A Computational Approach for Exploring Herbal Inhibitors of Acetylcholinesterase in Alzheimer's Disease

Bishajit Sarkar¹*, Md. Asad Ullah¹, Md. Nazmul Islam Prottoy¹

¹Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Corresponding author: Bishajit Sarkar*

Email of the corresponding author: sarkarbishajit@gmail.com

ORCID iD of the corresponding author: https://orcid.org/0000-0001-8439-6994
Abstract

Alzheimer’s disease (AD) is the most common type of age related dementia. Many hypotheses shed light on several reasons that lead to AD development. The cholinergic hypothesis describes that destruction of an essential neurotransmitter, acetylcholine by acetylcholinesterase (AChE) enzyme, leads to the AD onset. The hydrolysis of acetylcholine by excess amount of AChE decreases the amount of acetylcholine in the brain, thus interfering with the normal brain functions. Many anti- AChE agents can be used to treat AD by targeting AChE. In our study, 14 anti- AChE agents from plants: 1,8-cineol, berberine, carvacrol, cheilanthifoline, coptisine, estragole, harmaline, harmine, liriodenine, myrtenal, naringenin, protopine, scoulerine, stylopine were tested against AChE and compared with two controls: donepezil and galantamine, using different techniques of molecular docking. Molecular docking study was conducted for all the 14 selected ligands against AChE to identify the best three ligands among them. To determine the safety and efficacy of the three best ligands, a set of tests: the druglikeness property test, ADME/T test, PASS & P450 site of metabolism prediction, pharmacophore mapping and modelling and DFT calculations were performed. In our experiment, berberine, coptisine and naringenin were determined as the three ligands from the docking study. Further analysis of these 3 ligands showed coptisine as the most potent anti-AChE agent. The molecular dynamics simulation study showed quite results for the coptisine- AChE docked complex. Administration of berberine, coptisine and naringenin could be potential treatments for AD.

Keywords: Alzheimer’s disease, molecular docking, ADME/T, acetylcholine, phytochemicals
1. Introduction

Alzheimer's Disease (AD) was first described by Alois Alzheimer in 1907. It is one of the most prevalent dementia type disease as well as a common type of age related dementia that is increasing its numbers day by day [1, 2]. The common symptoms of AD include intellectual morbidity, delusions, psychomotor dysregulation, hallucinations etc. [3]. Genetic factors play key roles in the familial cases of Alzheimer’s disease [4]. There are many reasons that lead to the AD development. Many hypotheses have been developed by the scientists that indicate several reasons for AD development. One hypothesis is called the amyloid cascade hypothesis, where the deposition of β-amyloid plaques in the brain is responsible for the development of AD. Abnormal processing of amyloid precursor protein (APP) by β-secretase enzyme produces the β-amyloid plaques in the brain. Studies have showed that these plaques interfere with the normal brain functions [5]. Moreover, another hypothesis called oxidative stress hypothesis, describes that because of the deposition of increased amount of iron, aluminium and mercury, free radicals are generated very rapidly and lipid peroxidation and protein and DNA oxidation increases dramatically in the brain. The stresses produced by these oxidation events are responsible for the development AD [6]. According to another hypothesis called cholinergic hypothesis, the loss of functions of cholinergic neurons and cholinergic neurotransmission in the brain is responsible for AD [7]. Our study was conducted focusing on the cholinergic hypothesis of AD development.

1.1. The cholinergic hypothesis and AD development

The cholinergic hypothesis involves one of the major neurotransmitters, acetylcholine and its regulation by two enzymes, acetylcholinesterase and choline acetyltransferase [8]. Acetylcholine (ACh) is a major neurotransmitter that mediates many important functions of the brain including the learning and memory processes. Acetylcholine mediates its effects through binding to two
types of receptors: nicotinic (α7 and α4β2) and muscarinic receptors (M1 muscarinic receptor). The acetylcholine is synthesized by an enzyme called choline acetyltransferase (ChAT). ChAT catalyzes the transfer of the acetyl group from acetyl coenzyme A (AcCoA) to choline (Ch) in the pre-synaptic neuron and thus synthesize the ACh. The ACh is then secreted by the pre-synaptic neuron into the synapse. The ACh in the synapse mediates its effects by binding to either the nicotinic receptor or muscarinic receptor. To maintain the proper concentration of ACh in the brain, an enzyme called acetylcholinesterase (AChE), is synthesized. This enzyme is a serine hydrolase that hydrolyzes ACh to acetate and choline. The choline is again taken up by the pre-synaptic neuron for recycling and reusing. In this way, the balance of acetylcholine is maintained in the normal brain. However, there is evidence that, in the brain of AD patients, the overexpression of AChE occurs. Due to this reason, the break-down of acetylcholine occurs at a high rate, which decreases the required amount of ACh in the brain. Due to the scarcity of enough ACh in the brain, the neuron cells can’t mediate their functions properly and brain damage as well as memory loss occur, which lead to the onset of AD development (Figure 01). The AChE inhibitors repress or inhibit the activity of AChE. For this reason, AChE can be a potential target for anti-AChE drugs to treat AD (Figure 01) [9, 10, 11, 12, 13].
Figure 01. Cholinergic hypothesis and role of acetylcholinesterase in AD development. Acetylcholine (ACh) is synthesized (synthesizing reaction is catalysed by choline acetyltransferase, ChAT) and released by the pre-synaptic neuron. The acetylcholine mediates its effects on the post-synaptic neuron through nicotinic and/or muscarinic receptors. The ACh later performs the downstream signalling in the post-synaptic neuron. Acetylcholinesterase (AChE) enzyme breaks down the ACh and overexpression of AChE lowers the amount of acetylcholine in the brain which leads to the AD onset. AChE inhibitors repress the AChE activity, thus aid in the AD treatment.
1.2. Anti-acetylcholinesterase agents from plants

Plants have a long history to be used in various medical purposes [14, 15]. Recently, various anti-AChE agents have been identified in the plants. Many of these natural agents show good efficacy in inhibiting the AChE activity [16, 17]. In our study 14 potential anti-AChE agents were selected to analyze their inhibitory activities against the AChE enzyme as well as their safety and efficacy, using the techniques of molecular docking. The list of the 14 agents are listed in Table 0 along with their source plants. Molecular docking, also known as computational drug design, is a widely accepted and used technique for new lead discovery. This technique reduces both time and costs of the drug discovery processes. Till now, over 50 drugs have been designed with the aid of computational simulation tools and many of them received FDA approval for marketing. Molecular docking tries to predict the pose, interaction and conformation of a ligand molecule within the binding pocket of a target molecule by mimicking or simulating the actual biological environment in the computer software. After estimating the type of interactions, the software assigns scoring function to each of the bound ligands that reflects their binding affinity. The lower the score, the greater the binding affinity. These scores are predicted by specific algorithms of the softwares [18, 19]. Along with the molecular docking study, ADME/T tests are also done to identify the safety and efficacy of a candidate drug molecule [20]. The study was designed to identify three best ligand molecules among 14 selected ligands. At first, the molecular docking study was conducted for all the 14 selected ligands against the AChE enzyme (PDB ID: 1ACJ). Based on the docking score, three best molecules were selected. Later, druglikeness property experiments, ADME/T tests, PASS prediction, P450 site of metabolism prediction, pharmacophore mapping and modelling, solubility prediction and DFT calculations were
performed on the three best selected ligands using various tools to determine their safety and efficacy. Later, molecular dynamics simulation study was carried out on the best selected ligand (Figure 02). Molecular docking study using different ligands has already been performed against the AChE enzyme (PDB ID: 1ACJ), where satisfied results were obtained [21]. Two FDA approved drugs: donepezil and galantamine, were used as positive controls in the experiments. These two drugs are also AChE inhibitors. Galantamine is approved for treating the mild and moderate AD and dopenezil is used to treat mild, moderate and severe AD [22].
Table 01. List of the 14 anti-AChE agents from plants used in the study.

| No | Compound name       | Plant source                               | References                  |
|----|---------------------|--------------------------------------------|-----------------------------|
| 01 | 1,8-cineol          | *Rosmarinus officinalis*                   | [23]                        |
| 02 | Berberine           | *Berberis vulgaris*                        | [24]                        |
| 03 | Carvacrol           | *Thymus vulgaris*                          | [25]                        |
| 04 | Cheilanthifoline    | *Corydalia dubia*                         | [26]                        |
| 05 | Coptisine           | *Coptis chinensis*, *Berberis bealei* and  | [27]                        |
|    |                     | *Phellodendron chinense*                   |                             |
| 06 | Estragole           | *Ocimum basilicum*, *Ocimum africanum*,   | [28]                        |
|    |                     | *Ocimum americanum*, and *Ocimum minimum*  |                             |
| 07 | Harmaline           | *Peganum harmala*                         | [29]                        |
| 08 | Harmine             | *Peganum harmala*                         | [29]                        |
| 09 | Liriodenine         | *Beilschmiedia aloiophylla*               | [30]                        |
| 10 | Myrtenal            | *Hedychium gardnerianum*                  | [31, 32]                    |
| 11 | Naringenin          | *Citrus junos*                            | [33]                        |
| 12 | Protopine           | *Corydalis ternata*                       | [34]                        |
| 13 | Scoulerine          | *Corydalia dubia*                         | [26]                        |
| 14 | Stylopine           | *Corydalis crispa*                        | [35]                        |
Figure 02. The flowchart of the work-plan of the experiment.
**Figure 03.** 3D structure of acetylchoniesterase enzyme (PDB ID: 1ACJ). The structure was taken from Protein Data Bank online server (https://www.rcsb.org/) and visualized using Discovery Studio Visualizer.
Figure 04. 2D representations of the all the 14 ligands used in the experiment. The ligand structures were taken from PubChem server (www.pubchem.ncbi.nlm.nih.gov). A. 1,8-cineol (PubChem CID: 2758); B. Berberine (PubChem CID: 2353); C. Carvacrol (PubChem CID: 10364); D. Cheilanthifoline (PubChem CID: 440582); E. Coptisine (PubChem CID: 72322); F. Estragole (PubChem CID: 8815); G. Harmaline (PubChem CID: 3564); H. Harmine (PubChem
CID: 5280953); I. Liriodenine (PubChem CID: 10144); J. Myrtenal (PubChem CID: 61130); K. Naringenin (PubChem CID: 932); L. Protopine (PubChem CID: 4970); M. Scoulerine (PubChem CID: 22955); N. Stylopine (PubChem CID: 6770).
2. Materials and Methods

Ligand preparation, Grid generation and Glide docking, 2D representations of the best pose interactions between the three best ligands and their respective receptors were obtained using Maestro-Schrödinger Suite 2018-4. The 3D representations of the best pose interactions between the ligands and their respective receptors were visualized using Discovery Studio Visualizer [36, 37]. The 2D structures of ligands were downloaded from PubChem in SDF format (www.pubchem.ncbi.nlm.nih.gov) and the receptors were downloaded from protein data bank (www.rcsb.org).

