ranges from 40-70 per 100,000 in most developed countries and from 100-190 per 1000,000 in developed countries. Thus, close to 80% of epilepsy cases worldwide are found in developing countries alone. India is home to about 10 million people with epilepsy with an overall prevalence of 1% [2]. Convulsions can also be accompanied by muscle spasms and a loss of consciousness depending on the type of epilepsy. These are thought to result from an imbalance, occurring in discrete anatomical pathways in the brain, between the major excitatory and inhibitory systems, glutamate and g-amino-butyric acid (GABA), which leads to abnormal electrical discharges. Convulsions induce alterations in neurons, glia, and neuronal circuits that include alterations in membrane receptors and neurotransmitter uptake sites, both neurogenesis and apoptosis, astrocyte proliferation, and axonal sprouting [3]. The introduction of anticonvulsant therapy has significantly contributed to the management of epilepsy. 60-70% of patients with epilepsy achieve control of their seizures with the use of conventional anti-epileptic drugs (AED). However, seizure control is not achieved in nearly one-third of the epileptic patients, even with the continued use of AED. Moreover, the use of AEDs is associated with a vast array of side effects, dose-related and chronic toxicity as well as teratogenic effects. with a vast array of side effects, dose-related and chronic toxicity as well as teratogenic effects. As such, there is an increased need to discover drugs which are effective in refractory epilepsy and have lesser adverse effects. The use of medicinal plants, which are popular in developing countries, offer important sources of new chemical substances with potential therapeutic benefits [4].

Ricinus communis, commonly known as castor oil/amudham, is a perennial shrub with a small soft wooded tree that grows up to 6 meters with varying stem pigmentation, belonging to the family Euphorbiaceae. It is found not only in tropical and subtropical regions but also as a wild sprouting or ornamental plant essentially around the world. The plant has been shown to

possess essential and beneficial biological properties such as antioxidant, antimicrobial, antihelmintic, insecticidal, diuretic, anti-inflammatory, laxative; in the treatments of hypoglycemia, edema, rheumatism, headache, asthma, dermatitis, etc., has been documented for various medicinal properties (5). The present study aimed to evaluate the anti-epileptic activity of the methanolic root extract of *Ricinus communis* on Maximal electroshock (MES) induced convulsions and Pentylene tetrazole (PTZ) induced convulsion and an attempt is made to establish the *in silico* studies of active constituents of the extract using molecular docking and Ramchandran plot .

2. MATERIALS AND METHODS

Plant collection and drying

Roots of *Ricinus communis* were identified, collected, authenticated by botanist P. Suresh babu, government degree college, Kukatpally, Medchal District. *Ricinus communis* roots were cleaned and dried under shade for about six days and powdered. The powdered material was stored.

Preparation of methanolic extract of *Ricinus communis* (Soxhlet)

300 gm powdered material of roots of *Ricinus communis* were dried and extracted with 1500 ml of methanol by soxhlation technique at a temperature of $45\pm 5^{\circ}\text{C}$. As to get efficient extraction, this method allows a continuous extraction process; it is nothing but a series of short macerations. The organic extracts obtained were evaporated to dryness by keeping at room temperature. Large amounts of drug can be extracted with a much smaller quantity of solvent. This process of extraction is economical in terms of time, energy and consequently financial investments [6].

Preliminary phytochemical analysis of the extract

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the methanolic extract of roots of *Ricinus communis*.

Acute toxicity testing

The acute toxicity studies were carried out using OECD 425 guidelines. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

Animal housing

The animals (mice) were housed in poly acrylic cages with not more than six animals per cage, with 12 h light/12 h dark cycle. Animals have free access to standard diet and drinking water ad

libitum. The animals were allowed to acclimatize the laboratory environment for a week before the start of the experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

***In vivo* methods for evaluation of Antiepileptic activity**

In vivo evaluation of antiepileptic activity of the methanolic extract of flower heads of *Ricinus communis* was carried out in following models.

- a) Maximal electroshock Induced convulsions.
- b) Pentylentetrazole Induced convulsions.

Maximal Electroshock Induced Convulsions

24 healthy Albino rats of either sex weighing 200-250 gm were selected for the study. They are divided into 4 groups, each consisting of 6 animals (n=6).

