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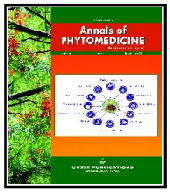

Original article: Open access

In silico identification of novel immunostimulating phytochemicals with acetylcholinesterase inhibition activity from *Piper betle* L. and *Vitex negundo* L. for the treatment of Alzheimer's disease (AD)

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Abstract

Alzheimer's disease (AD) is the most prevalent type of dementia and a worsening neurodegenerative disease. Drugs for AD has severe side effects and the development of new anti-AD medicines are in high demand. The use of medicinal herbs and plants in the treatment of a variety of diseases has become increasingly common in recent years. Thus, the present study focuses on identification of novel phytochemical compounds from *Piper betle* L. and *Vitex negundo* L. showing anti-AD by *in silico* analysis. Human acetylcholinesterase is used as the target enzyme. The compounds were preliminarily screened for druglikeness analysis (RO5). Molecular docking, protein-ligand interaction and ADMET analysis were carried out to identify the potential compounds. Total of 42 compounds were identified and 34 compounds showed druglikeness properties. About 22 compounds showed higher binding energies, *i.e.*, >-7 Kcal/mol and these compounds were analysed for interactions on binding sites of AChE. ADMET analysis was performed and compared with a standard drug rivastigmine. Total of 20 novel compounds, Piperine in *P. betle*, beta-Sitosterol, beta-Caryophyllene, Acerosin, Casticin, Mearnsetin, 5,3'-Dihydroxy-6,7,4'-trimethoxyflavanone, 5,3'-Dihydroxy-7,8,4'-trimethoxyflavanone, Detetrahydroconidendrin, Negundin A, Negundin B, Vitrofolal E, Vitrofolal F, Vitedoamine A, Vitedoin A, Vitedoin B, Vitexdoin A, Vitexdoin B, Vitexdoin C, Vitexdoin D and Vitexdoin E present in *Vitex negundo* were identified to be the effective acetylcholinesterase inhibitors. These compounds have potential to be developed as anti-AD drugs with higher efficacy and lesser side effects.

1. Introduction

Alzheimer's disease (AD) is the most prevalent type of dementia and a worsening neurodegenerative disease that causes patients to experience cognitive dysfunction, psychosis, hyperactivity, physical aggression, and anxiety (Lane *et al*., 2018). The global dementia population was estimated to be 35.6 million in 2010, and it is expected to almost doubled over the past 20 years, to be nearly 65.7 million in 2030 and will be 115.4 million in 2050. Every year, almost 7.7 million new cases of dementia are diagnosed worldwide, meaning one case every four seconds. Most of the rise will occur in developing countries, with China, India, and their south Asian and western Pacific neighbours seeing the highest growth in the elderly population. According to the United Nations, Europe had an estimated 10 million disease cases in 2010, and this number is expected to grow to 14 million by 2030. Looking at these statistics, it is obvious that something has to be done now. As the world's population ages, Alzheimer's disease (AD) has become a significant public health issue. People aged 60 and up are expected to make up 22% of the global population by 2050, with four-fifths residing in Asia, Latin America or Africa (Prince *et al*., 2015).

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While the exact immunological event that triggers Alzheimer's disease (AD) has yet to be identified, some studies suggest that AD as an autoimmune disease. A common denominator associated with various levels of dementia, including Alzheimer's disease, is age-related vascular disorders, which have an impaired blood brain barrier (BBB). A crucial discovery recently revealed not only the unusual existence of immunoglobulin detection in the subarachnoid space of AD tissues, but also that certain neurons with degenerative, apoptotic features contained these vascular-derived antibodies. In addition, classical complement constituents C1q and C5b-9 were found in these Ig-positive neurons, which were also spatially more correlated with reactive microglia than the Ig-negative neurons. Thus, the increased presence of anti-neuronal autoantibodies in the serum, whose significance had previously been overlooked, could be without pathological impact before the BBB is disrupted, allowing the autoantibodies' deleterious effects to reach their targets. As a result, these findings point to autoimmunity-induced cell death in Alzheimer's disease (D'Andrea, 2005; Schnabel, 1993).

Acetylcholinesterase (AChE) inhibitors including rivastigmine, galantamine and donepezil block AChE's effects in the synapse, causing, bradycardia, nausea, vomiting, anorexia and diarrhoea as side effects. Memantine is an N-methyl D-aspartate potent inhibitor that has been approved for the treatment of moderate-to-severe Alzheimer's disease in Europe and the United States, but it can cause headaches, dizziness, nausea, insomnia, constipation, mild allergies and auditory hallucinations. The development of new anti-

AD medicines is in high demand. The use of medicinal herbs and plants in the treatment of a variety of diseases has become increasingly common in recent years. According to the World Health Organization (WHO), 85 per cent to 90 per cent of the global population depend on natural medicine to handle their healthcare needs (Das *et al*., 2017; Kareti and Pharm, 2020).

Therefore, the present study concentrates on identification of novel phytochemical compounds from *P. betle* and *V. negundov* showing anti-Alzheimer's disease by *in silico* analysis. Human acetylcholinesterase is used as the target enzyme. The compounds were preliminarily screened for druglikeness analysis based on Lipinski's rule of Five (RO5). Molecular docking, protein-ligand interaction and ADMET analysis were carried out.

2. Materials and Methods

2.1 Preparation of target proteins

The Human acetylcholinesterase (PDB ID: 4M0E) was used as target proteins in this research and the 3D structures were retrieved from the Protein Data Bank (http://www.rcsb.org/). Using PyMol tool, the protein is visualized and then the protein bound water molecules, ligands and co-crystal ligands were eliminated (Figure 1). Further, protein was prepared in Auto Dock Tools, an open source software by introducing charges and energy minimization in Swiss PDB viewer and then converted to pdbqt format.

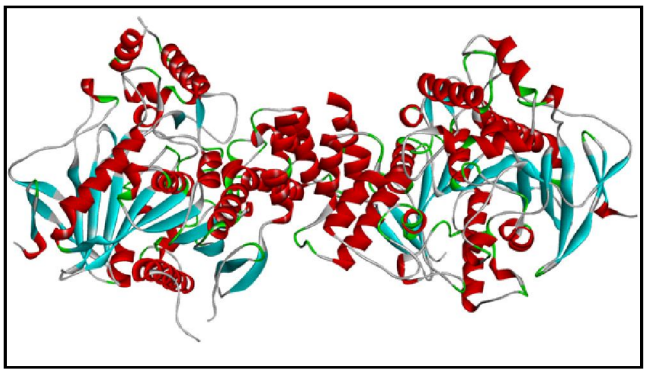

Figure 1: 3D structures of the human acetylcholinesterase.

