Integrative Network Pharmacology Approach for the Rational Design of a Polyherbal Anticonvulsant Therapy

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Abstract
Epilepsy, affecting 50 million people worldwide, involves recurrent seizures due to abnormal brain activity. Since existing antiepileptic drugs may be insufficient, exploring alternative therapies is crucial. This study investigated the anticonvulsant effect of traditionally exploited plants namely; *Vacha, Gulvel, Brahmi, Heeng, Jatamansi, Gotu Kola, and Sendha Namak.* The herbs were extracted using water and ethanol and screened for phytoconstituents. Various extract compositions were formulated and assessed using the mouse model for acute toxicity and anticonvulsant activity. The most potent combination was identified and further analyzed through network pharmacology to explore its underlying mechanisms. The phytochemical study showed the presence of saponins, flavonoids, alkaloids, and tannins are responsible for therapeutic benefits. Pharmacological evaluations, including acute toxicity studies and seizure phase analysis, revealed that specific herbal combinations were safe at a dose of 2000mg/kg body weight and significantly reduced seizure intensity and mortality rates compared to the control group. Network pharmacology analysis further identified key target proteins (HTR1B, DRD2, CYP2D6, GLRA1, and NR3C1) involved in seizure modulation, reinforcing the role of serotonergic, dopaminergic, and adrenergic systems in epilepsy pathogenesis. This article highlights the anticonvulsant potential of selected herbal extracts, encouraging further research into their mechanisms and clinical applications for epilepsy treatment.

Keywords: Epilepsy, Anticonvulsants, Polyherbal, Soxhlet method, Animal studies, Network pharmacology.

Introduction

About 50 million people worldwide suffer from epilepsy, a chronic, noncommunicable brain condition. Recurrent seizures, which are short bursts of uncontrollable movement that can affect a portion or the whole body, are its defining feature. Loss of awareness and control over bladder or bowel movements is occasionally also present. Almost 80% of individuals with epilepsy reside in nations with poor and moderate incomes (https://www.who.int/news-room/fact-sheets/detail/epilepsy) [1]. Also in India, 12 million individuals suffer from epilepsy; this field is still incredibly understudied in the area of sociology of health and practice [2]. The prevalence of epilepsy was 5.59-10 per 1000 rural children [3]. Whereas, studies verify that 2.4 million individuals are detected with epilepsy every year and remain untreated [4]. Epilepsy treatment includes antiseizure medications (ASMs), a ketogenic diet, neurostimulation, and surgery for drug-resistant cases. Despite significant advancements in ASMs, a substantial proportion of patients exhibit drug resistance or experience severe side effects, necessitating alternative therapeutic approaches.

Herbal medicine, deeply rooted in traditional healing systems like *Ayurveda*, offers a promising avenue for the development of anticonvulsant formulations due to its multi-targeted mechanisms, neuroprotective benefits, and minimal side effects [5]. Herbs like *Acorus calamus (Vacha)* have been widely used in traditional medicine for cognitive enhancement and neuroprotection. Its bioactive constituents, such as asarones, have shown potential in modulating GABAergic transmission, reducing neuronal hyperexcitability, and exerting antioxidant effects, which are crucial in seizure control [6]. *Tinospora cordifolia (Gulvel)* is a well-known adaptogenic herb with neuroprotective and immunomodulatory properties. Rich in alkaloids and terpenoids, making it beneficial in epilepsy management [7]. *Bacopa monnieri (Brahmi)* is a renowned nootropic herb extensively studied for its cognitive-enhancing and anticonvulsant properties. It exerts its effects by modulating GABAergic and serotonergic pathways and reducing oxidative stress, which helps in mitigating seizure activity [8]. *Ferula asafoetida (Heeng)* has long been used as an anticonvulsant in traditional medicine. It contains sulfur-rich bioactive compounds that potentially reducing seizure susceptibility and protecting neurons from excitotoxic damage [9]. *Nardostachys jatamansi (Jatamansi)* possesses sedative and anxiolytic properties. It modulates oxidative stress markers, and provides neuroprotection, contributing to its role as an anticonvulsant agent [10]. *Centella asiatica (Gotu kola)* is a neuroprotective adaptogen that improves synaptic plasticity, reduces neuronal excitability, and possesses antioxidant properties. These mechanisms help in maintaining neuronal stability and preventing seizures [11]. *Sendha namak (Rock salt)* is traditionally used for its role in electrolyte balance and neurostabilization. Proper electrolyte homeostasis is essential in maintaining neuronal function and preventing abnormal excitability that may lead to seizures.

But when single herbs are used, it shows limited efficacy due to the presence of only a few active compounds, whereas polyherbal formulations leverage synergistic effects, enhancing therapeutic potency and reducing side

effects. Combining multiple herbs can target different pathways, improve bioavailability, and provide broader pharmacological actions, making the treatment more effective [12]. Network pharmacology is a viable technique for mapping the identified interactions and evaluating networks between herbs and target genes to find potential targets based on multi-compound and multi-target theory, according to a recent trend in the study of herbal medicine processes [13,14]. This approach provides a rational, evidence-based foundation for developing optimized herbal formulations, making it a crucial tool in modern phytopharmacology and epilepsy research [15].

Given the complexities of epilepsy and the limitations of current treatments, this study explores the antiepileptic potential of polyherbal formulations through animal studies and network pharmacology**.** The primary objective is to investigate the anticonvulsant properties of traditionally used herbs**,** providing insights into their mechanisms and pathways. By integrating network pharmacology analysis**,** this research goals to assist in the growth of more effective and standardized herbal therapies for epilepsy management.

Materials and methods

The following section outlines the chemicals and procedures used in this research.

Materials and reagents

For this study, various herbs were procured from the Shabbar Ddawasaaz local market in Aurangabad, including *Vacha* (*Acorus calamus*), *Gulvel* (*Tinospora cordifolia*), *Brahmi* (*Bacopa monnieri*), *Heeng* (*Ferula asafoetida*), *Jatamansi* (*Nardostachys jatamansi*), *Gotu Kola* (*Centella asiatica*), and Sendha Namak (rock salt). The Clonazepam was obtained from Abbott, India. Other reagents such as ethanol, chloroform, conc. H_2SO_4, ferric chloride, lead acetate, conc. HCl, Dragendorff's reagent, and deionized water were purchased from Merck Company. Loba Chemie Pvt. Ltd., Mumbai supplied bromine water and Mayer's reagent. All solvents used for research work were of analytical grade.

Animals

The Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy in Aurangabad approved the use of 35 Swiss albino mice under proposal number CCSEA/IAEC/P'ceutics/57/2023-24/182.

Extraction of raw materials using different solvents

The collected herbs were individually extracted using simple decoction and Soxhlet extraction techniques. In the decoction method, approximately 250 g of dried herbal material was coarsely ground and added to 3–5 liters of distilled water (DW) in a boiling vessel, then heated at 80–100°C and simmered for 8–10 hours with occasional stirring. The solvent was gradually evaporated until a thick concentrate formed, which was then filtered using vacuum filtration to isolate the liquid extract from herb residues. The concentrated extract was transferred to a hot air oven at 50–60°C for complete drying, then ground into a fine powder by utilizing a mechanical grinder. Finally, the powdered extract was kept in an airtight, moisture-free, and light-protected container for future use [16]. The same procedure was repeated for all herbs using 1.5 liters of ethanol as the solvent, utilizing the Soxhlet extraction method to enhance the extraction of bioactive compounds [17]. Following extraction, the resulting powders were subjected to preliminary tests for further analysis.

