Original Article

Molecular Modeling Studies Approach Against Enzymes Causing Alzheimer's Disease Using *Hancorniaspeciosa* Linn By Molecular Docking And Molecular Dynamics Simulations Techniques

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Abstract

Alzheimer's disease affects fifty million people globally, with four million living in India. According to research, the APOE4 gene plays a significant role in the disease's development. The docking structures with the lowest docking energies were examined for protein stability and complex motion using molecular dynamic modelling. The moles server (http://imods.chaco nlab.org) was used to run the molecular dynamics simulation. Investigating such modes is made simpler by iMODS. The 3D interaction of the DNA gyrase and 14-alpha sterol demethylase inhibitor with squash and kunitz receptor and ligand complexes.The prospective use of these drugs against acetylcholinesterase and beta-secretase in therapy might be extremely beneficial. Further research might provide useful information into the efficacy of these drugs in vivo. Natural chemicals may have a synergistic effect on Alzheimer's disease, according to research.

Keywords: Molecular Modeling, Alzheimer's Disease, Hancornia Speciosa Linn, Molecular Docking, Molecular Dynamics

INTRODUCTION:

Alzheimer's disease (AD) is the most prevalent type of dementia which is a complex illness marked by the buildup of b-amyloid (Ab) plaques and neurofibrillary tangles made of tau amyloid fibrils, which are linked to synapse loss and neurodegeneration and cause memory loss and other cognitive issues [1]. Sporadic AD is the type of AD, which has no clear familial connection. It is complicated interplay of our environment, lifestyle, and genes. In Sporadic AD aging is the single biggest risk factor [2].Rather of focusing only on the difficulties these clinical conditions face, AD as a social phenomenon raises considerably more significant [3]. Numerous connections between AD and blood-brain barrier breakdown (BBBb), vascular risk factors (VRF), neurovascular unit dysfunction (NVUd), that suggest a pathogenetic continuum between AD and vascular dementia [4].

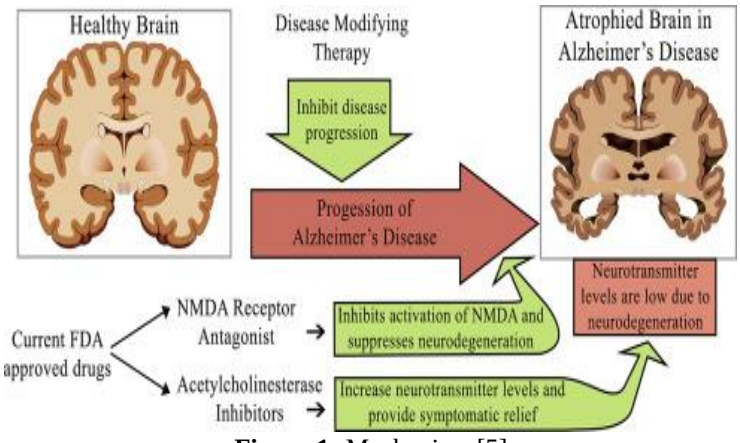


Figure 1: Mechanism [5]

Recent research suggests that it may occur before the age of 65. Alzheimer's disease affects fifty million people globally, with four million living in India. According to research, the APOE4 gene plays a significant role in the disease's development. Cognitive impairment, memory loss, trouble learning, and brain cell degeneration worsen with time, making living a normal life difficult.

Patients have memory loss to the point of forgetting significant events in their lives as a result of the brain beginning to shrink (atrophy). Psychopathic illnesses might also develop [6].
A key enzyme needed for the destruction of the neurotransmitter acetylcholine is acetylcholinesterase (Fig. 2). In a healthy brain, acetylcholinesterase is required to hydrolyze acetylcholine to acetic acid and choline, but this process becomes problematic in Alzheimer's disease [7]. The reason for this is the low levels of acetylcholine, which drop even further if acetylcholinesterase is not inhibited.
By acting on this enzyme, a variety of patient-accessible medications can stop it. Acetylcholinesterase is blocked by medications like donepezil, rivastigmine, and galantamine, increasing the amount of acetylcholine in neural synapses. There is a need for improved medications because these ones have unfavourable side effects. The development and repair of neurons are carried out by the amyloid precursor proteins (APP) [8]. Enzymes cleave the APP, turning it into soluble protein fibres that will ultimately break down and be recycled. It is known that the secretase enzymes -secretase, -secretase, and -secretase all contribute to the cutting of APP.