2.2 Selection and preparation of ligands

The phytochemical compounds present in *P. betle* and *V. negundo* were identified and retrieved using KNApSAck database (http://www.knapsackfamily.com/KNApSAcK/). Total of 42 phytochemical compounds were used for the study. The preparation of ligand is carried out by detecting their torsion root, assigning charges, correcting the torsion angle, optimizing using UFF (universal force field) and then finally converted into pdbqt format to generate 3D atomic coordinates of the molecules (Rédei, 2008).

2.3 Identification of active sites of target proteins

Potential docking analysis requires accurate assessment of the active site. The amino acids in the active pocket site formation for target proteins were identified using the CASTp server (Computed Atlas for Surface Topography) (Sanjay Prasad and Shanthi, 2020; Tian *et al*., 2018). CASTp is a simple and useful online tool to analyze the protein topology and active site pockets. Active site determination is a vital part to set the grid box before docking.

2.4 Screening of the of the ligands based on druglikeness

The druglikeness of the compounds are evaluated using the online server Swiss ADME (http://swissadme.ch/index.php). Druglikeness of a compound is a necessary parameter to validate them as potential ligands against therapeutic targets (Daina *et al*., 2017). 42 phytochemical compounds were screened using the Lipinski's Rule of Five and compounds showing druglikeness were used for docking studies.

2.5 Molecular docking and protein-ligand interaction analysis

The molecular docking of all the compound libraries was conducted using the PyRx tool by autodock wizard as the docking engine. Throughout, the docking process the ligands were assumed to be flexible and the protein was expected to be rigid. The grid parameter configuration file is generated using the grid box for 4M0E (x = 0.07, y = -44.36, z = 11.67) in PyRx, respectively (Dallakyan and Olson, 2015). After docking, the highest biding energy (most negative) was identified as the ligand with maximum binding affinity. The ligands exhibiting higher binding energy (<-7 Kcal/mol) were recognized and the ligand-protein interaction on the binding sites were analysed using Biovia Drug discovery studio 2020.

2.6 ADMET analysis of the selected ligands

ADMET analysis involves evaluation of absorption, distribution, metabolism, excretion and toxicity levels of the selected compounds using online based algorithms. There are numerous online database and offline software applications which helps in predicting the drug candidates behaviour. In this study, we have used admet SAR (Cheng *et al*., 2012) for ADMET predictions. The compounds showing higher binding energies were examined for its Human intestinal absorption, *in vivo* blood-brain barrier penetration, *in vitro* Caco-2 cell permeability, CYP450 2C9 substrate and toxicity parameters like mutagenicity by AMES test and carcinogenicity on rat were determined. Broad spectrum antibacterial drugs, amoxicillin and ciprofloxacin are used as a standard drug to compare with the compounds.

3. Results

3.1 Druglikeness profiling of compounds

The molecular and physical properties of the compounds play a key role in the identification of certain agents as a drug candidate. The compounds were filtered *via* Lipinski's five (Ro5) law to predict druglikeness. Lipinski's rule of five (Ro5) is a valuable parameter for determining the molecular properties of drug compounds and to estimate the essential pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion for drug design and development (Lipinski *et al*., 2012). From this analysis, 42 out of 36 phytochemical compounds satisfied Lipinski's rule of five and the values were presented in the Table 1.The screened 46 compounds were subjected to molecular docking analysis.

3.2 Binding site analysis and Molecular docking

Active site pocket in Human acetylcholinesterase was determined using CASTp. CASTp is a web-based tool to determine the amino acid residues in the active pocket of the proteins. CASTp results are depicted in Figure 2 for Human acetylcholinesterase. From CASTp results, only the amino acids in the active site and their positions are listed as Table 2. Grid box were generated covering the binding sites of the target protein.

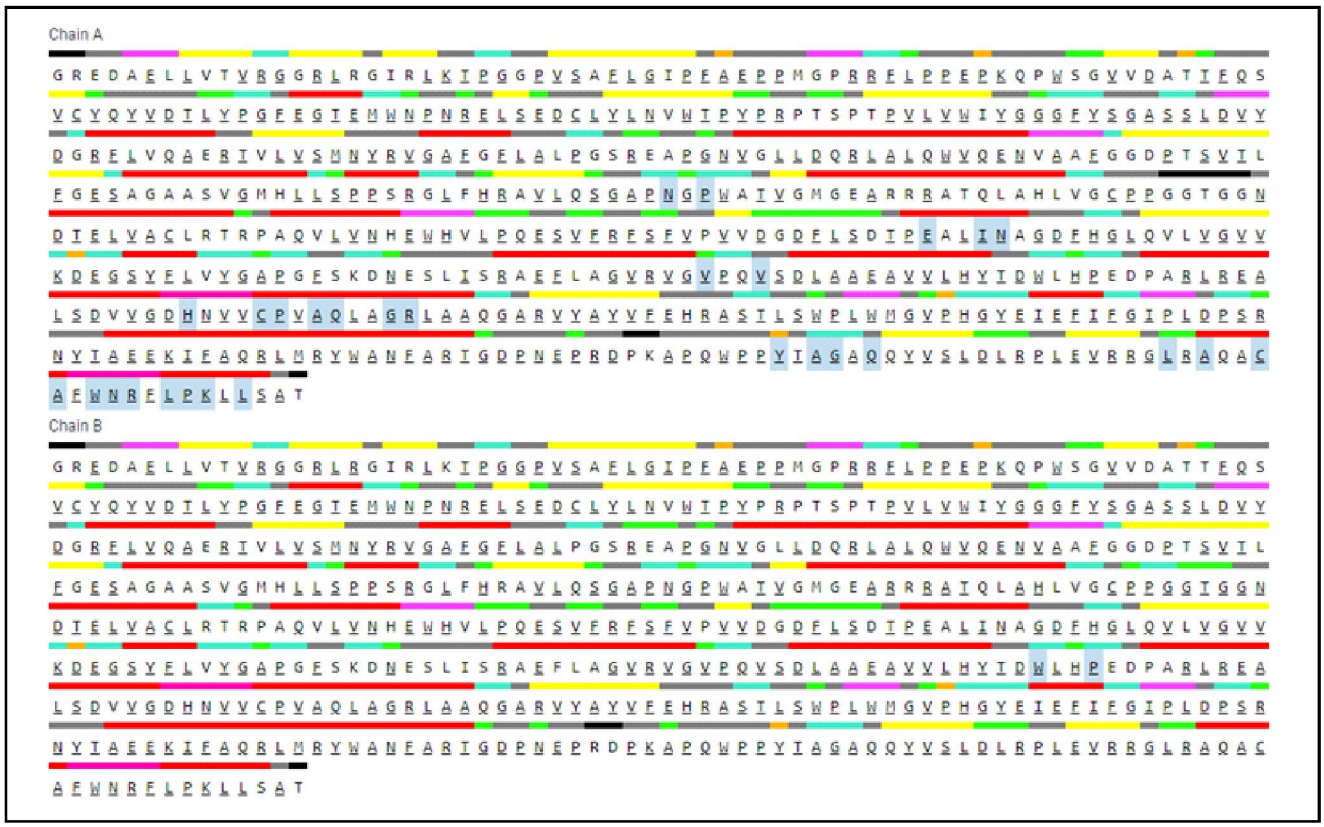

Figure 2: Binding sites of human acetylcholinesterase analysed using CASTp.

