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Assessing Inhibitory potential of natural compounds against BACE1 in Alzheimer's disease: A molecular docking and molecular dynamics simulation approach

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10% of people over 65 are affected by Alzheimer's disease (AD), worldwide health issue. It is believed that genetic, cellular, and multifactor pathophysiological pathways are responsible for the development of AD and other neurodegenerative diseases. BACE1, or Beta-site amyloid precursor protein cleaving enzyme 1, is an enzyme that plays a key role in the formation of amyloid-beta (Aβ) peptides in the brain. Aβ peptides are a hallmark characteristic of AD. The current work used highly developed computational biology technologies to find ligands that could bind to protein targets effectively in the context of AD. The aim of the current investigation was to determine the efficiency of a small chemical library against the BACE1. Our study enlightens the inhibitory role of phytocompounds against BACE1 through *in silico* approaches including molecular docking using Autodock Vina and molecular dynamics simulation. Thus in this study, we have elucidated the potential of best reported phytocompounds as potent inhibitors of Alzheimer's disease. Molecular docking was performed between 163 potent compounds to identify best potential inhibitor which could inhibit the BACE1. Out of 163 compounds Subtrifloralactone A and B, two natural chemicals, have demonstrated sufficient interactions with the active site residues depending on the AutoDock Vina binding affinity. These compounds' respective binding affinity were discovered to be -11.0 kcal/mol and -11.0 kcal/mol. Virtual screening of 1031 chemical compounds revealed that only two chemical compounds are having best interactions with BACE1 and might be the potential inhibitor(s) of the BACE1. Further, molecular dynamics simulation of these two chemical compounds revealed that subtrifloralactone A and subtrifloralactone B which belongs to the steroid group of compounds shows the stable activity with the BACE1.Additional experimental validation could confirm the inhibitory activity of the potential candidates.

Keywords: Alzheimer's disease, BACE1, *In silico* analysis, Molecular docking, Virtual screening

Dementia is the progressive loss of a person's cognitive abilities, such as their ability to reason, memorize, recall, solve problems, and think[1,2]. The critical global health issue of Alzheimer's disease (AD) has a huge negative societal and economic impact[1,2]. The key etiological signs of the disease are a gradual reduction in cognitive function and irreversible neuronal death[3]. AD is a long-term neurodegenerative disorder in which irreversible neuron loss causes progressive declines in cognition and memory formation. There is still much unknown about the precise process of AD. Etiopathogenesis, on the other hand, points to it as a complex, multi-factorial condition with a variety of aberrant cellular/molecular processes occurring in many brain regions and gradual neurodegeneration[4]. The majority of the amyloid precursor protein (APP) is cleaved by the amyloidogenic route, which involves the sequential cleavage of APP by β-secretase and γ-secretase, resulting in the development of amyloid plaques in the brains of people with Alzheimer's disease[5-7]. It is proposed that the formation of amyloid oligomers plays a significant role in the disease's genesis in addition to the down-regulation of insulin receptors, inhibition of the Wnt signaling pathway[8,9].

BACE1, or Beta-site amyloid precursor protein cleaving enzyme 1, is an enzyme that plays a key role in the formation of amyloid-beta (Aβ) peptides in the brain. Aβ peptides are a hallmark characteristic of Alzheimer's disease (AD). These peptides are produced when the amyloid precursor protein (APP)

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Abbreviations: AD, Alzheimer's disease; BACE1, Beta-site amyloid precursor protein cleaving enzyme 1; Aβ, Amyloid-beta; APP, Amyloid precursor protein; FDA, Food and Drug Administration; ADME, Absorption; Distribution, Metabolism, and Excretion; PDB, Protein data bank; SDF, Structure Data Files; NPACT, Naturally Occurring Plant-based Anti-cancer Compound-Activity-Target Database; PSVS, Protein Structure Validation Software suite; GROMACS, GROningenMAchine for Chemical Simulations; CASTp, Computed Atlas of Surface Topography of proteins; MD, Molecular dynamic; RMSD, Root mean square deviation

is cleaved by BACE1 and another enzyme called γ-secretase. The resulting Aβ peptides can then aggregate and form amyloid plaques, which are a central pathological feature of Alzheimer's disease. Given the critical role of BACE1 in the production of Aβ peptides, inhibiting the activity of BACE1 has been a target of interest for potential therapeutic interventions for Alzheimer's disease. Several BACE1 inhibitors have been developed and tested in preclinical and clinical studies. By inhibiting BACE1, it is hoped that the production of Aβ peptides will be reduced, which could potentially slow down or halt the progression of Alzheimer's disease. However, it is important to note that BACE1 has other substrates in addition to APP, and inhibiting its activity may have unintended consequences. For example, BACE1 has been implicated in the processing of other proteins involved in synaptic plasticity and neuronal function. Therefore, the development of BACE1 inhibitors as a treatment for Alzheimer's disease remains an area of active research and investigation.