Preliminary phytochemical studies

The first stage in maintaining the quality of herbal extracts or plants is phytochemical (PC) screening [18]. Using a distinct qualitative chemical test, described in Table 1, the PC analysis of 6 herbs extracts was carried out to identify the major phytoconstituents.

Test	Procedure	Observation
Steroids		
Salkowski	2.0 mL Filtrate of plant extract mixed with + 2.0 mL of chloroform + 2.0 mL of conc. H_2SO_4 + shake well for a few minutes	Greenish yellow fluorescence
Tannins		
Ferric chloride ($FeCl_3$)	Extracts + few drops of $FeCl_3$ solution	Blue, green, or black color
Lead acetate [$Pb(C_2H_3O_2)_2$]	Extracts + a few drops of $Pb(C_2H_3O_2)_2$ solution	White precipitate (ppt)
Bromine water	Extracts with bromine water	Decolorization
Alkaloids A few mL of extract + 5.0 mL of 1.5%v/v HCl + filtered. The sample was further processed for the following test:		
Dragendorff's	2-3 mL of Filtrate + few drops of Dragendorff's reagent	Orange-brown ppt
Mayer's	2-3 mL filtrate + a few drops of Mayer's reagent	Ppt
Saponins		
Foam test	20 mL water + 50gm extract (vigorously shaken for 15 min.)	A formation of a foam layer on the top of the test tube
Flavonoids		

Lead acetate	Filtrate + a few drops of $Pb(C_2H_3O_2)_2$	Yellow or yellowish-green ppt
Sulphuric acid	Filtrate + a few drops of sulphuric acid	A color change, such as from orange to red color,

Table 1. Identification test for phytoconstituents [19–21].

Preparation of various combinations of extracts

Various combinations of herbal extracts were prepared by systematically blending different extracts in precise quantities to enhance their therapeutic potential and synergistic effect [22]. The extracts included *Brahmi, Gotu Kola, Jatamansi, Heeng, Sendha Namak, Gulvel,* and *Vacha,* in different compositions mentioned in Table 2, and are named Extract/ test Combinations (EC) I-V.

Herbs	EC–I	EC-II	EC-III	EC-IV	EC–V
Brahmi	0.03g	0.06g	------	-------	0.05g
Gotu Kola	0.03g	--------	0.06g	-------	0.05g
Jatamansi	0.01g	0.01g	0.01g	0.03g	------
Heeng	0.006g	0.006g	0.006g	0.01g	-------
Sendha Namak	0.002g	0.002g	0.002g	0.002g	-------
Gulvel	0.01g	0.01g	0.01g	0.03g	------
Vacha	0.01g	0.01g	0.01g	0.03g	-------

Table 2. Various combinations of herbal extracts.

Animal study of extract combinations

After preparing extracts with different compositions of herbs, they were subjected to animal studies, which included an acute oral toxicity study and a pharmacological study. Swiss Albino mice of both sexes were used for these experiments to determine the safety and efficacy of the ECs. The details of these studies are described in the following section.

Preparation of experimental animals

The study followed ethical guidelines, with approval from the IAEC of Y.B. Chavan College of Pharmacy, Aurangabad. Swiss Albino mice of either sex (weighing 20–30 grams) were housed in an Animal Research Facility under standard laboratory conditions, with a 12-hour light/dark cycle and controlled room temperature, for one week to ensure proper acclimatization before the experiments [23]. Throughout the study, the mice were provided with adequate food, care, and water *ad libitum*, and housed in well-ventilated, spacious cages to maintain their well-being [24]. The experimental protocol was thoroughly reviewed and approved by the IAEC, ensuring compliance with ethical research standards.

Acute oral toxicity study

For this study, five groups of Swiss Albino mice (n=3 per group, both sexes, a total of 15 mice) were used. The study involved administering extract combinations (I–V) containing *Brahmi, Vacha, Gulvel, Jatamansi, Heeng, Rock Salt,* and *Gotu Kola* via oral gavage using an oral feeder needle. The standard dose for acute toxicity assessment was 2000 mg/kg BW. Given the average BW of the mice (25–26 grams), the calculated dose per mouse was 150 mg. According to that, 0.33 ml of this solution was administered to each mouse. Following administration, the animals were closely observed for any mortality signs on days 1, 3, 5, 7, and 14 to assess toxicity [25].

Pharmacological activity

Dose: In the pharmacological study, doses were administered based on the BW of the mice. The Pentylenetetrazol (PTZ) was employed to induce convulsions at a standard dose of 80–100 mg/kg BW. As a standard group, clonazepam, a well-established anticonvulsant, was administered at 0.5 mg/kg BW to evaluate its efficacy in preventing PTZ-induced seizures. Additionally, the extract combinations were given 100 mg/kg BW to assess their potential anticonvulsant properties. This dosing regimen enabled a comparative analysis of the extract's effects against both PTZ-induced convulsions and clonazepam's anticonvulsant action, providing insight into its therapeutic potential.

Procedure for anticonvulsant activity

The study involved seven groups of mice (each consisting of n=5): five test groups, one control group, and one standard group. Each mouse was individually weighed and assigned a unique identification number. The control group received an intraperitoneal injection of PTZ to induce seizures, and the progression of seizure stages—including tail stiffening, muscle jerks, clonus (rapid muscle contractions), stupor, death, and recovery outcomes

were carefully monitored and recorded. Clonazepam was administered orally as a reference anticonvulsant. The test groups received different doses of the experimental extract, also given orally. After a 30-minute interval, PTZ was administered to both the test and standard groups to induce seizures. Observations focused on assessing the extract's effects, including delays in seizure onset or complete seizure prevention, compared to the control and the clonazepam-treated group [26,27].

Statistical analysis

GraphPad Prism 9.5.1 was used to perform statistical analysis. The means, standard deviations (SD), and standard errors (SE) of the data are displayed. Analysis of variance (ANOVA) using a one-way test, Dunnett's multiple, and Tukey's multiple comparison tests were used to evaluate group comparisons. Statistical significance was defined as a p-value < 0.05*, extreme significance as ****, and non-significance as 'ns'.

Network pharmacology

Network pharmacology explores multi-target and multi-pathway interactions. The complete step-by-step procedure is briefly described in the following section.

Protein-protein interaction (PPI) network

A PPI analysis is an essential method for comprehending the intricate role of proteins in various metabolic cascades. Biological processes, functional modalities, and cellular architecture can all be thoroughly understood with the use of this method. STRING 11.0, an advanced virtual screening tool (https://stringdb.org/) [28,29], was used to investigate the complex web of interrelated proteins. The pertinent genetic elements were meticulously added to the STRING database, offering crucial information on complex PPIs. The PPI network's development was carefully adapted to the "*Homo sapiens*" setting, and the parameters used to evaluate the degree of confidence in interactions between target proteins were carefully calibrated for maximum reliability, surpassing the data confidence threshold of 0.9. Individual nodes in this complex network depiction stand in for different proteins, and the connecting edges deftly depict relationships between various protein types [30].

