In Silico Approach of Invistigation of Phytoconstituent from *Azadirachta indica* L, *Caricapapaya*, *Curcuma longa* L, *Mangifera indica* and *Psidium guajava* (guava) as Inhibition of CYP3A5 Enzyme through Molecular Docking for the Treatment of Malaria

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Abstract- Malaria is one of the life threatening infectious diseases caused by protozoan parasite and spread by the bite of female anopheles mosquito and its species. A family of enzyme called Cytochrome P450s has the ability to break down certain drugs. Cytochrome P450 enzyme make the medicine either more or less active, depending upon the medicine. Cytochrome P450 3A5 (CYP3A5) is part of CytochromeP450 family of protein in the body. It is responsible for breaking down medicines. It shows a trend for gametocytemia, parasitemia clearance rates. The structure of all selected chemical constituents of Azadirachta indica L. (Neem) (Nimbin, Gedunin, Salanin, Quercetin), Caricapapaya (Papain, Tocopherol), Curcuma longa L. (Tumerone, Zingeberene, Curcumine, Curlone) Mangifera indica (Mangiferin, Mangoleanone, Manglupenone, Mangostin), Psidium guajava (guava) (Guajavarin, Ascorbic acid, Citric acid, Limonene). The updated elucidated crystal structure of CYP3A5 was obtained from the RCSB Protein Data Bank (PDB) as entry 6MJM, 5VEU.

Keywords: ***Azadirachta indica*** **L.,** ***Caricapapaya, Curcuma longa*** **L.,** ***Mangifera indica, Psidium guajava*** **(guava), PyRx.**

INTRODUCTION

Malaria is one of the life threatening infectious diseases caused by protozoan parasite and spread by the bite of female anopheles mosquito and its species. Out of the five human Plasmodium species (Plasmodium falciparum, P. vivax, P. ovale, P. knowlesi, and P. malaria). Plasmodium falciparum which appears to be more virulent. (Iyamah et al., 2017) According to the most recent World Malaria Report, released on 30 November 2020, there were 229 millioncases of malaria in the year of 2019 compared to 228 million cases in 2018. The estimated figure of malaria deaths stand at 409 000 in 2019, compared with 411 000 deaths in 2018. The WHO African section was home to 94% of all malaria cases and deaths. In 2019, 6 countries accounted for about half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4%). Children under age of 5 years are the most susceptible group affected by malaria; in 2019 they accounted for 67% (2741

000) of all malaria deaths worldwide. (*Malaria 7.1*, n.d.) Chloroquine (CQ) and artemisinin (ART) derivatives are the two major classes of antimalarial drugs. However, repetitive and improper use of CQ caused drug resistance of malaria parasites. The epidemiological proof predicts the "tsunami" of ART resistance within the world, called "super malaria". In this condition, subsequent treatment failures with artemisinin-based combination therapy (ACT) have raised concerns about the loss of the only highly-efective treatment currently available to treat malaria. (Tahghighi et al., 2020) A family of enzyme called Cytochrome P450s have the ability to break down certain drugs. Cytochrome P450 enzyme make the medicine either more or less active, depending uponthe medicine. Cytochrome P450 3A5 (CYP3A5) is part of Cytochrome P450 family of protein in the body. (*CYP3A5 and Medicines*, n.d.) It is responsible for breaking down medicines. It shows a trend for gametocytemia, parasitemia clearance rates.

Natural products offer significant complementary opportunities in drug discovery. In this study, we present in-silico efforts at natural product drug discovery for the parasitic protozoaldisease (Malaria); molecular docking of phytochemical ligands with potential parasitic proteintargets. (Santos & Sp, 2016).

MATERIALS AND METHODS

Ligand preparation

The structure of all selected chemical constituents of Azadirachta indica L. (Neem) (Nimbin, Gedunin, Salanin, Quercetin) (Alzohairy, 2016) Caricapapaya (Papain, Tocopherol) (Vyas et al., 2014) Curcuma longa L. (Tumerone, Zingeberene, Curcumine, Curlone) (Leela et al., 2002) Curcuma longa L. (Tumerone, Zingeberene, Curcumine, Curlone) (Leela et al., 2002) Mangifera indica (Mangiferin, Mangoleanone, Manglupenone, Mangostin) (D[zbreve]amić et al., 2010) Psidium guajava (guava) (Guajavarin, Ascorbic acid, Citric acid, Limonene) (Naseer et al., 2018) And native ligand (SDF File) were downloaded from the official website of the U.S. National Library of Medicine PubChem(https://pubchem.ncbi.nlm.nih.go).Then Structures imported into PyRx 0.8 using an open babel tool and energy minimization (optimization) were performed by in a view of essential parameters based on the element, its hybridization, and connectivity i.e., by Universal Force Field (UFF).These ligands were then converted to Auto Dock Ligand format (PDBQT).

Targate preparation

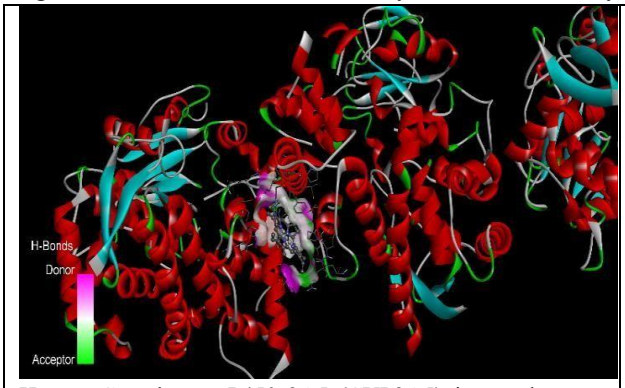
To perform the docking studies of all the chemical constituents of *Azadirachta indica* L.(Neem) (Nimbin, Gedunin, Salanin, *Quercetin), Caricapapaya (Papain, Tocopherol), Curcuma longa* L. *(Tumerone, Zingeberene, Curcumine, Curlone) Mangiferaindica (Mangiferin, Mangoleanone, Manglupenone, Mangostin), Psidium guajava (guava) (Guajavarin*, Ascorbic acid, Citric acid, Limonene) against the crystal structure of CYP3A5 Enzyme.(Khan et al., 2020) The updated elucidated crystal structure of CYP3A5 was obtainedfrom the RCSB Protein Data Bank (PDB) as entry 6MJM, 5VEU. (https://www.rcsb.org/structure/6MJM), (https://www.rcsb.org/structure/5VEU). Organism: Homo sapiens. The native ligand for 6MJM and 5VEU is protoporphyrin ix The viral protein structure was optimized, purified, and prepared for docking with the help of Discovery Studio Visualizer 2019 by removing unwanted water molecules, bound ligands from the proteins Structure and saved again in a pdb file format to the same folder. The details of CYP3A5 Enzyme using (PDB ID- 6MJM and 5VEU) are given in Table 1.

Table 1- The information of the crystal structures of the CYP3A5 Enzyme (PDB ID: 5VEU) and (PDB ID: 6MJM) enzyme

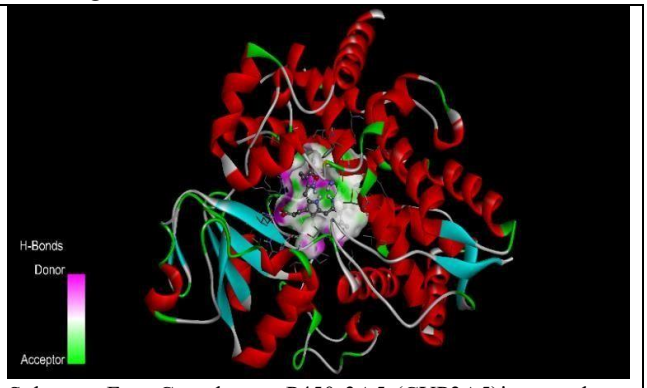
Particulars	5VEU	6MJM
Title	Human Cytochrome P450 3A5 (CYP3A5)	Substrate Free Cytochrome P450 3A5 (CYP3A5)
DOI	10.2210/pdb5VEU/pdb	10.2210/pdb6MJM/pdb
Authors	Hsu, M.-H., Johnson, E.F.	Hsu, M.H., Johnson, E.F.
Deposited on	2017-04-05	2018-09-21
Resolution	2.91 Å (reported)	2.20 Å (reported)
Classification	OXIDOREDUCTASE/OXIDOREDUCTASE inhibitor	OXIDOREDUCTASE
Organism(s)	Homo sapiens	Homo sapiens
ExpressionSystem	Escherichia coli	Escherichia coli DH5[alpha]
Method	X-Ray Diffraction	X-Ray Diffraction

The entry composition of the CYP3A5 Enzyme is represented in Figure **1**. There were 3 uniquetypes of molecules in this entry. The entry contains 3740 atoms, with hydrogens and 0 deuteriums that is why we need to add hydrogen in the protein purification process under targetpreparation for docking.

Fig. 1. The allosteric sites of the enzymes with the co-crystallize ligand molecules



Human Cytochrome P450 3A5 (CYP3A5) in complex with an inhibitor (PBD ID: 5VEU)



Substrate Free Cytochrome P450 3A5 (CYP3A5)in complex with an inhibitor (PBD ID : 6MJM)

Molecular Docking (MD)

The docking studies were performed to identify preferred orientation and molecular interactions of natural compounds with targeted proteins. The purified 6MJM and 5VEU file was loaded to docking software PyRx 0.8 using the load molecule option from the file toolbar. Chain-A was used to perform the docking as it contains the active amino acid residues. The receptor structure is then converted to Autodock macromolecule (pdbqt format) by using right- click option. Binding affinity studies were performed by using Vina Wizard Tool in PyRx 0.8.Molecules (PDBQT Files), both ligands (all the chemical constituents of Azadirachta indicaL.(Neem) (Nimbin, Gedunin, Salanin, Quercetin), Caricapapaya (Papain, Tocopherol), Curcuma longa L.(Tumerone, Zingeberene, Curcumine, Curlone) Mangiferaindica (Mangiferin, Mangoleanone, Manglupenone, Mangostin), Psidium guajava (guava) (Guajavarin, Ascorbic acid, Citric acid, Limonene) and native ligand*)* as well as the target(6MJM and 5VEU) were selected one by one for docking study. For 5VEU molecular docking simulation, the three-dimensional grid box (size_x = 142.892804379Ao; size_y = 146.001907524Ao; size_z = 80.883429613Ao) and for 6MJM molecular docking simulation, the three-dimensional grid box (size_x = 53.1904763183Ao; size_y = 77.06975438Ao; size_z= 63.4757003829Ao) was designed using Autodock tool 1.5.6 with exhaustiveness value of 8. After selecting molecules, the active amino acid residues were selected to define the cavity with the help of= the Toggle Selection Spheres option given in PyRx. (Patel & Kumar, 2010) To occupy all the active binding binding sites and important residues, the grid box was aligned properly. All the ligands and Enzyme were then subjected for docking to get the finding affinity with each other.

