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Drugs for Alzheimer's Disease from *Salix Alba* Discovered Computationally Utilizing Virtual Screening and Docking Against 1b41 Protein

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Abstract

Objective: The objective of this research is to evaluate the feasibility of using molecular docking techniques to identify novel acetylcholinesterase, a key enzyme implicated in the pathogenesis of Alzheimer's disease, a neurodegenerative disorder with a gradual but steady progression to death, due to the gradual accumulation of beta-plaques and neurofibrillary tangles, as well as a decline in acetylcholine levels. Due to its function in converting acetylcholine into acyl and choline molecules, acetylcholinesterase is an enzyme that could potentially be the target of future Alzheimer's disease treatments. Methods: In the current research, findings of an in-silico study were examined or developing a novel remedy to combat AD. IMPPAT database was employed to retrieve the phytocompounds from Salix alba. Then the chosen molecules were screened on SwissADME for drug-likeness. Pyrx software was used for the final screening, which revealed the binding affinity between the ligands and the 1b41 target protein. 3d and 2d interactions were examined using Biovia and ligplus respectively. Results: Potential drug candidates for blocking the acetylcholinesterase enzyme include amentoflavone, astragalin, fragile, and salidroside. Conclusion: According to Lipinski's parameters, none of the compounds are violated (amentoflavone, astragalin, fragile, and salidroside), rendering them all suitable for inhibition activities against the AChE enzyme in AD.

Keywords: *Salix alba,* Alzheimer's disease, Acetylcholine, Acetylcholinesterase, Molecular docking, ADME analysis, Lipinski rule of 5.

INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that results in progressive cognitive deterioration and memory impairment, eventually interfering with daily activities. Alzheimer's disease symptoms frequently resemble dementia symptoms. The term dementia refers to a range of cognitive deficits, such as memory loss and difficulty with critical thinking, which can substantially impede daily activities [1]. In general, the prevalence of Alzheimer's disease (AD) increases with age, with a peak prevalence in individuals in their late 60s. AD is initially characterised by relatively moderate memory loss, but as the disease progresses, symptoms worsen [2]. The characteristic features of AD are the accumulation of Aβ plaques and neurofibrillary tangles, which contribute to neuronal loss and brain atrophy. The entorhinal cortex and hippocampus, two brain regions important for memory formation, initially appear to be injured and gradually spreads throughout the brain, resulting in widespread neuronal death and synapse loss [3]. When a greater number of neurons die, the brain begins to atrophy. As Alzheimer's disease (AD) progresses, there is a widening of intracranial voids and subsequent

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atrophy of brain tissue, resulting in a further reduction in the brain's overall volume [4]. The severity of the disease correlates with the rate at which synapses and neurons are lost, resulting in a diminished brain volume. A complex interaction of genetic and environmental factors controls the onset of AD [5]. Individuals with Alzheimer's disease (AD) often have mutations in several genes, including the amyloid precursor protein (APP), presenilin 1 and 2, and apolipoprotein E (ApoE) [6]. Exposure to air pollution and high-calorie diets high in saturated fatty acids are two more environmental risk factors associated with an elevated risk of AD [7].

Despite the fact that the pathophysiology underlying Alzheimer's disease (AD) is complex and incompletely understood, several hypotheses attempt to explain its aetiology. The hypothesis of beta-amyloid protein accumulation suggests that the anomalous accumulation of these proteins may cause Alzheimer's disease. In the brain, all neurons express APP, which is indispensable for synaptic plasticity, synaptogenesis, and neuronal function. APP is degraded and recycled as any other protein in the body, and this process is mediated by APP-cleaving enzymes such as alpha- and gamma-secretase. However, a faulty cleavage by a separate group of enzymes, however, can generate a sticky fragment that accumulates and deposits outside the cell as amyloid plaque, which is considered to promote neuroinflammation [8].

The tau hypothesis postulates that aberrant aggregation of tau protein, may also contribute to AD, resulting in the formation of tangles within brain nerve cells [9]. In a healthy brain, tau proteins contribute to the elongation and support of the microtubule network. Microtubules are essential for the movement of information and nutrition molecule within neurons [10]. In cases where tau proteins are phosphorylated, they lose their microtubules-binding capacity and instead aggregate together to form neurofibrillary tangles. As a result, tau dissociation impairs microtubule assembly, thereby disrupting the neuronal transport system and impeding their biological communication [11]. Plaques form externally to the neuron, whereas neurofibrillary tangles are present internally [12]. Lastly, the cholinergic theory proposes that AD is affected by a deficiency of acetylcholine, a neurotransmitter important for memory and learning [13].

Neurotransmitter: Acetylcholine in Alzheimer’s

The exchange of information between neurons, also known as brain cells, is essential for numerous functions, including muscle contraction and memory. A neurotransmitter called acetylcholine is produced during this process by an enzyme known as choline acetyltransferase [14]. The release of acetylcholine into the synapse, where it binds to acetylcholine receptors on postsynaptic neurons, results in the propagation of an electrical signal. The central cholinergic nervous system controls acetylcholine levels by regulating acetylcholine synthesis and release [15]. Once acetylcholine has served its purpose, there comes acetylcholinesterase, a dimeric enzyme with the specific ability to cleave acetylcholine, which is cleaved by a trio of particular amino acids (Ser, his, and glu) to produce acetic acid. During the degradation process, water molecule attacks the acyl enzyme nucleophilically with the aid of the histidine 440 group. The by-product of this reaction is acetic acid, and the enzyme is regenerated so that it can continue degrading acetylcholine. This full the cycle of signal transmission, ensuring that neuronal interactions can take place normally [16]. The loss of cholinergic neurons with age, however, is linked to lower acetylcholine levels and the onset of dementias like Alzheimer's.

Cholinesterase inhibitors could potentially be useful for treating diseases like Alzheimer's that are linked to low acetylcholine levels. By preventing cholinesterase enzymes from degrading acetylcholine, these inhibitors boost the concentration and duration of the neurotransmitter's effects. Increasing acetylcholine levels is the intended effect of cholinesterase inhibitors, which work by blocking the enzymatic activity of cholinesterase enzymes. Improved cognitive performance and a slowed progression of Alzheimer's disease may result from the use of cholinesterase inhibitors (Figure 1), which work by extending the duration of acetylcholine's effects.

