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Uncovering The Anticonvulsant Mechanisms of *Saussurea Lappa*: A Network Pharmacology and Molecular Docking Approach

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Abstract: *Saussurea lappa*, a traditional medicinal herb, has been explored for its potential anticonvulsant effects through a combination of network pharmacology and molecular docking approaches. This study aimed to uncover the molecular mechanisms underlying the anticonvulsant activity of *Saussurea lappa* by identifying key bioactive compounds and their interactions with epilepsy-related targets. The analysis revealed several bioactive compounds with favorable pharmacokinetic properties, including Costunolide, Curcumen, and Dehydrocostus lactone, which demonstrated high oral bioavailability (Costunolide: 0.55; Curcumen: 0.67; Dehydrocostus lactone: 0.61) and promising drug-likeness scores. Network pharmacology analysis identified significant interactions with critical epilepsy-related targets, such as SCN1A, GRIN2A, and GABRA1, which are associated with neuronal excitability and synaptic transmission. Functional enrichment analysis further supported the involvement of these compounds in key neurological processes, including neurotransmitter signaling pathways and ion channel activity. Molecular docking studies revealed strong binding affinities between the bioactive compounds and epilepsy-related proteins, with Costunolide showing the highest docking score against SCN1A (-8.2 kcal/mol), indicating a potential role in modulating voltage-gated sodium channels. The interaction between Curcumen and GABRA1 (-7.8 kcal/mol) also suggests potential modulation of GABAergic signaling. These findings suggest that *Saussurea lappa* contains bioactive compounds with significant anticonvulsant potential, offering insights into their molecular mechanisms of action. The study provides a strong foundation for further preclinical and clinical investigations, with the potential to develop novel anticonvulsant therapies based on *Saussurea lappa*.

Keywords: *Saussurea lappa*, Anticonvulsant activity, Epilepsy, Network pharmacology, Molecular docking, Bioactive compounds

1. Introduction

Epilepsy is one of the most common neurological disorders, affecting approximately 50 million people worldwide. [1] Characterized by recurrent, unprovoked seizures, epilepsy significantly impacts the quality of life of those affected, often leading to social stigmatization, psychological distress, and, in severe cases, increased mortality. [2] The disorder's burden is particularly pronounced in low- and middle-income countries, where access to healthcare and effective treatment options is often limited.

Despite advancements in pharmacotherapy, approximately one-third of epilepsy patients remain resistant to existing anticonvulsant drugs, leading to what is known as refractory or drug-resistant epilepsy. [3] Moreover, the long-term use of conventional anticonvulsants is frequently associated with adverse side effects, such as cognitive impairment, dizziness, fatigue, liver toxicity, and teratogenicity. [4] These limitations underscore the urgent need for safer and more effective anticonvulsant therapies, prompting the exploration of alternative treatments, including those derived from natural sources.

Saussurea lappa, commonly referred to as costus or kuth, is a perennial plant native to the Himalayan region. It has been revered in traditional medicine systems, such as Ayurveda,

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Traditional Chinese Medicine (TCM), and Unani, for its diverse therapeutic properties. [5] Historically, *Saussurea lappa* has been employed to treat a variety of ailments, including digestive disorders, respiratory conditions, and inflammatory diseases. Importantly, it has also been used for its neuroprotective and sedative properties, making it a potential candidate for managing neurological disorders, including epilepsy. [6] The plant's roots contain a rich array of bioactive compounds, such as sesquiterpene lactones, which are believed to contribute to its medicinal effects. Despite its traditional use in neurological treatments, scientific validation of its anticonvulsant potential remains limited, necessitating further investigation. [7]

In recent years, there has been growing interest in the potential of natural products as sources of novel therapeutic agents. Plants, in particular, are recognized for their vast chemical diversity and ability to produce compounds with unique biological activities. [8] Given the limitations of current anticonvulsant drugs, natural products like *Saussurea lappa* offer a promising alternative for discovering new anticonvulsant agents that may have fewer side effects and better efficacy. The exploration of such natural remedies is essential not only for expanding the therapeutic arsenal against epilepsy but also for providing options that are accessible and affordable, especially in resource-limited settings.

The advent of advanced computational tools has revolutionized the process of drug discovery, enabling more efficient identification of potential therapeutic agents. Network pharmacology, an emerging field that integrates systems biology with pharmacology, offers a holistic approach to understanding the complex interactions between bioactive compounds and their targets within a biological network. This approach is particularly valuable for studying the multifaceted effects of natural products, which often act on multiple targets simultaneously.[9] Complementing this, molecular docking techniques allow for the *in silico* assessment of the binding affinities between compounds and specific molecular targets, providing insights into the potential mechanisms of action. Together, these tools facilitate a more comprehensive exploration of *Saussurea lappa*'s anticonvulsant potential, helping to identify key compounds and pathways involved in its activity. [10]

Due to the emergence of advanced computational tools, the process of drug discovery has reached a new paradigm where accurate and effective modeling of intricate biological interactions is possible. Network pharmacology depicts the interactions of numerous target proteins, while molecular docking and dynamics simulate the binding of bioactive compounds to certain target proteins and the stability of the binding. These methods serve as integration of old concepts and modern science and consequently allow early virtual screening tests even before laboratory tests are conducted which can save time and costs [11].

