RESEARCH ARTICLE

Pharmacological Evaluation of Alcoholic Extract of *Clitoria ternatea* Root for Anxiolytic and Anticonvulsant Activity

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ABSTRACT

Recent scientific studies have indicated that some of the same medicinal herbs used in traditional medicine to treat epilepsy may also have anticonvulsant qualities, suggesting that they may be a possible source of novel anticonvulsants. Anticonvulsant properties have been studied in animal models. *Clitoria ternatea* Linn's ethanolic root extract was examined for antiepileptic, anxiolytic, and phytochemical properties. Phenytoin 25 mg/kg, as a reference medicine, the extract was tested against strychnine-induced convulsion in rats at oral doses of 200 and 400 mg/kg. The elevated plus maze and staircase test were used to examine mice's anxiolytic activity with lorazepam (the gold standard) at 0.05 mg/kg. When using a strychnine-induced convulsion model, the ethanolic extract markedly accelerated the start of convulsions. In the elevated plus maze test, *Clitoria ternatea* Linn ethanolic root extract at 200, 400, and standard doses significantly increased the time and number of the entry in open arms compared to the control group. In a staircase test, the ethanolic root extract of *Clitoria ternatea* increased the number of steps climbed and lowered rearing compared to the control group. Docking scores in mcule software indicate that interactions with the glycine agonist (PDB: 5I57) and the GABA agonist (PDB: 4MS3) receptors for epilepsy and anxiety, respectively, are highly effective. Evidence for the biochemical evaluation of GABA, anticonvulsant, and anxiolytic action, as well as *in-silico* activities, of the ethanolic extract of *Clitoria ternatea* was reported in this work.

Keywords: *Clitoria ternatea*, Strychnine, Elevated plus maze, Staircase, *In-silico.*

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INTRODUCTION

The neurological illness of epilepsy is characterized by aberrant brain activity, which causes repeated, spontaneous seizures (disturbance in brain function) or convulsions. Excessive neurotransmitter release from brain cells could be to blame for the disruption. Autonomic hyperactivity, loss of consciousness, and strong spasmodic contractions of skeletal muscles (convulsions) are all common symptoms. Spasms of muscles and unconsciousness frequently follow convulsions in epilepsy. The abnormal electric discharge is theorized to originate from a disproportion between the brain's two primary neurotransmitters, glutamate and gamma-aminobutyric acid (GABA), which travel separate anatomical paths.[1]

An excessive sense of apprehension, uncertainty, and fear is defined as anxiety. A sense of tension and impending peril has set in. One interpretation classifies it as a form of behavioral inhibition triggered by novel or unexpected stimuli. One-eighth of the world's population suffers from anxiety, which has grown significantly in importance as a research topic in psychopharmacology during the past ten years. Many theories attempt to explain the effectiveness of anti-anxiety drugs by pointing to the participation of different chemical mediators in the CNS. The majority of anxiolytic drugs act by making it easier for GABA-activated chloride channels to open, which improves the response to GABA.[2]

Clitoria ternatea, a member of the family Fabaceae, with white or blue flowers that can be found all across the tropical region of India. It is known as Butterfly pea in English, Koyal in Hindi, and Aparajita in both languages.[3] ShankaPushpi, an Ayurvedic treatment intended to improve neurological health, includes this plant as one of its traditional medicinal ingredients.

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The plant's biological effects are the result of a combination of inorganic and organic substances called active chemicals, which include alkaloids, flavonoids, taraxerol, taraxerone, and triterpenoids. It's beneficial on a neuropharmacological level in many different ways, including as a nootropic, antidepressant, stress reliever, anxiolytic, and anticonvulsant.[4]

Clitoria ternatea is well-known to have fascinating qualities in traditional medicine. However, its anxiolytic and anticonvulsant activity have not been investigated. Traditional medicine has long used EECT for the treatment of epilepsy and anxiety, and this study set out to provide experimental support for this practice (Table 1).

MATERIALS AND METHODS

Plant Collection and Drying

Botanists from the Government Degree School, the Kukatpally area, and the Medchal region all worked together to identify and confirm *Clitoria ternatea* roots. After being washed and dried (for around 14 days) under concealer, the powdered roots of *Clitoria ternatea* were made. The powdered substance was put away for later use.