Compound and disease-target genes

Finding the genes associated with the disease is the first important step in building the compound-target network. Relevant information regarding target genes linked to convulsants was systematically gathered from reliable sources, such as GeneCards (https://www.genecards.org/) [31]. Additionally, SwissTargetPrediction provided thorough details on the protein targets of *Bacopa Moneiri* and *Centella Asiatica* (https://www.swisstargetprediction.ch/) [32].

Construction of a compound-target network

Clarifying the intricate chemical pathways at play was the next step after performing the PPI study. This was accomplished by using Cytoscape, a visualization program (version 3.7.1), to create a comprehensive compound-target network. Comprehension and evaluating the interactions between bioactive substances and their particular targets requires a comprehension of this network structure. This method aids in identifying the pathways that make up this intricate biological network [33].

GO and KEGG pathway annotation

The ShinyGo platform was used to annotate the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathways. To improve the accuracy of the data prediction, the GO analysis sought to investigate the network's gene cluster. It offers a methodically arranged list of standardized terms for molecular operations, biological processes, and cellular constituents. It includes carefully chosen and anticipated gene annotations from these terms for a range of species [34]. By providing a resource for pathway enrichment analysis, the annotation of biological processes using GO makes it easier to identify crucial biological processes concerning the goals of the study. Additionally, KEGG (http://www.genome.jp/kegg/pathway.html) was utilized to explore the functions and metabolic pathways of the genes and molecules in the network under investigation [35]. This study contributes to the understanding of the complex interactions between molecular entities and disease mechanisms by clarifying the contributing pathways linked to disease phenotypes.

Results

Extraction of raw materials using different solvents

The extraction of active constituents from the selected herbal raw materials—*Vacha (Acorus calamus), Gulvel (Tinospora cordifolia), Brahmi (Bacopa monnieri), Heeng (Ferula asafoetida), Jatamansi (Nardostachys jatamansi), Gotu Kola* (*Centella asiatica*), and *Sendha Namak* (rock salt)—was performed using water and ethanol as solvents. Based on this study, the ethanolic extracts of the above-mentioned herbs showed better extraction yield (Fig. S1) compared to the water extracts.

Preliminary phytochemical studies

It is beneficial in finding bioactive substances that can be used in the manufacture of valuable pharmaceuticals, as well as revealing the components of plant extracts and the ones that predominate over the others. To identify the phytoconstituents in all extracts, a preliminary phytochemical analysis was conducted using different qualitative test; the findings obtained from these tests as shown in the following Table 3.

Test name	Herbs name

	Vacha	Brahmi	Heeng	Jatamansi	Gulvel	Gotu Kola
Steroids	+	-	-	+	+	+
Tannins						
Ferric chloride	+	+	+	+	+	+
Lead acetate	+	+	+	+	+	+
Bromine water	+	+	+	+	+	+
Alkaloids						
Dragendorff's	+	+	+	+	+	+
Mayer's	+	+	+	+	+	+
Saponins	+	++	+	+	+	++
Flavonoids						
Lead acetate	-	+	-	++	-	++
Sulphuric acid	-	+	-	++	-	++

Table 3. Bioactive screening of selected ethanolic extracts.

Table 3 revealed the phytochemical analysis of *Brahmi, Vacha, Heeng, Jatamansi, Gulvel,* and *Gotu Kola*, highlighting their potential anticonvulsant properties due to their diverse bioactive constituents, including alkaloids, tannins, steroids, saponins, and flavonoids.

Animal study of extract combinations

Extracts were combined to enhance therapeutic efficacy through synergistic interactions, where multiple bioactive compounds work together to improve pharmacological outcomes. In this study, combining *Brahmi, Gotu Kola, Jatamansi, Gulvel, Heeng, Vacha,* and *Sendha Namak* helps target multiple mechanisms, and hence different compositions of polyherbal extracts were prepared (Table 2).

Acute oral toxicity study

This study was done in 5 groups using 5 different extract combinations of herbs. After giving the oral dose, all the animals were monitored for mortality for 14 days. The outcomes obtained from the study are mentioned in following Table 4.

Extract combinations	**Day 1**	**Day 03**	**Day 05**	**Day 07**	**Day 14**
I	00	00	00	00	00
II	00	00	00	00	00
III	00	00	00	00	00
IV	00	00	00	00	00
V	00	00	00	00	00

Table 4. Toxicity study of different polyherbal extracts.

The outcomes of this study show that all extract combinations (*I-V*), prepared using the above herbs, exhibited no signs of toxicity or mortality throughout the 14-day observation period. Hence, these extracts were found to be safe at the given dose.

Pharmacological study

The pharmacological study also revealed the efficacy of herbal extracts in repressing the occurrence and intensity of the phases of the seizures, consisting of Straub's tail, the onset of jerks, clonus, tail extensor, stupor, and mortality (Fig. 2-6). Apart from that, the mean and SD of control, standard, and test combinations are mentioned in Tables S5-S11 (Supplementary materials).

Straub's tail

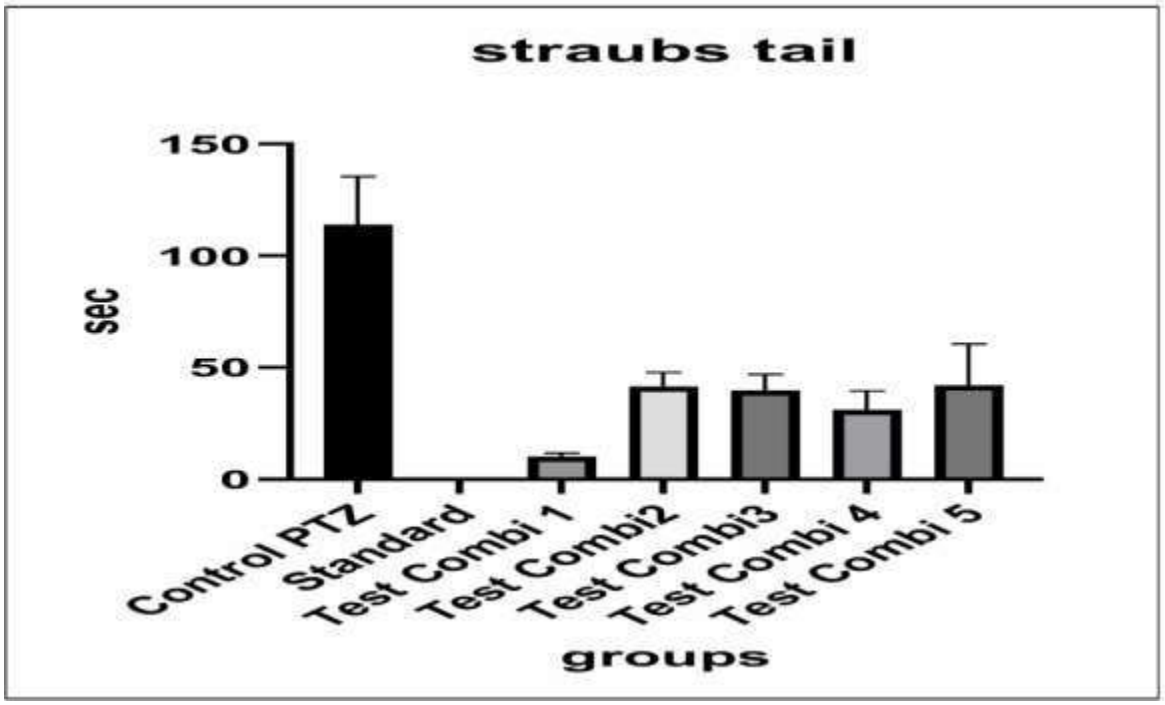

Figure 2. Results of Straub's tail response.

