https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(4s) (December 2024); 14232 - 14249
Research Article

Insilico Docking Study Of Prospitious Natural Compounds Of *Mitragyna Parvifolia* Effective On Hyperlipidemia Against Hmgcr, Npc1l1, Fxr And Pparα Targets

Umme Habiba[1*], Rounak Seal[2], Baban Majumder[3], Ansar Laskar[4], Soumya Deep Sinha[5], Sneha Ghosh[6], Kush Biswas[7]

[1*]Research Scholar, The Oxford College of Pharmacy, Bangalore -560068.
[2,5]Research Scholar, Maulana Abul Kalam Azad University of Technology, WB, 741249
[3]Assistant Professor, Suptendu Pharmacy Institute, West Bengal, Pin-712102
[4]Assistant Professor, Pandaveswar School of Pharmacy, WB, 713346
[6]Assistant Professor, Muktadhara Institute of Medical Science, WB, 722205
[7]Associate Professor, M.R. College of Pharmaceutical Sciences and Research, WB, 743234

***Corresponding Author:** Umme Habiba
*Department of Pharmacology, The Oxford College of Pharmacy, Bangalore, Karnataka, India-560068.
Email: ummehabibaself@gmail.com

ABSTRACT

Aim: The prevalence of hyperlipidemia is currently rising dramatically across the world. There are very few medications available for the treatment of hyperlipidemia and administering them for longer period could end up with unfavourable side effects.

Materials and Methods: In this study 4 targets i.e. HMG-CoA reductase (HMGCR) receptor, Niemann Pick C1 like1 protein (NPC1L1), Farnesiod X receptor (FXR), and Peroxisome proliferator-activated receptor α (PPARα) were selected on the basis of their mechanism of action in hyperlipidemia. The docking analysis was carried out by utilising PyRx 0.8 virtual screening and the visualization of protein-ligand interaction was investigated by utilising the BIOVIA Discovery Studio Visualizer.

Results: Docking simulation of active compounds from *Mitragyna parvifolia* against HMGCR receptor shows that 3-Isoajmalicine has the highest binding affinity of (-7.8 kcal / mole). In the characteristic binding to NPC1L1 receptor, Pteropodine showed highest binding affinity value of (-7 kcal / mole). Docking analysis of FXR receptor showed impressive binding affinities of Tetrahydroalstonine with a docking score of (-8.8 kcal / mole). Lastly docking studies of PPARα have revealed that Pteropodine (-7.7 kcal / mole) and Uncarine F (-7.7 kcal / mole) has shown highest binding energy.

Conclusion: On the basis of binding affinity and pharmacokinetic parameters our findings strongly support the potential use of phytoconstituents from *Mitragyna parvifolia* as a medicinal herb that might be taken in daily life to prevent higher level of cholesterol in blood because of their inhibitory effect on numerous proteins involved in hyperlipidemia. Further, additional research, i.e. in vitro and in vivo trials on the same constituents, is required to confirm our results and the clear picture of the mechanism of action by which the medicinal plant demonstrated their antihyperlipidemic effects.

Keywords: Hyperlipidemia, *Mitragyna parvifolia*, Phytoconstituents, Molecular docking, ADMET, Drug likeness.

Received: Accepted:

DOI: https://doi.org/10.53555/AJBR.v27i4S.7222

© 2024 The Author(s).
This article has been published under the terms of Creative Commons Attribution-Non-commercial 4.0 International License (CC BY-NC 4.0), which permits non-commercial unrestricted use, distribution, and reproduction in any

medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research

1. INTRODUCTION:

The term "hyperlipidemia' describes heterogeneous disorder that can be inherited or acquired that causes raised total blood cholesterol (TC), free fatty acids, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C) levels. Elevated low-density lipoprotein (LDL) build up in the extracellular subendothelial space of arteries where they are highly extremely toxic to vascular cells and atherogenic[1]. There is a general concept that each of these metabolic illnesses are considered to be linked with both micro- and macro-angiopathies by hyperinsulinemia and insulin resistance [2,3,4]. The prevalence of hyperlipidemia is currently rising dramatically across the world. It has been predicted that, the approximate number of individuals who are likely to suffer cardiovascular problems each year will surpass 24 million by 2030 [5]. According to Apollo's yearly report "Health of the Nation 2023" between 2019 and 2022, the prevalence for obesity increased by 50% and dyslipidemia increased by 18% among Indians. In developed countries, most of the hyperlipidemias are often due to their dietary habits and lifestyle [6]. High cholesterol, hypothyroidism, obesity, diabetes, age, alcohol consumption, lack of nutritious diet and smoking are of great significance and principle cause of dyslipidemia throughout the world [7]. Treatment of hyperlipidemia devided into two categories i.e. self-care and medication. Self-care involves maintaining healthy low fat diet, including nutritious food in daily meal, aerobic exercise, weight loss etc and the medication includes consumption of synthetic hypolipidemic drugs. Apart from their ability to decrease lipid level, these synthetic compounds have other positive effects as well. However, administering them for long period of time could end up with unfavourable side effects [8].