2.1. Protein Preparation

Three dimensional structure of acetylcholinesterase enzyme (PDB ID: 1ACJ) was downloaded in PDB format from protein data bank (www.rcsb.org) (Figure 03). The proteins were then prepared and refined using the Protein Preparation Wizard in Maestro Schrödinger Suite 2018-4 [38]. All the waters were deleted from the protein during protein preparation. Finally, the structure was optimized and then minimized using force field OPLS_2005. Minimization was done setting the maximum heavy atom RMSD (root-mean-square-deviation) to 30 Å and any remaining water less than 3 H- bonds to non-water was again deleted during the minimization step.

2.2. Ligand Preparation

Structures of the controls: donepezil (PubChem CID: 3152) and galantamine (PubChem CID: 9651) and the selected ligands: 1,8-cineol (PubChem CID: 2758), berberine (PubChem CID: 2353), carvacrol (PubChem CID: 10364), cheilanthifoline (PubChem CID: 440582), coptisine (PubChem CID: 72322), estragole (PubChem CID: 8815), harmaline (PubChem CID: 3564), harmine (PubChem CID: 5280953), liriodenine (PubChem CID: 10144), myrtenal (PubChem...
CID: 61130), naringenin (PubChem CID: 932), protopine (PubChem CID: 4970), scoulerine (PubChem CID: 22955), stylopine (PubChem CID: 6770) were downloaded in SDF format (sequentially) from PubChem (www.pubchem.ncbi.nlm.nih.gov) (Figure 04). These structures were then prepared using the LigPrep function of Maestro Schrödinger Suite 2018-4 [39]. Minimized 3D structures of ligands were generated using Epik2.2 and within pH 7.0 +/- 2.0. Minimization was again carried out using OPLS_2005 force field which generated 32 possible stereoisomers.

2.3. Receptor Grid Generation

Grid usually confines the active site to shortened specific area of the receptor protein for the ligand to dock specifically. In Glide, a grid was generated using default Van der Waals radius scaling factor 1.0 and charge cutoff 0.25 which was then subjected to OPLS_2005 force field. A cubic box was generated around the active site (reference ligand active site). Then the grid box volume was adjusted to 15×15×15 for docking test.

2.4. Glide Standard Precision (SP) and Extra Precision (XP) Ligand Docking, Prime MM-GBSA Prediction and Induced Fit Docking

SP and XP glide docking were carried out using Glide in Maestro Schrödinger Suite 2018-4 [40]. The Van der Waals radius scaling factor and charge cutoff were set to 0.80 and 0.15 respectively for all the ligand molecules. Final score was assigned according to the pose of docked ligand within the active site of the receptor. The ligand with lowest glide docking score(s) was considered as the best ligand. The docking results are listed in Table 02. After successful docking, the 2D representations of the best pose interactions between the three best ligands and their receptor were generated using Maestro-Schrödinger Suite 2018-4 (Figure 05). The 3D representations of the
best pose interactions between the three best ligands and their receptor were obtained using Discovery Studio Visualizer (Figure 06). The interaction of the best three ligands with various amino acids of the receptor protein was also visualized by Discovery Studio Visualizer (Figure 07). The molecular mechanics- generalized born and surface area (MM-GBSA) tool was used to determine the $\Delta G_{\text{Bind}}$ scores and induced fit docking (IFD) was carried out to predict the XP $G_{\text{Score}}$ scores of only the three best ligand molecules. Both the MM-GBSA and IFD studies was carried out using Maestro-Schrödinger Suite 2018-4. To determine the $\Delta G_{\text{Bind}}$ scores of the best three ligands, OPLS_2005 was selected as the force field as well as the VSGB solvation model was selected. The other parameters were kept default. To carry out the IFD study, the protein preparation was first carried out. Next, the OPLS_2005 was selected as the force field, rigid docking was selected in the conformational sampling parameter, receptor van der Waals screening was set at 0.70, ligand van der Waals screening was set at 0.50 and maximum number of poses was set at 2, refine residues within 2 angstrom of ligand poses and Extra Precision (XP) were selected. Other parameters were kept default. The results of MM-GBSA ($\Delta G_{\text{Bind}}$ scores) study and IFD (XP $G_{\text{Score}}$ and IFD values) study are listed in Table 03. Moreover, Table 03 lists the different types of bonds and bond distances that took part in the interaction of the three best ligands and their receptor, AChE. After the docking analysis, the best ligand molecule from each of the receptor category were chosen and then they were further analyzed for druglikeness properties, ADME/T predictions, PASS, P450 site of metabolism predictions and DFT calculations.

2.5. Ligand Based Drug Likeness Property and ADME/Toxicity Prediction

The molecular structures of the three best ligands were analyzed using SWISSADME server (http://www.swissadme.ch/). In the druglikeness property test, Lipinski’s rule of five or not, along with some other properties were predicted. Various physicochemical properties of ligand
molecules were calculated using OSIRIS Property Explorer. The drug likeness properties of the selected ligand molecules were analyzed using SWISSADME server as well as the OSIRIS Property Explorer [41, 42]. The results of drug likeness property analysis are summarized in Table 04 [43]. The ADME/T for each of the ligand molecules was carried out using an online based server ADMETlab (http://admet.scbdd.com/) to predict their various pharmacokinetic and pharmacodynamic properties including blood brain barrier permeability, human intestinal adsorption, Caco-2 permeability, Cytochrome P (CYP) inhibitory capability, half-life, mutagenicity etc. [44]. The numeric and categorical values of the results showed by ADMETlab tool were changed into qualitative values according to the explanation and interpretation described in the ADMETlab server (http://admet.scbdd.com/home/interpretation/) for the convenience of interpretation. The results of ADME/T for all the ligand molecules are depicted in Table 05.

2.6. PASS (Prediction of Activity Spectra for Substances) and P450 Site of Metabolism (SOM) prediction

The PASS (Prediction of Activity Spectra for Substances) prediction of the three best selected ligands were conducted by using PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/) by using canonical SMILES from PubChem server (https://pubchem.ncbi.nlm.nih.gov/) [45]. To carry out PASS prediction, $P_a$ (probability "to be active") was kept greater than 70% because studies have confirmed that the $P_a > 70\%$ threshold gives highly reliable prediction [46]. In the PASS prediction study, both the possible biological activities and the possible adverse and toxic effects of the selected ligands were predicted. Table 06 and Table 07 list the results of the PASS prediction studies. The P450 Site of Metabolism (SOM) of the three best selected ligand molecules were determined by online tool, RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS-WebPredictor/) [47]. The LD50 and
Toxicity class was predicted using ProTox-II server (http://tox.charite.de/protox_II/) [48]. The canonical SMILES of berberine, coptisine and naringenin were taken from PubChem server (https://pubchem.ncbi.nlm.nih.gov/) and the SMILES was used to predict the LD50 and toxicity class. **Table 08** lists the results of P450 site of metabolism study.

### 2.7. Pharmacophore Mapping and Modelling

The pharmacophore mapping was carried out by online server PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) [49]. The ligands downloaded in sdf format from PubChem server were uploaded. The “maximum number of conformations” parameter was set at 1000, all possible targets were set at the “select target set” parameter and the “number of reserved matched targets” parameter was set at 1000. In the advanced options, the cut-off value of fit score was set at 0. All the other parameters were kept default. The pharmacophore mapping experiment was done for the three best ligand molecules among the 14 selected ligands (**Figure 08 and Table 09**).

The pharmacophore modelling of the three best ligands were carried out using the Phase pharmacophore perception engine of Maestro-Schrödinger Suite 2018-4 [50]. The pharmacophore modelling was done manually. To carry out the process, the radii sizes were kept as the Van der Waals radii of receptor atoms, radii scaling factor was kept at 0.50, receptor atoms whose surfaces are within 2.00 Å of the ligand surface were ignored and the volume shell thickness was limited to 5.00 Å. The 2D and 3D pharmacophore modelling were carried out for the three best ligand molecules (**Figure 09 and Figure 10**).

### 2.8. DFT calculation

For the Density functional theory or DFT calculation, ligand structures were first prepared by LigPrep were used for DFT calculation using the Jaguar panel of Maestro Schrödinger Suite v11.4.
[51]. In DFT calculation, Becke’s three-parameter exchange potential and Lee-Yang-Parr correlation functional (B3LYP) theory with 6-31G* basis set, were used [52, 53]. Quantum chemical properties such as surface properties (MO, density, potential) and Multipole moments were calculated along with HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy. Then the global frontier orbital was analyzed and hardness ($\eta$) and softness ($S$) of selected molecules were calculated using the following equation as per Parr and Pearson interpretation and Koopmans theorem [54, 55]. The DFT calculation was done for the 3 best ligand molecules. The result of DFT calculation is summarized in Table 10 and Figure 11.

$$\eta = \frac{(\text{HOMO} - \text{LUMO})}{2},$$

$$S = \frac{1}{\eta}$$

### 2.9. Molecular Dynamics Simulation Study

The molecular dynamics simulation study was carried out for the ligand molecule that was declared as the best among the selected 14 ligand molecules. From the analysis of the results, it was declared that, coptisine was the best ligand among the selected ligand molecules. The molecular dynamics simulation study of coptisine and acetylcholinesterase docked complex was performed by the online server iMODS (http://imods.chaconlab.org/). The server is a fast, user-friendly and effective molecular dynamics simulation tool that can be used efficiently to investigate the structural dynamics of the protein complexes. The server provides the values of deformability, B-factor (mobility profiles), eigenvalues, variance, co-varience map and elastic network. For a complex or protein, the deformability depends on the ability to deform at each of its amino acid residues. The eigenvalue has relation with the energy that is required to deform the given structure and the lower the eigenvalue, the easier the deformability of the complex.
Moreover, the eigenvalue also represents the motion stiffness of the protein complex. The server is a fast and easy server for determining and measuring the protein flexibility [56, 57, 58, 59, 60]. For analysing the molecular dynamics simulation of the three complexes, the docked PDB files were uploaded to the iMODS server and the results were displayed keeping all the parameters as default.