Fig. 2 (Table S12) illustrates Straub's tail response across different experimental groups. The control group exhibits the highest response (~100 sec), indicating intense convulsant activity. The standard group shows minimal response, suggesting adequate protection. Test Combi 1 reduces the response significantly but still affects 60% of animals, implying partial protection. Test Combi 2, 3, 4, and 5 show moderate reductions (~40% of animals affected), suggesting varying degrees of anticonvulsant effects. Overall, the extract combinations reduce PTZ-induced effects, with Test Combi 1 offering better protection than the others except the standard. The ANOVA test confirms a statistically significant difference between groups, suggesting that the extract combinations have a measurable effect for the suppression of PTZ-induced convulsions.

Onset of jerks:

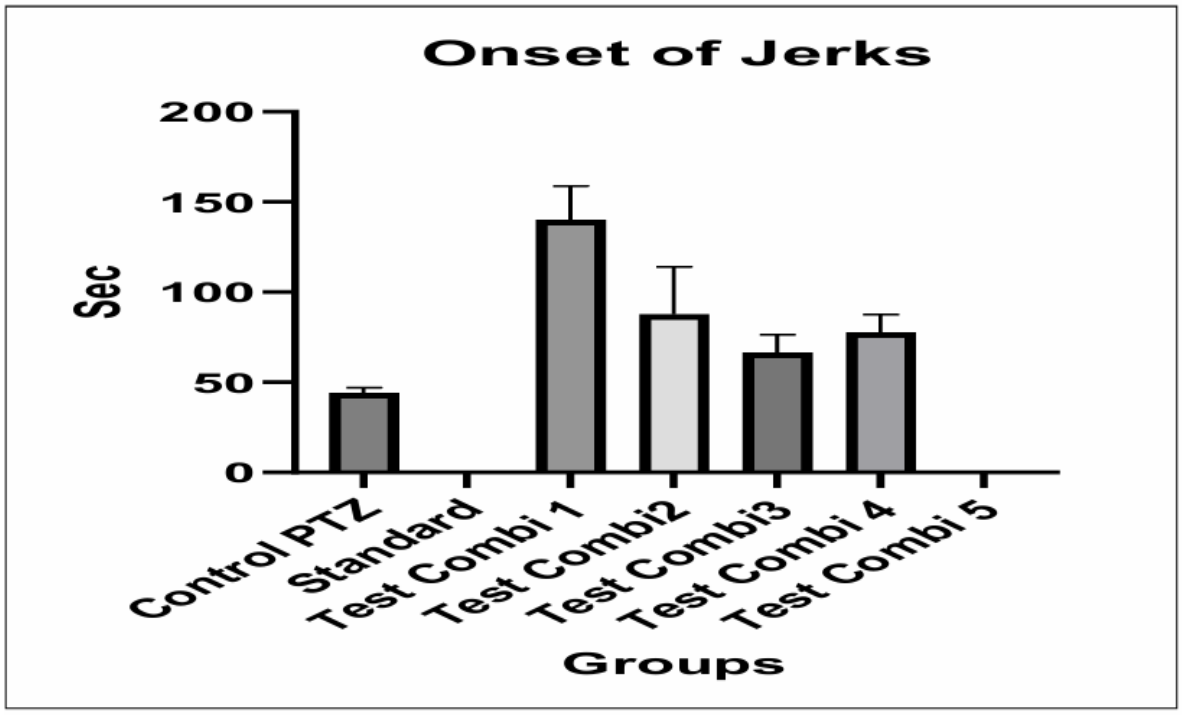

Figure 3. Onset of jerks findings.

Fig.3 represents the onset of jerks in a PTZ-induced seizure model, measured in seconds across different experimental groups. The PTZ group has the shortest onset time, indicating rapid seizure induction. The standard

group slightly delays seizure onset, showing a protective effect. Among the extract combinations, Test Combi 1 exhibits the most significant delay in seizure onset (~150 sec), suggesting strong anticonvulsant potential. Test Combi 5 (0 sec) failed to prevent seizures, while Test Combi 2, 3, and 4, also show delayed onset times compared to the control but with varying degrees of efficacy. The ANOVA results indicate a statistically significant difference among the groups, confirming that the treatments had a measurable effect on the onset of jerks.

Clonus phase:

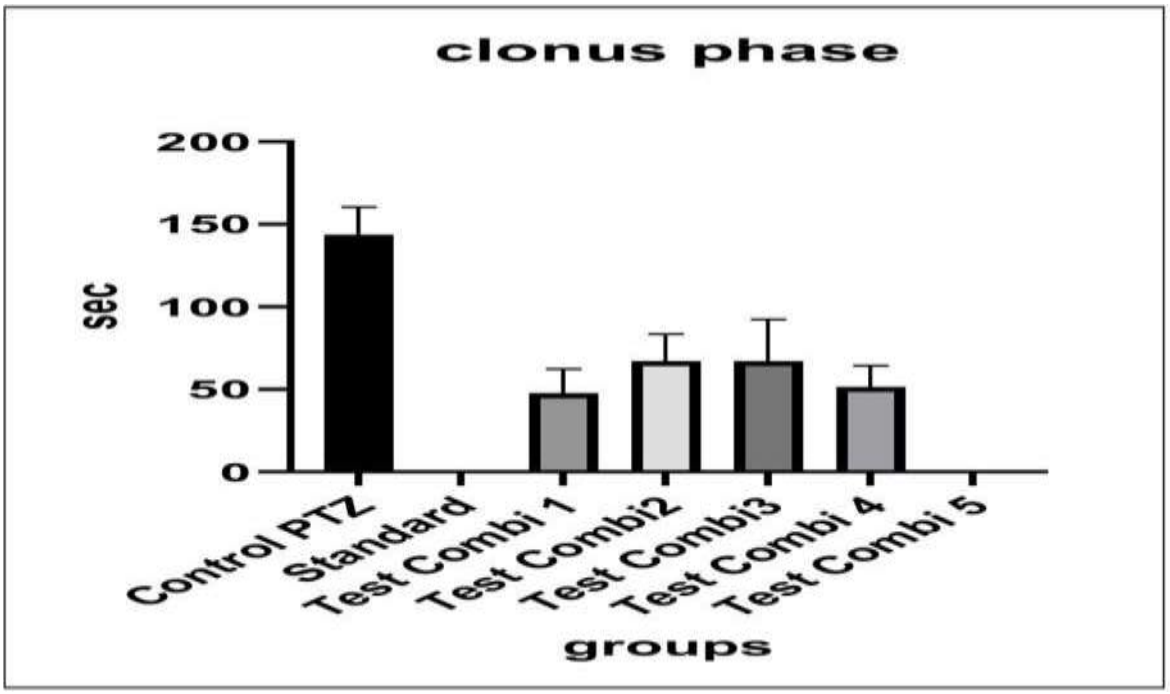

Figure 4. Clonus phase.