Mitragyna parvifolia is a plant belongs to the Rubiaceae family (commonly known as Kaim/kadam). A wide range of oxindolic and indolic alkaloids are documented in the leaves of *Mitragyna parvifolia*. It is abunded with chemical constituents viz. 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopteropodine, Isorhynchophylline, Mitraphylline, Rhynchophylline,, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroalstonine and Uncarine F [9,10,11]. Additionally, there are numerous therapeutic activity such as Analgesic, Anti-inflammatory, Antiarthritic activity, Antiproliferative, Antinociceptive, Anticonvulsant, Anxiolytic, Antibacterial, Antioxidant activity and many more properties have been reported from this plant but there are no scientific evidence available for antihyperlipidemic activity using *Mitragyna parvifolia*[12].

Molecular docking is a computer-aided approach which serves to identify specific molecules in accordance with their free binding energy and demonstrates structural hypotheses concerning the assessment of protein ligand interactions which plays a key role in determining molecular targets or receptors for various ligands. This approach has benefits in terms of decreasing errors in laboratory research, leads to lower costs and faster responses [13].

2. METHODOLOGY:

2.1 Software and Tools:

Protein Data Bank (PDB), PubChem, PyRx, Molegro Molecular Viewer, Discovery Studio Visualizer.

2.2 Ligand structure preparation:

The phytoconstituents viz. 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopteropodine, Isorhynchophylline, Mitraphylline, Rhynchophylline, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroalstonine and Uncarine F have been diagnosed from the chosen medicinal plant *Mitragyna parvifolia* and the 2D structures of the ligands were obtained from PubChem chemical libraries and saved as SDF format.

2.3 Target Prediction:

In order to identify the various possible targets based on the fit score, the Swiss Target Prediction tool was implemented. The canonical SMILES of chemical compounds has been presented in the website. The species was selected as homo sapiens, and all additional parameters have been retained as the default.

2.4 Protein structure preparation:

The 3D structures of targeted receptor proteins related to metabolism of lipid were recognized by Target Prediction and obtained from the Protein data bank (PDB). The three-dimensional (3D) coordinates of the crystal structure of HMG-CoA reductase receptor (HMGCR) in complex with fluvastatin, PDB ID: 1HWI (with a Resolution: 2.30 Å), Niemann Pick C1 were selected for molecular docking. In order to perform docking analysis the target was initially determined and afterwards 3D structure of the above receptors were obtained. It is commonly accepted that the PDB file format is unable to store bond order information and thus explicit hydrogen assignments are frequently incomplete or incorrect. Consequently, the Molegro Molecular Viewer was used to assign bond orders, correct bonds, hybridization and charges. During the protein preparation procedure, water molecules that were present in the crystal structure were removed.

2.5 Molecular docking:

The docking analysis was carried out by utilising Python Prescription (PyRx) 0.8 virtual screening tool along with BIOVIA Discovery Studio Visualizer (DS 2021). It is necessary to initially convert the updated receptor file from PDB to PDBQT file format before proceeding. All the converted receptor and ligands were uploaded to the

workspace of PyRx and minimization was done using the Open Babel program. All the receptors were closed in the grid box and all the grid points (i.e. X, Y, and Z axis) were assigned accordingly for docking. Grid box arrangement was carried out at a distance of 1 Å in order to enable the ligand to move freely and find the most suitable location to attach to the amino acid at the most convenient spot of the enzyme. For every single protein-ligand combination, a separate docking study was carried out. The analysis of the molecular docking simulation was acquired from parameter scoring of the affinity energy values. The outcomes were arranged in ascending order of docking energies. Each cluster's lowest binding energy was chosen as representative. The binding affinity in between the receptor and ligand is determined for all binding region and the result is reported as affinity (kcal/mol).