Alzheimer's disease (AD) currently has no known treatments, and only a few number of medications and nutritional supplements are used to treat AD patients' symptoms[10]. Rivastigmine, donepezil, galantamine, and memantine are the main medications that the US-FDA has approved, while CerefolinNAC®, Axona®, and Souvenaid® are dietary supplements that promise to help AD sufferers with their symptoms[10]. Sadly, there is currently no medicine on the market that precisely targets the major AD-related chemicals (*i.e.*, those responsible for amyloid plaques and tangles). Therefore, it is crucial to identify potential active substances (natural or synthetic) with few adverse effects that can inhibit the β-secretase enzyme in charge of producing deadly amyloid plaques. Inhibiting BACE1 activity could potentially mitigate the progression of the disease by reducing the production of Aβ peptides. However, further research is needed to better understand the role of BACE1 in Alzheimer's disease and to develop safe and effective BACE1 inhibitors for clinical use.

The approach of molecular docking is a new strategy in drug designing, which allows the characterization of the behavior of small molecules in the binding site of target proteins along with the elucidation of fundamental biochemical processes[11,12]. Natural products are the most valuable sources of medications and drug leads due to their structural variety and biological activity. A previous study identified that flavonoid and terpenoid compounds exhibit the following properties[2,5,13]. It prevents Aβ aggregation and has anti-apoptotic, antioxidant, anti-inflammatory, and anti-inflammatory properties. Previous studies identified that alkaloid compounds had the following characteristics (ameliorating memory and recognition, anti-oxidant effects, neutralizing tau-induced neurotoxicity, increasing acetylcholine effects, and neurite outgrowth and dendritic development)[2,5,13]. Terpenoids are a diverse class of natural compounds found in plants and some microorganisms. Some terpenoids, such as ginkgolides, have been shown to inhibit BACE1 activity *in vitro*[14,15]. Steroids are a class of lipophilic compounds with a core structure consisting of four fused rings. Some steroids, such as cholesterol and dehydroepiandrosterone (DHEA), have been reported to modulate BACE1 activity and Aβ production *in vitro* and in animal models of AD[16,17]. Terpenoids, flavonoids, alkaloids, and steroids are diverse classes of natural compounds that have been reported to modulate BACE1 activity and Aβ production *in vitro* and in animal models of AD[16,17]. These compounds have diverse structures and mechanisms of action, making them potentially valuable as drug candidates for BACE1 inhibition and the treatment of AD.

In the present study, we have targeted the BACE1 protein of Alzheimer's disease using available molecular modelling based methods by molecular docking, virtual screening, and molecular dynamics simulations[18,19]. Screened molecules were checked for ADME properties as per Lipinski rule of five[20]. Top docking complexes were further evaluated using Molecular Dynamics Simulations on 100 ns.

Materials and Methods

Protein preparation

The BACE1 structure was downloaded from the PDB-REDO with the PDB ID: 6EJ2 (resolution: 1.46 Å) in PDB format[21,22]. The Autodock Tools, which are part of the MGL Tools molecular visualization interface, was used to create the protein structure.The 6EJ2 structure file was stripped of water molecules and heteroatoms (Fig. 1). Using AutoDock MGL Tools 1.5.6, Kollman charges were added to the 6EJ2 structure during docking[23].

Preparation of Ligands

A list of 1031 chemicals (terpenoids 513 compounds, flavonoids 329 compounds, alkaloids 110