The revealed 100% clonus phase occurrence of a control group with the longest duration (~150 sec), while Clonazepam completely suppressed seizures**.** Among the extract combinations, Test Combi 1 and 5 (20%) showed the most protection, followed by other combinations**,** indicating slightly weaker protection. Although all test groups reduced seizure severity, comparatively less than clonazepam's efficacy.

Tail extensor:

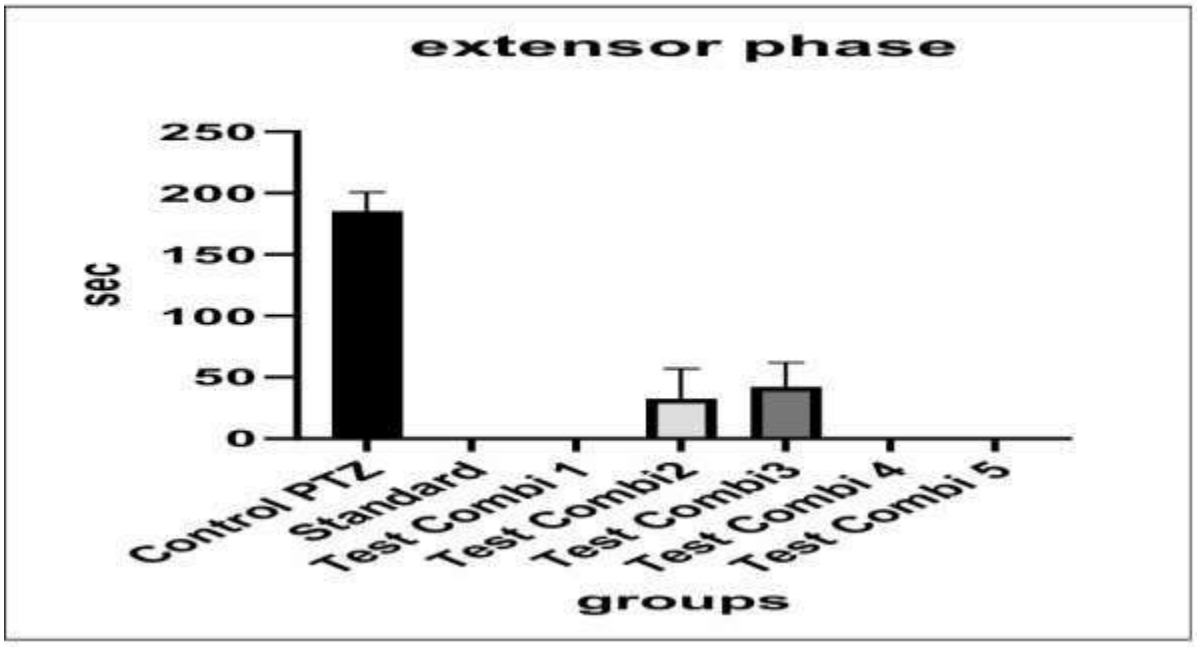

Figure 5. Extensor phase.

The data shows that the Control group exhibited prolonged extensor phase durations**,** confirming the severity of seizures. Clonazepam completely suppressed the extensor phase**,** demonstrating its strong anticonvulsant effect. Among test combinations, Test Combi 2 and 3 showed partial protection**,** with some animals experiencing reduced extensor duration, while Test Combi 1, 4, and 5 appear to be the most effective in preventing the extensor phase, similar to clonazepam. The ANOVA results confirm a statistically significant difference among groups,

suggesting that certain test combinations may have anticonvulsant potential.

Stupor phase:

The stupor phase refers to a postictal state characterized by immobility and impaired responsiveness following seizures. In the PTZ group**,** a 100% incidence rate was observed, indicating severe postictal effects. The Clonazepam group showed complete prevention of the stupor phase, confirming its strong anticonvulsant and neuroprotective effects. Among the test combinations, Test Combi 1, 2, 3, and 4 each suggest partial protection against PTZ-induced stupor. Test Combi 5 appears to be the most effective**,** showing no stupor phase and potentially offering better neuroprotection.

Mortality/recovery phase:

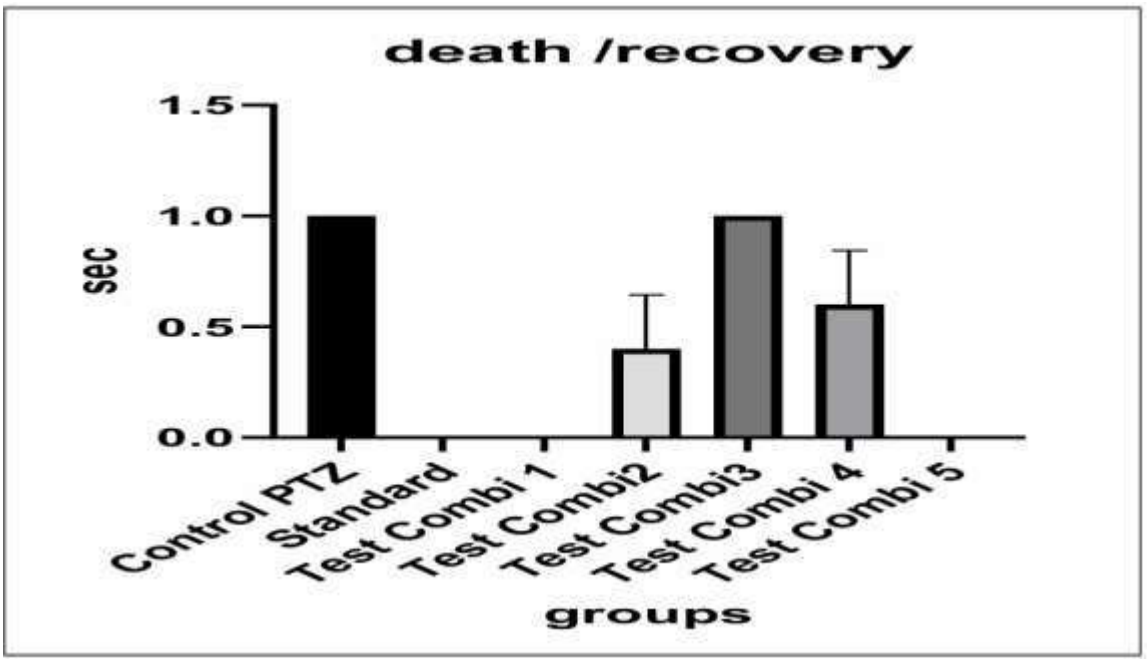

Figure 6. Mortality phase results.

The mortality/recovery phase data indicate that the control group had 100% mortality, while the standard group showed complete protection (0 mortality)**.** Test combinations varied in their protective effects: Test Combi 1 showed full recovery (0 mortality), Test Combi 2 had a 40% mortality rate, Test Combi 3 had 100% mortality, Test Combi 4 had 60% mortality, and Test Combi 5 had full recovery (0 mortality). The ANOVA results confirm a statistically significant difference among groups, suggesting that some test combinations offered partial protection, while Test Combi 3 was ineffective in preventing mortality.

