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Research Article

IN SILICO AND MOLECULAR DOCKING STUDIES: AIMING AMYLOID PRECURSOR-LIKE PROTEIN 2 USING ACTIVE PHYTOCHEMICALS FROM *WITHANIA SOMNIFERA*

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ABSTRACT

Objectives: Due to effective healing properties found in natural chemical compounds obtained from medicinal plants that are employed in curing several diseases, this study aims to exhibit the role of Indian ayurvedic plant *Withania somnifera* in the management of the Alzheimer's disease (AD) utilizing the molecular docking, drug-likeness and absorption, distribution, metabolism, and excretion (ADME) analysis.

Methods: Alzheimer's main protein was collected from the PDB database. Molecular docking is achieved using PyRx tool with the removal of the ligands possessing improper binding showing a significant effect on docking. Drug likeness and ADME analysis were evaluated using Swiss-ADME web server and ADMETlab 2.0 web tool. Ramachandran plot analysis for the target protein was achieved using SWISS-MODEL web server.

Results: In the protein structure, the distribution of torsion angles ϕ and ψ in a protein is visible. On the basis binding affinity ADME analysis, 27-Deoxywithaferin A is a safe medication and one of the most effective inhibitors of the amyloid precursor protein. It also has drug-like qualities.

Conclusion: According to the current research, 27-Deoxywithaferin A has a high affinity for binding, which makes it possible to suppress the major amyloid precursor protein while also managing therapeutic approaches for treating AD.

Keywords: *Withania somnifera*, Amyloid precursor protein, Alzheimer's disease, Cholinesterase inhibitor, Docking, Absorption, distribution, metabolism, and excretion analysis.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition named after Dr. A. Alzheimer impacting millions of populations worldwide with the increase in age accounting for the most prominent source of deaths every year affecting the various segments of the brain representing, namely, lewy body dementia, vascular dementia, frontotemporal conditions, etc. It is one of the indicative aspects of dementia which results in loss of cognitive power with reduced ability to think and remember anything and this progressively gets worse over time. The phrase "AD" is only used to refer to people exhibiting distinctive brain abnormalities. These alterations to the brain include inflammatory conditions, twisted fibers, protein aggregates, and disruption to and death of neurons and the links between them. Ten to twenty years before symptoms start to show, AD can start deep inside the brain and progressively spread to other regions [1]. Due to the disease's delayed onset and the complexity of the early changes in cognitive activity, it can be difficult to distinguish between aging-related changes and the early signs of AD [2]. Despite the fact that AD is not a form of rapid advancing age, it is distinguished by unique physiological and molecular changes in pathology, including the formation of plaques of amyloid, NFT accumulations, synaptic loss, and neuronal degeneration accompanied by substantial brain atrophy. The mild phase of the condition typically lasts the longest, sometimes up to 20 years. Individuals during this point struggle increasingly to recall previous occurrences, pick up novel data, organize complicated events, recall names, write and read, and deal with figures [3]. When the disease is at its most advanced stage, the patient no longer has the capacity to react to their surroundings, engage in interaction, or regulate their mobility [4].

Plaques of amyloid are accumulations of amyloid beta (Aβ) peptides which are mainly generated through the ordered activity of 2 aspartyl protease enzymes, β- and γ-secretases (amyloidogenic pathway), where the APP is first digested through β-secretase resulting in soluble APP as well as the remaining C-terminal regions, which is then absorbed further by γ-secretase to produce Aβ-40/42 segments [5]. Aβ is suspected of causing cell death by obstructing synaptic connections between neurons [6]. There are two main forms of AD: Familial and sporadic. A mere 5% of cases of AD are caused by early-stage familial AD, an autosomal-dominant illness caused by infrequent alterations in APP, PS1, and PS2 [7]. The majority of instances of AD are caused by a late-onset sporadic AD, which may be affected by a number of genetic sensitivity as well as risk factors related to the environment [8]. The degree of severity of this condition worsens as AD symptoms move from weak to moderate in nature including impaired cognitive and non-cognitive ability [9]. Thus, AD is categorized by the World Health Organization as a disease of public health priority [10].

The decline of cerebral tissue linked to a stroke enhances the probability of dementia and has a deteriorating impact that influences amyloid and tau pathology. Cardiovascular diseases (CVD) have been identified as an important trigger for AD [11]. An approach to prevent and delay AD can be developed by concentrating on the link between CVD, a reversible risk parameter, and AD [12]. As a result of rising amyloid-beta build-up, stress from oxidative damage, dysfunction of the mitochondria, and neurological inflammation, prolonged hyperglycemia may lead to cognitive decline. Obesity is a recognized contributory factor for developing Type 2 diabetes, tumors, and heart disease, which has been associated with dementia and AD [13].

