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Using *In-Silico* Studies To Identify The Antiepileptic Lead Compounds From Plants Using Swiss Adme And Swiss Dock

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Abstract:

Epilepsy is a complex neurological disorder that affects millions of people worldwide. Despite the availability of various antiepileptic drugs, there is still a need for more effective and safer therapeutic options. We employed a combination of in silico approaches to screen four phytochemical compounds (Apigenin, Cannabinol, Orientin, and Vitexin) for their potential as lead compounds in antiepileptic drug research The bioactivity prediction tool PASS (Prediction of Activity Spectra for Substances) suggested that these compounds may possess antiepileptic properties. Molecular docking studies against the target protein Carbonic Anhydrase (PDB ID: 3FSE) revealed that Cannabinol exhibited the strongest binding affinity, followed by Apigenin and Vitexin. Additionally, SwissADME predictions indicated that these compounds adhere to drug-likeness criteria, as well as pharmacokinetic and bioavailability guidelines.

Keywords: PASS, Carbonic Anhydrase, Apigenin, Cannabinol, Orientin, and Vitexin

Introduction

Epilepsy is the second most common and frequently encountered neurological disorder identified by recurrent seizures which are the result of uncontrollable neural excitation in the brain, often accompanied by cognitive deficits and mood disorders that impose a heavy burden on individuals, families, and also on healthcare systems (Borham *et al*., 2016; WHO, 2005 & Duncan *et al*., 2006). According to the International League Against Epilepsy (ILAE), epilepsy is a chronic brain disorder operationally defined by the occurrence of two unprovoked seizures more than 24 h apart, or one unprovoked seizure when the risk for another is known to be high (>60%) (International League of Epilepsy, 2014). Seizures can manifest in various clinical presentations with motor, sensory, autonomic or psychic origin (Shakirullah *et al*., 2014).

Epileptic state represents a dramatic imbalance between excitatory and inhibitory activity; a seizure activity due to altered γ-amino butyric acid type-A receptor (GABAAR) trafficking and/or subunit expression in animal models of temporal lobe epilepsy (TLE), Status epilepticus (SE) and in patients (Benarroch, 2007 & Coulter, 2001). The GABAAR is a ligand gated ion channel receptor which mediates quick inhibitory synaptic transmission into the central nervous system (CNS) and is a potential target of numerous essential neuroactive drugs (Macdonald *et al*., 1994 & Wallner *et al*., 2003). Patients with epilepsy are treated through drugs that work on membrane ion channels or on gamma amino butyric acidergic (GABA) or glutamatergic transmission. All the currently available antiepileptic drugs are synthetic molecules (Hema *et al*., 2009). In

many patients, the presently available antiepileptic drugs (AED) such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione etc., are unable to control seizures efficiently

The SwissADME web tool presented here is freely accessible at http://www.swissadme.ch and meant for user-friendly submission and easy analysis of the results, also for nonexpert in CADD. Compared to the state-of-the art of free web-based tools for ADME and pharmacokinetics and apart from unique access to proficient methods SwissADME strong points are, non-exhaustively: different input methods, computation for multiple molecules, and the possibility to display, save and share results per individual molecule or through global intuitive and interactive graphs.

Molecular docking is a computational tool that attempts to predict the structure of interaction between a protein and a molecule. Roughly, docking programs are a combination of a search algorithm and a scoring function. The search algorithm aims to find the precise ligand 3D geometry, also called poses, within a given targeted protein. The scoring function's purpose is to predict the binding affinity, evaluating how well ligands bind to the protein. The molecular docking of small molecules to protein binding sites was pioneered during the early 1980s (Kuntz *et al*., 1982). SwissDock is a docking web server that addresses the limitations described above. The structure of the target protein, as well as that of the ligand, can be automatically prepared for docking. In addition, the cumbersome syntax of the docking engine is hidden behind a clean web interface providing reasonable alternative sets of parameters and sample input files. All calculations are performed on the server side so that docking runs do not require any computational power from the user.

Materials and methods

Epilepsy is a serious neurologic condition associated with a social stigma, psychiatric discomfort and high cost of economy. The WHO's 2010 Global Burden of Disease study ranks epilepsy as the second most burdensome neurologic disorder worldwide in terms of disability-adjusted life years. The current treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side effects. Although herbal medicines are less potent in comparison to synthetic drugs in some cases these are still considered less toxic or have fewer side effects in contrast to synthetic drugs.

These compounds were screened for their biological activity by using the tool PASS (Alexey *et al*.,2000) The structure and other details of these natural compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. The standard drug Ethoxolamide was also obtained from the database. All the above four compounds were downloaded in the available file (.sdf) format and they were converted into (.pdb) format by using OpenBabel software (http://openbabel.org/)software). This file conversion was necessary because the structure of the ligand/protein could be loaded into the docking software only in the “pdb” file format. The screenshots of the tools used in this study are shown in Figure 2.

