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Molecular docking study of *Andrographis paniculata* L. compounds targeting against *Anopheles* mosquito acetylcholine esterase

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Abstract
Andrographis paniculata is a popular medicinal plant, to treat various diseases for centuries worldwide. This plant contains several phytochemical compounds with distinctive biological properties. Phyto-compounds present in *A. paniculata* leaves extract were concluded to be Benzyl alcohol, Dibutyl Phthalate, Diethyl phthalate, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Phytol, n-Hexadecanoic acid, L-Lyxose, Hexadecanoic acid, Octadecanoic acid, Dodecanoic acid, 1,3-Dioxolane and 2-Propyl-. These compounds have different biological activities based on their functional groups. Therefore, we have analysed the phyto-compound-based control of Anopheles mosquitoes, and their inhibitory action against AChE of *A. stephensi* was investigated using molecular docking studies through PyRx 0.8 software. AChE model was retrieved from the Uniprot database and phytocompounds sourced from *A. paniculata* L, were evaluated for their binding affinity. The phytocompounds binding exact site with the AChE of Anopheles mosquitoes also analysed.

Keywords: *A. paniculata*, Target protein, Molecular Docking, cholinergic system, Acetylcholine esterase

Introduction

Andrographis paniculata, commonly referred to as Green Chiretta or King of Bitters, belongs to the Acanthaceae family. It is an herbaceous plant and branched like arrangement. It has a different bioactive compound includes flavonoids, diterpenes, xanthones, and no cardioides, (Mussard *et al.*, 2020 [15]; Hossain *et al.*, 2021) [10]. Traditionally, the leaf extract of this plant has been used as a remedy for various ailments, including infectious diseases, colic pain, irregular bowel movements, fever-related illnesses, and diarrhea. Previous studies have shown that this plant has anticancer, antihepatic, antihyperglycemic, anti-inflammatory, antimalarial, antioxidative, and sex-disinfectant properties, (Gulshan Kumar *et al.*, 2020 [8]; Nutan Sharma *et al.*, 2021 [16]; Tundis *et al.*, 2023 [23]; Adiguna *et al.*, 2023) [1].

Anopheles stephensi is a mosquito that causes mosquito-transmitted diseases in humans. In densely populated areas, *A. stephensi* is an efficient primary malarial vector. The nervous system of this mosquito is renowned for its operational robustness. The cholinergic system, comprising the pivotal enzyme acetylcholine esterase (AChE), plays a crucial role in the Anopheles mosquito's nervous system. The eradication of mosquito-borne diseases heavily relies on the indispensable presence of mosquitoes (Deng *et al.*, 2023) [5]. Anti-choline esterase pesticides are developed to paralyse insects by inhibiting the AChE enzyme. Anti-choline esterase pesticides are used to control insects, including mosquitoes and agricultural pests. AChE functions to break down the neurotransmitter acetylcholine (ACh) at cholinergic synapses, thereby halting nervous signalling (Rosenberry, 1975 [19]; Veronika *et al.*, 2017) [25]. The active site of AChE is characterised by its deep and narrow structure, with the bottom region referred to as the catalytic site and the opening regions as peripheral sites (Gholami *et al.*, 2023) [7]. The AChE enzyme, which is present in the nervous tissue of both humans and insects, regulates the transmission of nerve impulses. When there is an interruption in the regular transfer of nerve impulses, there will be an excess accumulation of the acetylcholine enzyme in the synapses, which results in muscular convulsions, paralysis, and self-poisoning (Céspedes *et al.*, 2015; Ryan *et al.*, 1988) [20].

In silico screening of conserved target sites, particularly those found in mosquito AChEs, is required for effective and safe insecticides to control mosquito-borne diseases (Vieira, *et al.*, 2021) [26]. Amino acid sequences in these regions can serve as specific targets for the development of novel pesticides. Molecular docking software can be used to find a 3D model of AChE and phytocompound binding sites. This approach facilitates the design of structure-based rational inhibitors and drugs through molecular docking techniques (Durairaj and Muthu., 2015) [6]. It can be used to determine the complex formation of small ligand molecules with proteins and the kinetics of binding forces with phytocompounds. Considering these, my present study identified phytocompounds obtained from *A. paniculata* leaves and evaluated their potential as inhibitors of AChE. This enzyme sample was obtained from the Uniprot database, and its inhibitory activity was analysed against the phytocompound. Molecular docking analysis was performed using PyRx0.8 software to estimate the AChE binding site. This investigation was designed by sequence analysis of *A. stephensi* AChEs from GenBank, and a three-dimensional model of the enzyme was generated by homology modelling.

