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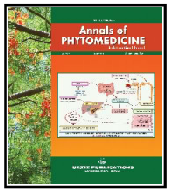

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Molecular docking studies of some natural phytochemical constituents as acetylcholinesterase and butyrylcholinesterase inhibitors

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Abstract

One of the ten major diseases that are the primary causes of death that cannot be slowed, treated, or prevented is Alzheimer's disease (AD); a form of neurodegenerative disease. According to the 2018 senses, AD affects persons over 60 years of age, and around 1 in 5 people worldwide have been diagnosed with the disease. It causes 60-70% of dementia cases and early senility, which is defined as having or exhibiting the vulnerability of an old age disease, particularly a loss of mental capabilities. These conditions lead to difficulties in later life. Pre-research investigations often employ molecular docking (MD), one of the most popular methods for analysing molecular interactions. The objective of this work is to perform molecular docking of phytoconstituents tomatine, guavanoic acid, isocaryophyllene, and beta-caryophyllene with the target enzymes, butylcholinesterase (BchE, 5DYT) and acetylcholinesterase (AchE, 4MoE), which have been associated in the progression of Alzheimer's disease. It also involves the comparison of phytoconstituents docking ability with the standard drugs used for treating the Alzheimer's disease (AD).

1. Introduction

Molecular docking (MD) is the 3-dimensional approach of predicting the strength of two molecules; namely, ligand and protein. MD now a days becoming an integral aspect in drug discovery and development area (Meng *et al.,* 2011; Mukesh and Rakesh, 2011). MD aims to find the active moiety that fits exactly to the target and produces the repose (Berry *et al.,* 2015**)**. MD plays a key role in finding the active constituent in various pharmaceutical (Perola *et al.,* 2004; Hartshorn *et al.,* 2007), pharmacological and pharmacognosy related field (Anthony *et al.,* 2016). In this, the Molegro Virtual Docker (MVD) system is used for docking. Hydrogen bonding and scoring function, search algorithm creates possible protein ligand complex and scoring function is employed to predict a ligand's affinity for binding to a protein (Khalid *et al.,* 2013; Lensink *et al.,* 2007; Robertson and Varani, 2007). AD is a neurodegenerative disease characterized by a decline in cognitive function of the brain (Goedert and Spillantini, 2006; Andreakou *et al.,* 2016; Qadi and Feldman, 2016). The pathology behind disease progression occurs through abnormal protein (beta-amyloid and tau) accumulation (Mrak and Microglia, 2012; Sheng *et al.,* 1997) inside the neuron lead to the cell death and loss of memory functions (Cohen and Kelly, 2003; Reitz, 2012). One more reason is declined level of acetylcholine in the synaptic cleft. In this, the main consideration is given to the declined level of acetylcholine. Both acetylcholinesterase and butylcholinesterase are selected as targets for the phytoconstituents to dock. The mechanism being cholinesterase enzyme acts on acetylcholine and degrades it causing its shortage in the synaptic cleft (Tabet, 2006). Docking studies verify the phytoconstituents' level antagonistic property towards the targeted enzyme and along with comparison with that of the standard drugs available in the market. The plants were selected based on the basis of traditional folklore, the plants are *Solanum lycopersicum, Psidium guajava and Eugenia caryophyllata*. The phytoconstituents obtained from *P. guajava* (Mittal *et al.,* 2010) and *E. caryophyllata* (Bhowmik *et al.,* 2012), *i.e.*, guavanoic acid and beta-caryophyllene, isocaryophyllene is rich antioxidants (Lobo *et al.,* 2010). The tomatine obtained from *S. lycopersicum* plant (Akhondzadeh and Abbasi, 2006) is found to have acetylcholine-sterase property according to the plant folklore. The plants with antioxidant, acetylcholinesterase inhibitor and neuroprotective properties can be used in Alzheimer's disease (Berg *et al.,* 1982). The standard drugs for reference docking are donepezil, galathanmine, hepurazine-A, tacrine, revastimine (Bolton *et al.,* 2008). These standard drugs selected are docked with same target molecule and their values are compared with the phytoconstituents selected for screening of anti-Alzheimer's activity. The molecular docking results are analysed and utilised to produce effective information about docking capability of the selected phytoconstituents.