Network pharmacology

Construction and analysis of the PPI network

The target genes linked with the respective constituents were meticulously studied using STRING v_11 to construct and visualize the PPI network (Fig. 7). The network topology, with a moderate average node degree (1.94) and clustering coefficient (0.388), suggests functional cooperation among these targets. The network consists of multiple interacting proteins, with HTR1B, DRD2, and CYP2D6 acting as central hubs, suggesting a significant role of the serotonergic and dopaminergic systems in convulsant mechanisms. The clustering of HTR1A, HTR2C, and NR3C1 indicates a regulatory interplay between neurotransmitter signaling and stress response pathways, which are known to influence seizure threshold. Additionally, the presence of growth factors (FGF2, EGF2, and COL18A1) in the network suggests neuroprotective roles that may counteract seizure-induced neuronal damage. Signaling proteins such as STAT3, PTPN1, and PPP2CA indicate involvement in neuroinflammation and synaptic plasticity, both of which are crucial in seizure propagation. Reduced glycine receptor (GLRA1) function leads to the disinhibition of neuronal circuits**,** increasing seizure susceptibility. The adrenergic receptors (ADRA1A, ADRA2A, ADRA2B, and ADRA2C) are also prominently connected, highlighting their role in modulating the excitatory-inhibitory balance in the brain. DRD1, a key excitatory dopaminergic receptor, contributes to proconvulsant activity by enhancing cortical excitability and seizure propagation.

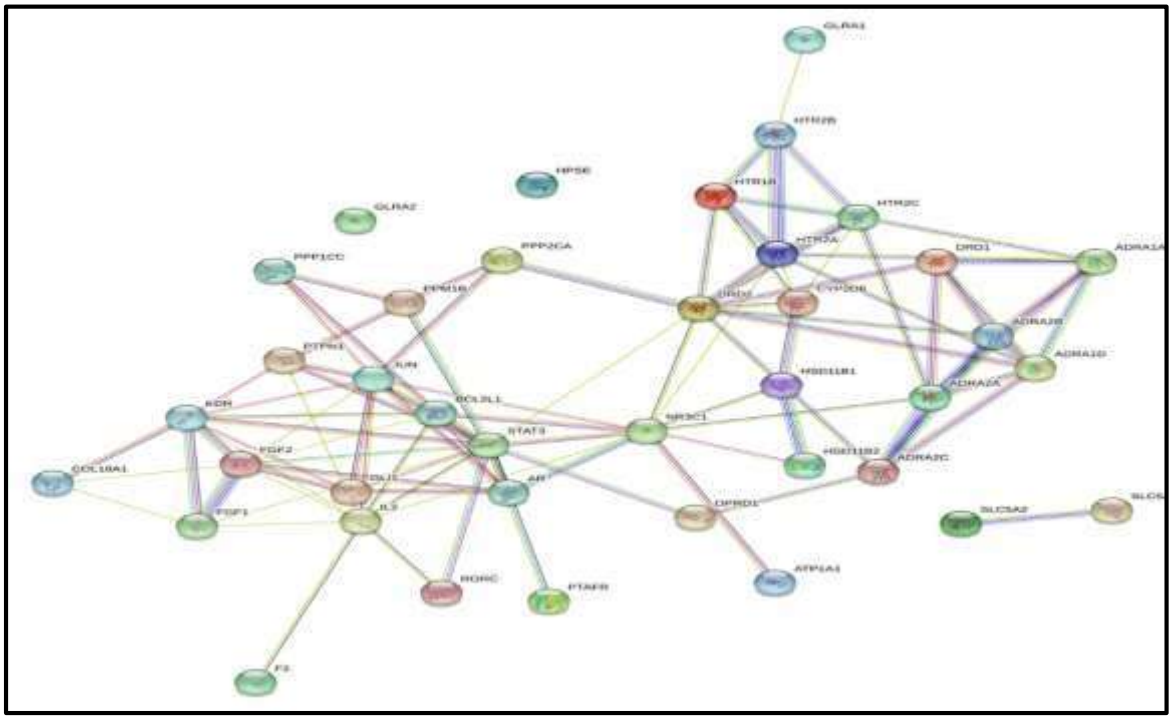

Figure 7. Representation of PPI network.

Compound and disease-target genes

A total of 5561 disease-related genes were retrieved from the GeneCards, with an additional 6 compound-specific targets identified. Through the intersection of these datasets, a final set of 39 overlapping targets was recognized, as depicted in the Venn diagram (Fig.8). These shared targets may represent key molecular mediators where the compound exerts its therapeutic effects in modulating disease pathology.

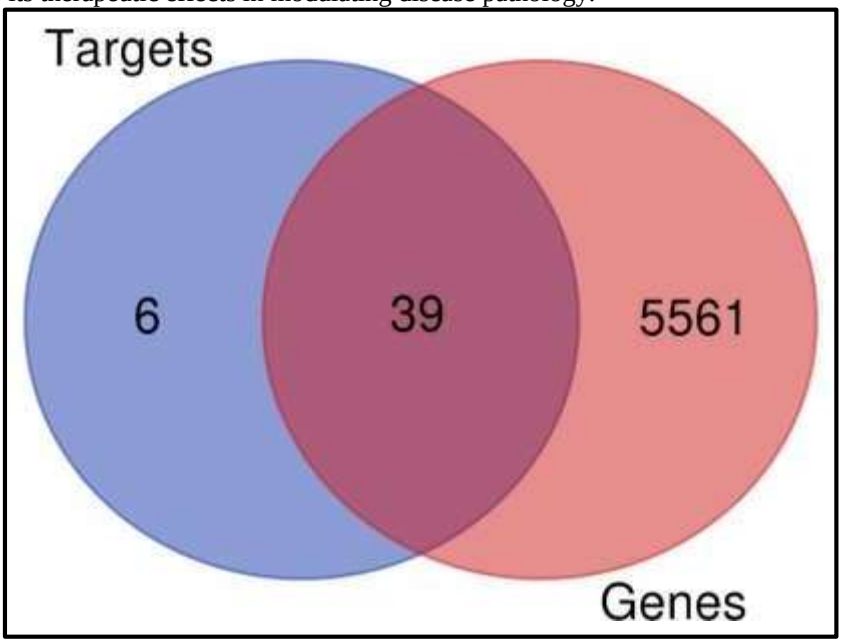

Figure 8. Venn diagram.

Construction of a compound-target network

The data was imported and analyzed using Cytoscape to investigate the signaling pathways and functional implications of the selected target genes. The network illustrates the interactions between bioactive compounds (Bacoside A, Bacoside A3, Asiaticoside B, and Bacosaponin C) and their target proteins, highlighting their potential role in modulating epilepsy-related pathways**.** From Fig.9, it is indicated that the key convulsant-related targets include HTR2A, HTR1B, HTR2B, DRD1, DRD2, and TACR2, which regulate neurotransmitter balance and may contribute to seizure susceptibility when dysregulated. Additionally, inflammatory and apoptotic mediators such as STAT3, BCL2L1, IL2, and VEGFA exacerbate seizure activity by promoting neuroinflammation. Conversely, neuroprotective and anticonvulsant targets such as GLRA1, GLRA2, NR3C1, and OPRD1 enhance inhibitory neurotransmission, regulate stress responses, and suppress seizures.