Analysis of Ligand–Targate Interaction

The active amino acid residues in the protein were identified and distinguished by using BIOVIA Discovery Studio Visualizer (version 20.1.0.19295). The selection of the amino acids in the active site was used to analyze the grid box and to define the cavity. All the docking poses, ligand, and protein interactions were studied by importing output files into Drug Discovery Studio, which enables us to identify the types of interactions. (Bhat et al., 2015) Discovery Studio is an offline life sciences software that offers toolsto study drug receptor interaction, docking poses visualization, and macromolecule preparations. Different output poses were analysed in Discovery Studio visualizer 2020for the formation of non-bonded hydrogen bonds. The best pose structure was analysed also by their binding affinity, inhibition constants and other supporting interactions.

RESULT

The ligand energies (kcal/mol), docking scores (kcal/mol), molecular formulas, and molecular weights(gm/mol) of all the docked phytoconstituents are tabulated in Table 2. The active amino residues, reactive atom of ligand, bond length (A^0), and type of

interactions of phytoconstituents with CYP3A5 enzyme are depicted in Table 3. The 2D- and 3D-docking poses of all the docked molecules are represented in Table 4.

Table 2- The ligand energies (kcal/mol), docking scores (kcal/mol), molecular formulas, and molecularweights (gm/mol) of all the docked phytoconstituents and native ligand/inhibitor.

Compound Name	PubChemCID	MolecularFormula	MolecularWeight (gm/mol)	Ligand Energy(kcal/mol)	Docking Score(kcal/mol)	
					CYP3A5(PDB ID:5VEU)	CYP3A5(PDB ID: 6MJM)
Ascorbic Acid	54670067	C6H8O6	176.12	210.27	-5.8	-5.5
Azadirachtin	5281303	C35H44O16	720.7	52764086261997744.00	-13.9	-15.5
Citric Acid	311	C6H8O7	192.12	102.43	-5.1	-5.1
Curcumin	969516	C21H20O6	368.4	1307.92	-7.4	-8.9
Curlone	196216	C15H22O	218.33	189.99	-6.8	-6.4
Gedunin	12004512	C28H34O7	482.6	2277.99	-9.5	-10.3
Guaijavarin	5481224	C20H18O11	434.3	583.00	-8.7	-8.2
Limonene	22311	C10H16	136.23	117.48	-6.3	-6
Mangiferin	5281647	C19H18O11	422.3	541.98	-8.3	-8.4
Manglupenone	131751009	C30H44O2	436.7	1533.98	-8.5	-9.5
Mangoleanone	101665782	C30H50O	426.7	1265.90	-9.9	-10.4
Mangostin(alphaMangostin)	5281650	C24H26O6	410.5	434.26	-9	-8.8
Nimbin	108058	C30H36O9	540.6	243441013673.93	-17.4	-14.8
Nimbolinin A	10580081	C37H44O10	648.7	2381164441207.82	-15.5	-14.1
Papain	3705436	C19H29N7O6	451.5	271.44	-7.4	-6.9
Quercetin	5280343	C15H10O7	302.23	229.64	-9	-8.5
Salannin	6437066	C34H44O9	596.7	913639849611.29	-11.6	-12.6
Tocopherol (Vitamin E)	14985	C29H50O2	430.7	6370783539.68	-9.5	-10.1
Turmerone	14367555	C15H22O	218.33	163.84	-7.4	-6.6
Zingiberene	92776	C15H24	204.35	230.85	-7	-5.8
Protoporphyrin (Native Ligand)	444097	C34H32N4O4	616.5	1085.75	-10.1	-10.3

Table 3- The active amino residues, reactive atom of ligands, bond length (A^0), and type of interactions ofphytoconstituents with CYP3A5 enzyme.

Active Amino Residue	Bond Length (A^0)	Bond Category	Bond Types
CYP3A5 (5VEU)			
Inhibitor (native ligand)			
LYS251	2.63721	Hydrogen Bond	Conventional Hydrogen Bond
LYS251	2.14253		
ASP244	2.63073		
ASP244	2.21899		
ARG255	4.42046	Electrostatic	Pi-Cation
PRO202	3.58867	Hydrophobic	Pi-Sigma
PHE248	5.28918		Pi-Pi T-shaped
PRO202	4.91801		Alkyl
PRO202	5.12158		Pi-Alkyl
PRO202	5.46474		
Ascorbic Acid			
LYS34	1.99465	Hydrogen Bond	Conventional Hydrogen Bond
THR42	2.58272		
TYR75	2.06692		
HIS30	2.0442		
LYS34	3.54493		Carbon Hydrogen Bond
Azadirachtin			
ILE301	3.58213	Hydrogen Bond	Carbon Hydrogen Bond
SER188	3.69778		
PHE271	4.70087	Electrostatic	Pi-Cation
PHE271	3.65376		
PHE271	4.41029		
Citric Acid			
LEU216	2.47434	Hydrogen Bond	Conventional Hydrogen Bond
GLY480	2.16478		
THR478	2.29366		
TYR53	2.22261		
Curcumin			
LYS208	2.48862	Hydrogen Bond	Conventional Hydrogen Bond
THR171	2.80824		
GLU163	4.60711	Electrostatic	Pi-Anion
VAL170	4.7011	Hydrophobic	Pi-Alkyl
VAL204	4.62963		
Curlone			
GLY443	2.51513	Hydrogen Bond	Conventional Hydrogen Bond

LEU133	4.14049	Hydrophobic	Alkyl
ILE184	4.92814		
ILE303	5.13417		
PHE137	5.30119		Pi-Alkyl
PHE189	4.97901		
PHE271	4.92824		
PHE271	3.78569		
PHE271	4.23678		
Gedunin			
ILE371	2.90661	Hydrogen Bond	Conventional Hydrogen Bond
LEU240	4.95824	Hydrophobic	Pi-Alkyl
Guaijavarin			
GLN200	2.04933	Hydrogen Bond	Conventional Hydrogen Bond
GLN200	2.44699		
PHE248	4.66925	Hydrophobic	Pi-Pi T-shaped
PRO202	5.36802		Pi-Alkyl
Nimbin			
ASP244	5.45941	Electrostatic	Attractive Charge
ASP244	5.06904		
GLU205	3.83406		
ASP244	3.11523		
SER206	2.36828	Hydrogen Bond	Conventional Hydrogen Bond
LYS251	2.15582		
PRO202	3.22033		Carbon HydrogenBond
GLU205	3.09318		
PHE248	4.8532	Electrostatic	Pi-Cation
PHE248	4.89397		
PHE248	3.8503		
PHE248	3.99225		Pi-Sigma
Nimbolinin A			
GLU205	5.39136	Electrostatic	Attractive Charge
ASP244	3.97949		
GLU205	5.27048		
ARG255	2.61622	Hydrogen Bond	Conventional Hydrogen Bond
ARG255	2.51481		
Papain			
GLU205	4.62659	Electrostatic	Attractive Charge
LYS251	2.2605	Hydrogen Bond	Conventional Hydrogen Bond
ARG255	2.37929		
ASP244	2.91103		
GLU205	1.97926		
GLN200	2.39345		
SER206	3.51071		
ASP244	3.75538		
LYS251	3.36603	Electrostatic	Pi-Cation
PHE248	4.21981	Hydrophobic	Pi-Pi Stacked
LYS209	2.41064	Hydrogen Bond	Conventional Hydrogen Bond
LYS251	2.02223		
ASP244	2.44729		
ASP244	2.65983		
PRO202	5.14889	Hydrophobic	Pi-Alkyl
LYS251	5.19879		
Salannin			
ASP174	4.55147	Electrostatic	Attractive Charge
ASP174	3.66781		
ASP174	4.26373		
GLU163	4.97051		
ASP174	4.99594		
LEU196	3.40109	Hydrogen Bond	Carbon HydrogenBond
LEU196	3.46064		
Tocopherol(Vitamin E)			
PHE271	4.30938	Electrostatic	Pi-Cation
PHE271	4.04617	Hydrogen Bond	Pi-Donor Hydrogen Bond
PHE137	5.15804	Hydrophobic	Pi-Alkyl
Turmerone			
PHE210	3.89012	Hydrophobic	Pi-Sigma
PHE213	3.95627		
PHE304	4.45802		Pi-Pi Stacked
PHE241	4.90089		Pi-Pi T-shaped
ILE300	5.3938		Alkyl
ILE301	4.61371		
LEU108	5.18476		

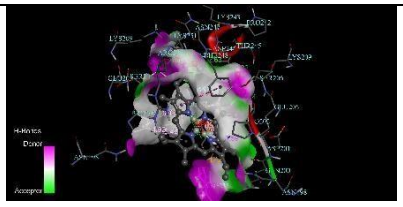
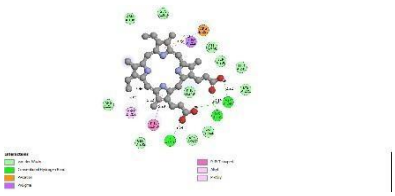
LEU240	4.76802		
PHE241	4.49231		Pi-Alkyl
PHE304	4.65279		
Zingiberene			
PHE241	3.71191		Pi-Sigma
Quercetin			
SER206	2.7675		
Limonene			
ILE184	4.64252		Alkyl
LEU133	4.16711		
PHE137	5.04308		
PHE189	5.1292		
PHE271	3.73434	Hydrophobic	Pi-Alkyl
PHE271	4.29461		
PHE271	5.09665		
Mangiferin			
PRO438	2.56611	Hydrogen Bond	Conventional Hydrogen Bond
SER299	2.69944		
ILE301	3.51421		Carbon Hydrogen Bond
PHE271	4.88264		Pi-Pi Stacked
PHE271	3.82616	Hydrophobic	
LEU133	4.95441		Pi-Alkyl
LEU133	4.5223		
Manglupenone			
No Amino Acid Present			
Mangoleanone			
PHE241	3.86754	Hydrophobic	Pi-Sigma
ALA370	5.09739		Alkyl
Mangostin(alpha-Mangostin)			
LYS251	2.25478	Hydrogen Bond	Conventional Hydrogen Bond
PHE248	4.70751		
PHE248	4.86416		
PHE248	4.97481		Pi-Pi T-shaped
PHE248	5.10289		
PRO202	4.06724		
PRO202	4.25356	Hydrophobic	Alkyl
LYS209	4.83243		
PHE203	4.87579		
PHE203	5.09877		Pi-Alkyl
PRO202	4.36713		
LEU240	5.4919		Alkyl
LEU240	5.26487	Hydrophobic	
PHE220	3.82539		Pi-Alkyl
PHE241	4.29392		
CYP3A5 (6MJM)			
Inhibitor (native ligand)			
ARG105	2.10576		Conventional Hydrogen Bond
CYS441	2.75323		
CYS441	2.96615		
ARG439	2.42666	Hydrogen Bond	
ALA305	3.52786	Hydrophobic	Pi-Sigma
CYS441	3.9875		
CYS441	4.66001	Other	Pi-Sulfur
PHE434	5.04821		Pi-Pi Stacked
PHE434, GLY435	4.96895		Amide-Pi Stacked
VAL313	4.36838		
LEU364	4.94956		Alkyl
VAL369	4.29616		
ILE184	5.24996		
PHE434	4.96234		
ALA370	4.76875	Hydrophobic	
CYS441	4.65818		
ALA305	4.31402		Pi-Alkyl
CYS441	4.33794		
ALA447	4.80502		
Ascorbic Acid			
ARG130	2.32176		Conventional Hydrogen Bond
SER134	2.05791		
ASN440	2.11199		
ASN440	2.43813	Hydrogen Bond	
CYS441	2.75902		
PHE137	2.47761		