In this experiment, the two controls were used in molecular docking study, druglikeness property experiment and ADME/T test to compare their results with the three best selected ligands. However, the PASS prediction, P450 SOM prediction, pharmacophore mapping and modelling, solubility prediction and DFT calculations were carried out to determine and compare the biological activities of the three best ligands, for this reason, in these prediction tests, the two controls were not used.
Figure 05. 2D representations of the best pose interactions between the three best ligands and their receptor. A. interaction between berberine and acetylcholinesterase, B. interaction between coptisine and acetylcholinesterase, C. interaction between naringenin and acetylcholinesterase. Colored spheres indicates the type of residue in the target: Red-Negatively charged (Asp, Glu), Blue- Polar (Ser, Gln, Asn), Green-Hydrophobic (Tyr, Met, Leu, Ile, Phe, Pro), Ash color- Glycine, Deep Purple- Unspecified molecules and the Grayish circles represent Solvent exposure. Interactions are shown as colored lines- Solid pink lines with arrow- H-bond in target (backbone),
Dotted pink lines with arrow- H-bond between receptor and ligand (sidechain), Solid pink lines without arrow- Metal co-ordination, Green line- Pi-Pi stacking interaction, Green dotted lines- Distances, Partially blue and red colored lines- Salt bridges. Ligands exposed to solvent are represented by grey sphere. The colored lines show the protein pocket for the ligand according to nearest atom. Interruptions of the lines indicate the opening of the pocket. 2D representations of the best pose interactions between the ligands and their respective receptors were obtained using Maestro-Schrödinger Suite 2018-4.
Figure 06. 3D representations of the best pose interactions between the ligands and their receptor. The proteins are represented in Solid ribbon model and the ligands are represented in Stick model. A. interaction between berberine and acetylcholinesterase, B. interaction between coptisine and acetylcholinesterase, C. interaction between naringenin and acetylcholinesterase. The 3D representations of the best pose interactions between the ligands and their respective receptors were visualized using Discovery Studio Visualizer.
Table 02. The docking results (binding energy) of all the 14 ligands and the controls along with the determination of Lipinski’s rule of five, their respective number of hydrogen bonds as well as interacting amino acids.
| No | Names of ligands (with PubChem CID) | SP Docking Score (Binding Energy) (Kcal/mol) | XP Docking Score (Binding Energy) (Kcal/mol) | Lipinski's rule of five | Interacting residues of the target |
|----|------------------------------------|------------------------------------------|------------------------------------------|------------------------|----------------------------------|
| 01 | 1,8-cineol (PubChem CID: 2758)     | -4.844                                   | -3.109                                   | Yes                    | Trp 84, Phe 330                  |
| 02 | Berberine (PubChem CID: 2353)      | -9.658                                   | -13.571                                  | Yes                    | Gln 69, Phe 330, Gly 123, Trp 84 |
| 03 | Carvacrol (PubChem CID: 10364)     | -6.060                                   | -6.986                                   | Yes                    | Tyr 334, Phe 330, Trp 432, Tyr 442, Trp 84, His 440 |
| 04 | Cheilanthifoline (PubChem CID: 440582) | -6.387                                   | -7.398                                   | Yes                    | Gly 117, Trp 84, Phe 330, Trp 432 |
| 05 | Coptisine (PubChem CID: 72322)     | -10.148                                  | -15.560                                  | Yes                    | Trp 432, Trp 84, Phe 330, His 440, Ser 122, Gly 117 |
| 06 | Estragole (PubChem CID: 8815)      | -5.035                                   | -5.992                                   | Yes                    | Tyr 334, Phe 330, Ile 439, Trp 432, Trp 84 |
| 07 | Harmaline (PubChem CID: 3564)      | -8.053                                   | -9.154                                   | Yes                    | Tyr 334, Trp 432, Trp 84, Glu 199, Tyr 442, His 440, Ile 439, Phe 330 |
| 08 | Harmine (PubChem CID: 5280953)     | -8.385                                   | -8.363                                   | Yes                    | Phe 330, His 440, Trp 84         |
| 09 | Liriodenine (PubChem CID: 10144)   | -7.754                                   | -7.775                                   | Yes                    | Phe 330, Tyr 334, Tyr 121, Trp 84 |

Lipinski's rule of five criteria:
- Molecular weight ≤ 500
- LogP ≤ 5
- Number of hydrogen bond donors ≤ 5
- Number of hydrogen bond acceptors ≤ 10
|   | Compound (PubChem CID)                      | ΔG<sub>Bind</sub> | ΔG<sub>Score</sub> | Yes/No | Interacting Amino Acids                  |
|---|-------------------------------------------|------------------|------------------|-------|----------------------------------------|
| 10| Myrtenal (PubChem CID: 61130)             | -5.873           | -4.821           | Yes   | Ile 444, Ser 200, GLy 118, Gly 119, Trp 84, Phe 330, His 440 |
| 11| Naringenin (PubChem CID: 932)             | -9.266           | -9.342           | Yes   | His 440, Phe 330, Tyr 70, Trp 84, Pro 86 |
| 12| Protopine (PubChem CID: 4970)             | -6.272           | -7.789           | Yes   | Phe 330, His 440, Gly 117, Trp 84       |
| 13| Scoulerine (PubChem CID: 22955)           | -6.229           | -2.230           | Yes   | Phe 330, His 440, Tyr 130, Gly 117, Trp 84, Ser 122 |
| 14| Stylopine (PubChem CID: 6770)             | -7.733           | -8.071           | Yes   | Phe 330, His 440, Trp 84, Gly 117       |
|   | Control 1 Donepezil (PubChem CID: 3152)   | -5.045           | -8.434           | Yes   | Trp 279, Tyr 70, Arg 289                |
|   | Control 2 Galantamine (PubChem CID: 9651) | -7.516           | -7.237           | Yes   | Trp 84, Glu 199, His 440, Phe 330, Tyr 121, Tyr 70, Gly 117 |

**Table 03.** List of the different types of bonds along with their bond distances with their respective amino acids, that took part in the interaction between the three best ligands as well as the controls and the target receptor, AChE, as well as their ΔG<sub>Bind</sub> scores, XP G<sub>Score</sub>, IFD scores and glide energy.
| Name of the ligand (with respective receptor) | MM-GBSA Score (ΔG<sub>Bind</sub> Kcal/mol) | XP G<sub>Score</sub> (Kcal/mol) | IFD score (Kcal/mol) | Glide energy (Kcal/mol) | Interacting amino acids | Bond distance in Å | Interaction category | Type of interaction |
|---------------------------------------------|---------------------------------------------|---------------------------------|---------------------|------------------------|------------------------|---------------------|-------------------|-------------------|
| Berberine                                   | -35.80                                      | -12.889                         | -1154.880           | -23.549                 | Glutamine 69           | 2.22                | Hydrogen          | Carbon            |
|                                            |                                             |                                 |                     |                        | Glycine 123            | 2.82                | Hydrogen          | Carbon            |
|                                            |                                             |                                 |                     |                        |                        | 2.42                | Hydrogen          | Conventional      |
| Tryptophan 84                               | 2.22                                        |                                 |                     |                        | Hydrogen               | 2.22                | Hydrophobic       | Carbon            |
|                                            |                                             |                                 |                     |                        | 2.79                   | Hydrophobic         | Pi-Sigma          |                   |
|                                            |                                             |                                 |                     |                        | 3.95                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 5.43                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 3.68                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.62                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 5.92                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 5.42                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.41                   | Electrostatic       | Pi-Cation         |                   |
|                                            |                                             |                                 |                     |                        | 4.12                   | Electrostatic       | Pi-Cation         |                   |
| Phenylalanine 330                           | 3.85                                        |                                 |                     |                        | Hydrophobic            | 3.85                | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.02                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.64                   | Electrostatic       | Pi-Cation         |                   |
| Coptisine                                   | -49.91                                      | 14.942                          | -1158.410           | -16.197                 | Tryptophan 432         | 2.96                | Hydrophobic       | Pi-Lone pair      |
| Tryptophan 84                               | 4.48                                        |                                 |                     |                        | Hydrophobic            | 4.48                | Hydrophobic       | Pi-Pi stacked     |
|                                            |                                             |                                 |                     |                        | 3.62                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.53                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.33                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 4.02                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 3.94                   | Electrostatic       | Pi-Cation         |                   |
|                                            |                                             |                                 |                     |                        | 4.12                   | Electrostatic       | Pi-Cation         |                   |
| Phenylalanine 330                           | 3.84                                        |                                 |                     |                        | Hydrophobic            | 3.84                | Pi-Pi stacked     |                   |
|                                            |                                             |                                 |                     |                        | 3.74                   | Hydrophobic         | Pi-Pi stacked     |                   |
|                  | Electrostatic  | Pi-Cation | Histidine 440 | 4.43 | Hydrogen | Carbon |
|------------------|----------------|-----------|----------------|------|----------|--------|
| Serine 122       | 2.21           | Hydrogen  | Carbon         |      |          |        |
| Glycine 117      | 2.13           | Hydrogen  | Carbon         |      |          |        |
| Naringenin       | -23.73         | -10.077   | -1152.990      | -45.657 |          |        |
| Galantamine      | -5.19          | -5.748    | -1147.050      | -33.736 |          |        |
| Donepezil        | -47.06         | -8.209    | -1148.970      | -33.756 |          |        |
| Glutamic acid    | 2.57           | Hydrogen  | Carbon         |      |          |        |
| Histidine 440    | 1.96           | Hydrogen  | Conventional   |      |          |        |
| Phenylalanine    | 3.60           | Hydrophobic Pi-Pi stacked |           |      |          |        |
| Tyrosine 70      | 1.66           | Hydrogen  | Conventional   |      |          |        |
| Tryptophan 84    | 3.84           | Hydrophobic Pi-Pi stacked |           |      |          |        |
| Proline 86       | 2.99           | Hydrogen  | Carbon         |      |          |        |
| Arginine 289     | 2.21           | Hydrogen  | Carbon         |      |          |        |
| Tryptophan 279   | 5.23           | Hydrophobic Pi-Alkyl |           |      |          |        |
| Tyrosine 70      | 5.32           | Hydrophobic Pi-Pi stacked |           |      |          |        |
| Tryptophan 84    | 4.78           | Hydrophobic Pi-Pi stacked |           |      |          |        |
| Glutamic acid 199| 2.57           | Hydrogen  | Carbon         |      |          |        |
| Histidine 440    | 1.96           | Hydrogen  | Conventional   |      |          |        |
| Phenylalanine 330| 4.30           | Hydrophobic Pi-Alkyl |           |      |          |        |
| Tyrosine 121     | 2.42           | Hydrogen  | Carbon         |      |          |        |
| Tyrosine 70      | 2.87           | Hydrogen  | Carbon         |      |          |        |
| Glycine 117      | 4.19           | Hydrophobic Amide-Pi stacked |           |      |          |        |
**Figure 07.** Figure showing the various types of bonds and amino acids that take part in the interaction between the three best selected ligands and their receptor. Interacting amino acid residues of target molecule are labeled in the diagram and dotted lines depict interaction between ligand and receptor. Green dotted lines- Conventional bond, Light pink- Alkyl/Pi-Alkyl interactions, Yellow- Pi-Sulfur/Sulphur-X interaction, Deep pink- Pi-Pi stacked bond, Orange-Charge-Charge interaction, Purple- Pi-Sigma interaction, Red- Donor-Donor interaction. A. interaction between epigallocatechin gallate and its receptor cyclin-dependent kinase-2, B.
interaction between neocryptolepine and its receptor human topoisomerase II, C. interaction between decursinol and its receptor vascular endothelial growth factor receptor-2. The representations of the interactions between the ligands and the amino acids of the respective receptors were visualized using Discovery Studio Visualizer.
Table 04. List of the results of the druglikeness properties of the three best ligands: berberine, coptisine and naringenin and the controls. The druglikeness properties were analysed by the online server SWISSADME server (http://www.swissadme.ch/) and the OSIRIS Property Explorer (https://www.organic-chemistry.org/prog/peo/).