Table 1: Compounds showing druglikeness properties

S.No.	Compound name	S.No.	Compound name
1	Carvacrol	18	Mearnsetin
2	Eugenol	19	(+)-Lyoniresinol
3	Chavicol	20	5,3'-Dihydroxy-6,7,4'-trimethoxyflavanone
4	Octadecanoic acid	21	5,3'-Dihydroxy-7,8,4'-trimethoxyflavanone
5	Triacontane	22	Detetrahydroconi-dendrin
6	Piperine	23	Negundin A
7	Methyl chavicol	24	Negundin B
8	beta-Sitosterol	25	Vitrofolal E
9	Cepharadione A	26	Vitrofolal F
10	Dotriacontanoic acid	27	Vitedoamine A
11	Piperlonguminine	28	Vitedoin A
12	(+)-alpha-Pinene	29	Vitedoin B
13	4-hydroxybenzoic acid	30	Vitexdoin A
14	Camphene	31	Vitexdoin B
15	beta-Caryophyllene	32	Vitexdoin C
16	Acerosin	33	Vitexdoin D
17	Casticin	34	Vitexdoin E

Table 2: Amino acid residues in the active sites

S.No	Target protein	Amino acid residues in binding sites
1	Human acetylcholi-nesterase (PDB ID: 4M0E)	A: ASN-233, PRO-235, GLU-313, ILE-316, ASN-317, VAL-367, VAL-370, HIS-405, CYS-409, PRO-410, ALA-412, GLN-413, GLY-416, ARG-417, TYR-503, ALA-505, GLY-506, 508-GLN, LEU-524, ALA-526, CYC-529, ALA-530, TRP-532, ASN-533, ARG-534, LEU-536, LEU-536, PRO-537, LYS-538, LEU-540B: TRP-385, PRO-388

PyRx was used for performing docking analysis for all 46 compounds against their target proteins tyrosyl-tRNA synthetase and DNA gyrase. Binding energies of the compounds were analyzed and the compounds showing higher binding energy (<-7.0 Kcal/mol) against the target enzyme acetyl cholinesterase were identified. 34 compounds showed significant binding energy (<-7.0 Kcal/mol) for both the targets and the compounds are shown in the Table 3.

3.3 Protein-ligand interaction analysis

The best-docked compounds were further analyzed for binding interactions with amino acid residues using Biovia Accelrys Discovery Studio Visualizer software. Bonding type, number of hydrogen bonds and hydrophobic interactions are a very important determinant of protein-ligand interactions as well as binding affinity.

Table 3: Binding energies of the compounds against the acetylcholinesterase

S. No.	Compound name	Binding energies (Kcal/mol)
1	Carvacrol	–5.9
2	Eugenol	–6.4
3	Chavicol	–5.8
4	Octadecanoic acid	–4.9
5	Triacontane	–4.2
6	Piperine	–7.0
7	Methyl chavicol	–5.5
8	beta-Sitosterol	–7.8
9	Cepharadione A	–7.5
10	Dotriacontanoic acid	–5.3
11	Piperlonguminine	–6.4
12	(+)-alpha-Pinene	–5.7
13	4-hydroxybenzoic acid	–5.9
14	Camphene	–5.3
15	beta-Caryophyllene	–7.0
16	Acerosin	–7.1
17	Casticin	–7.0
18	Mearnsetin	–7.1
19	(+)-Lyoniresinol	–6.5
20	5,3'-Dihydroxy-6,7,4'-trimethoxy-flavanone	–7.3
21	5,3'-Dihydroxy-7,8,4'-trimethoxy-flavanone	–7.3
22	Detetrahydroconidendrin	–7.9
23	Negundin A	–8.1
24	Negundin B	–7.2
25	Vitrofolal E	–7.4
26	Vitrofolal F	–7.2
27	Vitedoamine A	–7.8
28	Vitedoin A	–7.0
29	Vitedoin B	–7.2
30	Vitexdoin A	–7.4
31	Vitexdoin B	–7.4
32	Vitexdoin C	–7.4
33	Vitexdoin D	–7.5
34	Vitexdoin E	–7.3

The number of hydrogen bonds formed and amino acids involved in the interactions are tabulated in Table 4. The hydrogen bonds and other hydrophobic interactions of the ligands on the binding sites of the target proteins were shown in the Figures 3 to 24. All the 22 compounds showed H-bond formation on binding sites of the target proteins except beta-caryophyllene. This compound failed to show H-bond on binding sites of Human acetylcholinesterase. Therefore, the remaining 21 compounds were subjected to ADMET analysis to find out significant compounds for development of novel drugs for treatment of AD.

Table 4: Protein-ligand interactions

S.No.	Compound name	Ligand interaction on binding sites of target protein	
		No. of H-bond	Binding amino acid residue
1	Piperine	2	TYR-503, ARG-417
2	beta-Sitosterol	1	GLN-413
3	Cepharadione A	1	HIS-405
4	beta-Caryophyllene	-	-
5	Acerosin	4	GLN-413, ARG-417, TYR-503, ARG-534
6	Casticin	2	GLU-313, LYS-538
7	Mearnsetin	2	HIS-405, GLN-413
8	5,3'-Dihydroxy-6, 7,4'-trimethoxy flavanone	1	TYR-503
9	5,3'-Dihydroxy-7, 8,4'-trimethoxy-flavanone	1	GLU-313
10	Detetrahydroconi-dendrin	2	ASN-233, GLN-413, ASN-533
11	Negundin A	2	GLU-313, ASN-533
12	Negundin B	3	GLU-313, HIS-405, ASN-533
13	Vitrofolal E	4	GLU-313, HIS-405, GLN-413, ASN-533
14	Vitrofolal F	1	HIS-405
15	Vitedoamine A	1	HIS-405
16	Vitedoin A	2	TRP-532, ASN-533
17	Vitedoin B	1	HIS-405
18	Vitexdoin A	1	PRO-235
19	Vitexdoin B	1	GLU-313
20	Vitexdoin C	1	GLN-413
21	Vitexdoin D	1	HIS-405
22	Vitexdoin E	2	HIS-405, TRP-532