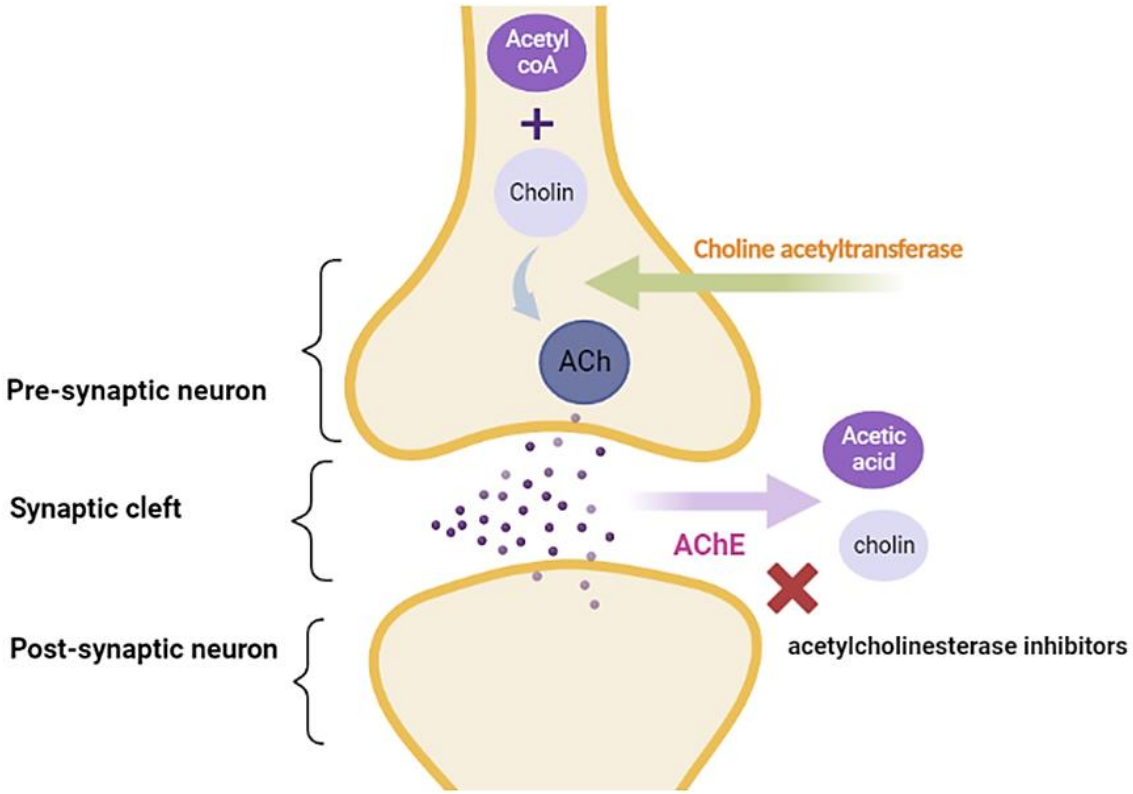

Figure 1. Image depicting the action of Acetylcholinesterase and inhibitor of acetylcholinesterase.

METHOD(S)

Retrieval of Target

In order to define the target for the ligand and the conformational space of the receptor being explored during the docking stimulation, retrieving the protein's structure is an important step in the molecular docking process. Human acetylcholinesterase complexed with fibrilin-II, glycosylated protein (1b41), was selected as the target protein after a review of the relevant literature. To obtain the protein's structure, a query was made to PDB, a repository for protein structure [17], a database of protein structures, was then queried to obtain the protein's structure. The overall resolution was 2.76 and there were 592 residues.

Purification of Target Protein

Following recovery, the protein was subsequently purified to eliminate impurities, prebound heteroatoms, and water molecules. This was accomplished using the BIOVIA Discovery Studio Visualizer [18]. Because water molecules could potentially affect the outcome of docking, they were eliminated entirely beforehand. To examine the various binding poses and binding affinities, chain A was retained, and all extra chains were completely removed. Only A chain was employed in the simulation with the ligand (Figure 2).

When the 1B41 was uploaded to the PDBsum generate tool [19], the protein's secondary structure and its motifs were obtained. With procheck server ramachandran plot was generated for the purpose of evaluating protein structure quality [20].

Retrieval of Phytoligands

The IMPPAT website, a digital archive for phytochemicals from Indian medicinal plants, was utilized to choose 20 phytocompounds from the *Salix alba* plant [21]. List of all 20 phytocompounds is given in Table 1. Their canonical SMILES and 3-dimensional SDS structure were obtained from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov/) [22].