Cholinesterase inhibitors (AChE) and butyrylcholinesterase are a class of medications that raise the amounts and potency of acetylcholine in the central and peripheral nerve systems by blocking its usual degradation into acetate and choline [14]. The cholinesterase enzyme has two active sites: A tryptophan-derived anionic site and a serine-derived

esteractic site. By binding with the serine esteractic site, compounds that inhibit cholinesterase like organophosphates prevent the enzyme from breaking down acetylcholine. Acetylcholine thus intends to keep to build up and turn on relevant receptors [15]. Neostigmine is a parasympathomimetic drug; it permanently blocks the AChE enzyme thus enhances the amount of ACh at the cholinergic regions [16]. It is not unexpected that from mild to serious AD symptoms are managed with AChE-Is like rivastigmine. In addition, rivastigmine exhibits selective suppression of the G1 type of AChE and offers a simultaneous reduction of BuChE and AChE [17]. The "first-line" treatments for Alzheimer's condition are drugs known as AChEs.

A notable example of India's health services system is ayurveda, which has been utilized in India for numerous years and is an integral component of the national medical system [18]. Ashwagandha, additionally referred to as *Withania somnifera*, is an Indian Ayurvedic herb that has been historically used for ages to treat a wide variety of human health issues. It has been demonstrated that ashwagandha's active ingredient is effective in healing a variety of neurological conditions, including AD. The roots of ashwagandha, which belongs to the nightshade (*Solanaceae*) family, are the component that is most frequently used. It is classified as a rasayana (rejuvenative) and is considered to have antioxidants, free radical scavenging, and immunity system-supporting properties. Therefore, this subtropical herb with Indian origins has been shown to enhance the functioning of neurons, lessen anxiety and stress, and foster mental serenity [19]. Considering *W. somnifera* a supportive candidate for the *in silico* study against AD, in the current research, an effort was undertaken to find novel, effective inhibitor against Alzheimer's main amyloid precursor protein from 17 different phytoactive compounds of *W. somnifera*.

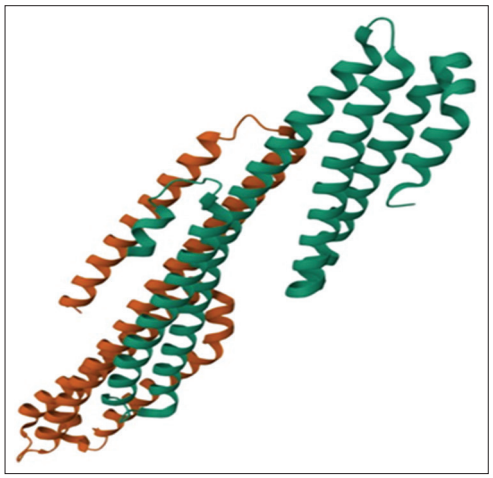

Fig. 1: 3D structure of amyloid precursor protein (5TPT)

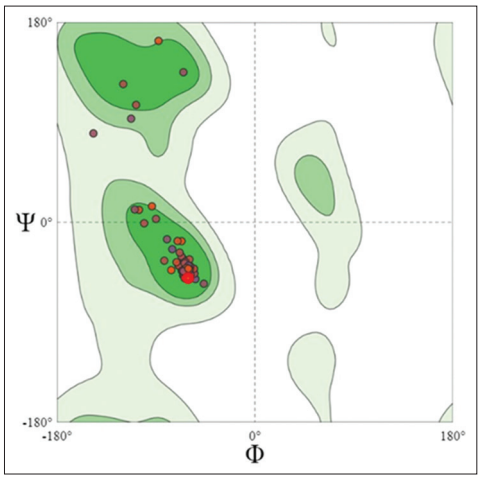

Fig. 2: Ramachandran plot analysis of amyloid protein

METHODS

Protein preparation

The three-dimensional crystal structure of a protein of the amyloid precursor protein (PDB ID: 5TPT) (Fig. 1) has been extracted using the Protein Data Bank maintained by the research collaboratory for structural bioinformatics. It is composed of A and B chains, both containing 193 residues of amino acids along with a resolution of 2.42 [20,21]. Just before the docking process, protein crystal structures are constructed to optimize hydrogen bonding and reduce atomic conflicts. The protein that was used was prepared according to the usual protocol of Discovery studio visualizer 21.1. The proteins' water molecules and heteroatoms got eliminated and then polarity (polar) hydrogen was introduced. Furthermore, the site of activity of the constructed protein was predicted.