Materials and Methods

Ligand selection

The three-dimensional model of the phytocompounds, which include Benzyl alcohol, Dibutyl Phthalate, Diethyl phthalate, diethyl phthalate, 2-hydroxy-1-(hydroxymethyl) ethyl ester, phytol, n-Hexadecanoic acid, L-Lyxose, Hexadecanoic acid, Octadecanoic acid, Dodecanoic acid and 1,3-Dioxolane, 2-Propyl- from *A. paniculata* were taken from the PubChem database.

Target protein selection and preparation

The three-dimensional structure of the target protein, Acetylcholine esterase was taken from the UniProt database (https://www.uniprot.org). Using Discovery Studio 2021 the three-dimensional structure of this target protein was prepared for in silico-docking studies.

Docking studies

Molecular docking studies were conducted for the modelled target protein AChEs and its corresponding compounds (ligands) using PyRx 0.8 software (Trott and Olson, 2010) [22]. The modelled target protein was further prepared for docking studies within the same software. Ligands were uploaded utilising the Open Babel option in PyRx 0.8, followed by grid generation and docking studies performed through the Vina wizard option. Binding affinity values were recorded in an Excel file. Subsequently, the results were analysed using Discovery Studio 2021, and both two-dimensional and three-dimensional docked images were captured.

Results and Discussion

The concept of molecular relationships is widely recognised and frequently applied in bioinformatics. Analysing molecules and their characteristics offers various avenues for utilization. This process aids in the discovery of novel molecules exhibiting desired properties and biological activities. The principles of similar properties (SPP) and structure-activity relationships (SAR) are based on the premise that molecules sharing structural similarities also possess similar properties and, consequently, exhibit comparable biological activities. Virtual screening operates on this principle by identifying similar molecules within a dataset and the phytocompounds with known binding affinity, thereby suggesting them as potential candidates for new active molecules.

Ligand modelling and Target protein selection

In this study, the three-dimensional structures of *A. stephensi* AChE ligands (compounds) were obtained from the PubChem database. Protein structure modelling facilitated the compounds capable of binding and inhibiting AChE (see Fig. 1). The three-dimensional structure of the target protein, AChEs (AF-P56161-F1-model_v3), was sourced from the UniProt database with the corresponding UniProt ID P56161. This protein model was used for predicting the AChE protein. The prediction of the *A. stephensi* AChE protein structure was based on the alignment with the target template. Hydrophobic contacts, hydrogen bonds, and π-stacking are widely used for ligand and protein interactions (Mohamed *et al.*, 2015) [14]. These parameters aid in determining the overall structural pattern of compounds and also in predicting and analysing the molecular docking of AChE, which is susceptible to convenient binding of the ligands. This same pattern of AChE parameters revealed an overall similar organisational pattern, displaying flexible areas of the proteins vulnerable for docking with ligands (Yadav *et al.*, 2017 [27]; Schmidt *et al.*, 2014) [21]. Binding affinity is crucial to understanding complex biochemical pathways and uncovering protein interaction networks.

Docking studies

Molecular docking studies of acetylcholine esterase and its ligands concluded that the binding efficiency. Ten compounds sourced from *Andrographis paniculata* were examined for their interaction with the modelled target protein. The outcomes are presented in Table 1, and the 2-dimensional and 3-dimensional interactions between the compounds and the modelled target are depicted in Figures 2–7. Notably, the highest binding affinity observed in the results signifies a favourable outcome.

Based on the results we obtained (Table 1) regarding the ten phytocompounds that we investigated, we discovered that benzyl alcohol had a very strong binding affinity (-5.6 kcal/mol) with the target protein Acetylcholine esterase's amino acid residues such as TRP 112, TYR 186, and GLU 260. Similarly, dibutyl phthalate established interactions with amino acid residues including PRO 293, PRO 467, HIS 462, ALA 427, ARG 599, LEU 595, and ILE 435, exhibiting an interesting binding capacity of -5.6 kcal / mol. With a binding ability about -5.1 kcal / mol, diethyl phthalate, another chemical, formed six bonds with the amino acid residues PHE 155, LEU 535, LEU 548, and GLU 549. It's essential to remember that diethyl phthalate is frequently used as a plasticizer, softener, medicinal coating, cosmetic substance, and exhibits antibacterial properties (Premjanu and Jaynthy, 2014) [18]. and is commonly used as a plasticizer, softener, pharmaceutical coating, cosmetic additive, and insecticide (Page *et al.*, 1995) [17].

