Original Article

Network Pharmacological Study of *Papaver Somniferum* to Explore the Potential Compounds to Treat Epilepsy

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ABSTRACT

OBJECTIVE: This study aims to discover potential compounds of *Papaver somniferum* for epilepsy treatment using network pharmacology and molecular docking tools.

METHODOLOGY: *Papaver somniferum* compounds were obtained from KNApSAcK and the IMPPAT database. Potential epilepsy targets were obtained from DisGeNET and GeneCards. After identifying common targets using a Venn diagram, DAVID analysis was performed. STRING database was used for protein-protein interactions, and Cytoscape was used for visualization and construction of target-compound-pathway network. Molecular docking was performed to identify potential active ingredients against epilepsy.

RESULTS: Fifteen active ingredients and 343 therapeutic targets were obtained from *Papaver somniferum*, and 7784 and 1215 epilepsy disease genes resulted in 79 common targets. Hub genes were identified using topological analysis. Molecular docking studies demonstrated that 15 active compounds possess the potential to bind the epilepsy targets.

CONCLUSION: Network pharmacology and molecular docking identified potential multitargeting compounds such as salutaridine, scoulerine, gamma-isomorphine, morphine, Codeine, Codeinone, (S)-Scoulerine and (-)-Codeinone for Epilepsy treatment.

KEYWORDS: *Papaver somniferum*, epilepsy, network pharmacology, Molecular docking, Medicinal plants, PPI network.

INTRODUCTION

Medicinal plants and herbs are used to treat various diseases[1]. People have been using plants for thousands of years, as evidenced by the oldest record of Nagpur, which is approximately 5000 years old[2][2]. In various regions of Pakistan, including Balochistan, Gilgit-Baltistan, Punjab, Sindh, and Pakhtunkhwa, herbal remedies are sold in many markets[3,4]. The largest herbal market is in Rawalpindi, Pakistan[4,5].

Papaver somniferum, a poppy plant belonging to the family *Papaveraceae*, grows in warm and temperate areas, is 100 cm tall, and has antidiabetic, antioxidant, antimicrobial, and anticancerous properties[6](3). *P. Somniferum* has been used as a medicinal plant for many decades because of its compounds, such as alkaloids, which have gained substantial commercial value. Different parts of this plant contain various pharmacological properties[7,8]. Minerals such as magnesium, calcium, potassium and iron are obtained from *Papaver somniferum* seed. Moreover, it also contains many carbohydrates, Vitamins (B and E), and trace elements like copper and zinc[9].

Epilepsy is a chronic brain disease that affects both sexes and is characterized by seizures, central nervous system insults, and cognitive, neurological, and psychological consequences[10]. Epilepsy accounts for 1% of the global disease burden, and its prevalence in Pakistan is 9.99 out of 1000 individuals, while in rural areas, 14.8 out of 1000 individuals[11]. The prevalence of depression in patients with epilepsy is 5–30%, according to population-based studies and 50% in tertiary centres[12]. 3-4% of people develop epilepsy out of 10% of people who experience seizures in industrialized countries[13,14]. The risk of epilepsy after Traumatic Brain injury, cerebrovascular accident, and acute infections is divided into early seizures (≤ 7 days) that carry a 25% risk of developing epilepsy and late seizures (≥ seven days) after injury if undue, 70-90% recurrence is considered[15]. Over 20 antiepileptic drugs are used for the management of seizures, but one-third of individuals with epilepsy do not respond to these medications[16]. One-third of people with epilepsy are unable to completely control their seizures despite taking anti-seizure medications (ASDs) either alone or in various combinations[17].

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Theoretically, resistance against epileptic drugs can manifest in four clinical patterns: 1) delayed resistance, in which patient becomes seizure-free at first, but seizures recur and uncontrollable after some time; 2) de novo (or ab initio) ASD resistance, in which patient never enters a valuable period of seizure freedom despite treatment with antiseizure drugs; 3) waxing-and-waning pattern, in which epilepsy alternates between being uncontrolled and controlled; or 4) epilepsy is initially drug-resistant but eventually responds to treatment. The mechanism of drug resistance is still unknown despite extensive research on epilepsy[16].