Ramachandran plot

Ramachandran plot is one of the most realistic hypotheses for studying protein structure, with few differences among the experiments and simulations [22]. When developing a simulation, the phi and psi angles are displayed for each pair of polypeptide chains in an individual protein structure to determine if the primary chain torsion angles are stereochemically possible. A web server called the Swiss model (https://swissmodel.expasy.org/) was utilized for the evaluating Ramachandran plot to model a variety of protein sequences and 3D structures of proteins in an attempt to provide useful insights into how they work on a molecular level concurrently [23]. The FASTA (canonical) sequence of the protein (5TPT) was fetched from the UniProt Knowledgebase (UniProtKB) (www.uniprot.org) which integrates validated UniProtKB/Swiss-Prot records, to enrich protein information with biological information and related data. The sequence was uploaded on the Swiss model server and a study of the Ramachandran plot was conducted with outliers that are labeled by the residue number and type as well as by chain [24].

Ligand selection

Altogether 17 phytoactive compounds from *W. somnifera* (Ashwagandha) were gathered from various pieces of literature to gather information on amyloid precursor inhibitors. Structures of compounds were obtained in the three-dimensional SDF file format from PubChem (https://pubchem.ncbi.nlm.nih.gov), a chemical information substance database at National Library of Medicine, a division of the U.S. national institutes of health [25] which supports several medical research organizations with contributors of data resource from research facilities at universities, governmental organizations, pharmaceutical firms, chemical suppliers, publishing houses, and many chemical biology sources [26]. Utilizing the PyRx tool, the ligand preparation process involved ligand optimization, energy minimization, and transformation of the ligands to 3D PDB format [27].

Molecular docking

In structural biology and computer-assisted drug design, molecular docking is a crucial tool. Predicting the dominant interaction mode(s) of the ligand with a protein having a known three-dimensional structure is the primary goal of ligand-protein docking [28]. For the purpose of the molecular docking research, PyRx-virtual screening tool software was employed. All 17 vital phytochemicals of *W. somnifera* were docked with the amyloid precursor protein using an open-source PyRx tool (PDB ID: 5TPT) [29]. Prepared receptors and ligand records were chosen as the goal for the docking investigation. The ligands were converted using the open-babel tool and a protein was loaded and transformed into a macromolecule [30]. After identifying the protein and ligand molecules, the grid box was established by maximization to examine binding of all ligands with protein. After making the necessary adjustments, docking was initiated by pressing the forward key. We received a table listing each ligand's binding affinity after the docking process was finished. Depending on which ligands had the highest binding affinities, the best five ligands were chosen for future investigation and saved as PDB files. The Discovery Studio Visualizer 21.1 was used to complete a 2D/3D interactive visualization analysis [31].