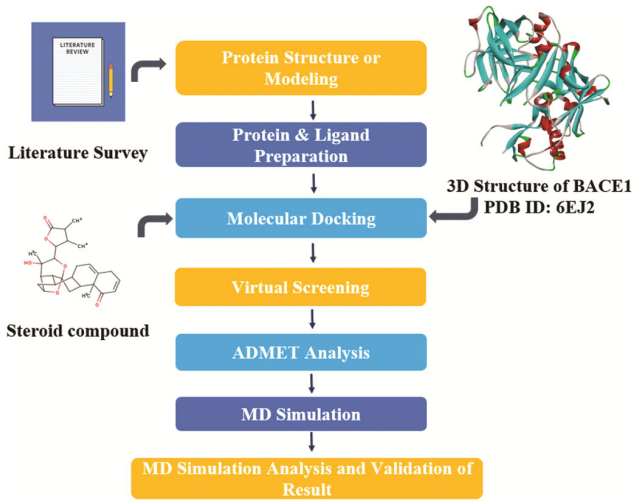

Fig. 1 — Graphical representation of the approach used in study

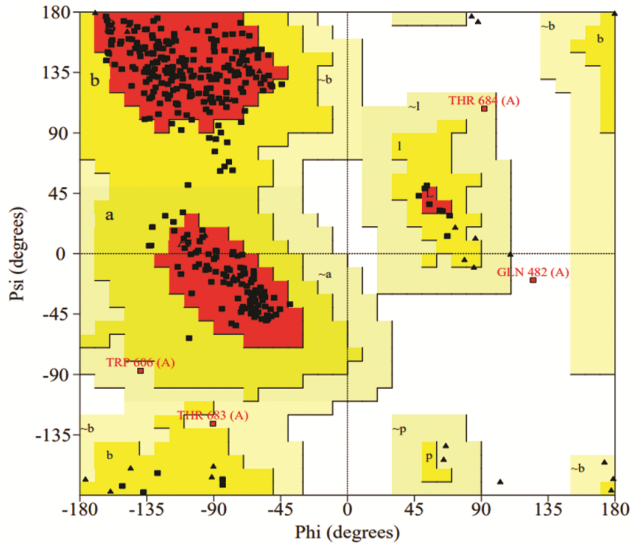

Fig. 2 — Ramachandran plots of 3D structure using SAVES and PSVS server

compounds, and steroids 79 compounds) was assembled for this study using the NPACT database. Out of 1031 compounds, 163 satisfy the requirements of the Ghose, Veber, and Muegge filters in addition to Lipinski's rule of five. The three-dimensional structures of all 163 naturally occurring chemicals (ligands) were retrieved in SDF format from the PubChem database and then translated into PDB format using PyMol[24-27].The AutoDock tools were used to convert each of the natural compounds (ligands) to PDBQT format.

Structure evaluation

By looking at residue-by-residue geometry, PROCHECK was used to assess the stereo-chemical characteristics of the produced 3D structure. By examining the psi (Ψ) and phi (Φ) torsion angles in the Ramachandran plot, it was feasible to determine the BACE1 enzyme's overall stereochemical quality and backbone conformation. The importance of the Ramachandran plot in analyzing protein structure has been well established and supported by prior research[28]. For theBACE1(PDB ID: 6EJ2) protein structure, the dihedral statistics of Ramachandran plot demonstrated a superb geometry combination. The ProsaII (-ve) 0.34 and total G-score of -0.36 indicated that the anticipated structure was suitable. The BACE1 protein structure was found to be of excellent quality by ProSA, and energy profiling revealed that the protein residues were positioned in the negative angles as depicted in (Fig. 2).

ProSA quality check showed the modelled protein to be of good quality, wherein the energy profiling showed the residues of the protein placed in the negative angles. A model is known to be of optimum quality if the 90% residues lie within the most favored regions. The Ramachandran plot created by Richardson's team for the current investigation revealed that 97.8, 1.4, and 0.8% of the residues were found in the most favored, allowed areas, and disallowed regions, respectively. In total, there were 87.8, 10.9, 0.9, and 0.3% residues in the most favored, furthermore allowed, and generously allowed categories, according to dihedral statistics of the Ramachandran plot predicted by PROCHECK, respectively, (Fig. 2). Utilizing VERIFY 3 D and MolProbity, the 3D structure of BACE1 was further confirmed utilizing the properties of the local environment. It was clear from the clash scores of 0.28 and 3.93, respectively, that the stereochemical quality of the BACE1 (PDB ID: 6EJ2)protein structure was good (Table 1)[29].

Virtual screening and molecular docking

Virtual screening is a computational method used in drug discovery to identify potential drug candidates from large compound libraries. It involves the use of computational algorithms to search through these libraries for molecules that are likely to bind to a specific target protein, such as BACE1, which is a key enzyme involved in the production of amyloid-beta peptides implicated in Alzheimer's disease. All 163 natural compounds were screened out against the BACE1(PDB ID: 6EJ2) structure using AutoDock Vina[30-32]. By locating active site residues and target structures, it is feasible to create powerful therapeutic drugs via ligand-protein docking. The active site was

Table 1 — Dihedral Statistics of 6EJ2 structure (BACE1) using different tools

Structure Quality Factors - overall statistics	Mean score	SD	Z-score g
Procheck G-factor e (phi / psi only)	-0.36	N/A	-1.10
Procheck G-factor e (all dihedral angles)	-0.16	N/A	-0.95
Verify3D	0.28	0.0000	-2.89
ProsaII (-ve)	0.34	0.0000	-1.28
MolProbityclashscore	3.93	0.0000	0.85
Ramachandran Plot Summary from Procheck			
Most favoured regions	87.8%		
Additionally allowed regions	10.9%		
Generously allowed regions	0.9%		
Disallowed regions	0.3%		
Ramachandran Plot Statistics from Richardson's lab			
Most favoured regions	97.8%		
Allowed regions	1.4%		
Disallowed regions	0.8%		

