



Identification of potential inhibitors of cholinergic and β -secretase enzymes from phytochemicals derived from *Gongronema latifolium* Benth leaf: an integrated computational analysis

Gideon Ampoma Gyebi^{1,2} · Oludare M. Ogunyemi³ · Ibrahim M. Ibrahim⁴ · Olalekan B. Ogunro⁵ · Saheed O. Afolabi⁶ · Rotimi J. Ojo⁷ · Gabriel O. Anyanwu¹ · Gaber El-Saber Batiha⁸ · Joseph O. Adebayo⁹

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Abstract

Neurodegenerative disorders (NDDs) are associated with increased activities of the brain acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and β -secretase enzyme (BACE1). Inhibition of these enzymes affords therapeutic option for managing NDDs such as Alzheimer's disease (AD) and Parkinson's disease (PD). Although, *Gongronema latifolium* Benth (GL) has been widely documented in ethnopharmacological and scientific reports for the management of NDDs, there is paucity of information on its underlying mechanism and neurotherapeutic constituents. Herein, 152 previously reported *Gongronema latifolium* derived-phytochemicals (GLDP) were screened against *hAChE*, *hBChE* and *hBACE-1* using molecular docking, molecular dynamics (MD) simulations, free energy of binding calculations and cluster analysis. The result of the computational analysis identified silymarin, alpha-amyrin and teraxeron with the highest binding energies (-12.3, -11.2, -10.5 Kcal/mol) for *hAChE*, *hBChE* and *hBACE-1* respectively as compared with those of the reference inhibitors (-12.3, -9.8 and -9.4 for donepezil, propidium and aminoquinoline compound respectively). These best docked phytochemicals were found to be orientated in the hydrophobic gorge where they interacted with the choline-binding pocket in the A-site and P-site of the cholinesterase and subsites S1, S3, S3' and flip (67–75) residues of the pocket of the BACE-1. The best docked phytochemicals complexed with the target proteins were stable in a 100 ns molecular dynamic simulation. The interactions with the catalytic residues were preserved during the simulation as observed from the MMGBSA decomposition and cluster analyses. The presence of these phytocompounds most notably silymarin, which demonstrated dual high binding tendencies to both cholinesterases, were identified as potential neurotherapeutics subject to further investigation.

Keywords Neurodegenerative disorders · Acetylcholinesterase · Butyrylcholinesterase · Molecular docking · Molecular dynamic simulation · Silymarin

✉ Gideon Ampoma Gyebi
gideonagyebi@gmail.com

¹ Department of Biochemistry, Faculty of Science and Technology, P.M.B 005, Karu, Nasarawa State, Nigeria

² Natural Products and Structural (Bio-Chem)-informatics Research Laboratory (NpsBC-RI), Bingham University, Nasarawa, Nigeria

³ Nutritional and Industrial Biochemistry Unit, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

⁴ Department of Biophysics, Faculty of Sciences, Cairo University, Giza, Egypt

⁵ Department of Biological Sciences, KolaDaisi University, Ibadan, Nigeria

⁶ Faculty of Basic Medical Sciences, Department of Pharmacology and Therapeutics, University of Ilorin, Ilorin, Nigeria

⁷ Department of Biochemistry, Faculty of Computing and Applied Sciences, Baze University, Abuja, Nigeria

⁸ Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira 22511, Egypt

⁹ Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria

Introduction

Neurodegenerative disorders (NDDs) are a cluster of diseases characterized by gradual damage and loss of susceptible group of neurons predominantly those associated with the central nervous system [1]. Alzheimer's disease (AD), Parkinson's disease (PD) and other PD-related disorders such as Spinal muscular atrophy (SMA), Spinocerebellar ataxia (SCA), Huntington's disease (HD), Motor neuron diseases (MND), Prion disease etc belongs to this cluster of diseases and have been reported to affect millions of people worldwide [2]; and thereby considered as a major cause of about 1 billion death and disability globally [3]. From a global perspective, the number of people diagnosed of AD and PD alone has risen over 5.4 and 10 million respectively, further indicating that, NDDs may continue as one of the foremost causes of death [1, 4]. A recent report on dementia indicated that, the number of people living with this condition would rise from the 57.4 million cases globally in 2019 to (130.8–175.9) million cases in 2050 [5]. Currently, there is no ideal therapy for NDDs as patients continue to experience impaired mental functions (dementias) and movement (ataxias) through the progressive degeneration and/or death of nerve cells [6]. The multiple etiological factors associated with NDDs including aggregation of misfolded proteins, decline in the brain neurotransmitters, inflammation, oxidative stress, mitochondrial dysfunction, apoptosis, and other genetic factors are promising therapeutic targets in these conditions [7]. In particular, cholinergic enzymes which may cause decline in important neurotransmitters such as neuroactive amines acetylcholine, butyrylcholine, are associated with the prevalence of neurological disorders; and thereby dubbed important drug targets [8]. Therefore, compounds that can bind strongly to both acetyl choline esterase (AChE) and butyryl choline esterase (BChE) with inhibitory activities are potential agents in the line for drug discovery and design in the fight against neurodegenerative disorders which underscores the importance of both cholinesterase enzymes in clinical of research for the treatment of neurological disorders [9, 10]. Strategies that involve the inhibition of these enzyme will increase the concentration of butyrylcholine and acetylcholine in the brain and by this means improve neural communications in the brain. Using this approach, some cholinesterase inhibitors have been developed as therapeutics for managing NDDs [11]. The β -secretase enzyme (BACE1) is another emerging drug target in NDDs. The β -amyloid (A β) fibrils are the main component of amyloid plaques in brain of AD patients and probably play a key role in the pathogenesis of neurodegenerative disorders [12–14]. The BACE1, which is a transmembrane aspartyl protease, saddled with the major function of cleaving the transmembrane amyloid

protein with the resultant production of the β -stubs, has been indicated to be involved in the rate-limiting catalytic step that eventually produces A β [15]. Hence, inhibitors of the BACE1 enzymes may also afford therapeutic option for the treatment of AD.

Recently, bioactive molecules from natural products are being screened as cholinesterase inhibitors using different models for the management of cognitive degeneration and neurological disorders. The search for inhibitors of cholinesterase from medicinal plants has been on the increase because the usual orthodox drugs are inefficacious, expensive, non-selective with poor bioavailability and discouraging cholinergic side effects (such as hepatotoxicity, short half-life, moderate to low effectiveness nausea, gastrointestinal disorders, dizziness, diarrhea, and vomiting) in the periphery [14]. *Gongronema latifolium* Benth, a food plant, have folkloric claims of being able to manage neurological disorders [16]. It is an edible green-leafy tropical plant with yellow flower belonging to the Asclepiadaceae family [17]. It is common in Nigeria and most of the West African countries. In Nigeria, it is often referred to as *Arokeke* by the Yorubas; *Utazi* by the Igbos; *Utasi* by the Ibibios, Quas and Efiks. *Akan-Asante aborode* is the local name in Ghana while the Senegalese call it *Sever gasule*. *G. latifolium* is common for both its nutritional and medicinal values. Different parts for the plants have been used in folk medicine for treating different types of medical condition health benefits of different parts of this plant cannot be over emphasized especially in traditional medicine. Several pharmacological activities attributed to bioactive compounds of the leaf extract of *G. latifolium* includes: antihyperglycemic/hypoglycemic/hypolipidemic/antidiabetic [18–28], nephroprotective [29, 30] hepatoprotective [30, 31], antioxidant [32, 33], anticancer [34, 35], antimalaria [36, 37], neuroprotection [38], antihaematopoietic [39] anti-inflammatory [39] antiulcer [40] immunomodulatory [41], antibacterial [42], antimicrobial [43], tissue regeneration [29] and learning and memory enhancer [44]. Although, the polypharmacology potential of the plant in the prevention and management of NDDs has been reported by previous studies that include computational screening [45, 46], there is still dearth of scientific information on the major constituent of leaf of *G. latifolium* that is responsible for the management of neurodegenerative diseases. Thus, the present study is aimed at computationally screening previously reported bioactive compounds from *Gongronema latifolium* for their inhibitory potential against acetylcholinesterase, butyrylcholinesterase, and beta-secretase 1, (BACE1) enzymes.

Methods

Preparation of proteins

The 3D crystallographic structures of *human* acetylcholinesterase (hAChE) complexed with donepezil (PDBID: 4EY7), *human* butyrylcholinesterase (hBChE) complexed with propidium (PDBID: 6ESJ) and *human* β -secretase (BACE-1) in complex with aminoquinoline compound 1 (PDBID: 5I3V) were accessed from the <http://www.rcsb.org> (Protein Data Bank). The co-crystallized ligand and water molecules accompanying the respective proteins were deleted, while the hydrogen atoms that were missing added using MGL-AutoDock Tools (ADT, v1.5.6). The Kollam charges were added as the partial atomic charge [47]. All proteins were subjected to the same procedure.

Ligands preparation

The Structure Data Format of one hundred and fifty two (152) phytochemicals that were previously identified in various extracts from *Gongronema latifolium* through gas chromatographic flame ionization analysis [48] as well as the co-crystallized and/or reference inhibitors (Donepezil and propidium) were assessed from www.pubchem.ncbi.nlm.nih.gov a PubChem database. Structures that were not available on the database were drawn using ChemDraw 19.0. The SDF format of the ligands converted to the mol2 format in Openbabel [49]. The AutoDock function tools as implemented in PyRx 0.8 was used to convert the ligands to PDBQT format [50].

Validation of docking protocol

The reference compounds which are the co-crystallized with the proteins were retrieved from the respective crystal structures and saved in PDB format using BIOVIA Discovery Studio Visualizer 20.1.0, San Diego, CA, USA 2020. The retrieved ligands were docked into the reference active site of their respective proteins. The docked pose having the least minimal energy was selected and superimposed on the extracted native ligand using BIOVIA Discovery Studio Visualizer 20.1.0. Further analyses were performed using the same software.

Targeted Molecular Docking

After validating the protocol to be used for the docking studies, the *Gongronema latifolium* derived phytochemicals (152) (GLDP) and reference inhibitors (donepezil and propidium) were docked in a targeted manner into the active site of (hAChE) and (hBChE) using an incorporated AutoDock

Vina function in PyRx 0.8 [50]. The ligands (phytochemicals and reference compounds) were imported via the OpenBabel section of PyRx 0.8 and minimized with OpenBabel [51]. The energy minimization parameter that was employed was Universal Force Field (UFF), while the optimization algorithms employed was the conjugate gradient descent. The catalytic pockets of the respective enzymes were mapped by selecting interacting residues with the native ligands in the binding pockets of the enzymes. The mapped-out region was defined by a grid box size and centre (Table S1) and employed for the docking studies. The default exhaustiveness of 8 was used.

Molecular dynamics

The apo proteins (4EY7, 6ESJ and 5I3V), and the best docked phytochemical in their complexed form with the respective enzymes were further investigated for the stability of the bound complexes using Molecular Dynamic (MD) simulation for a period of 100 ns on GROMACS 2019.2 employing the GROMOS96 43a1 forcefield implemented on WebGRO [52–54]. The topology files for the ligands were gotten from <http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg> (PRODRG webserver) [55]. The protein and the protein complexed with the ligands were solvated in a cubic box with a four-point (TIP4P) water model. A 0.154 M (physiological concentration) was set for neutralized NaCl ions to which the periodic boundary conditions employed. A 10,000 steps minimization for each of the systems was executed using the steepest descent algorithm for 0.3 nanoseconds in constant number of atoms, volume, and temperature ensemble (NVT) ensemble. An equilibration for 0.3 nanoseconds in a constant no. of atoms, pressure and temperature (NPT) was further applied. Pressure and temperature were set to 1 atm and 310 K; and were maintained throughout the period of the simulation using Parrinello-Rahman barostat, and velocity rescale respectively. Also, with a time step of 2 femtoseconds the Leap-frog integrator was employed. The 100 ns simulation run was completed for each of the systems with a total of 1000 frames gotten from 0.1 nano snapshot that was saved. The obtained MD trajectories were evaluated using the scripts on TK console in VMD [56]. The thermodynamic parameters including number of H-bond, SASA, RMSD, RoG and RMSF were then analysed.