2.6 Protein–Ligand Interactions:
The visualization of protein-ligand interaction was investigated by utilising the BIOVIA Discovery Studio Visualizer. 3D and 2D arrangement of the interaction was also displayed for the final docking output. Further details from the interaction of protein-ligand can be utilise to understand the nature of hydrophobics and other forms of hydrogen bonds, as well as which amino acids are crucial for bond formation.

2.7 Lipophilicity of the ligands:
Lipophilicity is a vital physical property of chemical compound which affects drug uptake, potency, distribution and metabolism. It can also defined as the partition coefficient (log Po/w) between water and n-octanol. The lipophilicity attribute of a chemical compound plays a significant role by dictating the success and failure rate in drug discovery. Higher lipophilicity is correlated with larger log P values. SwissADME provides admittance to five freely accessible predictive models, namely iLOGP, XLOGP, MLOGP, WLOGP and SILICOS-IT to determine the lipophilicity property of a molecule.

2.8 Water solubility:
A compound's solubility entirely depends on the solvent employed, the surrounding air temperature, and atmospheric pressure. When a medicine can dissolve at its maximum dose strength in 250 millilitres or less of aqueous medium with a pH range of 1 to 7.5, it is said to be very easily soluble. The estimated volume of 250 ml comes from standard bioequivalence study procedures in which the drug is administered to the participants on an empty stomach along with a glass of water. Each drug substance has been categorised into 4 classes; class I, refers to high solubility and high permeability; class II, indicates lower solubility and higher permeability; class III, which is poorly soluble and highly permeable; lastly, class IV signifies lower solubility and lower permeability. Swiss ADME utilises three topological approaches for water solubility estimation, the first model is ESOL, the second one is based on Ali et al, 2012 and the third one designed by SILICOS-IT. The molar solubility in water (log S) is expressed by the decimal logarithm for all anticipated values.

2.9 In silico Pharmacokinetic Studies:
Pharmacokinetic study is essential to evaluate ADME properties of chemical compound. The pharmacokinetic property of chemical constituents were assessed by using the Swiss ADME tool. The molecular configurations of the compounds were uploaded in the form of canonical SMILES in order to predict various pharmacokinetic parameters. The measured parameters include, passive gastrointestinal absorption (HIA) and penetration of blood-brain barrier (BBB), which are comprised within the BOILED-Egg model, Cytochromes P450 (CYP450), prediction of skin permeability coefficient (Kp), P-glycoprotein (P-gp), and bioavailability (F) score.

2.10 Drug-likeness of the ligands:
The phytochemicals viz. 3 isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopterodine, Isorhynchophylline, Mitraphylline, Rhynchophylline, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroelstonine, Uncarine F were selected as ligands.

2.11 Prediction of Toxicity:
ProTox-II webserver has been utilised to estimate the ligand's organ toxicities, toxicological end points, and LD50. The structure of the active compounds were found by using the compound names in the PubChem. The server analysed the toxicity and selected the toxicity of the targets after choosing the models to be utilised. It was also assessed whether the chemical compounds possess any potentially harmful side effects. Drug induced hepatoxicity is a type acute or chronic liver injury and drug withdrawal is a primary reason for hepatotoxicity. The term "Carcinogenicity" describes the induction of tumours in cells by chemicals or we can say a carcinogenic compound has the ability or tendency to cause cancer. Data analysis is done by using two databases: and the CEBS database and the Carcinogenic Potency Database (CPDB).

3. RESULTS AND DISCUSSION
3.1 Ligand structure preparation:
The phytoconstituents viz. 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopteropodine, Isorhynchophylline, Mitraphylline, Rhynchophylline,, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroalstonine and Uncarine F were selected as ligands and the structures of compounds.