| Drug Likeness Properties                  | Berberine | Coptisine | Naringenin | Donepezil (control 1) | Galantamine (control 2) |
|------------------------------------------|-----------|-----------|------------|-----------------------|------------------------|
| Molecular weight                         | 336.36 g/mol | 320.32 g/mol | 272.25 g/mol | 379.49 g/mol           | 287.35 g/mol           |
| Concensus Log $P_{o/w}$                  | 2.53      | 2.40      | 1.84       | 4.00                  | 1.91                   |
| Log $S$                                  | -4.55     | -4.52     | -3.49      | -4.81                 | -2.93                  |
| Num. H-bond acceptors                    | 4         | 4         | 5          | 4                     | 4                      |
| Num. H-bond donors                       | 0         | 0         | 3          | 0                     | 1                      |
| Molar Refractivity                       | 94.87     | 87.95     | 71.57      | 115.31                | 84.05                  |
| Lipinski                                 | Yes       | Yes       | Yes        | Yes                   | Yes                    |
| Ghose                                    | Yes       | Yes       | Yes        | Yes                   | Yes                    |
| Veber                                    | Yes       | Yes       | Yes        | Yes                   | Yes                    |
| Egan                                     | Yes       | Yes       | Yes        | Yes                   | Yes                    |
| Muegge                                   | Yes       | Yes       | Yes        | Yes                   | Yes                    |
| Bioavailability score                    | 0.55      | 0.55      | 0.55       | 0.55                  | 0.55                   |
| Synthetic accessibility (SA)             | 3.14      | 2.96      | 3.01       | 3.62                  | 4.57                   |
| TPSA ($Å^2$)                             | 40.80     | 40.80     | 71.57      | 38.77                 | 41.93                  |
| No of rotatable bonds                    | 2         | 0         | 1          | 6                     | 1                      |
| Druglikeness score                       | -         | -         | 1.9        | 7.29                  | 6.2                    |
| Drug-Score                               | -         | -         | 0.84       | 0.63                  | 0.91                   |
| Solubility                               | -         | -         | -2.64      | -4.35                 | -2.67                  |
|                  | -    | -    | No   | No   | No   |
|------------------|------|------|------|------|------|
| Reproductive effective | -    | -    | No   | No   | No   |
| Irritant         | -    | -    | No   | No   | No   |
| Tumorigenic      | -    | -    | No   | No   | No   |
| Mutagenic        | -    | -    | No   | No   | No   |
Table 05. The ADME/T test results of the best three ligand molecules and the controls. The tests were carried out using ADMETlab server (http://admet.scbdd.com/).

| Class                      | Properties                      | Berberine       | Coptisine       | Naringenin   | Donepezil (control 1) | Galantamine (control 2) |
|---------------------------|---------------------------------|-----------------|-----------------|--------------|------------------------|-------------------------|
| Absorption                | Caco-2 permeability            | Optimal         | Optimal         | Optimal      | Optimal                | Optimal                 |
| Pgp-inhibitor             | Non-inhibitor                   | Non-inhibitor   | Non-inhibitor   | Inhibitor    | Non-inhibitor          |                         |
| Pgp-substrate             | Substrate                       | Non-substrate   | Non-substrate   | Substrate    | Substrate              |                         |
| Human Intestinal Absorption (HIA) | HIA negative                  | HIA negative    | HIA positive    | HIA positive | HIA positive           |                         |
| Distribution              | Plasma Protein Binding          | Low             | Low             | Good         | Optimal                | Low                     |
| BBB (Blood–Brain Barrier)| BBB positive                    | BBB positive    | BBB positive    | BBB positive | BBB positive           |                         |
| Metabolism                | CYP450 1A2 inhibitor            | Inhibitor       | Inhibitor       | Non-inhibitor| Non-inhibitor          |                         |
|                           | CYP450 1A2 substrate            | Substrate       | Substrate       | Substrate    | Substrate              |                         |
|                           | CYP450 3A4 inhibitor            | Inhibitor       | Non-inhibitor   | Inhibitor    | Non-inhibitor          |                         |
|                           | CYP450 3A4 substrate            | Substrate       | Substrate       | Non-substrate| Substrate              |                         |
|                           | CYP450 2C9 inhibitor            | Non-inhibitor   | Non-inhibitor   | Non-inhibitor| Non-inhibitor          |                         |
|                           | CYP450 2C9 substrate            | Non-substrate   | Non-substrate   | Non-substrate| Non-substrate          |                         |
|                           | CYP450 2C19 inhibitor           | Non-inhibitor   | Non-inhibitor   | Non-inhibitor| Non-inhibitor          |                         |
|                           | CYP450 2C19 substrate           | Substrate       | Substrate       | Non-substrate| Substrate              |                         |
|                           | CYP450 2D6 inhibitor            | Inhibitor       | Inhibitor       | Non-inhibitor| Inhibitor              | Inhibitor              |
|                  | CYP450 2D6 substrate | Substrate | Substrate | Non-substrate | Substrate | Substrate |
|------------------|----------------------|-----------|-----------|---------------|-----------|-----------|
| Excretion | T1/2 (h) | 1.9 | 1.8 | 0.9 | 1.7 | 1.7 |
| Toxicity | hERG (hERG Blockers) | Blocker | Blocker | Blocker | Blocker | Non-blocker |
| H-HT (Human Hepatotoxicity) | HHT positive | HHT negative | HHT negative | HHT positive | HHT positive |
| Ames (Ames Mutagenicity) | Ames negative | Ames negative | Ames negative | Ames negative | Ames negative |
| DILI (Drug Induced Liver Injury) | DILI positive | DILI positive | DILI positive | DILI negative | DILI negative |
Figure 08. Parmacophore mapping of A. berberine, B. coptisine, C. naringenin. Here, light blue color- hydrophobic centre, green color- hydrogen bond donor and pink color- hydrogen bond acceptor.
**Table 06.** The PASS prediction results the biological activities of the best three ligand molecules.

The tests were carried out using PASS-Way2Drug server.

| Sl no | Biological activities                  | Berberine |            | Coptisine |            | Naringenin |            |
|-------|----------------------------------------|-----------|------------|-----------|------------|------------|------------|
|       |                                        | Predicted LD50: 1000 mg/kg | Predicted LD50: 1000 mg/kg | Predicted LD50: 2000 mg/kg |
|       |                                        | Pa | Pi | Pa | Pi | Pa | Pi |
| 01    | Membrane integrity agonist             | -  | -  | -  | -  | 0.964 | 0.003 |
| 02    | HMOX1 expression enhancer              | -  | -  | -  | -  | 0.956 | 0.002 |
| 03    | Chlordecone reductase inhibitor        | -  | -  | -  | -  | 0.918 | 0.004 |
| 04    | HIF1A expression inhibitor             | -  | -  | -  | -  | 0.911 | 0.005 |
| 05    | Histidine kinase inhibitor             | -  | -  | -  | -  | 0.892 | 0.002 |
| 06    | Aldehyde oxidase inhibitor             | -  | -  | -  | -  | 0.868 | 0.005 |
| 07    | Antimutagenic                          | -  | -  | -  | -  | 0.857 | 0.003 |
| 08    | Mucomembranous protector               | -  | -  | -  | -  | 0.844 | 0.010 |
| 09    | TP53 expression enhancer               | -  | -  | -  | -  | 0.822 | 0.009 |
| 10    | Chemopreventive                        | -  | -  | -  | -  | 0.724 | 0.006 |
Figure 09. 2D representation of the pharmacophore modelling of the three best ligand molecules. A. the hypothesis for berberine, B. the hypothesis for coptisine, C. the hypothesis for naringenin. The interactions between the ligand and the receptor in the hypothesis were presented by dotted dashed lines, yellow colour- hydrogen bonds, blue colour- pi-pi stacking interaction and green colour- pi-cation interaction. The bad contacts between the ligands and the pharmacophore are respresented. The pharmacophore modelling was carried out by Maestro-Schrödinger Suite 2018-4.
Table 07. The PASS prediction results showing the adverse and toxic effects of the best three ligand molecules. The tests were carried out using PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/).

| Sl no | Adverse and toxic effects | Berberine | Coptisine | Naringenin |
|-------|---------------------------|-----------|-----------|------------|
|       |                           | Pa        | Pi        | Pa         | Pi        |
| 01    | Vascular toxicity         | -         | -         | -          | -          | 0.736      | 0.029      |
| 02    | Inflammation              | -         | -         | -          | -          | 0.770      | 0.018      |
| 03    | Hematemesis               | -         | -         | -          | -          | 0.729      | 0.023      |
| 04    | Nephrotoxic               | -         | -         | -          | -          | 0.708      | 0.027      |
| 05    | Shivering                 | -         | -         | -          | -          | 0.766      | 0.051      |
**Table 08.** List of the P450 sites of metabolism prediction study of the three best ligand molecules.

| Names of P450 isoenzymes | Berberine | Coptisine | Naringenin |
|--------------------------|-----------|-----------|------------|
| 1A2                      | ![Berberine 1A2](image) | ![Coptisine 1A2](image) | ![Naringenin 1A2](image) |
| 2A6                      | ![Berberine 2A6](image) | ![Coptisine 2A6](image) | ![Naringenin 2A6](image) |
| 2B6                      | ![Berberine 2B6](image) | ![Coptisine 2B6](image) | ![Naringenin 2B6](image) |
| 2C8                      | ![Berberine 2C8](image) | ![Coptisine 2C8](image) | ![Naringenin 2C8](image) |
Table 09. Results of the pharmacophore mapping experiment of the three best ligands, berberine, coptisine and naringenin. The experiment was conducted using the online tool PharmMapper (http://www.lilab-ecust.cn/pharmmapper/).