This study aims to bridge the gap between traditional knowledge and modern science by employing network pharmacology and molecular docking to uncover the anticonvulsant mechanisms of *Saussurea lappa*. By doing so, it seeks to provide a scientific basis for the plant's traditional use and to contribute to the development of novel, plant-based anticonvulsant therapies.

2. Materials and Methods

2.1. Identification and ADME Evaluation of Potential Compounds

The identification of active chemicals from *Saussurea lappa* was conducted by utilizing database such as Indian Medicinal Plants, Phytochemistry and Therapeutics Database (IMPPAT), Chemical Entities of Biological Interest (ChEBI), KNApSAcK, and Dr. Duke's Databases. The Absorption, Distribution, Metabolism, and Excretion (ADME) qualities of these substances were assessed based on factors such as bioavailability through the mouth and drug-like characteristics. Compounds that had an oxygen balance (OB) of 30% or higher and a detonation velocity (DL) of 0.18 or higher were chosen for additional examination.[12]

2.2. Prediction and Screening of Target Genes

The identification of target genes linked to the bioactive substances was accomplished through the utilisation of STITCH and Swiss Target Prediction databases. Disease-associated targets were obtained by searching the GeneCards database utilising the phrase "autism spectrum disorder." Shared targets between chemicals and epilepsy were determined employing a Venn diagram and subsequently analysed further.[13]

2.3. GO Enrichment and KEGG Pathway Analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) database and shinygo server were utilised to conduct gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway studies. For detailed analysis, we picked the ten most significant GO keywords and KEGG pathways that are related with epilepsy [14].

2.4. Protein-Protein Interaction (PPI) Analysis

PPI analysis was conducted using the STRING database to identify and evaluate interactions among proteins related to epilepsy pathways. The network was visualized in Cytoscape, and hub genes were identified using the CytoHubba plugin. This step allowed us to prioritize genes most central to the epilepsy pathway. [15]

2.5. Network Construction

A network diagram was created in Cytoscape to illustrate the relationships between bioactive compounds, target genes, and pathways. The degree of relevance for each component was calculated using the Network Analyzer and mapping of complex

interactions to better understand *Saussurea lappa*'s multi-targeted effects [16].

2.6. Molecular Docking Analysis

The process of molecular docking was carried out utilising the cb-dock 2 server protocol. The 3D configurations of the target proteins as well as bioactive chemicals were obtained using the PDB and PubChem databases in addition respectively. The most well-arranged complexes were visualised using Discovery Studio Visualizer, where detailed ligand-receptor interactions, such as hydrogen bonds, hydrophobic contacts, and electrostatic interactions, were analyzed [17].

2.7. Molecular Dynamics Simulation

It is carried out using the https://imods.iqf.csic.es/. The dynamic behavior of the compound-receptor complexes was analyzed over a 100 ns simulation period to observe the conformational changes, flexibility, and stability of each complex. Trajectories were also processed [18].

3. RESULTS

3.1. Identification and Selection of Bioactive Compounds

Bioactive compounds were identified from *Saussurea lappa*. All were mentioned in table 1 with their class. These compounds showed high oral bioavailability and drug-likeness scores, making them suitable for further analysis.

These compounds exhibit bioavailability and permeability properties related to their structural features. Costunolides, dihydrocostus lactones, and curcumin are sesquiterpenes with stable rings and water-absorbing properties that promote membrane permeability and drug-likeness. Lincidomine and spermine hydrochlorides, with low Log P values, are suitable for water absorption around us to increase the solubility [19]. Myristicin, sirigaresinol, and alpha terpineol have moderate lipophilicity, supporting good membrane permeability. High lipid concentrations of pristimarin, stigmasterol, betulin, and ethyl linoleate indicate strong membrane affinity but may reduce solubility. Saussureamines A-D show potential interactions between receptors due to their hydrogen bonds, while the water solubility of Xanthosine supports cellular interactions. Cinnamic acid and diethyl di maleate for a balanced lipophilicity for absorption. The unique molecular properties of each compound influence its bioavailability, solubility, and cellular permeability [20].

The physiochemical parameters, oral bioavailability, and drug-likeness scores presented in Table 2 provide crucial insights into the potential of various chemical constituents as oral drugs. Compounds like Costunolide, Curcumen, and Dehydrocostus lactone exhibit favorable drug-likeness and good oral bioavailability, characterized by appropriate molecular weight, a low number of rotatable bonds, and suitable Log P values. These properties suggest that these compounds are likely to be absorbed well and have potential as orally administered drugs. In contrast, some larger molecules, such as the Saussureamines and Betulin, exhibit poor oral bioavailability and unfavorable drug-likeness scores, likely due to their higher molecular weights, increased hydrogen bond donors/acceptors, and Log P values, which could hinder their absorption and overall drug-like behavior. These findings underscore the importance of physiochemical properties in drug design and highlight Costunolide as a particularly promising candidate for further development [21].

