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Original Article

IN SILICO SCREENING AND DFT ANALYSIS OF *NELUMBO NUCIFERA* PHYTOCHEMICALS AS POTENTIAL BACE-1 INHIBITORS FOR ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) stands as one of the primary neurological disorders affecting humans. This condition is marked by deterioration of central nervous system function. The enzyme ß-secretase (BACE-1) plays a crucial part in AD pathogenesis by initiating the destruction of the amyloid precursor protein (APP). Consequently, BACE-1 has become a significant focus for therapeutic interventions in AD. *Nelumbo nucifera* phytochemical structures have been virtually scrutinized for the expansion of potent and selective therapeutic compounds. In silico analysis of 24 different phytochemical structures from *Nelumbo nucifera* have been carried out against the BACE-1 to explore the anti-Alzheimer potential. Anonaine, asimilobine, dehydroanonaine, liriodenine, and roemerin were found to be prominently binding with BACE-1 and found to be more stable in DFT analysis, which indicated physicochemical exploration of the *Nelumbo nucifera* may result in potent and selective anti-Alzheimer agents.

KEYWORDS: Alzheimer; *Nelumbo nucifera*; ß-secretase; In Silico; Docking and DFT analysis.

1. Introduction

Alzheimer's disease (AD) is a state manifest by rational deterioration, including the loss of memory and other mental functions. AD is one of the leading diseases which is affecting the human population. It was observed that the number of people who are living with AD usually doubles after five years in people above 65 years of age. The treatment for AD is generally focused on the management of the symptoms, which has a limited rate of success. Development of molecules against AD is a tough job due to the complexity of the disease. Phytochemicals are a rich source of medicinal compounds; several pharmaceutical medicaments are generated on the phytochemical structures. In the current decade, scientists have turned their focus on the development of medicinal compounds based on phytochemical pharmacophores. Several biological targets have been identified in the current decade for the potent and selective anti-Alzheimer agents. BACE-1 is a significant factor in the etiology of AD as it brings the destruction process of the APP. BACE-1 has been recognized as an important target for Alzheimer's disorder. BACE-1 is one of the major beta-secretase that has a profound utility in the production of amyloid-ß peptides in neurons. Amyloid-ß peptides have been the important initiators in the progression of AD. Inhibition of the BACE-1 might be a useful strategy to combat the progressing AD. Development of the selective BACE-1 inhibitors has been recently explored for the identification of potent anti-Alzheimer compounds [1,2]. Phytochemical structures can be an attractive scaffold for the development of BACE-1 inhibitors [1,2]. The aquatic plant *Nelumbo nucifera* (Padma) belongs to the *Nelumbonaceae* family. *Nelumbo nucifera* have been explored for various biological potentials like antimicrobial, hypoglycemic activity, antiviral, skin aging, antioxidant, and antitumor. Anti-Alzheimer potency of the *Nelumbo nucifera* has been exhaustively analyzed by various researchers. UthaiwanSuttisansanee reported the utility of *Nelumbo nucifera* on the key enzymes that are

relevant to Alzheimer's disease [3]. Khan and co-workers reported the anti-Alzheimer effect of *Nelumbo nucifera* [4]. Mitra et al. reported the protective effect of *Nelumbo nucifera* on beta-amyloid protein-induced apoptosis [5].

To analyze the possible biological mode of action of the various phytoconstituents from *Nelumbo nucifera* here we report in silico and DFT analysis of exploration of the phytoconstituents from the *Nelumbo nucifera* against the BACE-1 to identify the possible phytochemical structures. Molecular docking and in silico ADME profiling of 24 different phytoconstituents from the *Nelumbo nucifera* have been carried out. Anonaine, asimilobine, and dehydroanonaine were found to be prominently binding with BACE-1 which indicated physicochemical exploration of the *Nelumbo nucifera* may result in potent and selective anti-Alzheimer agents [6-26].

2. Materials and Methods

2.1. Ligand preparation

The phytochemical structures from Padma were identified from the reported literature and downloaded from the PubChem database. BIOVIA Discovery Studio was employed to adjust the tautomeric and ionization states of the structures. Energy minimization of the modified phytochemical structures was done with the MMFF94 force field, utilizing the steepest descent method [21, 27-30].