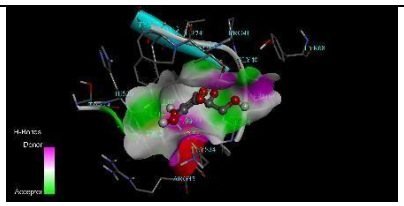
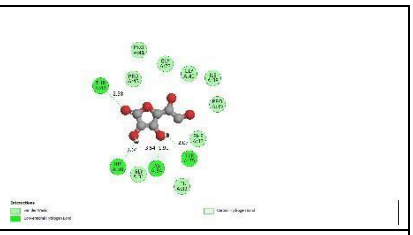
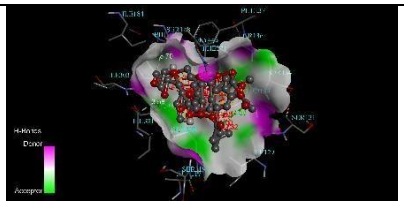
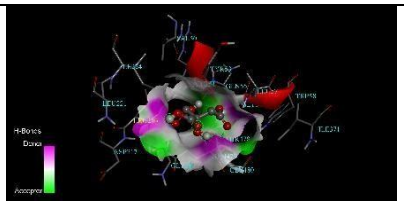
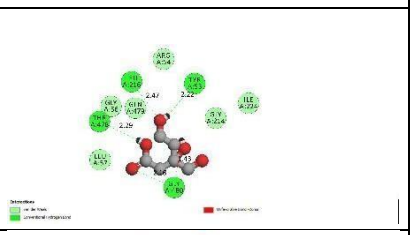
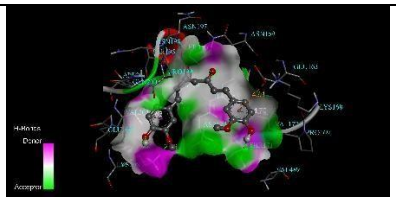
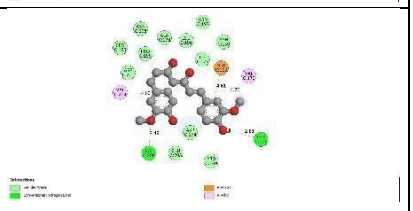
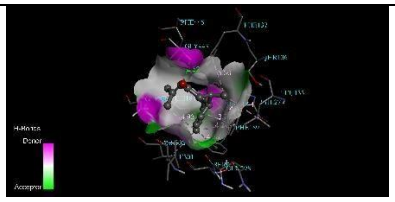
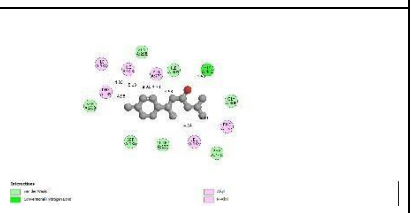
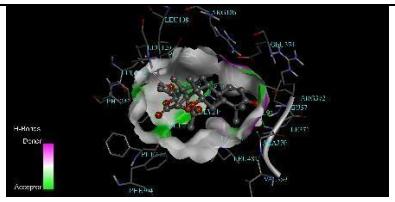
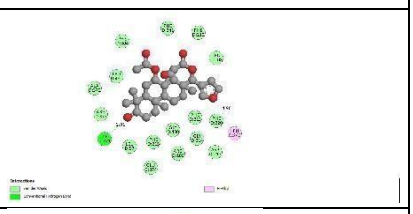
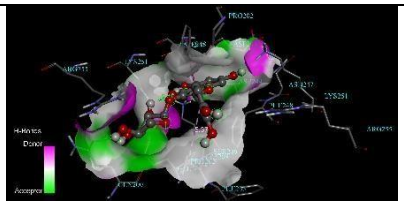
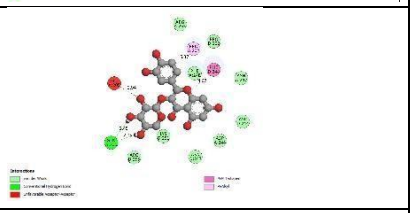
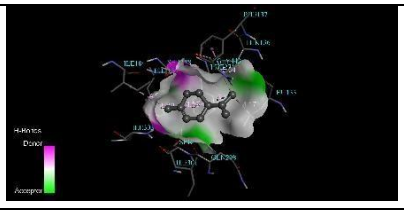
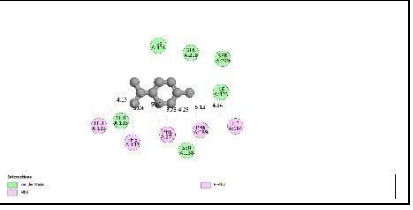
Azadirachtin			
ARG105	5.26044	Electrostatic	Attractive Charge
ILE442	2.39646	Hydrogen Bond	Conventional Hydrogen Bond
THR309	3.69431		
ALA305	3.90205	Hydrophobic	Alkyl
ILE118	5.129		
ILE301	4.38458		
Citric Acid			
THR42	2.63062	Hydrogen Bond	Conventional Hydrogen Bond
GLY73	2.51271		
HIS30	2.3623		
Curcumin			
ARG105	2.95417	Hydrogen Bond	Conventional Hydrogen Bond
ARG105	2.03183		
CYS441	3.42999		Carbon Hydrogen Bond
ILE442	3.02942		Pi-Donor Hydrogen Bond
THR309	3.79057	Hydrophobic	Pi-Sigma
CYS441	5.29256	Other	Pi-Sulfur
CYS441	4.39215	Hydrophobic	Alkyl
VAL313	4.05823		
LEU364	4.76307		
MET451	5.18393		
PHE434	4.54112		Pi-Alkyl
ILE118	5.44695		
ALA305	4.9536		
ILE442	5.27718		
CYS441	4.56808		
ALA447	5.25591		
Curlone			
VAL111	4.71022	Hydrophobic	Alkyl
LEU120	4.9306		
LEU240	4.8974		
PHE213	4.84799		Pi-Alkyl
PHE220	5.0171		
PHE220	5.0539		
PHE241	4.17701		
Gedunin			
LEU216	2.09578	Hydrogen Bond	Conventional Hydrogen Bond
GLU374	2.77061		
THR478	2.75651		
PHE220	5.15879	Hydrophobic	Pi-Pi T-shaped
Limonene			
ALA305	4.92135	Hydrophobic	Alkyl
ALA305	3.75007		
ILE442	5.45908		
ILE184	5.16973		
PHE271	4.86598		Pi-Alkyl
PHE302	4.85989		
PHE446	5.00716		
Mangiferin			
ARG439	2.68514	Hydrogen Bond	Conventional Hydrogen Bond
ARG372	2.17043		
PHE304	2.48259		
PRO433	3.59812		Carbon Hydrogen Bond
ALA370	3.79943	Hydrophobic	Pi-Sigma
LEU481	3.59127		
LEU481	3.66964		
LEU481	3.85768		
PHE304	4.64503		Pi-Pi Stacked
ALA370	5.18471		Pi-Alkyl
Manglupenone			
LEU57	2.04818	Hydrogen Bond	Conventional Hydrogen Bond
THR478	2.15683		
Mangoleanone			
PHE241	3.86754	Hydrophobic	Pi-Sigma
ALA370	5.09739		Alkyl
Mangostin(alpha-Mangostin)			