3.2. Results of Prediction and Screening of Target Gene

The predicted targets for the bioactive compounds in Table 3 highlight their potential to modulate key receptors and ion channels associated with epilepsy. Notably, compounds like Costunolide and Dehydrocostus lactone target critical components such as GABA receptors, voltage-gated sodium channels, and NMDA receptors, which are central to controlling neuronal excitability and synaptic transmission. The widespread targeting of GABA receptors across many compounds underscores their potential to enhance inhibitory neurotransmission, a crucial mechanism in anticonvulsant therapy. The Venn diagram (Figure 1) further illustrates the overlap between bioactive targets and known disease-associated genes, such as GRIN2A, SCN1A, and GABRA1, reinforcing the relevance of these compounds in modulating pathways implicated in epilepsy. These findings suggest that the bioactive constituents studied not only interact with key epilepsy-related targets but also hold promise for the development of novel therapeutic strategies aimed at modulating these critical pathways [22].

3.3. Target Genes and Pathway Analysis

Figure 2 provided a network analysis focusing on protein-protein interactions (PPIs), The PPI network with 20 nodes and 100 edges shows a high level of connectivity, with an average node degree of 10, indicating dense interactivity among proteins. The average local clustering coefficient of 0.789 reflects strong clustering within the network, suggesting that proteins form tightly-knit groups or functional modules. The observed number of edges (100) significantly exceeds the expected number (4), highlighting that the network is substantially more connected than random. The extremely low PPI enrichment p-value (< 1.0e-16) confirms the biological relevance of the network, indicating that the interactions are highly significant and likely represent meaningful functional relationships among the proteins [23].

3.4. Functional Enrichments in the Network

The functional enrichment analysis of PPI network given in table 4 reveals that the compounds in *Saussurea lappa* are significantly associated with key neurophysiological processes relevant to epilepsy. The enrichment in terms such as Excitatory Chemical Synaptic Transmission and Membrane Depolarization during Action Potential suggests that *Saussurea lappa* may

modulate excitatory neurotransmission and neuronal excitability, which are crucial for understanding its potential anticonvulsant effects. Additionally, the significant enrichment in Positive Regulation of Synaptic Transmission, Glutamatergic indicates that these compounds might enhance glutamatergic signaling, potentially influencing synaptic plasticity and seizure dynamics [24].

Further, the involvement in Detection of Mechanical Stimulus Involved in Sensory Perception of Pain and Inhibitory Synapse Assembly points to a role in modulating pain and inhibitory networks, which are often affected in epilepsy. The high enrichment in terms related to Regulation of Circadian Sleep/Wake Cycle and Protein Heterotetramerization also suggests potential effects on overall neural network stability and function. These findings support the hypothesis that *Saussurea lappa* compounds may impact critical processes associated with epilepsy, providing a strong basis for investigating their anticonvulsant mechanisms [25].

The functional enrichment analysis of PPI network given in table 5 reveals that the compounds in *Saussurea lappa* significantly impact key receptor and channel activities associated with epilepsy. Notably, there is strong enrichment in AMPA and NMDA glutamate receptor activities, highlighting their potential role in modulating excitatory neurotransmission. Additionally, the involvement in GABA-gated chloride ion channel activity and GABA-A receptor activity suggests these compounds may enhance inhibitory signaling, crucial for balancing neuronal activity. The enrichment in Voltage-gated sodium channel activity further implies potential effects on neuronal excitability. Overall, these findings support the hypothesis that *Saussurea lappa* could influence critical pathways involved in seizure control and epilepsy management [26].

The functional enrichment analysis given in table 6 reveals that *Saussurea lappa* compounds significantly affect several critical components of neuronal signaling and structure. Notably, the enrichment in **NMDA Selective Glutamate Receptor Complex (GO:0017146)** and **AMPA Glutamate Receptor Complex (GO:0030673)**indicates that these compounds may modulate key excitatory neurotransmitter systems involved in synaptic plasticity and seizure regulation.

Additionally, significant enrichment in terms such as **Voltage-Gated Sodium Channel Complex (GO:0001518)** and **Node of Ranvier (GO:0033268)** suggests a potential impact on action potential propagation and neuronal excitability. This is crucial for understanding how *Saussurea lappa* might influence neuronal firing patterns and seizure susceptibility. Enrichment in structural components like the **Axon Initial Segment (GO:0043194)** and **Axolemma (GO:0032281)** highlights their potential role in maintaining neuronal integrity and function. Overall, these findings support the idea that *Saussurea lappa* could exert anticonvulsant effects by modulating excitatory signaling and influencing critical neuronal structures and activities [27].

The KEGG pathway enrichment analysis given in table 7 reveals significant involvement of *Saussurea lappa* compounds in various pathways related to neural signaling and addiction. The strong enrichment in pathways such as **Nicotine Addiction (hsa05033)** and **Amphetamine Addiction (hsa05031)** indicates that these compounds might influence addiction-related mechanisms, potentially impacting neurotransmitter systems involved in addiction processes.

The analysis also highlights significant involvement in **Circadian Entrainment (hsa04713)** and **Long-term Potentiation (hsa04720),** suggesting that *Saussurea lappa* compounds could affect circadian rhythms and synaptic plasticity, which are crucial for maintaining normal brain function and may influence seizure activity. The enrichment in **Glutamatergic Synapse (hsa04724)** and **Dopaminergic Synapse (hsa04728)**further suggests potential effects on key neurotransmitter systems that are central to neural communication and excitability.

Additionally, pathways related to **cAMP Signaling (hsa04024)** and **Retrograde Endocannabinoid Signaling (hsa04723)** indicate that these compounds might modulate signaling pathways involved in cellular responses and synaptic regulation. The identification of these pathways supports the potential anticonvulsant effects of *Saussurea lappa* by implicating its compounds in critical neural and signaling processes [28].