Table 1: Docking scores with binding affinity and bonds Interaction.

S. No.	PubChem (CID)	Compound Name	Binding Affinity (Kcal/mol)	No. of Bonds	Interacting Residues	Bond Length (Å)
1.	244	Benzyl alcohol	-5.6	4	TRP 112 TRP 112 TYR 186 GLU 260	4.00 3.67 2.66 2.12
2.	3026	Dibutyl phthalate	-5.6	10	PRO 293 PRO 467 HIS 462 HIS 462 ALA 427 ALA 427 ARG 599	5.33 5.40 4.40 4.53 4.79 3.68 2.14

					ARG 599 LEU 595 ILE 435	2.02 4.18 5.20
3.	6781	Diethyl phthalate	-5.1	6	PHE 155 PHE 155 LEU 535 LEU 535 LEU 548 GLU 549	4.83 3.61 3.70 4.96 4.73 2.23
4.	5280435	Phytol	-4.8	7	GLY 60 ASP 87 TYR 124 PRO 62 PRO 62 ALA 79 LYS 65	2.99 3.56 5.10 5.34 4.31 4.51 4.39
5.	644176	L-Lyxose	-4.6	6	ARG 531 GLN 156 ASP 153 ARG 53 ARG 53 ALA 193	1.87 2.25 2.96 3.62 2.12 2.33
6.	123409	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	-4.5	7	ASP 87 ASP 87 GLY 60 GLY 60 PRO 93 PRO 93 PRO 93	2.24 2.30 3.56 3.44 4.09 3.66 4.15
7.	985	n-Hexadecanoic acid	-4.3	10	PRO 92 PRO 93 TYR 124 TYR 124 TYR 124 PRO 62 ALA 64 GLU 80 LEU 86 LEU 86	4.69 5.37 3.88 5.10 5.29 4.21 2.69 2.21 5.10 5.20
8.	5281	Octadecanoic acid	-4.1	11	GLY 60 ASN 59 ARG 90 ARG 90 LEU 86 LEU 86 TRP 82 TRP 82 ALA 79 PRO 62 PRO 62	2.99 2.32 2.60 2.86 5.47 5.29 5.33 5.19 4.15 5.24 5.39
9.	3893	Dodecanoic acid	-4	4	GLN 438 PHE 437 ALA 434 LEU 598	2.74 3.79 3.92 5.09
10.	179832	1,3-Dioxolane, 2-Propyl-	-4	4	ARG 53 PHE 149 VAL 194 GLN 156	2.57 5.03 3.81 2.54

Moreover, among the ten phytocompounds analysed, Dodecanoic acid, 1,3-dioxolane, and 1,3-Dioxolane-2-Propyl- displayed the most favourable binding affinity (-4 kcal/mol) with the amino acid residues GLN 438, PHE 437, ALA 434, LEU 598, and ARG 53, PHE 149, VAL 194, and GLN 156, respectively, within the target protein. Consequently, this study suggests that Benzyl alcohol, Dibutyl phthalate, and Diethyl phthalate, among others, exhibit highly favourable interactions with the target protein AChE, potentially contributing to the regulation of the *A. stephensi* population. This observation aligns with the findings of a previous study on *Plectranthus amboinicus* (Spreng) semi-purified fractions, which demonstrated selective β-Glucuronidase inhibition through in silico docking (Alvarez *et al*., 2021) [2]. Benzyl alcohol and nicotine both act on the brain, and because so many people use and abuse both drugs, it is likely that both drugs act on at least some of the same brain structures (UNODC, 2011) [24]. A covalent bond is formed with the reactive group, which is considered to form in molecular docking experiments.