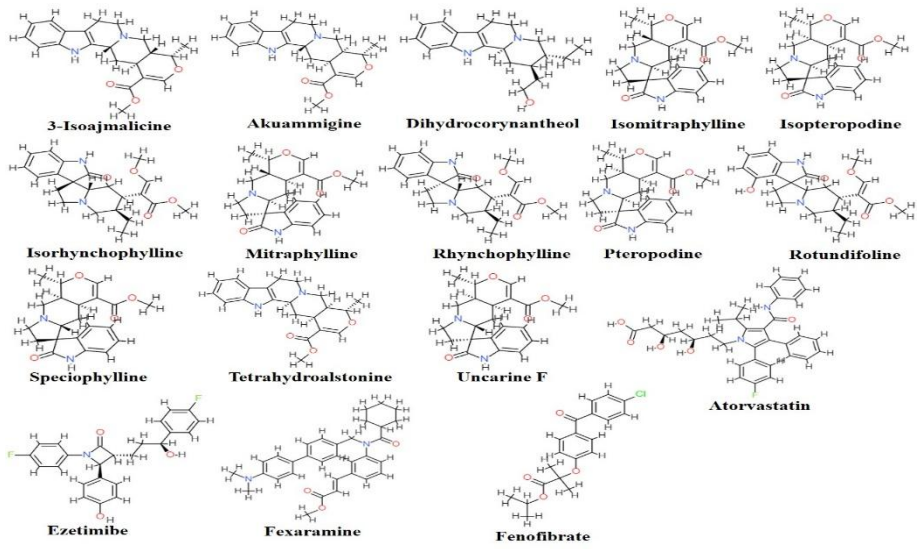


Figure 1. Chemical structure of ligands

Table 1. Displays the canonical SMILES (Simplified molecular input line entry specification) of ligands along with PubChem CID and Molecular formula

Ligand	SMILES	PubChem CID	Molecular Formula
3-Isoajmalicine	CC1C2CN3CCC4=C(C3CC2C(=CO1)C(=O)OC)NC5=CC=CC=C45	11416867	$C_{21}H_{24}N_2O_3$
Akuammigine	CC1C2CN3CCC4=C(C3CC2C(=CO1)C(=O)OC)NC5=CC=CC=C45	1268096	$C_{21}H_{24}N_2O_3$
Dihydrocorynantheol	CCC1CN2CCC3=C(C2CC1CCO)NC4=CC=CC=C34	164952	$C_{19}H_{26}N_2O$
Isomitraphylline	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	11726520	$C_{21}H_{24}N_2O_4$
Isopteropodine	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	9885603	$C_{21}H_{24}N_2O_4$
Isorhynchophylline	CCC1CN2CCC3(C2CC1C(=COC)C(=O)OC)C4=CC=CC=C4NC3=O	3037048	$C_{22}H_{28}N_2O_4$
Mitraphylline	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	94160	$C_{21}H_{24}N_2O_4$
Rhynchophylline	CCC1CN2CCC3(C2CC1C(=COC)C(=O)OC)C4=CC=CC=C4NC3=O	5281408	$C_{22}H_{28}N_2O_4$
Pteropodine	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	10429112	$C_{21}H_{24}N_2O_4$
Rotundifoline	CCC1CN2CCC3(C2CC1C(=COC)C(=O)OC)C4=C(C=CC=C4O)NC3=O	5321000	$C_{22}H_{28}N_2O_5$
Speciophylline	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	168985	$C_{21}H_{24}N_2O_4$
Tetrahydroalstonine	CC1C2CN3CCC4=C(C3CC2C(=CO1)C(=O)OC)NC5=CC=CC=C45	72340	$C_{21}H_{24}N_2O_3$
Uncarine F	CC1C2CN3CCC4(C3CC2C(=CO1)C(=O)OC)C5=CC=CC=C5NC4=O	12304288	$C_{21}H_{24}N_2O_4$
Atorvastatin	CC(C)C1=C(C(=C(N1CCC(CC(CC(=O)O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4	60823	$C_{33}H_{35}FN_2O_5$
Ezetimibe	C1=CC(=CC=C1C2C(C(=O)N2C3=CC=C(C=C3)F)CCC(C4=CC=C(C=C4)F)O)O	150311	$C_{24}H_{21}F_2NO_3$
Fenofibrate	CC(C)OC(=O)C(C)(C)OC1=CC=C(C=C1)C(=O)C2=CC=C(C=C2)Cl	3339	$C_{20}H_{21}ClO_4$
Fexaramine	CN(C)C1=CC=C(C=C1)C2=CC=C(C=C2)CN(C3=CC=CC(=C3)C=CC(=O)OC)C(=O)C4CCCCC4	5326713	$C_{32}H_{36}N_2O_3$

3.2 Protein structure Preparation:
The three-dimensional structures of the targeted receptor proteins associated with lipid metabolism were obtained from protein data bank. In this study 4 selected molecular targets related to Hyperlipidemia, explained below

3.2.1 HMG-CoA reductase receptor:
The most frequently employed drug for treatment of hyperlipidemia is HMG-CoA reductase inhibitors, also known as statin (i.e. atorvastatin, simvastatin). These drugs mostly work by competitively inhibiting HMGCR, the rate-controlling enzyme in the mevalonate pathway, therefore it reduce synthesis of cholesterol. From the protein data bank, HMG-CoA reductase receptor, PDB ID: 1HWI (with a Resolution: 2.30 Å), was retrieved. The protein (PDB ID: 1HWI) was selected on the basis of native ligand fluvastatin's capability to form a compound with HMG-CoA reductase. As determined by in vitro tests using human and mouse microsomes, the IC50 for fluvastatin is 100 nM. The 3D structure of the targeted receptor (PDB ID: 1HWI).