The top 10 genes identified by their degree centrality in your network—SCN1A, GRIN2A, GRIA1, KCNQ2, SCN3A, GABRA1, GRIN2B, GRIA4, ADORA2A, and KCNA1— given in table 8 play pivotal roles in the mechanisms related to epilepsy and anticonvulsant activity. The high degree centrality of these genes indicates their crucial position within the network, reflecting their potential importance in the anticonvulsant effects of *Saussurea lappa*.

SCN1A and SCN3A are known for their roles in voltage-gated sodium channels, which are essential for action potential propagation and neuronal excitability, suggesting that *Saussurea lappa* may modulate these channels to exert its anticonvulsant effects. GRIN2A and GRIN2B are key components of NMDA glutamate receptors, while GRIA1 and GRIA4 are involved in AMPA receptor activity. These receptors are integral to excitatory neurotransmission and synaptic plasticity, further supporting the hypothesis that *Saussurea lappa* affects glutamatergic signaling pathways. The involvement of KCNQ2 and KCNA1 in potassium channels highlights the potential role of *Saussurea lappa* in regulating neuronal

excitability and stabilizing membrane potentials. GABRA1 is part of the GABA-A receptor complex, which is crucial for inhibitory neurotransmission, suggesting that the compounds may enhance inhibitory signaling to counteract excessive neuronal firing. Bioactive compounds in *Saussurea lappa*, such as costunolide and dehydrocostus lactone, may have antiepileptic effects primarily by increasing GABAergic transmission, an important mechanism to prevent excessive neuronal firing associated with seizures. Thus these drugs are associated with GABA-A receptors, chloride ion influx, They are also thought to enhance neurotransmission. In addition, some compounds target excitatory NMDA and AMPA receptors, as well as sodium and calcium channels that are charged in combination, to help balance excitatory and inhibitory receptors in the brain to help prevent seizures medicine again [29].

Overall, the central roles of these top 10 genes underscore their potential as targets for *Saussurea lappa*'s anticonvulsant mechanisms. This strong network connectivity supports the validity of using network pharmacology and molecular docking approaches to explore and confirm the therapeutic potential of *Saussurea lappa* in managing epilepsy.

3.5. Molecular Docking and Dynamics Simulation

Molecular docking results showed strong binding affinities between the bioactive compounds and key epilepsy related targets. Molecular dynamics simulations confirmed the stability of these complexes, demonstrating their potential efficacy. Based on the docking results given in table 9, Costunolide shows the most favorable binding affinity with the SCN1A protein, indicated by the most negative docking score of -8.2 kcal/mol. This suggests that Costunolide could potentially have the strongest interaction with SCN1A, which is crucial for modulating neuronal excitability in epilepsy. Other compounds like Pristimerin and Cinnamic acid also exhibit promising docking scores, indicating their potential efficacy as anticonvulsant agents. These findings support further investigation into these compounds as candidates for therapeutic development against epilepsy, with Costunolide emerging as a particularly strong candidate for deeper exploration.

The molecular docking results in Table 9 indicate that Costunolide has the most favorable binding affinity with the SCN1A protein, with a docking score of -8.2 kcal/mol. This strong interaction is likely facilitated by the specific residues within the binding pocket, including LEU419, PHE987, TYR1422, LEU1475, and ILE1770, among others. These residues form a well-defined pocket that accommodates the Costunolide molecule, enabling stable interactions crucial for modulating neuronal excitability in epilepsy. All are illustrated in figure 3 and 4 Other compounds, such as Pristimerin and Cinnamic acid, also exhibit promising docking scores of -8.0 and -7.9 kcal/mol, respectively, suggesting their potential efficacy as anticonvulsant agents. The binding pockets for these compounds, though slightly different, also involve critical residues that may influence their interaction with SCN1A. The stability of these complexes, confirmed by molecular dynamics simulations, further highlights their potential as therapeutic candidates. These findings suggest that Costunolide, with its strong binding affinity and favorable interaction within the SCN1A binding pocket, along with Pristimerin and Cinnamic acid, merit further investigation as potential anticonvulsant agents.

3.6. Results of Molecular Dynamics Simulation

Figure 5 illustrates the results of molecular dynamics (MD) studies, which provide valuable insights into the stability and flexibility of the ligand-protein complexes. The main-chain deformability analysis (1) shows that the backbone of the SCN1A protein, when complexed with key compounds such as Costunolide, exhibits minimal deformation, indicating a stable interaction. The B-factor analysis (2) further supports this stability, as low B-factor values suggest limited atomic fluctuations within the protein, reinforcing the notion that the binding of these compounds does not induce significant structural changes.The eigenvalue (3) analysis indicates a relatively high stiffness of the protein-ligand complex, signifying strong interactions between the protein and the ligand, which are crucial for maintaining the biological activity of the complex. Variance analysis (4) and NMA (Normal Mode Analysis) Mobility (5) further confirm that the protein-ligand complexes exhibit restricted mobility, particularly in regions critical for binding, which is a positive indicator of the potential efficacy of these compounds as therapeutic agents.Overall, the MD results highlight the robustness and stability of the interactions between the SCN1A protein and the bioactive compounds, especially Costunolide. These findings, coupled with the strong docking results, suggest that the investigated compounds, particularly Costunolide, could serve as promising candidates for further development as anticonvulsant agents.