Clitoria ternatea Ethanolic Root Extract Preparation (Soxhlet)

Clitoria ternatea root was dried and separated with ethanol by a soxhlation strategy

Phytochemical Screening of the Extract

A number of primary phytochemical analyses were performed on the ethanolic concentrate of *Clitoria ternatea* root to identify the various phytoconstituents contained in the concentrate.[5]

Flavonoids are Separated using a Chromatographic Technique, Including Paper Chromatography

Flavonoids Sample Preparation

Paper chromatography was utilized to separate the flavonoids from the other components. In a mortar and pestle, 2 g of roots were homogenized with 20 mL of 80% methanol in 1% HCl. To reach a final volume of 25 mL, 80% for an additional 24 hours, the homogenate was kept in the dark to ensure that all of the flavonoids were removed. To further purify the extract, it was centrifuged for 20 minutes at 4,000 rpm. In a water bath, the supernatant was concentrated at 5 mL. After that, containers were used to keep the samples at 20°C until chromatography could be performed (Table 2).

Paper Chromatography for Flavonoid Separation

A mixture of n-butanol, acetic acid, and water was utilized as the solvent system for ascending paper chromatography (4:1:5). Flavonoids were spotted with the use of a UV transilluminator. After separating the flavonoids by chromatography, the sharpness of the image was improved by exposing the chromatogram to ammonia fumes for a whole night (Figure 1).[6]

Acute Toxicity Testing

The intense poisonousness studies were completed utilizing OECD 425 rules. The present review was done in the CPCSEA-endorsed creature place of GRCP.

Habitats for Animals

There were no more than six rodents or mice per enclosure, and each cage had a light on for 12 hours and a light off for 12 hours. Animals do not need to be restrained when it comes to following their regular diet and water is optional. The animals were given seven days to acclimatize to the conditions of the facility before the tests began. To regulate and supervise animal experiments, the advisory group's guidelines were followed throughout the process of considering and supporting the creatures (CPCSEA).

Analyzing Anticonvulsant and Anxiolytic Activity *In-vivo*

Clitoria ternatea ethanolic root extract's anticonvulsant efficacy was tested *in-vivo* using the following animal models.

Anticonvulsant Activity Assessment In-vivo

- Convulsions Caused by Strychnine

Twenty-four Wistar Albino rats of any sex and weight between 150 and 200 g are chosen for this experiment. There are 12 people total, and they are split into four groups of 6. As a disease-control measure, saline (0.9% NaCl) is given to group I. In group II, subjects were given 200 mg/kg of EECT, while in group III, they were given 400 mg/kg. Phenytoin 25 mg/kg was given to group IV. After one hour, each animal in the group received 4 mg/kg, bd wt. of strychnine intraperitoneally, causing them to experience strong opisthotonus tonic convulsions of the body and limbs. For every animal, we recorded when convulsions started and what percentage of them died.[7]

In-vivo Methods for Evaluation of Anxiolytic Activity

Elevated Plus Maze (EPM) Test

The most common model used to evaluate the anxiolytic action of new drugs is the EPM. A 25cm tall EPM has two open arms (16 by 5 cm) and two enclosed arms (16 by 5 by 12 cm) with an open roof, all of which are linked to a central platform (5 by 5 cm). Twenty-four albino mice (both sexes) weighing 25–30 gm are chosen and split into four groups of six. NaCl (0.9% solution) serves as the placebo for group I. EECT 200 mg/kg, to group II, EECT 400 mg/kg, treatment for group III. Regular dosing of Lorazepam 0.05 mg/kg, *p.o.* is used for Group IV. After the initial 30 minutes, the animals are placed in the center of the maze with their heads facing an open arm and allowed 5 minutes to find their way out (Table 3 and 4).[8]

Staircase Test

The study uses 24 healthy swiss albino mice weighing 25 to 30 gm. Group I received 0.9% NaCl. EECT 200 mg/kg,*p.o.*to group II.EECT 400 mg/kg, *p.o.* to group III. lorazepam, 0.05 mg/kg, to group IV. It has five identical steps, 10 cm broad, 7.5 cm deep, 2.5 cm high. Wall height is constant along the steps. One hour after oral delivery, the animal was placed on the floor of the box with its back to the stairs. Over 3 minutes, steps climbed and rearings are counted. To simplify the observation, only steps climbed by mice with all four paws are counted. If you want to make sure the next animal doesn't become influenced by the previous one's test results, you'll need to clean the box between each one (Table 5).[9]