Group I - (control) normal saline.

Group II - MERC at dose of 200 mg/kg, b. w. *p.o.*

Group III - MERC at dose of 400 mg/kg, b. w. *p.o.*

Group IV - standard phenytoin 25 mg/kg b. w. *i.p.*

All these drugs were administered 1-hour prior to induction of seizures by MES. After 1-hour, electric current of 150 mA for 0.2 seconds was administered through ear electrodes to induce convulsions in all the animals with the help of an electro convulsimeter. The different phases of convulsions were noted down along with the duration of each phase for recording various parameters. Rats were placed in a clear rectangular polypropylene cage with an open top permitting full view of the animal motor responses to seizure. Various phases of convulsions like tonic flexion, extension, stupor and mortality due to convulsions were observed. Abolition or reduction in the duration of hind limb tonic extensor (HLTE) phase will be taken as a measure of protection against MES induced seizures [7].



Figure 1: Extension phase of Wistar Albino rat.

Pentylentetrazole Induced Convulsions

24 healthy Albino mice of either sex weighing 20-25 gm were selected for the study. They are divided into 4 groups, each consisting of 6 animals (n=6).

Group I - (Disease control) normal saline + PTZ 90 mg/kg, b. w., *i.p*

Group II - MERC at dose of 200 mg/kg, b. w. *p.o.* + PTZ 90 mg/kg, b. w., *i.p*

Group III - MERC at dose of 400 mg/kg, b. w. *p.o.* + PTZ 90 mg/kg, b. w., *i.p*

Group IV - Standard Clonazepam 1 mg/kg b. w. *i.p.* + PTZ 90 mg/kg, b. w., *i.p*

30 minutes after treatment, mice in all the groups receive PTZ (90 mg/kg, b. w. *i.p*). Later the latency of convulsions, duration of the convulsions, and mortality protection (percentage of deaths in 24h) will be recorded. Absence of an episode of clonic spasm of at least 5seconds duration indicates the extract or a compounds ability to abolish the effect of PTZ on seizure threshold. [8].



Figure 2: Pentylentetrazole Induced Convulsions in Swiss albino mice

INSILICO ANALYSIS

Molecular Docking Studies

a. Structure based drug design

Initially the protein downloaded from PDB was prepared by removing chain B. Water molecules

present in both the chains are removed. Energy minimization was done. Later molecules drawn using chemdraw were converted to mol format and ligprep was created. Grid generation was done by removing crystal ligand and the structures were docked against protein 4MS4, 3WFG, 5HCV and 6J8G.

b. Schrodinger XP-docking results

XP docking indicates that some of our compounds have good binding ability with GABA agonists (PDB ID: 4MS4), Voltage gated sodium channel antagonist (PDB ID: 6J8G), Glutamate antagonist (PDB: 3WFG) and Aspartate antagonist (PDB ID: 5HCV).

Ramachandran plot Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot [9].

Statistical analysis

Values are expressed as Mean \pm SEM, (n=6). All the groups were compared with control, negative control, and standard by using Dunnett's test. Significant values are expressed as control group (**=p<0.01, *=p<0.05), negative control (a=p<0.01, b=p<0.05) and standard (A=p< 0.01, B=p< 0.05), ns- nonsignificant.

3. RESULTS

Methanolic extract of roots of *Ricinus communis* was explored for its *in vivo* antiepileptic activity using Maximal Electroshock (MES) induced convulsions and Pentylenetetrazole induced convulsions. All the results obtained in the study were included below.

Preparation of methanolic extract of roots of *Ricinus communis*

The methanolic extract of roots of *Ricinus communis* was prepared by soxhlation technique. The percentage yield of methanolic extract was calculated by using the following formula.

$$\% \text{ of yield obtained} = \text{Amount of extract obtained (gm)} / \text{Total amount powder used} \times 100$$

$$\% \text{ Yield of extract} = 55.5/300 \times 100 = 18.5 \% \text{ w/w}$$

Preliminary phytochemical analysis

The preliminary phytochemical investigation of methanolic extract of roots of *Ricinus communis* revealed the presence alkaloids, flavonoids, glycosides, terpenoids, carbohydrates, tannins, saponins (Table 1).

