Research article

Investigating the molecular mechanisms of *Ashwagandha* phytocompounds in epilepsy through differential gene expression and pathway analysis

Srivarshini Govinda Srinivasan[1], Susha Dinesh[2], Sameer Sharma[2], Bhavana Sunkadakatte Venugopal[1], Martin Lucas A.[3], Dinesh Sosalagere Manjegowda[1]

[1]Department of Human Genetics, School of Basic and Applied Sciences, Dayananda Sagar University, Bangalore, 560078, Karnataka, India

[2]Department of Bioinformatics, BioNome, Bangalore, 560078, Karnataka, India

[3]Department of Anatomy, Dr. Chandramma Dayananda Sagar Institute of Medical Education & Research, Devarakaggalahalli, Harohalli, Kanakapura Road, Ramanagara Dt., 562 112, Karnataka, India

(Received: May 2023 Revised: August 2023 Accepted: September 2023)

Corresponding author: **Dinesh Sosalagere Manjegowda.** Email: dineshmgowda1@gmail.com

ABSTRACT

Introduction and Aim: *Withania somnifera (Ashwagandha)* is a traditional Indian herb used in Ayurveda and Unani medicine, particularly in anti-inflammatory, anti-cancer, anti-stress, antioxidant, immune-boosting, and rejuvenating effects. Epilepsy is a severe neuropsychological condition that occurs sporadically and has a long-term effect on the electrical signals that travel between brain cells. The disorder is characterized by recurrent seizures that are brought on by a sudden increase in brain electrical activity. This is the outcome of abnormal neuronal discharges or coordinated neuronal hyperexcitability. The study's main objective is to find out the therapeutic phytocompound in treating Epileptic disorder.

Materials and Methods: This study investigated the potential use of phytochemicals from the *Ashwagandha* plant as epileptic seizure treatments that target key genes strongly associated with the disease. To forecast the binding affinity between the phytochemicals and the receptors, molecular docking simulations (PyRx) were used for the virtual screening.

Results: The preliminary screening of the twenty-two phytocompounds from *Withania somnifera* was based on their affinity for epilepsy. The results showed that withasomnine exhibited great binding affinity to the receptors, indicating their potential as targeted epileptic seizure therapeutics. The ligands revealed stronger binding with the epilepsy targets, and the binding score less than -7 kcal/mol was taken into consideration for further exploration. This research lays the groundwork for upcoming in-vitro and in-vivo studies to confirm the effectiveness of these phytochemicals as cancer therapies.

Conclusion: The results suggest that withasomnine derivatives from *Withania somnifera* could be a promising source of epilepsy therapies.

Keywords: KCNQ2; SCN1A; SCN9A; epilepsy; Ashwagandha root.

INTRODUCTION

Epilepsy is a condition affecting the central nervous system, marked by recurrent episodes of altered consciousness, potentially accompanied by seizures, and linked to unusual electrical activity in the brain. It is regarded as the clinical expression of an abnormal and excessive firing of a specific group of neurons in the brain (1-3). It is one of the most common neurological diseases worldwide, with an estimated 50 million people experiencing it globally. Regular seizures, unconsciousness, panic, jerks, blackouts, spasms, and shaking are typical symptoms of epilepsy (4). The disorder is characterized by recurrent seizures that are brought on by a sudden increase in brain electrical activity. This is the outcome of abnormal neuronal discharges or coordinated neuronal hyperexcitability. However, the incidence of these seizures differs from person to person.

Epileptic seizures, which result in unusually jerky or quivering motions in the body, can injure the brain or other areas of the body. Even one seizure has the power to impact cognition, behaviour, and brain development. Clinically, seizures from epilepsy are harmful. These seizures negatively influence patients' lives, particularly those who have them regularly (5). Seizures are influenced by a variety of factors, including the site of seizure onset in the brain, patterns of propagation, brain maturity, complicating disease processes, sleep-wake cycle, medications, and a host of other variables (6). Epileptogenesis is the process through which an intact brain becomes capable of generating spontaneous, recurrent seizures. Over time, this disturbs regular neural functioning and might affect other neuronal networks.

It is not necessarily an increase in excitation or a loss of inhibition that causes the imbalance between excitation and inhibition that gives rise to epileptogenic networks; in some circumstances, such as when there are no seizures or limbic epilepsies in the developing brain, an abnormal upsurge in inhibition as well be pro-epileptogenic (7). The

identification of known targets which are available from the 3 databases such as OMIM, GeneCards, and DisGeNET are retrieved and further analysis is carried out to find out the potential targets of epilepsy. *W. somnifera*, often known as ashwagandha, is a traditional Indian herb used in Ayurveda and Unani medicine. Its well-known plant is utilized in conventional medicine and treatments. Steroid alkaloids and steroidal lactones with an ergostane structure (with anolides) are the main components of Ashwagandha extracts from a variety of sections. According to numerous toxicological tests, ashwagandha is a safe herb that can be consumed. Ashwagandha helps the brain and the neurological system work well and boost memory. There are 13 alkaloids in *W. somnifera*'s biochemical makeup. This herb's medical benefit is classified as an adaptogen, which is a nontoxic herb that acts generally to normalize physiological function by affecting the neuroendocrine system and HPA axis.