2.2. Protein preparation

BACE1, a key beta secretase that plays a significant role in the production of amyloid-ß peptides in neurons so inhibition of the BACE1 might be a useful strategy to combat the progressing AD. The crystal structure of BACE1 bound to a 2-aminooxazoline-3-azaxanthene inhibitor 2 (PDB 5I3W), with a resolution of 2.15 Å, was obtained from the RCSB Protein Data Bank. The downloaded protein structure was refined via addition of polar hydrogen and removal of the water molecules. The refined protein structure was utilised for the docking analysis.

2.3. Molecular docking

Phytochemical structures were subjected to a docking study against the crystal structure of BACE1 in a complex with 2-aminooxazoline-3-azaxanthene inhibitor 2 (PDB 5I3W). The docking protocol was executed using the PyRx 0.8 program. Prepared protein and ligand structure were imported and selected in the PyRx 0.8 Grid box selected as X: 60.0429, Y: 62.7651, Z: 25.0000 coordinates. By default, the exhaustiveness was set to 8. Each compound's maximum negative binding affinity docked pose was stored in pdb format, and binding interactions were examined using BIOVIA Discovery Studio.

2.4. Insilico ADME Prediction

The promising lead from the docking analysis was further analyzed using Swiss ADME to explore the pharmacokinetic behaviour of the selected phytoconstituents. In-silico ADME Prediction was carried out using http://www.swissadme.ch/ [31].

2.5. DFT calculation

The structures of compounds anonaine, asimilobine, dehydroanonaine, liriodenine, and roemerin were downloaded from the PubChem database [32-46]. Subsequent energy minimization and optimization were done using established computational methods [33-35]. DFT calculations with the B3LYP functional and DEF2-SVP basis set were performed using ORCA 5.0.4 software [36-42] with ORCA-enhanced Avogadro facilitating input and output file generation [43]. FMO analysis and chemical reactivity descriptor calculations were conducted to examine the electronic properties, stability, and reactivity of the target compounds.

3. Results and Discussion

The potential of the chosen phytoconstituents from Padma to bind to BACE1, a key beta-secretase that plays a significant role in the production of amyloid-ß peptides in neurons. Amyloid-ß peptides have been the important initiators in the progression of AD. Inhibition of the BACE1 might be a useful strategy to combat the progressing AD. Docking analysis was utilized as initial scrutiny to identify the potential phytoconstituent candidates from Padma, which can be further explored for advanced remedies against AD. Molecular docking analysis was performed using PyRx 0.8 Crystal structure of BACE1 in complex with 2-aminooxazoline-3-azaxanthene inhibitor 2 (PDB 5I3W). A total of 24 phytoconstituents from Padma were identified and virtually analyzed against BACE-1. All phytoconstituents showed excellent binding affinity towards BACE1 ranging from -8 kcal/mol to -9.9 kcal/mol. Five phytochemical structures was found to be desirable pharmacokinetic property. Anonaine, which is one of the prominent alkaloids observed in Padma, showed binding affinity of -9.9 kcal/mol with BACE1 which indicated stronger binding potential. The key interactions like alkyl interaction with ILE118 and pi cation interaction with ASP32, pi-pi T shaped interaction with TYR71 as shown in Figure 1. Asimilobine is a aporphine alkaloid was found to be showing binding affinity of -9.3 kcal/mol and showed hydrogen bond interaction with TYR198, carbon hydrogen bond with ASN37, alkyl interaction with VAL69, TRP76 and pi cation interaction with ASP32, pi-pi T shaped interaction with TYR71 as shown in Fig. 1. Dehydroanonaine is also an alkaloid observed in Padma showed to be showing binding affinity of -9.6 kcal/mol and showed hydrogen bond interaction with TYR198, alkyl interaction with VAL69, ILE 118 and pi cation interaction with ASP32, pi-pi T shaped interaction with TYR71 while Liriodenine which another key phytochemical constituent of Padma was found to be showing binding affinity of -9.5 kcal/mol and showed Pi cation interaction with ASP32, Pi alkyl interaction with TYR71. Roemerin was found to be showing binding affinity of -9.6 kcal/ mol and showed Pi cation interaction with ASP32, Pi alkyl interaction with TYR71. Molecular interactions of the phytoconstituents from PADMA are shown in Table 1.