Binding free energy calculation using MM-GBSA

For further analysis of the interactions of the best docked phytochemicals and the reference compound with respective protein targets, free energy of binding using Molecular Mechanics Generalized Born Surface Area (MM-GBSA) computation was implemented. The `gmx_MMPBSA`

package was used to compute the free energy of binding using MM-GBSA algorithm for each complex systems. The binding energies were decomposed to get the of amino acids within 10 Å around the ligand [57, 58]. The concentration of salt and solvation (igb) were set to 0.154 M and 5 respectively, while the internal and external dielectric constant were set to 1.0 and 78.5 respectively. The MM-GBSA method is depicted in Eq. 1

$$\Delta G = G_{\text{complex}} - G_{\text{receptor}} - G_{\text{ligand}} \quad (1)$$

Different energy terms were calculated according to Eq. 2 to 6>

$$\Delta G_{\text{binding}} = \Delta H - T\Delta S \quad (2)$$

$$\Delta H = \Delta E_{\text{gas}} + \Delta E_{\text{solv}} \quad (3)$$

$$\Delta E_{\text{gas}} = \Delta E_{\text{ele}} + \Delta E_{\text{vdW}} \quad (4)$$

$$\Delta E_{\text{solv}} = E_{\text{GB}} + E_{\text{SA}} \quad (5)$$

$$E_{\text{SA}} = \gamma \cdot \text{SASA} \quad (6)$$

Where:

ΔH is the calculated enthalpy from solvation-free energy (E_{sol}) and gas-phase energy (E_{gas}).

The $T\Delta S$ which represent entropic contribution to the free binding energy was not computed in this study.

E_{gas} comprises of van der Waals (E_{vdW}) and electrostatic (E_{ele}) terms.

E_{sol} was computed from the polar solvation energy (E_{GB}) while (E_{SA}) nonpolar solvation energy was assessed from the accessible solvent surface area [59, 60].

Clustering of molecular dynamic trajectory

After a 100 ns atomistic MD simulation analysis, the MD trajectories for the protein-ligand systems were subjected to cluster analysis using TTClust V 4.9.0 that employed the the elbow method. After the conformation clustering, conformers that serve as representative structures were chosen. Protein-Ligand Interaction profiler (PLIP) [61] was used for the interaction analysis. Unlike the downloaded crystalized structures with gaps in the numbering of amino acid residues, During the cluster analysis the amino acid residues were automatically re-numbered so that the numbering starts from number 1 until the last number of the protein the sequence.

Results and discussion

Validation of docking protocol

Before the commencement of docking experimentation, the validation step is a very important step that is used to predict the precision, reliability and accuracy of the docking protocol [62]. In this study, the generated docked poses of the reference compounds having the least energetic conformation were superimposed on the native ligand that was co-crystallized with the protein so as to validate the protocol to be used for the molecular docking investigation of the GLDPs (152). After the superimposition, the root mean square deviation (RMSD) was computed. The RMSD for the for donepezil, propidium and aminoquinoline compound 1 were 0.5761, 0.6005 and 0.000 Å respectively. The low RMSD shows that the docking protocol was suitable for the docking of phytochemicals (Figure S1).

Molecular docking study

Molecular docking technique has developed over the time into an important tool that is used in the discovery of drugs [63]. This techniques has been used to identify novel compounds of therapeutic interest from large data libraries and to model or predict the interaction between small molecules and protein; and thereby enables characterization of how small molecules will behave at the binding site of target protein [64]. The results of binding energies of the GLDP (152) and reference compounds (donepezil, propidium and aminoquinoline (compound 1) against *hAChE*, *hBChE* and *hBACE-1* are presented in the supplementary data (Table S2). Ten hit phytochemicals were selected on the bases of low binding energy, orientation in binding site and interaction with catalytic residues. The binding energy ranges for the ten hit phytochemicals are: -11.2 to -12.3, -10.6 to -11.2 and -9.4 to -10.4 Kcal/mol for *hAChE*, *hBChE* and *hBACE-1* respectively. The results showed that donepezil had binding energies of -12.3 and -11.2 Kcal/mol for *hAChE* and *hBChE* respectively, while and propidium had -10.2 and -9.8 Kcal/mol for *hAChE* and *hBChE* respectively. Aminoquinoline (compound 1) had a binding energy of -9.8 Kcal/mol. It was further observed that silymarin was the only phytochemical that exhibited dual high docking tendencies to both the *hAChE* and *hBChE*. It is also worthy of note that, although all the top ten phytochemicals had comparable scores with the reference inhibitor, silymarin and sakuranin presented the same binding energy as donepezil to *hAChE* while all the selected 10 had binding energy higher than the propidium in their interaction to *hBChE* (Table 1). The structures of the top docked phytocompounds is presented in Fig. 1.

Table 1 Binding energies of the reference compounds (S1-S3) and 10 hit phytochemicals and that were docked to the active sites of *human* Acetylcholinesterase, *human* butyrylcholinesterase and *human* β -secretase

	Ligands	Binding energy (Kcal/mol) <i>hAChE</i>	Ligands	Binding energy (Kcal/mol) <i>hBChE</i>	Binding energy (Kcal/mol) <i>hBACE-1</i>
S1	Donepezil (E = 306.68)	-12.3	Donepezil (E = 306.68)	-10.2	
S2	Propidium (E = 668.97)	-	Propidium (E = 668.97)	-9.8	
S3		-		-	Aminoquinoline comp. 1 (E = 291.99) -9.4
1	silybin A uff (E = 444.88)	-12.3	Alpha amyrrin (E = 681.59)	-11.2	Taraxeron (E = 1187.93) -10.5
2	Sakuranin uff (E = 435.11)	-12.3	silybin A (E = 444.88)	-11.1	Solagenin (E = 816.94) -10.4
3	Epigallocatechin-3-gallate (E = 325.39)	-11.9	Paclitaxel_ (E = 2435.27)	-11.1	Naringin (E = 615.77) -10.4
4	Epigallocatechin (E = 325.39)	-11.9	Camptothecin (E = 403.24)	-11	Rutin_uff (751.29) -10.3
5	Ellipicine (E = 463.99)	-11.5	Taraxeron (E = 1187.93)	-11	Isoquercetin (E = 609.42) -10.1
6	Dehydroabietic acid (E = 439.63)	-11.5	Diosgenin (E = 743.33)	-10.6	Hispigenin (E = 770.07) -10.1
7	Naringin (E = 615.77)	-11.2	Rutin (E = 751.29)	-10.6	Alpha_ amyrrin (E = 681.59) -10
8	Epieudesmin (E = 680.46)	-11.1	Hecogenin (E = 835.92)	-10.6	Diosgenin (E = 743.33) -10
9	5-avenasterol (E = 590.17)	-11	Yanogenin (E = 727.49)	-10.6	Neochlorogenin (E = 739.41) -10
10	Chicoric acid (E = 272.31)	-10.9	Lupeol (E = 928.20)	-10.6	Baicalin (E = 439.91) -9.9

S: reference compound; *hAChE*: *human* Acetylcholinesterase; *hBChE* *human* butyrylcholinesterase and *hBACE1*: *human* β -secretase (values in brackets are the energy in KJ/mol after minimization step). The two best docked compounds are in boldface

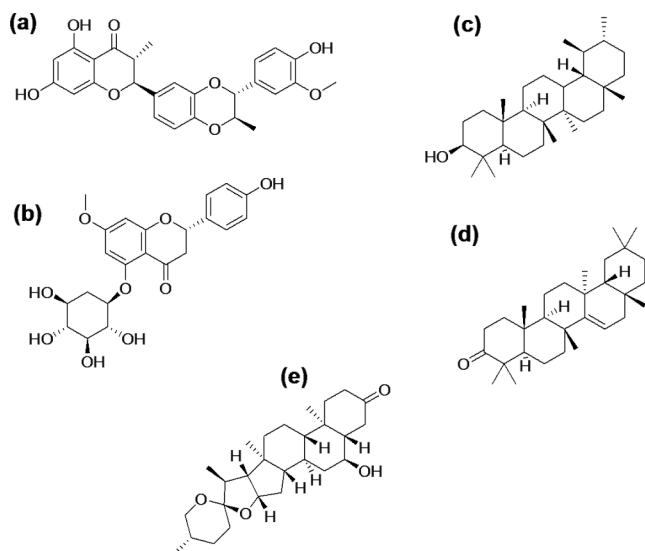


Fig. 1 Chemical Structure of top docked compounds (a) silybin A (CID: 31,553) (b) Sakuranin (CID 73,607) (c) α -amyrrin (CID 73,170) (d) Taraxerone (CID 92,785) (e) Solagenin (CID 155,860)

Interactions of selected phytochemicals with the amino acid in the active site of *human* acetylcholinesterase, *human* butyrylcholinesterase and *human* β -secretase

Although molecular docking is now majorly employed in

the virtual screening of compounds from small and large databases, it was initially developed to provide understanding of the mechanisms of molecular interaction between large and small molecules [64], the knowledge of such interactions can be used to predict the catalytic mechanism of a known or unknown ligand especially those that are involved in the inhibition or activation of a particular protein [65]. In this study, the best docked phytochemicals (silymarin and sakuranin for *hAChE*, α -amyrrin and silymarin for *hBChE*, taraxerone and Solagenin for *hBACE-1*), and reference compounds from the docking studies were selected for interaction analysis. Table 2 shows the various interactions in the active groove of the enzymes. The interactions between the respective groups of the ligand and the amino acids of the active site of donepezil co-crystallized *hAChE* revealed that donepezil was docked into the gorge active site (Fig. 2M). The revalidated docked conformation revealed that donepezil was stretched in the long, narrow, hydrophobic gorge of *hAChE* in similar fashion as the native ligand [66]. Only one hydrogen bond was observed between the carbonyl oxygen of the indenone ring and Phe295. The piperidine ring of donepezil formed 2 Pi-Alkyl contacts with Tyr337 and Tyr341 of *hAChE*. The 1-benzyl unit of donepezil interacted through an aromatic Pi-Pi stacking to Trp86 and His447. Two Pi-sigma bonds were observed between the 5-methoxy unit of inden-1-one, the piperidine ring and

Table 2 Amino acid interactions of *human* acetylcholinesterase and *human* butyrylcholinesterase with the two best docked GLDPs and reference inhibitors

Compounds	Protein	Hydrogen bonds Bond distance (Å)		Hydrophobic Interaction	
		Numbers	Interacted amino acid residues	Numbers	Interacted amino acid residues
Donepezil		1	Phe295 (2.88)	2	His447 Trp86 Trp286 Tyr341 Trp286 Phe338 Tyr337
Sakuranin	<i>hAChE</i>	5	Gly122 (2.81) Ser203 (2.77) Gly120 (3.00) Gly121 (3.40) Trp86 (3.35)	3	Phe338 Tyr341 Trp286
Silymarin		4	Tyr133 (2.86) Gly126 (3.47, 3.47) Glu202 (2.98) Val294 (3.16) Phe294 (2.56) Ser293 (3.08)	3	Trp86 Tyr337 Phe338 Trp286 Tyr341
Propidium		2	Ser ¹⁹⁸ (2.81) Trp ²³¹ (3.03)	7	Tyr332 Phe329(2) Leu 286 Trp231 Trp82 Glu197
Amyrin	<i>hBChE</i>			3	Trp82 Ala277 Try332 Pro285 Phe329 Ala328
Silymarin		2	Asn289 (2.16) Ser287 (2.67) Tyr128 Glu197 (2.34) Trp82 His483	6	Trp82 His483
Compound 1	BACE-1	5	Asp228(2) Asp32(2) Gly34	8	Tyr71(5) Phe108 Val69(2)
Teraxeron				10	Val69 Phe108 Ile110 Tyr71(5) Tyr198 Ile126
Solagenin		2	Ser35 Gly230	12	Leu30 Val69 Tyr71(3) Trp76 Trp115 Ile126 Tyr198 Ile118(2) Phe108(2)

hAChE: *human* Acetylcholinesterase; *hBChE* *human* butyrylcholinesterase and *hBACE-1*: *human* β -secretase

