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Glabridin From Glycyrrhizha Glabra Linn. As Multi-Targeted Therapeutic Agent Against Alzheimer's Disease: An In-Silico Study

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease mainly occurring in old age adults. Its pathophysiology involves accumulation of tau protein in brain leading to the intracellular neurofibrillary tangles (NFTs) and amyloid β protein accumulation leads to extracellular amyloid β plaques in the central nervous system. Existing treatments for such neurodegenerative conditions do not slow-down disease progression, and this will cause an overwhelming future burden on our healthcare system and immense suffering for many more patients and their families. The current researches for developing potential drugs for the Alzheimer's disease mainly focusing on the therapeutic targets such as acetylcholine esterase (AChE), Butrylcholine esterase (BuChE), β-site APP-cleaving enzyme1 (BACE1), Glycogen synthase kinase-3β (GSK3β) and Kelch-like ECH Associated Protein 1 (Keap1). To halt the progression of the disease, discovery of the molecules that potentially interferes with these therapeutic targets is paramount important. The ADMET property analysis showed that 5 flavonoid compounds from the plant Glycyrrhiza glabra Linn. were biologically active with drug-likeliness nature. The molecular docking and molecular dynamic simulation studies evidenced that among these phytochemicals glabridin can be a potential inhibitor of the Alzheimer's therapeutic target proteins AChE, BuChE, BACE-1, GSK3β and Keap1. This may have a role in controlling the pathogenesis and thereby curing the disease. Further in vivo investigations is required to validate the compound as a new therapeutic agent for the Alzheimer's disease.

Keywords: Alzheimer's Disease, Glycyrrhiza glabra Linn., Glabridin, Therapeutic agent, In-silico study

Introduction

Alzheimer's disease is the most common cause of dementia among the elderly adults and is characterized by loss of memory and other cognitive functions. Alzheimer's disease is histopathologically characterized by slow and progressive neurodegeneration. Accumulation of tau protein in AD brain leading to the intracellular neurofibrillary tangles (NFTs) and amyloid β protein accumulation leads to extracellular amyloid β plaques in the central nervous system (Maccioni et al., 2001). Prevalence of AD includes 13% of people older than 65 years and 45% of those older than 85 years. Worldwide, more than 50 million people have dementia; every year, nearly 10 million new cases are reported. The total number of people with dementia is projected to reach 82 million in 2030 and 152 million in 2050 (WHO, 2019). Current medications can't cure the degeneration of brain cells of AD patients. Instead, they will only help to lessen

or stabilize symptoms for a limited time. Current Drugs to treat AD symptoms in early to moderate stages are cholinesterase inhibitors. Cholinesterase inhibitors are prescribed to treat symptoms, but unfortunately cause undesirable side effects (Reddy, 2006).

Some of the widely discussed therapeutic targets of Alzheimer's disease are acetylcholine esterase (AChE), Butrylcholine esterase (BuChE), β-site APP-cleaving enzyme1 (BACE1), Glycogen synthase kinase-3β (GSK3β) and Keap1-Nrf2. Inhibition of these proteins are believed to slow down the disease progression and thereby providing a permanent cure to the neurodegenerative conditions.

The neurotransmitter enzyme acetylcholine esterase (AChE) is a specific esterase that belongs to the carboxyl esterase family of enzymes. It predominantly hydrolyzes the neurotransmitter acetylcholine (ACh). AChE is found in high concentrations mainly in the red blood cells as well as in the brain at neuromuscular junctions and cholinergic brain synapses. The choline esterase inhibitors (ChE-Is) attenuate the cholinergic deficit underlying the cognitive and neuro-psychiatric dysfunctions in patients with AD. Inhibition of brain acetylcholinesterase (AChE) has been the major therapeutic target of ChE-I treatment strategies for Alzheimer's disease. AChE positive neurons project diffusely to the cortex, modulating cortical processing and responses to new and relevant stimuli (Mushtaq et al., 2018).