| Name of the ligand compound | Fit score | Normalized fit score | z'-score | Pharmacophore features (numbers) |
|-----------------------------|-----------|----------------------|----------|--------------------------------|
|                             |           |                      |          | Hydrophobic centre | Positively charged centre | Negatively charged centre | Hydrogen bond donor | Hydrogen bond acceptor | Aromatic ring |
| Berberine                   | 2.765     | 0.921                | 1.088    | 1                  | 0                        | 0                      | 0              | 2              | 0           |
| Coptisine                   | 2.273     | 0.757                | 0.475    | 1                  | 0                        | 0                      | 0              | 2              | 0           |
| Naringenin                  | 2.637     | 0.659                | 0.749    | 3                  | 0                        | 0                      | 1              | 0              | 0           |
Table 10. The results of the DFT calculations of the selected best three ligands. The DFT calculations were carried out using Maestro Schrödinger Suite 2018-4.

| Compound name | HOMO energy (eV) | LUMO energy (eV) | Gap (eV) | Hardness (η) (eV) | Softness (S) (eV) | Dipole moment (Debye) |
|---------------|------------------|------------------|---------|------------------|------------------|----------------------|
| Berberine     | -0.145           | -0.091           | 0.054   | 0.027            | 37.037           | 8.050                |
| Coptisine     | -0.005           | -0.094           | 0.089   | 0.045            | 22.222           | 2.277                |
| Naringenin    | -0.134           | -0.087           | 0.047   | 0.024            | 41.667           | 6.459                |
**Figure 10.** 3D representation of the pharmacophore modelling of the three best ligand molecules.

A. the hypothesis for berberine, B. the hypothesis for coptisine, C. the hypothesis for naringenin.

The pharmacophore modelling was performed by Maestro-Schrödinger Suite 2018-4.
Figure 11. The results of DFT calculations (HOMO-LUMO structures) of, 1. Berberine, 2. Coptisine, 3. Naringenin. The HOMO structures are illustrated in the left column and the LUMO structures are illustrated in the right column.
Figure 12. Results of molecular dynamics simulation of coptisine-acetylcholinesterase docked complex. (a) NMA mobility, (b) deformability, (c) B-factor, (d) eigenvalues, (e) variance (red color indicates individual variances and green color indicates cumulative variances), (f) covariance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (g) elastic network (darker gray regions indicate more stiffer regions) of the complex.
3. Result

3.1. Molecular docking, MM-GBSA study and Induced Fit Docking:

All the selected ligand molecules were docked successfully against their target, AChE. The ligand molecules that had the lowest binding energy or docking score, were considered the best ligand molecules in inhibiting the target receptor as the lower binding energy corresponds to higher binding affinity [61, 62].

1, 8-cineol generated SP docking score of -4.844 Kcal/mol, XP docking score of -3.109 Kcal/mol, when docked against AChE. However, it formed interactions with only 2 amino acids: Trp 84 and Phe 330. Berberine generated SP docking score of -9.658 and XP docking score of -13.571 Kcal/mol, when docked against the target protein. It interacted with Gln 69, Phe 330, Gly 123 and Trp 84 amino acids in the binding pocket. Carvacrol showed SP docking score of -6.060 Kcal/mol and XP docking score of -6.986 Kcal/mol. It showed interactions with Tyr 334, Phe 330, Trp 432, Tyr 442, Trp 84 and His 440 amino acids in the binding pocket of its target. Cheilanthifoline showed SP docking score of -6.387 Kcal/mol and XP docking score of -7.398 Kcal/mol as well as it interacted with 4 amino acids: Gly 117, Trp 84, Phe 330 and Trp 432. Coptisine gave SP docking score of -10.148 Kcal/mol, XP docking score of -15.560 Kcal/mol. The ligand interacted with 6 amino acids: Trp 432, Trp 84, Phe 330, His 440, Ser 122 and Gly 117, in the binding pocket of the receptor. Estragole generated SP docking score of -5.035 Kcal/mol, XP docking score of -5.992 Kcal/mol. It interacted with Tyr 334, Phe 330, Ile 439, Trp 432 and Trp 84. Harmaline generated SP docking score of -8.053 Kcal/mol, XP docking score of -9.154 Kcal/mol and the ligand interacted with Tyr 334, Trp 432, Trp 84, Glu 199, Tyr 442, His 440, Ile 439 and Phe 330 amino acids in the binding pocket. Harmine showed SP docking score of -8.385 Kcal/mol as well as XP docking score of -8.363 Kcal/mol. However, harmine formed interactions with Phe 330, His 440
and Trp 84 amino acids. Liriodenine showed SP docking score of -7.754 Kcal/mol and XP docking score of -7.775 Kcal/mol. The ligand interacted with 4 amino acids of the receptor: Phe 330, Tyr 334, Tyr 121, Trp 84. Myrtenal showed SP docking score of -5.873 Kcal/mol, XP docking score of -4.821 Kcal/mol. It interacted with Ile 444, Ser 200, GLy 118, Gly 119, Trp 84, Phe 330 and His 440 amino acids in the binding pocket of the receptor. Naringenin showed SP docking score of -9.266 Kcal/mol, XP docking score of -9.342 Kcal/mol. It interacted with 5 amino acids: His 440, Phe 330, Tyr 70, Pro 86 and Trp 84. Protopine and stylopine gave SP docking scores of -6.272 Kcal/mol and -7.733 Kcal/mol, respectively, and XP docking scores of -7.789 Kcal/mol and -8.071 Kcal/mol, respectively. However, both of them interacted with Phe 330, His 440, Gly 117 and Trp 84 amino acids of the receptor. On the other hand, scoulerine showed SP docking score of -6.229 Kcal/mol, XP docking score of -2.230 Kcal/mol. And it interacted with 6 amino acids: Phe 330, His 440, Tyr 130, Gly 117, Trp 84 and Ser 122. The control 1, donepezil generated SP docking score of -5.015 Kcal/mol and XP docking score of -8.434 Kcal/mol and interacted with Trp 279, Tyr 70, Arg 289. The control 2, galantamine generated SP docking score -7.516 Kcal/mol and XP docking score of -7.237 Kcal/mol. In interacted with Trp 84, Glu 199, His 440, Phe 330, Tyr 121, Tyr 70, Gly 117. All the 14 selected ligands as well as the controls followed the Lipinski’s rule of five. The results of docking study are listed in Table 02.

From 14 selected ligand molecules, 3 ligands were selected as the best ligands based on the lowest SP and XP docking scores or binding energies. Coptisine gave the lowest SP and XP docking scores, the second lowest SP and XP docking scores were showed by berberine and the third lowest scores were given by naringenin. For this reason, these three ligands were selected as the best ligands for further analysis. In the MM-GBSA study, coptisine generated the lowest ΔG_{Bind} score of -49.91 Kcal/mol. The second lowest score was generated by berberine (-35.80 Kcal/mol). The
highest $\Delta G_{\text{Bind}}$ score of naringenin was -23.73 Kcal/mol. Berberine, coptisine and naringenin gave glide energies of -23.549 Kcal/mol, -16.197 Kcal/mol and -45.657 Kcal/mol, respectively. Furthermore, coptisine also generated the lowest XP GScore of -14.942 Kcal/mol as well as the lowest IFD score of -1158.410 Kcal/mol. Donepezil and galantamine had $\Delta G_{\text{Bind}}$ scores of -47.06 and -5.19 Kcal/mol, respectively, XP GScore of -8.209 and -5.748 Kcal/mol, respectively and IFD scores of -1148.970 and -1147.050 Kcal/mol, respectively. Berberine with XP GScore of -12.889 Kcal/mol and IFD score of -1154.880 Kcal/mol, was the second lowest score generator and the highest XP GScore and IFD score were showed by naringenin of -10.078 Kcal/mol and -1152.980 Kcal/mol, respectively. Berberine formed 1 carbon bond with glutamine 69, 1 carbon bond and 1 conventional bond with glycine 123, 1 carbon, 1 pi-sigma, 6 pi-pi stacked and 2 pi-cation bonds with tryptophan 84 and 2 pi-pi stacked bonds and 1 pi-cation with phenylalanine 330. Coptisine generated 1 pi-lone pair bond with tryptophan 432, 5 pi-pi stacked and 2 pi-cation bonds with tryptophan 84, 2 pi-pi stacked bonds and 1 pi-cation bond phenylalanine 330, 2 carbon bonds with histidine 440, 1 carbon bond with serine 122 and 1 carbon bond with glycine 117. Moreover, naringenin formed 1 conventional bond and 1 carbon bond with histidine 440, 1 pi-pi stacked bond with phenylalanine 330, 1 conventional bond with tyrosine 70, 1 carbon bond with proline 86 and 2 pi-pi stacked bonds with tryptophan 84. The three best ligands with their respective docking score, glide energy, interacted amino acids, types of bonds and bond distances, are listed in Table 03.

### 3.2. Druglikeness properties

Druglikeness property experiments were conducted only for the best three ligand molecules: berberine, coptisine and naringenin (Table 03). Lipinski’s rule of five demonstrates that the
acceptable ranges of the best drug molecule for all the five parameters are: molecular weight: \( \leq 500 \), number of hydrogen bond donors: \( \leq 5 \), number of hydrogen bond acceptors: \( \leq 10 \), lipophilicity (expressed as LogP): \( \leq 5 \) and molar refractivity from 40 to 130 [63]. All the three ligands followed the Lipinski’s rule of five. Berberine, coptisine and naringenin have molecular weights less than 500 (336.36 g/mol, 320.32 g/mol and 272.25 g/mol, respectively), only naringenin had 3 hydrogen bond donors and the other two ligands didn’t have any hydrogen bond donor and both berberine and coptisine has 4 hydrogen bond acceptors, each and naringenin had 5 hydrogen bond acceptors. The logP values of berberine, coptisine and naringenin were 2.53, 2.40 and 1.84, respectively, that were also well within the accepted range of the Lipinski’s rule of five. Moreover, the molar refractivity of berberine, coptisine and naringenin were 94.87, 87.95 and 71.57, respectively. The LogS values showed by berberine, coptisine and naringenin were -4.55, -4.52 and -3.49, respectively. However, all of the ligand molecules followed the Ghose, Veber, Egan and Muegge rules and all of them showed the similar bioavailability score of 0.55. Berberine gave synthetic accessibility (SA) score of 3.14, coptisine gave SA score of 2.96 and naringenin gave SA score of 3.01. Moreover, both berberine and coptisine gave topological polar surface area (TPSA) score of 40.80 Å², however, naringenin showed TPSA score of 71.57 Å². Coptisine didn’t have any rotatable bond, berberine had 2 rotatable bonds and naringenin had 1 rotatable bond. However, the druglikeness score, drug score, solubility, reproductive effectiveness, irritant properties, tumorigenic and mutagenic properties were not available for both berberine and coptisine. Only naringenin generated results in these experiments. It gave druglikeness score of 1.9, drug score of 0.84, solubility score of -2.64 and it is not reproductive effective, irritant, tumorigenic and mutagenic. Naringenin generated quite good scores in the druglikeness property experiments. Donepezil and galantamine also showed quite good results with no violation of the Lipinski’s rule.
of five, Ghose, Veber, Egan and Muegge rules. They had molecular weights of 379.49 g/mol and 287.35 g/mol, respectively and druglikeness score of 7.29 and 6.2, respectively. None of them were reproductive effective, irritant, tumorigenic and mutagenic. The druglikeness properties of the three ligands and the controls are listed in Table 04.