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2. Materials and Methods

2.1 Preparation of ligand

The 3-D structure of phtoconstituents, isocaryophyllene betacaryophyllene tomatine and guavanoic acid standard drugs huperzine A, galanthamine, donepezil, rivastigmine and tacrine were obtained from Pubchem (ACD/ChemSketch Freeware, 2012) chemical data bases (Mao *et al.,* 2010) and saved it in the mol2 format and converted to Pdb format.

2.2 Preparation of protien

The enzymes selected as targeted molecule is 4MoE (pdb Id: 1B41) and 5DYT (pdb Id: 4BDS). The protein data bank provides the 3D structure in a pdb format (Bernstein *et al.,* 1997). The structure that is received from the Protein Data Bank is frequently not aligned correctly; therefore, utilising MVD, it is aligned appropriately in terms of proper bond, bond order, hybridization, and other alterations. Using the built-in cavity detection feature of MVD, the binding sites of both targets were computed. Additionally, the existing water molecules are taken into account and substituted with another water molecule with assigned score of 0.50. The simulation's search space surrounding the active cleft, which is occupied by the subset region of 25.0 **A°**.

2.3 Molegro virtual docker : Scoring functions and docking search algorithm

The MVD is used to complete the docking for this research. MVD is the most often used docking programme having ligand binding to a target with high conformation. The basic idea of the MolDock software is a unique search algorithm that combines the cavity prediction method and differential evolution (Thomsen and Christensen, 2006). The search algorithm is an interactive optimisation tool based on Darwinian Evolution Theory. The molecule is subjected to competitive selection in the poor solution in this scenario. To create new solutions, recombination and mutation are employed. Piecewise linear potential (PLP), an amplified potential whose parameters are suited to protein-ligand structure and yield scoring function, is the source of inspiration for MolDock's scoring function (Gehlhaar *et al.,* 1995), and GEMDOCK (Generic Evolutionary Method For Molecular DOCK) gives this even more extension in this aspects (Yang and Chen, 2004).

2.4 Moldock optimizer

The guided differential evolution algorithm in MVD was utilised with the following parameters: population size = 50, maximum interactions = 2000, cross over rate = 0.9, scaling factor = 0.5 and number of runs = 5 by checking the constrain poses. Rather than using the root mean square deviation (RMSD), an **A°** variance-based termination technique was chosen. Pose clustering was used, resulting in several binding modes, to guarantee the best binding mode in the binding cavity.

2.5 The scoring function parameters

2.5.1 MolDock score

Ignoring the impact of atoms located distant from the binding location distance atom in MDV system is used. The directionality of hydrogen bond helps to determine whether or not H-bonding between possible donors and acceptors is possible or not. The enzyme's binding site was separated extending in the directions of X, Y and Z, and a radius of 25.0 **A°** around the cavity of choice is selected.

2.5.2 Rerankscore

This rerankscore helps creating and predicting models for understanding chemical properties (*e.g.,* QSAR). Determines and select the ideal position from a variety of poses that result from the same ligand. It provides estimation about the strength of interaction. The rerankscore can be less effective in ranking poses of various ligands, even while it is successful in ranking different poses of the same ligand. As a result, the rankscore is used to generate the virtual scoring ranking outcomes. The highest scored postures' approximate value is measured by the binding affinity (Preety Dubey *et al.,* 2020; Mamta Arya *et al.,* 2022).

3. Results

The phytochemicalconstituents tomatine, fuavaoic acid, isocaryophyllene and β-caryophyllene of plants along with the selected standard drugs such as galanthamine, rivastigmine, donepezil, isocaryophyllene, huperzine A, and tacrine, the following substances are docked with acetylcholinesterase and butylcholineterase enzymes independently. The binding efficacy between the two is then obtained and analysed in terms of MolDock score, rerankscore, and hydrogen binding energy (Sumithra and Ancy *et al.,* 2022).

The MVD docking analysis based on Moldock score and hydrogen bonding score of all ligands with acetylcholinesterase and butylcholinesterase target is given in Table 1and Table 2, respectively.