Trp286 and Phe338 respectively. (Fig. 2a). Silymarin, the best docked phytochemical to *hAChE* was orientated in the active site of gorge. The 4-hydroxy-3-methoxyphenyl unit formed 2 hydrogen bonds between its hydroxyl substituent to Tyr133 and Glu202, the methoxyl unit received a hydrogen atom from Gly126. The 3-methoxyphenyl unit made aromatic Pi-Pi stacking contact with Trp86. The phenyl unit of benzodioxine ring made a Pi-Pi T-shaped and a Pi-Pi stacking with Try337 and Phe338 respectively, while the phenyl unit of chromen-4-one ring made 2 Pi-Pi stacking hydrophobic contacts with Trp286 and Tyr341 (Fig. 2b). Sakuranin was docked into the active site of *hAChE* in a pattern similar to the binding of donepezil. The 4-hydroxyl unit of the oxan-2-yl ring formed hydrogen bonds with Gly120 while the 6-hydroxymethyl interacted with Gly121 and Tyr86 of *hAChE*. The oxygen atom of the 7-methoxyl unit of the chromen-4-on ring received 2 hydrogen atoms from Ser203 and Gly122 to form 2 hydrogen bonds. The phenyl ring of the chromen-4-on moiety made a Pi-Pi aromatic stacking while the 4-hydroxyphenyl unit interacted in a Pi-Pi T-shaped stacking with Tyr341 and Trp286 (Fig. 2c).

It can be observed that the best docked phytocompounds were found to interact with the catalytic triad consisting of Ser203, His447, and Glu334 [67] and other aromatic residues that project into the catalytic gorge of *hAChE*. The aromatic moieties of these phytochemicals interacted via pronounced

π - π stacking to the aromatic amino acids especially of peripheral anionic site (P-site) residues that is situated at the rim of the narrow gorge which offers both allosteric and substrate binding site [68]. The interaction with catalytic residues was stretched to anionic choline-binding site comprising residues Phe331, Trp86, and Tyr334. Among these residues, the aromatic residues, mainly Try337 and Trp86 are very essential in the inhibitory mechanism of the human AChE [69, 70]. The mode of interaction with the amino acid residues of P-site has been reported as a major factor that differentiate between the inhibitory mechanism of AChE and BChE enzymes [67]. From the catalytic mechanism of AChE enzyme, the P-site residues play important role in the orientation and for sliding down of the substrate on their way to the A-site where it is properly aligned with the catalytic serine. When the substrate is correctly aligned for catalysis, the acetyl part of the substrate is tightly accommodated by the acyl-binding residues (Trp236, Phe295 and Phe297) [71].

In the case of *hBChE*, propidium was docked deep into the active site gorge aligning in the same pattern as the native ligand. The phenanthridinium ring was accommodated in the groove of the acyl-binding pocket (Fig. 3M). The 6-phenyl moiety linked to the phenanthridinium ring also made a Pi-Pi T shaped contact with Tyr332 a P-Site residue. The first and second rings made Pi-sigma and Pi-Pi

Fig. 2 Interactions of reference compound and best docked GLDPs with the amino acid residues of the active site of *hAChE*. The ligands presented in stick conformation are shown by different colours (a) Green: propidium (reference inhibitor) (b) blue: sakuranin (c) purple: silymarin, while (S) surface representation

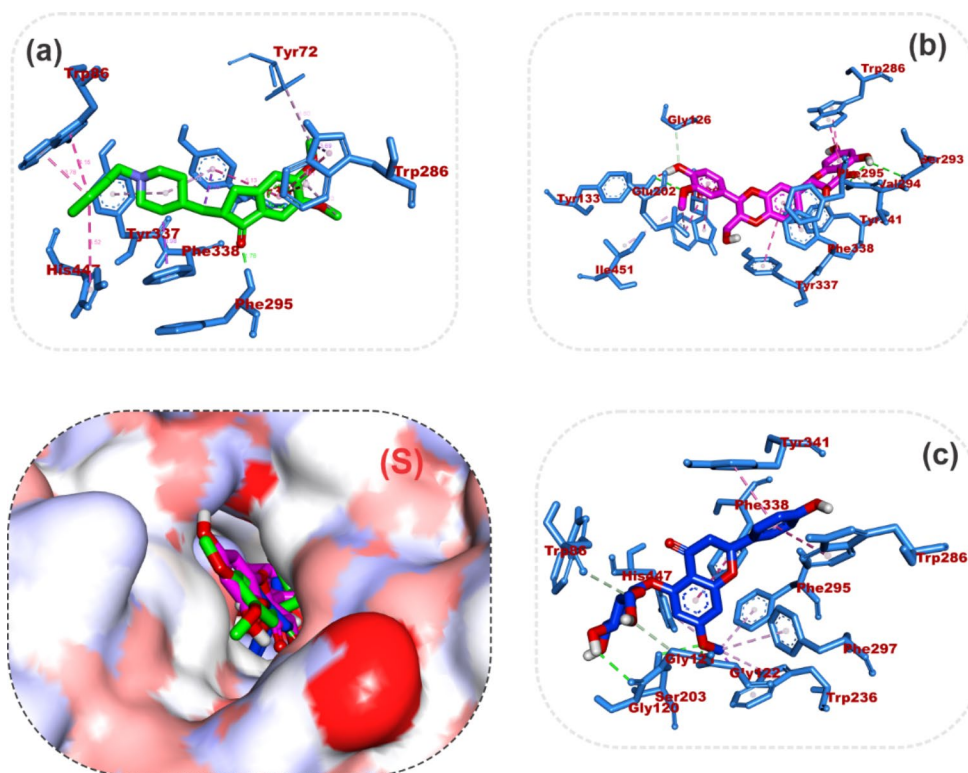
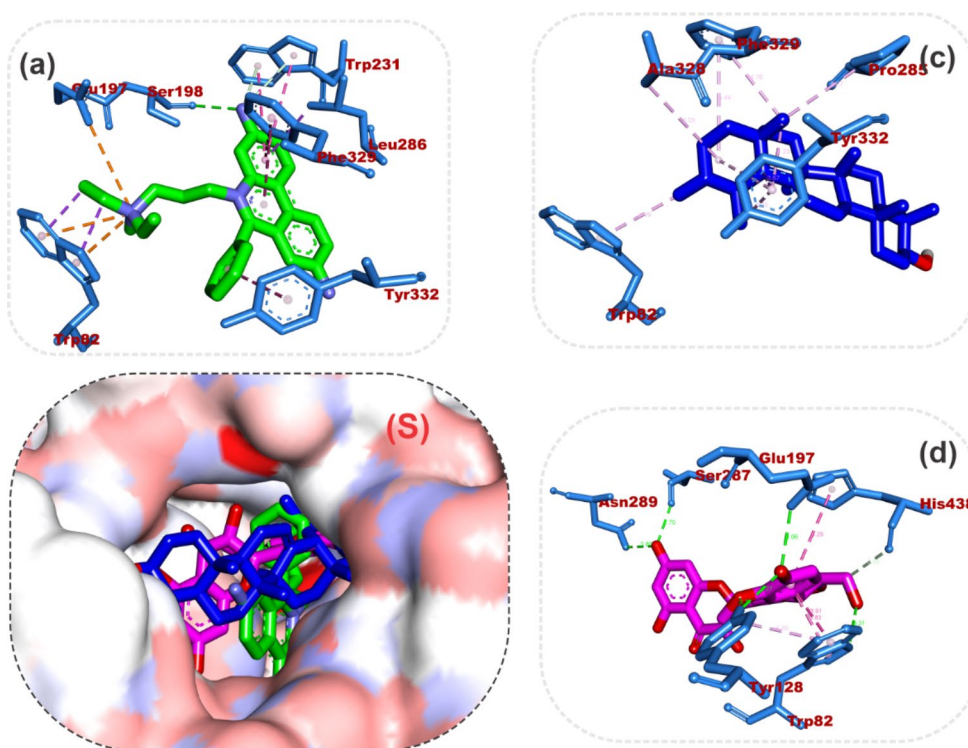


Fig. 3 Interactions of reference compound and best docked GLDPs with the amino acid residues of the active site of *hBChE*. The ligands presented in stick conformation are shown by different colours. Stick representations of the Ligands are shown by colours (a) Green: propidium (reference inhibitor) (b) blue: alpha amyrrin (c) purple: silymarin, while (M) surface representation



T shaped contacts with Leu286 and Phe329. The first ring of Pphenanthridine made both Pi-Pi T shaped and a Pi-donor Hydrogen bond to Trp231, while the amino group of the same ring interacted with a H-bond to Ser198. Two Pi-sigma contacts were observed between one of the ethyl

groups attached to the azanium moiety of propidium while the nitrogen atom of azane formed an attractive charged and Pi-cation contact with Glu197 and Trp82 respectively (Fig. 3a). In the case of α - Amyrrin, the Picen-3-ol ring was orientated into the groove with 11-methyl group directed

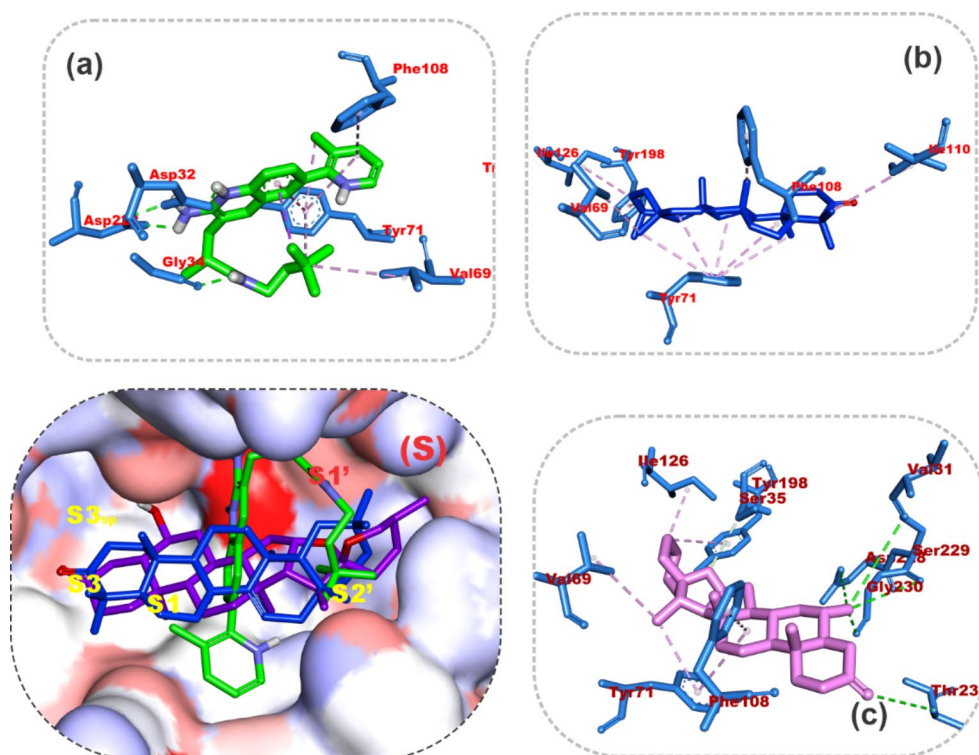
towards the A-site and interacting with Trp82 residue. The last ring of Picen-3-ol made stacking interactions with Try332, Ala328 and Phe329. Try332 and Phe329 made 3 aromatic Pi-Alkyl stacking, while Ala277 interacted with one of the 6-methyl group of α -Amyrin (Fig. 3b). The 4-hydroxy-3-methoxyphenyl group of silymarin was oriented toward the A-site forming aromatic Pi-Pi stacking interaction with residue Trp82. The 4-hydroxy, 3-methoxy of the same ring formed H-bonds with Glu197 and Tyr120 respectively. The 3-hydro group of dibenzodioxinyl moiety interacted via a H-bond to Trp82, while the 3-hydroxyl unit of chromen-4-one moiety formed 2 hydrogen bonds with Asn289 and Ser287 (Fig. 3c).