Network pharmacology, developed by Hopkins in 2007, combines principles from bioinformatics, pharmacology, and network science to understand the complex interactions between compounds and their target proteins[18]. With molecular docking, small molecules are docked with macromolecules to obtain the docking sores, which helps determine the compounds with solid affinity[19]. The study findings will provide a foundation for future pharmacological studies, which may contribute to developing novel effective anti-epileptics.

METHODOLOGY

Screening of Chemical Constitutes of *Papaver somniferum*

P. somniferum compounds were screened through KNApSAcK database (http://kanaya.naist.jp / KNApSAcK_Family) and IMPPAT database (https:// cb.imsc.res.in /imppat/help). *P. somniferum* compounds screening has been carried out by Lipinski Rule Five, Drug likeness (DL≥0.18) and Oral Bioavailability (OB≥0.30). Lipinski's rule of five assesses a compound's drug-likeness based on hydrogen bond donors and acceptors, molecular weight, and lipophilicity. Oral bioavailability (OB) refers to the bioavailability of pharmacological constituents, whereas drug-component similarity (DL) can suggest a possible drug. ADMET profiling was performed using ADMETlab 2.0 (https://admetmesh.scbdd.com/) and Swissadme (http://www.sib.swiss/). The blood-brain-barrier permeability of the compounds was confirmed by ADMET profiling. PROTOX II (https://tox -new.charite.de/) was used to exclude toxic compounds related to carcinogenicity, mutagenicity, cytotoxicity, hepatotoxicity, and immunogenicity.

Prediction of Potent Targets for *Papaver somniferum* Against Epilepsy Disease

The disease-gene targets of *P. somniferum* were identified using SwissTargetPrediction (http:// www.swisstargetprediction.ch/) while SMILES obtained from PubChem (https:// pubchem.ncbi.nlm.nih.gov/canonical). Homo sapiens was chosen as the species, and the predicted target results were collected. GeneCards and DisGeNET were used to retrieve genes involved in epilepsy using the keyword "Epilepsy". A Venn diagram (https:// bioinformatics.psb.ugent.be/webtools/Venn/) was constructed to identify the mutual disease and compound targets.

Gene Function Annotation and Pathway Analysis

Common Venn genes were entered into DAVID (https://david.ncifcrf.gov/). DAVID database provides gene ontology analysis covering molecular functions, biological processes, cellular components and KEGG pathways analysis. Seventy-nine common Venn genes were entered into the DAVID database for gene ontology terms and KEGG pathway analysis. "Homo sapiens" was chosen as a species. The top 10 gene ontology terms, KEGG pathways, and the highest counts of genes were selected (p-value <0.05). Hiplot software (https://hiplot.cn/) was used to visualize the results.

Networks Construction

Compound-Target Network Construction

Papaver somniferum active compounds and targets of epilepsy were entered into Cytoscape (version 3.9.1) (https://cytoscape.org/). Nodes represent the active compounds and targets, whereas edges represent interactions. Based on the degree of network node properties, the network was filtered. According to a degree network, the number of nodes connected to a particular network represents its degree.

PPI Network and Hub Genes Prediction

A protein-protein interaction network was constructed with the help of The String Database (https://string-db.org/). Cytoscape (version 3.9.1) was used to generate the PPI network using a PPI file into it. "Homo sapiens" was used as an organism, and the combined score was ≥0.4. Hub genes and higher-degree nodes were identified using the cytoHubba plugin.

Target -Compound- Pathway Network

DAVID was used to assess the top 20 KEGG pathways and design a target compound pathway network using Cytoscape.