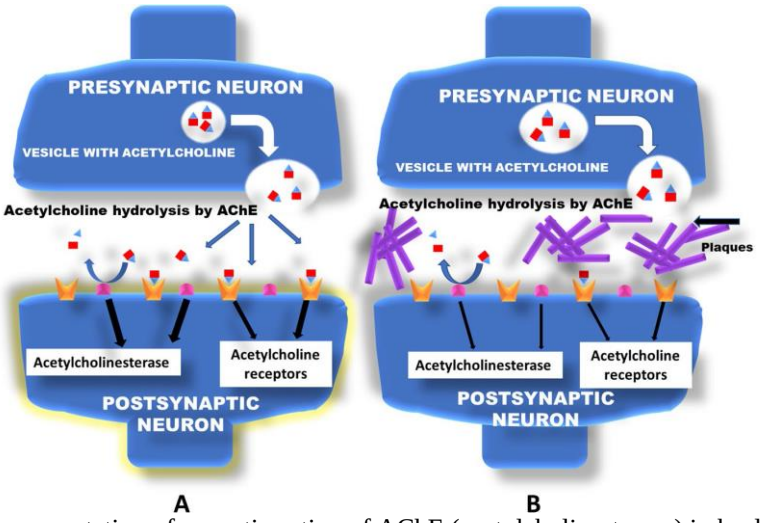


Figure 2 Diagrammatic representation of synaptic action of AChE (acetylcholinesterase) in healthy (**A**) anddiseased (**B**) neuronal cells

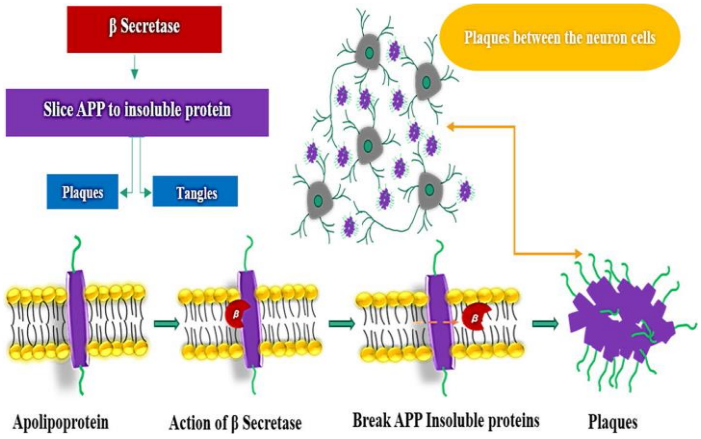


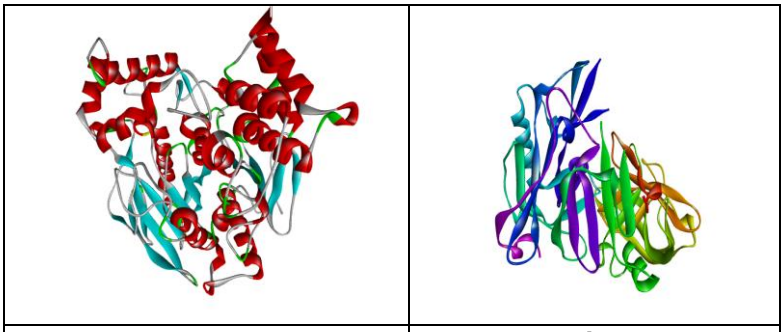
Figure 3 Image showing the involvement of β-secretase in causing plaques and tangles.