Table 1: Chemical components of *Clitoria ternatea*

S. No.	*RT*	*Compound Name*	*MolWt*	*%Peak Area*
i	31.649	Stigmasterol	412.690	0.900
ii	32.079	β-Sitosterol	414.710	4.530
iii	32.221	Kaempferol	286.230	12.250
iv	32.550	Lupeol	426.720	1.500
v	21.180	n-Hexadecanoic acid	256.40	1.250
vi	25.416	Heptacosane	380.70	0.620
vii	1.478	Taraxerone	424.70	26.710
viii	1.983	Chelidonine	353.40	3.110
ix	2.454	Taraxerol	426.720	30.160
x	26.237	Gallic acid	170.120	1.090
xi	27.769	Rutin	610.5170	4.370
xii	30.485	Delphinidin	303.240	1.280

Table 2: R_f values of flavonoids from *Clitoria ternatea* root extract

S. No	*Name of the Plant*	*Spot No*	*R_f value*	*Active constituent*
1	*Clitoria ternatea* root extract	A	21	Unidentified compound
		B	32	Glycosyl Flavones
		C	45	Flavonols (Myrcetin)
		D	61	Unidentified compound
		E	84	Kaempferol

Table 3: Effect of EECT on Strychnine-Induced Convulsions

Groups	*Convulsion Onset (Min)*	*%Mortality*
Disease control	3.60 ± 0.310	0
200 mg/kg of EECT	7.00 ± 0.340^{*A}	0
400 mg/kg of EECT	14.0 ± 0.480^{*A}	0
Phenytoin 25 mg/kg	25.2 ± 0.56*	0

The values are expressed as mean ± SEM (n=6) analysis.ANOVA followed by post hoc test against standard (^{A} = p < 0.0001) and against Disease control (*p < 0.0001).

Reducing Power Assay *In-vitro*

Increased reaction mixture absorbance suggests anti oxidant activity. To create a ferrous complex with maximal absorbance at 700 nm, reducing chemicals combine with $[K_3Fe(CN)_6]$ to produce $[K_3Fe(CN)_6]$, which is then reacted with ferric chloride. 1-mL of each *Clitoriaternatea* ethanolic root extract solution (250–1000µg) was combined with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. Twenty minutes at 50°C incubated the mixture. 10 minutes at 3000rpm with 2.5 mL of 10% trichloroacetic acid. 2.5 mL of the upper solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) 700 nm absorption. The experiment standard was ascorbic acid (Table 6 and Figure 2).[10]

$$\% \text{ reducing power increase} = \frac{Abs_{sample} - Abs_{control} \times 100}{Abs_{sample}}$$

Where Abs_{sample}= Test sampleabsorbance

$Abs_{control}$= Controlabsorbance

Estimating Brain GABA

When convulsions started, PTZ (85 mg/kg)-treated animals were killed. PTZ (85 mg/kg) was given after 45 minutes of EECT (200 mg/kg) and sacrificed at the commencement of convulsions. Homogenized in 5 mL of cold 0.01 N HCL, the brain was separated as quickly as possible. Brain homogenate was placed in 8 mL of ice-cold pure alcohol and refrigerated at 0°C for an hour. The supernatant was collected in a petri dish after 10 min of centrifugation at 16000 rpm. The precipitate was washed three times with 3–5 ml of 75% alcohol and mixed with the supernatant. A water bath with an air stream dried petridish contents at 70 to 90°C. After mixing 1-mL water and 2 mL chloroform, the dry mass was centrifuged at 2000 rpm. After separation, 10 µL of the top phase, which included GABA, was observed on the Whatman paper (No. 41).

Conditions

Acetic acid (12 mL), n-butanol (50 mL), and water made up the mobile phase (60 mL). For 30 minutes, a mobile phase saturated the compartment. The paper chromatogram was developed ascendingly. A 0.5% ninhydrin solution diluted in 95% ethanol was applied to the paper after drying in hot air. At 90°C, the paper dried. A paper patch with a blue tinge was cut out and heated for 5 minutes in a 2 mL ninhydrin solution. The solution was left for an hour with 5 cc of water. Absorbance at 570 nm was measured after decanting the supernatant (Figure 3 and 4).