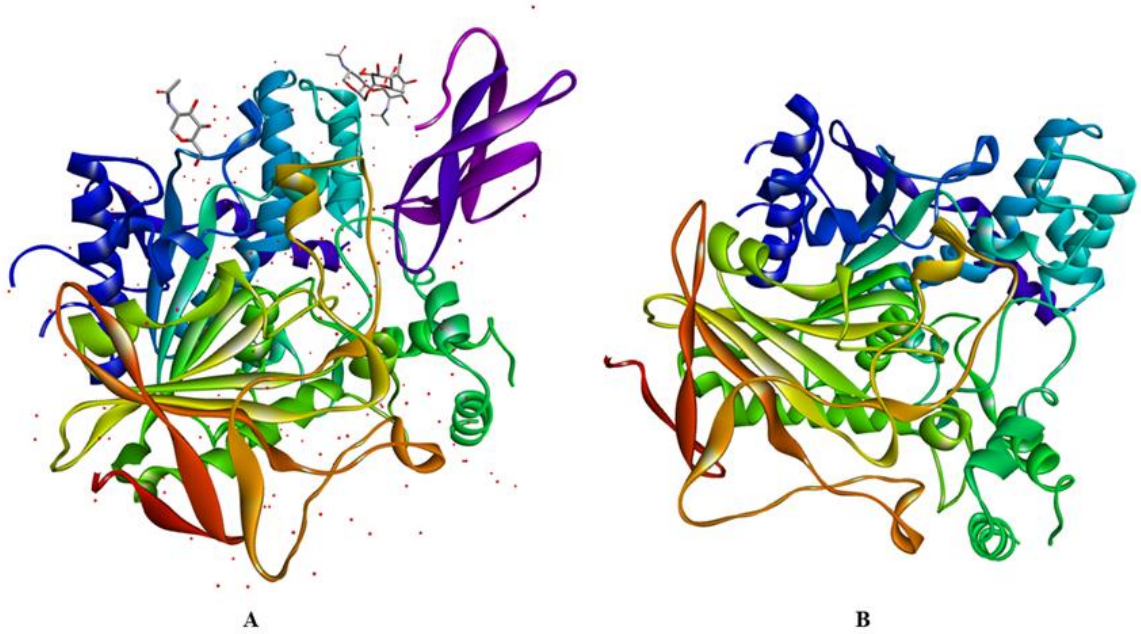

Figure 2. 'A' represents unpurified protein, 'B' is purified protein.

Table 1. Phytoligands extracted from IMPPAT.

Molecule number	Ligand name
1	Salicortin
2	Salireposide
3	Salicin
4	Picein
5	Salicylic acid
6	4-Hydroxycinnamic acid
7	Cianidanol
8	Quercimeritrin
9	Astragalin
10	quercetin 3,7-di-O-glucoside
11	Alboside I
12	Fragilin
13	Apigenin
14	Cupressuflavone
15	Amentoflavone
16	Echinacin
17	(+)-Gallocatechin
18	isorhamnetin-3-O-glucoside
19	Narcissin
20	Rutin

Drug Likeness Studies

Drug likeness studies are important before docking, and this can be achieved using SwissADME. This enables the computation of physicochemical descriptors, predict ADME parameters, pharmacokinetic characteristics, and druglike nature. Identification of substances that are anticipated to have favourable pharmacological properties and can be employed as drug candidates [23]. Canonical SMILE formats which were previously downloaded from PubChem were then used as input on SwissADME.

Molecular Docking

The optimal ligand-receptor binding posture and binding affinity can be determined using docking studies, which are based on insilco analyses. It provides information on many interactions. Based on their binding energy in kcal/mol, prospective medication candidates are to be identified. Open Babel, Autodock Wizard, and Vina Wizard are just a few of the many embedded programs available through Pyrx [24]. The ligand molecule that complied with the Lipinski rule and the purified structure obtained by the Biovia discovery studio were both uploaded to PyRx. The energy of the ligands and proteins was reduced, and grid dimensions were optimized with dimesions (X: 61.1353, Y: 68.4508, Z: 68.8675) for effective blind docking. CSV file containing binding energy was downloaded [26]. Two-dimensional structures of ligand-receptor interactions were generated in Ligplot plus [25] for the ligands with the lowest binding energy. Based on their binding energy in kcal/mol, prospective medication candidates are to be identified.

RESULTS

Retrieval of Target Protein and Purification

After loading the protein molecule in Biovia discovery studio visualizer chains B, C, heteroatoms and water molecules were removed to prevent any steric clashes and non-specific interactions that could impair the exactitude of the docking results and lead to inaccurate predictions of ligand binding. Hence before docking purification is a principal step. The secondary structure of the protein comprises 531 amino acids and features diverse range of structural elements including 4, 6, 3, 1, 3, 18, 18, 24, 24, 45, 5, 3; beta sheets, beta-alpha-beta motifs, beta hairpins, psi loop, beta bulges, strands, helices, helix-helix contacts, beta turns, gamma turns, and disulfides respectively. The alpha helices, beta sheets, left-handed helices, and loops together make up the protein's secondary structure are represented by the four quadrants of the Ramachandran plot. This is a method is used to visualise the range of allowed energies for backbone dihedral angles as a function of protein residues. The plot shows that 83.3% of all residues fall inside the preferred zone, whereas 11.8% fall within the region with some restrictions, indicating the overall quality of the protein structure (Figures 3 and 4)

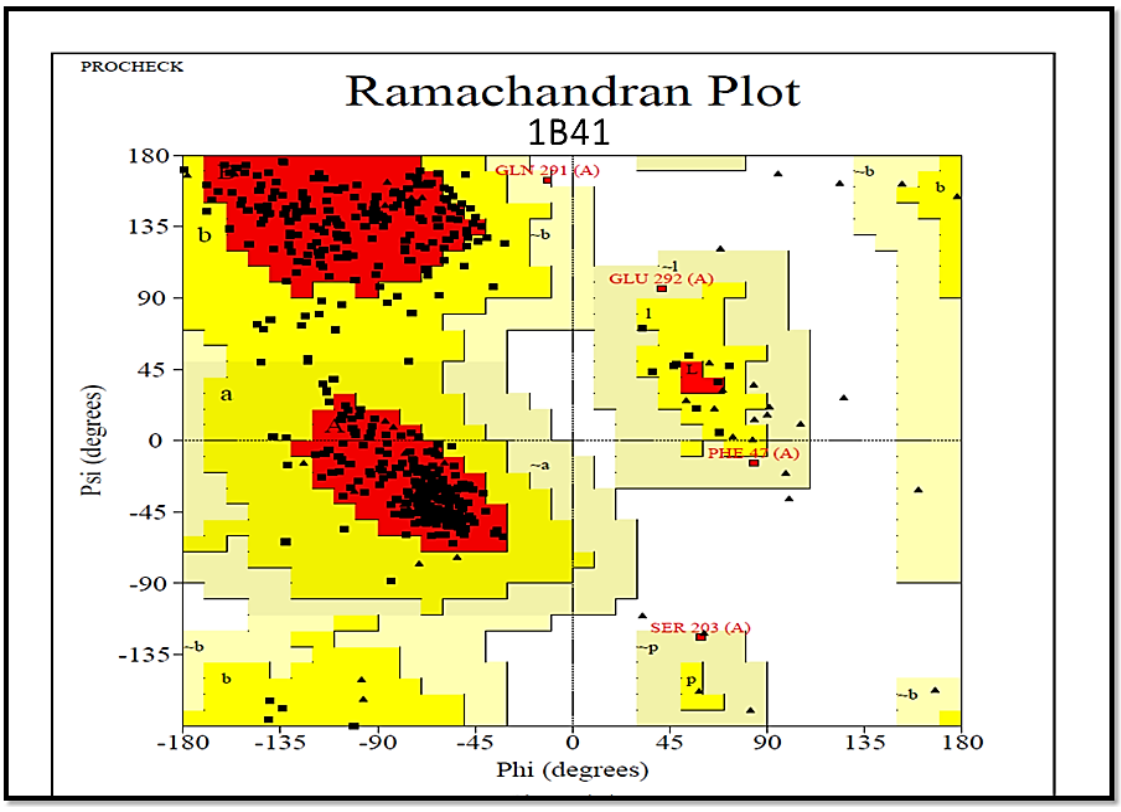

Figure 3. Ramachandran plot of 1B41 protein using Procheck.