Table 5: ADME analysis of the selected compounds

S.No.	Compound name	*In vivo* blood-brain barrier penetration (C.brain / C. blood)	Human intestinal absorption (%)	*In vitro* Caco-2 cell permeability (nm/sec)	Distribution	CYP450 2C9
1	Rivastigmine	0.99	0.99	0.77	0.82	0.84
2	Piperine	0.99	1.00	0.64	0.68	NS (0.88)
3	beta-Sitosterol	0.97	1.00	0.79	0.46	NS (0.84)
4	Cepharadione A	0.91	0.98	0.65	0.42	NS (0.80)
5	Acerosin	0.68	0.96	0.87	0.63	NS (0.75)
6	Casticin	0.59	0.98	0.89	0.79	NS (0.78)
7	Mearnsetin	0.63	0.97	0.88	0.64	NS (0.73)
8	5,3'-Dihydroxy-6,7,4'-trimethoxyflavanone	0.61	0.96	0.84	0.79	NS (0.75)
9	5,3'-Dihydroxy-7,8,4'-trimethoxyflavanone	0.61	0.96	0.84	0.79	NS (0.75)
10	Detetrahydroconidendrin	0.70	0.99	0.78	0.85	NS (0.75)
11	Negundin A	0.63	0.98	0.71	0.81	NS (0.74)
12	Negundin B	0.84	0.98	0.66	0.82	NS (0.79)
13	Vitrofolal E	0.71	0.99	0.88	0.90	NS (0.77)
14	Vitrofolal F	0.65	0.99	0.87	0.87	NS (0.79)
15	Vitedoamine A	0.84	1.00	0.50	0.76	NS(0.76)
16	Vitedoin A	0.92	1.00	0.78	0.84	NS (0.80)
17	Vitedoin B	0.92	0.99	0.56	0.75	NS (0.78)
18	Vitexdoin A	0.92	0.98	0.61	0.64	NS (0.78)
19	Vitexdoin B	0.96	0.99	0.61	0.82	NS (0.78)
20	Vitexdoin C	0.71	0.99	0.88	0.90	NS (0.77)
21	Vitexdoin D	0.65	0.99	0.87	0.87	NS (0.79)
22	Vitexdoin E	0.65	0.99	0.87	0.87	NS (0.79)

*NS-non substrate

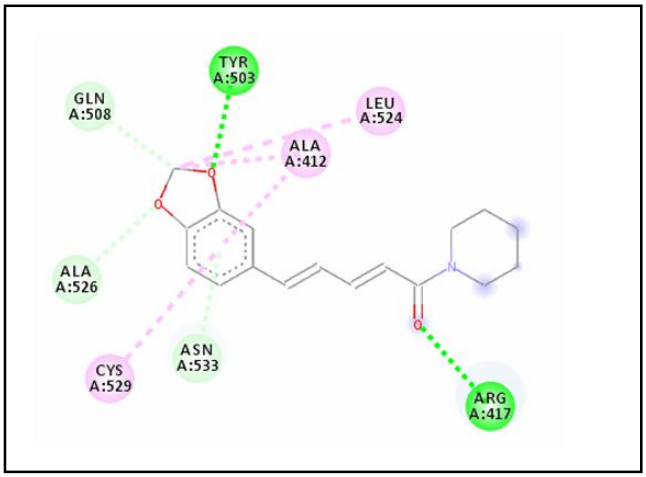

Figure 3: Interaction of piperine on acetylcholinesterase.

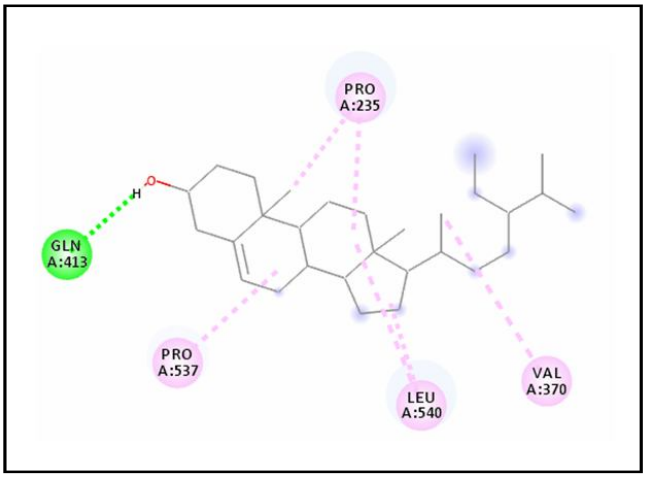

Figure 4: Interaction of beta-sitosterol on acetylcholinesterase.

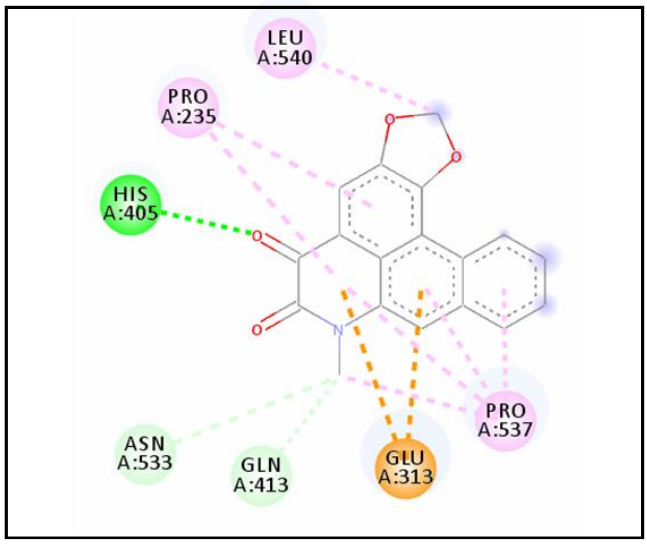

Figure 5: Interaction of cepharadione A on acetylcholinesterase.

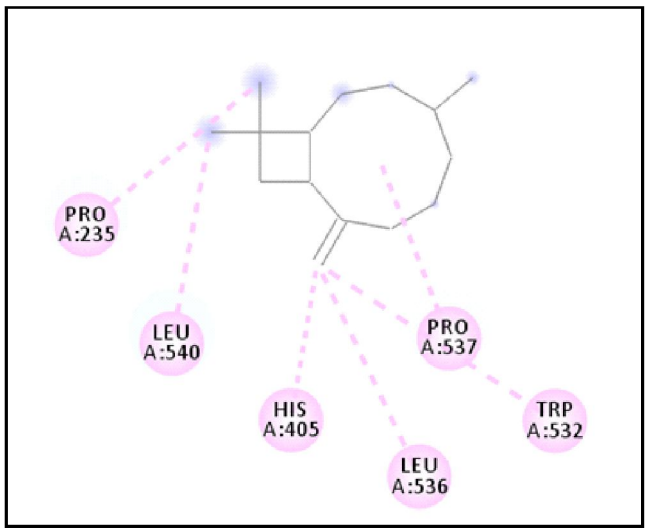

Figure 6: Interaction of beta-caryophyllene on acetylcholinesterase.