Standards

A stock solution of 1-mg/mL standard GABA in 0.01 N HCl was made. Serial dilutions yielded concentrations from 1 to 1000 ng/10 µl. Instead of brain homogenate, standard GABA solutions were employed to generate a standard concentration curve for GABA (Table 7).[11]

Table 4: Effect of ethanolic root extract on *Clitoria ternatea* on elevated plus maze test

Groups	*Time spent in arms (Secs)*		*No of Entries*	
	Open	*Closed*	*Open arm*	*Closed arm*
Control	21.8 ± 0.47	204.6 ± 0.66	3.1 ± 0.47	10 ± 0.36
200 mg/kg of EECT	52.8 ± 0.60^{*A}	153.8 ± 0.72^{*A}	8 ± 0.57^{*A}	6.1 ± 0.47^{**B}
400 mg/kg of EECT	131.5 ± 0.76^{*A}	78.5 ± 0.76^{*A}	12.6 ± 0.66^{*A}	5.1 ± 0.60^{*C}
0.05 mg/kg, bd.wt., p.o of Lorazepam	217.1 ± 0.87*	41.3 ± 0.84*	20 ± 0.73*	2.8 ± 0.70*

Mean ± SEM (n = 6),the values are expressed. ANOVA followed by post hoc test against standard (^{A} = p <0.0001, ^{B} = p <0.005, ^{C} = p <0.05), and control (*p <0.0001, **p <0.001).

Table 5: Effect of ethanolic root extract on *Clitoria ternatea* on staircase test

Groups	*No of steps climbed in 3 min*	*No of rearing in 3 min*
Control	2.80 ± 0.30	11.160 ± 0.47
200 mg/kg of EECT	6.60 ± 0.4[70]**A	7.30 ± 0.490[*A]
400 mg/kg of EECT	12.60 ± 0.66[0*]B	5.50 ± 0.420[*C]
0.05 mg/kg of Lorazepam	17.10 ± 0.870[*]	2.80 ± 0.60[*]

Mean ± SEM (n=6),the values are expressed.ANOVA followed by post hoc test against and against standard (^{A} = p <0.0001, ^{B} = p = 0.0001, ^{C} = p <0.005), control (*p <0.0001, **p <0.001).

Table 6: Anti-oxidant activity of *Clitoria ternatea* by using reducing power assay

S. No	*Name of the compound*	*Concentration (µg/mL)*	*Percentage Inhibition (Mean ± SEM)*	*IC_{50} value (µg/mL)*
1	Ascorbic acid	10.0	27.5 ± 0.300	20.490
		20.0	48.8 ± 0.360	
		30.0	62.9 ± 0.490	
		40.0	71.6 ± 0.520	
		50.0	78.3 ± 0.720	
2	EECT	10.0	26.3 ± 0.320	24.50
		20.0	46.2 ± 0.400	
		30.0	61.0 ± 0.570	
		40.0	69.2 ± 0.630	
		50.0	75.6 ± 0.820	

Table 7: Calibration curve of GABA and Effect of EECT on PTZ induced convulsion in rats

S. No	*Concentration (*ng/µL*)*	*Absorbance*
i	200.0	0.120
ii	400.0	0.230
iii	600.0	0.350
iv	800.0	0.410
v	1000.0	0.450
vi	Sample	0.030

Table 8: Docking scores with PDB: 5I57

S. No	*Ligands*	*Docking score*
1	Kaempferol	-7.1
2	n-Hexadecanoic acid	-6.3
3	Delphinidin	-6.1
4	Gallic acid	-5.6
5	Heptacosone	-4.9
6	Phenytoin	-7.2

Table 9: Docking scores with PDB: 4MS3

S. No	*Ligands*	*Docking score*
1	Delphinidin	-6.2
2	Chelidonine	-5.7
3	Rutin	-5.6
4	Kaempferol	-5.4
5	Stigmasterol	-5.2
6	Lorazepam	-5.6

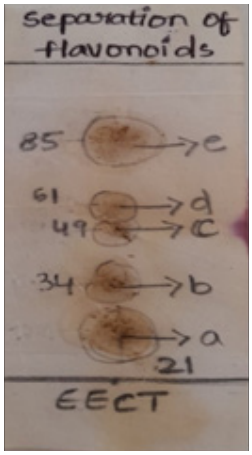

Figure 1: Separation of flavonoids by paper chromatography.