Table 1: Preliminary phytochemical analysis

Phytoconstituents	Results
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Terpenoids	+
Fatty acids	+
Steroids	-

Acute toxicity studies

Methanolic extract of roots of *Ricinus communis* was tested on Swiss albino mice up to a dose of 2000 mg/kg, b. w. The animal did not exhibit any signs of toxicity or mortality up to 2000 mg/kg b. w. various morphological and behavioral characters were observed during the study. Hence the extract was found to be safe up to 2000 mg/kg, b. w.

In vivo anti-epileptic activity

The methanolic extract of roots of *Ricinus communis* was screened for its antiepileptic activity using the following models and the results are depicted in the table 2.

a) Maximal electroshock induced convulsions

Table 2: Effect of MERC on Maximal Electro Shock (MES) Induced Convulsions

Groups	Flexion (seconds)	Extension (seconds)	Jerky movements (seconds)
Control	23±0.67	14±0.79	14.6±0.88
(MERC 200 mg/kg, b. w.)	8±0.57 ^{*A}	6.5±0.61 ^{*A}	7.83±0.54 ^{*A}
(MERC 400 mg/kg, b. w.)	4±0.36 ^{*B}	3.5±0.42 ^{*B}	4.66±0.21 ^{*B}
(Phenytoin 25 mg/kg, b. w.)	1.83±0.30 [*]	1.33±0.21 [*]	4±0.25 [*]

Values are expressed as Mean \pm SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Significant values were expressed as when compared to control group (* = p<0.0001) and when compared to standard group (A = p<0.0001, B = p<0.001).

Pentylentetrazole Induced Convulsions (PTZ)

Table 3: Effect of MERC on Pentylentetrazole Induced Convulsions (PTZ).

Groups	Latency of Convulsions (secs)	Duration of Convulsions (secs)	% Protection
Disease control	91.1 \pm 0.86	166 \pm 0.87	–
(MERC 200 mg/kg, b. w.)	128 \pm 0.89 ^{*A}	148.66 \pm 0.73 ^{*A}	24
(MERC 400 mg/kg, b. w.)	205.16 \pm 0.92 ^{*A}	128 \pm 0.99 ^{*A}	33
(Clonazepam 1 mg/kg, b. w.)	386 \pm 0.80 [*]	118.16 \pm 0.79 [*]	40

Values are expressed as Mean \pm SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Significant values were expressed as when compared to control group (* = p<0.0001) and when compared to standard group (A = p<0.0001).

In silico analysis

1) Molecular docking

Table 4: Schrodinger XP Docking Scores

Compounds	4MS4	3WFG	5HCV	6J8G
Catechin	-8.01	-7.92	-7.01	-7.43
Quercetin	-	-9.74	-10.03	-6.84
Gallic acid	-9.27	-7.25	-8.11	-6.60
Vitexin	-	-	-	-6.34
Luteolin	-8.47	-12.21	-9.92	-5.73
Gentisic acid	-8.22	-6.95	-7.83	-5.56
Vanillic acid	-3.79	-7.36	-6.77	-4.78
Ricinine	-	-6.19	-6.05	-
Ricinoleic acid	-8.91	-	-	-3.60
Quercetin-3-O- β-D- xylopyranoside	-	-	-	-7.81
Stigmasterol oleate	-	-	-	-5.22
Clonazepam	-2.40	-9.30	-10.25	-6.76
Phenytoin	-8.44	-7.96	-7.43	-5.16

G score = glide score, The more negative the Glide score, the more favorable the binding.

2) Ramachandran plot Analysis

Protein 4MS4, 3WFG, 5HCV and 6J8G were analyzed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 5 and pictorial representation by figure below.