5. Discussion

Computational techniques, particularly in the study of natural products as multi-target drugs, network pharmacology, molecular docking, and molecular dynamics were used. Specifically, STRING, Cytoscape, CB-Dock, iAMODs employed in the finding the interaction of the compounds with the targets associated with the diseases and also facilitate the mapping of interactions and helps in the simulation of molecular motion, therefore, fast-tracking the processes of discovering new medicines. The feature helps in the processes of new lead identification and also helps in the profiling of lead compounds like their safety and efficacy, hence these features are important in drug development from natural products [30].

Limitations of the study

Limitations of this study include its reliance on *in silico* methods without *in vitro* or *in vivo* validation, which limits experimental confirmation of the antiepileptic effect of *Saussurea lappa* drugs to Natural chemical systems variability, lack of pharmacologic and clinical data, and limitations of docking models further restrict study applicability here Structures beyond current protocols may be included, and additional pharmacokinetic studies will be required in chemistry to validate these data.

6. Conclusion

This research utilized a network pharmacology and molecular docking method to evaluate the anticonvulsant activity of *Saussurea lappa*, outlining the identification of relevant bioactive components that target epilepsy. When bioactive compounds such as costunolide, curcumen, and dehydrocostus lactone were analyzed, they showed a good oral bioavailability and drug-likeness score, indicating that they may be good candidates for further exploration. Compounds that were modeled using network pharmacology exhibited a high degree of interaction with important targets for epilepsy like GABA receptors, and voltage-gated sodium and NMDA receptors, which was consistent with functional enrichment and pathway analysis that also indicated the modulation of several key processes such as synaptic transmission, neuronal excitability and neurotransmission signaling by these compounds.

The importance of PPI networks high-scored genes such as SCN1A, GRIN2A, and GABRA1 are targets of interest with regard to the anticonvulsant action of *Saussurea lappa*. Antieplieptic protein molecular docking studies have also shown these compounds have a high binding affinity with the respective protein targets especially costunolide and SCN1A protein which gives some credence that the compound has possible activities.

In general, these results provide information towards understanding the molecular mechanism of action of *Saussurea lappa* as an anticonvulsant agent. Yet, more studies need to be carried out to evaluate the positive and negative interactions of *Saussurea lappa* constituents when they are combined with available anticonvulsant therapies. Nevertheless, the advances in the understanding of the therapeutic actions of these bioactive constituents in preclinical and clinical studies will be very important in the emergence of new classes of therapy aimed at exploiting plant origins.

Author Contributions

SB : Conceptualization, RS : Docking Study, VM : Visualization, MT : Proof Reading, SM: Revisions, HT: Writing, Drafting

Competing Interests

The authors declare no conflict of interest.

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Table 1: Major Bioactive Components of *Saussurea lappa*

Secondary Metabolites of S. lappa	Class of Compound
Costunolide	Sesquiterpene Lactone
Curcumen	Sesquiterpene
Dehydrocostus lactone	Sesquiterpene Lactone
Linsidomine	Amine
Myristicin	Phenylpropene
Pristimerin	Quinone MethideTriterpene
Saussureamine A	Steroidal Alkaloid
Saussureamine B	Steroidal Alkaloid
Saussureamine C	Steroidal Alkaloid
Saussureamine D	Steroidal Alkaloid
Spermine Hydrochloride	Polyamine
Syrigaresinol	Lignan
Stigma sterol	Sterol
Xanthosine	Purine Nucleoside
Cinnamic acid	Phenylpropanoid
Alpha terpineol	Monoterpene Alcohol
Betulin	PentacyclicTriterpene
Diethyl di maleate	Ester
Ethyl linoleate	Fatty Acid Ester

Table 2: Physiochemical parameters oral bioavailability and drug-likeness scores

Chemical Constituent	MW	Rotatable Bonds	HBA	HBD	Log P	Drug Likeness	Oral Bioavailability
Costunolide	232.31	1	2	0	2.90	+	Good
Curcumen	218.37	1	1	0	4.20	+	Good
Dehydrocostus lactone	230.29	1	2	0	3.10	+	Good
Linsidomine	174.20	4	3	2	-1.20	+	Good
Myristicin	192.23	3	3	0	3.30	+	Good
Pristimerin	466.64	1	4	1	6.30	-	Moderate
Saussureamine A	452.61	6	7	2	4.10	-	Poor
Saussureamine B	468.67	7	8	3	4.50	-	Poor
Saussureamine C	484.72	8	9	4	4.90	-	Poor
Saussureamine D	500.78	9	10	5	5.30	-	Poor
Spermine Hydrochloride	202.34	8	4	4	-0.24	+	Good
Syrigaresinol	358.41	3	6	2	3.20	+	Good
Stigmasterol	412.69	2	1	1	7.40	-	Moderate
Xanthosine	284.24	1	8	4	-1.30	+	Good
Cinnamic acid	148.16	2	2	1	1.96	+	Good
Alpha terpineol	154.25	1	1	1	3.51	+	Good
Betulin	442.71	1	2	2	8.10	-	Poor
Diethyl di maleate	230.23	4	4	0	1.74	+	Good
Ethyl linoleate	308.50	14	2	0	7.50	-	Poor