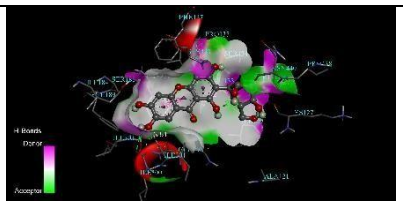
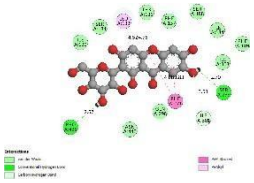
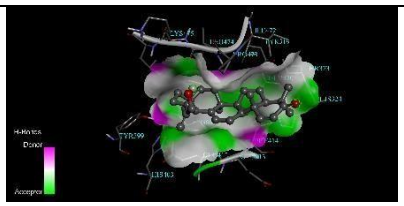
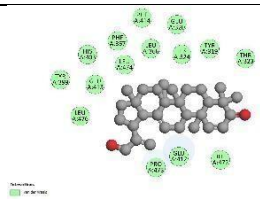
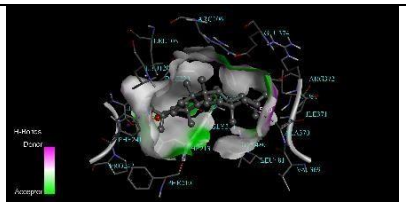
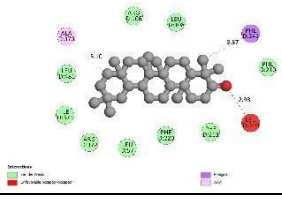
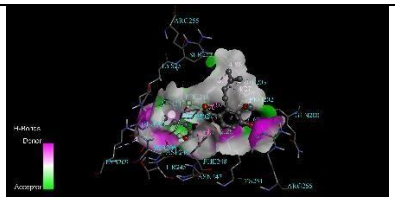
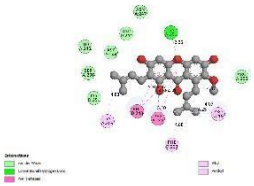
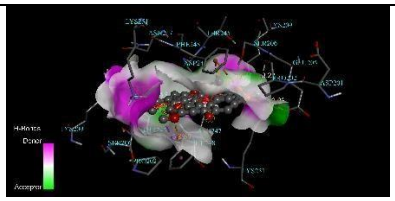
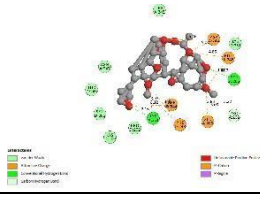
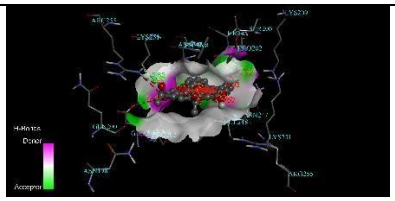
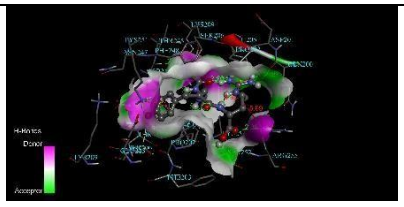
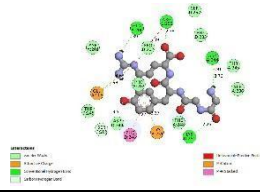
No Amino Acid Present			
Nimbin			
ARG105 (Unfavorable)	5.56747	Electrostatic	Attractive Charge
Nimbolinin A			
ASN450	2.78838	Hydrogen Bond	Conventional Hydrogen Bond
SER119	3.67623		Carbon Hydrogen Bond
CYS441	4.4152	Hydrophobic	Alkyl
Papain			
ARG106	2.40746	Hydrogen Bond	Conventional Hydrogen Bond
LEU481	2.92802		
GLU374	2.6682		
PHE210	2.27375		
GLU308	2.54715		
ARG106	2.06019		
SER107	2.47651		
LEU481	3.4979	Hydrophobic	Pi-Sigma
PHE304	4.05397		Pi-Pi Stacked
Quercetin			
THR310	2.82287	Hydrogen Bond	Conventional Hydrogen Bond
ASN450	2.36283		
ALA305, GLY306	4.14611	Hydrophobic	Amide-Pi Stacked
ALA305, GLY306	5.54522		
ALA305	4.74156		Pi-Alkyl
CYS441	5.25994		
ALA447	4.29742		
ALA447	4.85095		
CYS441	5.30153		
ALA447	4.09785		
Salannin			
GLY443	3.14669	Hydrogen Bond	Conventional Hydrogen Bond
GLY443	3.27135		
CYS441	4.42149	Hydrophobic	Alkyl
Tocopherol(Vitamin E)			
ALA370	4.00195	Hydrophobic	Alkyl
CYS441	4.72257		
ALA447	4.14009		
LEU373	5.30481		
PHE434	5.06941		Pi-Alkyl
Turmerone			
LEU216	1.91277	Hydrogen Bond	Conventional Hydrogen Bond
TYR53	4.71473	Hydrophobic	Pi-Pi T-shaped
ILE224	3.74905		Alkyl
LEU216	5.13823		
LEU221	4.3472		
TYR53	5.16502		Pi-Alkyl
Zingiberene			
LEU221	5.49275	Hydrophobic	Alkyl
ILE224	5.48683		
TYR53	4.65292		Pi-Alkyl

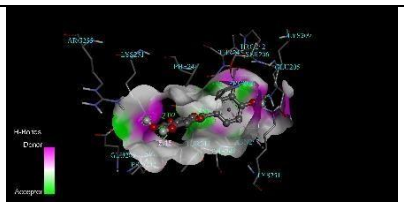
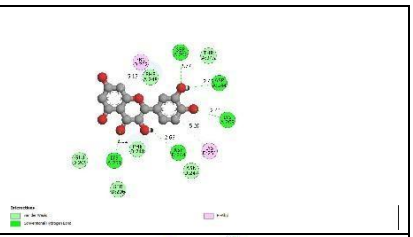
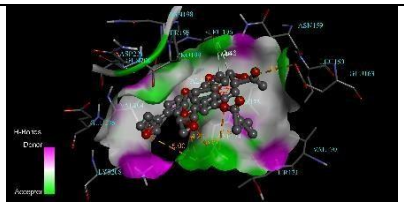
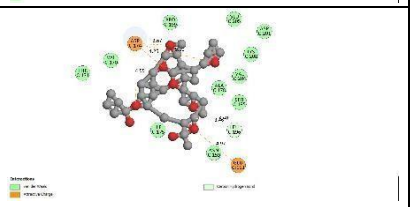
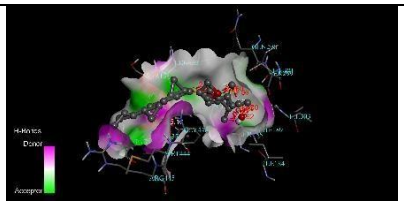
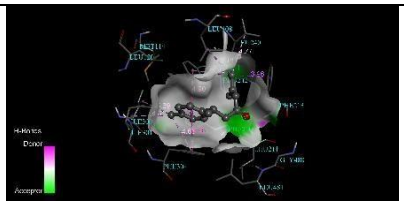
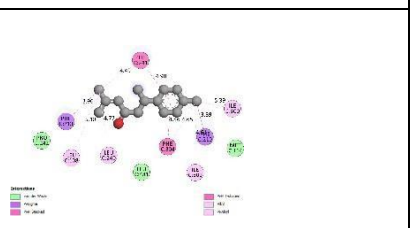
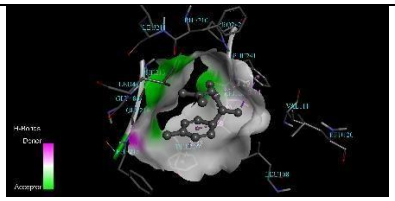
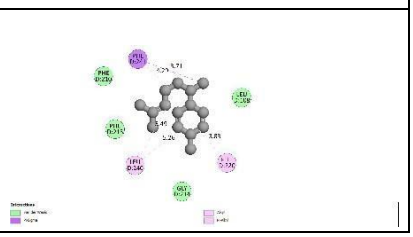
Table. 4- The 2D- and 3D docking poses of the ligands in allosteric site of the enzyme/target.

For 5VEU

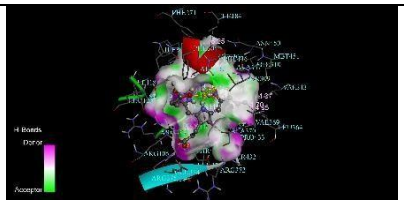
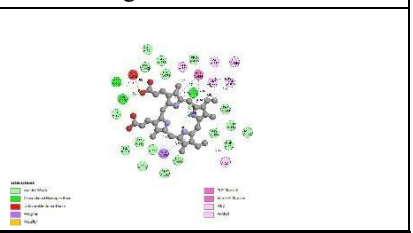
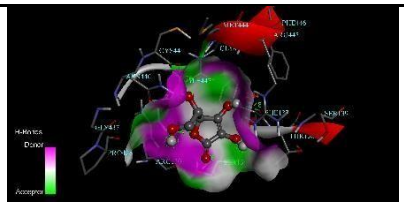
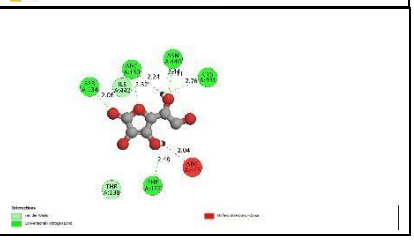
Name of Ligand	3D-docking Pose	2D-docking Pose
Protoporphyrin (Heme) Native Ligand		

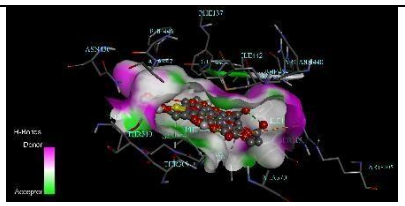
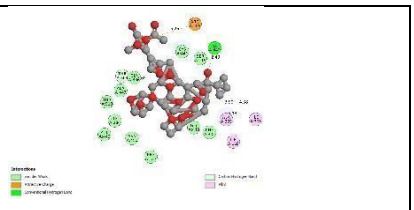
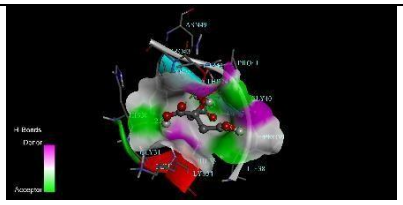
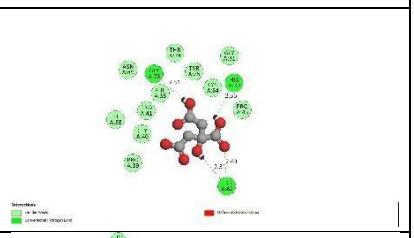
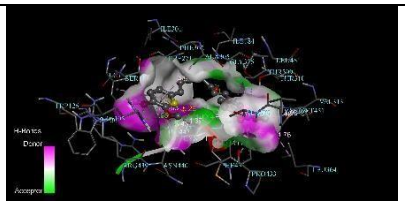
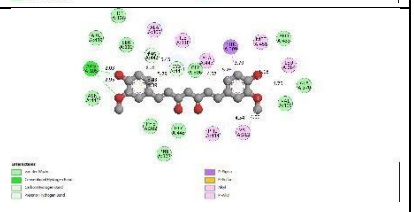
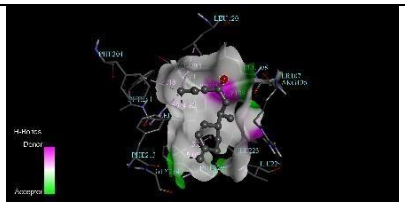
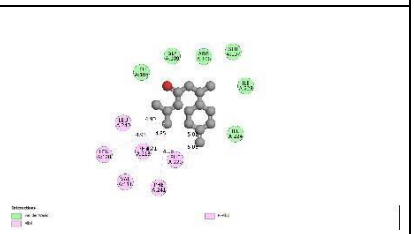
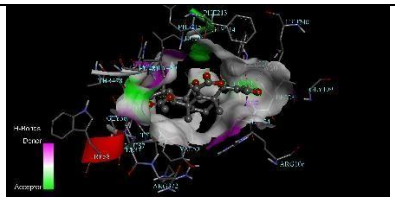
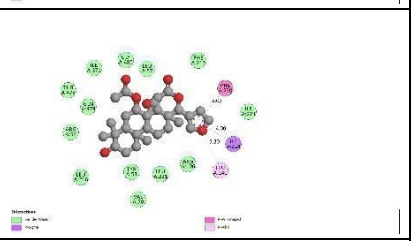
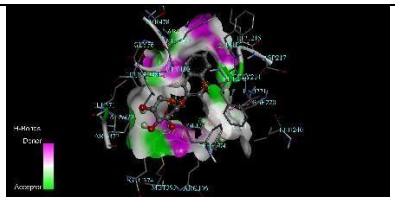
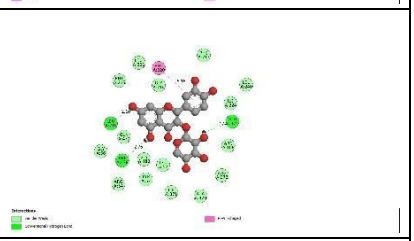
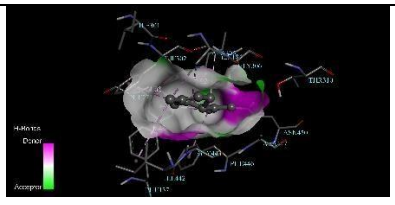
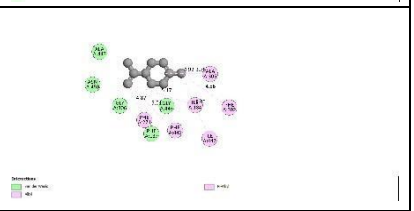
Ascorbic Acid		
Azadirachtin		
Citric Acid		
Curcumin		
Curlone		
Gedunin		
Guaijavarin		
Limonene		