Molecular Docking

Molecular docking confirmed the interaction between the ligands and the corresponding protein. Molecular docking was used to validate the findings of Papaver somniferum compounds against epilepsy. The PubChem database provided SDF file formats for 3D structures of the active components, which were then optimized. Protein Data Bank (RCSB) PDB provided the PDB file format for the hub genes (https:// www.rcsb.org/?ref=nav_home). Chimera X 1.4 version and Discovery Studio Visualizer 21.1.0.20298 were used to remove attached ligands and water molecules from protein structure preparation. The 3D structure of the chemical compound was downloaded in the SDF format using PubChem (https:// pubchem.ncbi.nlm.nih.gov/). Molecular docking was performed using the PyRx-Virtual screening tool, and the binding energy was calculated using the Lamarckian genetic algorithm. PyRx and Chimera X version 1.4 were used for visualization of molecular docking. The binding affinities between compounds and their corresponding targets were evaluated based

on their binding energies. Low binding energies suggest a strong affinity between a ligand and its corresponding protein.

RESULTS

Screening of Bioactive Compounds in *Papaver somniferum*

A total of 269 compounds were screened through literature review and databases. After further screening through ADMET analysis and Lipinski's rule of five, fifteen compounds were finalized as potential drug candidates (**Table I**).

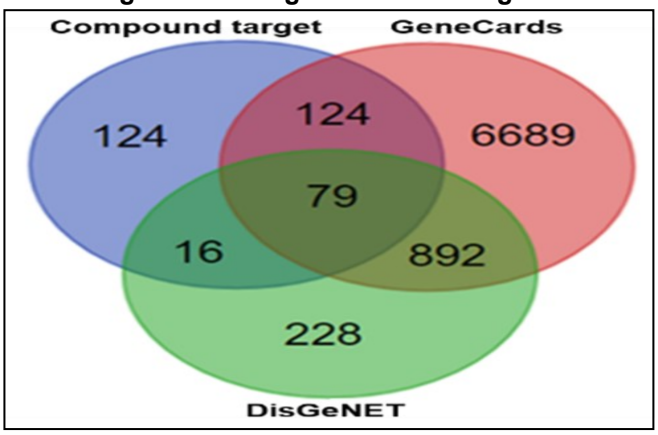
Identification of Potent Epilepsy Targets

Fifteen active compounds were used to retrieve 343 targets. A total of 7784 genes involved in epilepsy were retrieved from GeneCards and 1215 genes from DisGeNET, respectively. Through a Venn diagram, 79 common genes were identified between compound target genes and disease genes (**Figure I**).

Table I: Selected compounds with drug-likeness, oral bioavailability, class category and degree score

Compounds	Drug-likeness (DL ≥0.18)	Oral Bioa-vailability (OB ≥0.30)	Class category of compounds	Degree
Morphine	0.73	0.55	Alkaloid	26
Codeine	0.86	0.55	Alkaloid	25
Reticuline	1.13	0.55	Isoquinolinol	26
(S)-Scoulerine	1.1	0.55	Alkaloid	26
Salutaridine	1.13	0.55	Alkaloid	22
(4S,4aR,7S,7aR,12bS)-7,9-dimethoxy-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline	0.67	0.55	Alkaloid	26
gamma-Isomorphine	0.81	0.55	Alkaloid	26
Codeinone	0.73	0.55	Alkaloid	23
3,6-Dimethoxy-17-methyl-5,6,8,14-tetradehydro-morphinan-4,7-diol	1.05	0.55	Alkaloid	21
Coclaurine	0.89	0.55	Alkaloid	23
Scoulerine	1.1	0.55	Benzyli-soquinoline alkaloid	23
(-)-Codeinone	0.73	0.55	Isoquinoline alkaloid	22
7-O-Acetylsalutaridinol	1.15	0.55	Alkaloid	26
Salutaridinol	1.05	0.55	Morphinane alkaloid	8
Norcodamine	0.79	0.55	Alkaloid	23