One of the most effective revitalizing ingredients in Ayurveda is ashwagandha. Ayurvedic and Unani treatments utilize their roots, seeds, and leaves. The medicine made from the ashwagandha root is effective in treating epilepsy, neurological problems, joint inflammation, and rheumatoid arthritis (8). In this present study the 4 major phytocompounds of *W. somnifera* i.e., Ashwagandha act as potential ligands for docking with 3 key potential therapeutic targets of epilepsy.

MATERIALS AND METHODS

Identification of known therapeutic targets acting on epilepsy

The GeneCards, OMIM, and DisGeNET databases were mined for Epilepsy associated target genes. GeneCards(https://www.genecards.org/) is accessible via search, comprehensive, user-friendly information on every human gene that has been predicted or annotated is provided by an integrated database. The knowledge base is automatically updated with genomic, transcriptomic, proteomic, genetic, clinical, and functional data from 150 web sources (9). DisGeNET(https://www.disgenet.org/home/) is one of these resources, with the goals of covering all disease domains (Mendelian, complex, and environmental disorders), with specific attention to the integration and standardization of data, and enabling free access to knowledge of the genes connected to human diseases (10). OMIM®(http://omim.org), or Online Mendelian Inheritance in Man, is a comprehensive, reliable, and current research tool containing curated descriptions of human genes, phenotypes, and their connections (11).

Identification of common genes

The common genes were identified by Bioinformatics & Evolutionary Genomics Venn Diagram (https://bioinformatics.psb.ugent.be/webtools/Venn/) online tool. The Venn diagram showed common genes between Gene Cards, OMIM, and DisGeNET databases. At the end of the analysis, 28 genes were found common between Gene Cards and DisGeNET databases, and no common genes were found between all three databases. The 28 genes were considered to find the potential target genes through the String database.

PPI network construction and identification of key genes

The STRING database (https://string-db.org/) systematically collects and integrates protein–protein interactions, physical interactions, and functional associations (12). The Shiny Go (http://bioinformatics.sdstate.edu/go/) database is used to retrieve data about gene enrichment analysis. The preliminary screening of 28 common genes was done using the STRING database to determine the potential targets for the disease. The screening was done using the string database from which 3 genes were found to the therapeutic targets. The gene enrichment analysis gives us detailed information about the pathways that are involved in each gene.

Ligand retrieval

IMPPAT 2.0 contains curated data on the 17,967 phytocompounds and 1095 therapeutic applications of 4010 Indian medicinal plants (13). PubChem, a well-liked repository of chemical data, is available at https://pubchem.ncbi.nlm.nih.gov and has a variety of applications for AI and machine learning research (14). Secondary metabolites of Ashwagandha were derived from IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) (https://cb.imsc.res.in/imppat/home) database. A total of 21 phytocompounds were derived excluding the duplicates and the compounds which had longer phytochemical names, especially from only root sources. The canonical SMILES (Simplified Molecular Input Line Entry System) and the 2D SDF (Standard Data Files) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database for further research.

Protein retrieval and purification

In UniProtKB, there are two sets of proteins: UniProtKB/Swiss-Protein which has been verified through experimental methods or computational predictions and summarized in collaboration with experts for each entry; UniProtKB/Tremble which includes primarily computationally annotated entries created automatically (15). RCSB PDB((https://www.rcsb.org/) is unrestricted access to almost 200,000 experimentally determined 3D structures of biological macromolecules and their interactions with one another and small molecule ligands that have been persistently archived, thoroughly confirmed, and professionally curated (16). The 3-Dimensional structure of the proteins can be

downloaded from the RCSB PDB database. The crystal structures of KCNQ2 (PDB ID: 7CR1), SCN1A (PDB ID: 7DTD), and SCN9A (PDB ID: 7W9L) were resolved through X-Ray diffraction techniques. The crystal structures were purified in DS Biovia Studio Visualizer by removing the water molecules and heteroatoms. Main protein groups were retained for the current investigation to evade the complexity of the structure which can be viewed in the UniProt (https://www.uniprot.org/) database. The structure can be further enhanced by adding polar hydrogen atoms and the purified structure can be used for further study.