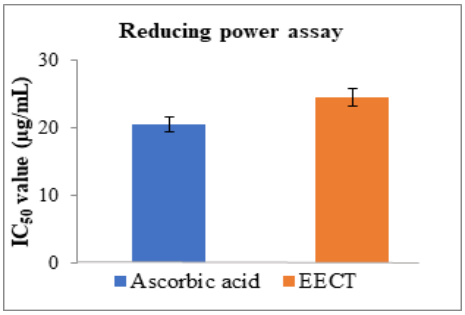

Figure 2: Anti-oxidant activity of *Clitoria ternatea* by using reducing power assay.

GABA Estimation from EECT

On ascending paper chromatography, plant extracts at 1000, 100, and 10 µg/mL and standard GABA at 1000 µg/mL were found. The mobile phase was prepared by combining 35 parts N-butanol, 35 parts acetone, 35 parts glacial acetic acid, and 20 parts water. The amount of GABA in the extract under study is compared to a gold standard. Amino acids were seen after heating a solution of 0.3% ninhydrin in n-butanol with 3% acetic acid for 10 minutes (Figure 3).[12]

$$R_f = \frac{\text{Component Distance}}{\text{Solvent Distance}}$$

In-silico/Molecular Docking

Molecular docking, which simulates how protein-ligand complexes interact by calculating the shape complementarities between the two molecules, has been applied to a variety of problems ranging from ligand design to drug discovery. The basic idea of the docking procedure is that ligands binding with high affinity to protein-binding sites tends to possess a molecular shape complementary to the shape of the binding site, which is usually described by the shape and the spatial distribution of the hydrophobic and hydrogen bonding residues that surround the binding site. PDB ID: 5I57 (Glycine Agonist) and PDB ID: 4MS3(GABA Agonist) proteins are docked against 12 ligands (Phytocompounds) obtained from GC-MS studies for anticonvulsant and anxiolytic activities.

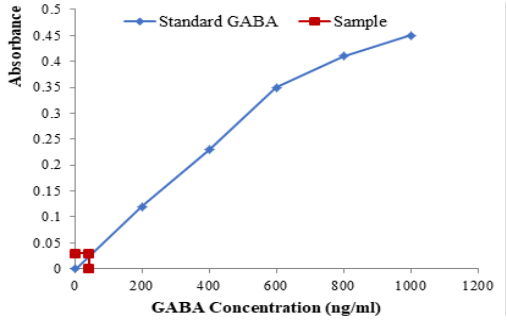

Figure 3: Calibration curve of Standard GABA.

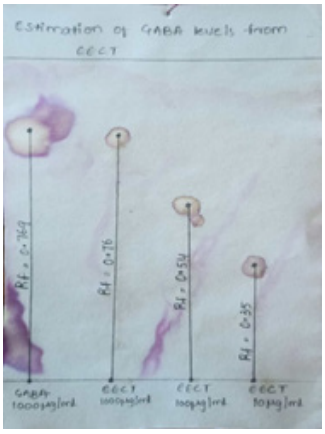

Figure 4: GABA concentration prediction using sample extract (EECT).

Docking Simulations

GluN1/GluN2A agonist binding domains on 5I57:Excitatory synaptic transmission and synaptic plasticity are both regulated by NMDA receptors in the central nervous system, but abnormal regulation of these receptors has been related to a wide variety of neurological disorders. Here, we detail GluN2A-selective negative allosteric modulators (NAMs) that inhibit NMDA receptors by maintaining the inactive apo form of the GluN1 ligand-binding domain (LBD), which prevents gating of the receptor's ion channels. Glycine agonists are also used to treat other symptoms associated with neurodegenerative diseases such as epilepsy and dementia (Table 8).

Docking Simulation on 4MS3

GABA agonists prevents seizures and antagonists cause them. The brain's G-protein-coupled GABA(B) receptor is essential for inhibitory neurotransmission. Numerous neuropsychiatric disorders appear to be caused by GABA (Table 9).[13]

Ligand Preparation

Chemical structures of molecules are drawn, and ligand preparation was created. The 2D ligands sketched in Mcule docking in the ligand imported side.