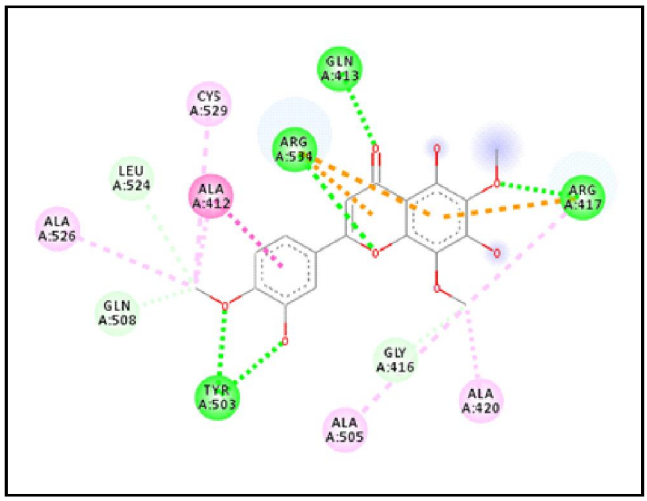

Figure 7: Interaction of acerosin on acetylcholinesterase.

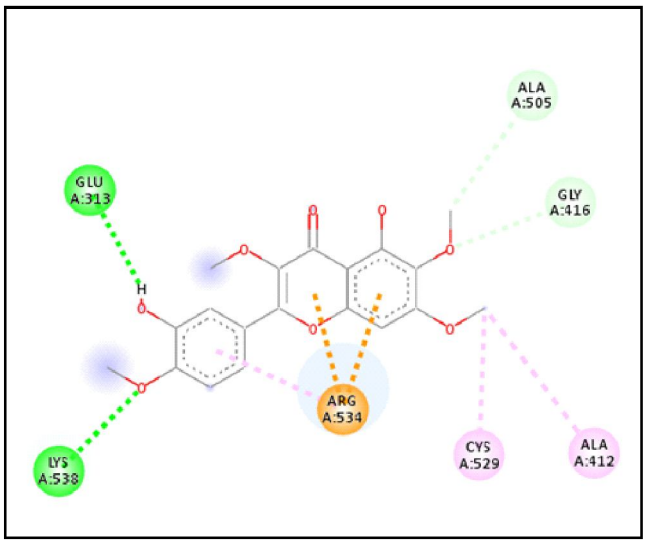

Figure 8: Interaction of casticin on acetylcholinesterase.

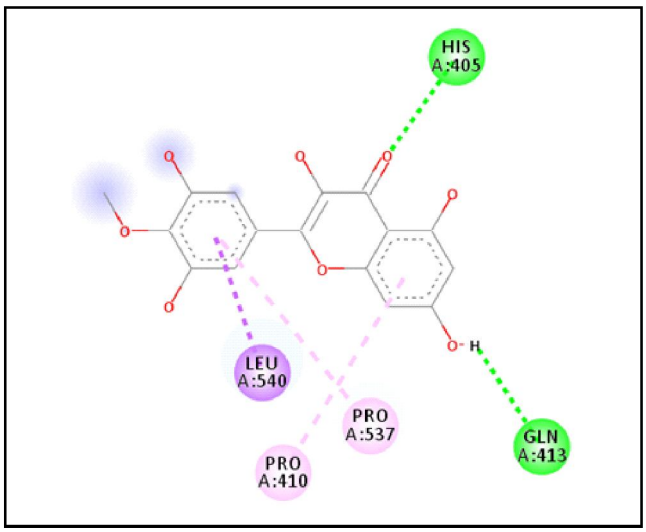

Figure 9: Interaction of mearnsetin on acetylcholinesterase.

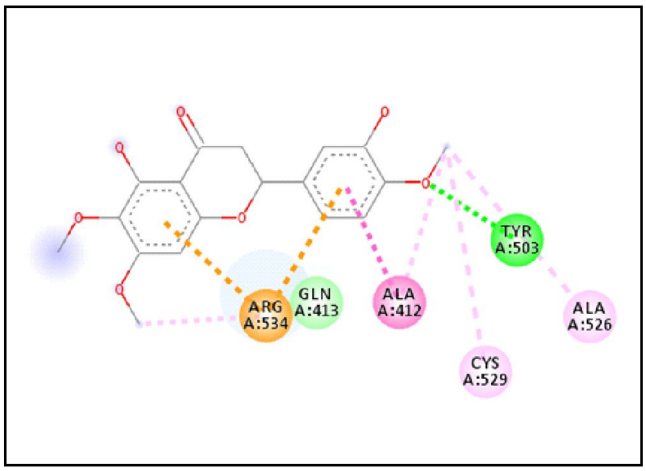

Figure 10: Interaction of 5,3'-dihydroxy-6,7,4'-trimethoxyflavanone on acetylcholinesterase.

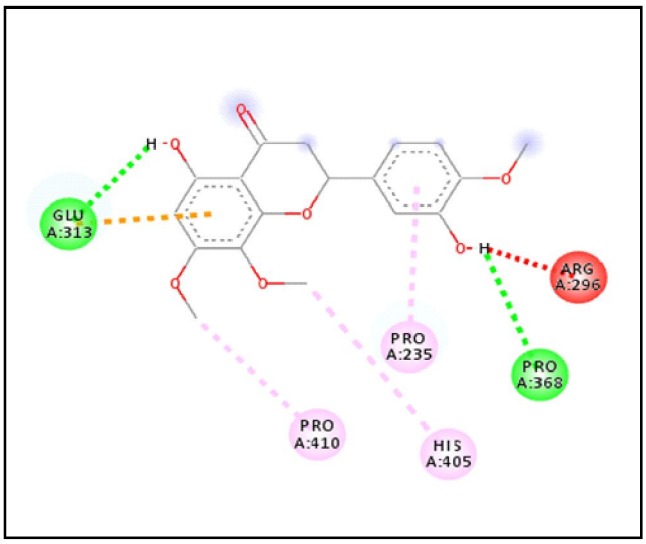

Figure 11: Interaction of 5,3'-dihydroxy-7,8,4'-trimethoxyflavanone on acetylcholinesterase.

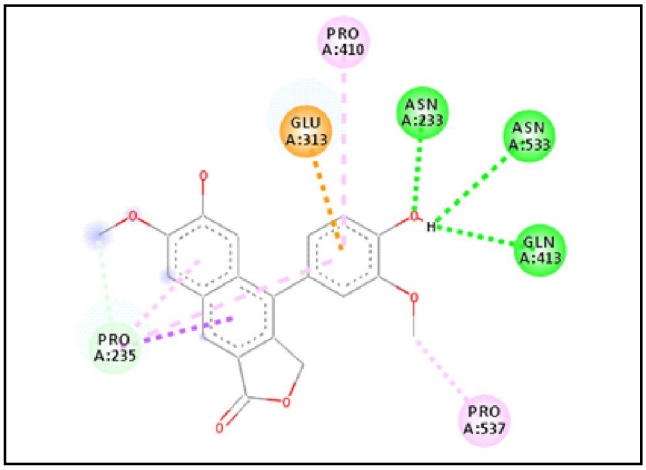

Figure 12: Interaction of detetrahydroconidendrin on acetyl cholinesterase.