Figure I:
Venn Diagram showing 79 common targets



Network Pharmacology Analysis

PPI Network analysis

A protein-protein interaction (PPI) network was constructed using 79 targets in the STRING database and was visualized in Cytoscape. The "Network analyzer" plugin revealed the network consists of 77 nodes and 635 edges. The hub genes were identified using the cytoHubba plugin. The Degree method was used as a topological analysis method to predict 10 Hub genes: alpha serine/threonine-protein kinase (AKT1)(46), Interleukin 6 (IL6)(45), Estrogen receptor 1 (ESR1)(41), Epidermal growth factor receptor (EGFR)(39), Jun Proto-Oncogene (JUN)(37), Glycogen Synthase Kinase 3beta (GSK3B)(35), Heat shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1)(34), Mechanistic Target Of Rapamycin Kinase (MTOR)(33), Prostaglandin-Endoperoxide Synthase 2 (PTGS2)(31), and Matrix metalloproteinase 9 (MMP9)(30).

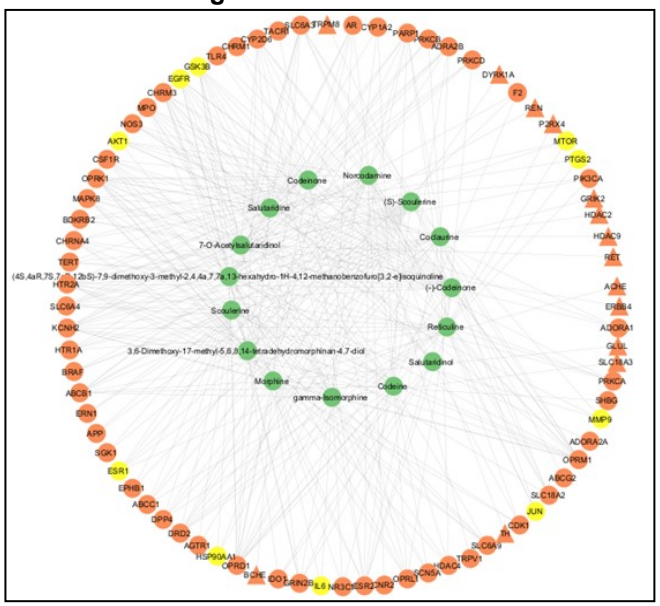
Compound Target Network

Cytoscape created a compound-target network between the fifteen active compounds and 79 common targets and analyzed their interactions. The network analysis revealed 94 nodes and 346 edges, as shown in **Figure II**. Each of the 15 targets was further selected for molecular docking against hub genes.

GO and KEGG Pathways Analysis

GO annotations for 79 epilepsy targets revealed that genes were involved in 391 biological processes (BP), including signal transduction, response to xenobiotic stimulus, inflammatory response, and protein phosphorylation; 65 cellular components (CC), including an integral component of the plasma membrane, plasma membrane, nucleus, and cytosol; 96 molecular functions (MF), including protein binding, ATP binding, identical protein binding, and enzyme binding. A total of 133 pathways were predicted using KEGG analysis. The top 20 GO annotations and KEGG pathways were selected with $p < 0.05$. Bubble plot graphs of GO annotations and KEGG pathways were generated using the Hiplot (**Figure III**).

Figure II: Compound-target network; green-coloured nodes depict active compounds, orange-coloured nodes represent the epilepsy targets, yellow-coloured nodes show the hub genes, and triangle-shaped nodes represent the least interacted genes



Compound-Target-KEGG Pathway Network

A compound-target-pathway network was constructed to identify the interaction of compounds, target genes and their respective pathways. "Network analyzer" revealed 114 nodes and 589 edges in the network with fifteen active compounds, twenty relevant pathways, and 79 potential targets (**Figure IV**).