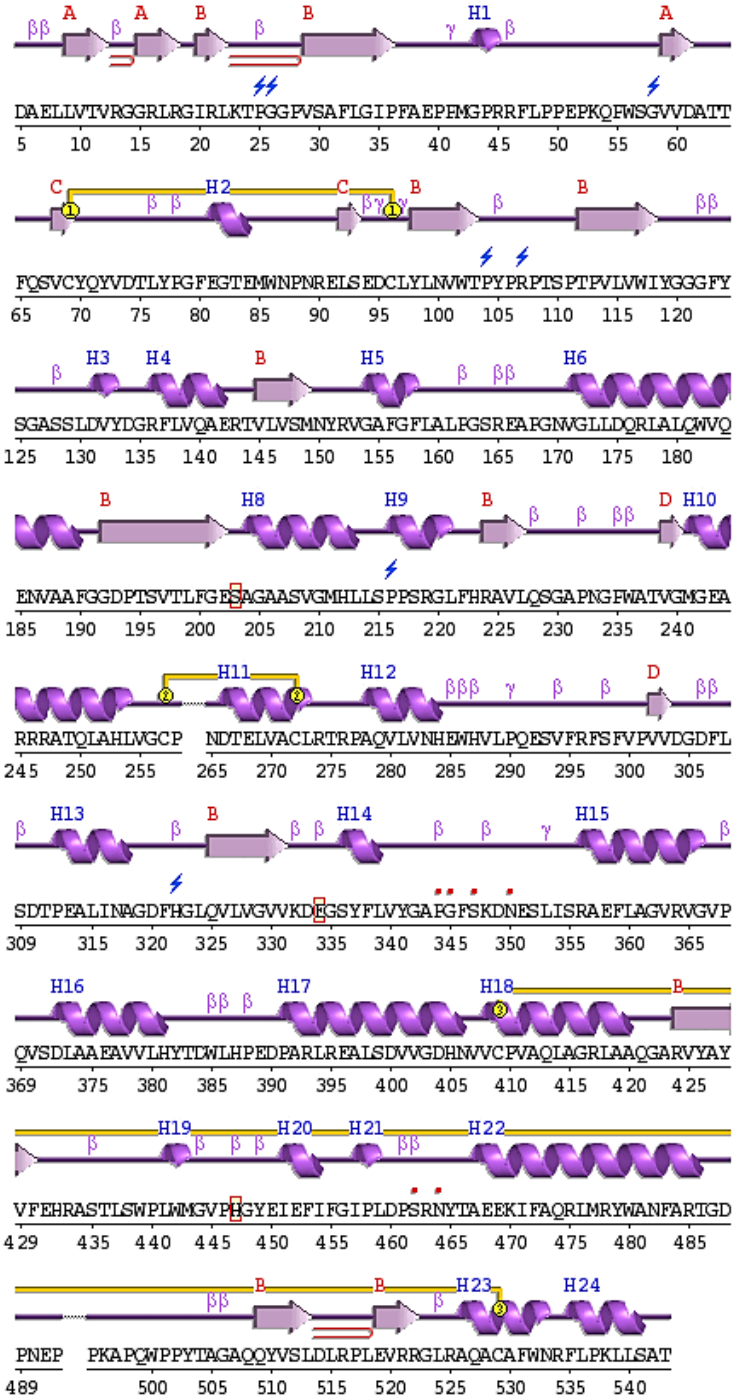

Figure 4. Secondary structure of 1b41 protein.

Retrieval of Ligands

Compounds used as ligands collected from the imppat database were then evaluated with the Swiss ADME programme. Docking ligands for the target protein were narrowed down to those molecules that made less than three violations. Phytochemical compounds were screened, and the ones that made it through were chosen for future investigation. The compounds Salicortin, Salireposide, Salicin, Picein, Salicylic acid, 4-hydroxycinnamic acid, Cianidanol, Quercimeritrin, Astragalin, Alboside I, Fragilin, Apigenin, Cupressuflavone, Amentoflavone, (+)-Gallocatechin, and isorhamnetin-3-O-glucoside were all included. The open-Bable programme was used to transform the 3D structures of these ligands from the SDF format into the pdbqt format necessary for docking simulations.

ADMET Analysis

The pharmacokinetics and bioavailability of medications are largely determined by their absorption, distribution, metabolism, and elimination (ADME) properties. Human pharmacokinetic studies of drug-like substances are another way to reduce the likelihood of clinical trial failure, hence are crucial for improving the drug development process. Computational methods like SWISS ADME is able used to predict the pharmacokinetic features of drug candidates, allowing for more accurate assessments of their potential. The blood-brain barrier (BBB), and gastrointestinal (GI) absorption analysis was made. Table 2 summarises the findings, and Figure 5 uses a boiled-egg design to show how well each ligand crosses the BBB and is absorbed by the gastrointestinal tract. Particularly notable are the ligand molecules exhibited in the yellow-yolk part, which indicate BBB crossing capabilities, and the white section, which depicts increased GI absorption.

Drug Likeness Analysis

To verify that the chosen compounds have the desired pharmacological properties, drug likeness analysis is a crucial stage in the drug design process. It specifies that the hydrogen bond donor should be equal to or less than 5, the hydrogen bond acceptor should not be more than 10, the lipophilicity (log p) value shouldn't be higher than 5, and the molecular weight shouldn't be higher than 500 Da rule. The ligands (molecules 10, 16, 19, and 20) in Table 3 all fail to adhere to the Lipinski Rule of 5. In contrast, Molecules 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, and 18 are appropriate for future drug design considerations since they match the Lipinski criteria.