The amyloid precursor protein (APP), on the other hand, is broken down by the beta-secretase enzyme into the insoluble monomer -amyloid protein. The protein fibres of the insoluble monomer are tacky inside the neuronal cells, where they tangle and cause beta-amyloid plaques to form. These plaques disrupt the communication between neurons, obstruct the blood arteries in the brain, and result in amyloid angiopathy, bleeding, and cell rupturing. The cytoskeleton is protected from harm by the tau protein that is situated in between the microtubules.

Researchers are continually looking for a cure for the condition because there is no final cure.
In light of the enormous diversity of therapeutic chemicals found in plants, natural sources of substances such as plants are also being investigated [9]. India is recognised for its diverse flora and availability of medicinal herbs. Earlier research with natural substances found that plant molecules are possible therapeutic candidates for a variety of disorders, including Alzheimer's [10]. Natural chemicals derived from medicinal plants are being researched because they are less poisonous and have less adverse effects than synthetically produced molecules.In this work, we used GC-MS to identify possible chemicals from the fruits of the *HancorniaSpeciosa*tree. The docking affinities of these drugs against two protein targets, acetylcholinesterase and -secretase, were next investigated. The compounds demonstrated good binding scores and ADME qualities, indicating that they might be used as medicines to treat Alzheimer's disease.

MATERIALS AND METHODS

Retrieval and Preparation of Protein: Acetylcholinesterase and beta-secretasewere the target proteins. The proteins were obtained from the Protein Data Bank (PDB) (http:// www. rcsb. org/ pdb/ home/ home. do). Acetylcholinesterase has the protein ID 1ACJ (https://www.rcsb.org/structure/1acj) and beta-secretase has the protein ID 1TQF (https://www.rcsb.org/structure/1tqf). On the Discovery studio visualisation tool, the proteins were created independently. Polar hydrogens and Gasteiger charges were introduced.



a b

Figure 4: Enzyme structure a. acetylcholinesterase b. β-secretase

Retrieval of Ligands

The ethanolic extract of *HancorniaSpeciosa*yielded twenty-two potential ligands. The PubChem and Chemspider databases were utilised to find ligands. These ligands were selected for docking against target proteins. These ligands 3D structures were retrieved for docking and ADME investigations.

Table 1: Chemical constituents of *HancorniaSpeciosa*

Sr. No.	Chemical constituents	Molecular formula	Pub Chem ID	IUPAC Names
1	Querecetin	$C_{15}H_{10}O_7$	5280343	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
2	Phlorizin	C21H24O10	6072	1-[2,4-dihydroxy-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-(4-hydroxyphenyl)propan-1-one
3	Kaempferol	C15H10O6	5280863	3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one
4	Eriodictyol	C15H12O6	440735	(2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one
5	Quinic acid	C7H12O6	6508	(3R,5R)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid
6	Rutin	C27H30O16	5280805	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one
7	Obtusalin	$C_{30}H_{50}O_2$	14414398	(1*S*,3*aR*,5*aR*,5*bR*,7*aR*,9*S*,11*aR*,11*bR*,13*bS*)-5*a*-(hydroxymethyl)-3*a*,5*b*,8,8,11*a*-pentamethyl-1-propan-2-yl-1,2,3,4,5,6,7,7*a*,9,10,11,11*b*,12,13*b*-tetradecahydrocyclopenta[a]chrysen-9-ol
8	Phloretin	$C_{15}H_{14}O_5$	4788	3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one
9	Luteolin	C15H10O6	5280445	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one
10	Rosamarinic acid	$C_{18}H_{16}O_8$	5281792	(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid
11	Chlorogenic acid	C16H18O9	1794427	(1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid
12	Erithrodiol	$C_{30}H_{50}O_2$	101761	(3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-(hydroxymethyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-ol
13	β-Amirin	$C_{30}H_{50}O$	73145	(3S,4aR,6aR,6bS,8aR,12aR,14aR,14bR)-4,4,6a,6b,8a,11,11,14b-octamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-ol
14	Caffeic Acid	$C_9H_8O_4$	689043	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid
15	Bornesitol	$C_7H_{14}O_6$	440078	(1R,2S,4R,5R)-6-methoxycyclohexane-1,2,3,4,5-pentol
16	Lupeol	$C_{30}H_{50}O$	259846	(1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol
17	β-Sitosterol	$C_{29}H_{50}O$	222284	(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol
18	Geraniol	$C_{10}H_{18}O$	637566	(2E)-3,7-dimethylocta-2,6-dien-1-ol
19	Protocatechuic acid	$C_7H_6O_4$	72	3,4-dihydroxybenzoic acid
20	Catechin	$C_{15}H_{14}O_6$	9064	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol
21	Apigenin	$C_{15}H_{10}O_5$	5280443	5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one