The neurotransmitter enzyme butrylcholine-esterase (BuChE) is a non-specific type of cholinesterase enzyme that hydrolyzes different types of choline esters and it ubiquitously exists in the body, including human liver, blood serum, pancreas and the central nervous system. In the brain, BuChE is primarily associated with glial cells and endothelial cells (Mushtaq et al., 2018). Butryl cholinesterase (BuChE) project specifically to the frontal cortex, and may have roles in attention, executive function, emotional memory and behaviour. Furthermore, BuChE activity progressively increases as the severity of dementia advances. Therefore, inhibition of BuChE may provide additional benefits to the AD therapy (R. M. Lane et al., 2005).

To reduce the level of cerebral amyloid plaques, reducing and eliminating Aβ production are being considered. These agents typically had three different target sites: direct targeting of Aβ and the β or γ-secretase enzymes involved in APP cleavage. β-site APP-cleaving enzyme1 (BACE1) inhibitors showed reduced cerebrospinal fluid (CSF) beta-amyloid small molecules; suggests that BACE1 enzyme is essential for the production of β-amyloid (Cao et al., 2018). The fact that BACE1 triggers the formation of β-amyloid and the observation of elevated BACE1 levels in the Alzheimer's disease provides a direct and compelling reason for the development of therapies that directly inhibit BACE1, thereby reducing β-amyloid and its toxicity (Das and Yan, 2019). Currently, one of the AD-modifying therapies follows the amyloid cascade theory. Hence, discovery of a novel molecule that inhibits the aggregation of Aβ protein and acts as BACE1 inhibitors is a therapeutic target of interest.

Glycogen synthase kinase-3β (GSK3β) is a proline-directed kinase mainly expressed in central nervous system that phosphorylates tau protein (Terwel et al., 2008). Over expression of GSK3β increases tau protein phosphorylation, allowing it to disassemble from microtubules, leading to axonal transport alterations, neurodegeneration and learning impairment (Avila, 2006). GSK3β is able to phosphorylate tau at 42 sites; its activity correlates with the level of NFTs in AD brains (Leroy et al., 2002).Reduction of tau phosphorylation via inhibition of kinases is a major therapeutic strategy for AD. So, GSK3β has emerged as an interesting therapeutic target candidate of Alzheimer's disease (Barten and Albright et al., 2008).

Nrf2 is a gene that normally protects cells from stressful conditions and it is considered as an attractive therapeutic target for the prevention of neurodegenerative diseases, including Alzheimer's disease. An

understanding of the mechanisms mediating Nrf2 inhibition in neurodegenerative conditions may therefore direct the design of drugs targeted for the prevention of these diseases with minimal side-effects. Direct Keap1-Nrf2 disruptors can specifically target the defects in Nrf2 activity observed in neurodegenerative diseases, and supports the further development of such compounds as potential new drugs to prevent neuronal decline AD and other neurodegenerative conditions (Kerr et al., 2017).

Ayurveda, the traditional treatment method of India attained a greatest attention and recognition worldwide. 'Medhyarasayana' is a group of medicinal plants described in Ayurveda with multiple benefits, specifically to enhance memory and intellect. Glycyrrhiza glabra Linn. (Licorice) is one among the Medhya plants used in Ayurveda. Roots of licorice have many medicinal properties like antipyretic, anti-microbial, anxiolytic and memory enhancing activity. There is much literature for the memory enhancing activity of the plant, still there is no specific active phytocompound from the plant is developed as a drug (Kalani et al., 2013).

Virtual screening is coming to its importance in drug discovery since it can be tested computationally in order to reduce the number of compounds that need to be tested experimentally (Liao et al., 2013). High-throught put virtual screening (HTVS) computationally screens large databases of virtual compounds that either possesses similarity towards a known inhibitor (ligand based) or complementary towards the solved receptor structure (Shoich et al., 2004).). To deal with such a large set of data and to design new therapeutic compounds, the drug discovery process requires virtual screening of drugs, molecular docking and molecular dynamics simulations studies (Seniya et al., 2014). To deal with such a large set of data and to design new therapeutic compounds, the drug discovery process requires virtual screening of drugs, molecular docking and molecular dynamics simulations studies (Seniya et al., 2014). Molecular docking is a powerful computational modelling tool in evaluating binding of a ligand to the active site of a protein or receptor. The amount of energy liberated upon docking of a ligand with a receptor is a measure of stability of the complex, and indicates how strongly a ligand may inhibit a protein (Torres et al., 2019). Molecular dynamics (MD) simulation is a powerful computational method for delineating motions of proteins at an atomic-scale via theoretical and empirical principles in physical chemistry. Recent advances in the hardware and software for biomolecular simulation have rapidly improved the precision and performance of this technique. Consequently, MD simulation is quickly extending the range of applications in biology, helping to reveal unique features of protein structures that would be hard to obtain by experimental methods alone. MD simulation currently allows us to investigate the structural dynamics of proteins on timescales of nanoseconds to microseconds, and will probably allow investigation to milliseconds in the future (Ode et al., 2012).