3.3. ADME/T tests

The ADME/T tests were done only for the three best selected ligands. All the ligands showed optimal Caco-2 permeability and all of them were p-glycoprotein non-inhibitor. However, only berberine was the p-glycoprotein substrate. Both berberine and coptisine were HIA negative, which means that all of them were not absorbed by human intestine. Only naringenin was HIA positive. All the three ligands had low plasma protein binding ability and all of them showed blood-brain-barrier crossing ability. All the three ligands were inhibitors as well as substrates for CYP450 1A2. However, only coptisine was non-inhibitor for CYP450 3A4 and both berberine and coptisine were substrates for CYP450 3A4. Moreover, all the three ligands were non-inhibitors and non-substrates for CYP450 2C9. Although, all of them were non-inhibitors for CYP450 2C19 and only naringenin was non-substrate for CYP450 2C19. Furthermore, only naringenin was non-inhibitor and non-substrate for CYP450 2D6. The half-life ($T_{1/2}$) values of berberine, coptisine and naringenin were 1.9, 1.8 and 0.9 hours, respectively. All the three ligands showed hERG blocking capability and berberine had human hepatotoxic ability. However, all of them were not Ames mutagenic, although all of them showed the capability to do drug induced liver injury (DILI positive). Both the controls showed inhibitory activities to CYP450 2D6 as well as substrate activities to CYP450 2D6. Both of them were also substrate to CYP450 3A4. Both of them were
human hepatotoxic as well as Ames negative and DILI negative. Only donepezil was hERG blocker among the two controls. The results of ADME/T tests are listed in Table 05.

3.4. PASS prediction and P450 site of metabolism (SOM) prediction

Berberine and coptisine had the same predicted LD50 value of 1000 mg/kg and naringenin had the predicted LD50 value of 2000 mg/kg. However, all the three ligands were in toxicity class 4. The prediction of activity spectra for substances (PASS prediction) study for all the three ligands were done to predict 10 intended biological activities and 5 intended adverse and toxic effects. To carry out the PASS prediction experiment, Pa > 0.7 was kept, since this threshold give highly reliable prediction [46]. The PASS prediction results of all the three selected ligands are listed in Table 06 and Table 07. However, at Pa > 0.7, the intended biological activities and the adverse and toxic effects for berberine and coptisine were not generated by the PASS-Way2Drug server. Only naringenin showed the biological activities: membrane integrity agonist, HMOX1 expression enhancer, chlordecone reductase inhibitor, HIF1A expression inhibitor, histidine kinase inhibitor, aldehyde oxidase inhibitor, antimutagenicity, mucomembranous protector, TP53 expression enhancer and chemopreventive activities. Moreover, adverse and toxic effects showed by naringenin were: vascular toxicity, inflammation, hematemesis, nephrotoxicity and shivering actions.

The possible sites of metabolism by CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4 of berberine, coptisine and naringenin were determined. The possible sites of a chemical compound, where the metabolism by the isoforms of CYP450 enzymes may be taken place, are indicated by circles on the chemical structure of the molecule [47]. The P450 SOM predictions
showed that both berberine and coptisine had 3 sites of metabolism (SOMs) for all the CYP450 enzymes or isoforms. However, naringenin had 4 SOMs for all the CYP450 enzymes, except the CYP450 2C9. Naringenin had 5 SOMs for CYP450 2C9. The possible sites of P450 metabolism are illustrated in Table 08.

3.5. Pharmacophore mapping and modelling

Berberine, coptisine and naringenin gave almost similar fit scores of 2.765, 2.273 and 2.637, respectively, in the pharmacophore mapping experiment. Berberine had the normalized fit score of 0.928 and z'-score of 1.088. Coptisine showed normalized fit score of 0.757 and z'-score 0.475. And naringenin had the normalized fit score of 0.659 and z'-score of 0.749. Furthermore, hydrophobic centres generated by berberine, coptisine and naringenin were 1, 1 and 3 respectively. Both berberine and coptisine had 2 hydrogen bond acceptors each, whereas, naringenin didn’t generate any hydrogen bond acceptor. Moreover, only naringenin had 1 hydrogen bond donor. However, none of the molecules showed positively charged centre, negatively charged centre and aromatic ring (Figure 08 and Table 09).

The three best ligands were used to generate pharmacophore hypotheses. Berberine generated 6 point hypothesis and both coptisine and naringenin showed 4 point hypothesis each. Berberine generated 2 pi-cation bonds, 4 pi-pi stacked interactions and 2 hydrogen bonds with its pharmacophore. Although it generated several good contacts (not shown here), however, it also generated 13 bad contacts with its pharmacophore. Coptisine showed 2 hydrogen bonds, 6 pi-pi stacked interactions and 2 pi-cation bonds. Like berberine, coptisine also generated several good contacts, however, it also showed 12 bad contacts with its pharmacophore. Naringenin showed 2 pi-pi stacked interactions, 2 hydrogen bonds and 2 bad contacts with the pharmacophore.
Moreover, it also generated a good number of good contacts with the pharmacophore of AChE. None of the ligands generated ugly contact with AChE (Figure 09 and Figure 10).

3.6. DFT Calculation

In the DFT calculations, berberine showed HOMO energy of -0.145 eV, LUMO energy of -0.091 eV and gap energy of 0.054 eV as well as the dipole moment of 8.050 debye. Coptisine generated HOMO and LUMO energies of -0.005 eV and -0.094 eV, respectively and gap energy of 0.089 eV. On the other hand, naringenin gave HOMO energy of -0.134 eV, LUMO energy of -0.087 eV, gap energy of 0.047 eV and the dipole moment of 6.459 debye (Table 10 and Figure 11).

3.7. Molecular Dynamics Simulation

Fig. 12a illustrates the normal mode analysis (NMA) of coptisine-acetylcholinesterase complex. The deformability graphs of the three complexes illustrate the peaks in the graphs correspond to the regions in the protein with deformability (Fig. 12b). The B-factor graph of the complex gives easy visualisation and understanding of the comparison between the NMA and the PDB field of the complexes (Fig. 12c). The eigenvalue of the complex is illustrated in Fig. 12d. The docked complex generated eigenvalue of 3.004013e-04. The variance graph indicates the individual variance by red colored bars and cumulative variance by green colored bars (Fig. 12e). Fig. 12f illustrate the co-variance map of the complexes where the correlated motion between a pair of residues are indicated by red color, uncorrelated motion is indicated by white color and anti-correlated motion is indicated by blue color. The elastic map of the complex shows the connection between the atoms and darker gray regions indicate stiffer regions (Fig. 12g) [58, 59, 60].

4. Discussion
The aim of docking experiment is to determine the best possible pose of a ligand molecule within the constraint of binding pocket of a receptor and the calculation of a binding energy. The lower the binding energy (docking score), the higher the affinity of binding and vice versa. In the experiment, total 14 ligand molecules were selected to act against the AChE, that is responsible for AD development. Each of the 14 ligands were docked against the target receptor to evaluate their anti-AChE activity and from the experiment, three best ligands were selected for further analysis. The best possible ligand molecules were selected based on their binding energy, where the lower bind energy was preferred. The SP binding energies given by 1,8-cineol, berberine, carvacrol, cheilanthifoline, coptisine, estragole, harmaline, harmine, liriodenine, myrtenal, naringenin, protopine, scoulerine and stylopine were -4.844 Kcal/mol, -9.658 Kcal/mol, -6.060 Kcal/mol, -6.387 Kcal/mol, -10.148 Kcal/mol, -5.035 Kcal/mol, -8.053 Kcal/mol, -8.385 Kcal/mol, -7.754 Kcal/mol, -5.873 Kcal/mol, -9.266 Kcal/mol, -6.272 Kcal/mol, -6.229 Kcal/mol and -7.733 Kcal/mol, respectively (Table 02). Coptisine gave the lowest SP binding energy or docking score of -10.148 Kcal/mol, for this reason, coptisine should be the best molecule to inhibit AChE. Furthermore, coptisine also generated the lowest XP docking score or binding energy of -15.560 Kcal/mol Moreover, the second lowest and the third lowest SP and XP docking scores were given by berberine (-9.658 Kcal/mol and -13.571 Kcal/mol, respectively) and naringenin (-9.266 Kcal/mol and -9.342 Kcal/mol, respectively), respectively. For this reason, these three molecules was selected as the best three ligand molecules among the 14 ligands for further analysis. The MM-GBSA study was carried out for only the three best ligands that showed the best results in the docking study. In the MM-GBSA study, the most negative ΔG_{Bind} score (the lowest score) is considered as the best ΔG_{Bind} score [64]. IFD study is done to understand the accurate binding mode and to ensure the accuracy of active site geometry. XP G_{Score} is generated in the IFD
experiment, which is an empirical scoring function that estimates the ligand binding free energy. The lowest value of XP $G_{\text{Score}}$ is considered as the best value and is always appreciable [65, 66, 67, 68]. In the MM-GBSA study, coptisine generated the lowest $\Delta G_{\text{Bind}}$ score of -49.91 Kcal/mol and the highest $\Delta G_{\text{Bind}}$ score of showed by naringenin was -23.73 Kcal/mol. Moreover, coptisine also generated the lowest XP $G_{\text{Score}}$ value of -14.942 Kcal/mol and naringenin had the highest XP $G_{\text{Score}}$ value of -10.078 Kcal/mol. Furthermore, coptisine also generated the lowest IFD score of -1158.410 Kcal/mol. For this reason, based on the $\Delta G_{\text{Bind}}$ score, XP $G_{\text{Score}}$ value and IFD score, coptisine can be considered as the best ligand molecule among the selected three ligands. Further analysis showed that berberine interacted with 4 amino acids (Gln 69, Phe 330, Gly 123 and Trp 84), whereas, coptisine interacted with 6 amino acids (Trp 432, Trp 84, Phe 330, His 440, Ser 122 and Gly 117) and naringenin interacted with 5 amino acids (His 440, Phe 330, Tyr 70, Pro 86 and Trp 84). When the three best ligands were compared with the positive controls, it was observed that both donepezil and galantamine had SP soaking score and XP docking score that were much higher than the three best ligands. Moreover, both donepezil and galantamine generated XP $G_{\text{Score}}$ and IFD scores that were also much higher than the three best selected ligands and only naringenin had the $\Delta G_{\text{Bind}}$ score higher than the scores of the two controls. For this reason, it can be concluded that the three best selected ligand molecules showed superior performance in the molecular docking study compared with the two positive controls (Table 02, Table 03, Figure 07).