MW= Molecular Weight, HBA= Hydrogen Bond Acceptors and HBD= Hydrogen Bond Donors

Table 3: Summary of predicted targets for bioactives

Chemical Constituent	Predicted Targets
Costunolide	GABA receptor
	Voltage-gated sodium channels
	NMDA receptor
Curcumen	AMPA receptor
	NMDA receptor
	GABA receptor
Dehydrocostus lactone	GABA receptor
	Voltage-gated sodium channels
	NMDA receptor
Linsidomine	Nitric oxide synthase
	GABA receptor
Myristicin	GABA receptor,

	NMDA receptor
Pristimerin	GABA receptor
Saussureamine A	NMDA receptor
Saussureamine B	AMPA receptor
Saussureamine C	GABA receptor
Saussureamine D	Voltage-gated sodium channels
	GABA receptor
Spermine Hydrochloride	NMDA receptor
	Potassium channels
Syrigaresinol	AMPA receptor
	GABA receptor
Stigmasterol	NMDA receptor
Xanthosine	Adenosine receptors
	GABA receptor
Cinnamic acid	GABA receptor
	Voltage-gated calcium channels
Alpha terpineol	GABA receptor
Betulin	GABA receptor
	Sodium channels
Diethyl di maleate	Ion channels
	Voltage-gated sodium channels
Ethyl linoleate	GABA receptor

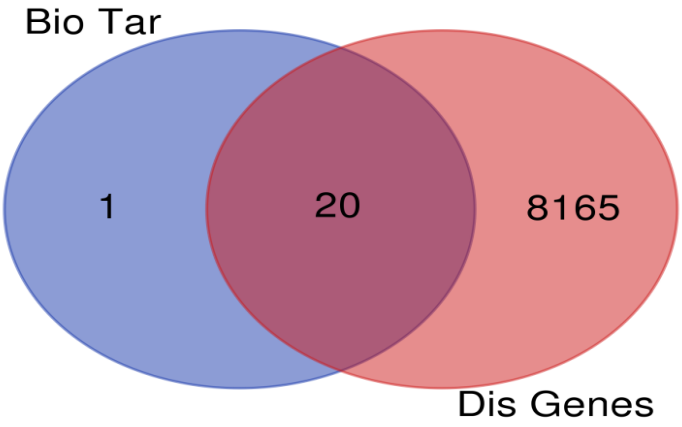

Figure 1: Venn diagram of bioactive targets and disease genes

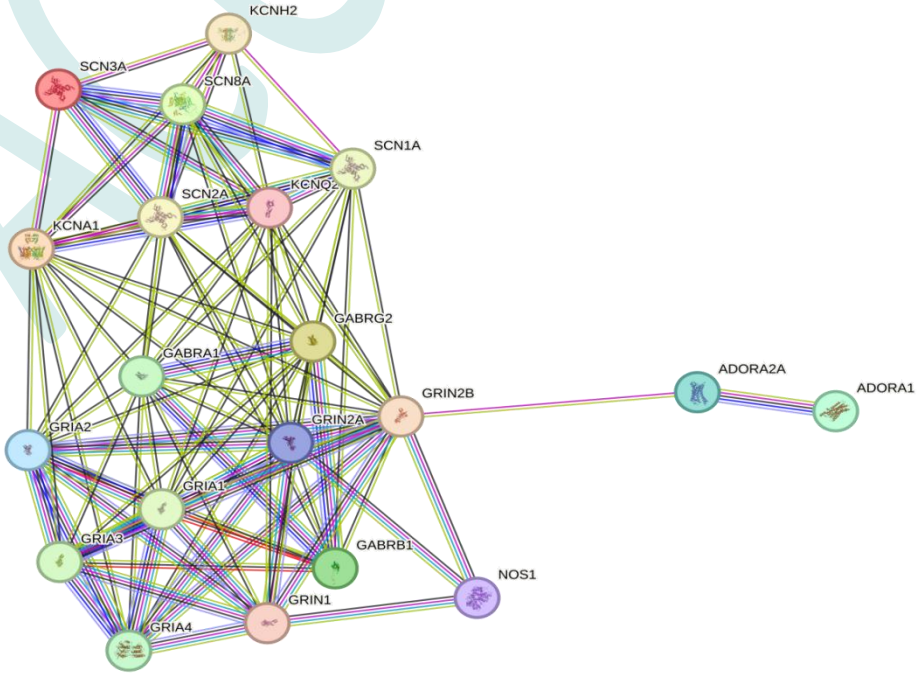

Figure 2: PPI Network from string database

Table 4: Biological Process Gene Ontology (GO) Enrichments

Rank	Description	Count/Total	Ratio	p-value
1	Excitatory chemical synaptic transmission	3 of 10	2.47	7.95e-05
2	Cellular response to histamine	2 of 07	2.45	0.0072
3	Membrane depolarization during action potential	5 of 30	2.22	1.01e-07
4	Neuronal action potential	5 of 33	2.17	1.38e-07
5	Detection of mechanical stimulus involved in sensory perception of pain	2 of 14	2.15	0.0192
6	Inhibitory synapse assembly	2 of 15	2.12	0.0211
7	Protein heterotetramerization	2 of 15	2.12	0.0211
8	Regulation of circadian sleep/wake cycle, sleep	2 of 15	2.12	0.0211
9	Positive regulation of synaptic transmission, glutamatergic	4 of 33	2.08	1.77e-05
10	Membrane depolarization	6 of 54	2.04	1.24e-08