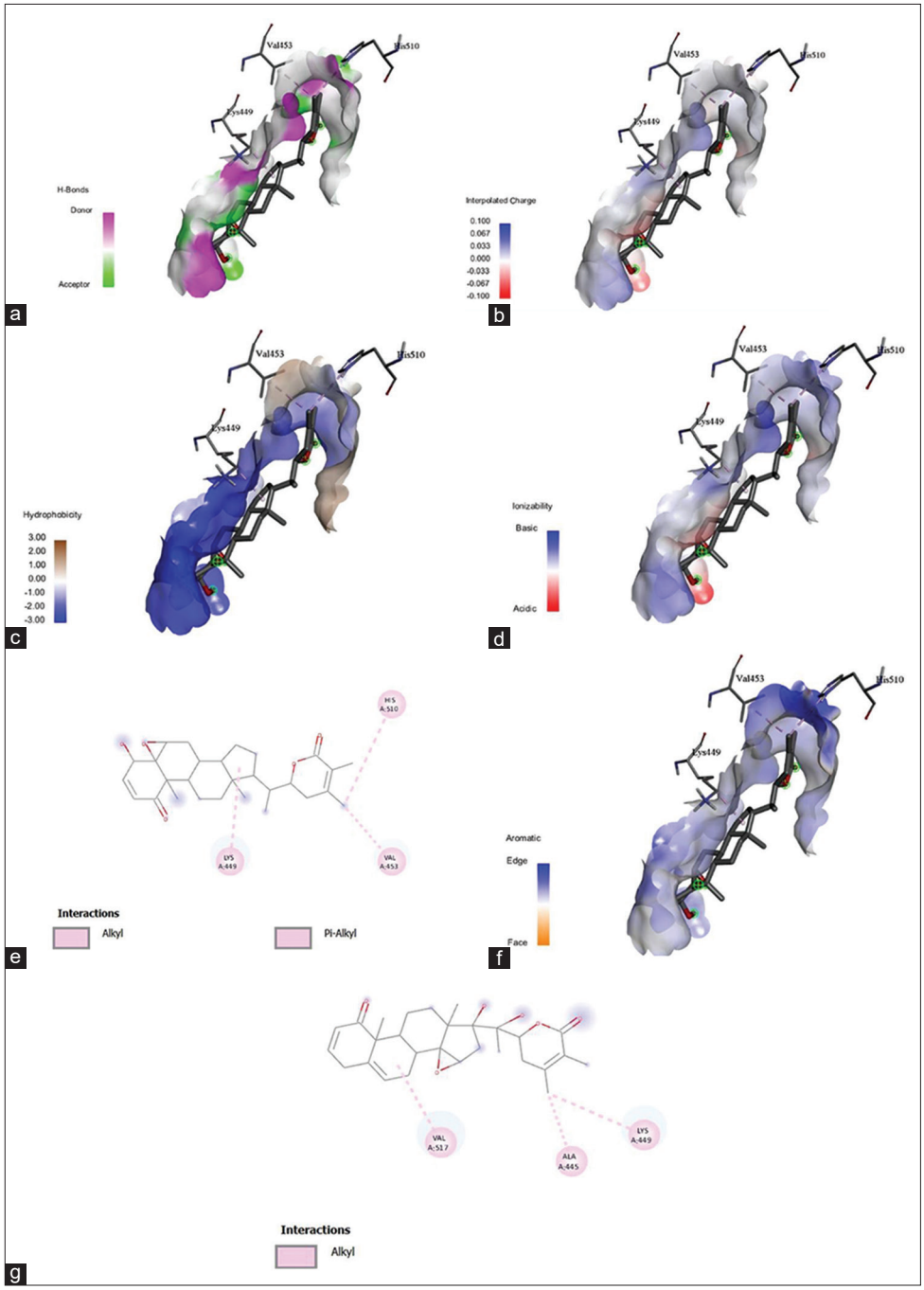

Fig. 3: 2D and 3D interaction of 27-deoxywithaferin A with 5TPT (a) 2D diagram (b) Aromatic (c) H-bond (d) Charge (e) Hydrophobicity (f) Ionizability (g) SAS

Absorption, distribution, metabolism, and excretion (ADME) analysis

The SwissADME web tool is readily accessible at http://www.swissadme.ch and is designed for friendly-to-use submission and quick result analysis [32]. The SwissADME web tool provides open access to reliable models that predict physical and chemical characteristics, pharmacokinetics, and drug-likeness, among which are in-house effective methods like the BOILED-Egg; to determine the passive gastrointestinal absorption human intestinal absorption (HIA) and brain access blood-brain barrier (BBB), two important ADME parameters [32]., iLOGP, and Bioavailability Radar [32]. In this study, the top five compounds with their greatest binding affinity were chosen for the Absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis and drug-likeness test. Using SwissADME and ADMETLAB (https://admetmesh.scbdd.com/service/evaluation/index) an online tool called to aid in the ADMET analysis., drug-likeness and ADMET analyses were performed. The SWISS-ADME technology was also used for boiled egg analysis. For ADME analysis, the Lipinski rule of five was taken into account [33]. If a molecule meets two or more of the criteria, Lipinski's rule of five predicts whether a drug-likeness will be effective or not and also determines if a particular compound has a probability to be administered by mouth fulfilling the following criteria: Molecular mass <500 Dalton, logP <4.15, H-bond donor <5, H-bond acceptor <10 and 40 < molar refractivity <130.

RESULTS

Ramachandran plot

We take into account the following protein geometry in the Ramachandran plot. We take into account the following protein geometry in the Ramachandran plot. Out of the 50 templates discovered