ADME and Toxicity Analysis

Clinical studies of promising substances that fail might waste time, money, and other resources. When the ligands are examined for pharmacological and pharmacokinetic features, the likelihood of such failures is greatly decreased. The results of computer research on the ligand's structure might indicate whether effective it would be in vivo.Swiss ADME (http://www. swissadme. ch) was utilized to screen the ligands for the most pharmacokinetically relevant substances. ADME is an acronym that stands for absorption, distribution, metabolism, and excretion. Other characteristics addressed include oral bioavailability, blood-brain barrier permeability, and lead similarity. Lipinski's rule of five was applied to assess the drug-likeness of the selected ligands.

Molecular dynamic simulation

The docking structures with the lowest docking energies were examined for protein stability and complex motion using molecular dynamic modelling. The moles server (http://imods.chaco nlab.org) was used to run the molecular dynamics simulation. Investigating such modes is made simpler by iMODS, which also generates workable transition paths over two homologous structures [11]. The iMOD server evaluates the protein stability by computing its internal coordinates in normal mode (NMA). The main-chain deformability plot, B-factor values, eigen value, covariance matrix, and elastic network model all serve as illustrations of the protein's stability.

VIRTUAL SCREENING AND VISUALIZATION

Acetylcholinesterase: It is an enzyme that hydrolyzes acetylcholine to produce reusable derivatives that are recycled as transmitters [8]. PyRx[12] was used to dock the ligands against the target protein. PyRx is a virtual screening software that can evaluate the binding affinity of various ligands to a target protein. PyRx is a handy, easy-to-use virtual screening tool. The AutoDockVina plugin was used to dock candidate chemicals from PubChem against the acetylcholinesterase target protein.The grid box was placed over the whole protein, and docking was completed. Predicting the level of successful interaction between ligands and the target protein uses the score function of the virtual screening tool extensively. The AutoDockVina tool from PyRx 0.8 is utilised as the scoring function. The docked compounds were seen using the Discovery Studio visualisationprogramme [13].

Beta-Secretase: The enzyme beta-secretase is responsible for improper cleavage of the amyloid precursor protein, resulting in beta-amyloid particles that create plaques and impair brain signaling [15]. Using the PyRx virtual screening programme [14], the ligands were docked against the protein. The AutoDockVina plugin was used to dock candidate chemicals from PubChem against the beta-secretase target protein. The whole protein was covered by a grid box, and docking was performed. The docked ligands were visualised using the Discovery Studio visualisation tool.

RESULTS AND DISCUSSION

ADMET Analysis for Drug Likeness Prediction

Computational approaches were employed to predict the drug similarity and pharmacokinetic features of the compounds. The compounds that were chosen were evaluated for ADME study, and the best compounds were explored further for binding scores. ADME is an acronym that stands for absorption, distribution, metabolism, and excretion. Pharmacokinetic features of prospective medicines to reduce failure in in vivo experiments will be examined prior to clinical trials.

The evaluation of the compounds' drug similarity is determined by looking at parameters including molecular weight, the amount of hydrogen bond donors and acceptors, rotatable bonds, and Log P value. To analyse the drug likeness and foretell the likelihood of a successful trial under in vivo circumstances, Lipinski's rule of five is applied [16]. Additionally, blood-brain barrier permeability, bioavailability, and gastrointestinal absorption were examined. A key factor in a possible compound's success is its log P values. The ideal Log P scores are under 5.
The solubility and oral absorption of the substances are poor and their metabolic turnover is high, which is not ideal for prospective therapeutic candidates. The best bioavailability ratings are 0.55. TPSA, molecular charge, and Lipinski's rule of five are used to determine bioavailability ratings. The Verber's rule scores also show if a substance is palatable or not orally.