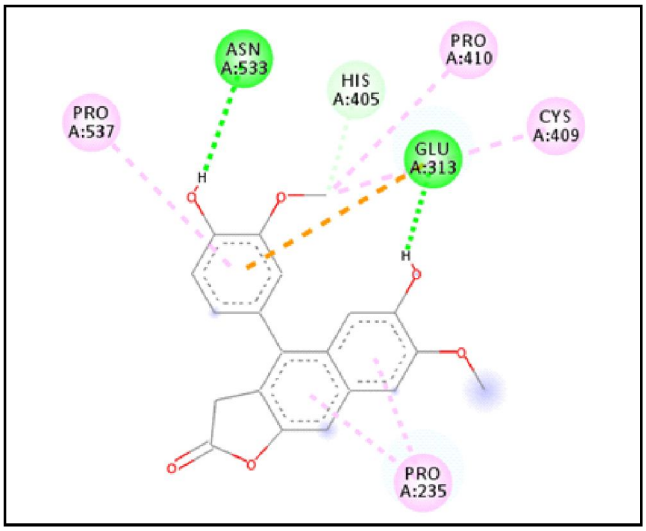

Figure 13: Interaction of negundin A on acetylcholinesterase.

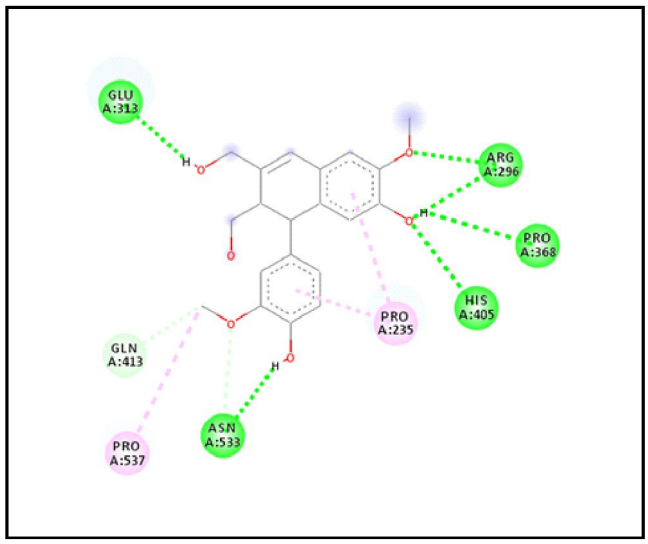

Figure 14: Interaction of negundin B on acetylcholinesterase.

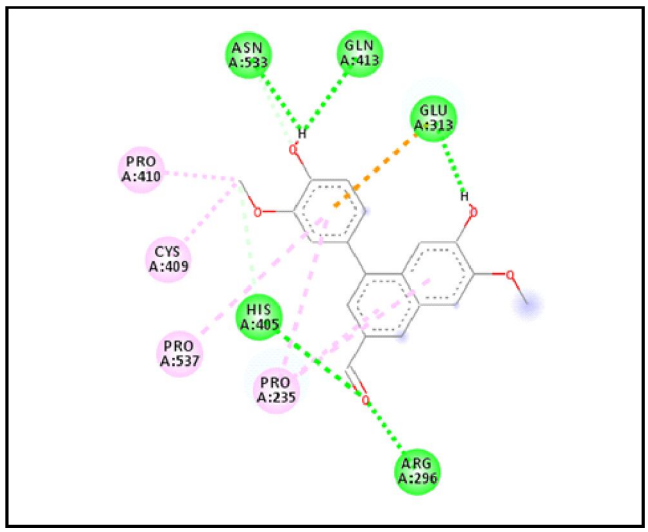

Figure 15: Interaction of vitrofolal E on acetylcholinesterase.

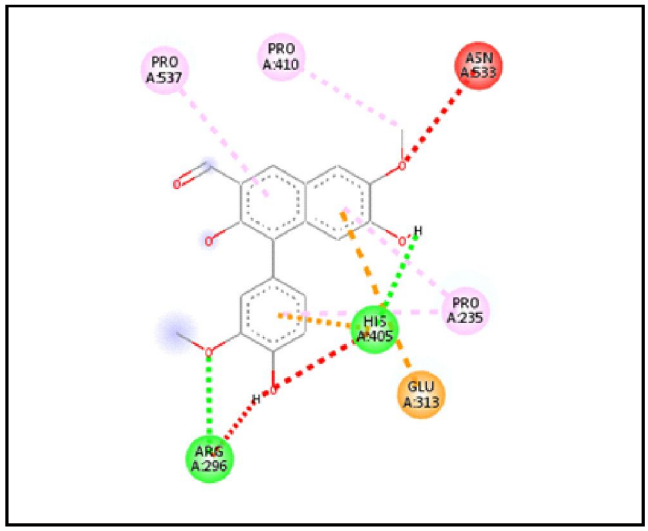

Figure 16: Interaction of vitrofolal F on acetylcholinesterase.

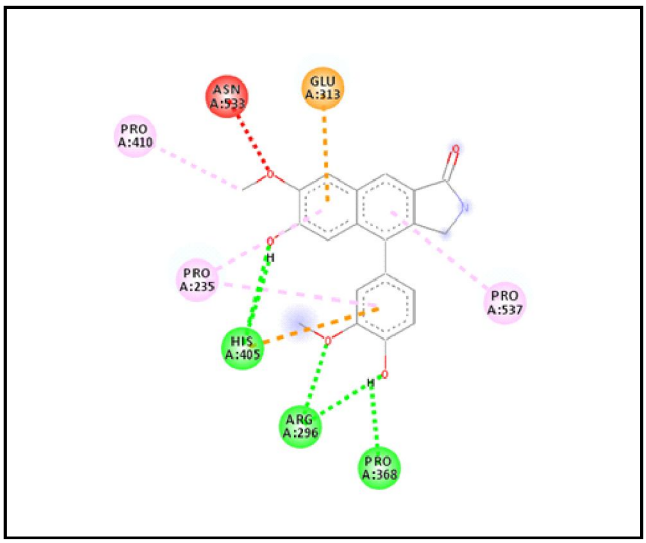

Figure 17: Interaction of vitedoamine A on acetylcholines terase.

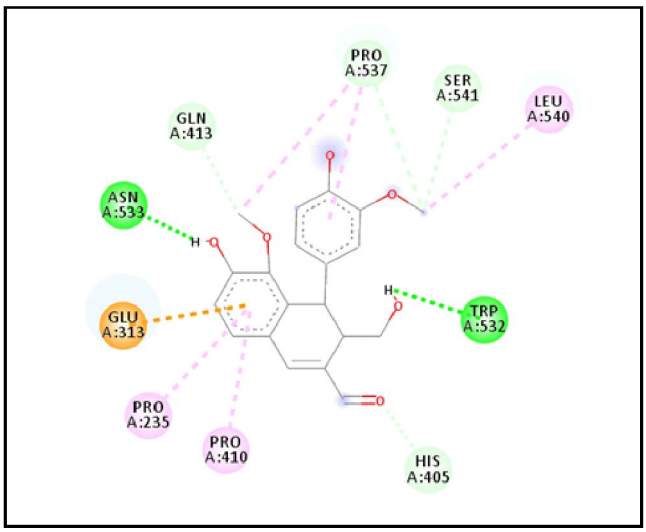

Figure 18: Interaction of vitedoin A on acetylcholinesterase.