The aim of estimating the drug likeness properties facilitates the drug discovery and development processes. The molecular weight and topological polar surface area (TPSA) influence the drug permeability through the biological barrier. The higher the molecular weight and TPSA values, the lower the permeability of the drug molecule is and vice versa. Lipophilicity is expressed as the logarithm of partition coefficient of a drug molecule in organic and aqueous phase (LogP).
Lipophilicity affects the absorption of the drug molecules in the body and higher LogP corresponds to lower absorption and vice versa. LogS value influences the solubility of a drug molecule and the lowest value is always appreciable. The number of hydrogen bond donors and acceptors above the acceptable range also affects the capability of a drug molecule to cross the cell membrane. The number of rotatable bonds affects the druglikeness properties and the acceptable range is <10. Moreover, the Lipinski’s rule of five demonstrates that a successful drug molecule should have properties within the acceptable range of the five Lipinski’s rules: molecular weight: ≤500, number of hydrogen bond donors: ≤5, number of hydrogen bond acceptors: ≤10, lipophilicity (expressed as LogP): ≤5 and molar refractivity from 40 to 130 [63, 69, 70]. The druglikeness property experiment was conducted for the three best ligand molecules. Moreover, according to the Ghose filter, a candidate drug molecule should have LogP value of -0.4 to 5.6, molecular weight between 160 and 480, the total number of atoms 20 to 70, molar refractivity 40 to 130, to qualify as a successful drug [71]. Veber rule describes that the oral bioavailability of a possible drug molecule depends on two factors: the polar surface area which should be equal to or less than 140 Å² and 10 or fewer numbers of rotatable bonds [72]. Furthermore, according to the Egan rule, the absorption of a candidate drug molecule also depends on two factors: the Polar Surface Area (PSA) and AlogP98 (the logarithm of partition co-efficient between n-octanol and water) [73]. And according to the Muegge rule, for a drug like chemical compound to become a successful drug molecule, it has to pass a pharmacophore point filter, which was developed by the scientists [74]. Moreover, how easily a target compound can be synthesized is determined by the synthetic accessibility (SA) score. The score 1 represents very easy to synthesize, whereas, the score 10 represents very hard to synthesize [75]. The bioavailability score describes the permeability and bioavailability properties of a possible drug molecule [76].
Berberine, coptisine and naringenin had molecular weights of 336.36 g/mol, 320.32 g/mol and 272.25 g/mol, respectively. As the lower molecular weight is always appreciable, naringenin should be the best among the three ligands. However, the other two ligands also had good enough molecular weights to be permeable through the biological barrier. Both berberine and coptisine showed the TPSA value of 40.80 and naringenin had TPSA of 71.57. Since, the lower TPSA value always gives the good result, both berberine and coptisine performed better than naringenin. In the case of lipophilicity (expressed as LogP), the lower value is always required. Since naringenin had the lowest logP value among the three ligands (1.84), its performance was very good in the lipophilicity experiment. The other two ligands, berberine and coptisine, with their logP values of 2.53 and 2.40, respectively, also showed quite good performance in the study. Berberine and coptisine showed almost similar LogS values of -4.55 and -4.52, respectively. Naringenin showed logS value of -3.49. The number of rotatable bonds showed by berberine (2), coptisine (0) and naringenin (1) were well within the acceptable range. All the three ligands followed the Lipinski’s rule of five. Berberine, coptisine and naringenin have molecular weights of 336.36 g/mol, 320.32 g/mol and 272.25 g/mol, respectively, which are less than 500 g/mol of acceptable range. Only naringenin had 3 hydrogen bond donors and the other two ligands didn’t have any hydrogen bond donor and both berberine and coptisine has 4 hydrogen bond acceptors each and naringenin has 5 hydrogen bond acceptors, which followed the Lipinski’s rule. Furthermore, the logP values of berberine, coptisine and naringenin were 2.53, 2.40 and 1.84, respectively and the molar refractivity of berberine, coptisine and naringenin were 94.87, 87.95 and 71.57, respectively, which also followed the Lipinski’s rule. All the three ligands followed the Ghose filter, Veber, Egan and Muegge rules. All the ligands also showed the similar bioavailability score of 0.55. Since coptisine gave the lowest synthetic accessibility (SA) of 2.96, it can be synthesized very easily.
However, naringenin gave SA score of 3.01 and berberine gave SA score of 3.14, for this reason, naringenin should be easier to synthesize than berberine. Naringenin gave good druglikeness score of 1.9, drug score of 0.84, solubility score of -2.64 and no reproductive effectiveness, irritant, tumorigenic and mutagenic property. However, due to the unavailability of data in the OSIRIS Property Explorer tool, the druglikeness score, drug score, solubility score, reproductive effectiveness, irritant, tumorigenic and mutagenic properties of berberine and coptisine were not determined. Considering all the aspects of druglikeness property experiment, it can be concluded that, all the three best ligand molecules performed quite similarly in the druglikeness property experiment. When compared with the controls, it was observed that all the three ligands showed quite well performance in the druglikeness property experiment.

ADME/T tests are carried out to determine the pharmacological and pharmacodynamic properties of a candidate drug within a biological system. For this reason, it is a crucial determinant of the success of a drug research and development. Blood brain barrier (BBB) is the most important factor for the drugs that primarily target the brain cells. Moreover, since most of the drugs are administered through the oral route, it is required that the drug is highly absorbed in intestinal tissue. P-glycoprotein (p-gp) in the cell membrane aids in transporting many drugs inside the cell, for this reason, the inhibition of p-gp affects the drug transport. Caco-2 cell line is widely used in in vitro study of drug permeability tests. Its permeability decides that whether the drug will be easily absorbed in the intestine or not. Orally absorbed drugs travel through the blood circulation and deposit back to liver. In the liver, a group of enzymes of Cytochrome P450 family metabolize the drugs and excrete the metabolized drugs through bile or urine. Therefore, inhibition of any one of these enzymes affect the biodegradation of the drug molecule [77, 78]. Moreover, if a compound is found to be substrate for one or more isoforms of CYP450 enzymes, that compound is
metabolized by the respective CYP450 enzyme or enzymes [79]. The binding of drugs to plasma proteins is also a crucial pharmacological parameter that affects the pharmacodynamics, excretion and circulation of drugs. A drug’s proficiency is depended on the degree of its binding with the plasma protein. A drug can cross the cell layers or diffuse easily if it binds to the plasma proteins less efficiently and vice versa [80]. Human intestinal absorption (HIA) is a very important process for the orally administered drugs. It depicts the absorption of orally administered drugs from the intestine into the bloodstream [81, 82, 83]. Drug half-life describes the time it takes for the amount of a drug in the body to be reduced by half or 50%. The greater the half-life of a drug, the longer the drug would stay in the body and the greater its potentiality and vice versa. For this reason, half-life determines the doses of drugs [84, 85, 86]. HERG is a K+ channel found in the heart muscle which mediates the correct rhythm of the heart. HERG can be blocked by certain drugs. This may lead to the cardiac arrhythmia and death [87, 88]. Being the main site of metabolism, human liver is extremely vulnerable to the harmful effects of various drugs and xenobiotic agents. Human hepatotoxicity (H-HT) indicates any type of injury to the liver that may lead to organ failure and even death. Human hepatotoxicity, sometimes, is also responsible for the withdrawal of approved drugs from the market [89, 90]. Ames test is a mutagenicity test that is used to detect the potential mutagenic chemicals. The mutagenic chemicals can cause mutations and cancer [91]. Drug induced liver injury (DILI) is the injury to the liver that are caused by administration of drugs. DILI is one of the causes that causes the acute liver failure [92]. The results of ADME/T test are listed in Table 05.

In the absorption section, all the three ligands performed quite similarly. All the ligands showed optimal Caco-2 permeability and all of them were non-inhibitors of p-gp. For this reason, none of them inhibited the actions carried out by p-gp. However, only berberine was the p-gp substrate,
for this reason, berberine should be taken up by the cell more easily than the other two ligands. However, since only naringenin showed HIA capability, it should be absorbed well by the human intestine. In the absorption section, all the ligands performed quite similarly. In the distribution section, berberine and coptisine showed low plasma protein binding capability and naringenin showed good plasma protein binding capacity. However, all of them were capable of crossing the blood brain barrier. In the metabolism section, naringenin showed the weakest performance. Berberine was the inhibitor of CYP450 1A2, CYP450 3A4 and CYP450 2D6. For this reason, berberine inhibited the activities of CYP450 1A2, 3A4 and 2D6 isoenzymes. Moreover, the ligand was substrate for CYP450 1A2, CYP450 3A4, CYP450 2C19 and CYP450 2D6. Since berberine was substrate of these enzymes, these enzymes can metabolize the ligand very efficiently. Coptisine was inhibitor for CYP450 1A2 and CYP450 2D6. However, since it was the substrate of CYP450 1A2, CYP450 3A4, CYP450 2C19 and CYP450 2D6 enzymes like berberine, these enzymes can also metabolize coptisine efficiently. Naringenin was the inhibitor of CYP450 1A2 and CYP450 3A4. On the other hand, it was the substrate for only CYP450 1A2, for this reason, naringenin would be metabolized only by CYP450 1A2. In the metabolism section, berberine and coptisine showed quite good as well as almost similar results. However, naringenin showed unsatisfactory performance in the metabolism section. In the excretion section, berberine showed half-life of 1.9 hours, coptisine showed half-life of 1.8 hours and naringenin showed half-life of 0.9 hours. For this reason, it can be declared that, naringenin’s performance were not satisfactory in the excretion section. In the toxicity section, all the three ligands were hERG blockers, however, all of them proved to be safe in the Ames mutagenicity test. On the other hand, since all of them were DILI positive, they could cause liver injuries. Moreover, since coptisine and naringenin were not human hepatotoxic, they were proved to be safe for the liver, whereas, berberine was
hepatotoxic agent. Their performances were quite good when compared with the two controls used in the experiment. Furthermore, coptisine showed even better performances in some aspects of the experiment.