Validation of protein structure

Understanding the 3-Dimensional structures for molecular docking is essential as they determine how the interaction occurs between ligands and proteins. Therefore, the purified structures of Epilepsy key genes were validated using the servers PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/), ProSA (https://prosa.services.came.sbg.ac.at/prosa.php), ProQ (https://proq.bioinfo.se/ProQ/ProQ.html).

Pharmacological studies

The process of discovering drugs is an uneven road, filled with challenges that cause a very small number of prospects to move from phytochemical compounds into commercially available products due in part to factors that include low bound affinity, unintended effects, or physicochemical properties like solubility and stability. Early in the drug development process, after hit compounds have been found, features including absorption, distribution, metabolism, excretion, and toxicity (ADMET) should be taken into account and optimized. Small molecules' physicochemical, drug-likeness, and ADMET properties were assessed using the ADMETlab 2.0 webserver (https://admetmesh.scbdd.com/) (17). A total of 21 ligands were initially screened for BBB yes and Lipinski is yes in SWISS ADME (http://www.swissadme.ch/index.php). After the initial screening, only 4 ligands were used for further investigation in ADMET LAB 2.0, PyRx, and DS Biovia Discovery Studio.

Molecular docking and visualization

In the world of drug development, molecular docking is a precise, efficient, and productive approach that is used to look at the intermolecular interactions that have been established between the candidate medication and the desired protein (18). The present study has used the virtual screening software PyRx to perform molecular docking of Ashwagandha phytocompounds. The molecular interaction in the binding pocket of the target proteins was further visualized in DS Biovia Discovery Studio Visualizer for the best-docked complexes.

RESULTS

Identification of known therapeutic targets acting on epilepsy

The study's initial methodology used DEGs, but since there were only a few datasets available, additional analysis was conducted. The samples were gathered from the GEO2R database. Later, we retrieved the known therapeutic genes from OMIM, GeneCards, and DisGeNET, three databases. 114 genes from the DisGeNET database, 133 genes from Gene Cards, and 200 genes from OMIM. To look into the potentially significant genes of epilepsy, additional research was done with 447 genes from all three databases where common genes were detected.

Identification of common genes

The common genes identified for Epilepsy using the online tool for Venn diagram between Gene Cards and DisGeNET is 28 genes which are SCN1A, GRIN2A, SCN2A, GABRG2, SCN9A, GABRB3, CNTNAP2, LGI1, KCNQ2, CACNB4, SLC6A1, PIGQ, PCDH1, SCN8A, STX1B, POLG, STXBP1, CDKL5, HCN1, MECP2, PRRT2, ABCB1, SLC12A5, CHD2, NEXMIF, GRIN2B, SYNGAP1, KCND2 genes were further examined for finding the key genes for Epilepsy.

PPI network construction and identification of key genes

Using the string database 3 potential target genes such as KCNQ2, SCN1A, and SCN9A genes were identified at 0.7 confidence by hiding the disconnected nodes from the pathway analysis depicted below in Fig. 1. The functional enrichment analysis for all the genes performed in the database is shown in (Table 1) Gene enrichment analysis is done using the Shiny Go software depicted below in Figs. 2 and 3.

Ligand retrieval

The ligands from Ashwagandha were retrieved from the IMPPAT database and the structure of the ligands which have exhibited better binding with the protein structure of genes associated with epilepsy was used for molecular docking and visualization.