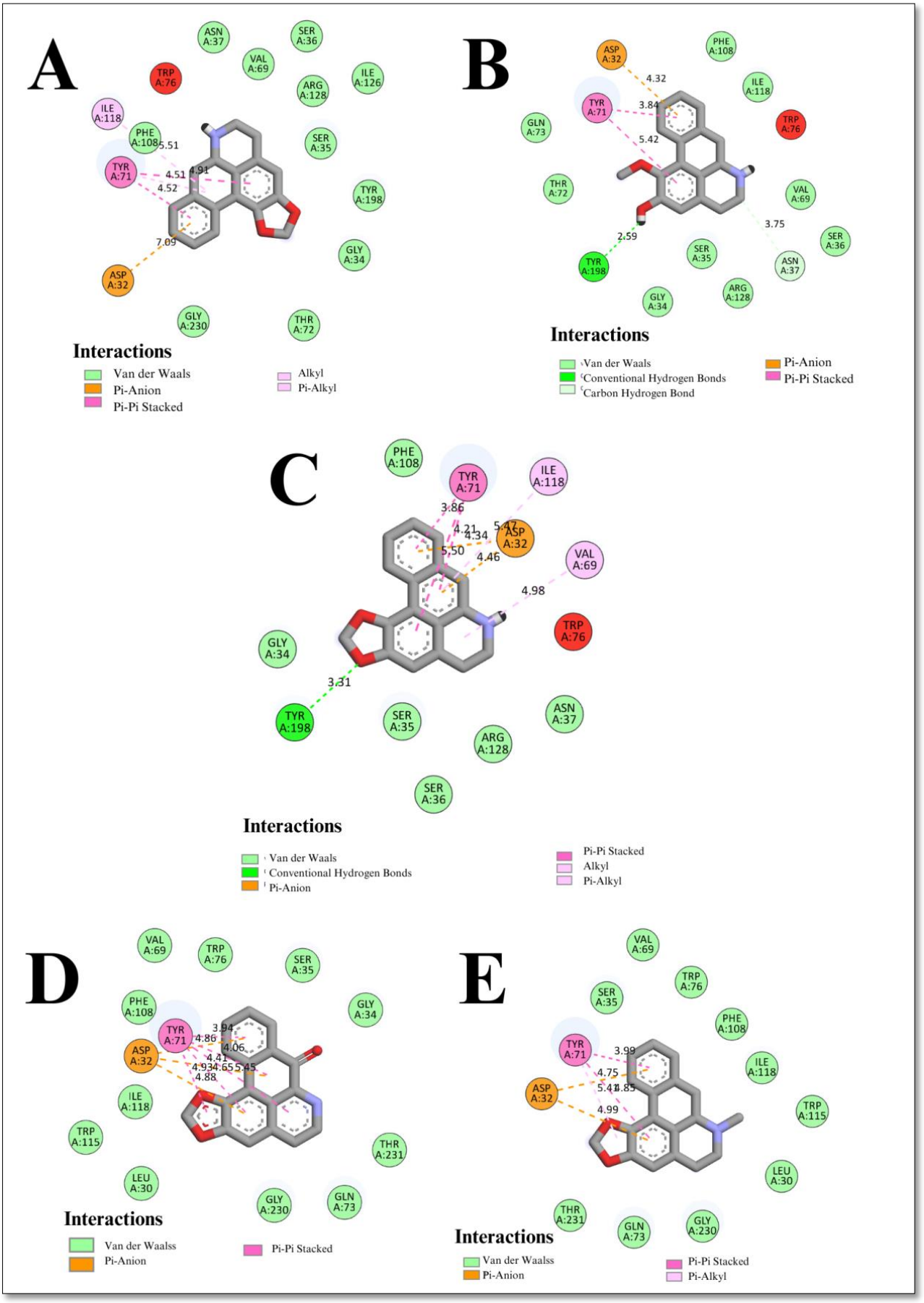

Fig. 1. Docking interaction of anonaine (A), asimilobine (B), dehydroanonaine (C), liriodenine (D), and roemerin (E).

Table 1. Molecular Interactions of the phytoconstituents from Padma.