The selected compounds for this investigation lack reactive groups, prompting an extensive exploration of their hydrogen bond interactions with AChE. Acetylcholine served as the standard in the docking experiment, forming hydrogen bond interactions with compounds. Previous studies have highlighted the impact of AChE on diverse ion channels, including ligand-gated ion channel receptors (Veronika *et al.*, 2017 [25]; Deng *et al.*, 2023 [5]; Gholami *et al.*, 2023) [7]. Benzyl alcohol, dibutyl phthalate, and diethyl phthalate demonstrated robust interactions with AChE based on docking scores, while the remaining seven compounds exhibited moderate interactions with AChE, contributing to lethality against *A. stephensi*.

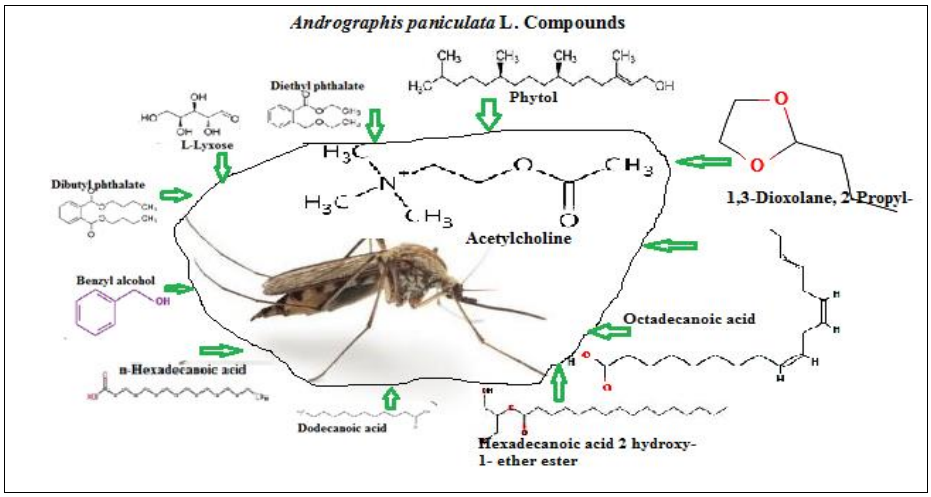

Fig 1a: Molecular docking study of *A. paniculata L* phytocompound against mosquito AChE