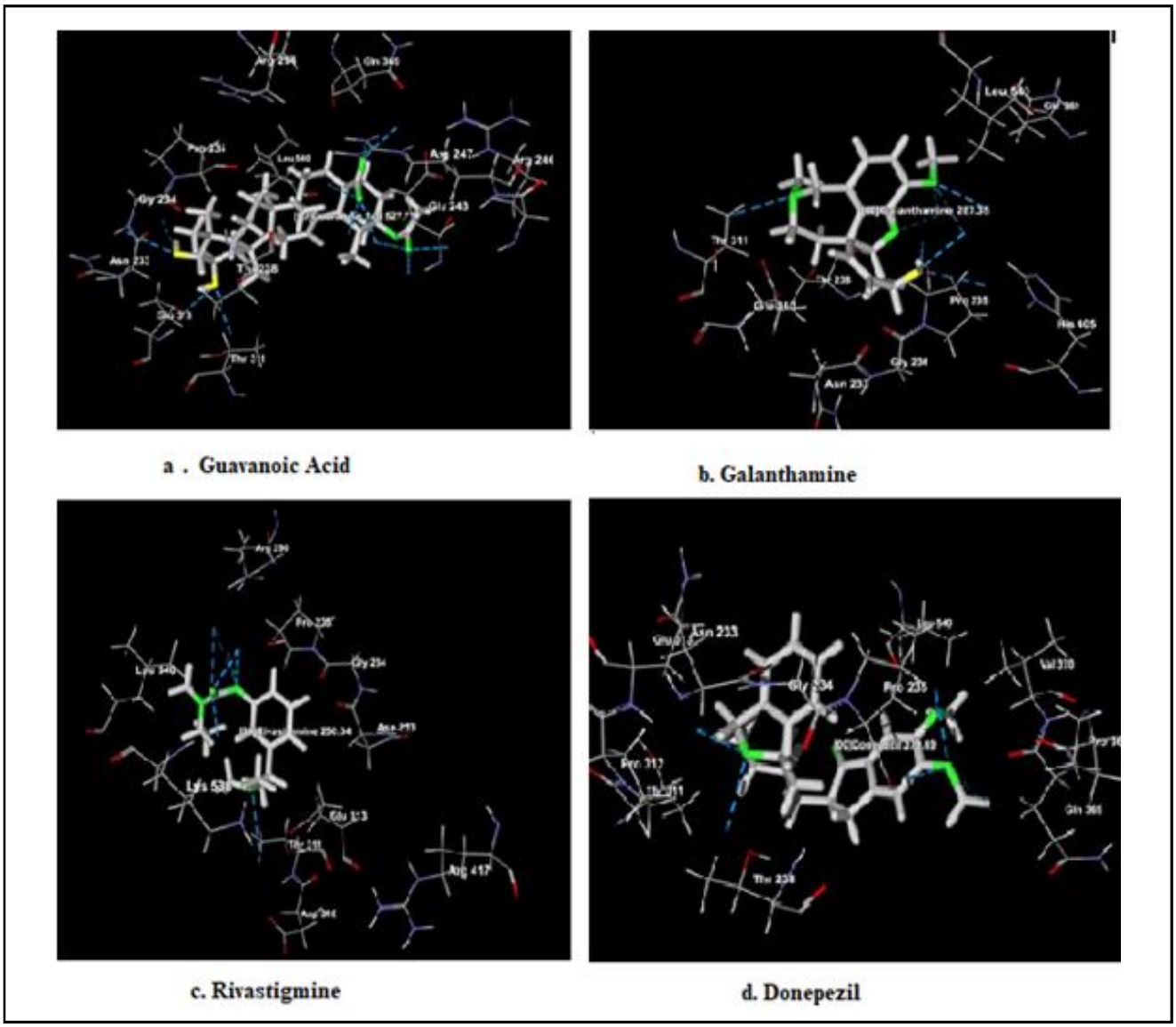
Table 1: *In silico* docking analysis of phytoconstituents with target 4MoE (Ranking based on MolDock score)