Figure IV: Compound-Target-KEGG pathway network: green nodes represent compounds, yellow nodes depict KEGG pathways, pink nodes are hub genes and orange nodes show potential targets

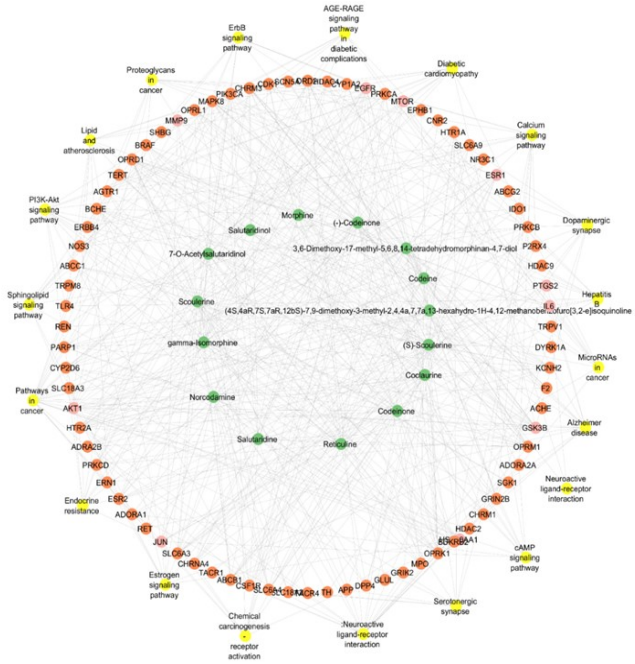
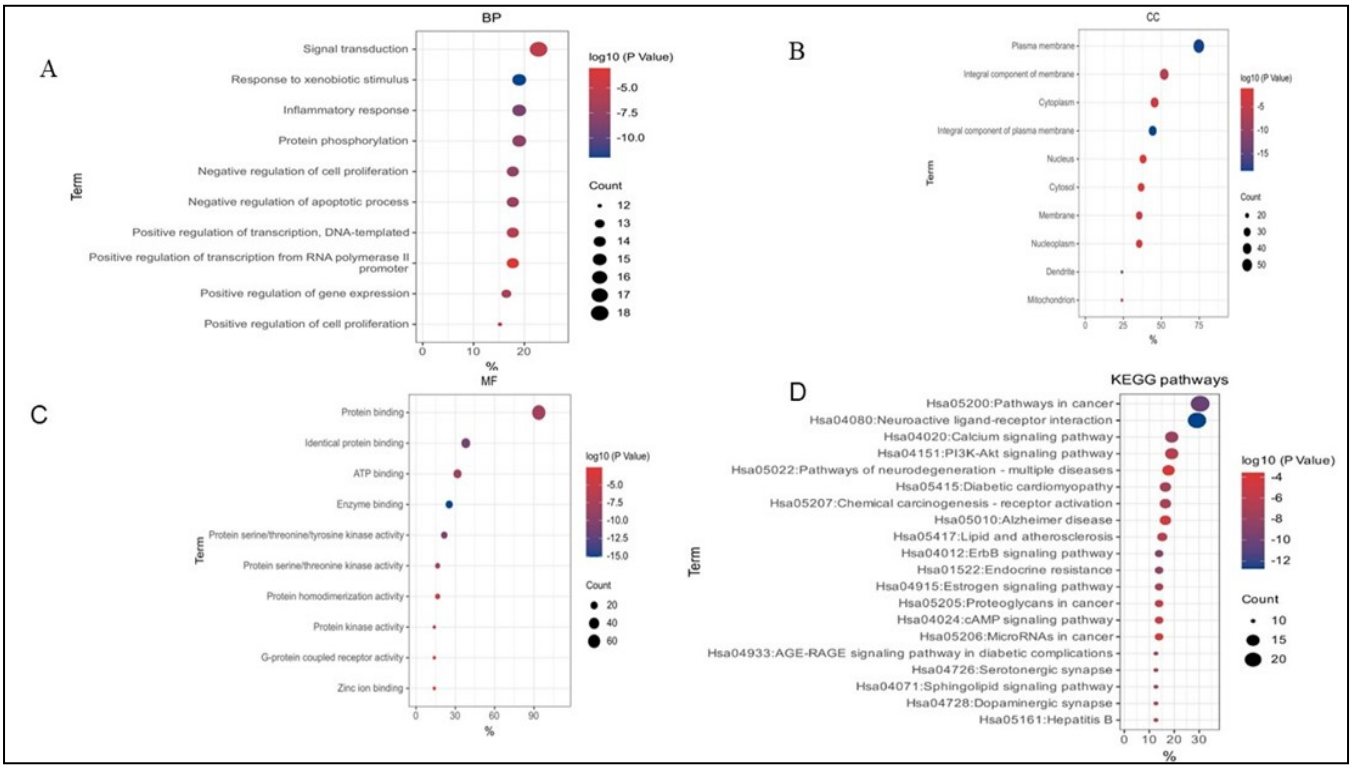