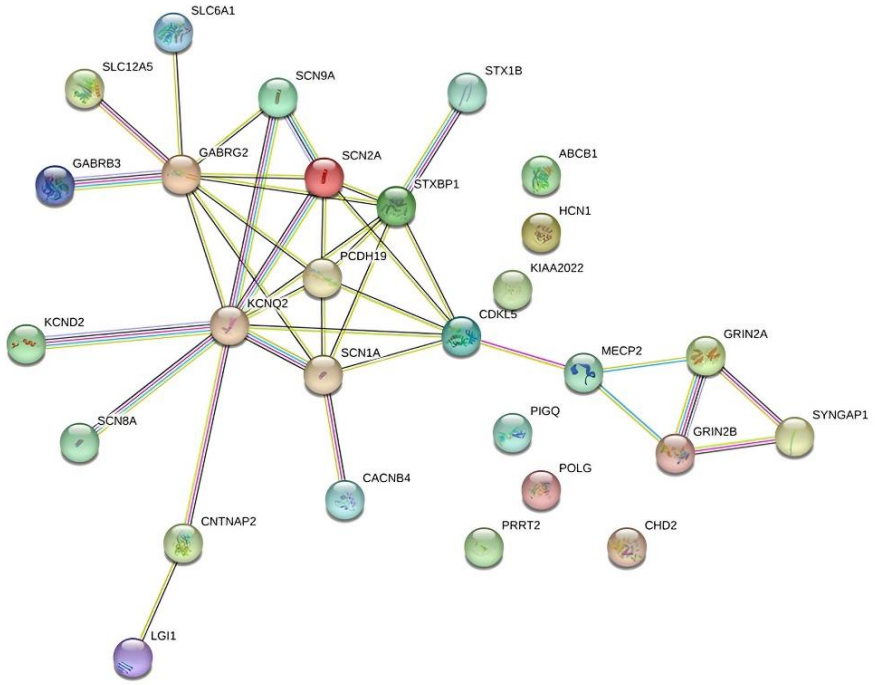

Fig.1: PPI Network Pathway from STRING

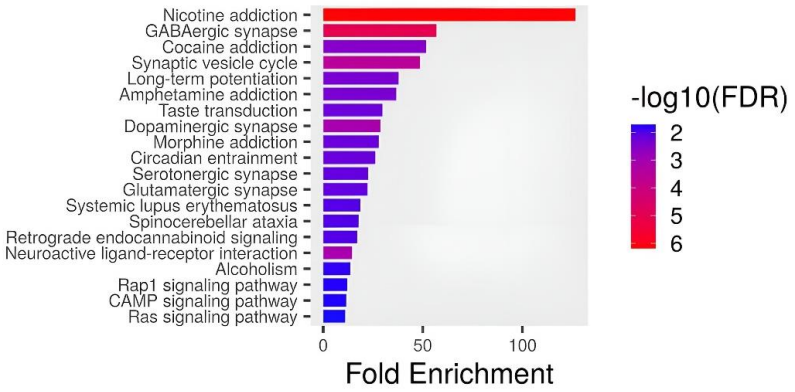

Fig.2: KEGG Fold Enrichment from Shiny GO

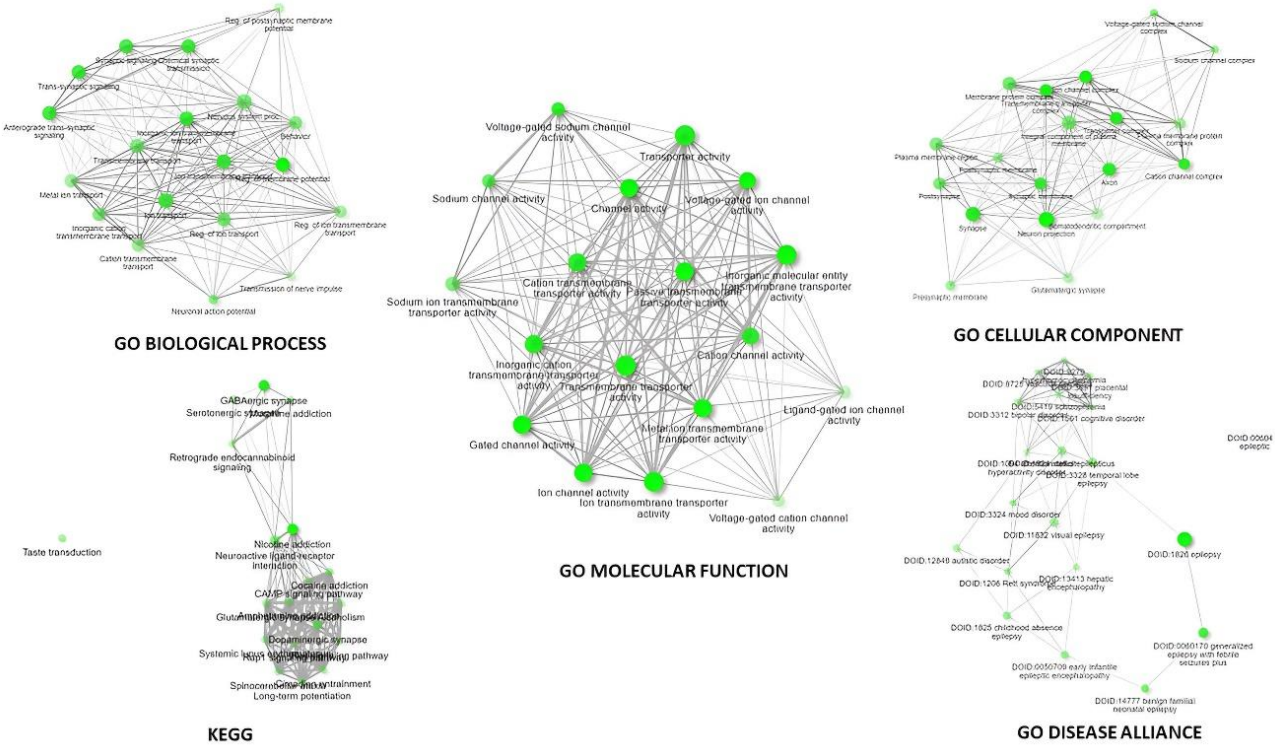

Fig. 3: Pathway database network from Shiny GO

Table 1: Functional enrichment network from string database

	GO-term	**Description**	**Count in Network**	**Strength**	**False Discovery Rate**
Biological process-gene ontology	GO:0032229	Negative regulation of synaptic transmission	2 of 7	2.3	0.0115
	G0:0010807	GABAergic Regulation of synaptic vesicle priming	2 of 7	2.3	0.0115
	G0:0071420	Cellular response to histamine	2 of 8	2.24	0.0138
	G0:0098976	Excitatory chemical synaptic transmission	2 of 9	2.19	0.0159
	G0:0050884	Neuromuscular process controlling posture	3 of 16	2.12	0.0006
Molecular function-gene ontology	G0:0022849	Glutamate-gated calcium ion channel activity	2 of 5	2.45	0.0044
	GO:0004972	NDA glutamate receptor activity	2 of 7	2.3	0.0071
	G0:0005248	Voltage-gated sodium channel activity	5 of 23	2.18	8.47E-08
	GO:0022851	GABA-gated chloride ion channel activity	2 of 3	2.03	0.0195
	GO:0015377	Cation: chloride symporter activity	2 of 17	1.91	0.0298
Cellular component-gene ontology	GO:0033268	Node of Ranvier	4 of 15	2.27	1.20E-06
	G0:0001518	Voltage-gated sodium channel complex	4 of 17	2.22	1.76E-06
	60:0017146	NDA selective glutamate receptor complex	2 of 11	2.1	0.0056
	GO:0043194	Axon initial segment	3 of 18	2.07	0.00019
	GO:1902711	GABA-A receptor complex	2 of 13	2.03	0.0072
.	**Pathway**	**Description**	**Count in Network**	**Strength**	**False Discovery Rate**
Kegg pathway	hsa05033	Nicotine addiction	3 of 38	1.74	0.009
	hsa04721	Synaptic vesicle cycle	3 of 74	1.45	2.99E-02