Molecule Name	Interactions						Binding Affinity
	Conventional Hydrogen bond	Carbon hydrogen bond	pi cation	Alkyl	pi-pi T shaped	PI-Sigma	
Anonaine			ASP32	ILE118,	TYR71		-9.9
Armepavine	THR72	GLN73	ASP32	VAL69, TRP76	TYR71		-8
Asimilobine	TYR198	ASN37	ASP32		TYR71		-9.3
Dehydroanonaine	TYR198			ILE118, VAL69	TYR71		-9.6
Dehydronuciferine			ASP32		TYR71		-8.8
Demethylcoclaurine	GLY230, ILE126	SER35	ASP32	ILE118	TYR71		-8.4
Hyperin	GLY34, HR231, ASP228, HE108		ASP32	ILE118	TYR71		-9.3
Isoliensinine		GLN73, GLY230		ILE110, VAL69, ARG128, LEU30			-9.6
Kaempferol	LYS107, TYR71		ASP32	ILE118,	TRP115		-8.2
Liensinine	ARG235,SER325	PHE108, ASN37		ILE110, TRP115, TRP76, VAL69, ARG128, ILE118,		TYR71	-9.4
Lirinidine			ASP32	TRP76, VAL69			-8.9
Liriodenine			ASP32		TYR71		-9.5
Lotusine			ASP228, ASP32	ILE118	TYR71		-8.3
Luteolinglucoside	THR329, GLUY230	LYS107	ASP32		TYR71		-9.2
Neferine		THR231, GLY230		ARG128, VAL69, TRP76, ILE118, ILE110			-9.6
N-methylasimilobine	GLY230, THR231		ASP32	ILE118	TYR71		-9.1
N-methylcoclaurine	LYS107, TRP76,	ASP228, GLY230, ASP32		ARG128, VAL69, TRP76, ILE118, ILE110	TYR71		-8.5
N-methylisococlaurine	GLY230, THR72		ASP32	ILE118	TYR71		-8.6
N-norarmepavine	TRP76	GLN73	ASP32	ARG128, VAL69, ILE118	TYR71		-8.1
Nornuciferine		ASN37, ILE126	ASP32	ARG128, VAL69, ILE118	TYR71		-8.6
Nuciferine			ASP32		TYR71		-8.9
Pronuciferine		GLY11, LYS107				ILE110	-6.4
Roemerin			ASP32		TYR71		-9.6
Rutin	GLY34, ASP228, TYR71, THR231, LYS107, GLN73, LYS321, THR232, GLY230			ILE118			-8.9

3.1. In silico ADME Prediction

In silico ADME prediction was performed for all the 24 selected phytochemical structures, the seven phytochemicals showed excellent ADME properties for anti-Alzheimer potential. Anonaine, asimilobine, dehydroanonaine, liriodenine, N-methylasimilobine, roemerin was found to show acceptable ADME parameters. Major parameters like Molecular weight, Rotatable bonds, H-bond acceptors/ donors are all within the acceptable limits what makes them very good drug like candidates. BBB permeability of all above molecules is good thus makes them very good candidates for CNS potentials. The phytochemicals like anonaine, asimilobine, dehydroanonaine, liriodenine, N-methylasimilobine, roemerin can be further explored for development of anti-Alzheimer medications as shown in Table 2.

3.2. DFT calculation

Performed DFT study aimed to evaluate the energies of the frontier molecular orbitals (FMOs), namely the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which are key indicators of a molecule's reactivity. This method was applied to assess the electronic structure of the compounds. Estimating the FMO energies gained insights into their electronic properties. Furthermore, reactivity descriptors were calculated using Koopmans' theory

Table 2. Predicted ADME properties of the selected phytochemicals.

Molecule Name*	MW	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	GI absorption	BBB permeant	Pgp substrate
Anonaine	265.31	0	3	1	30.49	High	Yes	Yes
Asimilobine	267.32	1	3	2	41.49	High	Yes	Yes
Dehydroanonaine	263.29	0	2	1	30.49	High	Yes	Yes
Liriodenine	275.26	0	4	0	48.42	High	Yes	Yes
N-methylasimilobine	281.35	1	3	1	32.7	High	Yes	Yes
Roemerin	279.33	0	3	0	21.7	High	Yes	Yes

equations, which provided important information about the reactivity and stability of these compounds. The analysis of the FMOs (HOMO and LUMO) and chemical reactivity descriptors reveals significant data on the reactivity and stability of the title compounds. A smaller HOMO-LUMO energy gap indicates higher reactivity but lower kinetic stability, as seen with compound liriodenine, which has the smallest energy gap (3.512 eV) and is the most reactive. In contrast, compounds anonaine and asimilobine, with more significant energy gaps (4.512 eV and 4.685 eV, respectively), are more kinetically stable but less reactive. The reactivity order based on the HOMO-LUMO energy gap is liriodenine > dehydroanonaine > roemerin > anonaine > dehydroanonaine. Furthermore, chemical reactivity descriptors, including ionization potential (IP), electron affinity (EA), electronegativity (x), chemical potential (μ), chemical hardness (η), and electrophilicity (ω), provide a deeper understanding of the compounds' behavior. Compound liriodenine exhibits the highest IP, EA, and electrophilicity, indicating a strongtendency to engage in reactions despite its lower stability. In contrast, compounds anonaine and dehydroanonaine, with higher chemical hardness and lower electrophilicity, are more resistant to charge transfer, making them more stable but less reactive. These insights guide the potential applications of these compounds, with compound liriodenine suited for highly reactive environments and compounds anonaine and dehydroanonaine better for applications requiring more excellent stability. The derived values for the descriptors of chemical reactivity are shown in Fig. 2 and Table 3.