All of the compounds had good pharmacokinetic qualities and appeared to be viable candidates for future investigation. These blood-brain barrier permeates are non-mutagenic, have good gastrointestinal absorption, and are great candidates for further investigation.

Virtual Screening of Ligands

The ligands were docked to two protein targets, acetylcholinesterase and β-secretase. Acetylcholinesterase is an enzyme that hydrolyzes acetylcholine to produce reusable derivatives. In normal settings, this enzyme function is required to recycle the acetylcholine following signal transmission. In an Alzheimer's patient, however, the synapses of the neurons are blocked by plaques, causing signals to be delayed or lost. Acetylcholinesterase inhibition can enhance signalling by slowing the breakdown of acetylcholine to the choline moiety.

Table 2: Amino acid interaction and docking score of chemical constituents with AchE

Sr. No.	Chemical constituents	Binding energy (kcal/mol)	Amino acid interaction with bond length
1	Querecetin	-7.9	2.57-HIS446, 2.89-TYR184, 2.88-TRP382
2	Phlorizin	-7.7	3.69-LYS353, 4.55-ALA351
3	Kaempferol	-7.9	2.99-TYR455, 3.58-MET316, 4.52-ASP447
4	Eriodictyol	-8.1	2.66-GLU312, 4.05-ASN309, 4.25-ASP305,
5	Quinic acid	-8.1	No interaction
6	Rutin	-8.5	2.47-ARG244, 2.11-HIS398, 4.37-PRO403, 5.30-TRP524, 4.61-PRO232, 4.89-PRO232, 4.16-HIS362
7	Obtusalin	-8.6	2.63-TRP382, 3.58-ASP447, 3.84-ALA351
8	Phloretin	-8.1	3.84-TYR184, 3.66-LEU315, 4.52-GLU380
9	Luteolin	-7.9	2.33-ASP350, ARG346, TYR455, ARG450
10	Rosamarinic acid	-7.8	1.55-ARG450, 2.38-TRP131, 2.28-HIS446
11	Chlorogenic acid	-7.8	No interaction
12	Erithrodiol	-7.8	2.48-TRP382, 2.69-LYS353, 3.52-LEU 315
13	β-Amirin	-9.2	2.88-GLU380, 2.89-ALA351, 3.50-ASP350
14	Caffeic Acid	-7.5	4.05-MET490, 5.28-SER461, 2.62-ILE459
15	Bornesitol	-7.9	1.98-ARG450, 2.20-GLU380, 2.58-ALA351
16	Lupeol	-8.2	5.50-TRP306, 5.05-TRP310, 2.78-TRP304
17	β-Sitosterol	-8.3	2.88-ASP447, 2.89-ALA351, 3.05-TRP382
18	Geraniol	-6.9	No interaction
19	Protocatechuic acid	-6.8	No interaction
20	Catechin	-6.9	3.69-TYR455, 2.65-MET316, 2.55-ASP447
21	Apigenin	-8.2	2.11-HIS398, 4.37-PRO403, 5.30-TRP524

As shown in Table 2, the findings for acetylcholinesterase ranged from -6.8 to -9.2, which are good results. Table 3 shows the results for beta-secretase, which ranged from -6.5 to -10.5 kcal/mol. The findings for both proteins suggested that substances are top candidates for more study. Only substances with scores greater than 8 kcal/mol were deemed to be the best ligands.