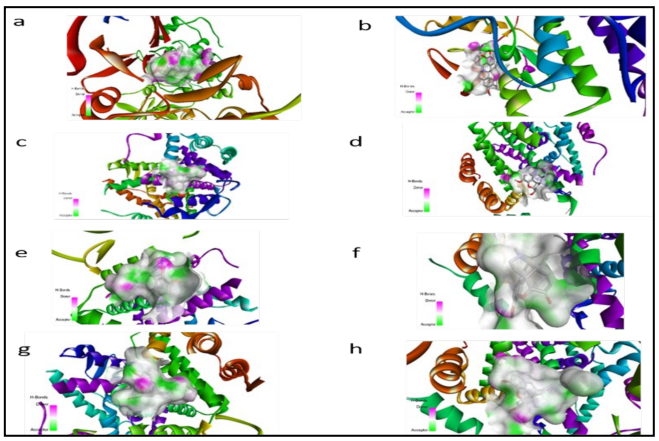
Figure III: Bubble graphs of GO analysis and KEGG pathways. (A) biological processes, (B) cellular components, (C) molecular functions, (D) KEGG pathways



Molecular docking

After removing tumor suppressor genes, the top five hub genes (AKT1, GSK3B, EGFR, JUN, and ESR1) were docked against fifteen active compounds. The binding affinities of bioactive molecules and their targeted genes ranged from -6.3 to -9.1 kcal/mole (Table II). Lowest docking score of compounds: Salutaridine with GSK3B (-8.1), scoulerine with EGFR (-8.1), ESR1 with gamma-Isomorphine (-9.1), ESR1 with Codeine (-8.7), (S)-Scoulerine (-8.7), Codeinone (-8.7), (-)-Codeinone (-8.7) and ESR1 with morphine (-9) are shown in **Figure V**.

Figure V: Docking of compounds: (a) Salutaridine with GSK3B (-8.1), (b) scoulerine with EGFR (-8.1), (c) ESR1 with gamma-Isomorphine (-9.1), (d) ESR1 with Codeine (-8.7), (e) (S)-Scoulerine (-8.7), (f) Codeinone (-8.7), (g) (-)-Codeinone (-8.7) and (h) ESR1 with morphine (-9)



DISCUSSION

Traditional medicines have been used for centuries to treat many diseases[20]. The drug development is a costly, time-consuming, and risky process, averaging $ 1.24 billion per drug. Preclinical investigations, clinical trials, and post-marketing safety monitoring take approximately 12-17 years due to constraints on safety, efficacy, and quantity in animal research and clinical trials[21]. Network pharmacology is an *in-silico* approach that helps us to predict potent compounds against disease targets; this helps us reduce the cost and time of *in vitro* and *in vivo* experiments[22].

Epilepsy can lower the quality of life and is associated with a higher risk of mental health issues, including cognitive impairment and migraine[23,24]. Although there are several epilepsy medicines, they also have side effects that are rare but serious; lamotrigine causes an allergic rash or Steven Johnson syndrome, Vigabatrin causes an irreversible peripheral field defect, lacosamide causes a prolonged PR interval, rufinamide causes arrhythmias and topiramate causes fatal hyperammonemic encephalopathy[25].

Papaver somniferum belongs to the plant family *Papaveraceae*. Although the plant is narcotic, as reported in the literature, *P. somniferum* cultivation is restricted in certain regions due to opium production, which is used in narcotic drug production; therefore, in many countries, laws are strictly followed to monitor and control its cultivation[26]. Despite its restriction on cultivation, *P. somniferum* is crucial for medicinal purposes; therefore, balancing medical benefits with proper regulatory controls is essential in ethical decision-making regarding its cultivation and use.