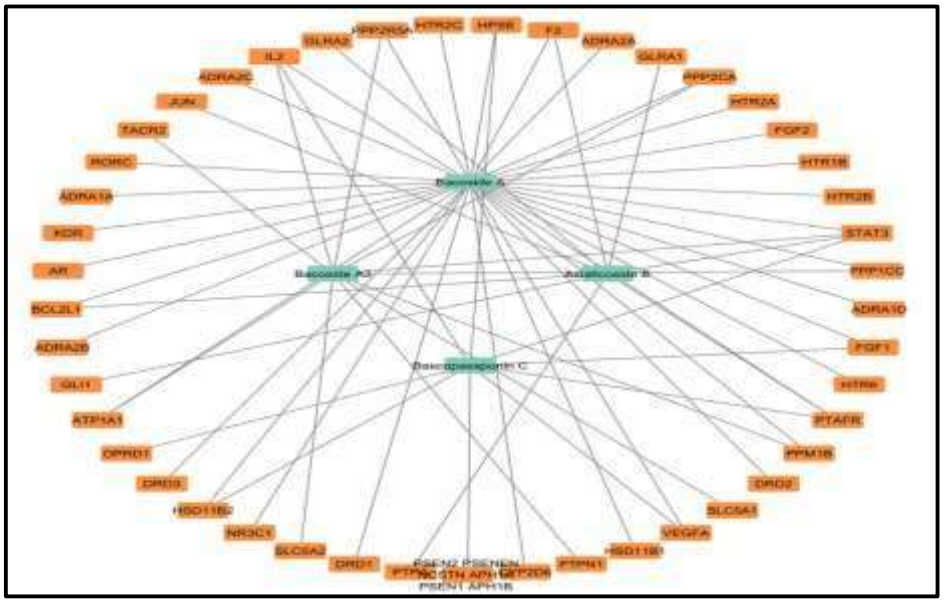

Figure 9. Compound target network.

GO and KEGG pathway annotation

For the underlying target proteins, GO enrichment analysis was used. Using the ShinyGo platform, the KEGG and GO pathways were annotated.

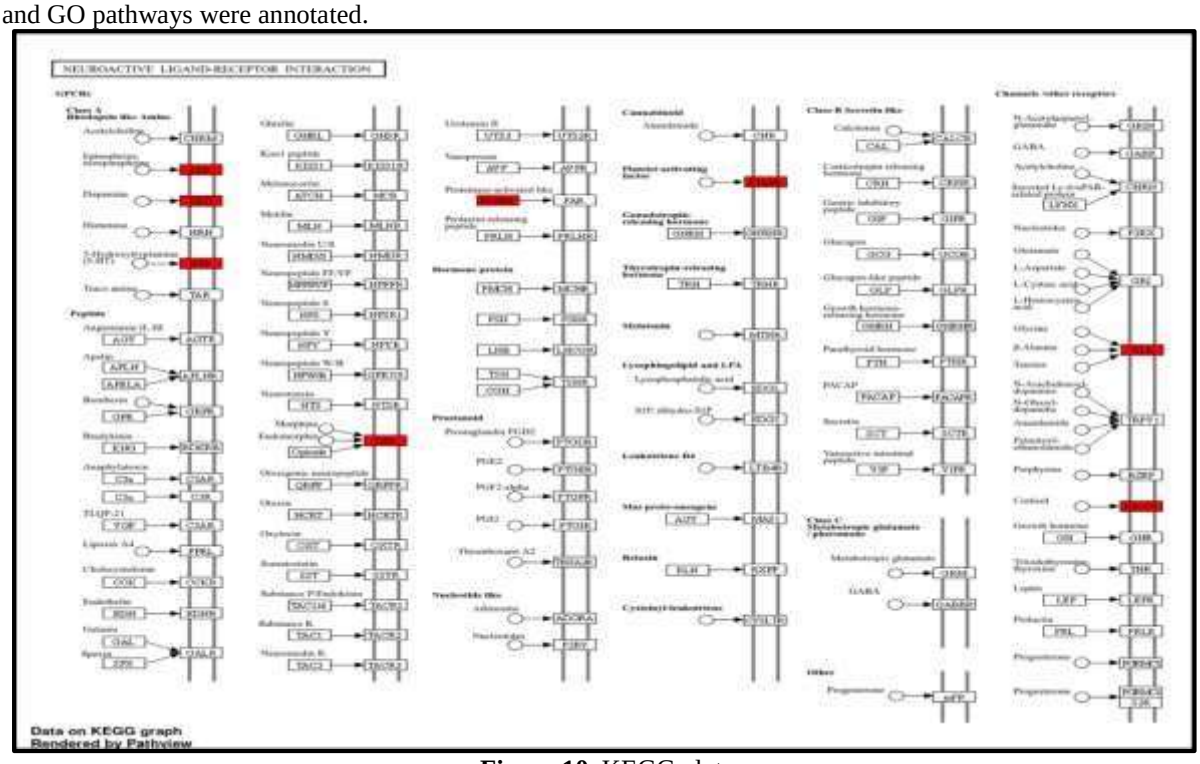

Figure 10. KEGG plot.

This KEGG pathway map illustrates neuroactive ligand-receptor interactions, emphasizing key neurotransmitters and their receptor targets. The highlighted (red) receptors indicate significant alterations, likely associated with dysregulated neurotransmission. These include dopamine (DRD1), histamine (HRH1), opioid (OPRD1), neuropeptide (NTSR1), and glutamate (GRIN2C) receptors, which are crucial in modulating neuronal excitability, pain perception, and mood regulation. Dysregulation of these pathways is implicated in neurological disorders, including epilepsy, depression, and neurodegenerative diseases (Fig.10). Key signaling pathways associated with convulsant activity are shown in figure S11.