Name	Ligand	MolDock score	Rerank score	H Bond
Guavanoic acid	Guavanoic acid	-83.8387	-72.5541	-1.41085
Galanthamine	Galanthamine	-70.1782	-65.0216	0
Rivastigmine	Rivastigmine	-69.7732	-63.2213	0
Donepezil	Donepezil	-69.5349	-53.8859	0
Isocaryophyllene	Isocaryophyllene	-61.4782	-53.6579	0
Huperzine A	Huperzine A	-60.5378	-58.6999	0
Beta-caryophyllene	Beta-caryophyllene	-56.8315	-48.8024	0
Tacrine	Tacrine	-43.4637	-45.0414	0
Tomatine	Tomatine	20673.6	833.648	-0.353885

Table 2: ***In silico*** **docking analysis of phytoconstituents with target 5DYT (Ranking based on MolDock score)**

Name	Ligand	MolDock score	Rerank score	H Bond
Tomatine	Tomatine	-162.081	-88.6603	-11.0039
Donepezil	Donepezil	-134.164	-113.489	0
Guavanoic acid	Guavanoic acid	-117.087	-91.4195	-5.39331
Galanthamine	Galanthamine	-103.672	-86.729	-3.40438
Rivastigmine	Rivastigmine	-96.5109	-80.3078	0
Isocaryophyllene	Isocaryophyllene	-95.3016	-72.7855	0
Huperzine A	Huperzine A	-94.806	-80.9463	-2.4817
Beta-caryophyllene	Beta-caryophyllene	-90.2763	-72.3881	0
Tacrine	Tacrine	-88.154	-81.1884	0

The docked view of all the ligands with the two significant targets are given in Figures 1 and 2. In this, all images are captured using ligand energy inspector tool in MVD.