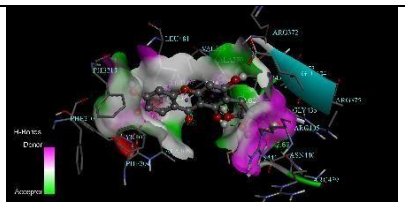
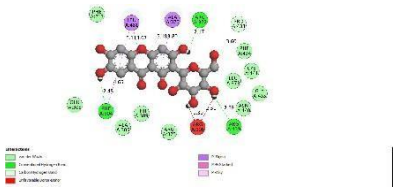
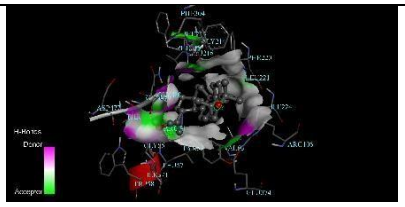
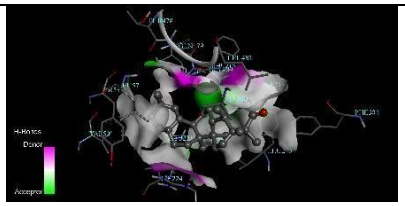
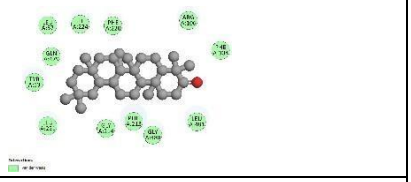
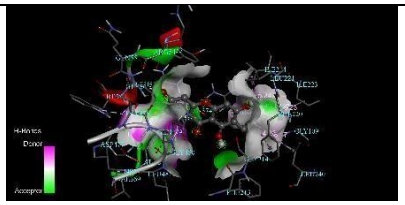
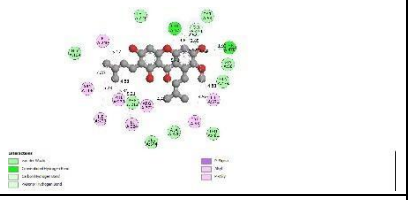
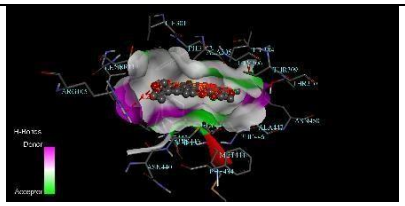
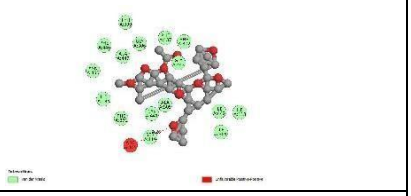
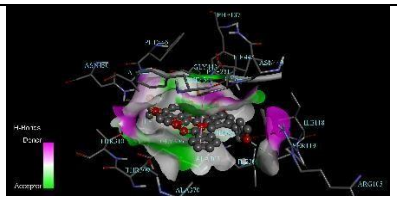
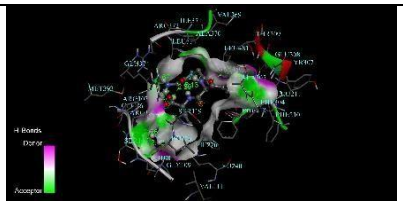
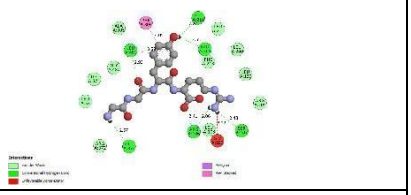
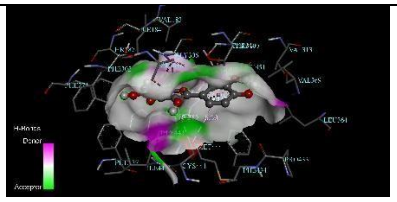
Mangiferin		
Manglupenone		
Mangoleanone		
Mangostin(alpha-Mangostin)		
Nimbin		
Nimbolinin A		
Papain		

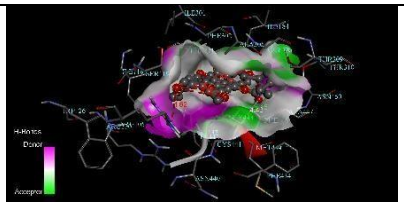
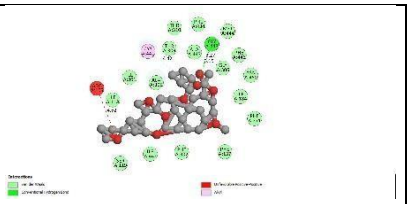
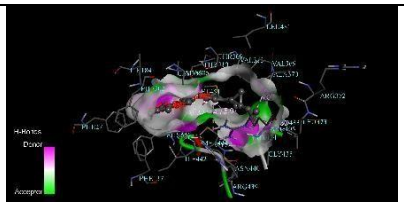
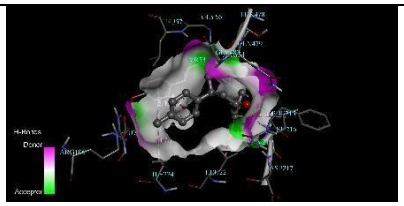
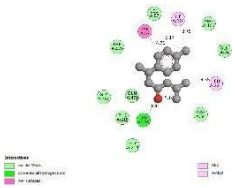
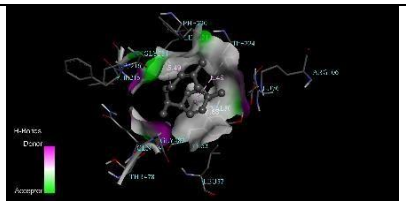
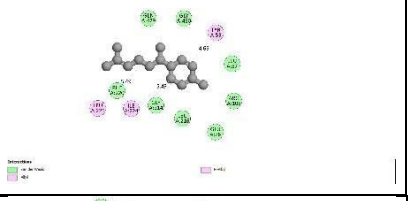
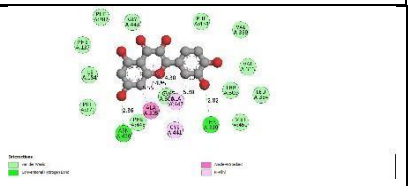
Quercetin		
Salannin		
Tocopherol(Vitamin E)		
Turmerone		
Zingiberene		

For 6MJM

Name of Ligand	3D-docking Pose	2D-docking Pose
Protoporphyrin (Heme) Native Ligand		
Ascorbic Acid		

Azadirachtin		
Citric Acid		
Curcumin		
Curlone		
Gedunin		
Guaijavarin		
Limonene		

Mangiferin		
Manglupenone		
Mangoleanone		
Mangostin(alpha-Mangostin)		
Nimbin		
Nimbolinin A		
Papain		
Quercetin		

Salannin		
Tocopherol(Vitamin E)		
Turmerone		
Zingiberene		
		

DISCUSSION NATIVE LIGAND

The native ligands of PDB structures were used as reference molecule for the validation of results obtained from MD. In case of CYP3A5 enzyme (PDB ID 6MJM and 5VEU) the native ligand was protoporphyrin (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoic acid) is a derivative of porphyrin which has propionic acid groups, specifically a porphyrin that plays an important role in living organisms as a precursor to other critical compounds like hemoglobin and chlorophyll. (Ix et al., 1975) It exhibited -10.1 kcal/mol binding affinity with CYP3A5 enzyme (PDB ID5VEU) and formed 2 Hydrogen bond withASP244 (2.63073A^0, 2.21899A^0) and one electrostatic interaction with AGR255 (4.42046A^0) and It has shown 5 Hydrophobic interaction with PHE202 (3.58867A^0, 4.91801A^0, 5.12158A^0, 5.46474A^0), PHE248 (5.28918A^0), by the Pi-Cation, Pi-Pi-sigma,Pi-Pi T-shaped, Alkyl, and Pi-alkyl orbitals of orbitals of aromatic ring system. In 6MJM it exhibited -10.3 kcal/mol binding affinity with CYP3A5 enzyme and formed 4 hydrogen bonds (4 conventional) with ARG105, (-HH21 2.10576A^0), CYS441, (-SG 2.75323A^0, 2.96615A^0), ARG439 (2.42666A^0).

It has developed hydrophobic interactions with ALA305, (3.52786A^0), CYS441 (3.9875A^0), PHE434 (5.04821A^0), PHE434, GLY435 (-C 4.96895A^0), VAL313 (4.36838A^0), LEU364 (4.94956A^0), VAL369 (4.29616A^0), ILE184 (5.24996A^0), PHE434 (4.96234A^0), ALA370 (4.76875A^0), through Pi-Sigma, Pi-Pi Stacked, Amide-Pi Stacked and Alkyl. The other bonds also form with CYS441 (4.66001A^0) by Pi-Sulfur. In terms of binding affinity. The 2D- and

3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Ascorbic Acid

Ascorbic acid (vitamin C) plays a role as a redox cofactor and catalyst in a broad array of biochemical reactions and processes. Ascorbic acid Ascorbic Acid is a natural water-soluble vitamin (Vitamin C). This acid is a potent reducing and antioxidant agent that functions in fighting bacterial. The IUPAC–IUB Commission on Biochemical Nomenclature changed vitamin C (2-oxo-Ltheo-hexono-4-lactone-2,3-enediol) to ascorbic acid or L-ascorbic acid in 1965. Ascorbic acid has two chiral centers, which contain four stereoisomers. (Keith, 2005) It exhibited -5.8 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 5 hydrogen bonds (4 conventional hydrogen bonds and 1 carbon hydrogen bonds) with LYS34 (-HN, 1.99465A^0), THR42 (-HG1, 2.58272A^0), TYR75 (-OH, 2.06692A^0), HIS30 (-O, 2.0442A^0) and LYS34 (-CE, 3.54493A^0). As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -5.8 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 6 Hydrogen bond (total 6 conventional hydrogen bonds) with AGR130 (-HH21, 2.32176A^0), SER134 (-HG 2.057912A^0), ASN440 (-HD22, 2.11199A^0, - OD, 2.43813A^0), CYS441 (-O, 2.75902A^0), and PHE137 (-O, 2.47761A^0). In terms of binding affinity, it is less effective than the native ligand, and it is also less stable since its ligand energy was 1355.52 kcal/mol. With CYP3A5 enzyme (PDB ID 6MJM) enzyme, it has shown less binding free energy than the native ligand i.e. -5.5 kcal/mol. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table4.