The differences in the specificity of ligand binding between BChE and AChE have been shown to develop from different number of aromatic residues that is situated in the gorge, only 4 of the aromatic residues present in the gorge of the active site of hAChE are found in the gorge of hBChE. In a similar binding pattern as propidium, the best docked phytocompounds (alpha amylin and silymarin) were stretched in the narrow gorge, interacting with the P-sites catalytic residue Tyr332 (that corresponds to Tyr341 in hAChE) and A-sites residues (Phe329, Trp231 and Trp82) of hBChE (that corresponds to Phe338, Trp236 and Trp86 in hAChE). Substrates with positive charge like butyrylcholine are guided down the catalytic gorge through interaction with Tyr 332 and Asp70 which are situated at the lip portion of the hBChE active site gorge [72]. Also, residues Trp82 and Asp70 of the cysteine Ω -loop, known to establish direct

linkage between the choline-binding pocket in the P-site and A-site are involved in the catalytic activities of hBChE. Hence, compounds like reference compound (propidium) that strongly interact with these catalytic residues will inhibit the substrate catalytic activity of the enzymes.

The docked aminoquinoline (3-(2-Amino-6-(3-methylpyridin-2-yl) quinolin-3-yl)-N-(3,3-dimethylbutyl)-2-methylpropanamide) compound (1) exhibited the similar pose with the exact pattern as the co-crystallized compounds. The compound was orientated in the S1' and S2' sub-pocket of the protein, while leaving S3 and S3' subpocket (sp) accessible (Fig. 4M). Two amino groups donated 2 hydrogen atoms to Asp228 while the same same moiety and that of the quinoline ring donated 2 hydrogen atoms to Asp32. The amino group of N-methylpropanamide donated one hydrogen atom to Gly34. The benzyl ring of the quinoline ring made a Pi-Pi T shaped stacking with Tyr71, while the 3-methylpyridin-2-yl and made 2 Pi-alkyl contact with Phe108 and Tyr71 (Fig. 4a). The 3,3-dimethylbutyl moiety made several alkyl contacts with Val69 and Tyr71 of BACE-1 enzyme. Although, the two best docked phytocompounds were docked into the substrate binding pocket of BACE-1, but they interacted in different binding mode. Taraxeron and solagenin were stretched into the subpocket (S3 and S3') of the substrate binding site. The B, C and D ring of taraxerone made Pi-alkyl contacts with Tyr71 while the 23, 26, 25, 27 and 30 methyl groups formed alkyl contacts with Ile110, Phe108, Tyr71, Val69 and Tyr198 of BACE-1 enzyme (Fig. 4b). The 19-hydroxyl group of solagenin formed the

Fig. 4 Interactions of reference compound and best docked GLDPs with the amino acid residues of the active site of BACE-1. The ligands presented in stick conformation are shown by different colours (a) Green: Compound 1 (reference inhibitor) (b) blue: taraxeron (c) purple: solagenin while (M) surface representation



only hydrogen bond with Gly230, while several alkyl and Pi-alkyl interaction were formed with the residues of the subsites: S1, S2 and S1' (Fig. 4c).

Besides the high binding propensities of the best docked phytochemicals to the ChE enzymes, they demonstrated comparative or higher affinity for the catalytic site residues of human beta-site APP cleaving enzyme 1 (BACE1) than the reference co-crystallized inhibitor. The blockage of the substrate binding cleft of the enzyme by interacting with the residues that forms the catalytic dyad (Asp32/Asp228) that is centrally positioned in the cleft and covered by a β -hairpin loop [73], has been reported as the best mode of inhibiting the catalytic activity of the enzyme [74]. The best docked phytochemicals were firmly accommodated in the binding cleft of the enzyme. Although they did not interact with the aspartic residues of the catalytic dyad, the best docked phytocompounds formed strong interactions with several residues of the subsites (S1, S3, S3') and those of the flip (67–75) region of the binding pocket. Interactions with amino acid residues that makes up the flip region of the active site have been shown to alter the conformation of the entrance to the substrate binding cleft, and by this means blocking the substrate from access to the cleft [75, 76].

Molecular dynamics simulation of best docked phytochemicals

Although molecular docking technique has been primarily used as a standalone technique in drug design and development, recently, the approach is integrated into workflows that comprise several other computational approaches such as MD simulation, and post MD simulation binding free energy calculation [77, 78]. In MD simulation analysis, the structural integrity and stability of the bound complexes are structurally compared to the apo proteins from the several structural fluctuations that occurs during the period of simulation [79]. In these studies, the RMSF, RMSD, RoG, SASA, and number of hydrogen bonds were calculated from the obtained trajectories. The plots for the RMSD reveals the extent at which each frame deviates from the initial structure, therefore, information assessed from the plots can provide insight into the stability of the protein systems [80]. Figure 5 shows the plots for the RMSD of the three enzyme systems. All the protein systems exhibited experienced equilibration around 10 ns with observed minimal fluctuations throughout the simulation period. The same pattern of RMSD were exhibited by *hAChE* systems with mean RMSD values of 3.9030, 4.2361, and 4.13430 Å for the apo enzyme, donepezil and silymarin complexes respectively (Fig. 5a). The *hBChE* systems had mean RMSD values of 3.9581, 4.111, 4.02335 Å for the unbound enzyme, propidium and amylin complexes respectively (Fig. 5b),

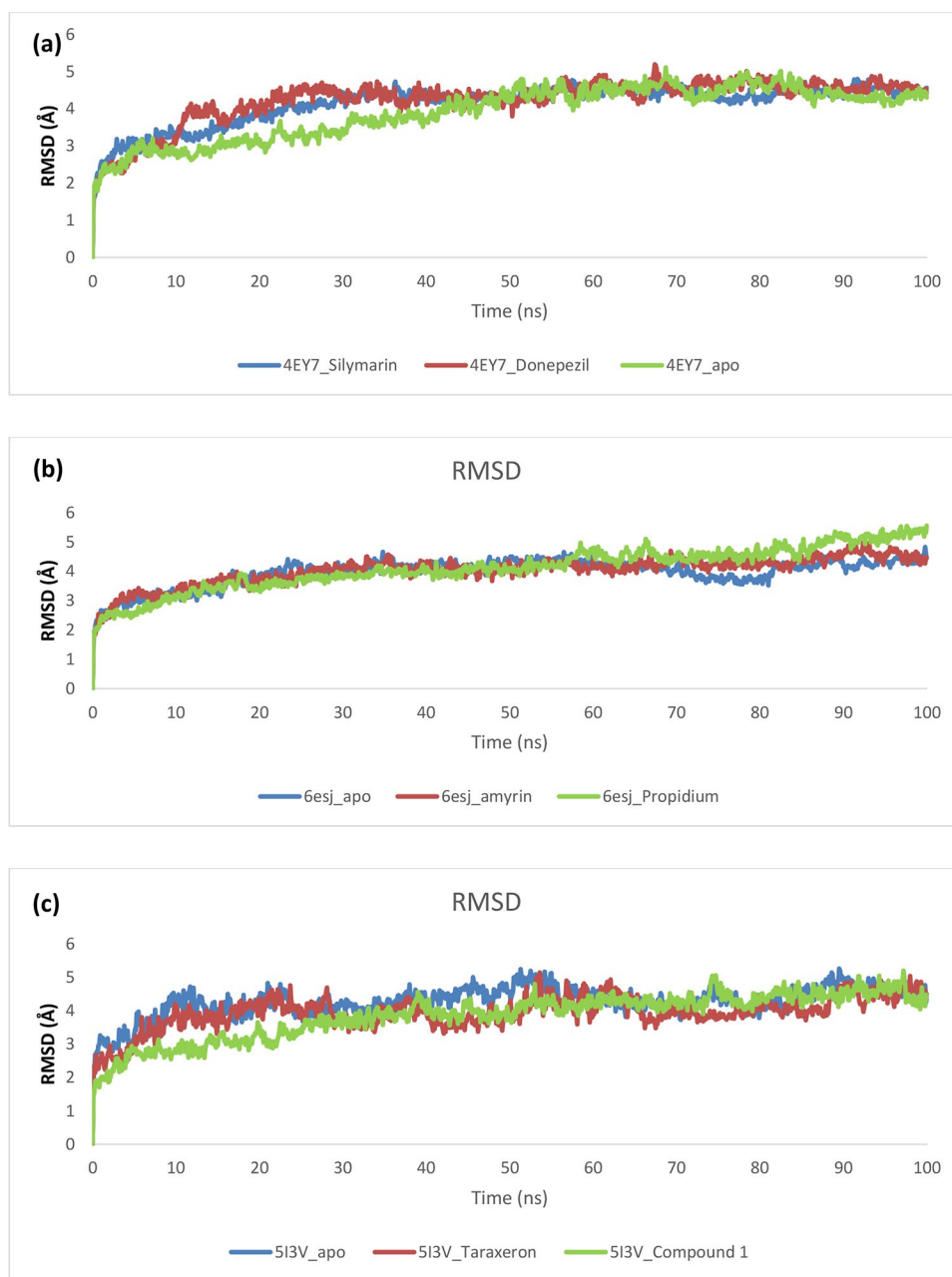
while the BACE-1 systems had 4.2591, 3.8524 and 3.9451 Å for the unbound protein, Compound 1 and taraxeron complex systems (Fig. 5c). It can be observed from the RMSD plots and the mean RMSD values that the binding of the best docked GLDP to the active regions of the proteins did not cause a major alteration in the structural integrity of the protein, the exhibited close mean RMSD values of the bound structures as compared to the apo proteins shows a more compacted complex structure without a major disruption of the integrity of the proeint [80].

The RMSF plots reveal how flexible different regions of the protein are [81]. The *hAChE* systems had mean RMSF values of 2.0213, 2.1693 and 1.9529 Å for the unbound enzyme, donepezil and silymarin complexes respectively (Fig. 6a). The *hBChE* systems had average RMSF values of 1.9993, 1.4947 and 1.8943 Å for the apo enzyme, propidium, and amylin enzyme complexes respectively (Fig. 6b), while the BACE-1 systems presented average RMSF values of 1.932470732 for the apo enzyme, 1.7285 and 1.9324 Å for the BACE-1 enzyme complexed with Compound 1, and taraxeron respectively (Fig. 6c). The reduction in mean RMSF values in the complex structures when compared to the unbound structures indicates more compacted complexes. The reduction in RMSF mean values in the complex structures compared to the unbound enzymes further indicates more compacted complexes [81].