Table 3: Amino acid interaction and docking score of chemical constituents with β-secretase

Sr. No.	Chemical constituents	Binding energy (kcal/mol)	Amino acid interaction with bond length
1	Querecetin	-7.2	2.69-MET490, 4.69-SER461, 4.85-ILE459
2	Phlorizin	-6.8	No interaction
3	Kaempferol	-8.4	2.69-LYS353, 2.55-ALA351
4	Eriodictyol	-7.5	3.99-TYR455, 2.58-MET316, 5.52-ASP447
5	Quinic acid	-8.0	4.57-HIS446, 3.89-TYR184, 3.88-TRP382
6	Rutin	-9.2	2.34-ASP228, 2.94-ASP322, 2.02-THR722, 3.69-ARG235, 1.86-GLY230, 3.49-ILE110, 2.93-THR232, 3.92-THR232, 2.12-GLY11
7	Obtusalin	-8.6	1.63-TRP382, 4.58-ASP447, 2.84-ALA351
8	Phloretin	-8.1	2.84-TYR184, 4.66-LEU315, 2.52-GLU380
9	Luteolin	-8.2	3.33-ASP350, 3.20-ARG346, 2.55-TYR455, 2.88-ARG450
10	Rosamarinic acid	-8.8	1.55-ARG450, 2.38-TRP131, 2.28-HIS446
11	Chlorogenic acid	-8.3	2.68-HIS245
12	Erithrodiol	-7.9	No interaction
13	β-Amirin	-8.9	3.48-TRP382, 3.69-LYS353, 4.52-LEU 315
14	Caffeic Acid	-7.5	4.88-GLU380, 3.89-ALA351, 2.50-ASP350
15	Bornesitol	-8.1	3.05-MET490, 4.28-SER461, 3.62-ILE459
16	Lupeol	-10.5	2.98-ARG450, 3.20-GLU380, 3.58-ALA351
17	β-Sitosterol	-8.5	4.50-TRP306, 4.05-TRP310, 3.78-TRP304
18	Geraniol	-8.3	3.88-ASP447, 3.89-ALA351, 2.05-TRP382
19	Protocatechuic acid	-6.5	No interaction
20	Catechin	-6.8	3.89-ALA351
21	Apigenin	-7.8	3.55-ASP447, 4.28-SER461, 4.62-ILE459

The cleavage of beta-amyloid protein into beta-amyloid plaques, the hallmark of Alzheimer's and dementia, is known to be caused by beta-secretase. It is possible to prevent the amyloid precursor protein from being converted into insoluble beta-amyloid by inhibiting the activity of beta-secretase [17].

Total 21 ligands are docked with both proteins. The compound obstusalin, rutin, sitosterol and apigeninexhibited the highest binding affinity.

All the chemical compounds of *HancorniaSpeciosa* docked with both protein except Quinic acid, Geraniol, Protocatechuic acid not interacted with AchE and Chlorogenic acid , Phlorizin, Erithrodiol, Protocatechuic acid does not interacted with beta-secretase enzymes[18].