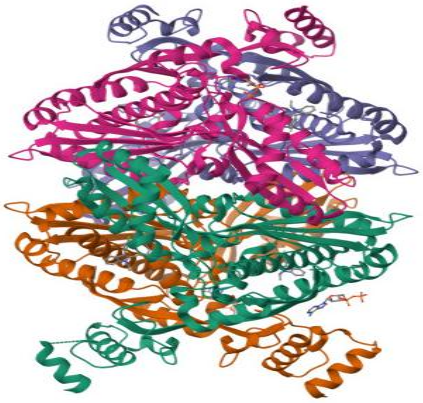


Figure 2. 3D structure of HMG-CoA reductase receptor

3.2.2 NPC1L1 receptor:
NPC1L1 is a polytopic intracellular protein and a renowned target for the cholesterol absorption inhibitor ezetimibe. The protein, PDB ID: 3QNT (with a Resolution: 2.83 Å) was downloaded from protein data bank. It promote absorption of cholesterol in the gut by transferring cholesterol throughout BBM via NPC1L1. Inhibition of NPC1L1 decreases the absorption of lipid in the gut, results in reduced cholesterol level in plasma. 3D structure of the targeted receptor (PDB ID: 3QNT).

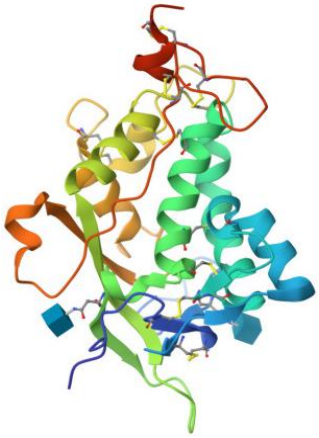


Figure 3. 3D structure of Niemann Pick C1 like1 protein

3.2.3 Farnesiod X-Receptor:
From the protein data bank 3D structure of targeted receptor FXR, PDB ID: 1OSH (with a Resolution: 1.80 Å) was obtained. Being a bile acid (BA) sensor, FXR regulates vitamin and dietary fat absorption, lipid balance, and cholesterol metabolism. In the maintenance of bile acid and cholesterol homeostasis, it plays a key role. Furthermore, plasma triglyceride (TG) levels are reduced when FXR is activated. For those reasons, FXR has become a desirable target molecule for passive lipid control. The 3D structure of targeted receptor (PDB ID: 1OSH).

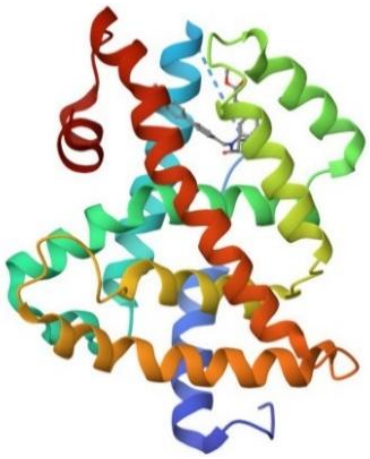


Figure 4. 3D structure of Farnesiod X-Receptor

3.2.4 Peroxisome proliferator-activated receptor:
The 3D crystal structure of PPARα, PDB ID: 2P54 (with a Resolution: 1.79 Å), was downloaded from protein data bank. PPARα was initially considered to be a type of steroid hormone receptor regulated by substances that induce peroxisomal proliferation. PPARα is a nuclear receptor that has a highly standardised DNA binding and ligand-binding domain (DBD and LBD) that might be activated by both synthetic and natural ligands. Based on genetic and functional analysis, PPARα seems to be involved in lipid metabolism in the intracellular and immune system. 3D structure of targeted protein (PDB ID: 2P54).