[f] Residues selected based on: dihedral angle order parameter, with S(phi)+S(psi)>=1.8

[g] With respect to mean and standard deviation for for a set of 252 X-ray structures < 500 residues, of resolution <= 1.80 Å, R-factor <= 0.25 and R-free <= 0.28; a positive value indicates a 'better' score.

Table 2 — Docking results of BACE1 (PDB ID: 6EJ2) with steroids compounds (Highlighted compounds show the best interaction with BACE1 based on AutoDock binding affinity)

S. No.	Compound Name	Binding Affinity(kcal/mol)
1.	17-alpha-H-digitoxigenin	-9.3
2.	17-alpha-H-periplogenin	-9.0
3.	Strophanthidin	-8.9
4.	Digoxigenin	-9.0
5.	Gitoxigenin	-9.0
6.	Guggulsterone	-9.4
7.	Z-Guggulsterone	-9.5
8.	Subtrifloralactone A	-11.0
9.	Subtrifloralactone B	-11.0
10.	Subtrifloralactone D	-10.7
11.	Subtrifloralactone E	-10.3
12.	Subtrifloralactone F	-10.2
13.	Subtrifloralactone G	-10.3

predicted once the target protein's three-dimensional structure was determined[33]. The conformations of the ligands were obtained and the complex with the lowest energy was considered. The compounds for chosen steroids, flavonoids, alkaloids, and terpenoids which are displayed in (Tables 2-5), respectively[34-36].

Table 3 — Docking results of BACE1 (PDB ID: 6EJ2) with flavonoids compounds

S. No.	Compound Name	Binding Affinity(kcal/mol)
1.	(-)-Epicatechin	-8.4
2.	Candenatenin A	-7.8
3.	4'-bromoflavone	-8.6
4.	Baicalein	-8.4
5.	Cajanol	-8.1
6.	Catechin	-7.8
7.	Curcumin	-8.6
8.	Daidzein	-8.1
9.	Dalrubone	-9.2
10.	Deguelin	-8.5
11.	4'-Hydroxy wogonin	-8.3
12.	Acacetin	-8.6
13.	Flavone	-8.4
14.	Galangin	-8.0
15.	Genistein	-8.4
16.	Hesperetin	-8.7
17.	Isobonducellin	-7.9
18.	Isocarthamidin	-8.2
19.	Isoliquiritigenin	-7.9
20.	Licochalcone A	-8.3
21.	Limonin	-10.4
22.	Liquiritigenin	-8.5
23.	Luteolin	-8.8
24.	Malvidin	-7.9
25.	Morin	-7.9
26.	Mucronulatol	-7.9
27.	alpha-Naphthoflavone	-9.4
28.	Naringenin	-8.6
29.	Pelargonidin	-8.0
30.	Candenatenin B	-7.6
31.	Petunidin	-7.9
32.	Pongavilleanine	-8.9
33.	Quercetin	-8.4
34.	Sativanone	-7.7
35.	Sinensetin	-8.0
36.	Tangeretin	-8.2
37.	Taxifolin	-8.1
38.	Tricin	-8.5
39.	Wogonin	-8.2
40.	Zapotin	-7.9
41.	Tephrosin	-8.4
42.	Artemetin	-7.9
43.	Hispidulin	-8.0
44.	Pseudobaptigenin	-8.2
45.	Sumatrol	-8.6
46.	Tectorigenin	-8.6
47.	Uvangoletin	-7.7
48.	Wighteone	-8.1
49.	Xenognosin A	-7.9
50.	7,4'-dihydroxyhomoisoflavanone	-8.4