a . Guavanoic Acid

b. Galanthamine

c. Rivastigmine

d. Donepezil

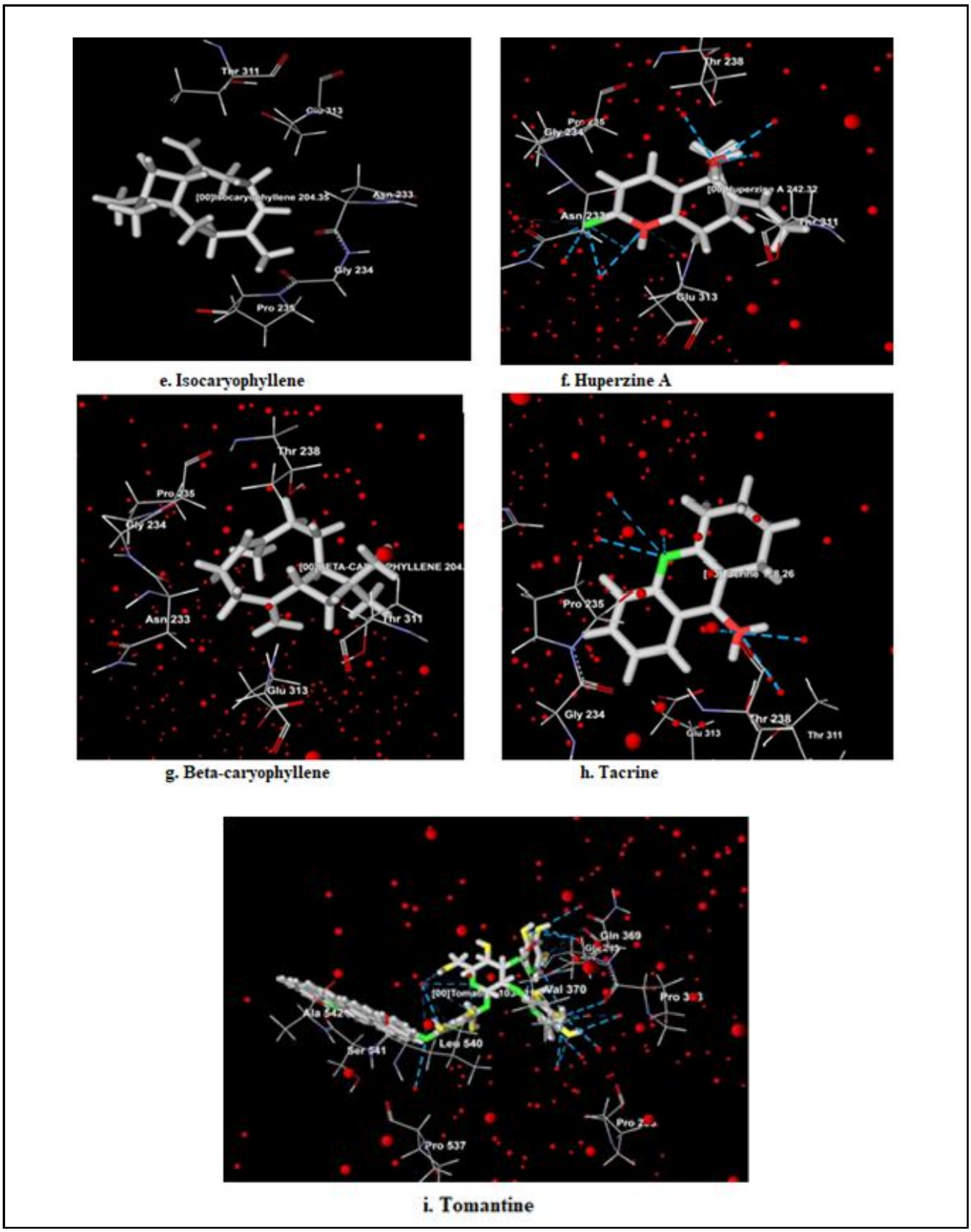
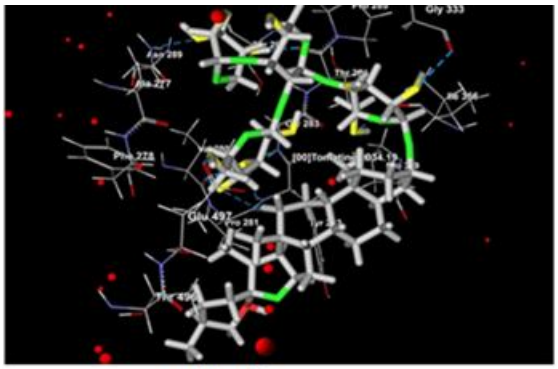
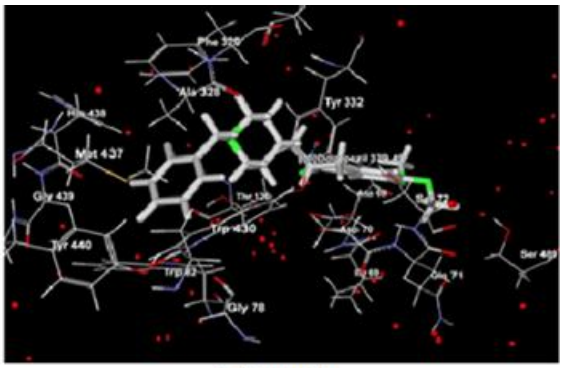


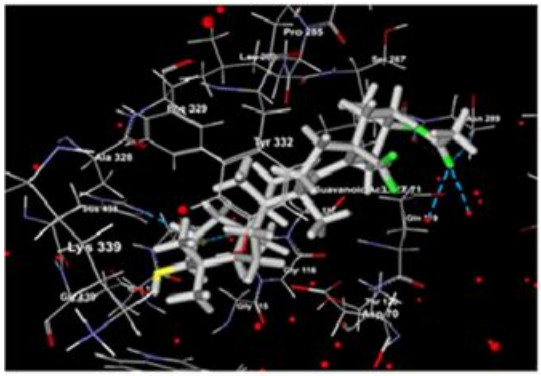
Figure 1: Docking interactions of phytochemical constituents with target acetylcholinesterase (4MOE).