Azadirachtin

Azadirachtin (AZA) is the most abundant and relevant compound present in Azadirachta indica. This compound can be found in various parts of the Neem tree (seeds, callus, fruits and leaves) but the concentrations are quite variable presenting values that range from ca. 0.25μgg−1 in callus to ca. 48,000μgg−1 in seeds. Azadirachtin is a tetranortriterpenoid of the class of limonoids that presents the chemical formula C35H44O16 and a molecular weight of 720.71 g mol−1. (Fernandes et al., 2019) It exhibited -13.9 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 2 hydrogen bonds (2 carbon hydrogen bonds) with ILE301 (-C, 3.58213A^0) and SER188 (-C, 3.69778A^0). It has developed electrostatic interactions with PHE271 (-C, 3.65376A^0) and PHE271 (-C, 4.41029A^0) through Pi-cation. As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. It exhibited -15.5 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 2 Hydrogen bond (2 conventional) with, ILE442 (-HN, 2.39646A^0), THR309 (-OG1, 3.69431A^0), and developed hydrophobic interactions with ALA305 (3.90205A^0), ILE118 (-A, 5.129A^{0,}), ILE301 (-A, 4.38458A^0), through Alkyl bond. It has shown electrostatic interactions with ARG105 (-NH2,5.26044A^0), by the Attractive charge of aromatic ring system. In terms of binding affinity, it is more potent than the native ligand. The 2D- and 3D-binding poses of both the native ligands are represented in table 4.

Citric acid

Citric acid (2-hydroxy-1,2,3-propane tricarboxylic acid, C6H8O7) is an acidulant, preservative, emulsifier, flavorant, sequestrant and buffering agent widely used across many industries especially in food, beverage, pharmaceutical, nutraceutical and cosmetic products. First crystallized from lemon juice and named accordingly by Scheele in Sweden in 1784, citric acid is a tricarboxylic acid whose central role in the metabolism of all aerobic organisms was undisclosed by Krebs. (Ciriminna et al., 2017) It exhibited -5.1 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 4 hydrogen bonds (4 conventional hydrogen bonds) with LEU216 (2.47434A^0), GLY480 (2.16478A^0), THR478 (2.29366A^0) and TYR53 (2.22261A^0). As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -5.1 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 3 Hydrogen bond (3conventional bond) with, THR42 (-HG1, 2.63062A^0), GLY73 (-O, 2.51271A^0), HIS30 (-O, 2.3623A^0). As compare to native legend, it is less potent than the native ligand. The 2D- and 3D-binding poses of both the native ligands are represented in table 4.

Curcumin

Curcumin [1, (1 E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a constituent (up to ~5%) of the traditional medicine known as turmeric. (Nelson et al., 2017) It exhibited -7.4 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 2 hydrogen bonds (2 conventional hydrogen bonds) with LYS208 (-HZ3, 2.48862A^0) and THR171 (-OG1, 2.80824A^0). It has developed electrostatic interactions with GLU163 (-OE1, 4.60711A^0) through Pi-Anion and hydrophobic interactions with VAL170 (4.7011A^0) and VAL204 (4.62963A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -8.9 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) cbond,1 Carbon hydrogen bond, 1 Pi donar hydrogen bond) with AGR105 (- HH22, 2.95417A^0, -HH22, 2.03283A^0), CYS441 (-CA, 3.42999A^0), ILE442 (-HN, 3.02942A^0). In terms of binding affinity, it is less effective than the native ligand, and it is also less stable since its ligand energy was 1355.52 kcal/mol. With CYP3A5 enzyme, kcal/mol. In terms of binding affinity, it is more potent than the native ligand. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Curlone

Curlone, also known as b-turmerone, belongs to the class of organic compounds known as sesquiterpenoids. These are terpenes with three consecutive isoprene units. Curlone is an extremely weak basic (essentially neutral) compound (based on its pKa). (Stanojevic et al., 2015) It exhibited -6.8 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 1 hydrogen bond (conventional hydrogen bond) with GLY443 (-HN2, 2.51513A^0). It has developed hydrophobic interactions with LEU133 (4.14049A^0), ILE184 (4.92814A^0), ILE303 (5.13417A^0), PHE137 (5.30119A^0) through Alkyl and PHE189 (4.97901A^0), PHE271(4.92824A^0), PHE271 (3.78569A^0), and PHE271 (4.23678A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -6.4 kcal\mol binding free energy with 6MJM enzyme. It has developed 7 hydrophobic interactions with VAL111 (4.71022A^0), LEU120 (4.9306A^0), LEU240 (4.8974A^0), PHE213 (4.84799A^0) PHE220 (5.0171A^0,5.0539 A^0), PHE241 (4.17701A^0) through Alkyl and Pi-Alkyl. As

compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table4.

Gedunin

Gedunin [C28H34O7 (MW: 482.55 g/mol)] is important limonoid present in several genera of the Meliaceae family, mainly in seeds. Gedunin is the most representative member of the ring D-seco class of limonoids. (Braga et al., 2020) It exhibited -9.5 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 1 hydrogen bond (conventional hydrogen bond) with ILE371 (-HN, 2.90661A^0). It has developed hydrophobic interactions with LEU240 (4.95824A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -10.3 kcal\mol binding free energy with 6MJM enzyme and It has developed 3 hydrophobic interactions with ILE224 (-CD1, 3.99688A^0), PHE220 (5.02028A^0), LEU240 (5.20009A^0), through Pi-Sigma, Pi-Pi T-shaped, Pi-Alkyl. As compared to native ligand, it has exhibited same potency in CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Guaijavarin

Guaijaverin (C20H18O11) is the 3-O-arabinoside of quercetin. It is found in the leaves of Psidium guajava, the common guava. (Naseer et al., 2018) It exhibited -8.7 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 2 hydrogen bonds (2 conventional hydrogen bonds) with GLN200 (-OE1, 2.04933A^0) and GLN200 (-OE1, 2.44699A^0). It has developed hydrophobic interactions with PHE248 (4.66925A^0) through Pi-Pi T-shaped and PRO202 (5.36802A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -8.2 kcal\mol binding free energy with 6MJM enzyme and forms 3 hydrogen bonds (3 conventional hydrogen bonds) with LEU216 (-HN, 2.09578A^0) and GLU374 (-OE2, 2.77061A^0), THR478 (-O, 2.75651A^0). It has developed hydrophobic interactions with PHE220 (5.15879 A^0) through Pi-Pi T-shaped. As compared to native ligand,

it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Limonene

Limonene (1,8-p-menthadiene = 1-methyl-4-(1-methylethenyl-cyclohexene) is one of the most common essential oil constituents of aromatic plants. It is widely found in several plant genera, which could be attributed to its precursory role from which several monocyclic monoterpenoids are derived. (Erasto & Viljoen, 2008) It exhibited -6.3 kcal\mol binding free energy with CYP3A5 enzyme (5VEU). It has developed hydrophobic interactions with ILE184 (4.64252A^0), LEU133 (4.16711) through Alkyl and PHE137 (5.04308A^0), PHE189 (5.1292A^0), PHE271 (3.73434A^0), PHE271 (4.29461A^0) and PHE271 (5.09665A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -6 kcal\mol binding free energy with 6MJM enzyme. It has developed 7 hydrophobic interactions with ALA305 (4.92135A^0, 3.75007A^0), ILE442 (5.45908 A^0), ILE184 (5.16973A^0), PHE271 (4.86598A^0), PHE302 (4.85989A^0), PHE446 (5.00716A^0) through Alkyl and Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Mangiferin

Mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one) is a xanthone present in significant levels in higher plants and in different parts of the mango fruit, such as the peel, stalks, leaves, barks, kernel, and stone. It is a promising antioxidant with tremendous health-related properties such as antiviral, anticancer, ant diabetic, anti-oxidative, antiaging, immunomodulatory, hepatoprotective and analgesic effects. (Imran et al., 2017) It exhibited -8.3 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 3 hydrogen bonds (2 conventional hydrogen bonds and 1 carbon hydrogen bond) with PRO438 (-O, 2.56611A^0), SER299 (-O, 2.69944A^0) and ILE301 (-C, 3.51421A^0). It has developed hydrophobic interactions with PHE271 (4.88264A^0), PHE271 (3.82616A^0) through Pi-Pi Stacked and LEU133 (4.95441A^0), LEU133 (4.5223A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5(6MJM) enzyme inhibition. It exhibited -8.4 kcal\mol binding free energy with CYP3A5 enzyme (6MJM) and forms 4 hydrogen bond (3 conventional hydrogen bond,1 Carbon hydrogen) with AGR439 (-O, 2.68514 A^0), AGR372 (- O, 2.17043A^0) PHE304 (-O, 2.48259 A^0), PRO433 (-O, 3.59812 A^0). It has developed hydrophobic interactions with ALA370 (-CB, 3.79943A^0, 5.18471A^0), LEU481 (-CD1, 3.59127A^0, -CD2, 3.66964A^0, -CD1, 3.85768A^0), PHE304 (4.64503A^0), through Pi-Sigma, Pi-Pi stacked, Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Manglupenone

The compound of Manglupenone is isolated from Mangifera indica. It's IUPAC name is 1-(3-hydroxyprop-1-en-2-yl)-3a,5a,5b,8,8,11a-hexamethyl2, 3, 4, 5, 6, 7, 7a, 11b,12,13 b-decahydro-1H-cyclopenta [a] chrysen-9-one and MW is 436.7 g/mol. (Shah et al., 2010) It exhibited -8.5 kcal\mol binding free energy with CYP3A5 enzyme (5VEU), but there is no amino acid present in manluoenone. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -9.5kcal\mol binding free energy with CYP3A5 enzyme (6MJM). And forms 2 hydrogen bond (2 conventional hydrogen bond,) with LEU57 (-HN, 2.04818 A^0), THR478 (-O, 2.15683 A^0), as compared to native ligand, it has exhibited less potent CYP3A5(6MJM) enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Mangoleanone

The compound of Manglupenone is isolated from Mangifera indica. It's IUPAC name is (4aR,6aR,6aR,6bR,8aR,12aS,14aR,14bR)-4,4,6a,6b,8a,11,11,14b-octamethyl 2,4a,5,6,6a,7,8,9,10,12,12a,13,14,14a-tetradecahydro-1H-picen-3-one and MWis 426.7 g/mol. (D[zbreve]amić et al., 2010) It exhibited -9.9 kcal\mol binding free energy with CYP3A5 enzyme (5VEU). It has developed hydrophobic interactions with PHE241 (3.86754A^0), through Pi-sigma and

ALA370 (5.09739A^0) through Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited - 10.4kcal\mol binding free energy with CYP3A5(6MJM) enzyme. It has developed hydrophobic interactions with PHE241 (3.86754A^0), through Pi-sigma and ALA370 (5.09739A^0) through Alkyl. As compared to native ligand, it has exhibited more potent CYP3A5(6MJM) enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table4.