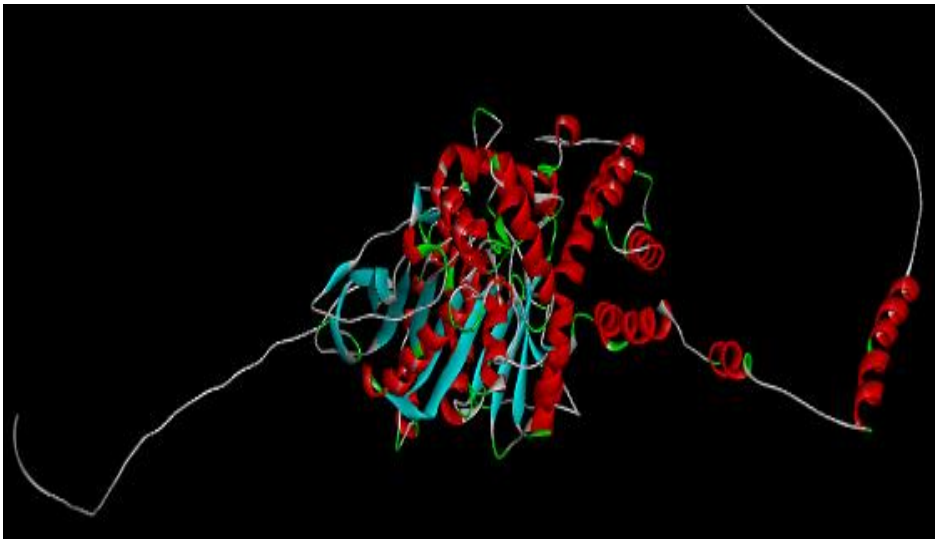

Fig 1: The 3D structure of the modelled target protein Acetylcholine esterase

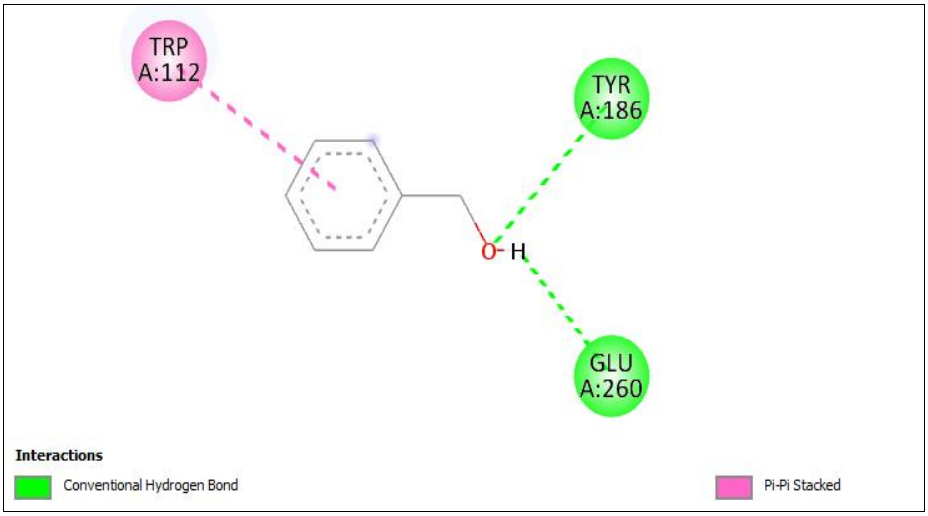

Fig 2: The 2D interaction of phyto-compound Benzyl alcohol with the modelled target protein

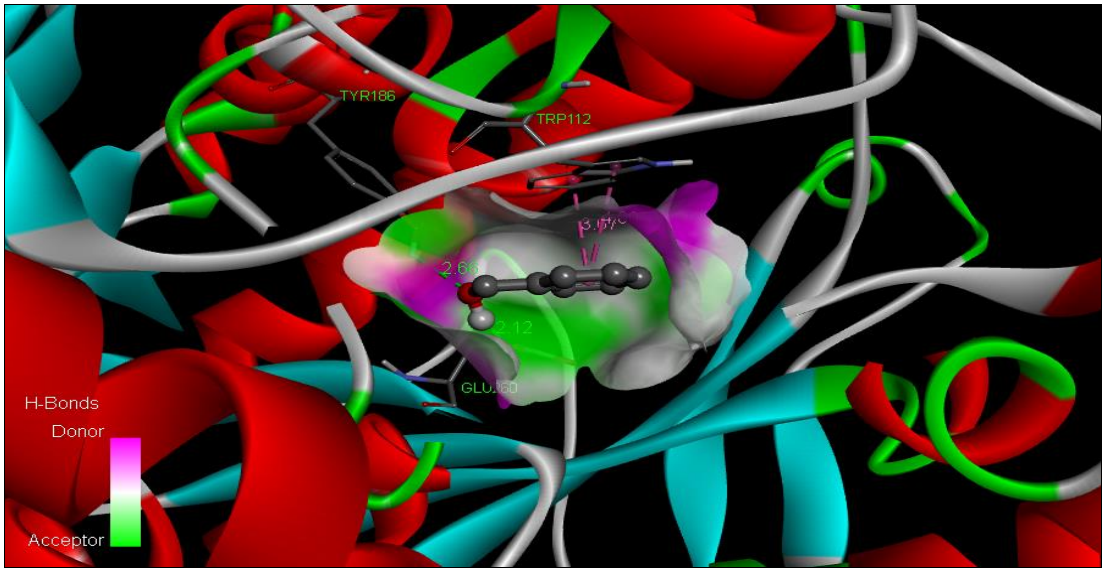

Fig 3: The 3D interaction of phyto-compound Benzyl alcohol with the modelled target protein