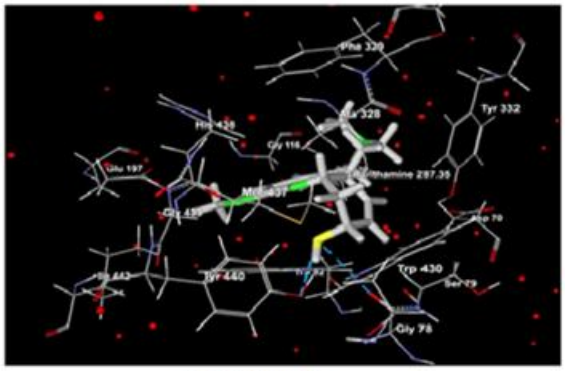
a. Tomatine



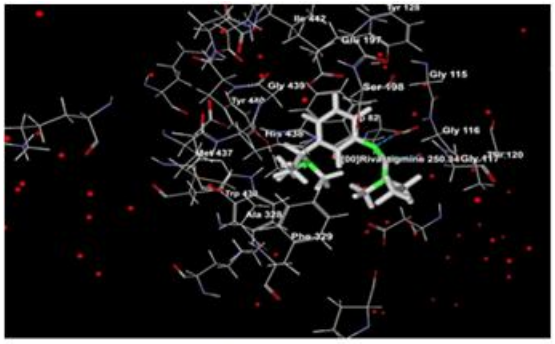
b. Donepezil



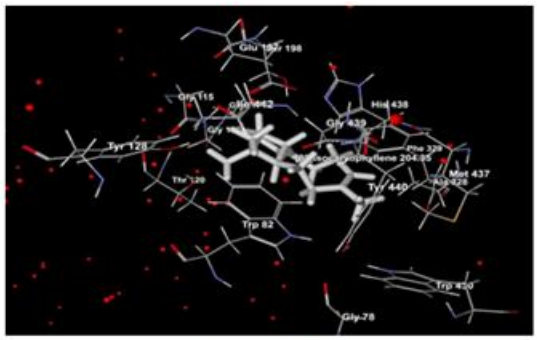
c. Guavanoic Acid



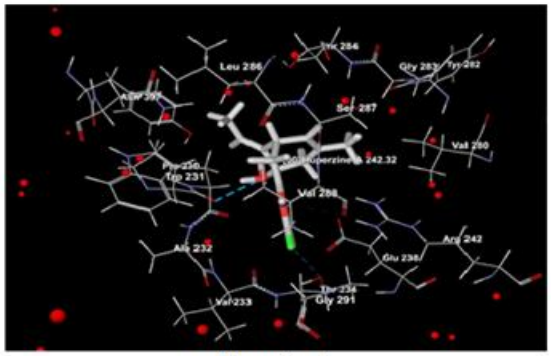
d.Galanthamine



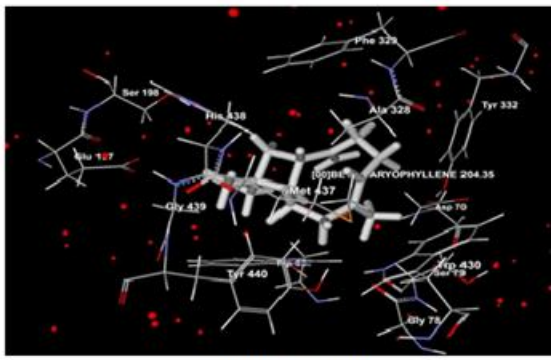
e. Rivastigmine



f. Isocaryophyllene



g. Huperzine A



h. Beta-caryophyllene

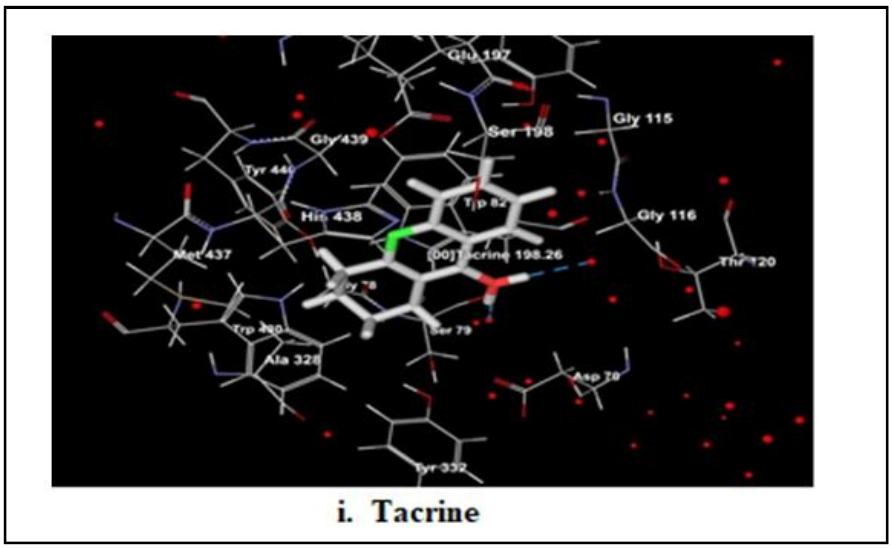


Figure 2: Docking interactions of phytochemical constituents with target butrylcholinesterase (5DYT).