Molecular Docking and Visualization

The PyRx system was utilized to generate a CSV file comprising the ligand docking results for the protein receptor 1b41. The CSV file showed various binding energies related to various poses of ligands involving protein. The ligands (Amentoflavone, Astragalin, Fragilin, Salireposide) with the lowest binding affinities were selected for visualisation in Biovia (3D) (Figure 6) and ligplot+ (2D). The Figures (7 to 10) depicts the interactions between hydrogen atoms and hydrophobic groups in the ligand-protein complex in two-dimensions, whereas Table 4 provides detailed information on the amino acids involved in these interactions and the type of interactions formed between 1b41 and selected phytoligands. Table 5 provides binding affinity of ligands with protein 1b41.

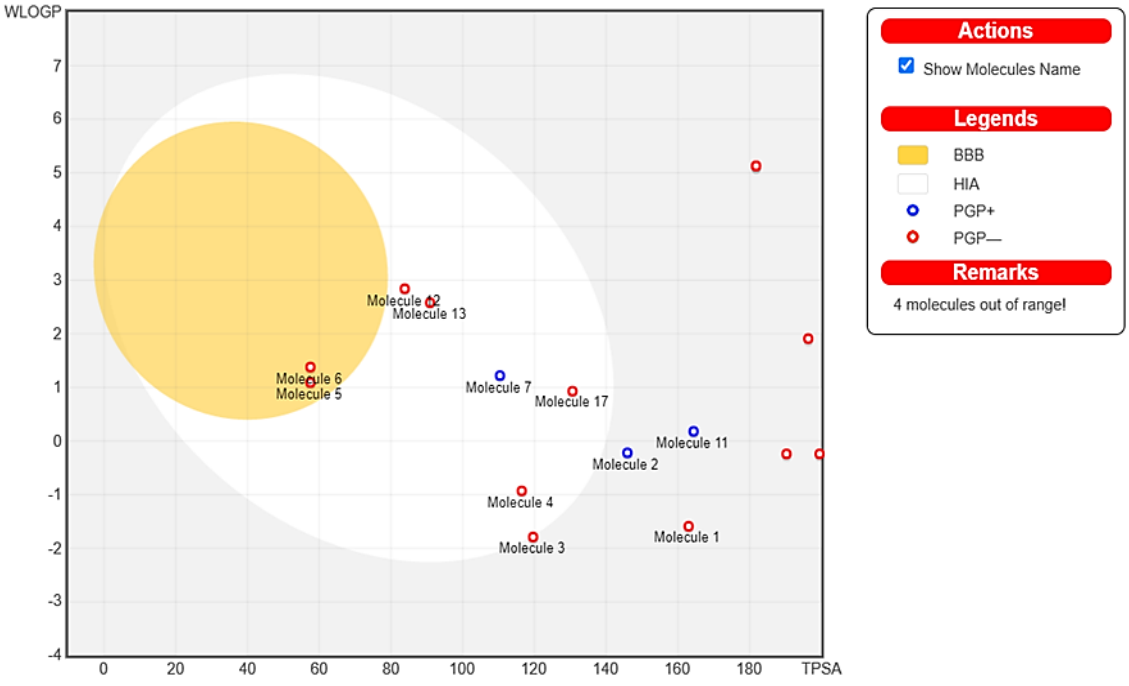

Figure 5. BOILED-egg generated on SwissADME.

Table 2. ADME Prediction.

Ligands	Blood brain barrier	GI absorption	p-GP substrate
Salicortin	-	↓	-
Salireposide	-	↓	+
Salicin	-	↓	-
Picein	-	↑	-
Salicylic acid	+	↑	-
4-Hydroxycinnamic acid	+	↑	-
Cianidanol	-	↑	+
Quercimeritrin	-	↓	-
Astragalin	-	↓	-
quercetin 3,7-di-O-glucoside	-	↓	+
Alboside I	-	↓	+
Fragilin	-	↑	-
Apigenin	-	↑	-
Cupressuflavone	-	↓	-
Amentoflavone	-	↓	-
Echinacin	-	↓	-
(+)-Gallocatechin	-	↑	-
isorhamnetin-3-O-glucoside	-	↓	-
Narcissin	-	↓	+
Rutin	-	↓	+

***In a table '-' indicates No, '+' is for Yes, '↑' and '↓' indicating high and low GI absorption*

Table 3. Lipinski filter analysis.

Molecule	Ligand	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	MLogP
1	Salicortin	424.4	5	10	-1.56
2	Salireposide	406.38	5	9	-0.33
3	Salicin	286.28	5	7	-1.48
4	Picein	298.29	4	7	-1.31
5	Salicylic acid	138.12	2	3	0.99
6	4-Hydroxycinnamic acid	164.16	2	3	1.28
7	Cianidanol	290.27	5	6	0.24
8	Quercimeritrin	464.38	8	12	-2.59
9	Astragalin	448.38	7	11	-2.1
10	quercetin 3,7-di-O-glucoside	626.52	11	17	-4.62
11	Alboside I	508.51	5	11	-0.57
12	Fragilin	318.71	2	5	1.12
13	Apigenin	270.24	3	5	0.52
14	Cupressuflavone	538.46	6	10	0.25
15	Amentoflavone	538.46	6	10	0.25
	Echinacin		6	12	-0.56
17	(+)-Gallocatechin	306.27	6	7	-0.29
18	isorhamnetin-3-O-glucoside	478.4	7	12	-2.37
19	Narcissin	624.54	9	16	-3.69
20	Rutin	610.52	10	16	-3.89