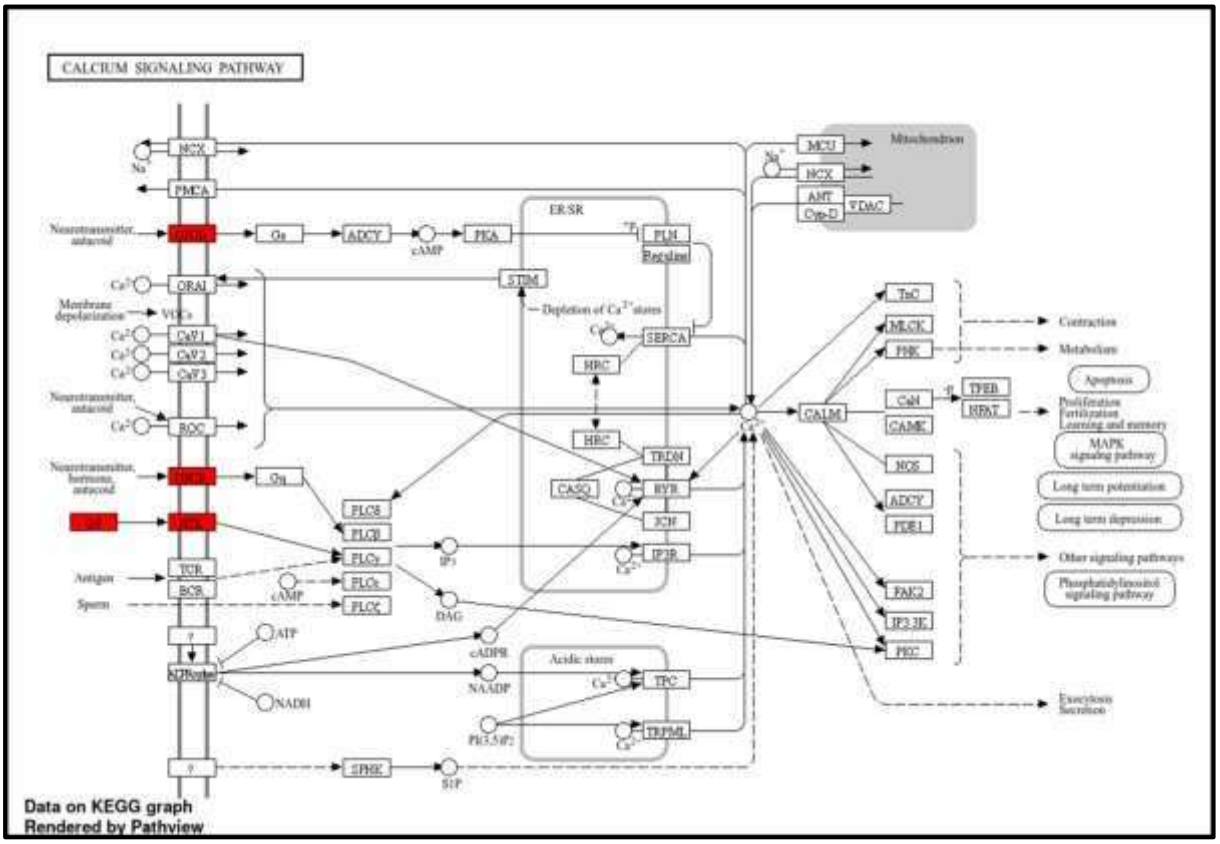

Figure 12. Calcium Signaling Pathway.

This Pathway highlights the dysregulation of calcium homeostasis, which plays a crucial role in neuronal excitability and seizure generation. Overactivation of GPCRs and RTKs (marked in red) can lead to excessive calcium influx through voltage-gated calcium channels (CaV1, CaV2, CaV3) and receptor-operated channels (ROC), triggering abnormal neurotransmitter release and hyperexcitability. Dysfunctional intracellular calcium regulation via IP_3R and RYR may contribute to excitotoxicity, leading to neuronal damage and prolonged seizures. Calcium-dependent signaling cascades involving calmodulin (CALM) further modulate apoptosis, synaptic plasticity, and long-term potentiation, potentially exacerbating seizure susceptibility (Fig.12).

Discussion

Epilepsy is a disorder of the brain characterized by repeated seizures [36]. Anyone can develop epilepsy. It affects both men and women of all races, ethnic backgrounds, and ages [37]. About one-third of the patients had drug-resistant epilepsy according to a definition proposed by Kwan & Brodie (2000) [38], and this fact makes us realize there is no other most immediate target for another therapeutic approach different than ASM. The present study was designed to evaluate anticonvulsant properties of herbal extracts *Vacha, Gulvel, Brahmi, Heeng, Jatamansi, Gotu Kola, and Sendha Namak*. These are traditionally employed for their anticonvulsant effects in systems like Ayurveda and Traditional Chinese Medicine (TCM). The extraction of these herbs indicated a higher yield in ethanol solvent. The extraction yields vary depending on solvent polarity and chemical composition of each herb [39]. Ethanol, being a moderately polar solvent, demonstrated higher extraction efficiency for phytoconstituents such as flavonoids, alkaloids, and terpenoids, and it can dissolve both polar and non-polar compounds. In contrast, water showed better extraction for polysaccharides and certain glycosides due to its highly polar nature [40]. These herbs were further screened for primary PCs like tannins, flavonoids, steroid alkaloids, saponins, and various others that have neuroprotective or anti-epileptic activity as per the report published by *Sridharan et al* [41]. An acute toxicity study was performed using a mouse model. The results revealed an absence of any toxic effects **(**00 values across all time points), suggesting that these herbal extracts, in their respective combinations, are well-tolerated and safe for oral administration at the 2000 mg/kg dose. These findings provide preliminary safety validation, allowing for further pharmacological evaluations to assess their anticonvulsant efficacy [42]. The pharmacological study also revealed the effectiveness of polyherbs in suppressing the occurrence and intensity of the phases of the seizures, consisting of Straub's tail, onset of jerks, clonus, tail extensor, stupor, as well as mortality. In various tests involving combinations of extracts of *Brahmi* and *Golu kota*, a marked reduction in the intensity of seizures and a higher percentage of convalescence was observed than in the control set with 100% convulsions administered. Further, by using network pharmacology, the complex interactions between drugs, targets, and disease-related pathways were analyzed. *Huang et al.* used this technique for exploring the anti-epileptic multi-target mechanism of *Rhizoma Coptidis* [43]. In another study, the key molecules in (*Brahmi Nei*) BN revealed their ability to modulate molecular targets implicated in memory, cognition, neuronal survival, proliferation, regulation of cellular bioenergetics, and oxidative stress using the same technique [44]. In our study,

in the PPI network**,** proteins like GLRA1, BCL2L1, and NR3C1 serve as potential therapeutic targets for epilepsy, whereas DRD1, HTR2A, and ADRA1A are implicated in seizure pathogenesis. This offers insights into novel therapeutic strategies aimed at modulating neurotransmitter systems to control seizures. The network illustrates the interactions between bioactive compounds (Bacoside A, Bacoside A3, Asiaticoside B, and Bacosaponin C) and their target proteins, highlighting their potential role in modulating epilepsy-related pathways**.** Whereas compound disease target genes showed the overlap of 39 targets that could serve as critical intervention points, supporting their potential role in drug repurposing, biomarker discovery, and precision medicine strategies for effective disease management. Although more controlled clinical trials should be conducted to confirm these results and to develop modern clinical guidelines for the use of these treatments in today's practice, which may offer an enhanced quality of life for patients with drug-resistant epilepsy.

Conclusions

This study highlights the significant anticonvulsant potential of traditionally used herbal extracts, demonstrating their ability to reduce seizure intensity and recovery in the PTZ-induced seizure model. The existence of bioactive such as flavonoids, saponins, alkaloids, and tannins contributes to their therapeutic efficacy. Acute toxicity studies confirmed the safety of the selected combination at a dose of 2000 mg/kg BW. Additionally, network pharmacology analysis identified key molecular targets (HTR1B, DRD2, CYP2D6, GLRA1, and NR3C1), reinforcing the involvement of serotonergic, dopaminergic, and adrenergic pathways in epilepsy modulation. These findings support the integration of polyherbal formulations as complementary therapeutic strategies, warranting further research for clinical translation.

Data availability

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request

Competing Interest

The authors declare no competing interests.

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