Methodology

Molecular details and structures (2D) of phytochemicals present in Glycyrrhiza glabra Linn. (G. glabra Linn.) were collected from PubChem database (Kim, 2019) for the ligand preparation and other experimental analysis (Table 1).

Sl. No.	PubChem CID	Compound Name	Formula	Molecular Weight
1.	11230	Terpinen-4-ol	$C_{10}H_{18}O$	154.25
2.	14982	Glycyrrhizin	$C_{42}H_{62}O_{16}$	822.93
3.	68071	Pinocembrin	$C_{15}H_{12}O_4$	256.25
4.	114829	Liquiritigenin	$C_{15}H_{12}O_4$	256.25

5.	124052	Glabridin	$C_{20}H_{20}O_4$	324.37
6.	197678	Shinflavanone	$C_{15}H_{26}O_4$	390.47
7.	442774	Hispaglabridin A	$C_{25}H_{28}O_4$	392.49
8.	637566	Geraniol	$C_{10}H_{18}O$	154.25
9.	638278	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.25
10.	5318591	Isoliquiritin	$C_{15}H_{12}O_4$	256.25
11.	5318998	Licochalcone A	$C_{21}H_{22}O_4$	338.40
12.	5318999	Licochalcone B	$C_{16}H_{14}O_5$	286.28
13.	6475724	Licuraside	$C_{26}H_{30}O_{13}$	550.51
14.	10076238	Liquiritin apioside	$C_{26}H_{30}O_{13}$	550.51
15.	10336244	Shinopterocarpin	$C_{20}H_{18}O_4$	322.35
16.	10473311	Licochalcone D	$C_{21}H_{22}O_5$	354.40
17.	11349817	Prenyllicoflavone A	$C_{25}H_{26}O_4$	390.47
18.	14218027	Licoflavanone	$C_{20}H_{20}O_5$	340.37
19.	74819335	Glucoliquiritin apioside	$C_{32}H_{40}O_{18}$	712.65

Table 1: List of phytochemicals from the plant Glycyrrhiza glabra Linn.

This study were conducted using Small-Molecule Drug Discovery Suite 2018-4, Schrodinger, LLC, New York, 2018.

2D structures of phytochemicals Glycyrrhiza glabra were prepared for the ADME (Absorption, Diffusion, Metabolism and excretion) analysis and docking experiment using the LigPrep module of the Schrodinger suite (LigPrep, Schrödinger, 2020). Hydrogen atoms are added and desalted during the preparation of ligands. Epik at pH 7.0 ± 2.0 was used to create possible ionization, and tautomeric states and energy minimized using force field OPLS3e. Ligands with the lowest energy obtained and saved in maestro (.mae) file format for further experiments.

The computational pharmacokinetics properties of the selected ligands were studied using the Qikprop module of Schrodinger suite 2018-4. ADME properties such as central nervous system activity (CNS), Caco-2 permeability (QPPCaco), brain/blood partition coefficient (QPlogBB), MDCK cell permeability (QPPMDCK) and percentage of human oral absorption (PHOA) and Lipinski's rule of five were analyzed. 3D structures of the Acetylcholine esterase (AChE) (PDB ID – 1QON) (Harel et al., 2000), Butrylcholine esterase (BuChE) (PDB ID – 5DYW) (Kosaks et al., 2016), Glycogen synthase kinase 3β (GSK3β) (PDB ID - 1J1B) (Aoki et al., 2004), β secretase enzyme-1(BACE1) (PDB ID -2WJO) and Kelch-like ECH Associated Protein 1(Keap-1) (PDB ID - 2FLU) (Lo et al., 2006) were retrieved from protein data bank (RCSB PDB) (Burley et al., 2019). Before molecular docking, the protein was prepared using the protein preparation wizard of Maestro 11.8.012 of Schrodinger software 18.04 (Maestro, Schrödinger). Protein pre-treatment was done with the OPLS3e force field (Shivakumar et al., 2010). Adding hydrogen bonds and side chains, deleting water, and heteromeric states were generated using Epik (Epik, Schrödinger, Greenwood et al., 2010, Shelley et al., 2007) at pH 7.0 ± 2.0. The structure was minimized until the root mean square deviation (RMSD) of the heavy atom reached a maximum value of 0.3 Å. All the protein structures were co-crystallized with ligands. So, those sites were used to determine the ligand-binding site of the protein. Grid box size of the protein was determined with active residues of the protein.