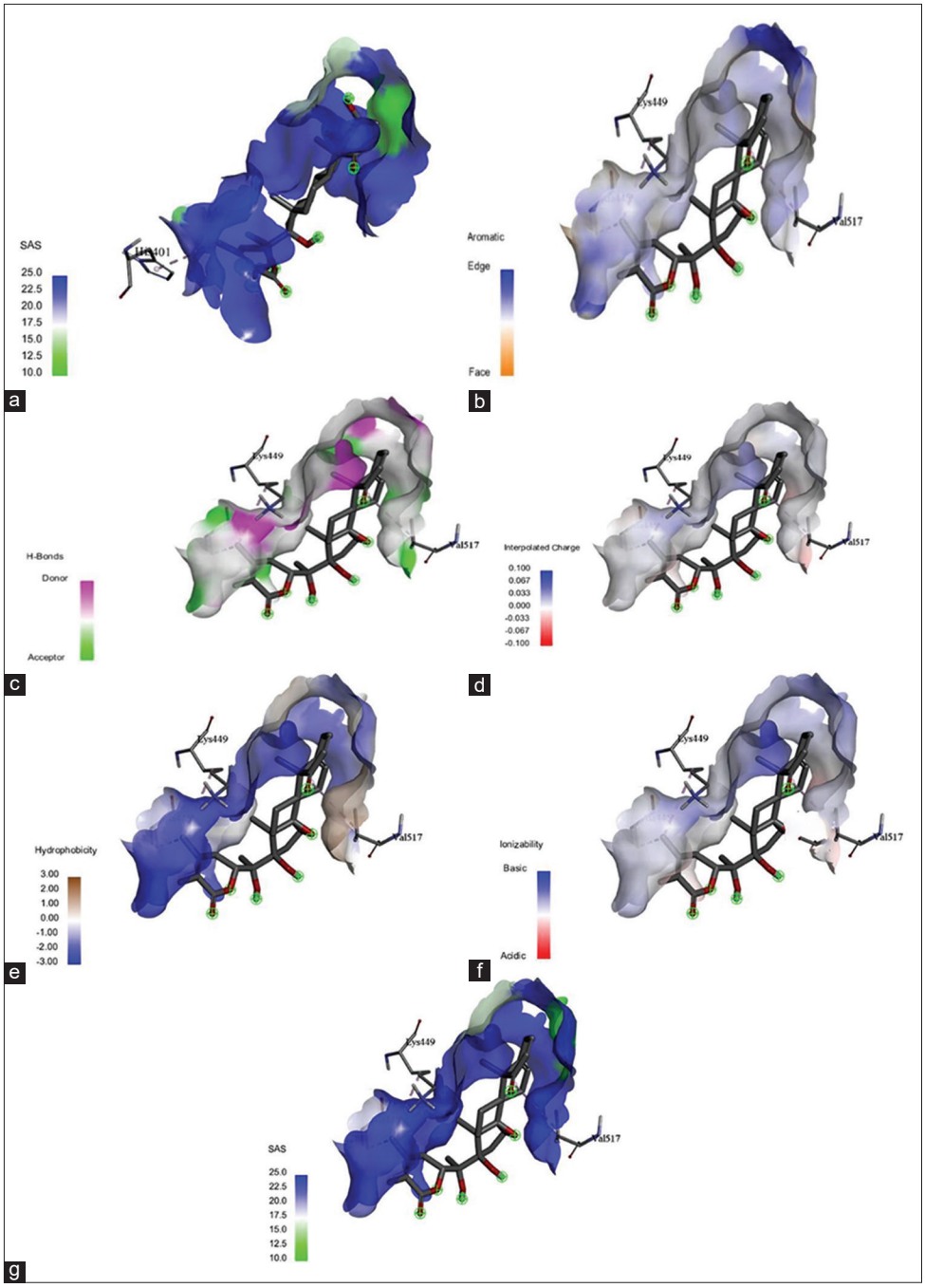

Fig. 4: 2D and 3D interaction of Withanolide M with 5TPT (a) 2D diagram (b) Aromatic (c) H-Bond (d) Charge (e) Hydrophobicity (f) Ionizability (g) SAS

for the 5TPT template, we chose 4yn0, which had a sequence identity of 63.51%, Ramachandran Favored status of 99.50%, Ramachandran Outliers status of 0.00%, no poor bonds with GMQE of 0.19, QMEAN Z-Scores of 2.43, and QMEANDisCo Global: 0.57 ± 0.06, all of which suggest acceptable protein quality (Fig. 2).

Molecular docking

The binding energy is a factor in determining the affinity for binding between ligands and receptors; the lower the energy, the higher the affinity. Several bioactive compounds from *W. somnifera* were discovered to have a strong affinity for the amyloid precursor protein through molecular docking. The top five phytochemicals from *W. somnifera* with the highest affinity for the amyloid protein are listed (Table 1). Based on findings from molecular docking analyses performed with PyRx, we discovered that seven of the 17 chemicals from *W. somnifera* have a considerable binding affinity (more than 8 Kcal/mol) with the amyloid protein that causes AD. We chose the top five compounds (Table 2) for

Table 1: Top five phytocompounds from *Withania somnifera* having the highest binding affinity with amyloid precursor protein

S. No.	PubChem compound ID	Name of phytocompound	Binding energy (Kcal/mol)
1.	CID_23266155	27-deoxywithaferin A	−8.5
2.	CID_21679023	Withanolide G	−8.5
3.	CID_25090669	Withanolide M	−8.3
4.	CID_101281364	Withanolide R	−8.3
5.	CID_23266161	17alpha-hydroxywithanolide D	−8.2