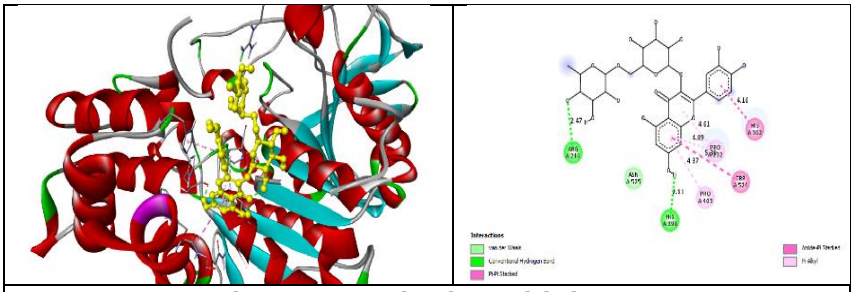


Fig. 5 Ligand rutin interacted with acetylcholiesterase enzyme

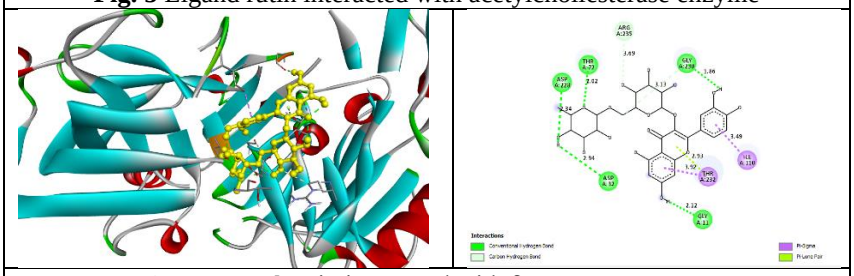
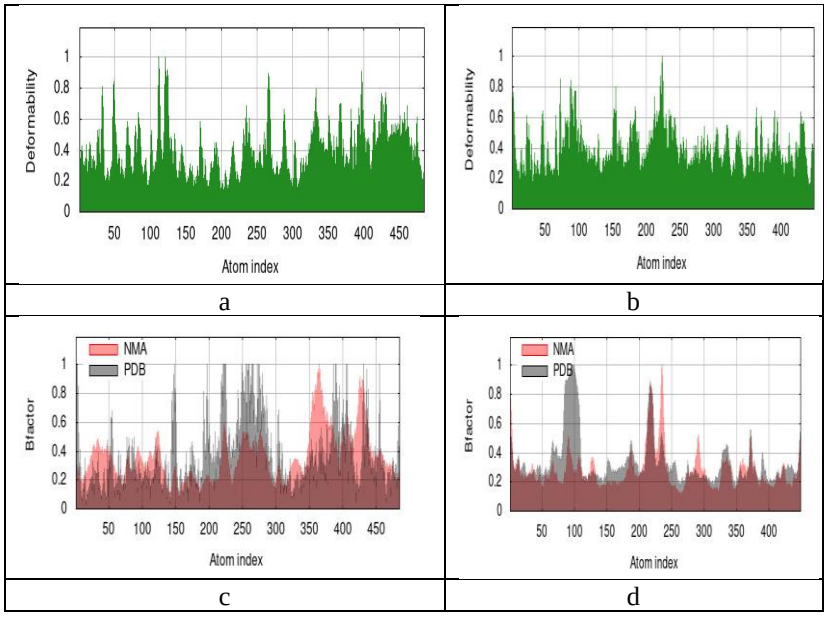
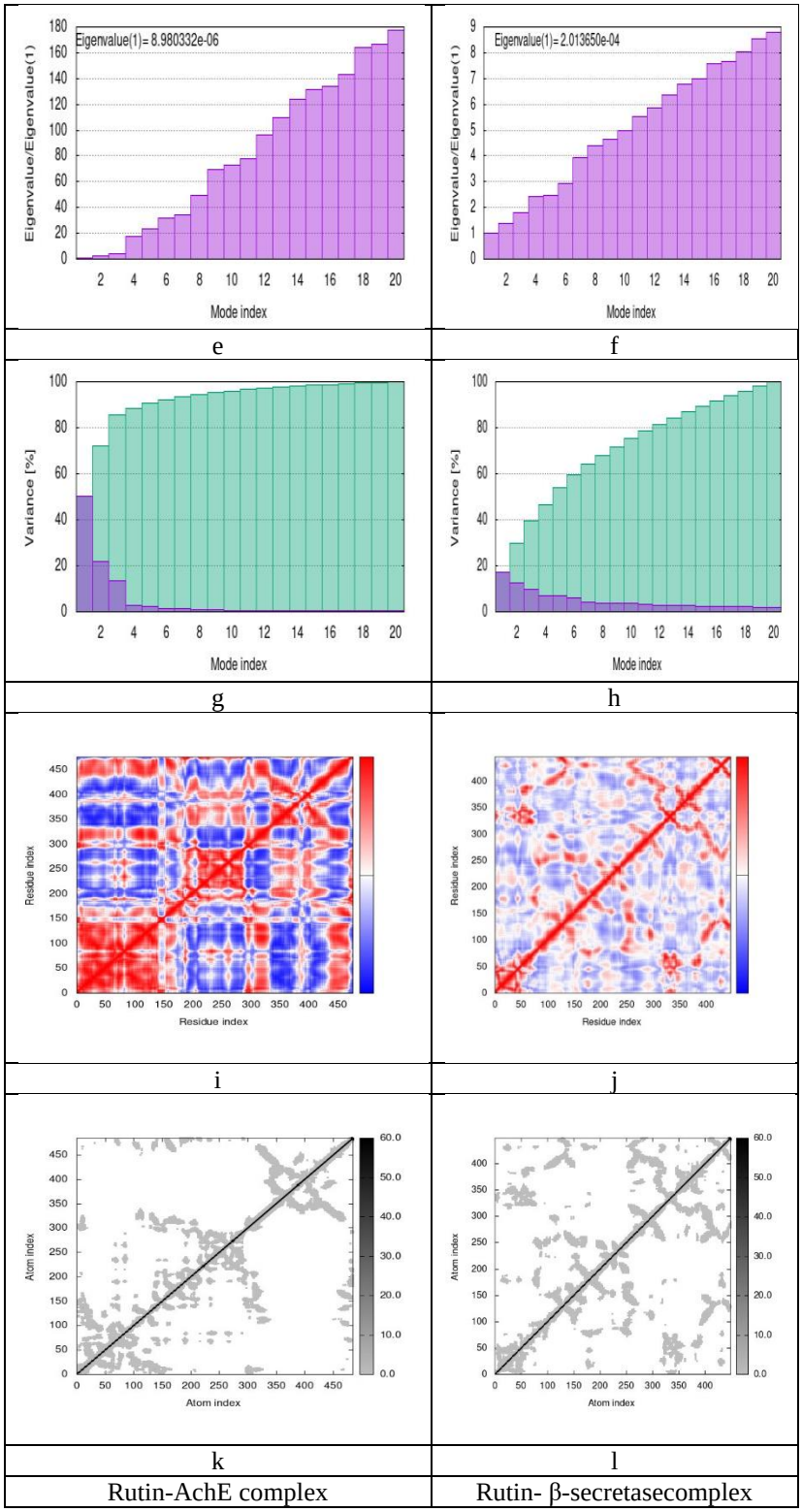