The compactness of a protein structure can be assessed from the RoG plots [82]. Usually, protein structures that are stably folded presents a steady RoG plot with minimal fluctuation during the period of simulation. Figure 7 shows the respective RoG plots for the 3 protein systems. A steady progression with minimal fluctuation was observed for the *hAChE* systems while the *hBChE* and BACE-1 systems presented minimal fluctuations. The three enzyme systems showed close range of average RoG values. The calculated mean RoG values for the *hAChE* systems are 23.209, 23.208 and 22.963 Å for the apo enzyme, donepezil and Silymarin complexed systems respectively (Fig. 7a). Those for the *hBChE* are 23.0294, 23.1665 and 22.9975 Å for the apo enzyme, enzyme complexed with propidium and enzyme complexed with amylin respectively (Fig. 7b). The BACE-1 enzyme systems had average RoG values of 21.9142, 20.8247 and 21.6558 Å for the apo, Compound 1, and taraxeron complexes with BACE-1 (Fig. 7c).

The extent of accessibility of solvent by the surface of proteins structure can be assessed from the SASA plots [82]. Both SASA and RoG plots indicates the extent of the unfolding of proteins structure, using the original structure as a reference. Figure 8 shows the SASA plots for the enzymes systems. After equilibration at the early stage of the simulation, the three enzyme systems showed a steady progression throughout the simulation period. The computed mean of

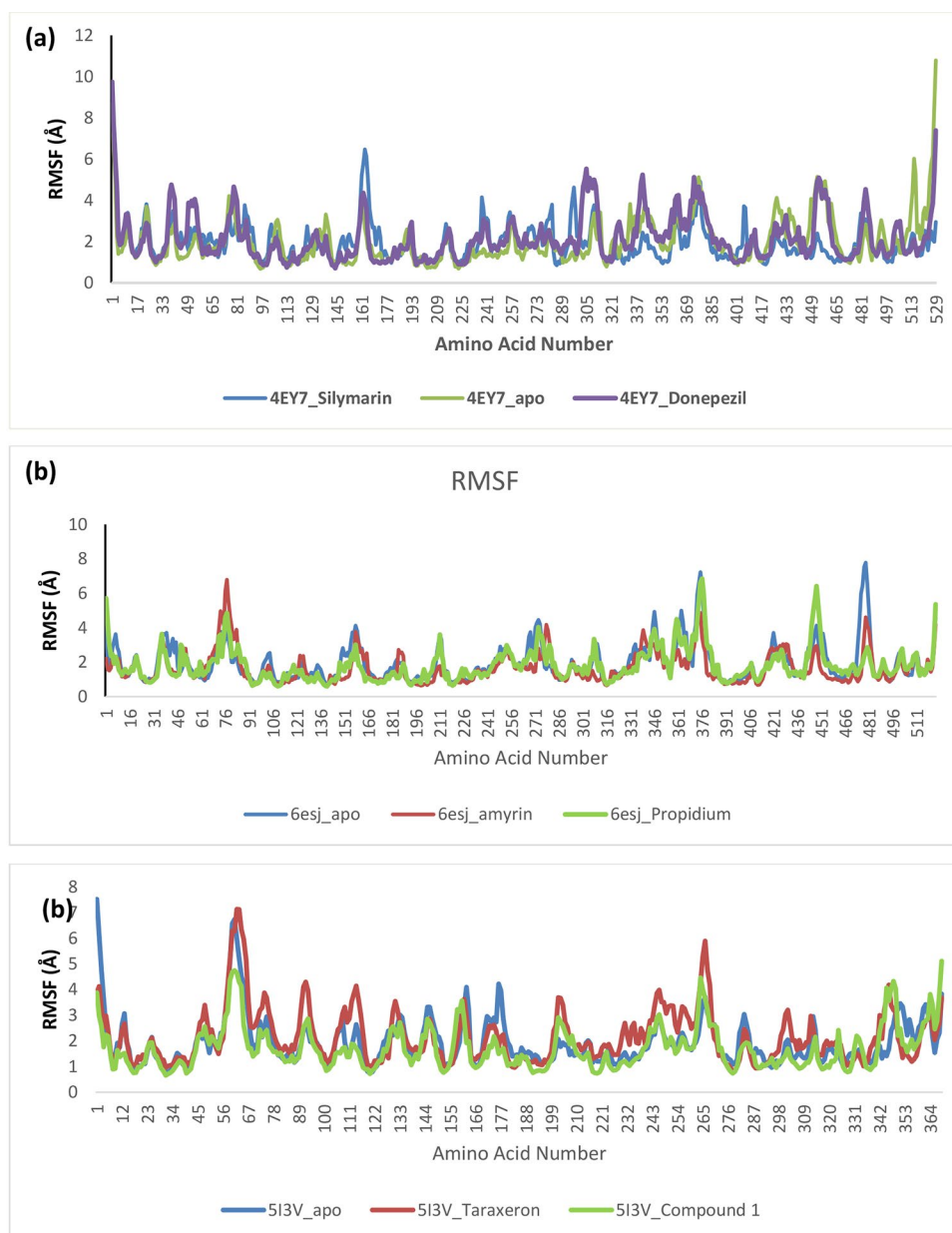
Fig. 5 The Backbone-Root Mean Square Deviation (RMSD) plots of molecular dynamics (MD) simulation of ligands complexed to (a) *human* acetylcholinesterase (*hAChE*) (b) *human* butyrylcholinesterase (*hBChE*) (c) *human* β -secretase (*BACE1*)



SASA values for the *hAChE* protein systems are 23615.65, 24332.76 and 23529.57 Å² for the apo enzyme, donepezil-, and silymarin- *hAChE* complexes respectively (Fig. 8a). The *hBChE* protein systems had mean SASA values of 24,252., 232731.04 and 23764.26 Å² for the unbound protein, propidium-, and amyryn- *hBChE* complexes (Fig. 8b), while the BACE-1 systems had lower mean SASA values of 18470.53, 17750.3 and 18463.40 Å² for apo enzyme, Compound 1, and taraxeron complexes respectively (Fig. 8c). The mean number of hydrogen bonds observed in the unbound *hAChE* system, donepezil- and silymarin- *hAChE* complexes are 71.709, 70.555 and 69.392 respectively (Fig. 9a). The *hBChE* protein systems had mean number of H-bonds

of 71.168, 68.765 and 74.259 for the unbound protein, propidium-, and amyryn- *hBChE* complexes (Fig. 9b), while the apo BACE-1 enzyme, Compound 1-, and taraxeron-BACE-1 complex systems had 42.33, 47.322 and 41.407 for mean number of H-bonds (Fig. 9c). In the *hAChE* systems, there was an observed slight reduction in the mean number of H-bonds in the bound complexes when compared to the apo protein, while there was an increase in the mean number of H-bonds in the complex structures when compared to the apo protein for the *hBChE* and BACE-1 systems. The binding of the best docked GLDP in the same manner as the reference compounds may have enhanced the intramolecular H-bonds in the *hBChE* and BACE-1 systems. This

Fig. 6 Per residue Root Mean Square Fluctuations (RMSF) plots of molecular dynamics (MD) simulation of ligands complexed to (a) human acetylcholinesterase (*hAChE*) (b) *human* butyrylcholinesterase (*hBChE*) (c) human β -secretase (BACE1)



further indicates that the structural integrity especially intermolecular hydrogen bonds in the enzymes were not altered [80].

Molecular mechanics generalized born surface area (MMGBSA) analysis

Simulation-based quantitation of free energy of binding of ligands to proteins in a dynamic environment has been demonstrated to be a more considerably reliable and accurate calculations of the binding affinity [83]. Thus, the ΔG_{bind} computations provides an in-depth information about the binding modes of best docked compounds at the initial stages of drug design and development [84]. The results for

hAChE systems showed that sakuranin had the lower binding free energy (-48.43 Kcal/mol) as compared to silymarin (-36.7 Kcal/mol). The two best docked compounds had lower binding free energy than the reference compounds donepezil (19.89 Kcal/mol). Taraxeron and silymarin had lower binding free energy to the *hBChE* and BACE-1 enzymes respectively. The results from the static docking calculation shows that the two best docked compounds to respective proteins had close and comparable binding energies. Except for solagenin and taraxeron with close binding free energies to *hBChE*, sakuranin and silymarin, the ligands demonstrated higher binding free energy compared to the other best docked phytocompounds. The analysis of various component of the total binding free energy is presented in

Fig. 7 The Radius of gyration (RoG) plots of molecular dynamics (MD) simulation of ligands complexed to (a) *human* acetylcholinesterase (*hAChE*) (b) *human* butyrylcholinesterase (*hBChE*) (c) *human* β -secretase (*BACE1*)