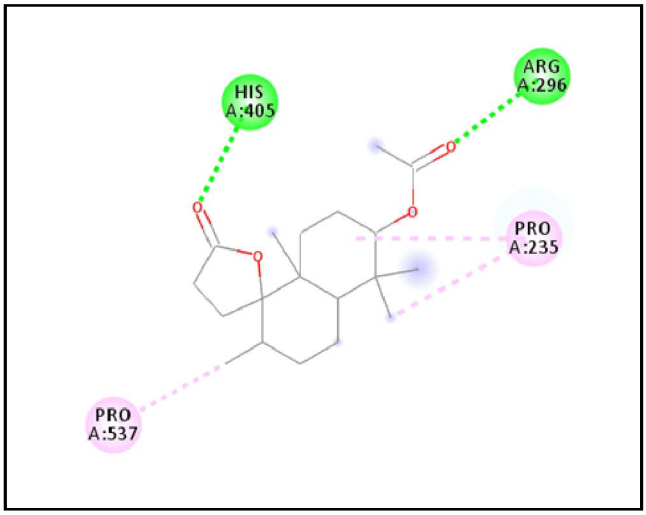

Figure 19: Interaction of vitedoin B on acetylcholinesterase.

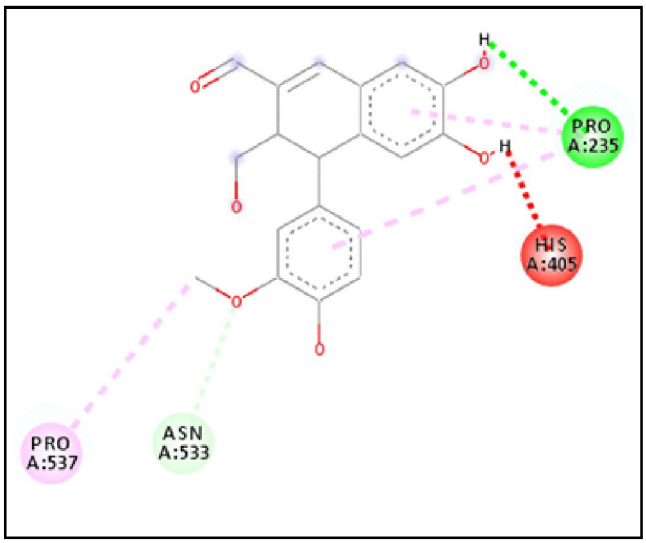

Figure 20: Interaction of vitexdoin A on acetylcholinesterase.

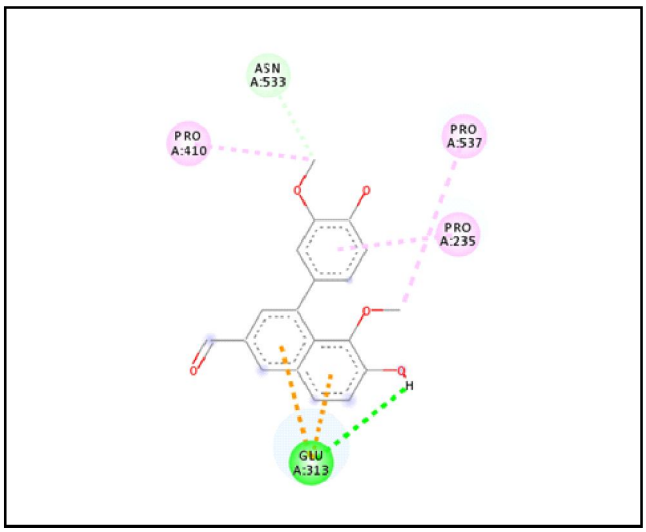

Figure 21: Interaction of vitexdoin B on acetylcholinesterase.

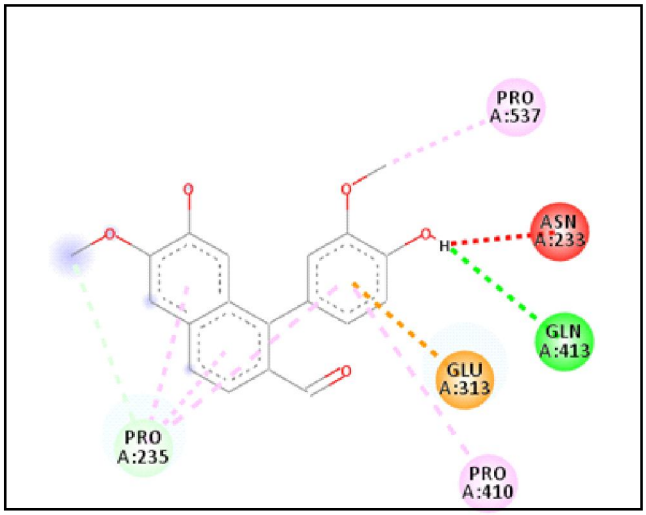

Figure 22: Interaction of vitexdoin C on acetylcholinesterase.

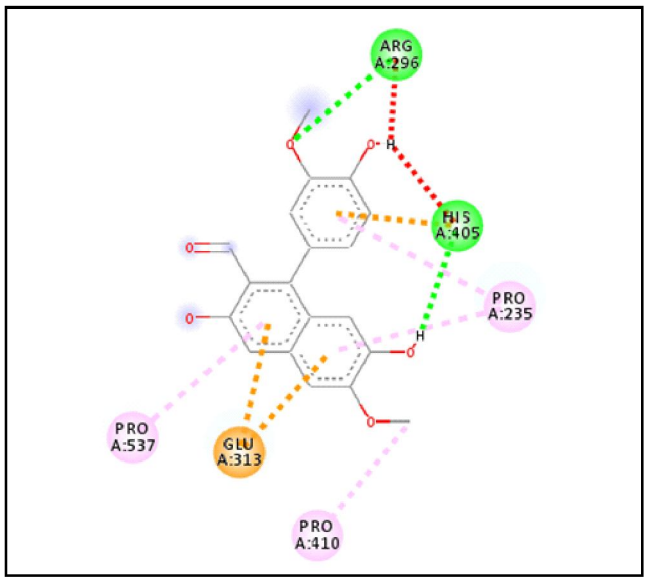

Figure 23: Interaction of vitexdoin D on acetylcholinesterase.