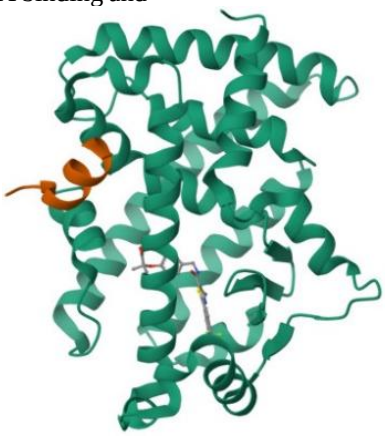


Figure 5. 3D structure of Peroxisome proliferator-activated receptor

3.3 Molecular docking:
3.3.1. Docking Analysis of HMG-CoA reductase receptor
Docking simulation of active compounds from *Mitragyna parvifolia* against HMGCR receptor shows that 3-Isoajmalicine has the highest binding affinity of (-7.8 kcal / mole) which is similar to standard drug Atorvastatin and Rhynchophylline shows the lowest binding affinity with a docking score of (-6.5 kcal / mole). Furthermore Akuammigine (-7.2 kcal / mole), Dihydrocorynantheol (-7.3 kcal / mole), Isomitraphylline (-6.8 kcal / mole), Isopterodine (-7.1 kcal / mole), Isorhynchophylline (-7.7 kcal / mole), Mitraphylline (-7.1 kcal / mole), Pteropodine (-7.7 kcal / mole), Rotundifoline (-6.6 kcal / mole), Speciophylline (-7.2 kcal / mole), Tetrahydroelstonine (-7.5 kcal / mole), Uncarine F (-7.4 kcal / mole) showed a slightly lower docking score than Atorvastatin.

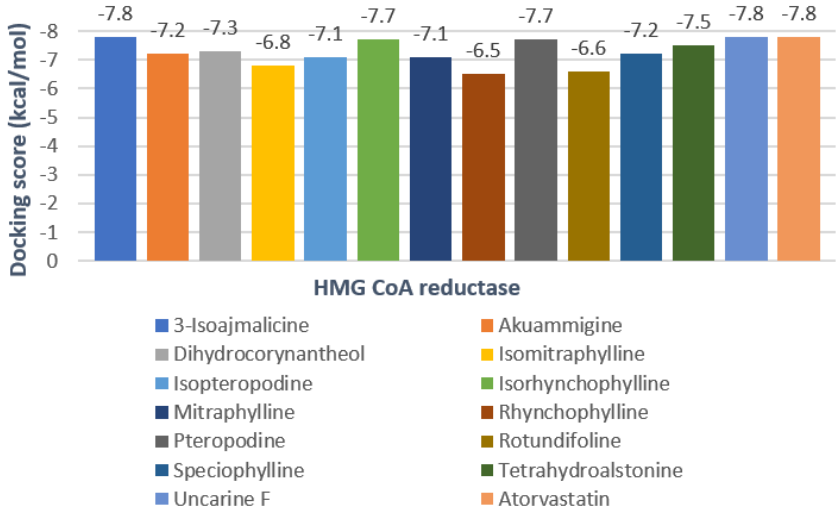
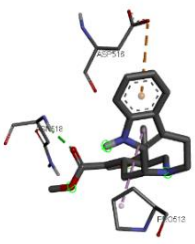
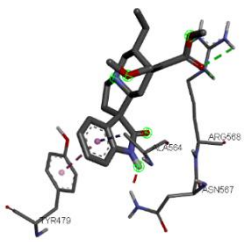
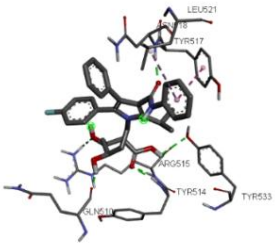


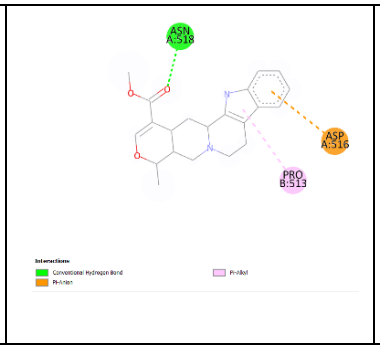
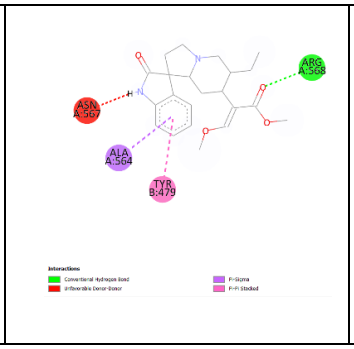
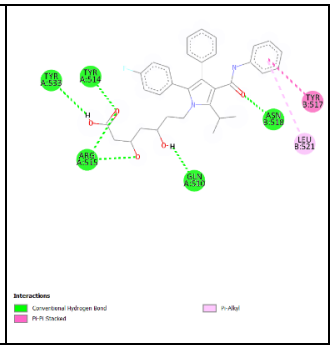
Figure 6. Binding affinity of HMGCR with active compounds of *Mitragyna parvifolia* and synthetic ligand