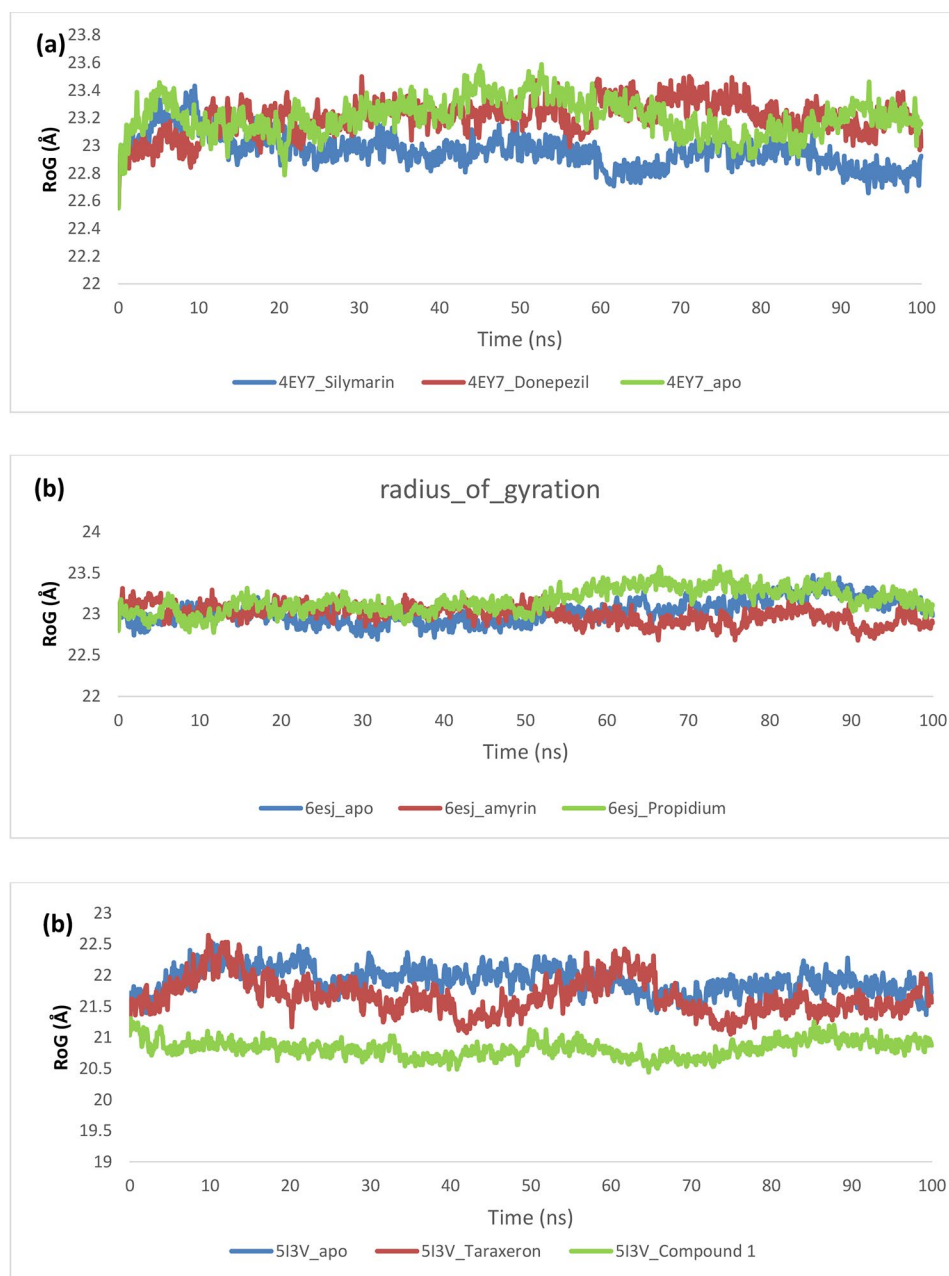


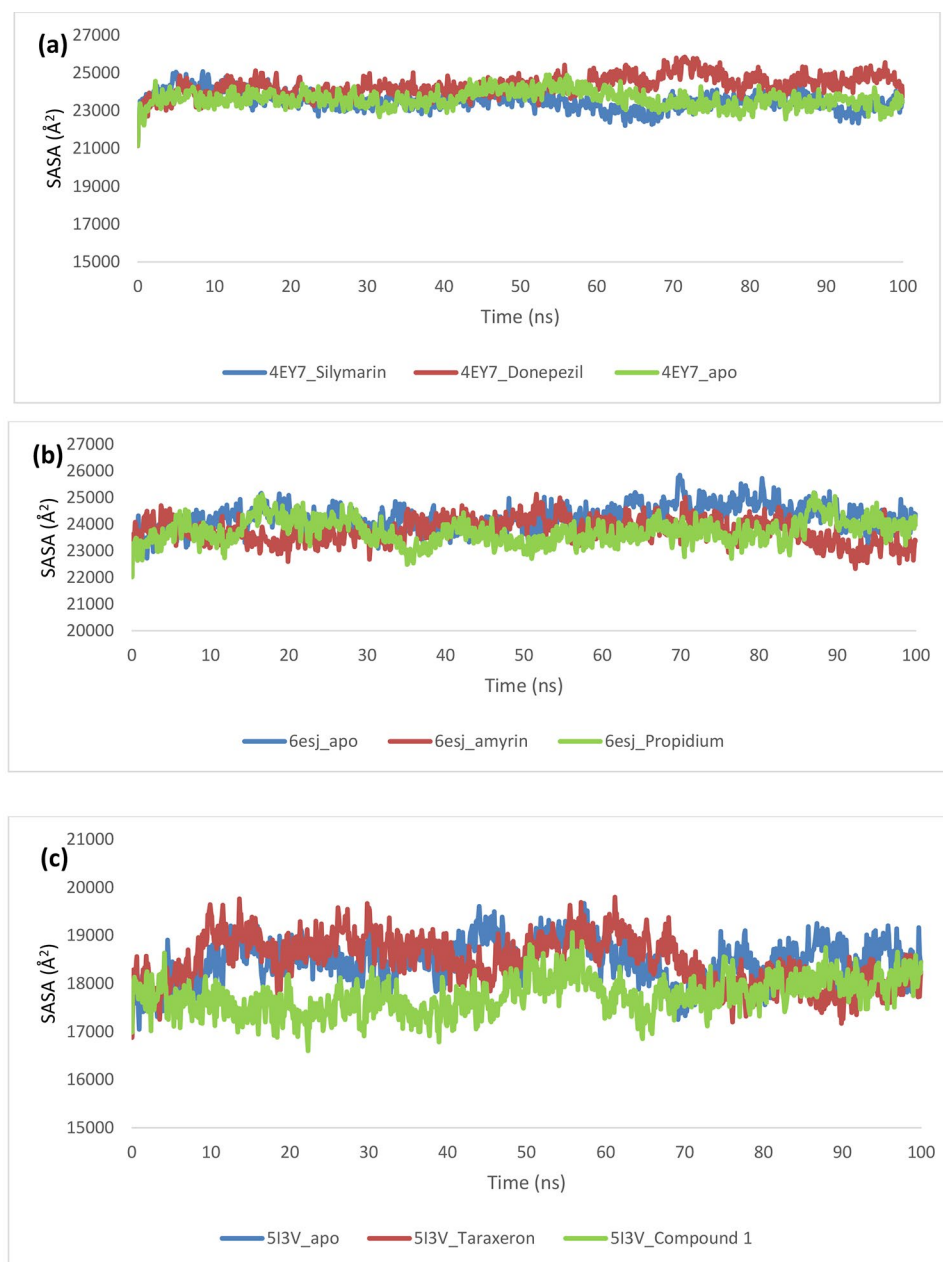
Table 3. The decomposition of the total binding free based on contributing amino acid and the energy changes of the system throughout the simulation period of 100 ns is presented as supplementary data (Figures S2–S4), while the Free Binding Energy change across the simulation is presented in Figure S5–S7 (supplementary data). The binding free energy computation based on the MMGBSA methods and the decomposition analysis of the total free energy to contributing amino acids shows that the post dynamics results corroborated the static docking calculations. The interactions with catalytic residues were preserved in the simulated dynamic environment. The binding free energy

for the best docked phytochemicals to the respective protein targets were computed using the MMGBSA methods [84].

Clustering analysis of MD simulation trajectories of the protein-ligand bound complexes

The trajectories of MD simulation of the protein-ligand systems were subjected to cluster analysis by employing the elbow method utilizing TTClust V 4.9.0. A representative conformer was selected after the conformation analysis that was subjected to further amino acid interactive analysis using by Protein-Ligand Interaction profiler (PLIP). After the cluster analysis, all gaps within the amino acid sequence

Fig. 8 The Surface Accessible Surface Area (SASA) plots of molecular dynamics (MD) simulation of ligands complexed to (a) *human* acetylcholinesterase (hAChE) (b) *human* butyrylcholinesterase (hBChE) (c) *human* β -secretase (BACE1)



were removed, hence the amino acids for each protein structure were re-numbered to start from number 1 until the end of the sequence, this was different from the number in the retrieved crystal structures. From the cluster analysis it was observed that the interactions with important catalytic residues observed in the static docking analysis were preserved during the course of the simulations. (Table S2-S4 & Figure S8-S10).

The cluster analysis of the trajectories obtained from the MD simulation and interaction analysis in representative conformers of the clusters reveals that the interactions (H-bonds and hydrophobic interaction) that were detected from the static docking experiment were majorly preserved

at different time during the period of simulation. This further suggests that the interactions described in the static environment can also be sustained in more dynamic environment; and thereby could be adopted for further experimental procedures. Overall, the mentioned interactions formed between the best docked phytochemicals to the enzymes may serve any of these roles: competitive inhibition of the catalytic activity of the enzymes or blocking the A β recognition zone at the P-site, thereby preventing amyloid fibrillogenesis by [85, 86]. It is worthy of note that the best docked phytochemicals (silymarin, sakuranin and alpha- amylin) identified from the 152 GLDP have various documented neuroprotective activities. Silymarin have been

Fig. 9 The changes in the number of H-bonds during the MDS trajectory of ligands complexed to (a) *human* acetylcholinesterase (hAChE) (b) *human* butyrylcholinesterase (hBChE) (c) *human* β -secretase (BACE1)

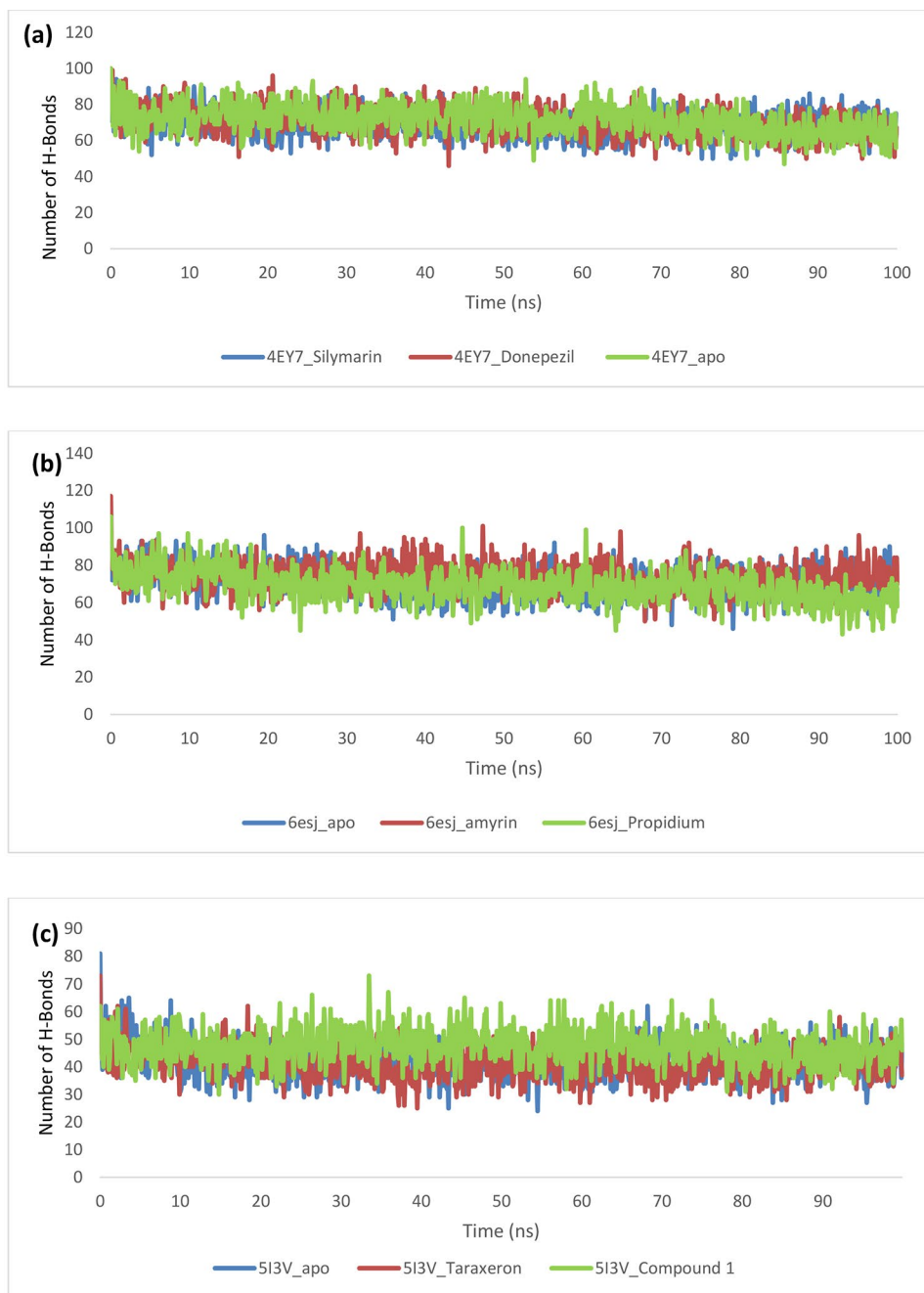


Table 3 Mean and SEM of different energy components of the Binding Free Energy of best docked phytochemicals to respective proteins