Table 5: Ramachandran plot status with protein with 4MS4, 3WFG, 5HCV and 6J8G

Residues	4MS4	3WFG	5HCV	6J8G
Most favorable region (%)	91.2	94.7	94.8	94.5
Additional allowed regions (%)	8.5	5.3	5.8	3.7
Generously allowed regions (%)	0.3	0.0	0.0	1.1
Disallowed regions (%)	0.0	0.0	0.0	0.7

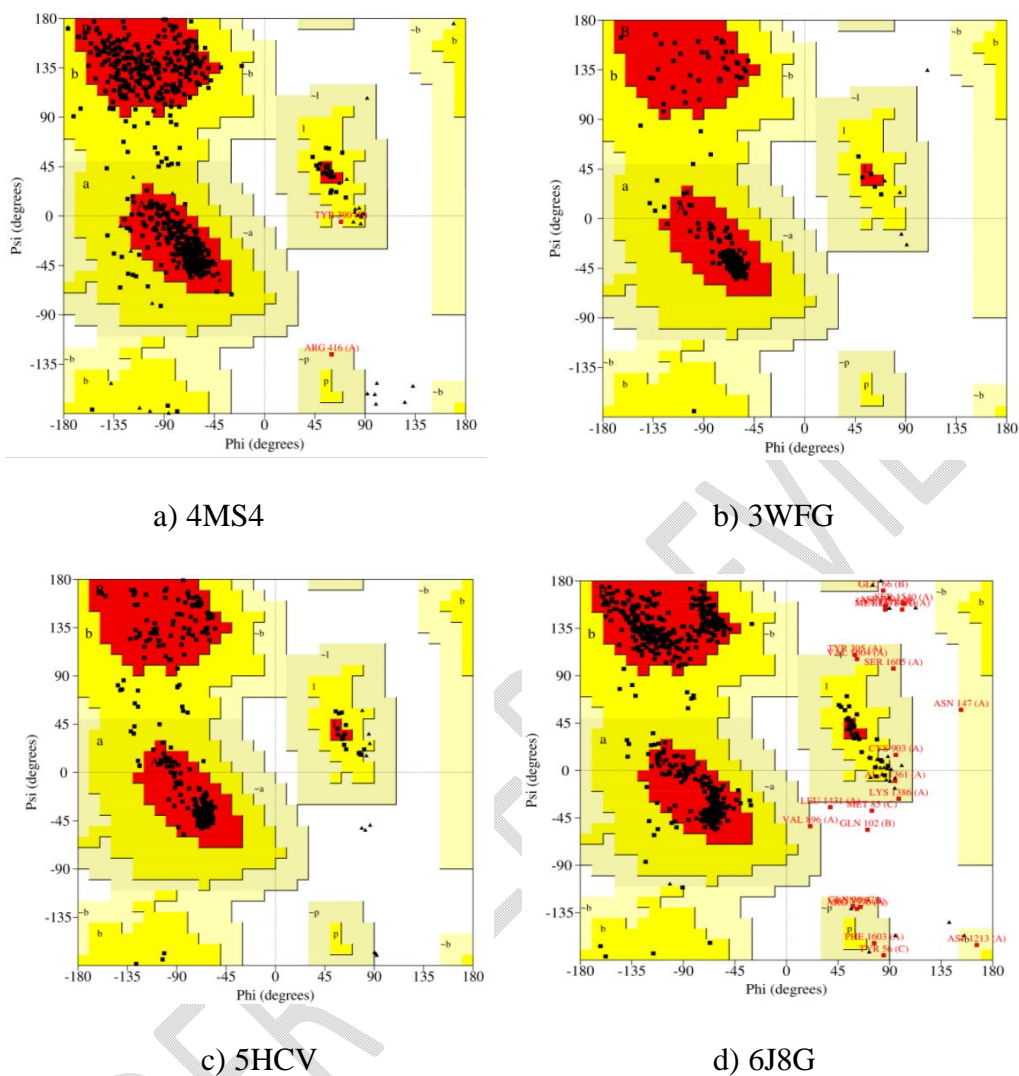


Figure 3: Ramachandran plot of protein 4MS4, 3WFG, 5HCV and 6J8G

4. Discussion

The preliminary phytochemical investigation of methanolic extract of roots of *Ricinus communis* revealed the presence alkaloids, flavonoids, glycosides, terpenoids, carbohydrates, tannins, saponins. The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance. These phytochemical constituents were reported to be safe as per the acute toxicity study. These did not induce any mortality or pre-terminal death. No changes were observed in salivation, lacrimation, perspiration, piloerection, micturition, or defecation. The animals were observed for ptosis, drowsiness, stereotypy, aggression, tremors,

convulsion, Straub's test, motor in-coordination, and writhing and no abnormalities were observed in any of the treated animals. Gait, righting reflex, and corneal reflex were normal. Skin, fur, eyes, and body weight of the animals were also normal. Tremors, lethargy, diarrhea, and coma were not observed during the study.