Table 3. Calculated FMO and global chemical reactivity descriptors for Selected Five Phytoconstituents.

Entry	HOMO (eV)	LUMO (eV)	HLG (eV)	DM (Debye)	IP (eV)	EA (eV)	x (eV)	μ (eV)	η (eV)	ω (eV)
1	-5.458	-0.946	4.512	1.070	5.458	0.946	3.202	-3.202	2.256	2.272
3	-5.621	-0.936	4.685	2.408	5.621	0.936	3.279	-3.279	2.343	2.294
4	-4.842	-0.959	3.883	2.077	4.842	0.959	2.901	-2.901	1.942	2.167
12	-6.155	-2.643	3.512	6.572	6.155	2.643	4.399	-4.399	1.756	5.510
23	-5.427	-0.995	4.432	1.012	5.427	0.995	3.211	-3.211	2.216	2.326

HOMO: highest occupied molecular orbital; LUMO: lowest unoccupied molecular orbital; HLG: HOMO-LUMO gap; DM: dipole moment; IP: ionization potential; EA: electron affinity; x: electronegativity; μ; chemical potential; η: chemical hardness; ω: electrophilicity.

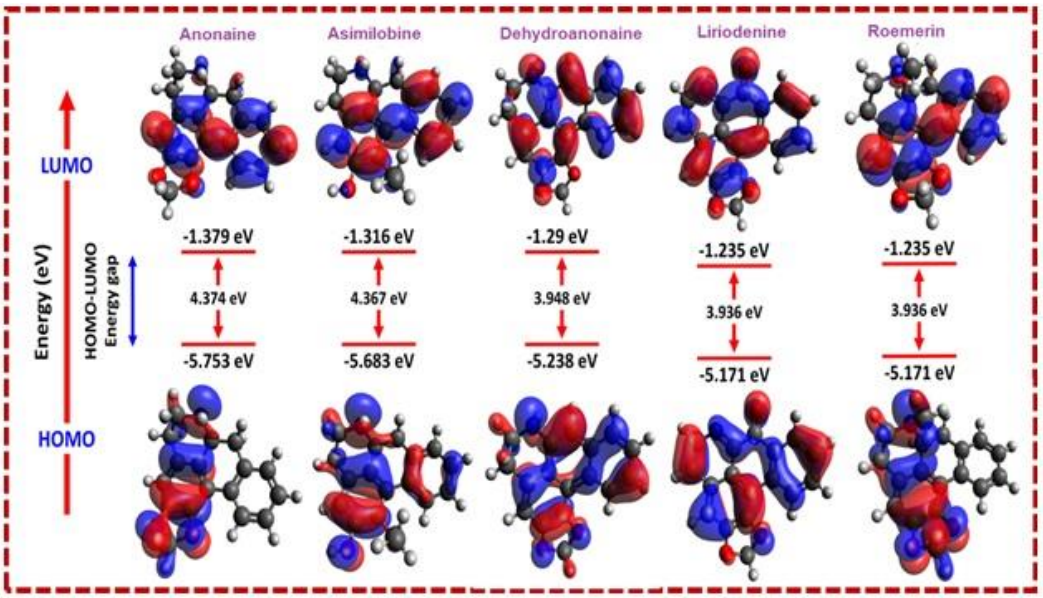

Fig. 2. HOMO-LUMO and energy gap (HLG) of selected five phytoconstituents.

4. Conclusions

BACE-1 is a significant factor in the etiology of AD as it brings the destruction process of the APP. BACE-1 has become a key target for Alzheimer's disease. To create a potent and specific therapeutic molecule, the phytochemical structures of *Nelumbo nucifera* have been virtually examined. In silico and DFT, analysis of 24 different phytochemical structures from *Nelumbo nucifera* was carried out against the BACE-1 to explore its anti-Alzheimer's potential. Anonaine, asimilobine, dehydroanonaine, liriodenine, N-methylasimilobine, and roemerin were found to be promising phytochemicals for the development of anti-Alzheimer's drugs.

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