The target protein used for docking was Carbonic anhydrase (PDB ID: 3F8E). Biological activity analyses were carried out using PASS. This structural data with “sdf” extension was submitted to the PASS server. The chemical properties, of these compounds are also given in the table (Table 1).

Table 1. Chemical properties of these compounds

S.No	Compound	Pubchem ID	Mol Formula	Mol Wt
1	Apigenin	5280443	$C_{15}H_{10}O_5$	270.24g/mol
2	Cannabinol	2543	$C_{21}H_{26}O_2$	310.4g/mol
3	Orientin	5281675	$C_{21}H_{20}O_{11}$	448.4g/mol
4	Vitexin	5280441	$C_{21}H_{20}O_{10}$	432.4g/mol

The analysis was carried out by the following protocol:

Prediction of biological activities by virtual screening

Step 1: Open the PASS home page of PASS software by typing the web address.

http:// www.ipmh.msk.su/pass/

Step 2: Activate the working window by entering the username and password.

Step 3: Click the "browse" button to open the file name in which the molecule is saved.

Step 4: Fix the threshold value to 70%.

Step 5: Click the 'prediction' button to run the program.

Step 6: The results will be displayed in the screen.

Step 7: Save the page.

Evaluation of compounds for their drug likeliness

The Structure, Physicochemical properties, Lipophilicity, water solubility, Pharmacokinetics, Drug likeleness and medicinal chemistry of the chemical compound were predicted using SwissADME. This smile format file can be submitted in the server taken from pubchem or the structure of the compound can drawn using Chem stetch for SwissADME. Lipinski's Rule of Five (Lipinski *et al*., 2001) is a general accepted standard for oral applicable drugs. The rule describes molecular properties important for a drug's absorption, distribution, metabolism and excretion in the human body. It is stated that an orally active drug has not more than 5 hydrogen bond donors, not more than 10 hydrogen-bond acceptors, a molecular weight below 500 g/mol and a logP less than 5.

Testing the molecule for with SwissADME.

Step 1: get connected to SwissADME server

(http://www.swissadme.ch/)

Step 2: Get the smile format of the molecule and import it

Step 3: Convert this into Marvin Sketch window

Step 4: Upload the file into the server

Step 5: Predict the result

Step 6: Analyze the result

Docking the ligand with a protein

Molecular docking is a computational tool that attempts to predict the structure of interaction between a protein and a molecule. Roughly, docking programs are a combination of a search algorithm and a scoring function. The search algorithm aims to find the precise ligand 3D geometry, also called poses, within a given targeted protein. The scoring function purpose is the prediction of the binding affinity to evaluate how well the ligands bind to the protein.

In our project work, docking was carried out using the Carbonic anhydrase (PDB ID: 3F8E) protein as the target molecule. The target protein was downloaded from the PDB database (URL:www.rcsb.org/pdb)

and the structure was downloaded in the PDB format. The structure of the protein have crystallized this protein and submitted the structure to the PDB database. This PDB file was opened in text format for editing.

The compounds (Apigenin, Cannabinol, Orientin and Vitexin) were treated as the ligands and were docked separately with Carbonic anhydrase (PDB ID: 3F8E) as the target protein using SwissDock

SwissDock is a web server dedicated to carrying out protein-ligand docking simulation intuitively and elegantly. SwissDock is based on the protein-ligand docking program EADock DSS and has a simple and integrated interface. The SwissDock allows the user to upload structure files for a protein and a ligand, and returns the results by e-mail. To facilitate the upload of the protein and ligand files, we can prepare these input files using the program UCSF Chimera

Results and discussion

According to the World Health Organization (WHO), around 80% of those living with epilepsy reside in developing countries, where access to adequate medical treatment is often limited. Epilepsy is a neurological disorder characterized by recurrent seizures that can vary in frequency and severity. These seizures result from abnormal electrical activity in the brain and can significantly impact the quality of life of those affected. Research has indicated that both flavonoid- and non-flavonoid-containing plants may possess therapeutic properties that can help improve morbidity associated with epilepsy. Flavonoids, which are a diverse group of phytonutrients found in many fruits, vegetables, and herbs, have been shown to exhibit neuroprotective effects, potentially through their antioxidant and anti-inflammatory properties. Similarly, non-flavonoid compounds from certain plants may also contribute to seizure control and neuroprotection.