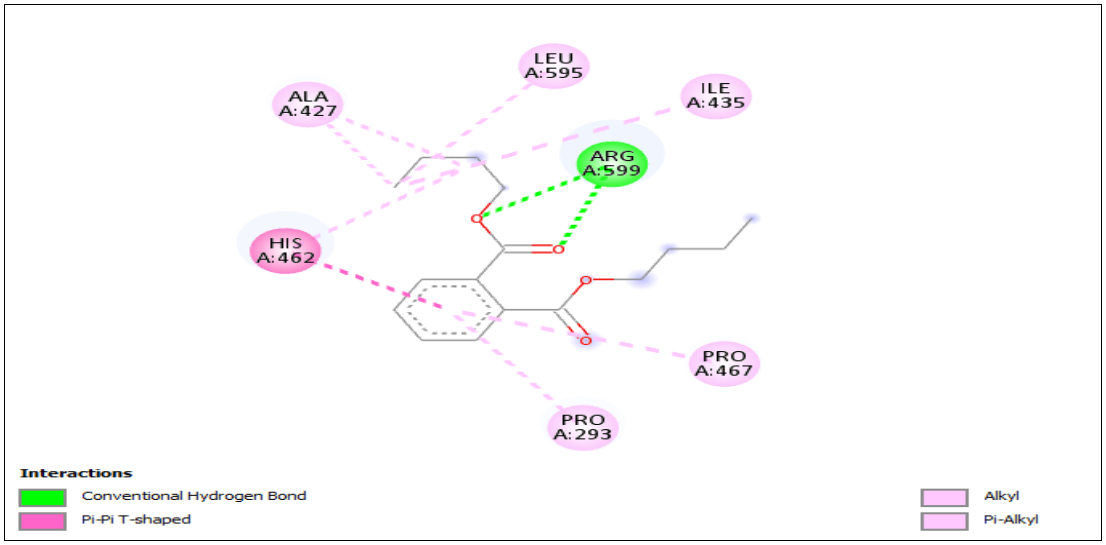

Fig 4: The 2D interaction of phyto-compound Dibutyl phthalate with the modelled target protein

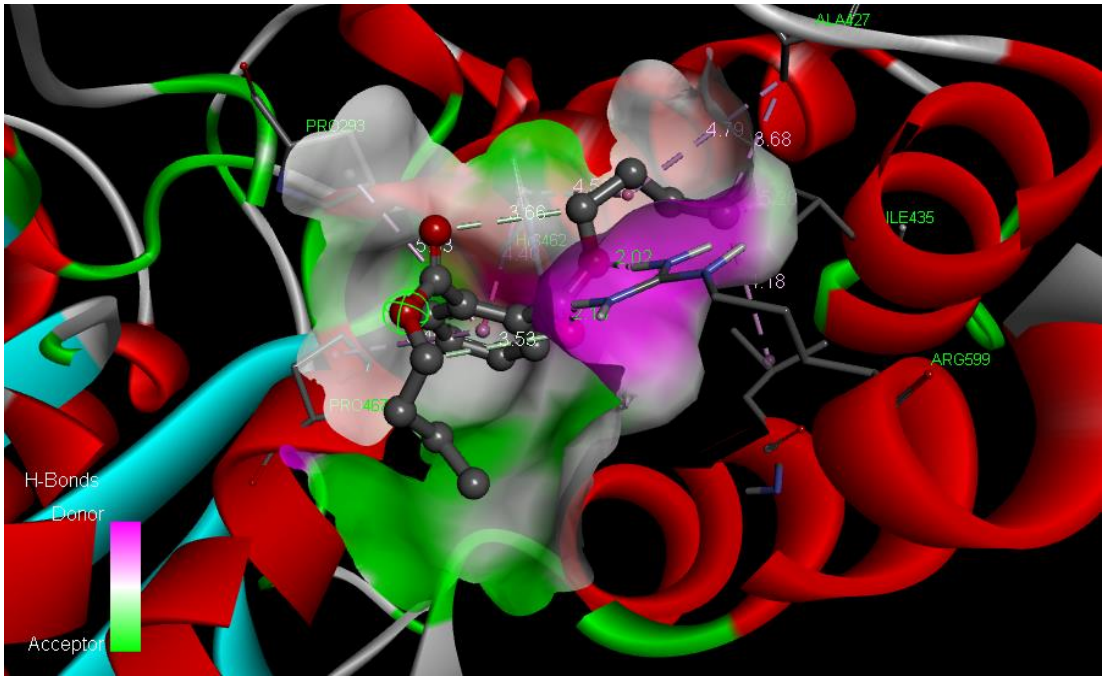

Fig 5: The 3D interaction of phytocompound Dibutyl phthalate with the modelled target protein