Protein Preparation

PDB ID: 5I57 (Glycine Agonist) and PDB ID: 4MS3 (GABA Agonist) proteins were initially downloaded from the RCSB protein bank website in PDB format were prepared by removing extra chains.

Protein-ligand Interactions

Drug candidates' protein targets' docking orientations are predicted *via* docking simulations. Docking simulation research using Mcule.

Ligand Docking and Scoring

Proteins are uploaded with sphere attributes for 5I57(X=-8.238400 Y=9.608000 Z=-36.613200); 4MS3 (X=-8.358500 Y=30.150833 Z=-40.605000). Docking indicated that some compounds have the good binding ability with 5I57 and 4MS3 proteins. The compounds docked display a docking score. The ligand interactions of compounds present in *Clitoria ternatea* with 5I57 and 4MS3 proteins follow.

Visualization and Analysis

Discovery Studio visualized docking stances. Visualizing ligand interactions revealed protein-ligand binding. The glide score determined the best-docked buildings. Binding improves with lower scores. Docked ligand poses and ligand-receptor interactions were also displayed.

Statistical Analysis

ANOVA and post hoc tests assessed all values as arithmetic mean ± SEM.

RESULTS

Initial Phytochemical Analysis

Clitoria ternatea's ethanolic root extract revealed alkaloids, flavonoids, proteins, phenols, triterpenoids, carbohydrates, and steroids.

GC-MS of *Clitoria ternatea* Crude Ethanolic Extract

Table 1 shows chemicals analyzed by GC-MS for *Clitoriaternatea*. The extract's GC-MS analysis revealed the following bioactive components.

Chromatographic Separation of Flavonoids

Paper chromatography results are in Table 5. Rf values detected flavonoids were found in *Clitoriaternatea* root extraction Figure 1.

Acute Toxicology

*Clitoria ternatea's*ethanolic root extract was tested on swiss albino mice at 2000 mg/kg bd. wt. During the review, the organisms showed no signs of poisonousness or death up to 2000 mg/kg bd. wt. They had diverse morphological and social traits. Thus, the dose was considered safe up to 2000 mg/kg bd. wt.

Dose Selection

Toxic investigations showed a section of 2000 mg/kg bd. wt. was protected, and the functional piece was 1/10th, or 200 mg/kg. Pharmacological tests were performed using 200 and 400 mg/kg, bd. wt.

Convulsions from Strychnine

EECT Linn significantly ($p < 0.05$) accelerated the onset of convulsions in the treated group (EECT 200 and 400 mg/kg) compared to the control group. The percent mortality in

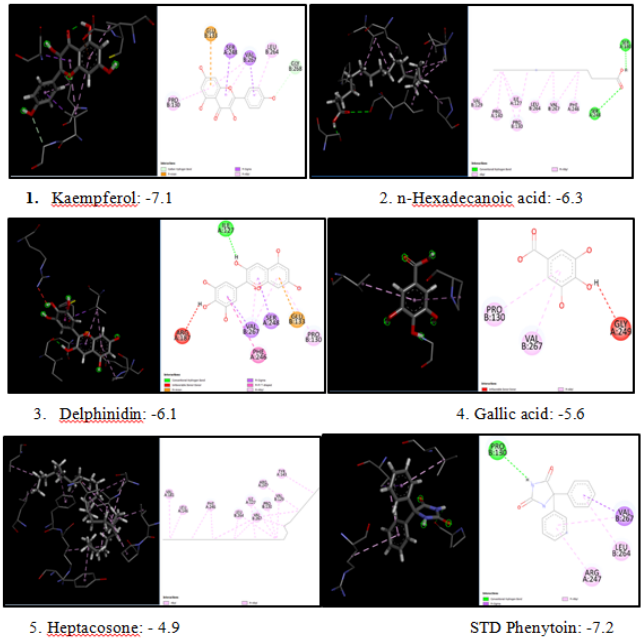

Figure 5: Docking scores with PDB: 5I57.