	hsa04727	GABAergic synapse	3 of 86	1.39	0.0306
	Disease	Description	Count in Network	Strength	False Discovery Rate
Disease gene association	DOID:0060169	Benign familial infantile epilepsy	3 of 3	2.84	9.90E-06
	DOID:2846	Bruxism	2 of 2	2.84	0.0021
	DOID:2538	Landau-Kleffner syndrome	2 of 2	2.84	0.0021
	DOID:0080422	Dravet syndrome	5 of 6	2.77	5.46E-10
	DOID:0050703	Infancy electroclinical syndrome	5 of 8	2.64	1.34E-09

Validation of protein structure

By predicting how ligands will interact with target proteins, molecular docking can identify potential therapeutic possibilities. Therefore, the following analysis was used to verify the pure protein structures where all the structures had residues has more than 90% Ramachandran most favoured regions, and Z-scores for all the Epilepsy key genes were found to be within the range of their native proteins, thereby confirming the protein models used are high quality and these structures were further considered for docking.

Molecular docking

The docking in PyRx takes the ligands to be flexible and the macromolecules to be rigid. The binding affinity at zero RMSD serves as a measure of the ligand's effectiveness. In the present research as the ligands demonstrated better binding with the epilepsy targets, a binding score less than -7.7 was considered for further investigation (Table 2). It was found that withasomnine has a better binding with all the target epilepsy genes.

Table 2: Binding affinity of *Withania somnifera* phytocompounds with target epilepsy genes

NO.	**LIGAND**	**PUBCHEM ID**	**KCNQ2**	**SCN1A**	**SCN9A**
1	Withasomnine	442877	-7.2	-7.7	-7.7
2	Nicotine	89594	-6.3	-5.9	-5.6
3	Anahygrine	12306778	-6.7	-6.1	-6.3
4	Cuscohygrine	1201543	-6.6	-5.5	-5.5