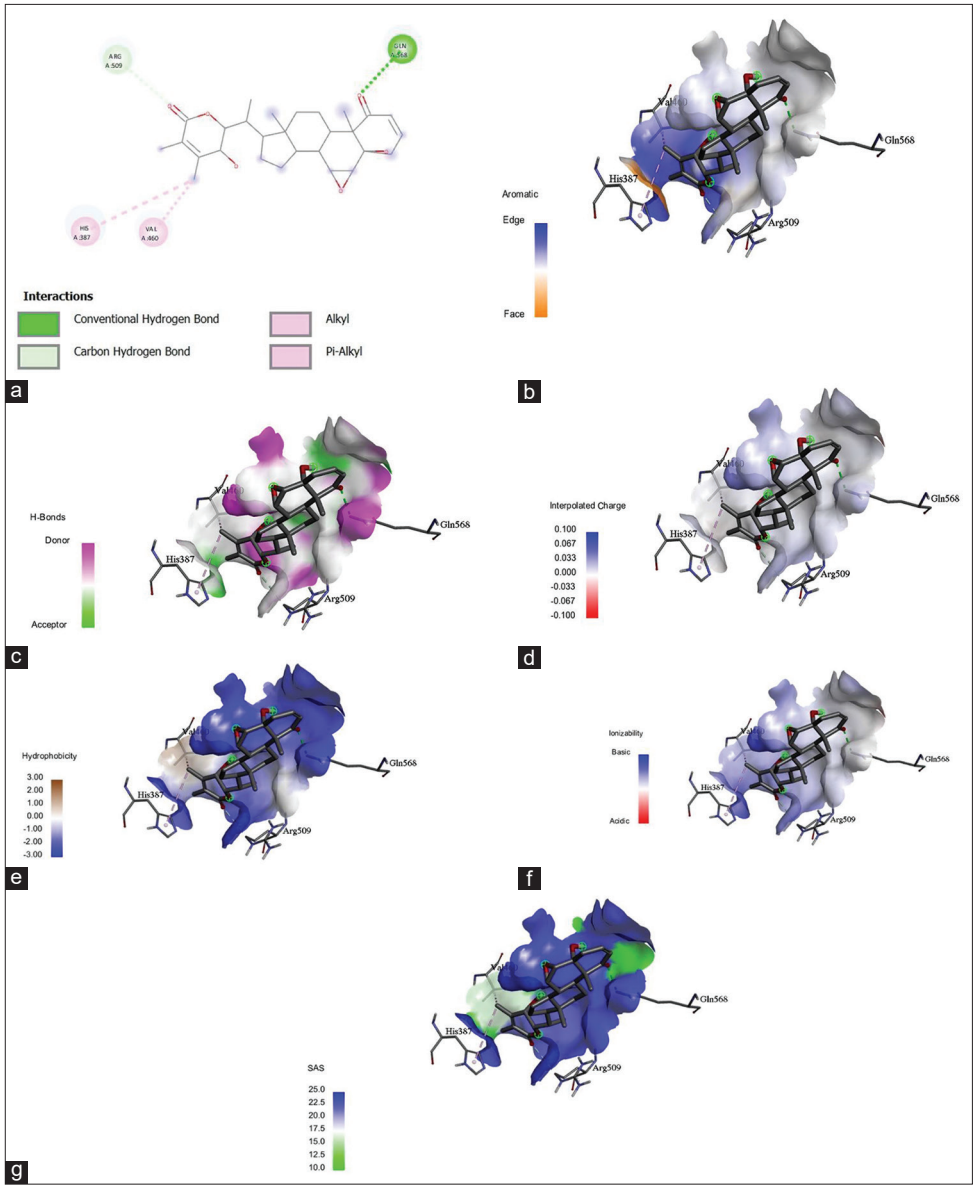

Fig. 5: 2D and 3D interaction of Withanolide R with 5TPT (a) 2D diagram (b) Aromatic (c) H-Bond (d) Charge (e) Hydrophobicity (f) Ionizability (g) SAS

Table 2: Following Lipinski's criteria, the ADME study of the best-docked compounds

S. No	Ligand name	Molecular weight (g/mol)	H-Bond donor	H-Bond acceptor	LogP	Molar refractivity
1.	27-deoxywithaferin A	454.60 g/mol	1	5	4.212	126.33
2.	Withanolide G	454.60 g/mol	2	5	3.78	128.08
3.	Withanolide M	468.58 g/mol	2	6	3.233	127.09
4.	Withanolide R	470.60 g/mol	2	6	2.955	127.49
5.	17 alphahydroxywithanolide D	486.60 g/mol	3	7	2.387	128.73

ADME: Absorption, distribution, metabolism, and excretion

drug-likeness estimation and ADME analyses based on the docking data. Significant associations between phytocompounds and 5TPT in 2D and 3D were seen.

Molecular visualization

Using Discovery Studio Visualizer 21.1, the interactions between the receptor and ligand of the top three phytocompounds with the highest binding affinity were viewed. Docked ligands were documented using the PDB file format. PyRx was subsequently accessed with Alzheimer's amyloid precursor protein. Significant interactions among phytocompounds and the main protein were seen in 2D and 3D. Van der Waal forces, carbon-hydrogen bonds, π-sulfur interactions, alkyl, and π-alkyl interactions, π-π-T-shaped contacts, and unfavorable interactions were all visible in the diagram of 2D interactions.

27-Deoxywithaferin A

27-Deoxywithaferin A forms various types of 2D-3D interactions with amyloid precursor protein including alkyl and π-alkyl bond with HIS 510, LYS 449, and VAL453. 3D interactions involve aromatic, H-bond, Charge, Hydrophobic, Ionizability, and SAS receptor surface on current receptors (Fig. 3).