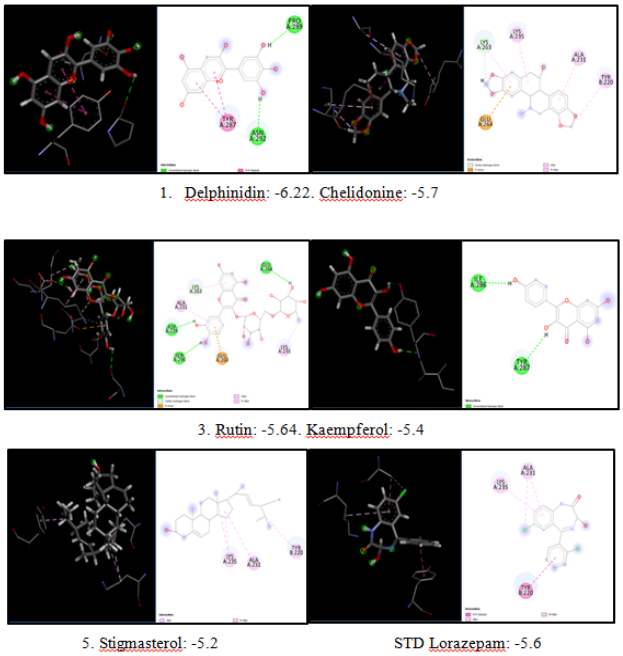

Figure 6: Docking scores with PDB: 4MS3.

animals receiving EECT at 200 and 400 mg/kg was 0%. Phenytoin had strong anticonvulsant efficacy at 25 mg/kg.

EPM Test

Animals' natural tendencies are investigated with the help of the raised plus maze test. Compared to the control group, the amount of time spent in open arms and the number of entries increased significantly after receiving an ethanolic root extract of *Clitoria ternatea* Linn at doses of 200 and 400 mg/kg. The number of open-arm entrances and total time spent in them were both significantly boosted by standard (Lorazepam), whereas closed-arm entries were reduced.

Staircase Test

Table 5 shows that a dose-dependent increase in the number of steps taken and a decrease in the number of rearing occurred after the administration of ethanolic root extract of *Clitoria ternatea* (200 and 400 mg/kg) compared to the control group. Anxiolytic effects were noticeable with a dose of 0.05 mg/kg of the commonly used drug lorazepam.

In-vitro Reducing Power Assay

Reducing power assays were used to measure *Clitoria ternatea's* anti-oxidant activity. The percentage of free radicals inhibited by *Clitoria ternatea* rose with the dose. The extract had an IC_{50} value of 24.50 µg/mL, which is comparable to that of regular ascorbic acid.

Biochemical Testing

Inferring GABA Activity in the Brain

The absorbance value for the disease sample was 0.0189. Disease samples were measured to have a concentration of 20 ng/mL. The absorbance of the sample used for testing was determined to be 0.03. The sample's concentration was calculated to be 40 ng/l. The amount of GABA in the brain of animals given PTZ after 45 minutes of treatment with EECT at 200 mg/kg was considerably higher in a PTZ-induced model. GABA levels are higher in the test concentration than they are in the illness control.

GABA Concentration Prediction using Sample Extract (EECT)

The RF value for GABA at 1000 ng/mL is 0.769, while the RF value for EECT at 1000 ng/mL is 0.76. Comparing the Rf value of EECT can confirm the presence of GABA in the test extract to Standard GABA.

Molecular Docking Studies

Repeating virtual docking studies for the best five hits from the mcule.com server allowed us to gain a deeper knowledge of ligand-target interactions.

Glycine Agonist (5I57)

(X = -8.238400 Y = 9.608000 Z = -36.613200) (Figure 5).

GABA Agonist (4MS3)

(X = -8.358500 Y = 30.150833 Z = -40.605000) (Figure 6).

DISCUSSION

Here, we investigated the anticonvulsant and anxiolytic effects of an ethanolic *Clitoria ternatea* root extract utilizing strychnine-induced convulsions and the EPM and staircase tests, respectively.

After the stroke, epilepsy is the most common brainv disorder. It puts a significant burden, hence it is essential to study newer anticonvulsant drugs with improved side effect profiles and increased pharmacological efficacy as a result of the long-term nature of epilepsy treatment and the wide variety of adverse effects and unpredictable pharmacological effects

of currently available antiepileptic drugs. Due to their mild bioreactivity, traditional herbal remedies are safer to use. A major focus of the study is the isolation and characterization of active chemicals from medicinal plants for application in the creation of novel therapeutics.

In this study, we used two dosages of strychnine to assess EECT's ability to prevent convulsions in rats. The usual dose of phenytoin was 25 mg/kg. When the seizures first started and how many people died were both noted.