We aimed to screen four phytochemical compounds for their potential as lead compounds in antiepileptic drug research. The selected natural plant compounds—Apigenin, Cannabinol, Orientin, and Vitexin—were used as ligands in docking studies against the target protein Carbonic Anhydrase (PDB ID: 3F8E). For comparison, the standard antiepileptic drug Ethoxzolamide was also included in the docking analysis. All compounds, including the standard drug, were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

To assess the biological activity of these compounds, we utilized the PASS (Prediction of Activity Spectra for Substances) web-based tool. PASS predicts the potential biological activities of a molecule based on its chemical structure, providing a probability score for various biological functions, such as enzyme inhibition and receptor activation. The tool analyzes the chemical features of the selected compounds and predicts their likelihood of exhibiting specific biological activities by comparing them to known active compounds.

The bioactivity predictions for the selected compounds were quantified using two key metrics: Pa (the probability of being an active compound) and Pi (the probability of being an inactive compound). These results are summarized in Table 2. In this context, a Pa value greater than Pi indicates a higher likelihood of biological activity, with the scale ranging from 0.000 to 1.000. Generally, the interpretation of the PASS prediction results is as follows:

- If $Pa > 0.7$, there is a high probability of finding the activity experimentally.
- If $0.5 < Pa < 0.7$, the likelihood of finding the activity experimentally is lower, and the compound may not closely resemble known pharmaceutical agents.
- If $Pa < 0.5$, the chance of finding the activity experimentally is minimal, although structural similarities may still exist (Filimonov et al., 2014).

Thus, the predicted activity spectrum serves as an intrinsic property of the compounds, guiding further experimental validation and exploration of their potential as antiepileptic agents.

Table 2a. Apigenin

SI.No	Pa	Pi	Activity
1	0,973	0,001	Chlordecone reductase inhibitor
2	0,967	0,002	Membrane integrity agonist
3	0,946	0,002	Membrane permeability inhibitor
4	0,946	0,004	CYP2C12 substrate
5	0,942	0,002	2-Dehydropantoate 2-reductase inhibitor
6	0,941	0,002	Kinase inhibitor
7	0,936	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
8	0,937	0,003	Aldehyde oxidase inhibitor
9	0,931	0,001	P-benzoquinone reductase (NADPH) inhibitor
10	0,931	0,003	Anaphylatoxin receptor antagonist

Table 2b. Cannabinol

SI.No	Pa	Pi	Activity
1	0,936	0,005	CYP2C12 substrate
2	0,855	0,015	Ubiquinol-cytochrome-c reductase inhibitor
3	0,766	0,017	Membrane permeability inhibitor
4	0,735	0,005	Reductant
5	0,722	0,011	Nitrate reductase (cytochrome) inhibitor
6	0,689	0,011	Vasoprotector
7	0,685	0,009	General pump inhibitor
8	0,685	0,010	HMOX1 expression enhancer
9	0,678	0,008	MMP9 expression inhibitor
10	0,678	0,021	Antidyskinetic

Table 2c. Orientin

SI.NO	Pa	Pi	Activity
1	0,974	0,003	TP53 expression enhancer
2	0,961	0,003	Membrane integrity agonist
3	0,955	0,001	Free radical scavenger
4	0,952	0,002	Cardioprotectant
5	0,940	0,004	HIF1A expression inhibitor
6	0,927	0,002	Hepatoprotectant
7	0,904	0,004	Membrane permeability inhibitor
8	0,892	0,003	UGT1A9 substrate
9	0,888	0,003	2-Dehydropantoate 2-reductase inhibitor
10	0,884	0,003	Vasoprotector

Table 2d. Vitexin

SI.No	Pa	Pi	Activity
1	0,973	0,003	TP53 expression enhancer
2	0,966	0,002	Membrane integrity agonist
3	0,950	0,002	Cardioprotectant
4	0,940	0,004	HIF1A expression inhibitor
5	0,901	0,002	Free radical scavenger
6	0,891	0,004	Membrane permeability inhibitor
7	0,890	0,004	Anaphylatoxin receptor antagonist
8	0,883	0,003	Hepatoprotectant
9	0,882	0,003	UGT1A9 substrate
10	0,877	0,004	2-Dehydropantoate 2-reductase inhibitor

The results obtained from from SwissADME indicated that these compounds can be a druggable substance with a minimum violation of any of drug-likeness rules, pharmacokinetics, bioavailability and medicinal chemistry. It was interesting to note that the results from the SwissADME predictor values of Log P, molar refractivity, and the total polar surface area in these molecules were in excellent agreement with the most important rules of drug-likeness. The results of the SWISS ADME for the selected compounds are shown in Figures 3.