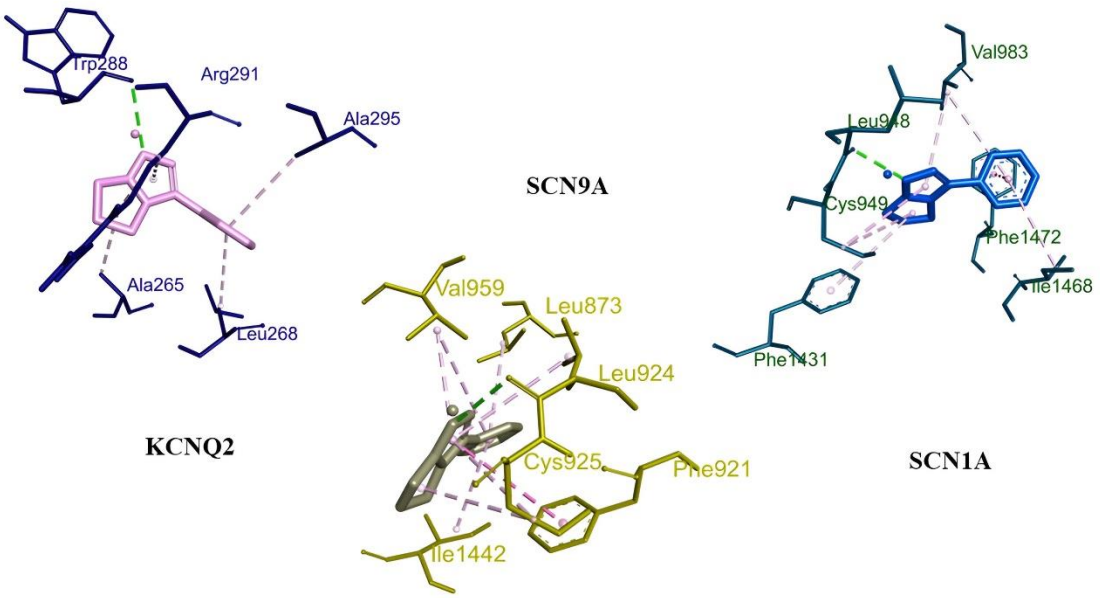

Fig. 4: Molecular interaction between key genes and ligands

Visualization

The binding affinity, RMSD/ub, and r/lb were measured after our ligands docked with the intended proteins. The 4 ligands that demonstrated the best binding in DS Biovia Discovery Studio Visualizer for each protein namely KCNQ2, SCN1A, and SCN9A were visualized, and the ligand as seen in Fig. 4.

Pharmacological studies

The ADMET qualities of *Withania somnifera* phytocompounds are researched since the bioactive compounds ought to have favourable drug-like effects. The Absorption and distribution (Table 3), medicinal chemistry and excretion metabolism properties (Table 4), and toxicity qualities (Table 5) of the four ligands that were evaluated using docking were examined. It is clear from the phrenological evaluation that every substance had beneficial pharmacological qualities.

Table 3: Absorption and distribution properties of *Withania somnifera* phytocompounds

Sl. No	PubChem CID	Caco-2 Permeability	Pgp-inhibitor	Pgp-substrate	HIA	VD	BBB Permeation
1	442877	-4.517	0.163	0.001	0.003	1.872	0.96
2	89594	-4.299	0	0.003	0.005	2.774	0.968
3	12306778	-4.79	0.431	0.012	0.093	1.129	0.517
4	1201543	-4.687	0.059	0.011	0.069	1.271	0.693

Caco-2: Caco-2 Permeability; Pgp inh/ Pgp-sub: the inhibitor and substrate of P-glycoprotein; HIA: Human intestinal absorption; BBB: Blood–brain barrier; VDss: Volume Distribution; Fu: fraction unbound in plasma; CL: Clearance rate; T1/2: Half-life of the small molecules.

Table 4: Medicinal and excretion metabolism properties *Withania somnifera* phytocompounds

Sl. No	PubChem CID	QED	SA Score	Lipinski rule	PAINS	CL	T (1/2)
1	442877	0.665	2.275	Accepted	0	10.351	0.195
2	89594	0.626	2.5	Accepted	0	15.401	0.672
3	12306778	0.787	3.398	Accepted	0	9.066	0.528
4	1201543	0.723	3.356	Accepted	0	10.62	0.746

QED: A measure of drug-likeness based on the concept of desirability; PAINS: Pan Assay Interference Compounds; Lipinski Rule of 5: Molecular weight less than 500 Daltons, nHD<5, nHA<10, and lipophilicity<4.15; SAScore: Synthetic accessibility score were accounted; CL: Clearance rate; T1/2: Half-life of the small molecules.

Table 5: Toxicity properties of *Withania somnifera* phytocompounds

No	PubChem CID	hEGR	DILI	AMES Toxicity	Carcinogenicity	IGC50	LC50 FM
1	442877	0.028	0.701	0.808	0.768	3.426	4.461
2	89594	0.049	0.04	0.013	0.235	2.486	3.024
3	12306778	0.049	0.044	0.263	0.635	2.081	2.378
4	1201543	0.088	0.038	0.114	0.821	2.191	2.459

hERG: The human ether-a-go-go related gene; DILI: Drug-induced liver injury; AMES: The Ames test for mutagenicity; LC50 FM: 96-hour fathead minnow LC_{50} were examined; IGC50: Tetrahymena pyriformis 50 percent growth inhibition concentration.