The descriptor calculation for the ligands in the ligand process includes the following.

3.1 Element count

Determines how many atoms there are of a specific element. H, C, N, O, P and S are counted by default. And "Other" refers to all other components.

3.2 Basic characteristics

A group of widely used characteristics, such as molecular weight, the number of hydrogen donors and acceptors count, and other simple descriptors. The descriptors that are at available are:

- Molecular weight, or MW
- **Atoms:** Count of atoms, including hydrogen atoms
- **Heavy atoms:** Total number of atoms (hydrogen not included)
- **Rot2:** The number of rotatable bonds (excluding bonds rotating terminal atoms)
- **Rot:** The number of rotatable bonds
- **HD:** The number of donors of hydrogen
- **HA:** The number of acceptors of hydrogen
- **Rings:** The number of rings;
- **Aro:** The number of aromatic

3.3 Terms of andrews affinity

Andrews affinity together with the necessary terms for the computation. Refer to "Drug-receptor interactions: Functional group contributions" published by PR Andrews, DJ Craik, JL Martin Journal of Medicinal Chemistry, The American Chemical Society 27:1212, 1648-1657, 1984.

3.4 Matrix of chemical feature distance

The topological distance between each pair of chemical characteristics is calculated to determine the minimum, maximum, and mean, which yields the CFDM descriptors. The minimal number of covalent links between the two features is known as the topological distance.

Investigated chemical properties include ring systems, positively and negatively charged atoms, hydrogen acceptors, and hydrogen donors. Observe that an atom must have a minimum charge of ± 0.2 in order to be classified as charged (need to adjust this threshold in the settings dialogue).

3.5 Wiener index

The total topological distance between every pair of heavy atoms is known as wiener index.

4. Discussion

In molecular docking study, the highly negative dock score indicates the best docking effect. The docking of each individual ligand molecules with target enzymes acetylcholinesterase and butylcholinesterase with Pdb id's (4MoE, 5DYT) is docked and ranking of results is based on MolDock score, rerankscore and H- bonding score is separately plotted in the Tables 1 and 2 (Yuva Bellik *et al.,* 2020; Siraj *et al.,* 2022; Sabeena Arif *et al.,* 2022). The molecular docking of all ligand molecules with 4MoE is analysed, among which guavanoic acid was found to have high negativity Moldock and rerank score with a values of -83.8387 and -72.5541, respectively. Hence, there is more chance of more anti-Alzheimer's activity. The MolDock score of guavanoic acid is even more better comparing to that of the standard drug galanthamine. The docking of ligands with 5DYT was analysed and gives a ranking in favour of phytoconstituent tomatine and was having comparative MolDock score and rerank score with values of -162.081 -88.6603, respectively, when compared to that of the standard drugs galanthanmine and donepezil. This suggests that tomatine also has capability of anti-Alzheimer's activity when compared to standard drugs. The H-Bond score of guavanoic acid and tomatine were comparable better than other chemical constitutents with 4MoE acetylcholinesterase target having the score of -1.41085 and -0.353885 and the 5DYT butylcholinesterase target having the score of -5.39331 and -11.0039 showing better interactions with anti-Alzheimer's activity.

5. Conclusion

The results of the molecular docking investigation of the chosen phytoconstituents for Alzheimer's activity have demonstrated that tomatine and guavanoic acid are beneficial phytoconstituents. In the docking analysis, it was discovered that the phytoconstituents, guavanoic acid and tomatine produced MolDock and rerankscores that were comparable to those of the standard drugs galanthamine and donepezil. Therefore, more research on the two phytoconstituents can be done utilising in *vitro* and *in vivo* investigations to assess them for anti-Alzheimer's activity is encouraged by the researchers to prove the results.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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