Table 2. Represents docking interaction of HMGCR with active compounds of *Mitragyna parvifolia* and synthetic ligand

Ligands	**Binding affinity**	**Interaction with Amino acids**
3-Isoajmalicine	**-7.8**	ASN-518, ASP-516, PRO-513
Akuammigine	-7.2	SER-493, TYR514, ARG515
Dihydrocorynantheol	-7.3	TYR-514, ARG-515, ASP-516, PRO-513
Isomitraphylline	-6.8	ARG-702, TRP-698, PRO-693
Isopteropodine	-7.1	ALA-564, TYR-479, ARG-568
Isorhynchophylline	**-7.7**	ASN-567, ALA-564, TYR-479, ARG-568
Mitraphylline	-7.1	ALA-694, ASN-697, LYS-692, VAL-772, ARG-702
Rhynchophylline	-6.5	ASN-518, TYR-511, MET-534, TYR-517, PRO-535
Pteropodine	**-7.7**	ASN-697, ARG-702, PRO-693
Rotundifoline	-6.6	ASN-518, TYR-511, MET-534, TYR-517, PRO-535
Speciophylline	-7.2	ASN-518, PRO-513, PRO-535
Tetrahydroalstonine	**-7.5**	ARG-515, TYR-514, PRO-513, LEU-812, PRO-813
Uncarine F	-7.4	PRO-693, VAL-772, ALA-754, ASN-697, ALA-694
Atorvastatin	-7.8	ARG-515, TYR-514, TYR-533, GLN-510, ASN-518, TYR-517, LEU-521

Table 3. 3D and 2D structure of the two higher binding affinities of active contituents and binding ligand on HMG-CoA reductase receptor

Structures	**Chemical constituents**		**Standard (Atorvastatin)**
	3-Isoajmalicine	**Isorhynchophylline**	
3D			

2D			

3.3.2. Docking analysis of NPC1L1 Receptor

In the characteristic binding to NPC1L1 receptor, all the reported compounds were found to have potential binding affinity. The target prediction suggests that Pteropodine has highest binding affinity value of (-7 kcal / mole) which is more negative compared to standard ligand Ezetimibe (-6.2 kcal / mole) and 3-Isoajmalicine shows the lowest binding affinity value of (-6.2 kcal / mole) that is similar to Ezetimibe. In addition Akuammigine (-6.4 kcal / mole), Dihydrocorynantheol (-6.3 kcal / mole), Isomitraphylline (-6.8 kcal / mole), Isopterodine (-6.7 kcal / mole), Isorhynchophylline (-6.3 kcal / mole), Mitraphylline (-6.9 kcal / mole), Rhynchophylline (-6.7 kcal / mole), Rotundifoline (-6.6 kcal / mole), Speciophylline (-6.5 kcal / mole), Tetrahydroelstonine (-6.8 kcal / mole), Uncarine F (-6.6 kcal / mole) ranked higher than the standard ligand Ezetimibe.