Table 4 — Docking results of BACE1 (PDB ID: 6EJ2) with alkaloids compounds

S. No.	Compound Name	Binding Affinity(kcal/mol)
1.	Camptothecin	-9.1
2.	Cryptolepine	-8.4
3.	Harmine	-7.2
4.	Hydrastine	-9.1
5.	Isostrychnine	-9.1
6.	Marcanine A	-8.2
7.	Matrine	-8.4
8.	N-(4-hydroxyundecanoyl)anabasine	-7.2
9.	Neocryptolepine	-8.5
10.	N-n-octanoylnornicotine	-6.9
11.	Noscapine	-8.5
12.	Papaverine	-8.1
13.	Physostigmine	-8.0
14.	Reserpic acid	-8.6
15.	Rhyncophylline	-8.2
16.	Rohitukine	-7.8
17.	Strychnine	-9.8
18.	Vincamine	-8.6
19.	(-)-anonaine	-8.7
20.	(+)-tylophorinidine	-9.5
21.	(R)-antofine	-9.5
22.	(R)-cryptopleurine	-9.8
23.	(R)-tylophorine	-9.8
24.	(S)-tyloindicine I	-9.1
25.	9-methoxycanthin-6-one	-8.4
26.	Brucine	-9.6
27.	9-methoxycanthin-6-one 3N-oxide	-8.2
28.	Beta-carboline-1-propionic acid	-7.7
29.	Canthin-6-one	-8.6
30.	Capsaicin	-7.2
31.	Corydaline	-8.8
32.	Ellipticine	-8.7
33.	Evodiamine	-8.9
34.	Holamine	-9.8
35.	Isocephalotaxine	-8.4
36.	Liriodenine	-9.4
37.	Secoantofine	-9.1
38.	Tylocrebrine	-9.3
39.	Tyloindicine F	-8.7
40.	Tyloindicine G	-9.6
41.	Tylophoridicine C	-9.9
42.	Tylophoridicine F	-9.9
43.	6-O-desmethylantofine	-9.5
44.	13(R)-antofine-N-oxide	-9.8
45.	13(R)-14(R)-hydroxyantofine-N-oxide	-9.9
46.	7-methoxy-beta-carboline-1-propionic acid	-7.7
47.	4-methoxycarbonyl-5,10-benzogquinolinequinone	-7.8
48.	6-O-desmethylsecoantofine	-8.8
49.	Saprosmine A	-7.6
50.	Saprosmine B	-7.4

Table 5 — Docking results of BACE1 (PDB ID: 6EJ2) with terpenoids compounds

S. No.	Compound Name	Binding Affinity(kcal/mol)
1.	12-Deoxyphorbol 20-acetate 13-angelate	-8.9
2.	12-Hydroxychiloscyphone	-6.6
3.	1-beta,2-alpha-epoxytagitinin C	-8.3
4.	1-beta,6-alpha-dihydroxy-4(15)-eudesmene	-7.0
5.	20-hydroxy-12-deoxyphorbol angelate	-8.4
6.	20-hydroxyresiniferol 9,13,-14-orthophenylacetate	-9.1
7.	28-deoxonimbolide	-8.6
8.	2-alpha-hydroxytirotundin	-8.0
9.	3,6-Epidioxy-1,10-bisaboladiene	-7.2
10.	3-alpha-acetoxydiversifolol	-7.9
11.	3-beta-acetoxy-8-beta-isobutyryloxyreynosin	-7.9
12.	4-alpha,10-alpha-dihydroxy-3-oxo-8-beta-isobutyryloxyguaia-11(13)-en-12,6-alpha-olide	-7.9
13.	4-alpha,6-alpha-dihydroxyeudesman-8-beta,12-olide	-8.0
14.	7-epi-7-O-methylrosmanol	-8.5
15.	7-O-methylrosmanol	-8.6
16.	Ailanquassin A	-8.9
17.	Alpha-Humulene	-6.8
18.	Altaicalarins C	-7.8
19.	Beta-Caryophyllene	-7.0
20.	Bigelovin	-7.9
21.	Bisabolene	-6.9
22.	Bisabolol	-7.5
23.	Bisabolone	-7.3
24.	Bucidarasin D	-7.7
25.	Buddledin A	-7.7
26.	Buddledin B	-6.8
27.	Buddledin C	-7.1
28.	Carnosol	-9.0
29.	Cedronolactones B	-8.8
30.	Cedronolactones D	-9.5
31.	Citronellyl propionate	-6.3
32.	Clerodane diterpenoid	-8.9
33.	Cnicin	-8.0
34.	Costunolide	-7.5
35.	Crocetin	-7.2
36.	Curcusone A	-8.2
37.	Curcusone B	-8.4
38.	Curcusone C	-8.4
39.	Curcusone D	-8.2
40.	Dehydrocostuslactone	-7.7
41.	Ent-14-beta-Hydroxykaur-16-en-15-one	-1.1
42.	Ent-7-alpha,14-beta-dihydroxykaur-16-en-15-one	-9.5

(*contd.*)

Table 5 — Docking results of BACE1 (PDB ID: 6EJ2) with terpenoids compounds (*contd.*)