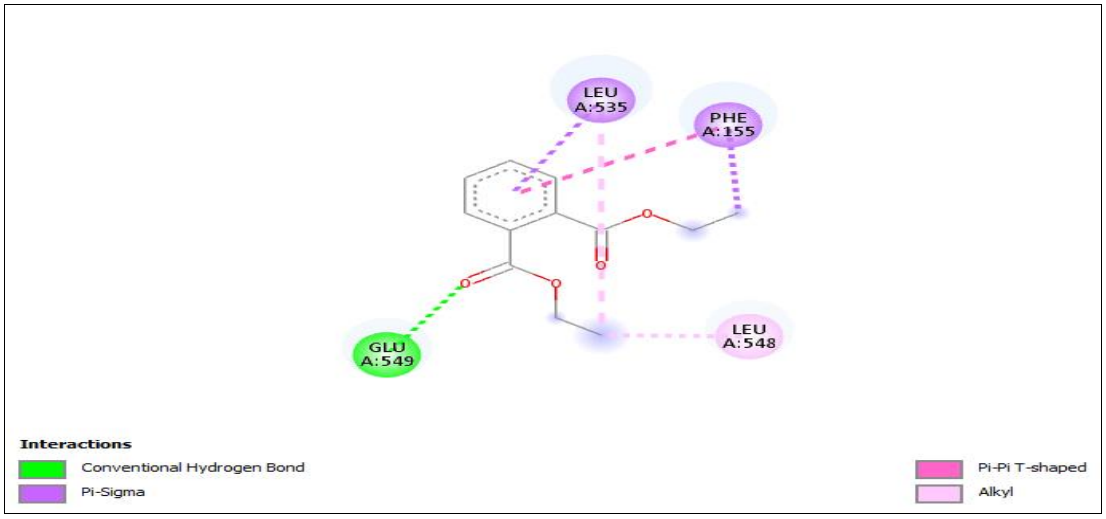

Fig 6: The 2D interaction of phytocompound Diethyl with the modeled target protein

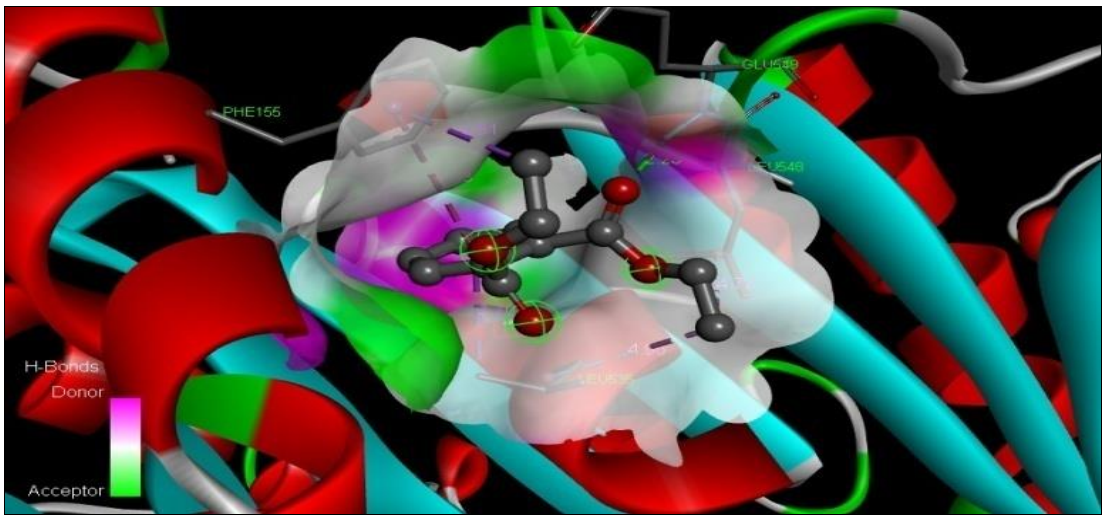

Fig 7: The 3D interaction of phytocompound Diethyl phthalate with the modelled target protein

Conclusion

In the present study, ten phyto-compounds from *A. paniculata* were obtained from PubChem database, and the 3D structure of the modelled AChE was taken from the UniProt database. Docking studies results were analysed using Discovery Studio 2021. As per results, the phytocompounds Benzyl alcohol, Dibutyl phthalate, and Diethyl phthalate showed very good interactions with Acetylcholine esterase. Hence, this study concludes that the phytocompounds Benzyl alcohol, Dibutyl phthalate, and Diethyl phthalate may have the capacity to control the population of *A. stephensi*.

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but phyto-compound-based control of Anopheles mosquitoes is still missing from the previous studies.
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