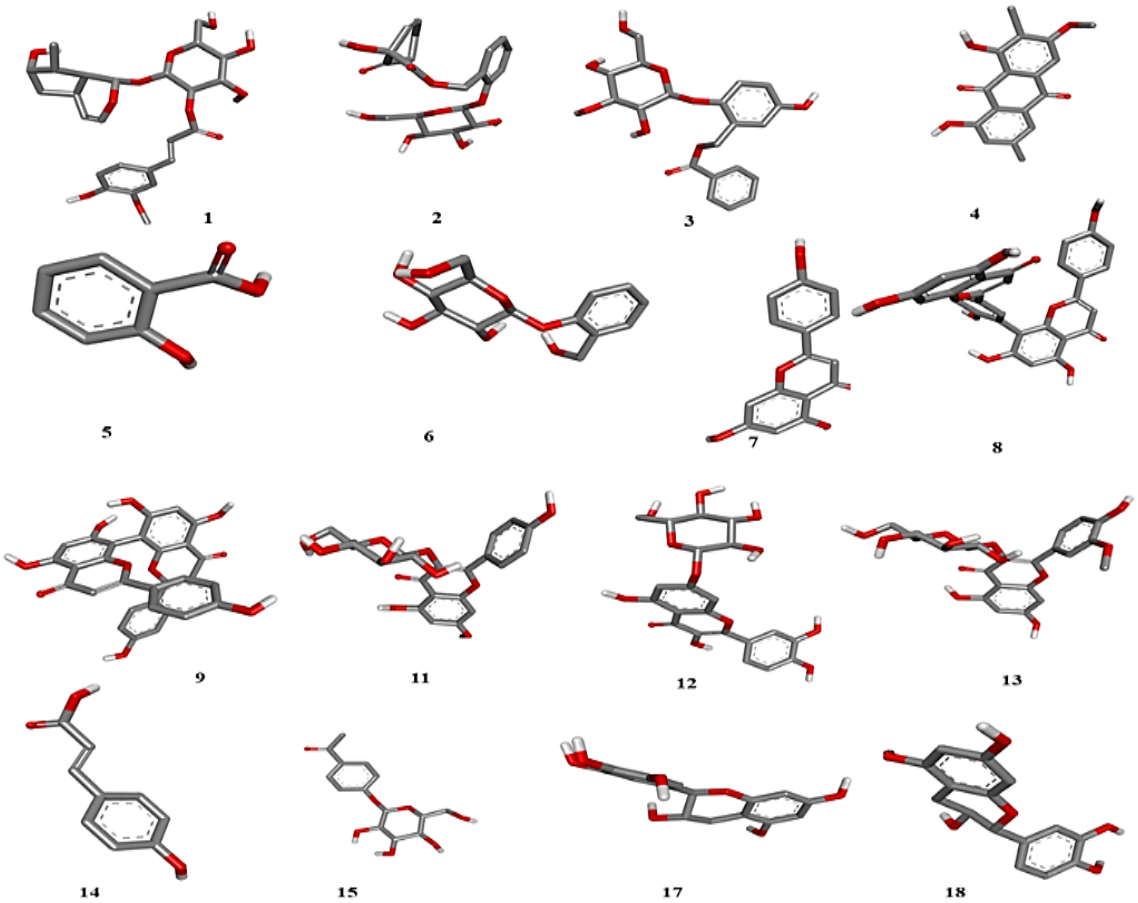

Figure 6. 3d structure of ligands generated in Biovia 1 to 18 represents molecules Salicortin, Salireposide, Salicin, Picein, Salicylic acid, 4-hydroxycinnamic acid, Cianidanol, Quercimeritrin, Astragalin, quercetin 3,7-di-O-glucoside, Alboside I, Fragilin, Apigenin, Cupressuflavone, Amentoflavone, (+)-Gallocatechin, isorhamnetin-3-O-glucoside, Narcissin, Rutin respectively.

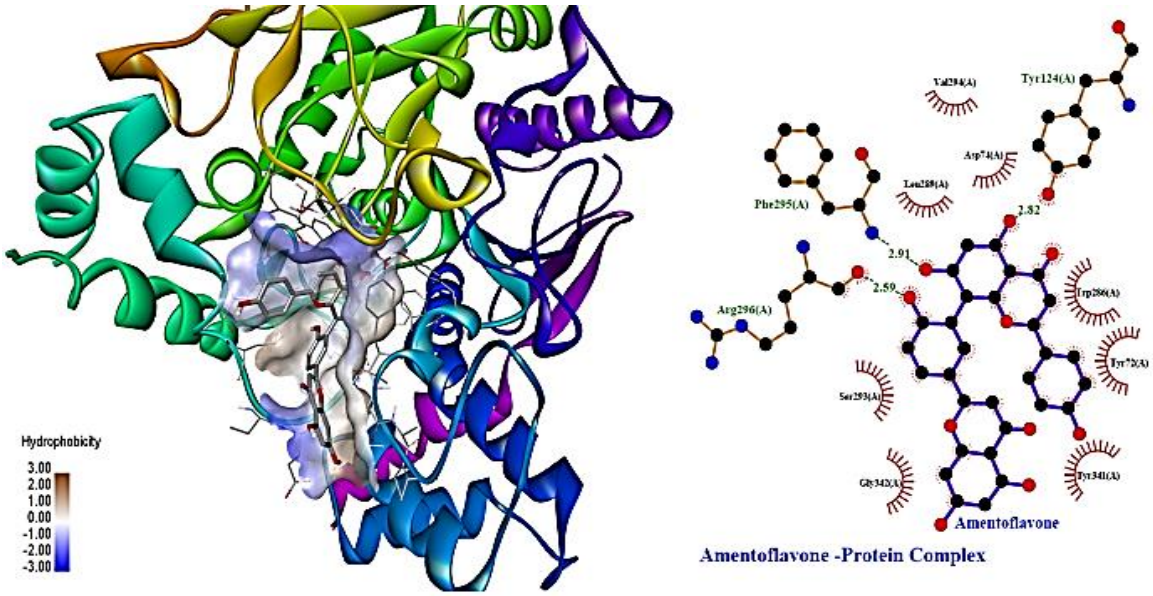

Figure 7. The distance between the hydrogen atoms in the Amentoflavone-1b14 complex (right) is marked along the green dashed line. Residues participating in hydrophobic interactions are shown by arcs with radiating spikes. Hydrophobic surfaces are shown in blue in the 3D construction on the left.