S. No.	Compound Name	Binding Affinity(kcal/mol)
44.	Ent-8,9-Seco-7-alpha,11-beta-dihydroxykaura-8(14),16-dien-9,15-dione	-8.4
45.	Ent-8,9-Seco-7-alpha-acetoxy-11-beta-hydroxykaura-8(14),16-dien-9,15-dione	-9.4
46.	Ent-8,9-Seco-7-alpha-acetoxykaura-8(14),16-dien-9,15-dione	-8.6
47.	Ent-8,9-Seco-7-alpha-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione	-8.0
48.	Ergolide	-7.9
49.	Escobarine A	-8.6
50.	Escobarine B	-8.5

Molecular dynamics (MD) simulations

In-depth analysis of the complex's protein-ligand interactions, stability, and conformational changes has been done using MD simulations. It can be done with a variety of tools. In this study, we investigated GROMACS, which makes use of the web server WEBGRO (accessed on 12 October 2022, 22 October 2022 and 3 November 2022)[37-38].GROMOS is a general-purpose molecular dynamics computer simulation package for the study of biomolecular systems. GROMACS supports the GROMOS force fields, with parameters provided in the distribution for 43a1. To perform the MD simulations, GROMOS96 43a1, SPC, Triclinic were taken as the force field, water model and box type, respectively. Sodium and chloride ions were added to neutralize the protein charge, followed by further additions of ions to mimic a salt solution concentration of 0.15 M.

Here, the PDB used (6EJ2) was constructed in accordance with the website's instructions, and the molecules' topology was used the PRODRG webserver to construct (accessed on 12 October 2022). According to the information that is currently available, the PRODRG server received the data that was extracted from the docked molecules and used it to create the zip file. To screen the molecules (Subtrifloralactone A and Subtrifloralactone B), the MD simulations were initially run at 300 K. It provides the trajectory: root mean square deviation (RMSD). These are the main markers that help us see the structural characteristics in relation to the movement trajectories. To compute these factors, several modules from the GROMACS package were utilized. These trajectories can be used to discuss the inhibition of BACE1 by various potent compounds (Subtrifloralactone A and Subtrifloralactone B).

Results and Discussion

Molecular docking

A list of 1031 chemicals (terpenoids 513 compounds, flavonoids 329 compounds, alkaloids 110 compounds, and steroids 79 compounds) was assembled for this study using the NPACT database[18]. Out of 1031 compounds, 163 satisfy the requirements of the Ghose, Veber, and Muegge filters in addition to Lipinski's rule of five[41]. For BACE 1 enzyme, The CASTp analysis reflected that Lys418, Ser419, Gly420, Gln421, Gly422, Tyr423, Leu439, Asp441, Gly443, Ser444, Ser445, Asn446, Ala448, Val478, Tyr480, Thr481, Gln482, Trp485, Lys516, Phe517, Ile519, Trp524, Ile527, Ile535, Arg537, Leu563, Cys564, Gly565, Ala566, Ala577, Val579, Tyr607, Lys633, Ile635, Asp637, Ser638, Gly639, Thr640, Thr641, Asn642, Arg644, Trp686, Gln712, Gln713, Leu715, Arg716, Pro717, Glu719, Asp720, Tyr729, Lys730, Ser734, Thr738, Val741, Ala744, Glu748, His769, and Val770 were the essential residues of amino acids that supported enzymatic action[33].

Among these 163 compounds, top 2 compounds were subtrifloralactone A and subtrifloralactone B. Subtrifloralactone A and B, two natural chemicals, have demonstrated sufficient interactions with the active site residues depending on the AutoDock Vina binding affinity. These compounds' respective binding affinity were discovered to be -11.0 kcal/mol and -11.0 kcal/mol. Figure 3A andB demonstrate docked ligand molecules with the 6EJ2 structure.

The binding affinity of Subtrifloralactone A against the BACE1 structure protein was found to be -11.0 kcal/mol.The residues involved in the protein-ligand interaction were GLY443, SER444, SER445, ASN446, VAL478, TYR480, THR481, GLN482, TRP485, LYS516, PHE517, TRP524, ILE527, ILE535, ALA536, ARG537, TYR607, and GLY639.There were two conventional hydrogen bond interaction with the residues SER445, ARG537 as shown in (Fig. 3A). It showed π-alkyl interaction with residues VAL478, TYR480, TRP485, and TYR607 of BACE1 protein.The binding affinity of Subtrifloralactone B against the BACE1 structure protein was found to be -11.0 kcal/mol.The residues involved in the protein-ligand interaction were GLY443, SER444, SER445, ASN446, VAL478,