Table II: Binding energies of the potential targets with the active compounds. The green colour indicates a high binding energy (>8) for different active compounds of *P. somniferum*

Compounds	Targets				
	AKT1	GSK3B	EGFR	JUN	ESR1
Codeine	-6.9	-7.9	-7.3	-6.3	-8.7
Reticuline	-7	-7.2	-7.1	-6.3	-7.2
(S)-Scoulerine	-7.3	-8.1	-7.3	-6.7	-8.7
Salutaridine	-7.4	-8.1	-7.8	-6.9	-7.2
(4S,4aR,7S,7aR,12bS)-7,9-dimethoxy-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline	-6.8	-6.7	-7.1	-6.5	-7.5
gamma-Isomorphine	-7	-8	-6.6	-6.5	-9.1
Codeinone	-7.1	-8.1	-7.3	-6.7	-8.7
3,6-Dimethoxy-17-methyl-5,6,8,14-tetradehydromorphinan-4,7-diol	-6.9	-7	-6.7	-6.7	-7.2
Coclaurine	-7	-7.1	-6.8	-6.3	-7.9
Scoulerine	-7.6	-7.7	-8.1	-7	-7.4
(-)-Codeinone	-7.1	-8.1	-7.3	-6.7	-8.7
7-O-Acetylsalutaridinol	-6.6	-7.1	-7	-6.5	-7
Salutaridinol	-6.9	-6.7	-6.7	-6.7	-7.2
Morphine	-7.3	-8.2	-7.1	-6.5	-9
Norcodamine	-6.9	-7.7	-7.1	-6.4	-6.9

P. somniferum is a valuable herb with antidiabetic, antioxidant, antimicrobial, and antiproliferative effects[27]. *Papaver somniferum* is a rich source of phytochemicals. In this study, we retrieved 269 compounds and fifteen compounds were further screened through ADEMT analysis (**Table I**). This work provides a baseline for the preliminary in silico screening of bioactive compounds in *Papaver somniferum* as potential drug candidates against epilepsy[28].

GO analysis for biological processes revealed that anti -epilepsy targets of *P. somniferum* were involved in signal transduction, response to xenobiotic stimulus, inflammatory response, and protein phosphorylation (**Figure IIIA**). Signal transduction plays a role in epilepsy; atypical glutamate release in epilepsy can cause excessive excitatory signaling, which in turn can trigger seizures by over-activating glutamate release channels and causing neuronal hyperexcitability[29]. Numerous studies suggested that the basis of seizures and the process of epileptogenesis are related to inflammatory mediators released by the brain and peripheral immune cells[30].

GO analysis for Cellular components shows that our hub genes were expressed in the cytoplasm, plasma membrane, and dendrite (**Figure IIIB**). These genes are integral components of the membrane and integral components of the plasma membrane. Dendrite arborization is essential for integrating information and synaptic plasticity, as it defines their connection. Many studies on humans and animals have shown that structural anomalies in dendrites may play a role in neuronal dysfunction, behavioral and cognitive impairments, and epileptogenesis[31].

GO Molecular function analysis revealed that hub genes are involved in ATP binding, protein binding, protein serine/threonine kinase activity (STPK), protein kinase activity, and G-protein coupled receptor activity (**Figure IIIC**). Signals between neurons and glial cells are transferred by activating P2X receptors, which are ligand-gated ion channels that activate ATP. During an epileptic episode, the concentration of potassium ions in the extracellular space increases. This change causes astrocytes and hippocampus neurons' Pannexin1 (Panx1) channels to open, releasing ATP. An epileptic seizure starts when ATP receptor activation causes neurons to become hyperactive[32]. The most significant membrane protein class is G-protein coupled receptors, which are the therapeutic targets of most medications[33].