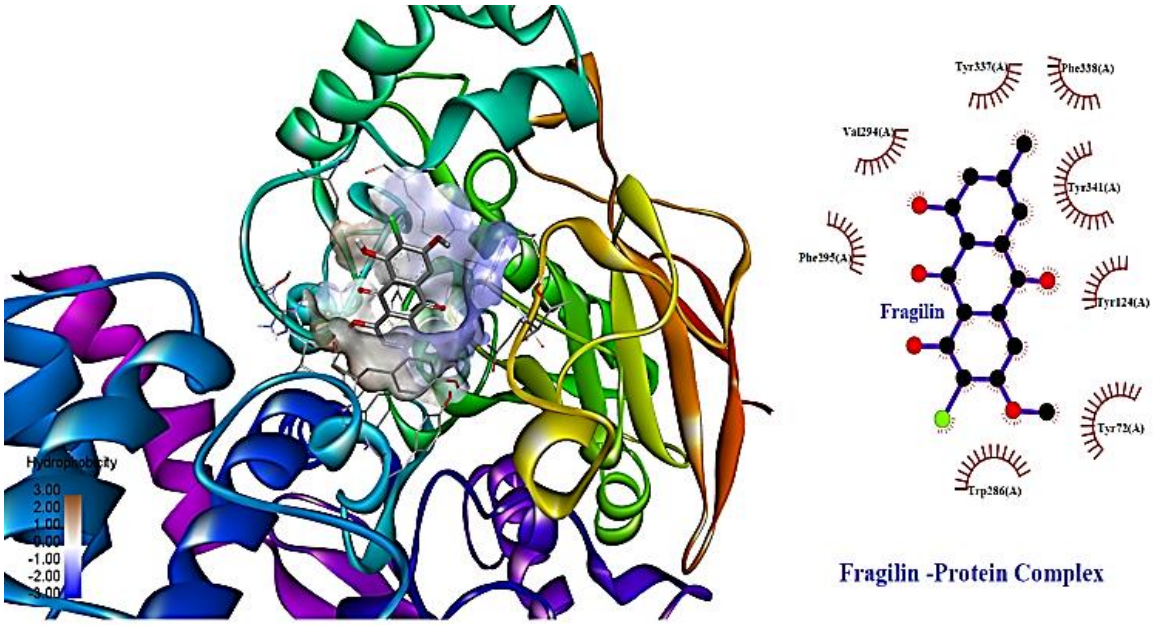

Figure 8. The distance between the hydrogen atoms in the fragllin-1b41 complex (right) is marked along the green dashed line. Residues participating in hydrophobic interactions are shown by arcs with radiating spikes. Hydrophobic surfaces are shown in blue in the 3D construction on the left.

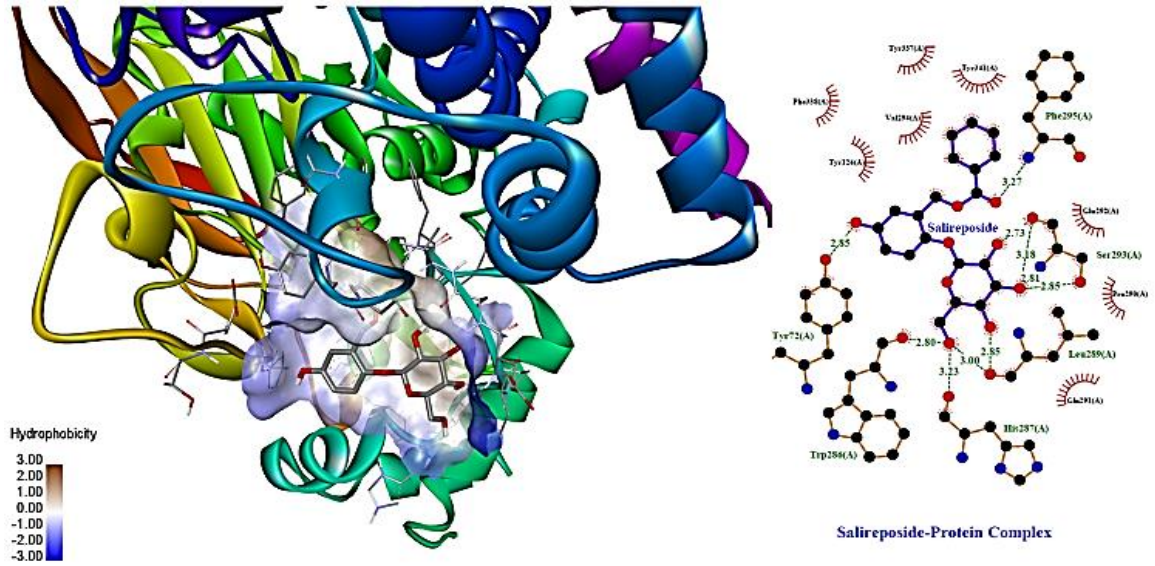

Figure 9. The distance between the hydrogen atoms in the salireposide-1b41 is marked along the green dashed line. Residues participating in hydrophobic interactions are shown by arcs with radiating spikes. Hydrophobic surfaces are shown in blue in the 3D construction on the left.

Table 4. Docking interaction of ligand-protein complex.

Molecule*	**Ligand name**	**Type of interaction**	**Amino acid residue**
15	Amentoflavone	Hydrogen Bond	A:Phe295, A:Tyr124, A:Arg296,
		Hydrophobic residue	A:Leu289, A:Asp74, A:Val294, A:Tyr72, A:Tyr341, A:Gly342, A:Ser293,A:Trp286
9	Astragalin	Hydrogen Bond	A:Phe295, A:Tyr337, A:Ser293, A:Tyr341, A:Arg296
		Hydrophobic residue	A:Val294, A:Tyr124, A:Tyr72, A:Trp286, A:Glu292, A:Phe297, A:Gln291, A:Leu289

12	Fragilin	Hydrophobic residue	A:Phe338, A:Tyr337, A:val294, A:Tyr341, A:Tyr124, A:Phe295, A:Tyr72, A:T286
2	Salireposide	Hydrogen Bond	A:Phe295, A:Ser293, A:Leu289 A:Hist287, A:Trp286, A:Tyr72
		Hydrophobic residue	A:Phe338, A:Tyr337, A:val294, A:Tyr341, A:Tyr124, A:Glu292, A:Pro290, A:Gln291

***Molecule number assigned to ligands while analysis in SwissADME.*

Table 5. Binding affinity of ligands with protein 1b41.