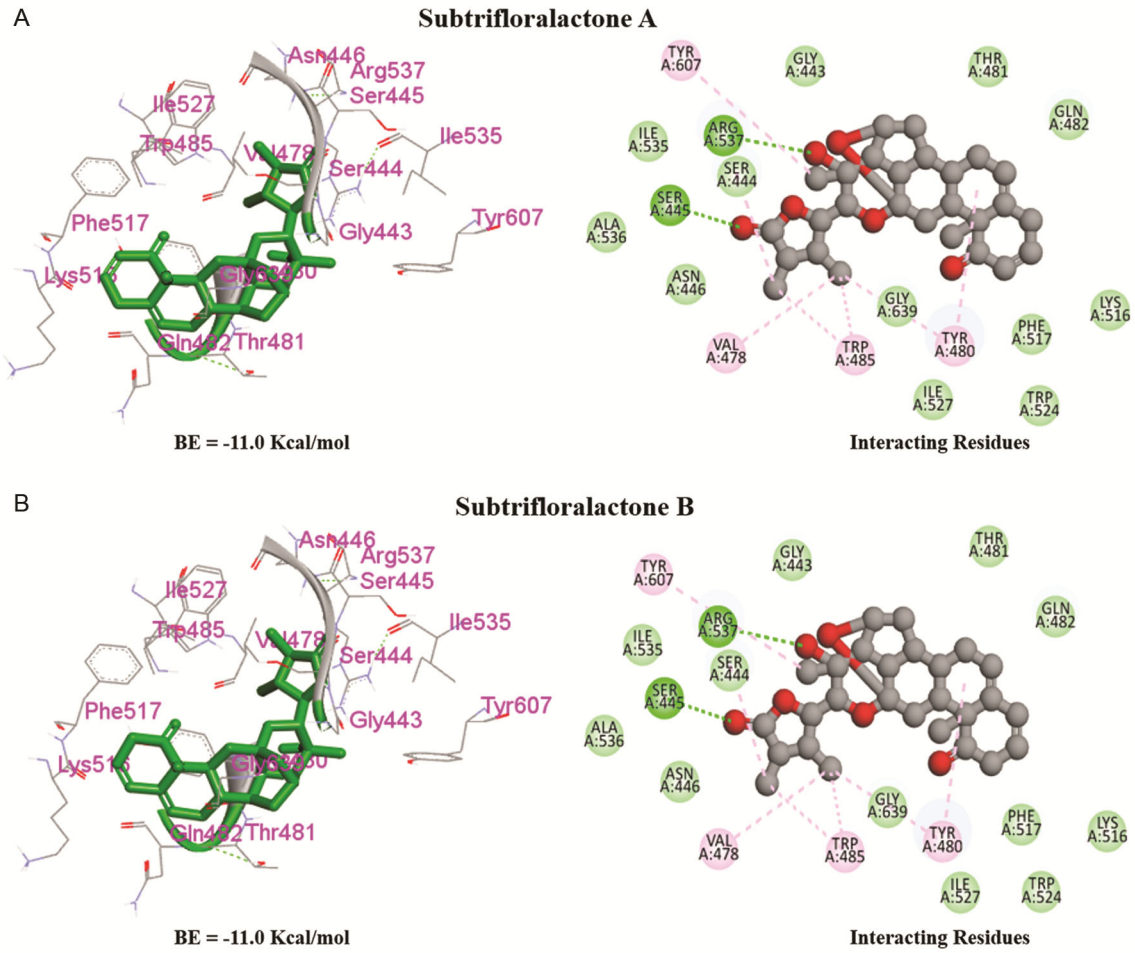

Fig. 3 — (A) 2-D interaction of Subtrifloralactone A; (B) 2-D interaction of Subtrifloralactone B with BACE1

TYR480, THR481, GLN482, TRP485, LYS516, PHE517, TRP524, ILE527, ILE535, ALA536, ARG537, TYR607, and GLY639.There were two conventional hydrogen bond interaction with the residues SER445, ARG537 as shown in (Fig. 3B). It showed π-alkyl interaction with residues VAL478, TYR480, TRP485, and TYR607 of BACE1 protein. As a result, this research study provides significant contributions to the field of targeted therapy development for Alzheimer's disease, emphasizing the importance of investigating natural compounds.

Molecular Dynamic (MD) Simulation

One of the most well-known *in silico* techniques for producing dynamic data with atomic spatial precision and picosecond or finer temporal resolution is MD simulation[42].Using a 100ns simulation duration, it was possible to examine the stability of docked phytochemicals such as subtrifloralactone A and subtrifloralactone B in the binding domain of BACE1.