Table 3: Admetlab 2.0 ADME analysis

S. No	Ligand name	LogS	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's rule
1.	27-deoxywithaferin A	−5.1	0.118	0.014	0.537	0.163	Accepted, 0 Violations
2.	Withanolide G	−4.659	0.027	0.001	0.356	0.821	Accepted, 0 Violations
3.	Withanolide M	−4.582	0.028	0.006	0.772	0.919	Accepted, 0 Violations
4.	Withanolide R	−4.399	0.204	0.378	0.9	0.04	Accepted, 0 Violations
5.	17alphahydroxywithanolide D	−4.18	0.545	0.633	0.902	0.683	Accepted, 0 Violations

ADME: Absorption, distribution, metabolism, and excretion, HIA: Human intestinal absorption, Pgp-sub: Permeability glycoprotein substrate, BBB: Blood-brain barrier

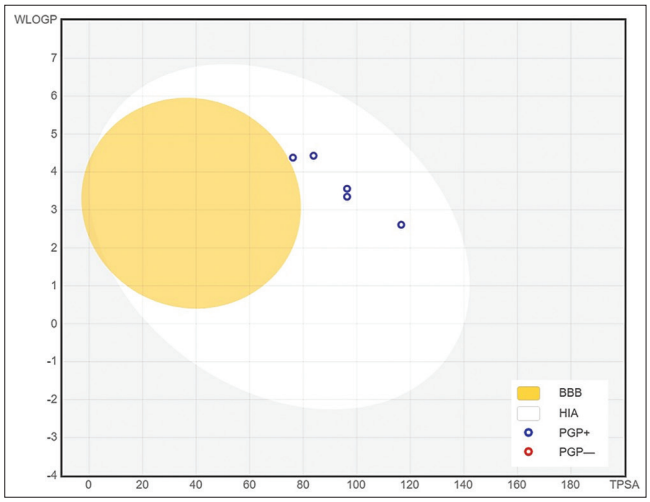

Fig. 6: Boiled-egg analysis

Withanolide M

Withanolide M forms 2D-3D interactions with amyloid precursor proteins such as alkyl bonds with VAL 517, ALA 445, and LYS 449 residues. 3D interactions involve aromatic, H-bond, charge, hydrophobic, ionizability, and SAS receptor surface on current receptors (Fig. 4).

Withanolide R

Withanolide R forms 2D interactions with amyloid 5TPT protein such as conventional bonding with GLN 568 residue, C-H bond with ARG 509, Alkyl, and pi-alkyl with HIS 387 and VAL 460 residues. 3D interactions involve aromatic, H-bond, charge, hydrophobic, ionizability, and SAS receptor surface on current receptors (Fig. 5).

Drug-likeness prediction and ADMET analysis

Lipinski's rule of five helps in the distinction of substances classified into drug-like and non-drug-like molecules. Lipinski's rule of five was used to predict the drug-likeness of the best-docked compounds, and ADME analysis was carried out using the web tool Swiss-ADME and ADMETLAB 2.0. The Swiss-ADME tool was also used to do a boiled egg analysis to forecast the passive brain access (BBB) and gastrointestinal absorption (HIA) of particular phytoactive compounds (Fig. 7). In addition, the water solubility (LogS), BBB, HIA, permeability glycoprotein substrate, carcinogenic effects, and Lipinski's rule validation of the best-docked compounds were examined within the accepted range (Table 3).

Bioavailability radar

Bioavailability radar evaluates a compound's drug-like characteristics. A radar plot of a phytoactive compound must lie within the pink-colored region when analyzing its properties for it to be taken into account as drug-like. Results depend on ligands that are present and are either expected to be in the oral form or not orally accessible through the radar plot. Flexibility and polar are two crucial characteristics that affect a compound's availability. Rotatable bonds, which measure FLEX, suggest which have rotatable bonds >10 will have poor oral availability, whereas topological polar surfaces area should be between 20 Å2 and 130 Å2 (Fig. 6).

DISCUSSION

One method to delay or cope with the decline in memory, reasoning, and daily functioning may be Alzheimer's medication. AChEs and memantine are two examples of the various medication types expressly authorized by the Food and Drug Administration to treat AD complications. Acetylcholine is a chemical messenger critical for consciousness, recall, and processes, levels get reduced in the brain as a result of AD. By halting the depletion of acetylcholine in the brain, inhibitors of cholinesterase increase the quantity of acetylcholine that is accessible to the nerve cells. The pathophysiology of AD is greatly influenced by the amyloid precursor protein. The Aβ peptide is produced during APP analyzing, and its buildup is linked to AD.