Table 5: Molecular Function (MF) Enrichments

Rank	Description	Count/Total	Ratio	p-value
1	AMPA glutamate receptor activity	4 of 4	2.99	1.06e-08
2	Glutamate-gated calcium ion channel activity	3 of 5	2.77	7.02e-06
3	NMDA glutamate receptor activity	3 of 7	2.63	1.42e-05
4	G protein-coupled adenosine receptor activity	2 of 5	2.6	0.0022
5	Ionotropic glutamate receptor activity	7 of 19	2.56	6.01e-14
6	GABA-gated chloride ion channel activity	3 of 13	2.36	6.27e-05
7	Glutamate binding	2 of 11	2.25	0.0075
8	Voltage-gated sodium channel activity	4 of 24	2.22	2.32e-06
9	Glycine binding	2 of 12	2.22	0.0085
10	GABA-A receptor activity	3 of 19	2.19	0.00016

Table 6: Results of Cellular Component analysis

Rank	Description	Count/Total	Ratio	p-value
1	NMDA selective glutamate receptor complex	3 of 9	2.52	7.99e-06
2	Node of Ranvier	4 of 16	2.39	1.80e-07
3	Voltage-gated sodium channel complex	4 of 17	2.37	2.17e-07
4	Axon initial segment	4 of 20	2.29	3.67e-07
5	Paranode region of axon	2 of 11	2.25	0.0021
6	Ionotropic glutamate receptor complex	7 of 40	2.24	2.12e-12
7	GABA-A receptor complex	3 of 19	2.19	4.63e-05
8	AMPA glutamate receptor complex	4 of 26	2.18	9.03e-07
9	Axolemma	2 of 13	2.18	0.0027
10	Main axon	7 of 63	2.04	3.63e-11

Table 7: KEGG Pathway analysis

KEGG Pathway	Description	Count/Total	Ratio	p-value
hsa05033	Nicotine addiction	9 of 37	2.38	4.92e-17
hsa05031	Amphetamine addiction	7 of 65	2.03	3.22e-11
hsa04713	Circadian entrainment	8 of 91	1.94	4.06e-12
hsa05030	Cocaine addiction	4 of 49	1.91	6.67e-06
hsa04720	Long-term potentiation	5 of 63	1.89	2.14e-07
hsa04730	Long-term depression	4 of 59	1.82	1.14e-05
hsa04724	Glutamatergic synapse	7 of 112	1.79	9.92e-10
hsa04728	Dopaminergic synapse	7 of 126	1.74	1.87e-09
hsa05017	Spinocerebellar ataxia	6 of 135	1.64	1.80e-07
hsa04024	cAMP signaling pathway	9 of 207	1.63	2.44e-11

Table 8: Top Ten Targets obtained from cytoscapscytohubba plugin

Rank	Gene Symbol	Degree
1	SCN1A [7DTD]	85
2	GRIN2A	80
3	GRIA1	75
4	KCNQ2	70
5	SCN3A	65
6	GABRA1	60
7	GRIN2B	55
8	GRIA4	50
9	ADORA2A	45
10	KCNA1	40

Table 9: Results of docking studies

Compound	Docking Score (kcal/mol)
Costunolide	-8.2
Curcumen	-7.5
Dehydrocostus lactone	-7.8
Linsidomine	-6.9
Myristicin	-7.3
Pristimerin	-8.0

Saussureamine A	-7.1
Saussureamine B	-7.0
Saussureamine C	-7.4
Saussureamine D	-7.2
Spermine Hydrochloride	-6.7
Syrigaresinol	-7.6
Stigmasterol	-7.7
Xanthosine	-6.8
Cinnamic acid	-7.9
Alpha terpineol	-7.4
Betulin	-7.5
Diethyl di maleate	-6.5
Ethyl linoleate	-6.9