DISCUSSION

Epilepsy is one of the most prevalent neurological conditions. Epilepsy has a wide range of clinical traits and substantial genetic variation. To maintain a child's quality of life and enable unhindered development, quick diagnosis, and suitable treatment are crucial. Childhood epilepsy frequently affects young individuals. The development and increased accessibility of advanced genomic techniques in the last ten years has caused a significant change in etiological diagnosis of epilepsy. Daily increases in the number of causative genes have increased diagnostic yield. The yield of diagnostic tests has increased as a result of the daily increase in the number of causative genes. Epileptic key genes KCNQ2, SCN1A, and SCN9A have a strong association with the disease. The central nervous system (CNS) expresses the transmembrane protein Kv7.2 (encoded by KCNQ2), which joins forces with Kv7.3 to produce hetero-multimeric channels. Together with the KCNQ3 subunit, KCNQ2 encodes a voltage-gated potassium channel subunit that can create neuronal M channels that transport slowly activating and non-inactivating potassium currents. These currents control the excitability of central and peripheral neurons and contribute to the resting membrane potential. Myokymia, benign new-born seizures, and epileptic and developmental encephalopathies are all brought on by KCNQ2 mutations. Through the production of NaV1.1 in inhibitory interneurons, the SCN1A gene, which has a high association with epilepsy, contributes significantly to maintaining the cortical excitation-inhibition balance. The SCN1A and SCN9A mutations cause shortened sodium channels, which change how depolarizing impulses are transmitted across the neurons (19).

Ashwagandha, also known as *W. somnifera*, has been used for millennia to treat a variety of health issues. This herbal supplement's numerous health advantages make it the ideal rejuvenator of mental and physical wellness (20). Numerous studies have examined the efficacy and safety of *W. somnifera* extract, which have revealed that it is safe for use by people of all ages, including those who are pregnant (21). According to a study, *W. somnifera* have anticonvulsant characteristics when used with the GABAergic regulation of the pentylenetetrazol (PTZ) seizure threshold paradigm. PTZ threshold rises in response to *W. somnifera*. The seizure threshold was raised when *W. somnifera* was also administered with GABA or benzodiazepines. *W. somnifera* elevates the seizure threshold via activating chloride channels, which alters the GABA receptor's activity and interferes with it. Therefore, the GABAergic neurotransmitter system is most likely the main mechanism by which the *W. somnifera* root extract raises the threshold of PTZ-induced seizure. Co-administration of *W. somnifera* and flax seed oil significantly shortens the convulsion phase in rats experiencing MES seizures. *W. somnifera* aqueous seed extracts have anticonvulsant activities by changing the levels of dopamine and serotonin in the hippocampus of pilocarpine-induced rat models (22). In recent decades, researchers and pharmaceutical companies have developed several methods for extracting natural chemicals that have been successfully utilized to treat epileptic seizures in preclinical animal models, including extracts, essential oils, and purified natural compounds. These discoveries, meanwhile, are still in their infancy, and further study is necessary before these medicines can be put on the market. These findings offer great hope for the management of cases of medication resistance in the treatment of epilepsy (23). As a result, because important genes play crucial functional roles in epilepsy, these were evaluated as possible targets in the current investigation. It was discovered that the phytocompounds from *Withania somnifera*, such as Withasomnine (442877), Nicotine (89594), Anahygrine (12306778), and Cuscohygrine (1201543), were among the top phytocompounds that had shown improved binding (Table 2 & Fig. 4) and beneficial pharmacological properties (Tables 3-5) with the target protein. Withasomnine was the main class of phytocompounds from *Withania somnifera* that effectively suppressed epilepsy genes. The substance withasomnine demonstrated substantially stronger inhibition than the other compounds, according to the findings of the current investigation.

CONCLUSION

Potential treatment targets include KCNQ2, SCN1A, and SCN9A because of their significant contributions to epileptic disease. Different forms of epileptic seizures may result from any changes to these essential genes. Through accurate and thorough