Anti -Epileptic Activity:

Epilepsy is the commonest neuropsychiatric disorder characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbance of consciousness, with or without characteristic body movements (convulsions) with sensory or psychiatric phenomena causing significant medical and social morbidity. GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders. The agents which increase the brain GABA content and administration of centrally active GABA mimetic agents have been used as effective therapeutic approach for treatment of anxiety and epilepsy [10]. In the present study *in-vivo* anti-epileptic activity was evaluated by using animal models involving gamma-amino butyric acid (GABA) ergic neurotransmission i.e., MES in rats and PTZ induced convulsions in mice. MES and PTZ models may be exerting their convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors. GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. [11]. The various phytochemical active constituents identified in the methanolic extract of *Ricinus communis* roots were alkaloids, flavonoids, terpenoids, tannins, steroids, carbohydrates, saponins. Ricinine (Alkaloid) possesses anticonvulsant property against MES and PTZ induced seizures which could be by interfering with GABA, glutaminergic mechanism and Na⁺, Ca²⁺ channels. [12]. Quercetin (Flavonoid) might also modulate intracellular signaling cascades through influence on some protein kinases and lipid kinases signaling cascades, such as the phosphatidylinositol 3-kinase (PI3K)/Akt, tyrosine kinase, protein kinase C (PKC) and mitogen-activated protein kinase (MAP kinase) signaling pathways [13]. These processes change the excitability of neurons and functions of the central nervous system, including its seizure susceptibility. Gallic acid (Phenolic acid) possesses anticonvulsant activity may be due to potentiation of GABAergic neurotransmission and/or increase in neuronal seizure threshold by decreased Na⁺ channel activity [14]. Vanillic acid (Phenol) anticonvulsant mechanism is not fully understood. However, the elevation of GABAergic transmission is the most prominent mechanism. The ellagic acid has neuroprotective action in both MES and PTZ models of seizures and its anticonvulsant effects

may be exerted through decreasing oxidative stress and in increasing GABA_A transmission in the brain [15].

Molecular docking

Molecular docking continues to hold great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. The docking analysis of isolated compounds from *methanolic root extract of Ricinus communis* and standard drug like *phenytoin*, *clonazepam* was carried out using Schrödinger software. The various constituents identified in the plant extract are Catechin, Quercetin, Gallic acid, Vitexin, Luteolin, Gentisic acid, Vanillic acid, Ricinine, Ricinoleic acid, Quercetin-3-O- β -D-Xylopyranoside, Stigmasterololeate and standard drugs phenytoin, clonazepam was subjected to docking against PDB ID: 4MS4, 3WFG, 5HCV, 6J8G. The highest glide scores were observed with Catechin, Quercetin, Gallic acid, Vitexin, Luteolin, Gentisic acid, Vanillic acid, Ricinine, Ricinoleic acid, phenytoin and clonazepam against all the selected PDB ID: 4MS4, 3WFG, 5HCV, 6J8G. The glide scores of the Catechin, Quercetin, Luteolin, Gallic acid were found to be more than the glide score of standard drug clonazepam and phenytoin stating that the compounds might have same affinity to bind to the proteins. These results clearly indicate that the chemical constituents mentioned above might have shown similar mechanism to that of the standard drug clonazepam and phenytoin as anti-epileptic.

The proteins identified namely PDB ID: 4MS4, 3WFG, 5HCV, 6J8G are modeled and the qualities of the 3D model were evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that predicted models have most favorable regions, additionally allowed regions, generally allowed regions and disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. According to Ramachandran plot a good quality model would be expected to have over 90% in the most favored region. Proteins like PDB ID: 4MS4, 3WFG, 5HCV, 6J8G showed 90% favored a region which clearly indicates that the selected models in the present study are of good quality [16].