An increase in β-amyloid accumulation and behavioral abnormalities were prevented by oral treatment of extraction of the roots of *W. somnifera*, which is mostly composed of withanolides and withanosides. Consequently, Amyloid protein 5TPT can be regarded as a key target. For additional studies to examine the stability between ligand and the target molecule binding affinity, interactions certain *in silico* methods such as molecular docking, ADME analysis, and toxicity modeling have been demonstrated to be effective. Researchers are particularly interested in the steroidal chemicals found in ashwagandha, including the withanolides A to Y, withasomniferin A, withanone, and others whose substituents aid in scavenging free radicals produced during the onset and evolution of AD. This plant has the capacity to promote neurite proliferation.

A study was made analyzing purity of amyloid protein (PDB ID: 5TPT) employing the Ramachandran plot to validate the purity of the protein and therefore observed no outliers and poor rotamers. Molecular docking was performed on the 17 phytocompounds that were selected and were examined for the highest binding affinity against amyloid precursor protein. Out of these, five compounds were chosen having the highest binding affinity and further analyzed for ADME analysis. Based on the binding affinity, 27-Deoxywithaferin A and Withanolide G are considered as the significant inhibitors of amyloid precursor protein. 27-Deoxywithaferin A complies with all the parameters required for a phytocompound at the time of ADME analysis for drug-likeness and toxicity detection and is considered safe against Alzheimer's condition for minimizing its harmful impacts. While analyzing Withanolide G results, it tends to be carcinogenic and cannot be adopted taking into account the criteria for further treatment of AD.

CONCLUSION

The purpose of this study is to evaluate *W. somnifera* phytoactive chemicals as possibly curative drugs for AD, one of the foremost causes of mortality annually impacting different parts of the human brain. The amyloid precursor protein has a lot of capacity and is an essential candidate for avoiding the progression of AD. In the present research, an attempt was made to find novel, efficient inhibitors contrary to AD's main amyloid precursor protein from 17 distinct phytoactive compounds of *W. somnifera*. This was done because *W. somnifera* is considered a supportive candidate for the *in silico* study against AD. These compounds have the highest binding affinity and high inhibitory efficacy. These phytochemicals have the highest amyloid precursor protein binding affinity and high suppressive efficacy. The application of Ayurveda in the management of AD will be improved by the

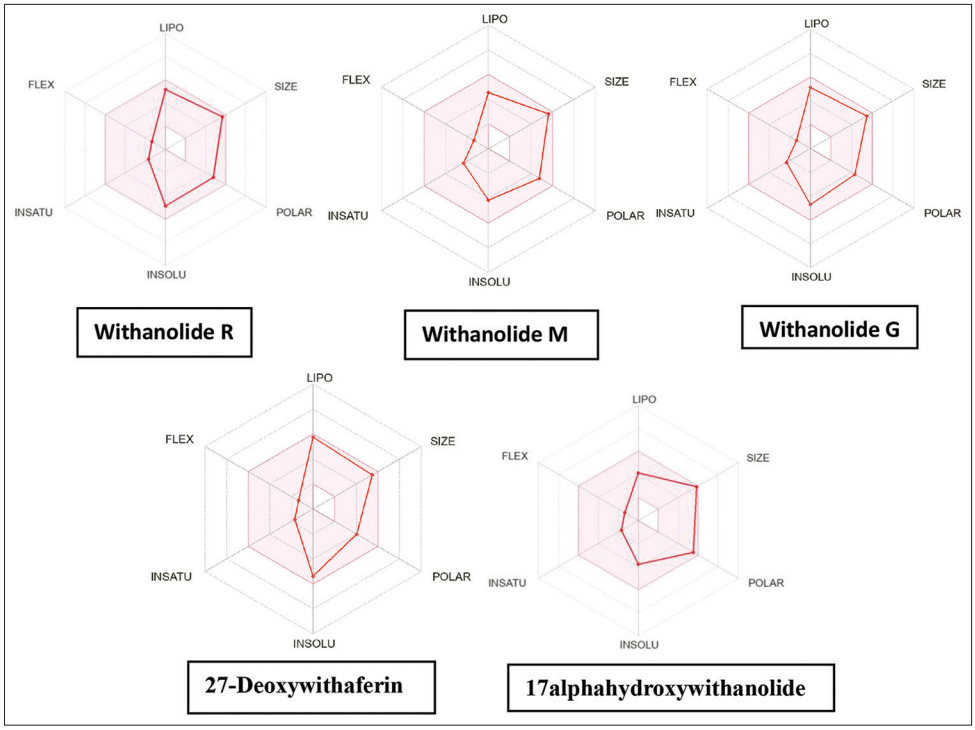

Fig. 7: Radar plots of Withanolide R, Withanolide M, Withanolide G, 27-Deoxywithaferin A 17alphahydroxywithanolide D

development of optimal amyloid precursor protein inhibitors and the use of the most effective plant substances with drug-like qualities, and safe ADMET toxic effects estimation.

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AUTHOR'S CONTRIBUTION

All the authors have contributed equally to the paper.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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