The data shown in Table 3 compares four compounds: Apigenin, Cannabinol, Orientin, and Vitexin, across various physicochemical and pharmacological properties. Apigenin and Cannabinol show high gastrointestinal absorption and favorable bioavailability scores, making them potentially effective for therapeutic applications. Both compounds also adhere to Lipinski's rule, indicating good drug-like properties. Orientin and Vitexin, with higher molecular weights and more hydrogen bond acceptors, exhibit lower GI absorption and bioavailability scores. Their higher TPSA values suggest they may have limited permeability, which could affect their efficacy as drugs. Cannabinol stands out with its ability to cross the blood-brain barrier, which may be beneficial for central nervous system-targeted therapies. However, it also has multiple CYP enzyme interactions, which could lead to drug-drug interactions. The presence of alerts for Orientin indicates potential issues with its drug-likeness, which may complicate its development as a therapeutic agent. Apigenin is the easiest to synthesize, while Orientin and Vitexin may pose challenges due to their higher values, suggesting more complex synthetic route.

Table 3. Result of SwissADME analysis

	Apigenin	**Cannabinol**	**Orientin**	**Vitexin**
Mol weight	270.24 g/mol	310.43 g/mol	448.38 g/mol	432.38 g/mol
Rotatable bonds	1	4	3	3
H bond Acceptors	5	2	11	10
H bond donor	3	1	8	7
Molecular refractivity	73.99	97.10	108.63	106.61
TPSA	90.90 Aº	29.46 Aº	201.28 Aº	181.05 Aº
Log S (ESOL)	-3.94	-5.74	-2.70	-2.84
Log S (Ali)	-4.59	-6.51	-3.62	-3.57
Log S (SILICOS-IT)	-4.40	-7.49	-1.79	-2.38
GI absorption	High	High	Low	Low
BBB permeant	No	Yes	No	No
P-gp substrate	No	Yes	No	No

CYP1A2 inhibitor	Yes	Yes	No	No
CYP2C19 inhibitor	No	Yes	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	No
CYP3A4 inhibitor	Yes	No	No	No
Log K_p (skin permeation)	-5.80 cm/s	-3.06 cm/s	-9.14 cm/s	-8.79 cm/s
Lipinski	Yes	Yes: 1 violation	No: 2 violations	Yes: 1 violation
Ghose	Yes	No: 1 violation	No: 1 violation	Yes
Veber	Yes	Yes	No: 1 violation.	No: 1 violation.
Egan	Yes	Yes	No: 1 violation	No: 1 violation.
Muegge	Yes	No: 3 violations	No: 3 violations	No: 2 violations
Bioavailability Score	0.55	0.56	0.17	0.55
PAINS	0 alert	0 alert	1 alert catechol A	0 alert
Brenk	0 alert	0 alert	1 alert calechol	0 alert
Leadlikeness	Yes	No: 1 violation	No:1 violation	No: 1 violation
Synthetic accessibility	2.96	3.39	5.17	5.12

Docking Studies

In this present study, the docking tool SwissDock was used to analyze the binding affinity of three different plant compounds (Apigenin, Cannabinol, Orientin and Vitexin) against Carbonic anhydrase (PDB ID: 3F8E). The commercial drug Ethoxolamide also was docked against the same target for a comparative study. The best binding conformation for the four phytocompounds into target proteins was determined by looking for the one having the lowest total binding energy among the different conformations generated. Cannabinol exhibited the strongest binding affinity with a score of -6.299 kcal/mol, suggesting it may have a significant interaction with the target protein. Apigenin and Vitexin also showed promising binding scores of -5.932 kcal/mol and -5.963 kcal/mol, respectively, indicating potential as effective ligands. Orientin had a slightly lower binding score of -5.302 kcal/mol, which still suggests some level of interaction but may be less favourable compared to the others. Ethoxzolamide, the standard drug, had the weakest binding score of -4.290 kcal/mol, indicating a lower affinity for the target protein compared to the natural compounds These binding scores can help prioritize which compounds to further investigate for their potential as antiepileptic agents based on their interaction with Carbonic Anhydrase (Table 4).

Table 4. The binding affinity of the selected compounds against the target protein

S.no	Compound name	Binding score (kcal/mol)
1.	Apigenin	-5.932
2.	Cannabinol	-6.299
3.	Orientin	-5.302
4.	Vitexin	-5.963
5.	Ethoxolamide	-4.290

The binding energy of receptor-ligand interactions is a critical factor in assessing the suitability of a compound as a potential drug. A more negative binding energy indicates a stronger and more favourable interaction between the ligand and the target protein, suggesting that the drug fits well into the binding site of the target molecule. This spontaneous binding process is essential for the efficacy of a drug, as it implies

that the compound can effectively engage with its target (Balavignesh *et al*., 2013). Phytochemicals have multifunctional properties such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, and immunomodulatory, and they act as antagonists and agonists for various types of inhibitory and excitatory receptors in the body, especially in the CNS (Akhtar *et al*., 2021). The efficacy of plant extracts and phytochemicals against various diseases related to the central nervous system (Ayeni *et al*., 2022 & Luthra and Roy, 2022).

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