5. CONCLUSION

According to *in vivo* and *in silico* investigations the methanolic root extract of *Ricinus communis* clearly possessed antiepileptic activity in rodent models. But more research is

required to identify specific phytochemical components of the extract and determine precise mechanism underlying its antiepileptic effect.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee of GRCP approved the research entitled "Neurological and molecular interactions of phytoconstituents from *Ricinus communis* root extract- A pharmacological and computational approach" with Regd number. 1175/PO/Re/S/08/CPCSEA. All animal experiments were carried out in accordance with CPCSEA guidelines.

REFERENCES:

1. Knutsen LJS, Williams M. Epilepsy. Comprehensive Medicinal Chemistry II. 2007;279–296.
2. Saikia A, Tigga PR. Anticonvulsant activity of the methanolic extract of *Lawsonia inermis* leaves in albino rats. 2016;7(7):3068-3072.
3. Rang, H.P., Dale, M.M., Ritter, J.M. (2016). Rang and Dale's pharmacology. (7); 536-549.
4. Hitendra SC, Gayatri S, Babita J. Medicinal properties of *Ricinus Communis*: a review. International Journal of Pharmaceutical Sciences and Research. 2021;12(7); 3632-3642.
5. Ahmad N, Mishra A, Ahsan F, Mahmood T, Hasan N, Khan Z. *Ricinus communis*. Pharmacological actions and marketed medicinal products. World Journal of Pharmaceutical and Life Sciences. 2016;2(6):178-188.
6. William N, Farhana SNI, Masrina MN. Comparative Studies on Different Extraction Methods of *Centella asiatica* and Extracts Bioactive Compounds Effects on Antimicrobial Activities. 2021;10(4):457.
7. Raju MG, Fernandes J, Narayana VK. Evaluation of antiepileptic activity of the alcoholic extract of *Adhatoda vasica* leaves in rats. Res J Pharm Biol Chem. 2011;2(3):5-10.
8. Reddy VNVLS, Raju GM, Sirisha S, Nikitha K. Neuropsychological Screening on *Acalypha indica* Whole Plant Extract. J Young Pharm. 2020;12(2): s82-86.
9. Pasupuleti SK, Yellapu N, Prasad UV. *In silico* designing and molecular docking of a potent analog against *Staphylococcus aureus* porphobilinogen synthase. Journal of Pharmacy and Bioallied Sciences. 2014;6(3): 158-166.

10. Pitchaiah G, Anusha VL, Hemalatha CH, Kumar AY, Sravani K. Anxiolytic and Anticonvulsant activity of methanolic extract of *Allium cepa* Linn (Onion) bulbs in Swiss albino mice. Journal of Pharmacognosy and Phytochemistry. 2015; 4(2); 131-135.
11. Sumlatha G, Sreedevi A. Evaluation of anti-epileptic activity of chloroform extract of *Acalypha fruticosa* in mice. Pharmacognosy Research, 2014;6(2):108-112.
12. Padma L. Screening of *Ricinus communis* Linn leaves for Anticonvulsant and Analgesic activity. Asian journal of Pharmaceutical and clinical research. 2014;7(3):110-114.
13. Dajas F, Andres AC, Florencia A, Carolina E, Felicia RM. Neuroprotective actions of flavones and flavonols: mechanisms and relationship to flavonoid structural features. Central Nerv Syst Agents Med Chem. 2013;13:30-35.
14. Vijayalakshmi V, Anbu RJ, Velraj M, Jayakumari S. Anticonvulsant and neurotoxicity profile of the rhizome of *Smilax china* Linn in mice. Indian Journal of pharmacology. 2011;43(1); 27-30.
15. Khazaei A, Mohammad S, Mansouri T, Siahpoosh A, Ghorbanzadeh B, Salehi S, Khodayar MJ. Ameliorative Effects of Ellagic Acid on Maximal Electroshock and Pentylentetrazole-Induced Seizures in Mice. 2019;14(13): 5-9.
16. Raju MG, Yadav EV, Reddy VNVLS, Nicholas M. Pharmacological and *in silico* Evaluations of methanolic flower extract of *Tagetes Patula* as anti-depressant and anxiolytic. Bulletin Environmental Pharmacological Life Sciences. 2021; 10(3): 29-35.