Phytochemicals of G. glabra. Linn. were subjected to docking using Glide (Grid-based Ligand Docking with Energetics) (Friesner et al., 2006, Glide, Schrödinger). Receptor grid for docking all receptors (Table

3.3) was generated using receptor grid generation panel of Schrodinger. All ligands were docked with AChE, BuChE, GSK3β, BACE1 and Keap1 with XP (Extra precision) mode (PrimeX, Schrödinger, 2020, Bell et al., 2012).

ΔG bind (The binding free energy) of phytochemicals and the therapeutic targets calculated using Prime MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) (Prime, Schrödinger, 2020). Ligands and target proteins prepared for Prime MM-GBSA using LigPrep and Protein Preparation Wizard. Best poses of protein-ligand complexes from Glide XP docking used to calculate binding free energy (ΔG bind). The equation is given below.

ΔGbind = Gcomplex – (Gprotein + Gligand)

Gcomplex – Free energy of complex

Gprotein – Free energy of protein

Gligand – Free energy of ligand

The phytochemical, Glabridin (PubChem CID-124052), is subjected to the molecular dynamics (MD) simulation studies against the Alzheimer's therapeutic target proteins in Maestro molecular modeling interface (Version 11.8.012) using TIP3P model. An orthorhombic box was generated in the System builder panel and the volume is minimized. Adequate numbers of Na+/Cl- ions were added to maintain charge neutrality of the system. Glabridin was subjected to MD simulation with each therapeutic target proteins for 100 ns. Detailed PDF report of the simulation studies for 100 ns is generated using the 'Simulation Interaction Diagram' window in Maestro.

Results

Phytochemicals from the plant Glycyrrhiza glabra Linn. were analysed for their pharmacological properties. In the study five phytochemicals from the plant, namely, glabridin, terpen-4-ol, pinocembrin, geraniol and shinopterocarpin, were showed drug likeliness properties. The ADME properties of the five eligible drug candidates are shown in the
Table 2.

Sl. No.	PubChem CID	Compound Name	MW	Donor HB	Accept HB	QP log Po/w	QPP Caco	QPlog BB	QPP MDCK	% HOA	CNS
1	124052	Glabridin	324.37	2	3	4.021	1156.887	-0.519	579.109	100	0
2	11230	Terpinen-4-ol	154.25	1	0.75	2.972	5735.879	0.247	3268.186	100	1
3	68071	Pinocembrin	256.25	1	3.25	2.391	428.969	-0.828	198.174	88.06	-1
4	637566	Geraniol	154.25	1	1.7	2.597	2823.603	-0.242	1519.201	100	0

5	10336244	Shinopterocarpin	322.36	1	3	4.309	3010.363	-0.019	1628.098	100	0

Table 2. Drug-like phytochemicals from Glycyrrhiza glabra Linn.

#**MW**-Molecular Weight, **Donor HB-** Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, **Accept HB-** Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. **QPlog Po/w-** Predicted octanol/water partition coefficient, **QPPCaco-** Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are model for the gut-blood barrier. QikProp predictions are for non-active transport, **QPlogBB-** Predicted brain/blood partition coefficient, **QPPMDCK-** Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier. Qik Prop predictions are for non-active transport. **%HOA-** Percent Human Oral Absorption, **CNS-** Predicted central nervous system activity. **CID-**Compound ID number.