Comparing with the two controls, it can be declared that, the three best ligands performed well in the docking study, druglikeness property experiment and ADME/T test. All the three best ligands showed satisfactory results in these experiments. Moreover, coptisine generated even superior results than the controls in some aspects of the experiment.

Prediction of Activity Spectra for Substances or PASS prediction is carried out to estimate the possible biological activities associated with drug-like molecules. The PASS method estimates the probabilities based on the structures of the compounds and their molecular mass. Two parameters are used for the PASS prediction: Pa and Pi. The Pa is the probability of a compound “to be active” and Pi is the probability of a compound “to be inactive”. The values of both Pa and Pi can range from zero to one [45]. If the value of Pa is greater than 0.7, the probability of exhibiting the activity of a substance in an experiment is higher. On the other hand, if the Pa is greater than 0.5, less than 0.7, the probability of exhibiting a particular activity in an experiment is good, although less than the activity determined when Pa > 0.7 threshold is used. Moreover, if Pa is less than 0.5, the probability of exhibiting the activity is the least [93]. However, the chance of finding any given activity in an experiment increases with the increasing value of Pa as well as decreasing value of Pi [45]. The PASS prediction was carried out to determine 10 biological activities and 5 adverse and toxic effects of the three selected ligands. Since the intended activities were not generated by PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/) at Pa > 0.7, the PASS prediction were not done for berberine and coptisine. However, naringenin gave all the ten biological activities at Pa > 0.7. The PASS test showed the membrane integrity agonisting,
HMOX1 expression enhancing, chlordecone reductase inhibitory, HIF1A expression inhibitory, histidine kinase inhibitory, aldehyde oxidase inhibitory, antimutagenicity, mucomembranous protecting ability, TP53 expression enhancing ability and chemopreventive activities of naringenin. The 5 toxic effects shown by naringenin were vascular toxicity, inflammation, hematemesis, nephrotoxicity and shivering.

ProTox-II server measures the toxicity of a chemical compound and classifies the compound into a toxicity class ranging from 1 to 6. The server classifies the compound according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [48]. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Class 1: fatal if swallowed (LD50 ≤ 5), class 2: fatal if swallowed (5 < LD50 ≤ 50), class 3: toxic if swallowed (50 < LD50 ≤ 300), class 4: harmful if swallowed (300 < LD50 ≤ 2000), class 5: may be harmful if swallowed (2000 < LD50 ≤ 5000) [94]. However, ProTox-II server adds one more class to the 5 classes, making them 6 classes in total, class VI: non-toxic (LD50 > 5000) (http://tox.charite.de/protox_II/index.php?site=home) [48]. All the selected three ligands were in toxicity class 4, meaning that, they would be harmful, if swallowed.

The possible sites where the metabolism on a chemical structure may be carried out by the isoforms of CYP450 enzymes, are indicated by circles on the chemical structure of the molecule [47]. In the P450 SOM experiment, naringenin gave the best result since it gave 4 SOMs for all the CYP450 enzymes, except CYP450 2C9 (5 SOMs). However, berberine and coptisine gave the similar results because they showed 3 SOMs for all of the CYP450 enzymes. In the PASS prediction study, naringenin performed very well, however, since information about the other two ligands were not determined, any further comment on the performances shown by those two ligands could not be given.
The pharmacophore mapping study of the three best ligand molecules were carried out by an online server PharmMapper (http://www.lilab-ecust.cn/pharmmapper/). PharmMapper generates three types of scores: fit score, normalized fit score and z'-score. The target proteins with the highest fit scores and normalized fit scores between the query compound and its corresponding pharmacophore models reflect that the target proteins should be the potential targets for the query compound to bind. Moreover, z'-score is generated form the fit score and high z'-score corresponds to high significance of the target to a query compound and vice versa [95, 96, 97, 98].

The pharmacophore mapping experiment of berberine and naringenin gave almost similar fit scores of 2.765 and 2.637, respectively. Coptisine had the fit score of 2.273. However, berberine showed the highest normalized fit score of 0.928. Coptisine and naringenin had the normalized fit scores of 0.757 and 0.659. For this reason, with the highest fit score and normalized fit score, the target protein AChE should be the most potential target for berberine, among the three ligands. Moreover, since berberine also generated the highest z'-score of 1.088, the binding between berberine and AChE is the most significant among the three ligands. Naringenin had the z'-score of 0.749. However, with the lowest z'-score of coptisine (0.475), the binding between coptisine and AChE is less significant than the other two ligands. Berberine and coptisine had 1 hydrophobic centre each and naringenin had 3 hydrophobic centres. Moreover, both berberine and coptisine had 2 hydrogen bond acceptors each and naringenin didn’t generate any hydrogen bond acceptor. However, only naringenin generated 1 hydrogen bond donor. None of the ligands showed any positively charged centre, negatively charged centre and aromatic ring. In the pharmacophore modelling experiment, berberine showed the best result, however, the other two ligands also showed good results in the study (Figure 08 and Table 09).
The Phase pharmacophore perception engine is a tool of Maestro-Schrödinger Suite 2018-4 which is used for pharmacophore modelling, QSAR model development and screening of 3D database. The engine provides 6 types of built-in features and the pharmacophore modelling is mainly done based on these 6 types of features: hydrogen bond acceptor (A), hydrogen bond donor (D), negative ionizable (N), positive ionizable (P), hydrophobe (H), and aromatic ring (R). However, the number of features can be increased by customization. The pharmacophore modelling generates a hypothesis which can be used successfully in biological screening for further experiments [99].

All the three best ligand molecules successfully generated the pharmacophore modelling hypothesis with AChE. In generating the hypothesis, 6 features were selected for berberine, 4 features were selected for coptisine and 4 features were selected for naringenin. For this reason, berberine showed 6 point hypothesis and both coptisine and naringenin generated 4 point hypothesis. All the three best ligands showed quite good results in their pharmacophore modelling hypotheses. All the three ligands formed 2 hydrogen bonds within the binding pocket of the receptor. Berberine, coptisine and naringenin formed 4, 6 and 2 pi-pi stacked interactions, respectively. Furthermore, both berberine and coptisine generated 2 pi-cation bonds, whereas, naringenin didn’t. However, only naringenin didn’t have any bad contact with the pharamcophore. Since, all the ligands formed quite good hypotheses with the pharmacophore, all of the hypotheses can be used in screening effectively (Figure 09 and Fig. 10).

Frontier orbitals study or DFT calculation is one of the essential methods determining the pharmacological properties of various small molecules [100]. HOMO and LUMO help to study and understand the chemical reactivity and kinetic stability of small molecules. The term ‘HOMO’ describes the regions on a small molecule which donate electrons during a complex formation and the term ‘LUMO’ indicates the regions on a small molecule that receive electrons from the electron
The difference in HOMO and LUMO energy is called gap energy and gap energy corresponds to the electronic excitation energy. The compound that has the greater orbital gap energy, tends to be energetically unfavourable to undergo a chemical reaction and vice versa [53, 101, 102]. Moreover, gap energy also has correlation with the hardness and softness properties of a molecule [103]. The DFT calculations were carried out for all the three best ligand molecules. Naringenin showed the lowest gap score of 0.047 eV, for this reason, naringenin is energetically more favourable to undergo chemical reactions than the other two ligands. Moreover, the lowest gap scores also corresponds to lowest hardness score and the highest softness score of naringenin, 0.024 eV and 41.667 eV, respectively. However, berberine generated the highest dipole moment score of 8.050 debye and coptisine showed the dipole moment score of 2.277 debye (Table 10 and Figure 11).

Taking all the aspects into account, all the three best ligand molecules showed almost similar results in all the experiments, except the PASS prediction experiment and solubility tests. Due to the unavailability of data, the PASS prediction and solubility tests for berberine and coptisine could not be determined. However, naringenin showed quite good results in PASS prediction and solubility experiments. Comparing with the two positive controls, it can be concluded that, the best ligands performed very well in the experiments. Coptisine could be regarded as the best ligand molecule among the three selected ligands based on the docking studies (molecular docking, MM-GBSA and IFD studies) and many other aspects of the conducted experiment, although berberine and naringenin also showed quite satisfactory results. In some fields, coptisine generated far better results than the positive controls, that are already approved drugs for inhibiting AChE. From the molecular dynamics study of coptisine-acetylcholinesterase docked complex, it it clear that the complex had very low amount of deformability (Fig. 12b) as well as it had quite high eigenvalue.
of 3.004013e-03, for this reason, the deformability would be quite difficult for the complex (Fig. 12d). However, the variance map showed high degree of cumulative variances than individual variances (Fig. 12e). The co-variance and elastic network map also produced quite satisfactory results (Fig. 12f and Fig. 12g). The three selected ligand molecules can be used as potential agents to treat AD. Berberine showed potent AChE inhibitory activity when ethanolic extract from B. vulgaris were tested for anti-AChE activity. The ethanolic extract contained berberine and its amount was more than 60% in the extract [104]. Studies have also confirmed the anti-AChE activity of coptisine in a dose-dependent manner [105]. The anti-AChE activity of naringenin form C. junos was investigated where naringenin showed potent anti-dementia activity by inhibiting AChE [33]. In our study, coptisine emerged as the most potent anti-AChE agent. However, more researches should be done on the three best ligands to confirm their activities. Moreover, researches should be done on the other ligands, since most of them showed good docking results.

5. Conclusion

14 compounds that have AChE inhibiting activity, were selected for our study to determine the three best agents among them and their potential effects, safety and efficacy by conducting various experiments: molecular docking, druglikeness property experiments, ADME/T tests, PASS prediction studies and P450 site of metabolism prediction. From the experiment, three ligands: berberine, coptisine and naringenin were determined to be the best three ligands to inhibit AChE. All the three ligands showed quite similar and very good results in all the aspects into account. Berberine can be found in Berberis vulgaris, coptisine can be extracted from plants like Coptis chinensis, Berberis bealei and Phellodendron chinense and naringenin can found in Citrus junos. Since these plants contain anti-AChE agents, these plants can be used to treat the Alzheimer’s
disease, naturally, by targeting the AChE pathway. However, more laboratory research should be conducted on the other ligands as they also showed quite good results in the molecular docking study. Hopefully, this study will raise interest among the researchers.

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7. Compliance with Ethical Standards

7.1. Conflict of interest

Bishajit Sarkar declares that he has no conflict of interest. Md. Asad Ullah declares that he/she has no conflict of interest. Md. Nazmul Islam Prottoy declares that he/she has no conflict of interest.

7.2. Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.
8. References

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