Mangostin (alpha-Mangostin)

The compound of Manglupenone is isolated from Mangifera indica. It's IUPAC name is 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl) xanthen-9-one and MW is 410.5 g/mol. (Shah et al., 2010) It exhibited -9 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 1 hydrogen bond (conventional hydrogen bond) with LYS251 (-HZ3, 2.25478A^0). It has developed hydrophobic interactions with PHE248 (4.70751A^0), PHE248 (4.86416A^0), PHE248 (4.97481A^0), PHE248 (5.10289A^0) through Pi-Pi T-shaped, PRO202 (4.06724A^0), PRO202 (4.25356A^0), LYS209 (4.83243A^0) through Alkyl and PHE203 (4.87579SA^0), PHE203 (5.09877A^0), PRO202 (4.36713A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme (6MJM) inhibition. It exhibited -8.8 kcal\mol binding free energy with CYP3A5 enzyme (6MJM), but there is no amino acid preent in manluoenone. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition.

Nimbin

Nimbin is a limonoid found in Azadirachta indicaand it's IUPAC name is Methyl (2R,3aR,4aS,5R,5aR,6R,9aR,10S,10aR)-5-(acetyloxy)-2-(furan-3-yl)-10-(2-methoxy-2-oxoethyl)-1,6,9a,10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2H-cyclopenta[b]naphtho[2,3-d]furan-6-carboxylate. (Gupta et al., 2019) It has a role as a plant metabolite and a pesticide. It is an acetate ester, a limonoid, a member of furans, a cyclic terpene ketone, an enone, a tetracyclic triterpenoid and a methyl ester. .(Sufiyan sk REF) It exhibited -17.4 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 4 hydrogen bonds (2 conventional hydrogen bonds and 2 carbon hydrogen bonds) with SER206 (-HG, 2.36828A^0), LYS251 (-HZ1, 2.15582A^0), PRO202 (-O, 3.22033A^0) and GLU205 (-OE1, 3.09318A^0). It has developed electrostatic interactions with ASP244 (-OD2, 5.45941A^0), ASP244 (-OD2, 5.06904A^0), GLU205 (-OE1, 3.83406A^0) and ASP244 (3.11523A^0) through attractive charge, PHE248 (4.8532A^0), PHE248 (4.89397A^0), PHE248 (3.8503A^0) through Pi- Cation and PHE248 (3.99225A^0) through Pi-Sigma. As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. It exhibited -14.8 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 1 Electrostatic bonds (1 attractive Charge) with ARG105 (Unfavorable) (5.56747A^0). As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Nimbolinin A

Nimbolinin A is a triterpenoid isolated from Neem and it's IUPAC name is [(1R,2R,4R,6S,8R, 11R,12S, 13R, 16R,17R,19S,20R)-17,19-diacetyloxy-8-(furan-3-yl)-4 hydroxy-1,9,11,16-tetramethyl-5, 14 dioxapentacyclo[11.6.1.02,11.06,10.016,20]icos-9-en- 12-yl] benzoate. (Gupta et al., 2019) It exhibited -15.5 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 2 hydrogen bonds (2 conventional hydrogen bonds) with ARG255 (-HH11, 2.61622A^0) and ARG255 (-HH12, 2.51481A^0). It has developed electrostatic interactions with GLU205 (-OE1, 5.39136A^0), ASP244 (-OD2, 3.97949A^0) and GLU205 (-OE1, 5.27048A^0) through attractive charge. As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. It exhibited -14.1 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 2 hydrogen bonds (1 conventional and 1 carbon) with ASN450 (-HD21, 2.78838A^0), SER119 (-CB, 3.67623A^0). It has developed hydrophobic interactions with CYS441 (4.4152A^0) through Alkyl. As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Papain

Papain is a plant proteolytic enzyme for the cysteine proteinase family cysteine protease enzyme in which enormous progress has been made to understand its functions. Papain is found naturally in papaya (Carica papaya L.) manufactured from the latex of raw papaya fruit. (Amri, 2016) Papain is made possible is through the cysteine-25 portion of the triad in the active site that attacks the carbonyl carbon in the backbone of the peptide chain freeing the amino terminal portion. (Amri & Mamboya, 2012) It exhibited -7.4 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 7 hydrogen bonds (7 conventional hydrogen bonds) with LYS251 (-NZ, 2.2605A^0), ARG255 (-HH11, 2.37929A^0), ASP244 (-O, 2.91103A^0), GLU205 (-OE1, 1.97926A^0), GLN200 (-O, 2.39345A^0), SER206 (-CB, 3.51071A^0) and ASP244 (-OD2,3.75538A^0). It has developed electrostatic interactions with GLU205 (-OE1, 4.62659A^0) through attractive charge and LYS251 (-NZ, 3.36603A^0) through Pi-Cation. It also developed hydrophobic interactions with PHE248 (4.21981A^0) through Pi-Pi Stacked. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -6.9 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 7 hydrogen bonds (7 conventional) with ARG106 (-HE, 2.40746A^0, -O, 2.06019A^0), LEU481 (-HN, 2.92802A^0,), GLU374 (-OE2, 2.6682A^o,), GLU308 (-H, 2.11627A^0), PHE210 (-O, 2.27375A^0), ARG106 (-O, 2.06019A^0), SER107 (-O, 2.47651A^0,). It has developed hydrophobic interactions with LEU481 (-CD1, 3.4979A^0), PHE304 (-N 4.05397A^0), through Pi-Sigma and Pi-Pi Stacked of aromatic system. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Quercetin

Quercetin is one of the major dietary flavonoids belonging to a group of flavonols. It occurs mainly as glycosides, but other derivatives of quercetin have been identified as well. Attached substituents change the biochemical activity and bioavailability of molecules when compared to the aglycone. (MATERSKA, 2008) It exhibited -9 kcal\mol binding free energy with CYP3A5 enzyme (5VEU) and forms 5 hydrogen bonds (5 conventional hydrogen bonds) with SER206 (-HG, 2.7675A^0), LYS209 (-HZ3, 2.41064A^0), LYS251 (-HZ3, 2.02223A^0), ASP244 (-OD2, 2.44729A^0) and ASP244 (-O, 2.65983A^0). It has developed hydrophobic interactions with PRO202 (5.14889A^0) and LYS251 (5.19879A^0) through Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. It exhibited -8.5 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 2 hydrogen bonds (2 conventional) with THR310 (-HG1, 2.82287A^0), ASN450 (-HD22, 2.36283A^0). It has developed hydrophobic interactions with ALA305, GLY306 (-C, 4.14611A^0, -C, 5.54522A^0, 4.74156A^0) CYS441 (5.25994A^0, 5.30153A^0) ALA447 (4.29742A^0, 4.85095A^0, 4.09785A^0) through Amide-Pi Stacked and Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Salannin

Salannin is a limonoid with insecticidal activity isolated from Azadirachta indica. It is an acetate ester, a member of furans, a limonoid, an organic heteropentacyclic compound and a methyl ester. It derives from a tiglic acid. (Jarvis et al., 1997) It exhibited -12.6 kcal/mol binding affinity with CYP3A5 enzyme (5VEU) and formed 2 Hydrogen bond(2 Carbon hydrogen bond) with LEU196 (-O 3.40109A^0, -O 3.46064A^0), and It has shown 5 electrostatic interactions with ASP174 (-OD2, 4.55147A^0, -OD2, 3.66781A^0, -OD1, 4.26373A^0, -OD1,
4.99594A^0), GLU163 (-OE2, 4.97051 A^0), by the Attractive charge. In terms of binding affinity, it is more effective than the native ligand, With CYP3A5 enzyme. In terms of binding affinity, it is more potent than the native ligand. It exhibited -12.6 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 2 hydrogen bonds (2 conventional) with GLY443, (3.14669A^0, 3.27135A^0). It has developed hydrophobic interactions with CYS441, (4.14611A^0) through Alkyl. As compared to native ligand, it has exhibited more potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Tocopherol (Vitamin E)

It is a nutrient that is important for many body processes. It helps your nerves and muscles work well, prevents blood clots, and boosts the immune system. Vitamin E is a type of antioxidant, a substance that protects cells from damage. , was characterized as (2*R*)-2,5,7,8- tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol. (Blasini et al., 2019) It exhibited -9.5 kcal/mol binding affinity with with CYP3A5 enzyme (5VEU) and formed 1 Hydrogen bond(1 Carbon hydrogen bond) with PHE271 (4.04617A^0), one hydrophobic bond with PHE137 (5.15804A^0) through Pi alkyl bond, and It has shown 1 electrostatic interactions with PHE271(4.30938 A^0), by the Pi-Cation. In terms of binding affinity, it is less effective than the native ligand, With CYP3A5 enzyme (6MJM). It exhibited --12.6 kcal/mol binding affinity with CYP3A5 enzyme and it has developed hydrophobic interactions with ALA370, (4.00195A^0), CYS441 (4.72257A^0), ALA447 (4.14009A^0), LEU373 (5.30481A^0), PHE434 (5.06941A^0) through Alkyl and Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Turmerone

Turmerone stimulates the proliferation of peripheral blood mononuclear cells and increases the production of TNF-α, IL-2, and IFN-γ.[1] It also promotes the maturation of dendritic cells and induces the proliferation of neural stem cells both *in vitro* and *in vivo*. Formal name is 2- methyl-6S-(4-methylphenyl)-2-hepten-4-one. (*Product Information*, 2014) It exhibited - 7.5kcal/mol binding affinity with CYP3A5 enzyme (PDB ID5VEU) and It has shown 10 Hydrophobic interaction with PHE210 (3.89012A^0), PHE213 (3.95627A^0), PHE304 (-N, 4.45802A^0, -N, 4.65279A^0), PHE241 (-N, 4.90089A^0, -N, 4.49231A^0), ILE300 (-4.61371A^0), LEU108 (5.18476A^0), LEU240 (4.76802A^0), by the Pi-sigma,Pi-Pi stacked,Pi-Pi T shapped,Pi alkyl orbitals of ring system. In terms of binding affinity, it is less effective than the native ligand, With CYP3A5 enzyme (6MJM). It exhibited -6.6 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and formed 1 hydrogen bonds (1 conventional) with LEU216 (1.91277A^0), It has developed hydrophobic interactions with TYR53 (4.71473A^0), ILE224 (3.74905A^0), LEU216 (5.13823A^0), LEU221 (4.3472A^0), TYR53 (5.16502A^0) through Alkyl and Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