Five selected drug candidates after ADME/Tox prediction analysis were docked with the AD therapeutic target proteins AChE, BuChE, BACE-1, GSK3β and Keap-1. The Glide XP Docking scores of the selected phytochemicals with therapeutic targets of AD are provided in table 3. The score function of the glide ligand docking indicates the ability of the ligand to bind a specific conformation of the protein receptor. The greater the negative value on the score function indicates better docking. Glabridin was the best phytochemical with less docking scores against the therapeutic targets. The respective docking scores of glabridin with target proteins were AChE (-8.454), BuChE (-6.402), BACE-1 (-6.509), GSK3β (-5.124) and Keap-1 (-3.399). The compound effectively bind with these targets and shows stable interactions with the active sites of the target proteins.

Phytochemical	**Glide XP Docking Score**				
	Acetylcholine esterase (AChE)	**Butrylcholine esterase (BuChE)**	**β secretase cleavage enzyme-1 (BACE-1)**	**Glycogen synthase kinase-3-beta (GSK3β)**	**Kelch-like ECH Associated Protein-1(Keap-1)**
Glabridin	-8.454	-6.402	-6.509	-5.124	-3.399
Pinocembrin	-9.105	-9.524	-6.357	-7.308	-6.371
Terpinen-4-ol	-6.574	-4.995	-4.182	-3.838	-3.386
Shinopterocarpin	-7.477	-5.848	-4.958	-6.126	-2.077
Geraniol	-6.725	-4.754	-4.817	-5.088	-1.947

Table 3. Glide XP Docking scores of selected phytochemicals with therapeutic targets of AD

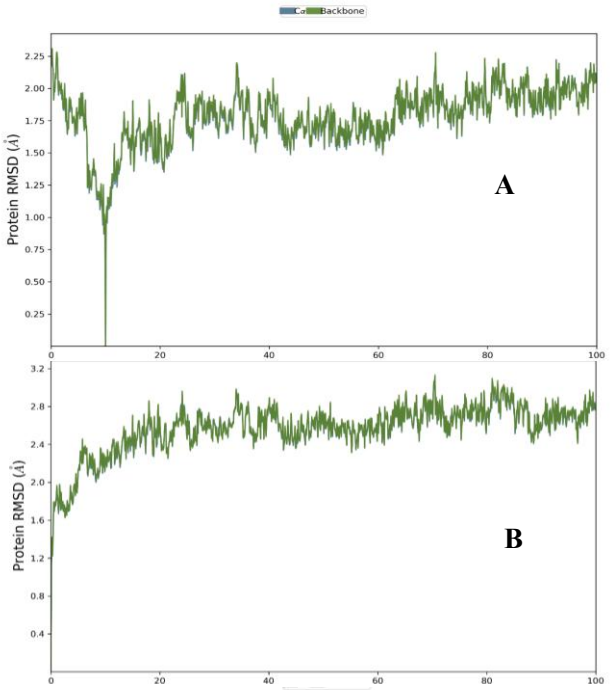
The Prime MMGBSA of the selected phytochemicals with therapeutic targets of AD are provided in table4. The more negative value of Prime - MMGBSA binding energy indicating stronger binding between ligand and receptor. From table 4 we can see that glabridin has the binding energy ranging between-32.22 and -44.84 kcal/mol with the therapeutic targets. This less binding energy indicates strong binding affinity of glabridin against these proteins. The respective free binding energies of glabridin with the target

proteins were AChE (-32.22 kcal/mol), BuChE (-32.22 kcal/mol), BACE-1(-40.2 kcal/mol), GSK3β (-34.66 kcal/mol) and Keap-1(-44.84 kcal/mol).

Phytochemical	Prime – MMGBSA (kcal/mol)				
	Acetylcholine esterase (AChE)	Butrylcholine esterase (BuChE)	β secretase cleavage enzyme-1 (BACE-1)	Glycogen synthase kinase-3 beta (GSK3β)	Keap-1
Glabridin	-32.22	-32.22	-40.2	-34.66	-44.84
Pinocembrin	-28.92	-28.92	-30.65	-33.01	-36.72
Terpinen-4-ol	-16.26	-16.26	-27.5	-27.01	-30.84
Shinopterocarpin	-32.18	-32.18	-39.8	-34.62	-37.78
Geraniol	-19.56	-19.56	-37.1	-31.19	-17.46

Table 4. Prime – MMGBSA of selected phytochemicals with therapeutic targets of AD