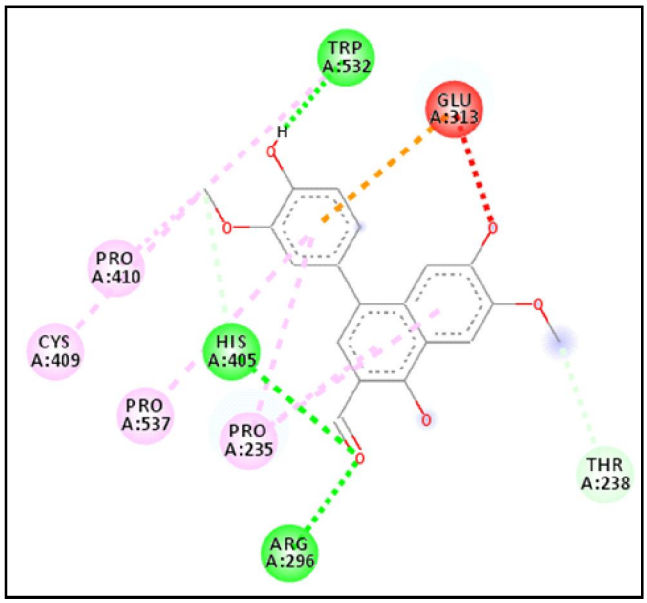

Figure 24: Interaction of vitexdoin E on acetylcholinesterase.

3.4 ADMET properties

ADMET properties of the compounds interact with the absorption, distribution, metabolism, excretion and toxicity in and across the human body. ADMET defines the pharmacokinetic properties of the drug molecule and it is very important in assessing its pharmacodynamic activity. Rivastigmine is used as control drug for comparison. Rivastigmine showed BBB, HIA, *In vitro* Caco-2 permeability, distribution and non-substrate of CYP450 2C9 values of 0.99, 0.99, 0.77, 0.82 and 0.84. Other compounds like piperine, beta-Sitosterol, negundin B, vitedoin A and vitexdoin B showed significant ADME values (Table 5). From the toxicity analysis, AMES toxicity and mutagenicity of the compounds were determined. All the compounds were found to be non-mutagenic and all the compounds except cepharadione A were non-AMES toxic.

4. Discussion

Reduced levels of acetylcholine (Ach) and the loss of cholinergic neurons in the brain are linked to the Alzheimer's disease (AD). Ach was the first neurotransmitter found in the central nervous system, and it transmits synaptic signals to all autonomic ganglia, including the neuromuscular junction and synapses. The signal transmission between preganglionic sympathetic and parasympathetic neurons in the autonomic nervous system is regulated by Ach. It is also responsible for the stimulation of muscles, including those of the gastrointestinal tract. The loss of Ach function has been linked to the progression of Alzheimer's disease (Kareti and Pharm, 2020). Natural neurotransmission is hampered by acetylcholinesterase (AChE), an enzyme that breaks down the neurotransmitter Ach into acetate and choline. According to the cholinergic theory of illness, inhibiting AChE activity may be one of the more practical approaches to symptomatic treatment of AD. AChE is one of the most important targets in the fight against Alzheimer's disease (Giacobini, 2004). Thus, AChE is used as the target protein in the present study. The 3D structure of the protein was downloaded from protein databank. The active sites of the proteins were identified by using CASTp online server. Since active sites are the functional sites of the proteins, it is necessary for a ligand docking to be on the binding sites. To evaluate this ideology, ligand interactions on binding sites of target proteins was examined using Discovery studio visualizer 2020.

P. betle and *V. negundo* were plants used for the study. *P. betle*, also known as betel leaf, is a plant with heart-shaped leaves. *Piperaceae*, also known as the pepper family, is a large flowering plant family found in tropical and subtropical regions of the world. *P. betle* is a native of Malaysia's central and eastern regions. India, Malaysia, Indonesia, Sri Lanka, the Philippines, China and Vietnam are all home to this species. Climbing herbs and shrubs make up the majority of the plants in this genus. Piper is the only genus in this family of commercial value. *P. betle* or Paan's leaves, fruits and roots are stimulant, carminative, antiseptic, and used to treat malaria. Antiseptic, antioxidant, analgesic, antibacterial, cardiotonic, antispasmodic, expectorant, tonic, carminative, contraceptive and litholytic properties are all found in betel leaf. An aromatic volatile oil is found in betel leaves. This essential oil has antihypertensive, anticardiac, antirespiratory, and cardiotonic properties (Baviskar *et al*., 2017; Dwivedi and Tripathi, 2014).

The Five-Leaved Chaste Tree (*V. negundo*) is a medicinal deciduous shrub with five leaves. It is a native of India, but it can also be found in the Philippines, Bangladesh, Sri Lanka, China and Japan. Ayurveda, Homeopathy, Unani, Allopathy and Siddha use various parts of the tree for medicinal purposes, including leaves, seeds, leaf oil, fruits and roots. It is used to treat headaches, skin irritations, bruises, inflammation, asthmatic pains, and both male and female sexual and reproductive issues. Leaves are used to treat inflammatory swellings of the joints caused by acute rheumatism, as well as swellings of the testes caused by gonorrhoeal epididymitis and orchitis. During a headache, a paste of leaves is added to the temples. The juice of the leaves was applied topically to wounds and ulcers to remove foul smelling discharge. Leaves were also be useful as muscle relaxants, pain relievers, antianxiety, anti-asthmatic, and phlegm reducers (Basri *et al*., 2014; Nishtha and Kaur, 2020). As these plants enhance the immune system and prevents from several disorders, these plants can also be used as immuno-stimulants.

Phytochemical compounds present in these plants were identified by using KNApSAck database. Total of 42 compounds were identified 34 compounds showed druglikeness based on the Lipinski's Rule of Five (RO5). Molecular docking was performed and compounds showing highest binding energy, *i.e.*, >-7 Kcal/mol was selected for further analysis. Nearly, 22 compounds showed higher binding energies. All the compounds except beta-caryophyllene showed H-bond formation on binding sites of the target protein (AChE). From the ADMET analysis, all the compounds showed significant ADME properties on comparison with rivastigmine. Cepharadione A was found to be non-AMES toxic.

5. Conclusion

From the analysis, 20 compounds were found to be significant and possess good ADMET properties. Piperine present in *P. betle* and beta-Sitosterol, beta-Caryophyllene, Acerosin, Casticin, Mearnsetin, 5,3'-Dihydroxy-6,7,4'-trimethoxyflavanone, 5,3'-Dihydroxy-7,8,4'-trimethoxyflavanone, Detetrahydroconidendrin, Negundin A, Negundin B, Vitrofolal E, Vitrofolal F, Vitedoamine A, Vitedoin A, Vitedoin B, Vitexdoin A, Vitexdoin B, Vitexdoin C, Vitexdoin D,Vitexdoin E present in *V. negundo* were identified to be the effective acetylcholinesterase inhibitors. Thus, these compounds can be developed as potent drugs and immuno-stimulants for the treatment of Alzheimer's disease.

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Conflict of interest

The author declares that there are no conflicts of interest relevant to this article.

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