Zingiberene

Zingiberene is 2-Methylcyclohexa-1, 3-diene. It is a sesquiterpene found in the dried rhizomes of Indonesian ginger, Zingiber Officinale. It is a sesquiterpene and a cyclohexadiene. (Rani, 1999) It exhibited -7 kcal/mol binding affinity with CYP3A5 enzyme (5VEU) and It has shown 5 Hydrophobic interaction with PHE241, (-N, 3.71191A^0, 4.49231A^0), LUE240 (-N, 5.4919A^0,-N, 5.26487A^0), PHE220 (-N, 3.83539A^0), by the Pi-sigma,Alkyl,and Pi-Pi alkyl orbitals of ring system. In terms of binding affinity, it is less potent than the native ligand, With CYP3A5 enzyme. It exhibited -5.8 kcal/mol binding affinity with CYP3A5 enzyme (6MJM) and it has developed hydrophobic interactions with LEU221, (5.48683A^0), ILE224 (4.72257A^0), TYR53 (4.65292A^0), through Alkyl and Pi-Alkyl. As compared to native ligand, it has exhibited less potent CYP3A5 enzyme inhibition. The 2D- and 3D-binidng poses of Ascorbic acid in allosteric cavity of both the enzymes are illustrated in table 4.

CONCLUSION

For several years, the testing of compounds through computational approach has been an important field of science. In recent years, electronic compound screening has been widely performed owing to the wearying and costly nature of the investigational screening procedures.The goal of this research was to discover, classify and evaluate novel drugs against Malaria in the relationship between drug-receptor interactions and their *in silico* examination. New drug candidates from the medicinal plant *Azadirachta indica L., Caricapapaya, Curcuma longa L., Mangifera indica, Psidium guajava (guava)* have been reported as potent CYP3A5 enzyme inhibitor. The present investigative research showed that Anti-malarial has important values in the recognized compounds included in the present analysis. The twenty compounds selected were evaluated on the basis of

CYP3A5 enzyme binding energy values. Except Ascorbic Acid, Citric Acid, Curcumin, Curlone, Gedunin, Guaijavarin, Limonene, Mangiferin, Manglupenone, Mangoleanone, Mangostin(alpha-Mangostin), Papain, Quercetin, Tocopherol(Vitamin E), Turmerone, Zingiberene, all the selected compounds have exhibited very potent on CYP3A5 enzyme of protein structure of 5VEU inhibitor than its native ligand. Azadirachtin, Gedunin, Mangoleanone, Nimbin, Nimbolinin A, Salannin have shown excellent CYP3A5 enzyme of protein structure of 6MJM inhibitor than its native ligand. As a result of present investigation, it has been concluded that these compounds can be used to treat the Malaria.

REFERENCES

[1] Alzohairy, M. A. (2016). Therapeutics role of azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*, *2016*. https://doi.org/10.1155/2016/7382506.

[2] Amri, E. (2016). *PAPAIN , A PLANT ENZYME OF BIOLOGICAL IMPORTANCE : A REVIEW PAPAIN , A PLANT ENZYME OF BIOLOGICAL IMPORTANCE : A REVIEW*. *January 2012*, 2–8. https://doi.org/10.3844/ajbbsp.2012.99.104.

[3] Amri, E., & Mamboya, F. (2012). Papain, a plant enzyme of biological importance: A review. *American Journal of Biochemistry and Biotechnology*, *8*(2), 99–104. https://doi.org/10.3844/ajbbsp.2012.99.104

[4] Bhat, H. R., Singh, U. P., Thakur, A., Ghosh, S. K., Gogoi, K., Prakash, A., & Singh, R. K. (2015). SC. *Experimental Parasitology*. https://doi.org/10.1016/j.exppara.2015.06.016.

[5] Blasini, L. V., Gardner, M., Braselton, M., Brosius, C. R., Caron, K. T., Chambers, D., Corstvet, J., Cowan, E., Jesús, V. R. De, Espinosa, P., Fernandez, C., Holder, C., Kuklenyik, Z., Kusovschi, J. D., Newman, C., Reis, G. B., Rees, J., Reese, C., Silva, L., … Pirkle, J. L. (2019). *Vitamin E Acetate in Bronchoalveolar-Lavage Fluid Associated with EVALI*. 1–9. https://doi.org/10.1056/NEJMoa1916433.

[6] Braga, T. M., Rocha, L., Chung, T. Y., Oliveira, R. F., Pinho, C., Oliveira, A. I., Morgado, J., & Cruz, A. (2020). Biological activities of gedunin—A limonoid from the Meliaceae family. *Molecules*, *25*(3). https://doi.org/10.3390/molecules25030493.

[7] Ciriminna, R., Meneguzzo, F., Delisi, R., & Pagliaro, M. (2017). Citric acid: Emerging applications of key biotechnology industrial product. *Chemistry Central Journal*, *11*(1). https://doi.org/10.1186/s13065-017-0251-y.

[8] *CYP3A5 and medicines*. (n.d.). https://www.stjude.org/treatment/patient-resources/caregiver-resources/patient-family-education-sheets/pharmacy-and-medicines/cyp3a5-and-medicines.html.

[9] D[zbreve]amić, A. M., Marin, P. D., Gbolade, A. A., & Ristić, M. S. (2010). Chemical composition of mangifera indica essential oil from Nigeria. *Journal of Essential Oil Research*, *22*(2), 123–125. https://doi.org/10.1080/10412905.2010.9700279

[10] Erasto, P., & Viljoen, A. M. (2008). Limonene - A review: Biosynthetic, ecological and pharmacological relevance. *Natural Product Communications*, *3*(7), 1193–1202. https://doi.org/10.1177/1934578x0800300728.

[11] Fernandes, S. R., Barreiros, L., Oliveira, R. F., Cruz, A., Prudêncio, C., Oliveira, A. I., Pinho, C., Santos, N., & Morgado, J. (2019). Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art. *Fitoterapia*, *134*(February), 141–150. https://doi.org/10.1016/j.fitote.2019.02.006

[12] Gupta, A., Ansari, S., Gupta, S., & Narwani, M. (2019). Therapeutics role of neem and its bioactive constituents in disease prevention and treatment. 8(3), 680–691.

[13] Imran, M., Arshad, M. S., Butt, M. S., Kwon, J. H., Arshad, M. U., & Sultan, M. T. (2017). Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipidsin Health and Disease, 16(1), 1–17. https://doi.org/10.1186/s12944-017-0449-y

[14] Ix, P., Ester, D., Caughey, W. S., & Ibers, J. A. (1975). Crystal and Molecular Structure of the Free Base. 6639(6), 6639–6645.

[15] Iyamah, P., Famuti, A., & Idu, M. (2017). GC-MS and Molecular Docking Studies for Identification of Anti-malarial Compounds in Agbo-Iba PMII-a Polyherbal Formulation. *Chemistry Research Journal*, *2*(1), 46–56.

[16] Jarvis, A. P., Johnson, S., Morgan, E. D., Simmonds, M. S. J., & Blaney, W. M. (1997). Photooxidation of nimbin and salannin, tetranortriter-penoids from the neem tree *(Azadirachta indica)*. *23* (12), 2841–2860.

[17] Keith, R. E. (2005). Ascorbic acid. Sports Nutrition: Vitamins and Trace Elements, Second Edition, July, 29–46. https://doi.org/10.29309/tpmj/18.4566.

[18] Khan, S. L., Siddiqui, F. A., Jain, S. P., & Sonwane, G. M. (2020). Discovery of Potential Inhibitors of SARS-CoV-2 (COVID-19) Main Pro- tease (M pro) from Nigella Sativa (Black Seed) by Molecular Docking Study. 2, 1–20.

[19] Leela, N. K., Tava, A., Shafi, P. M., John, S. P., & Chempakam, B. (2002). Chemical composition of essential oils of turmeric (Curcuma longa L.). *Acta Pharmaceutica*, *52*(2), 137–141.

[20] *Malaria 7.1*. (n.d.).

[21] MATERSKA, M. (2008). Quercetin and Its Derivatives : Chemical Structure and Bioactivity -a Review. *Polish Journal of Food and Nutrition Sciences*, *58*(4), 407–413.

[22] Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). *The phytochemistry and medicinal value of Psidium guajava (guava)*.

[23] Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The Essential Medicinal Chemistry of Curcumin. *Journal of Medicinal Chemistry*, *60*(5),1620–1637. https://doi.org/10.1021/acs.jmedchem.6b00975

[24] Patel, S. M., & Kumar, R. R. (2010). *Molecular Docking Studies of Antimalarial Drugs for Malaria*. *January*. https://doi.org/10.4172/jcsb.1000059

[25] *Product Information*. (2014). *11759*, 11759.

[26] Rani, K. (1999). *Cyclisation of farnesyl pyrophosphate into sesquiterpenoids in ginger rhizomes ž Zingiber officinale /*. 568–574.

[27] Santos, S. E. B., & Sp, L. (2016). *Pharmacogenomics*. *17*, 1903–1911.

[28] Shah, K. A., Patel, M. B., Patel, R. J., & Parmar, P. K. (2010). *Mangifera Indica (Mango)*. *4*(7).

[29] Stanojevic, J., Stanojevic, L., Cvetkovic, D., & Danilovic, B. (2015). Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (Curcuma longa L.). *Advanced Technologies*, *4*(2), 19–25.

[30] Tahghighi, A., Mohamadi-Zarch, S. M., Rahimi, H., Marashiyan, M., Maleki-Ravasan, N., & Eslamifar, A. (2020). In silico and in vivo anti-malarial investigation on 1-(heteroaryl)- 2-((5-nitroheteroaryl) methylene) hydrazine derivatives. *Malaria Journal*, *19*(1), 1–12. https://doi.org/10.1186/s12936-020-03269-7

[31] Vyas, S. J., Khatri, T. T., Ram, V. R., Dave, P. N., & Joshi, H. S. (2014). Biochemical Constituents in Leaf of *Carica papaya* - Ethnomedicinal Plant of Kachchh Region. *International Letters of Natural Sciences*, *12*(August 2015), 16–20. https://doi.org/10.18052/www.scipress.com/ilns.12.16