Molecular dynamic (MD) simulation studies with all the selected therapeutic targets with of glabridin phytocompound for a period of 100 ns were performed with the energy minimised docked structures the therapeutic target proteins. The graphs with RMSD values of the proteins during the simulation are shown above. The RMSD values of the respective proteins was in the range of **0 - 2.25 Å** (for AChE), **1.2 - 2.8 Å (**for BuChE), **1.2 - 2.4 Å** (for BACE-1), **1.2 - 3.6 Å** (for GSK3β) and **0.8 – 1.6 Å** (for Keap-1). We can see from the graphs that the proteins are undergoing conformational changes during simulations within a perfectly acceptable range. It is also important to note that the RMSD values of the proteins stabilize around a fixed value in all the five graphs. The value is not increasing or decreasing largely beyond a recommended range. The compound remains bounded with the target proteins during the simulations and the system was equilibrated throughout 100 ns. The results clearly evidence that the simulations were long enough for rigorous analysis.



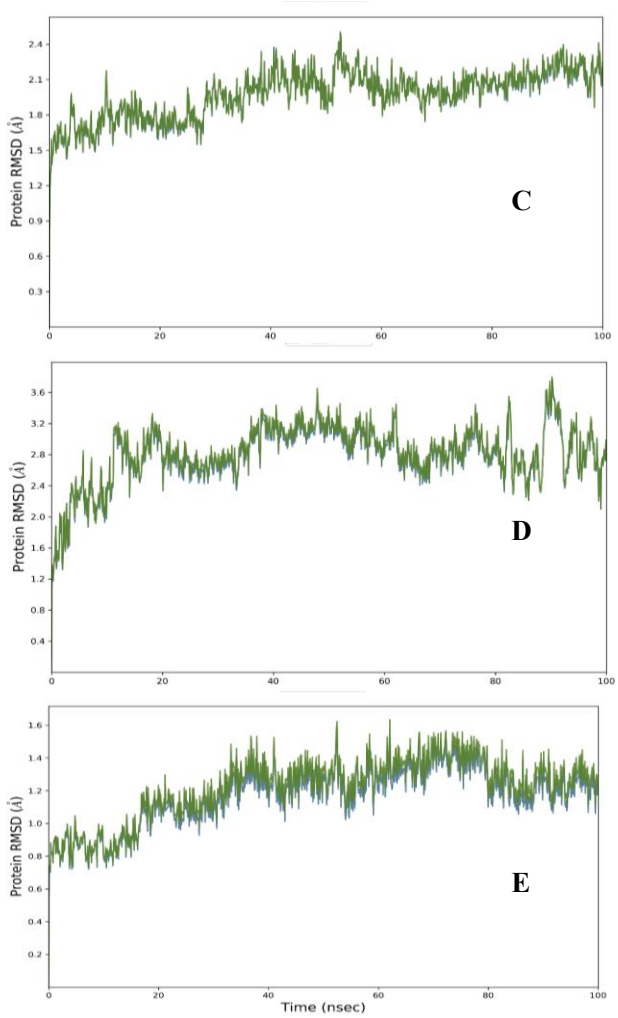


Figure 2: Molecular dynamics (MD) simulation analysis of AD therapeutic targets and glabridin: The Root Mean Square Deviation (RMSD) of glabridin with (A) AChE (B) Butrylcholine esterase (BuChE) (C) β secretase cleavage enzyme-1 (BACE-1) (D) Glycogen synthase kinase-3 beta (GSK3β) (E) Keap-1, complex in 100ns time.

Discussion and Conclusion

Natural isoflavonoids are mainly distributed in leguminous plants (Fabaceae) and play ecophysiological roles as defensive agents against other organisms ranging from animals to microbes (phytoalexins). Some isoflavonoids are considered symbiotic signals to rhizobial bacteria to form nitrogen-fixing root nodules. Glabridin, a prenylated isoflavonoid of G. glabra Linn. roots (European licorice, Fabaceae), has been associated with a wide range of biological properties such as antioxidant, anti-inflammatory, anti-atherogenic, regulation of energy metabolism, estrogenic, neuroprotective, anti-osteoporotic and skin-whitening (Simmler et al., 2013). It is considered as an effective agent for the treatment of atopic dermatitis, dementia, and a promising memory enhancer (Dayananda et al., 2010).