Strychnine brings on convulsions because it blocks the inhibitory neurotransmitter glycine from doing its job in the neurons of the spinal cord. To counteract the inhibitory effects of glycine on receptors, strychnine competes with it for binding sites. It has been discovered that strychnine and glycine both influence the same receptor in distinct ways. In rats, EECT significantly postponed the start of convulsions at 200 and 400 mg/kg. 200 and 400 mg/kg were similar to phenytoin in reducing the frequency of convulsions. There was no mortality in the treated animal of EECT groups. Researchers have found that some alkaloids and flavonoids can reduce the severity of convulsions.

Since flavonoids share molecular similarities with benzodiazepines, they often operate as anticonvulsants *via* altering the $GABA_A$-Cl channel combination. In experimental epilepsy models, the flavonoids quercetin, isoquercitrin, and rutin exhibited anticonvulsant effects. Quercetin demonstrated anticonvulsant qualities as well. It is suggested that it inhibits NMDA receptors and modulates GABA receptors. By reducing oxidative stress, rutin exhibited an anticonvulsant effect in kainic acid-induced convulsions. The $GABA_A$ receptor is positively allosterically modulated by rutin, making it a useful ligand for benzodiazepine receptors. The presence of different flavonoids in EECT can be ascribed to its anticonvulsant properties.[14]

In order to measure the effectiveness of "anxiogenic and anxiolytic" medications in rodents, the EPM is employed as a model. In EPM, rats' avoidance of open areas is interpreted as a sign of anxiety; therefore, they tend to spend more time where they feel safest. Thus, less anxiousness would lead to more open-armed embraces. When compared to the control group, the number of open-arm entrances and total time spent in the open arms for the EECT at doses of 200 and 400 mg/kg were considerably higher in this study. A larger dose of EECT (400 mg/kg) showed dose-dependent anxiolytic action, suggesting that this effect may require quite high levels. When compared to the gold standard medicine lorazepam, EECT's effect in this model is quite close at.[15]

The staircase test is a simple and reliable approach for screening anxiolytics that has been used in a number of laboratories. The anxiety that mice feel when they are placed in a new setting causes them to be more alert and active than usual. The staircase paradigm claims that step-climbing reflects exploratory or locomotor activity and that rearing behavior is an indicator of the subject's anxiety level. The number of rears and the number of steps taken in a three-minute period are both recorded.[16]

It has been found that EECT significantly increases the number of steps ascended and decreases the number of rearings. Anxiolytic effects were noticeable with a dose of 0.05 mg/kg of the commonly used drug lorazepam. The anti-anxiety effects of EECT can be traced back to the presence of several flavonoids and terpenoids in the substance.

Clitoria ternatea ethanol extract exhibited anti oxidant action in reducing power assay. Under oxidative stress, the *Clitoria ternatea* ethanolic extract may neutralize a wide variety of free radicals. Having a high reducing power correlates with anti-oxidant activity. For a chemical to be able to reduce, it needs to be in contact with reductants, which are substances with anti oxidative potential that can donate a hydrogen atom to stop a chain reaction caused by free radicals. Measuring the Fe^{3+}-Fe^{2+} complex provides information on a compound's reducing capability. The reduction of Fe^{3+} is a key mechanism of anti oxidant action, and it is caused by the electron-donating activity of EECT. As absorption rises, EECT's capacity to reduce pollution rises with it. One possible explanation for EECT's reducing power assay is that it contains hydroxyl groups. Possible causes include hydroxyl groups per cell and the presence of alkaloids and flavonoids.[17] *In-silico* docking studies represented the highest docking scores in both anticonvulsant and anxiolytic activities with respective receptors glycine and GABA.

CONCLUSION

Reports indicate that the antiepileptic and anxiolytic effects of an ethanolic root extract from *Clitoria ternatea* are particularly potent. Further isolation, identification, and confirmation of the exact mechanism is required.

ETHICAL APPROVAL

The IAEC of GRCP approved the research entitled "Pharmacological Evaluation of alcoholic extract of *Clitoria ternatea* root for anxiolytic and anticonvulsant activity" with Regd number. 1175/PO/Re/S/08/CPCSEA.

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CONFLICT OF INTERESTS

All authors have no conflicts of interest to declare.

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