Fig. 6 Ligand rutin interacted with β-secretase enzyme

Molecular dynamics simulation of docked receptor-ligand complexes

The 3D interaction of the DNA gyrase and 14-alpha sterol demethylase inhibitor with squash and kunitz receptor and ligand complexes is depicted in Fig. 7. The deformability graph in Fig. 7 (a, b) demonstrates that improved deformability is particularly noticeable at flexible hinge zones.The NMA B-factor in Fig. 7 represents the mobility profile and relative amplitude of the atomic displacements around the equilibrium conformation (c, d). The eigenvalues (e, f) in Fig. 8 represent motion stiffness, which is highly related to the energy required to deform the structure. The lower the energy, the better the structural deformation. Figure 8 (g, h) displays the variance map, which has the coordinates and is inversely proportional to the eigenvalue. The eigen values for squash and kunitzcomplexed with E protein are 8.980332e-06 and 2.013650e-04, respectively, indicating that squash-E complex is more stable. Figure 8 j) illustrates the covariance matrix between the pairs of residues, demonstrating their interactions (red: correlated, white: uncorrelated, blue: anti-correlated). Figure 7 depicts the elastic network model of amino acid residues (k,l).





Rutin-AchE complex | Rutin- β-secretasecomplex

Fig. 7. Molecular dynamics simulation of the squash and kunitz structures with coupled E proteins. Deformability graphs (a,b), B-factor plots (c,d), eigenvalue plots (e,f), variance map plots (g,h), correlation matrix plots (i,j), elastic network model plots (k,l). Individual (red) and cumulative (green) variances were represented by coloured bars in the correlation matrix. The dots in the elastic network graph are coloured based on their stiffness; darker greys imply stiffer springs and vice versa.

CONCLUSION

The docking results indicated that the possible compounds of *HancorniaSpeciosa*might be promising candidates for further research into Alzheimer's disease. Rutin compounds out of 21 docked to beta-secretase and acetylcholinesterase had the strongest effects against both proteins. Rutin had binding values are 8.5 kcal/mol for AchE and -9.2 kcal/mol for beta secreatase. These molecules also showed exceptional pharmacokinetic characteristics. The prospective use of these drugs against acetylcholinesterase and beta-secretase in therapy might be extremely beneficial. Further research might

provide useful information into the efficacy of these drugs in vivo. Natural chemicals may have a synergistic effect on Alzheimer's disease, according to research.

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