Root-mean-square deviation (RMSD)

The conformational shift that ligands and proteins experience upon binding was measured using the RMSD. RMSD plot of the ligand subtrifloralactone Abound to the protein BACE1 showed stable and consistent RMSD (0.23 nm to 0.30 nm) compared to subtrifloralactone B (Fig. 4).Between 4 ns and 45 ns, subtrifloralactone A RMSD remained stable at a range of 0.20 nm to 0.30 nm, but after 45 ns, it increased and stayed at 0.35 nm. The smaller the RMSD value, the smaller the fluctuation of the complex structure. The chemical compound subtrifloralactone B has displayed oscillations on a 100 ns timeline at two different intervals, indicating that it might have altered the BACE1 binding region's conformation. Between 4 ns and 30 ns, the first stable conformation is found, while the second is between 35 ns and 75 ns. The RMSD remained steady at 0.25 nm, but there was a considerable swing between 75 ns and 90 ns, with RMSD>0.25 nm. There was no influence on protein

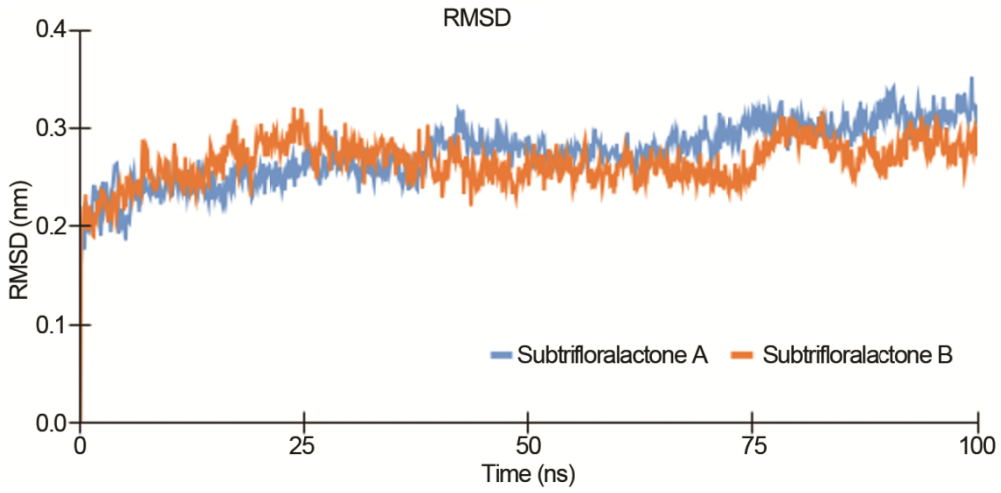

Fig. 4 — Root mean square deviation of the docked complexes of BACE1 with Subtrifloralactone A (blue) and Subtrifloralactone B (orange)

structure due to the significant variation in chemical (Fig. 4). Overall, subtrifloralactone A and subtrifloralactone B strongly binding with BACE1 as observed by the multiple interaction, strong binding conformation at 100 ns, and the high binding affinity compared to other natural compounds. Additionally, throughout the simulation, Subtrifloralactone A and Subtrifloralactone B demonstrated stable conformation. These two top compounds (Subtrifloralactone A and Subtrifloralactone B) have diverse structures and mechanisms of action, making them potentially valuable as drug candidates for BACE1 inhibition and the treatment of Alzheimer's disease (AD). As a result, this research study provides significant contributions to the field of targeted therapy development for Alzheimer's disease, emphasizing the importance of investigating natural compounds.

Conclusion

In summary, our present study attempted to explore the potential of effective natural compounds from steroids, flavonoids, alkaloids, and terpenoids compounds against the BACE1 (PDB ID: 6EJ2) structure. We have selected 163 effective compounds and out of 163 compounds, 2 compounds found as the most potent inhibitors against BACE1 target protein 6EJ2. Throughout the simulation run, the docked complexes showed constant interactions, according to an examination of 100 ns of MD simulations. During post-simulation analysis, the molecule subtrifloralactone A and subtrifloralactone B was simulated for 100 ns using molecular dynamics, and the results showed that the RMSD was most stable and consistent. The outcomes of the molecular docking and MD simulations analyses show that subtrifloralactone A and subtrifloralactone B can be further utilized as appropriate inhibitors against Alzheimer's disease, which is essential for reducing the β-secretase enzyme involved in the genesis of toxic amyloid plaques. These two compounds (Subtrifloralactone A and Subtrifloralactone B) will be used as a scaffold for future modifications. Our results from molecular docking exhibit that subtrifloralactone A and subtrifloralactone B showed the best binding efficacy against the BACE1 (PDB ID: 6EJ2) structure, which can further encourage us to examine its potential in pre-clinical and clinical studies. Additional experimental validation could confirm the inhibitory activity of the potential candidates.

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Conflicts of interest

Both the authors declare no conflict of interest.

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