SYSTEM	$\Delta_{VDWAALS}$	Δ_{EEL}	Δ_{EGB}	Δ_{ESURF}	Δ_{GGAS}	Δ_{GSOLV}	Δ_{TOTAL}
4ey7_ Donepezil	-42.78 ± 3.08	-9.71 ± 11.53	38.59 ± 10.65	-5.99 ± 0.37	-52.49 ± 12.24	32.6 ± 10.5	-19.89 ± 3.61
4ey7_ Sakuranin	-46.09 ± 3.49	-48.81 ± 6.6	53.76 ± 3.56	-7.28 ± 0.25	-94.9 ± 6.54	46.47 ± 3.5	-48.43 ± 4.85
4ey7_ Silymarin	-49.58 ± 2.98	-37.32 ± 6.96	56.99 ± 5.13	-6.78 ± 0.27	-86.9 ± 6.75	50.2 ± 5.02	-36.7 ± 3.13
5i3v_ Compound 1	-370.4 ± 32.86	364.83 ± 30.05	-4.99 ± 0.5	-397.78 ± 34.35	359.84 ± 29.76	-37.93 ± 6.41	-27.38 ± 5.22
5i3v_ Solagenin	-36.57 ± 2.85	-9.77 ± 5.07	39.88 ± 4.29	-5.01 ± 0.35	-46.34 ± 5.73	34.87 ± 4.08	-11.46 ± 2.87
5i3v_ Taraxeron	-35.58 ± 3.54	-4.33 ± 2.01	31.51 ± 3.53	-4.61 ± 0.55	-39.91 ± 4.08	26.91 ± 3.16	-13.01 ± 2.58
6ep4_ Propidium	-31.58 ± 3.06	-227.84 ± 15.38	246.11 ± 17.04	-5.09 ± 0.4	-259.42 ± 16.43	241.02 ± 16.85	-18.41 ± 2.85
6ep4_ Amyrin	-47.54 ± 2.06	-1.19 ± 1.06	44.68 ± 2.75	-5.56 ± 0.22	-48.72 ± 2.42	39.12 ± 2.65	-9.6 ± 2.38
6ep4_ Silymarin	-42.15 ± 4.66	-41.9 ± 14.05	58.01 ± 8.28	-6.23 ± 0.4	-84.04 ± 13.93	51.78 ± 8.19	-32.27 ± 7.36

reported to inhibit the production of A β by inhibiting the major precursor substance of A β formation (APP). Silymarin could also inhibit the polymerization of A β , aside the property of increasing the concentration acetylcholine in the nervous system by inhibiting cholinesterase activity. The effect of resisting oxidative stress and the inflammatory response of the nervous system has also been documented [87, 88]. Sakuranin, a flavanone glycosides has also been reported as acetylcholinesterase inhibitor in a computational study [89] while, alpha-amyrin, a triterpenoid has been reported to have anti-acetylcholinesterase activity [90, 91]. The presence of these phytochemicals most notably silymarin; that demonstrated dual high binding tendencies to both cholinesterases, may have contributed to the high cholinesterase inhibitory activity of the leaf extract that have been early reported.

Conclusion

In this study, several computational approaches including molecular docking screening, MD simulation analysis, MMGBSA binding free energy analysis and clustering analysis of trajectories of MD simulation to identify the best docked phytochemical with high binding tendencies to three protein targets (*hAChE*, *hBChE* and *hBACE-1*) that are implicated in neurodegeneration. Among the GLDPs, silymarin, alpha-amyrin and teraxeron were the best docked phytochemicals against *hAChE*, *hBChE* and *hBACE-1* respectively. Silymarin exhibited dual-high binding tendency with both a cholinergic protein target *hAChE* and the *hBACE-1*. These phytochemicals in part or in a synergetic association may be responsible for the anti-cholinesterase of the acclaimed ethnomedicinal and reported neuroprotective activities of *Gongronema latifolium* leaf. These compounds may serve as lead compounds for the development of drugs against neurodegenerative diseases, they are therefore recommended for further studies.

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Data Availability All data generated or analysed during this study are included in this published article [and its supplementary information

files].

Declarations

Conflict of interest The authors declare no potential conflict of interest.

References

1. Behl T, Makkar R, Sehgal A, Singh S, Sharma N, Zengin G et al (2021) Current Trends in Neurodegeneration: Cross Talks between oxidative stress, cell death, and inflammation. *Int J Mol Sci* 22:7432. <https://doi.org/10.3390/ijms22147432>
2. Dugger BN, Dickson DW (2017) Pathology of neurodegenerative diseases. *Cold Spring Harb Perspect Biol* 9:a028035
3. Feigin VL, Nichols E, Alam T, Bannick MS, Beghi E, Blake N et al (2019) Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the global burden of Disease Study 2016. *Lancet Neurol* 18:459–480
4. Bozoki A, Giordani B, Heidebrink JL, Berent S, Foster NL (2001) Mild cognitive impairments predict dementia in nondemented elderly patients with memory loss. *Archives of neurology*. ;58:411 – 6.10.1001/archneur.58.3.411
5. Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F et al (2022) Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*. ;7:e105–e25.10.1016/S2468-2667(21)00249-8
6. Hussain R, Zubair H, Pursell S, Shahab M (2018) Neurodegenerative diseases: regenerative mechanisms and novel therapeutic approaches. *Brain Sci* 8:177
7. Bar-Am O, Amit T, Kupersmidt L, Aluf Y, Mechlovich D, Kabha H et al (2015) Neuroprotective and neurorestorative activities of a novel iron chelator-brain selective monoamine oxidase-A/monoamine oxidase-B inhibitor in animal models of Parkinson's disease and aging. *Neurobiology of aging*. ;36:1529 – 42.10.1016/j.neurobiolaging.2014.10.026
8. Hampel H, Mesulam M-M, Cuello AC, Farlow MR, Giacobini E, Grossberg GT et al (2018) The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 141:1917–1933
9. Atatreh N, Al Rawashdah S, Al Neyadi SS, Abuhamdah SM, Ghattas MA (2019) Discovery of new butyrylcholinesterase inhibitors via structure-based virtual screening. *J Enzyme Inhib Med Chem* 34:1373–1379
10. Marucci G, Buccioni M, Dal Ben D, Lambertucci C, Volpini R, Amenta F (2021) Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology* 190:108352
11. Klafki HW, Staufenbiel M, Kornhuber J, Wiltfang J (2006) Therapeutic approaches to Alzheimer's disease. *Brain: a journal of neurology*. ;129:2840 – 55.10.1093/brain/awl280
12. Ravera E, Ciambellotti S, Cerofolini L, Martelli T, Kozyreva T, Bernacchioni C et al (2016) Solid-state NMR of PEGylated proteins. *Angew Chem* 128:2492–2495
13. Adewole KE, Gyebi GA, Ibrahim IM (2021) Amyloid β fibrils disruption by kolaviron: molecular docking and extended molecular dynamics simulation studies. *Comput Biol Chem* 94:107557
14. Brown MR, Radford SE, Hewitt EW (2020) Modulation of β -amyloid fibril formation in Alzheimer's disease by microglia and infection. *Front Mol Neurosci* 13:609073
15. Hampel H, Lista S, Vanmechelen E, Zetterberg H, Giorgi FS, Galgani A et al (2020) β -Secretase1 biological markers for Alzheimer's disease: state-of-art of validation and

- qualification. *Alzheimer's Research & Therapy.* ;12:130.10.1186/s13195-020-00686-3
16. Ekong MB, Peter MD, Peter AI, Eluwa MA, Umoh IU, Igiri AO et al (2014) Cerebellar neurohistology and behavioural effects of *Gongronema latifolium* and *Rauwolfia vomitoria* in mice. *Metab Brain Dis* 29:521–527
17. Balogun M, Besong E, Obimma J, Mbamalu O, Djibissie S (2016) *Gongronema latifolium*: a phytochemical, nutritional and pharmacological review. *J Physiol Pharmacol Adv* 6:811–824
18. Ugochukwu N, Babady N, Cobourne M, Gasset S (2003) The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J Biosci* 28:1–5
19. Nwanjo H, Okafor M, Oze G (2006) Anti-lipid peroxidative activity of *Gongronema latifolium* in streptozotocin-induced diabetic rat. *Nigerian J Physiological Sci.* ;21
20. Adebajo A, Ayoola M, Odediran S, Aladesanmi A, Schmidt T, Verspohl E (2012) P 29: insulinotropic constituents and evaluation of ethnomedical claim of *gongronema latifolium* root and stem. *Diabetes Metab* 38:S115
21. Ibegbulem C, Chikezie P (2013) Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi*, *Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattus norvegicus*). *Pharmacognosy Commun* 3:12
22. Robert A, Luke U, Udosen E, Ufot S, Effiong A, Ekam V (2011) Anti-diabetic and Anti-hyperlipidemic Properties of Ethanolic Root Extract of *Gongronema Latifolium* (Utazi) on Streptozotocin (STZ) Induced Diabetic Rats.
23. Ogunyemi O, Gyebi A, Adebayo J, Oguntola J, Olaiya C (2020) Marsectohexol and other pregnane phytochemicals derived from *Gongronema latifolium* as α -amylase and α -glucosidase inhibitors: in vitro and molecular docking studies. *SN Appl Sci* 2:1–11
24. Ogunyemi O, Gyebi G, Saheed A, Paul J, Nwaneri-Chidozie V, Olorundare O et al Inhibition mechanism of alpha-amylase, a diabetes target, by a steroidal pregnane and pregnane glycosides derived from *Gongronema latifolium* Benth. *Frontiers in molecular biosciences.* 2022;9
25. Akpaso M, Orie N, Ebong P (2019) Administration of combined methanolic leaf extracts of *vernonia amygdalina* and *gongronema latifolium* enhanced glut 2 expression in the pancreas and down-regulates serum caspase 3 activity of streptozotocin-induced diabetic wistar rats.
26. Ofoha PC, Nimenibo-Uadia RI Effect of Ethanolic Leaf Extract of *Gongronema Latifolium* on blood glucose and cholesterol levels in Alloxan-Induced Diabetic rats
27. Chukwudozie IK, Agbo MC, Ugwu KO, Ezeonu IM (2021) Oral administration of *Gongronema latifolium* Leaf Extract modulates gut microflora and blood glucose of Induced Diabetic rats. *J Pure Appl Microbiol* 15:346–355
28. Al-Hindi B, Yusoff NA, Ahmad M, Atangwho IJ, Asmawi MZ, Al-Mansoub MA et al (2019) Safety assessment of the ethanolic extract of *Gongronema latifolium* Benth. Leaves: a 90-day oral toxicity study in Sprague Dawley rats. *BMC Complement Altern Med* 19:1–10
29. SC O, Chinaka N (2013) Carbon tetrachloride induced renal toxicity and the effect of aqueous extract of *Gongronema latifolium* in Wistar albino rats. *Drug discovery* 4:15–16
30. Imo C, Uhegbu FO, Glory IN (2015) Histological and Hepatoprotective Effect of Ethanolic Leaf Extract of *Gongronema latifolium* Benth in Acetaminophen-Induced hepatic toxicity in male albino rats. *Int J Preventive Med Res.* ;1
31. Johnkennedy N, Adamma E (2011) The protective role of *Gongronema latifolium* in acetaminophen induced hepatic toxicity in Wistar rats. *Asian Pac J Trop Biomed* 1:S151–S4
32. Adekanle E, Omozokpia UM (2015) Antioxidant potentials of *Gongronema latifolium* (utazi) leaf extracts. *Biokemistri* 27:85–88
33. Usuh I, Akpan H (2015) Antioxidative efficacy of combined leaves extracts of *Gongronema latifolium* and *Ocimum gratissimum* on streptozotocin induced diabetic rats. *J Med Med Sci* 2:88–95
34. Gyebi GA, Adebayo JO, Olorundare OE, Pardede A, Ninomiya M, Saheed AO et al (2018) Iloneoside: a cytotoxic ditigloylated pregnane glycoside from the leaves of *Gongronema latifolium* Benth. *Nat Prod Res* 32:2882–2886
35. Iweala EEJ (2015) Anti-cancer and free radical scavenging activity of some nigerian food plants in vitro. *Int J cancer Res* 11:41–51
36. Adebayo J, Ceravolo I, Gyebi G, Olorundare O, Babatunde A, Penna-Coutinho J et al (2022) Iloneoside, an antimalarial pregnane glycoside isolated from *Gongronema latifolium* leaf, potentiates the activity of chloroquine against multidrug resistant *Plasmodium falciparum*. *Mol Biochem Parasitol* 249:111474
37. Idowu ET, Ajaegbu HC, Omotayo AI, Aina OO, Otubanjo OA (2015) In vivo anti-plasmodial activities and toxic impacts of lime extract of a combination of *Picralima nitida*, *Alstonia boonei* and *Gongronema latifolium* in mice infected with chloroquine-sensitive *Plasmodium berghei*. *Afr Health Sci* 15:1262–1270
38. Ekong MB, Nwakanma AA (2017) *Rauwolfia vomitoria* and *Gongronema latifolium* extracts influences cerebellar cortex. *Alzheimer's. Dement Cogn Neurol* 1:1–6
39. Akinnuga A, Bamidele O, Ekechi P, Adeniyi O (2011) Effects of an ethanolic leaf extract of *Gongronema latifolium* on haematological some parameters in rats. *Afr J Biomedical Res* 14:153–156
40. Owu D, Nwokocha C, Obembe A, Essien A, Ikpi D, Osim E (2012) Effect of *Gongronema latifolium* ethanol leaf extract on gastric acid secretion and cytoprotection in streptozotocin-induced diabetic rats. *West Indian Med J.* ;61
41. Egba S, Omeoga H, Njoku O (2014) Oral administration of methanol extract of *Gongronema latifolium* (utazi) Up-Regulates cytokine expression and influences the immune system in wistar albino rats. *World Appl Sci J* 31:745–750
42. Akani N, Nwachukwu C, Hakam I (2020) Evaluation of the antibacterial activity of *Gongronema latifolium* and *Costus afer* Leaf extracts on *E. coli* (ATCC 29455) and *S. aureus* (ATCC 25923). *Int J Pathogen Res.* :11–6
43. Enitan SS, Ehizibue OP, Adejumo EN, Akele YR, Owolabi TO (2017) Evaluation of the Antimicrobial potential of *Gongronema latifolium* extracts on some Wound-Associated Pathogens. *Open Sci J Pharm Pharmacol* 5:34–41
44. Ujong GO, Beshel JA, Nkanu E, Ubana OP, Ofem OE (2022) Ethanolic extract of *Gongronema latifolium* improves learning and memory in swiss albino mice. *J Drug Delivery Ther* 12:45–50
45. Nwanna EE, Oyeleye SI, Ogunsuyi OB, Oboh G, Boligon AA, Athayde ML (2016) In vitro neuroprotective properties of some commonly consumed green leafy vegetables in Southern Nigeria. *NFS J* 2:19–24. <https://doi.org/10.1016/j.nfs.2015.12.002>
46. Oyinloye BE, Iwaloye O, Ajiboye BO (2021) Polypharmacology of *Gongronema latifolium* leaf secondary metabolites against protein kinases implicated in Parkinson's disease and Alzheimer's disease. *Sci Afr* 12:e00826. <https://doi.org/10.1016/j.sciaf.2021.e00826>
47. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS et al (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 30:2785–2791
48. Imo C, Uhegbu FO (2015) Phytochemical analysis of *Gongronema latifolium* Benth leaf using gas chromatographic flame ionization detector. *Int J Chem Biomol Sci* 1:60–68

49. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: An open chemical toolbox. *Journal of cheminformatics*. ;3:33.10.1186/1758-2946-3-33
50. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. ;31:455–61.10.1002/jcc.21334
51. O'Boyle N, Banck M, James CA, Morley C, Vandermeersch T (2011) Hutchison GR Open Babel: an open chemical toolbox. *J Cheminf* 3:33
52. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B et al (2015) GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 1:19–25
53. Bekker H, Berendsen H, Dijkstra E, Achterop S, Vondrumen R, Vanderspoel D et al (eds) (1993) Gromacs-a parallel computer for molecular-dynamics simulations. 4th international conference on computational physics (PC 92); : World Scientific Publishing
54. Oostenbrink C, Villa A, Mark AE, Van Gunsteren WF (2004) A biomolecular force field based on the free enthalpy of hydration and solvation: the GROMOS force-field parameter sets 53A5 and 53A6. *J Comput Chem* 25:1656–1676
55. Schüttelkopf AW, Van Aalten DM (2004) PRODRG: a tool for high-throughput crystallography of protein–ligand complexes. *Acta Crystallogr Sect D: Biol Crystallogr* 60:1355–1363
56. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. *J Mol Graph* 14:33–38
57. Valdés-Tresanco MS, Valdés-Tresanco ME, Valiente PA, Moreno E (2021) gmx_MMPBSA: a new tool to perform end-state free energy calculations with GROMACS. *J Chem Theory Comput* 17:6281–6291
58. Miller IIIBR, McGee TD Jr, Swails JM, Homeyer N, Gohlke H, Roitberg AE (2012) MMPBSA. py: an efficient program for end-state free energy calculations. *J Chem Theory Comput* 8:3314–3321
59. Xue W, Yang F, Wang P, Zheng G, Chen Y, Yao X et al (2018) What contributes to serotonin–norepinephrine reuptake inhibitors' dual-targeting mechanism? The key role of transmembrane domain 6 in human serotonin and norepinephrine transporters revealed by molecular dynamics simulation. *ACS Chem Neurosci* 9:1128–1140
60. Tuccinardi T (2021) What is the current value of MM/PBSA and MM/GBSA methods in drug discovery? Taylor & Francis, pp 1233–1237
61. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M (2015) PLIP: fully automated protein–ligand interaction profiler. *Nucleic Acids Res* 43:W443–W7
62. Gyebe GA, Ogunyemi OM, Ibrahim IM, Afolabi SO, Adebayo JO (2021) Dual targeting of cytokine storm and viral replication in COVID-19 by plant-derived steroidal pregnanes: an in silico perspective. *Comput Biol Med* 134:104406
63. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des*. ;7:146–57.10.2174/157340911795677602
64. Pinzi L, Rastelli G (2019) Molecular Docking: Shifting Paradigms in Drug Discovery. *Int J Mol Sci*. ;20.10.3390/ijms20184331
65. Stanzione F, Giangreco I, Cole JC (2021) Chapter four - use of molecular docking computational tools in drug discovery. In: Witty DR, Cox B (eds) *Progress in Medicinal Chemistry*, vol 60. Elsevier, pp 273–343
66. Hines M, Blum J (1979) Bend propagation in flagella. II. Incorporation of dynein cross-bridge kinetics into the equations of motion. *Biophys J* 25:421–441
67. Ordentlich A, Barak D, Kronman C, Flashner Y, Leitner M, Segall Y et al (1993) Dissection of the human acetylcholinesterase active center determinants of substrate specificity. Identification of residues constituting the anionic site, the hydrophobic site, and the acyl pocket. *J Biol Chem* 268:17083–17095
68. Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L et al (1991) Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science* 253:872–879
69. Ashani Y, Grunwald J, Kronman C, Velan B, Shafferman A (1994) Role of tyrosine 337 in the binding of huperzine A to the active site of human acetylcholinesterase. *Mol Pharmacol* 45:555–560
70. Harel M, Schalk I, Ehret-Sabatier L, Bouet F, Goeldner M, Hirth C et al (1993) Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proceedings of the National Academy of Sciences*. ;90:9031–5
71. Colletier JP, Fournier D, Greenblatt HM, Stojan J, Sussman JL, Zaccari G et al (2006) Structural insights into substrate traffic and inhibition in acetylcholinesterase. *EMBO J* 25:2746–2756
72. Darvesh S, Hopkins DA, Geula C (2003) Neurobiology of butyrylcholinesterase. *Nature Reviews Neuroscience*. ;4:131–8.10.1038/nrn1035
73. Xu Y, Li M, Greenblatt H, Chen W, Paz A, Dym O et al (2012) Flexibility of the flap in the active site of BACE1 as revealed by crystal structures and molecular dynamics simulations. *Acta Crystallogr Sect D: Biol Crystallogr* 68:13–25
74. Mouchlis VD, Melagraki G, Zacharia LC, Afantitis A (2020) Computer-aided drug design of β -secretase, γ -secretase and anti-tau inhibitors for the discovery of novel Alzheimer's therapeutics. *Int J Mol Sci* 21:703
75. Gupta S, Parihar D, Shah M, Yadav S, Managori H, Bhowmick S et al (2020) Computational screening of promising beta-secretase 1 inhibitors through multi-step molecular docking and molecular dynamics simulations-pharmacoinformatics approach. *J Mol Struct* 1205:127660
76. Kumar A, Roy S, Tripathi S, Sharma A (2016) Molecular docking based virtual screening of natural compounds as potential BACE1 inhibitors: 3D QSAR pharmacophore mapping and molecular dynamics analysis. *J Biomol Struct Dynamics* 34:239–249
77. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD (2015) Molecular docking and structure-based drug design strategies. *Molecules* 20:13384–13421
78. Lavecchia A (2015) Machine-learning approaches in drug discovery: methods and applications. *Drug Discovery Today* 20:318–331
79. Karplus M, McCammon JA (2002) Molecular dynamics simulations of biomolecules. *Nat Struct Biol* 9:646–652
80. Cheng X, Ivanov I (2012) Molecular dynamics. *Computational toxicology*. :243–85
81. Dong Y-w, Liao M-l, Meng X-l, Somero GN (2018) Structural flexibility and protein adaptation to temperature: Molecular dynamics analysis of malate dehydrogenases of marine molluscs. *Proceedings of the National Academy of Sciences*. ;115:1274–9
82. Sinha S, Wang SM (2020) Classification of VUS and unclassified variants in BRCA1 BRCT repeats by molecular dynamics simulation. *Comput Struct Biotechnol J* 18:723–736
83. Perez A, Morrone JA, Simmerling C, Dill KA (2016) Advances in free-energy-based simulations of protein folding and ligand binding. *Curr Opin Struct Biol* 36:25–31
84. Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L et al (2000) Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Acc Chem Res* 33:889–897
85. Khare G, Andrew Symons R, Do D (2008) Common ophthalmic emergencies. *Int J Clin Pract* 62:1776–1784
86. Inestrosa NC, Alvarez A, Perez CA, Moreno RD, Vicente M, Linker C et al (1996) Acetylcholinesterase accelerates assembly of amyloid- β -peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* 16:881–891

87. Guo H, Cao H, Cui X, Zheng W, Wang S, Yu J et al (2019) Silymarin's inhibition and treatment effects for Alzheimer's disease. *Molecules* 24:1748
88. Haddadi R, Shahidi Z, Eyvari-Brooshghalan S (2020) Silymarin and neurodegenerative diseases: therapeutic potential and basic molecular mechanisms. *Phytomedicine* 79:153320
89. Remya C, Dileep K, Tintu I, Variyar E, Sadasivan C (2014) Flavanone glycosides as acetylcholinesterase inhibitors: computational and experimental evidence. *Indian J Pharm Sci* 76:567
90. de Almeida P, Boleti APdA, Rüdiger AL, Lourenço GA, da Veiga Junior VF, Lima ES (2015) Anti-inflammatory activity of triterpenes isolated from *Protium paniculatum* oil-resins. *Evidence-Based Complementary and Alternative Medicine*. ;2015
91. Gurovic MSV, Castro MJ, Richmond V, Faraoni MB, Maier MS, Murray AP (2010) Triterpenoids with acetylcholinesterase inhibition from *Chuquiraga erinacea* D. Don. subsp. *erinacea* (Asteraceae). *Planta Med* 76:607–610

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