Ligand	Binding affinity in kcal/mol
Alboside I	-8.3
Salicortin	-7.7
Salireposide	-8.4
Fragilin	-9.3
Salicylic acid	-6.5
Salicin	-7.3
Apigenin	-9
Amentoflavone	-10.4
Cupressuflavone	-9.6
Astragalin	-9.2
Quercimeritrin	-9.2
isorhamnetin-3-O-glucoside	-9.1
4-Hydroxycinnamic acid	-7.1
(+)-Gallocatechin	-8.2
Cianidanol	-8.3
Picein	-7.4

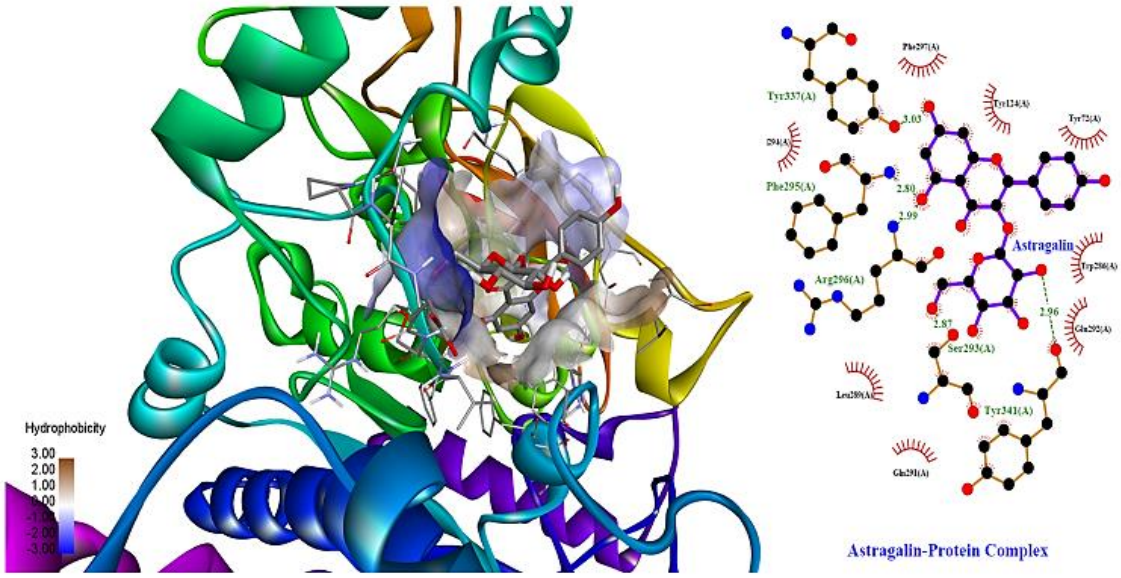

Figure 10. The distance between the hydrogen atoms in the Astragallin-1b14 complex (right) is marked along the green dashed line. Residues participating in hydrophobic interactions are shown by arcs with radiating spikes. Hydrophobic surfaces are shown in blue in in the 3D construction on the left.

DISCUSSION

In 1906, a German psychiatrist and neuropathologist named Dr. Alois Alzheimer discovered Alzheimer's disease while examining a patient named Auguste Deter. The patient's memory, language

skills, and behaviour all steadily declined over time. Amyloid plaques and neurofibrillary tangles, now widely recognised as markers of Alzheimer's disease, were found during a postmortem examination of her brain. The investigation's findings allowed for the distinction of Alzheimer's disease as a unique neurological condition [27]. This neurodegenerative condition that produces about 60-70% of cases of dementia and has no effective treatment at present. The hippocampus, a key part of the brain in making memories, is one of the first places to shrink in AD [3]. The rising prevalence of chronic diseases makes it pivotal to find effective treatments for AD. The cholinergic theory posits that Alzheimer's disease (AD) can originate from a deficiency of acetylcholine in the brain, which is caused by the loss of central cholinergic neurons. To combat this, cholinesterase inhibitors are used to prevent the breakdown of acetylcholine by cholinesterase enzymes [13]. With this research, we aimed to find novel cholinesterase inhibitors made from phytochemicals isolated from Salix alba (white willow), a plant with anti-inflammatory and analgesic characteristics [28]. Several compounds, including amentoflavone, astragalin, fragilin, and salireposide, were found to have shown preliminary success in blocking the AChE enzyme's normal function. 2D and 3D visualisation tools were used to study the ligand-protein interactions, and the results showed that these compounds form multiple hydrogen bonds and hydrophobic interactions with the AChE enzyme upon binding with target protein 1b41.

CONCLUSION

In this study, we looked at the interaction between phtyoligands from *Salix alba* and 1B41 was evaluated and concluded that, according to the biochemical features of the docking simulation and the chosen enzymes, there are considerable interactions between the two, including hydrogen bonds and hydrophobic contacts. None of the substances (amentoflavone, astragalin, fragile, and salidroside) failed to meet Lipinski's criteria for inhibitory actions against the AChE enzyme in AD. The results we discovered suggested they showed its potential as inhibitors of the AChE enzyme in Alzheimer's disease.

On the top of this more clinical trials are required to verify the effectiveness and safety of these phytoligands. Understanding the molecular mechanisms behind the interaction between Salix alba and AChE is important to explore possible therapeutic interventions for Alzheimer's disease, and these results lay the groundwork to accomplish so. To further understand their potential as lead compounds for the creation of new medications for the treatment of Alzheimer's disease, future research should examine the pharmacological activity of these compounds in vivo.

Acknowledgment

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Authors' Contribution

Each author has made an equivalent contribution to the overall work.

Conflicts of Interest

There are no competing interests that the authors categorically state might potentially influence the findings, results, or conclusions given in the study.

Abbreviations

AD	Alzheimer's disease
Ach	Acetylcholine
AChE	Acetylcholinesterase
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Amyloid-Beta
CAT	Choline acetyltransferase

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