ADMET is one of the key measures of the pharmacological activity of the chemical as a drug (Kalani et al., 2013). Screening of natural compounds for drug candidates is based on Lipinski's rule of five (Yu et al., 2017). This rule is formulated for orally administered drugs. It uses four criteria to determine if a molecule is drug like; to have a molecular weight of ≤500, a LogP (logarithm of partition coefficient) ≤5, five or fewer hydrogen bond donor sites, and ten or fewer hydrogen bond acceptor sites. Molecules

violating more than one of these rules may have problems with their bioavailability (Alam et al., 2017). In this study the ADMET properties for the 19 phytochemical compounds of G. glabra were analysed in silico by using the Qikprop module of Schrödinger suite 2018-4. From the 19 phytochemical compounds analysed, 5 were selected based on the drug-like properties of the compounds. The selected compounds were namely glabridin, terpen-4-ol, pinocembrin, geraniol and shinopterocarpin. For these compounds estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution of the compounds was in the range of 1-2. Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution of the compounds was in the range of 0.75-3.25. Percent human oral absorption was 88.06-100% which indicates high absorption of the phytocompounds (Li, 2001). Almost all the pharmacokinetics properties of the compounds were within the recommended ranges (Makhouri and Ghasemi, 2018; Table 2).

To ensure the interaction between the phytochemicals of G.glabra and Alzheimer's disease therapeutic targets (AChE, BuChE, BACE-1, GSK3β and KEAP-1), molecular docking analysis were performed. The 5 selected phytochemicals according to the ADMET results were docked with the active sites of the above therapeutic target proteins (Table 3).

The greater the negative value on the score function indicates better docking (Amudha and Rani, 2015). So the assigned ligands and proteins classified by score function of glide. The scoring function of the glide docking program is shown as G score, which indicates the ability of the ligand to bind a specific conformation of the protein receptor (Torres et al., 2019). Among the 5 selected phytochemicals of G. glabra Linn. analysed, glabridin showed better binding affinity and good docking scores against the active sites of the therapeutic target proteins AChE (-8.454), BuChE(-6.402), BACE-1(-6.509), GSK3β(-5.124) and KEAP1 (-3.399).

Prime - MMGBSA calculates the binding energy between the ligands and receptors. This method is one of the best techniques for prioritization of lead molecules (Vadivelu et al., 2015). The more negative value of Prime - MMGBSA binding energy indicating stronger binding between ligand and receptor. From table 4 we can see that glabridin has the binding energy ranging between-32.22 and -44.84 kcal/mol with the therapeutic targets AChE, BuChE, BACE-1, GSK3β and KEAP-1. This less binding energy indicates strong binding affinity of glabridin against these proteins.

To analyse the stability of the complexes forming by glabridin and the therapeutic target proteins molecular dynamic simulation studies were performed for 100 ns using Desmond. The Root Mean Square Deviation (RMSD) is used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame. It is calculated for all frames in the trajectory. All protein frames are first aligned on the reference frame backbone, and then the RMSD is calculated based on the atom selection. Monitoring the RMSD of the protein can give insights into its structural conformation throughout the simulation. RMSD analysis can indicate if the simulation has equilibrated or not — its fluctuations towards the end of the simulation are around some thermal average structure. Changes of the order of 1-3 Å are perfectly acceptable for small, globular proteins. Changes much larger than that, however, indicate that the protein is undergoing a large conformational change during the simulation. It is also important that the simulation converges — the RMSD values stabilize around a fixed value. If the RMSD of the protein is still increasing or decreasing on average at the end of the simulation, then the system has not equilibrated, and simulation may not be long enough for rigorous analysis.

In this study the conformational changes of the therapeutic target proteins throughout the simulation were in perfectly acceptable range and the system was equilibrated till the end of the simulation (Graph 1,2,3,4 and 5).

The results of the ADME/Tox predictions, molecular docking and molecular dynamic simulation studies clearly indicates that the phytochemical Glabridin can be a potential inhibitor of the Alzheimer's therapeutic target proteins AChE, BuChE, BACE-1, GSK3β and KEAP-1.This may have a role in controlling the pathogenesis and thereby curing the disease. Further in vivo investigations is required to validate the compound as a new therapeutic agent for the Alzheimer's disease.

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