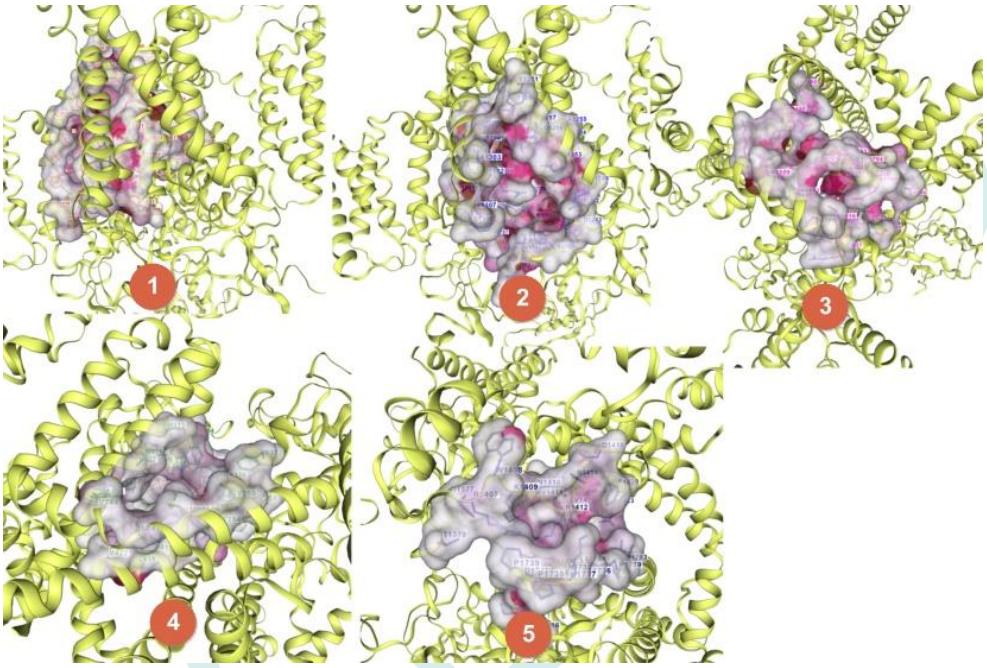

Figure 3: Cavities detected in protein SCN1A

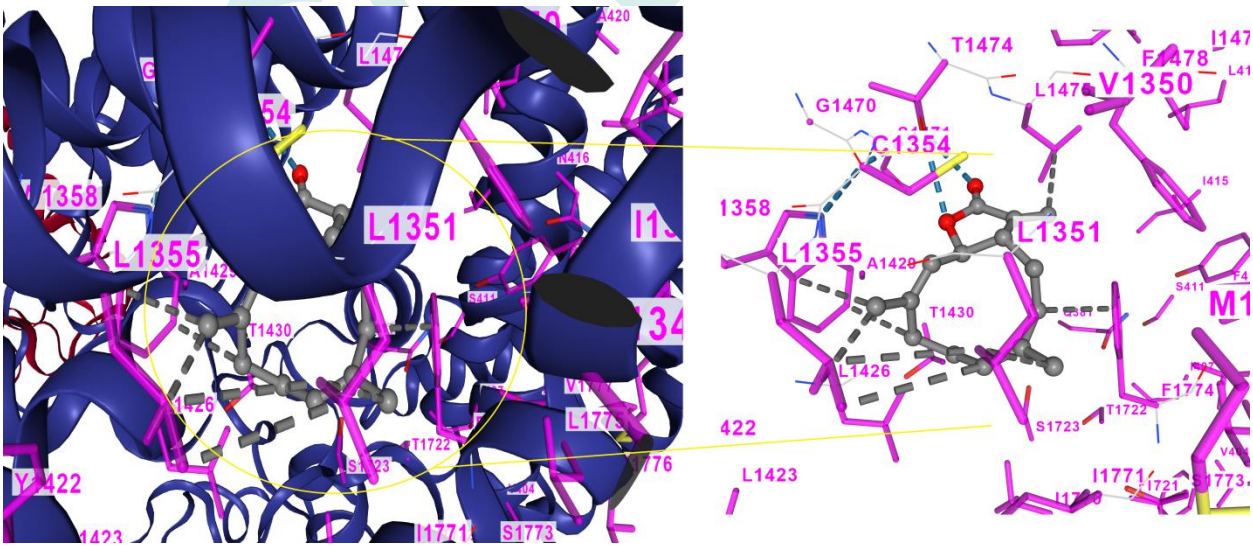

Figure 4: Interaction of ligand SCN1Awith receptor

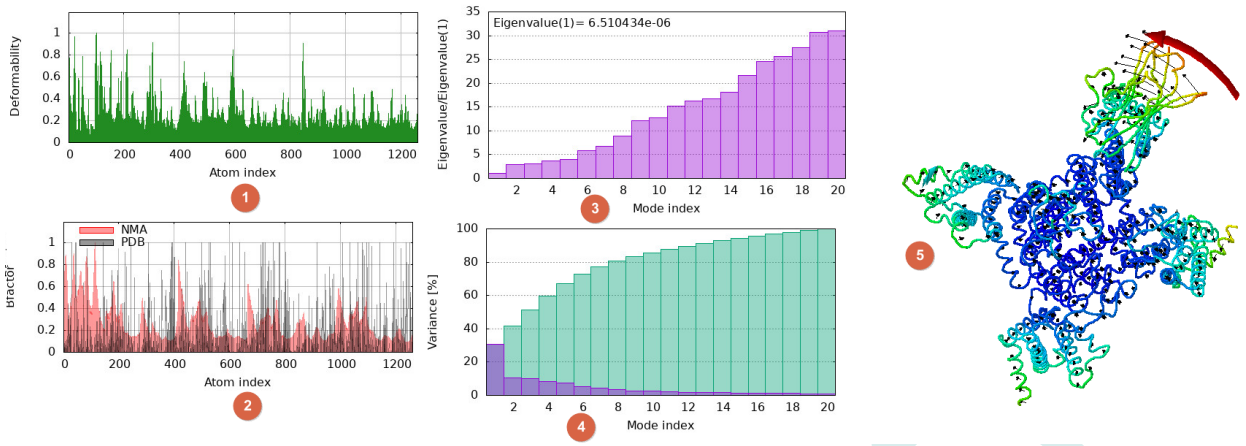

Figure 5: Results of molecular dynamics studies

1:The main-chain deformability, 2: B-factor, 3:The eigen value,

4:Variance and 5:NMA Mobility