According to KEGG pathway analysis, hub genes were expressed in calcium signaling pathways, neuroactive ligand-receptor interaction, pathways in cancer, the PI3K-Akt signaling pathway, pathways related to neurodegeneration and multiple diseases (**Figure IIID**). The calcium signaling pathway is a crucial KEGG pathway related to epilepsy because calcium signaling is essential in neuronal development. Still, if calcium signaling is disturbed, it results in neural development disorders[34].

After performing GO, KEGG pathway, and network analysis, AKT1, GSK3B, EGFR, JUN, and ESR1 were selected as the primary protein targets. Glycogen Synthase Kinase-3β (GSK-3β) has been demonstrated to control several essential processes in neurons that underlie structural and functional synaptic plasticity[35]. It has been found that brain tissue removed from epileptic patients exhibits either an increase or a decrease in GSK-3β phosphorylation at Ser9[36]. Pharmacological investigations to clarify the function of GSK-3β inhibition in epilepsy revealed that GSK-3β inhibition protects neurons from glutamate-induced toxicity in vivo and invitro[37]. GSK3B shows the lowest docking score (-8.1) with salutaridine (**Figure Va**).

The epidermal growth factor receptor (EGFR), the first receptor tyrosine kinase superfamily member, is essential for embryogenesis and adult tissues. It plays a role in developing, differentiating, maintaining, and repairing various tissues and organs[38]. In epileptic tissue, EGFR is associated with immune cell and mast cell infiltration. EGFR showed a negative correlation with CD56dim natural killer cells and a positive correlation with immature dendritic cells, mast cells, MDSCs, and plasmacytoid dendritic cells. These findings offer fresh perspectives on the etiology and development of epilepsy and new potential treatment avenues[29]. In animal models, deficiency or absence of EGFR in brain cells increases the susceptibility to seizures chemically induced by kainic acid[39]. EGFR has also shown the Lowest docking score (-8.1) with scoulerine (**Figure Vb**).

Estrogen receptor 1 (ESR1) is a significant estrogen signaling pathway transcription factor that plays a role in treating epilepsy because of its neuroprotective effects, which primarily affect neuronal plasticity[40]. Recent research indicates that ESR1 and ESR2 are distributed throughout the brain, including in the hypothalamus, hippocampus, and amygdala. They control the transcription and translation of genes related to the circadian clock, affecting circadian autonomic processes and the sleep-wake cycle[41]. Docking scores of ESR1 with gamma-isomorphine (-9.1), ESR1 with morphine (-9), with Codeine (-8.7), Codeinone (-8.7), (S)-Scoulerine (-8.7), (-)-Codeinone (-8.7) (**Figure V**).

The present study predicts the active compounds of *Papaver somniferum.* The study utilizes network pharmacology and bioinformatics analysis, which provides predictions but may not fully represent the actual *in-vivo* effects. However, future experimental work is required to confirm the antiepileptic potential of predicted compounds.

CONCLUSION

This study has identified the potential compounds of *Papaver somniferum* against epilepsy. Compounds with lowest docking scores salutaridine with GSK3B (-8.1), scoulerine with EGFR (-8.1), ESR1 with gamma-Isomorphine (-9.1), ESR1 with Codeine (-8.7), (S)-

Scoulerine (-8.7), Codeinone (-8.7), (-)-Codeinone (-8.7) and ESR1 with morphine (-9) belonged to alkaloids and benzylisoquinoline alkaloids classes. However, the current study has certain limitations; additional pharmacological and phytochemical research is required to confirm our results.

Conflict of Interest: No conflicts of interest, as stated by authors.

Financial Disclosure / Grant Approval: No funding agency was involved in this research.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically. The questionnaire used in this is given in the Annexure.

AUTHOR CONTRIBUTION

Bibi F: Writing an original draft, Formal analysis, Methodology
Rauf A: Formal analysis
Khan MA: Conceptualization, Supervision
Ashfaq UA: Methodology, Validation
Qasim M: Formal analysis, Validation
Masoud MS: Methodology
Zarmeena R: Methodology
Bhinder MA: Writing original draft
Afandi MH: Writing original draft
Bhatti R: Conceptualization, Supervision

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