analyses of disease-gene-target-drug interaction networks, we were able to pinpoint many biological processes and molecular functions of epilepsy. *Withania somnifera* Dunal, also referred to as ashwagandha, is a key component of the traditional Indian medical system known as Ayurvedic medicine. Ashwagandha is one of Ayurveda's most potent revitalizing components. Their roots, seeds, and leaves are used in Ayurvedic and Unani remedies. Ashwagandha root extract is a potent remedy for rheumatoid arthritis, epilepsy, neurological conditions, and joint inflammation. According to a recent study, withasomnine has the potential to treat epilepsy and related illnesses while also acting as a potential anti-epileptic drug. To fully understand its mechanisms of action and potential clinical applications, more research is needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Mayuri, B., Kumar, D.S., Kishore, P. A Review on epilepsy. Journal of Medical Science and Clinical Research. 2019; 7(3):1362-1369.
2. Raju, G.P., Dwajani, S., Rajendran, K., Adarsh, E. A study of rationale use of sodium valproate and levetiracetam as monotherapy in pediatric patients with epilepsy at tertiary care hospital. Biomedicine. 2022 Mar 5;42(1):160-164.
3. Mahmood, I.A., Muslim, A.T., Kaddoori, H.G. The utility of hematological indices in differentiation between general and focal onset epilepsy. Biomedicine. 2022 Mar 5;42(1):64-71.
4. Mishra, A., Batham, L., Verma, S., Mishra, S., Shrivastava, V. Management of epileptic seizures through an integrated approach including yagya therapy. Interdisciplinary Journal of Yagya Research. 2019;2(1):52-64.
5. Beghi, E. The epidemiology of epilepsy. Neuroepidemiology. 2020;54(2):185-191.
6. Bonilla-Jaime, H., Zeleke, H., Rojas, A., Espinosa-Garcia, C. Sleep disruption worsens seizures: neuroinflammation as a potential mechanistic link. International Journal of Molecular Sciences. 2021;22(22):12531.
7. Thijs, R.D., Surges, R., O'Brien, T.J., Sander, J.W. Epilepsy in adults. The Lancet. 2019;393(10172):689-701.
8. Yang, L., Wang, Y., Chen, X., Zhang, C., Chen, J., Cheng, H., *et al*., Risk factors for epilepsy: A national cross-sectional study from National Health and Nutrition Examination Survey 2013 to 2018. International Journal of General Medicine. 2021;14:4405-4411.
9. Safran, M., Rosen, N., Twik, M., BarShir, R., Stein, T.I., Dahary, D., *et al*., The GeneCards Suite. Pract. Guid. Life Sci. 2021;Databases 1, 27–56. https://doi.org/10.1007/978-981-16-5812-9_2.
10. Piñero, J., Ramírez-Anguita, J.M., Saüch-Pitarch, J., Ronzano, F., Centeno, E., Sanz, F., *et al*., The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic acids research. 2020;48(D1):D845-D855.
11. Hamosh, A., Amberger, J.S., Bocchini, C., Scott, A.F., Rasmussen, S.A. Online Mendelian inheritance in man (OMIM®): Victor McKusick's magnum opus. American Journal of Medical Genetics Part A. 2021;185(11):3259-3265.
12. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., *et al*., The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Research. 2023;51(D1):D638-D646.
13. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., *et al*., PubChem 2023 update. Nucleic Acids Research. 2023; 51(D1):D1373-D1380.
14. Vivek-Ananth, R.P., Mohanraj, K., Sahoo, A.K., Samal, A. IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS Omega. 2023;8(9):8827-8845.
15. UniProt Consortium. UniProt: The universal protein knowledgebase in 2023. Nucleic Acids Res. 2023;51(D1): D523-D531.
16. Burley, S.K., Bhikadiya, C., Bi, C., Bittrich, S., Chao, H., Chen, L., *et al*., RCSB Protein Data Bank (RCSB. org): Delivery of experimentally-determined PDB structures alongside one million computed structure models of proteins from artificial intelligence/machine learning. Nucleic Acids Research. 2023;51(D1):D488-D508.
17. Chang, Y., Hawkins, B.A., Du, J.J., Groundwater, P.W., Hibbs, D.E., Lai, F. A Guide to *in silico* drug design. Pharmaceutics. 2023;15(1):49.
18. Er-rajy, M., Imtara, H., Noman, O.M., Mothana, R.A., Abdullah, S., Zerougui, S., *et al*., QSAR, ADME-Tox, molecular docking and molecular dynamics simulations of novel selective glycine transporter type 1 inhibitor with memory enhancing properties. Heliyon. 2023;9(2): e13706.
19. Blazekovic, A., Gotovac, Jercic, K., Meglaj, S., Duranovic, V., Prpic, I., Lozic, B., *et al*., Genetics of pediatric epilepsy: Next-Generation Sequencing in Clinical Practice. Genes. 2022; 13(8):1466.
20. Umadevi, M., Rajeswari, R., Rahale, C. S., Selvavenkadesh, S., Pushpa, R., Kumar, K. S., *et al*., Traditional and medicinal uses of Withania somnifera. The Pharma Innovation.2012; 1(9):102-110.
21. Mandlik, D.S., Namdeo, A.G. Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects. Journal of Dietary Supplements. 2021;18(2):183-226.
22. Bhat, J.A., Akther, T., Najar, R.A., Rasool, F., Hamid, A. *Withania somnifera* (L.) Dunal (Ashwagandha); current understanding and prospect as a potential drug candidate. Frontiers in Pharmacology. 2022;13:1029123.
23. Pina, L.T., Guimarães, A.G., Sampaio, L.A., Guimarães, J.O., Rabelo, T.K., Savi, F.M., *et al*., Plant-based pharmacological alternatives in the seizure treatment: A patent review. Research, Society, and Development.2022;11(2): e40411225940.