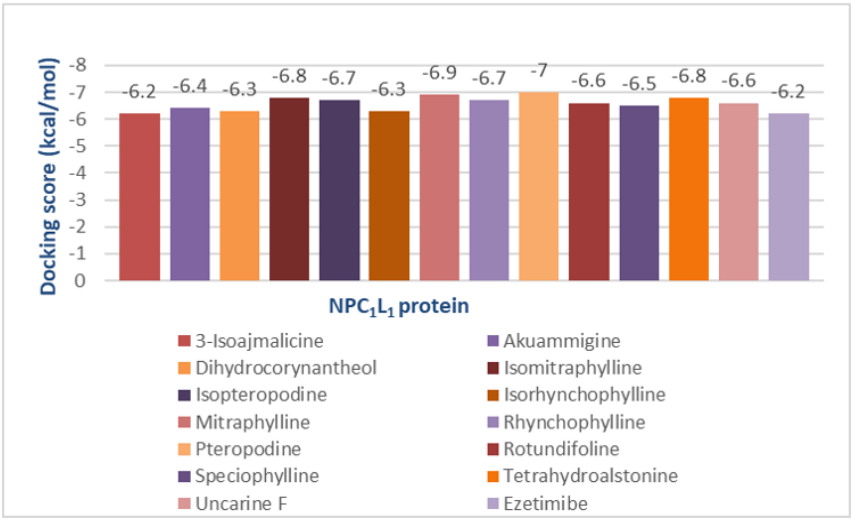


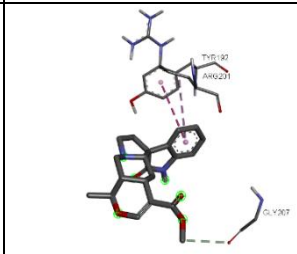
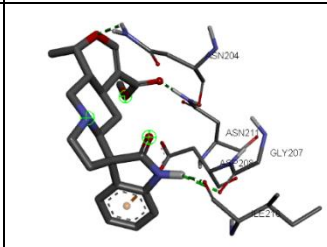
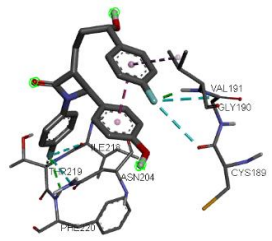
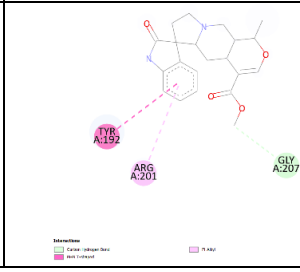
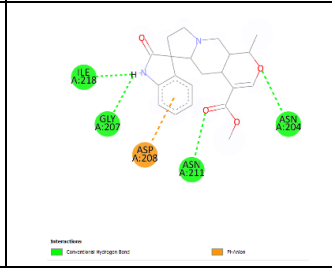
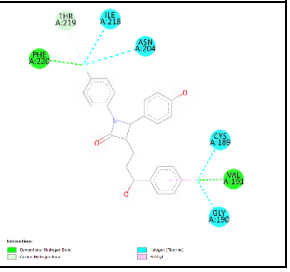
Figure 7. Binding affinity of NPC1L1 receptor with active compounds of *Mitragyna parvifolia* and synthetic ligand

Table 4. Represents docking interaction of NPC1L1 receptor with active compounds of *Mitragyna parvifolia* and synthetic ligand

Ligands	Binding affinity	Interaction with Amino acids
3-Isoajmalicine	-6.2	ARG-242, ALA-241, ARG-80
Akuammigine	-6.4	ASN-211, TYR-192
Dihydrocorynantheol	-6.3	PRO-225, LEU-236
Isomitraphylline	-6.8	ALA-260, ALA-261, CYS-243, ARG-242, ASN-244
Isopteropodine	-6.7	CYS-243, ARG-242, ALA-261, ASN-244
Isorhynchophylline	-6.3	ASP-208, ILE-218, ASN-204, TYR-192
Mitraphylline	-6.9	ILE-218, ASP-208, ASN-204, GLY-207, ASN-211
Rhynchophylline	-6.7	GLY-207, ASP-208, ILE-218, ASN-211
Pteropodine	-7	TYR-192, ARG-201, GLY-207
Rotundifoline	-6.6	GLY-207, ILE-218, ASP-208, ASN-211
Speciophylline	-6.5	CYS-243, ALA-261, ARG-242, SER-173
Tetrahydroalstonine	-6.8	GLN-161, PRO-235, ILE-233, GLN-234, ALA-228
Uncarine F	-6.6	ARG-201, GLY-207

Ezetimibe	-6.2	PHE-220, THR-219, CYS-189, ILE-218, ASN-204, VAL-191, GLY-190

Table 5. 3D and 2D structure of the two higher binding affinities of active contituents and binding ligand on NPCL1 Receptor receptor

Structures	Chemical constituents		Standard (Ezetimibe)
	Pteropodine	Mitraphylline	
3D			
2D			

3.3.3. Docking analysis of FXR Receptor

To compare the binding affinity of the cocrystallized ligand and the synthetic compound, we first redocked Fexaramine into the enzyme's active site and then substituted it with our chemical constituents. The results of binding affinity values indicate the proximity of docking scores between ligands and standard drug Fexaramine. Docking analysis showed impressive binding affinities of Tetrahydroalstonine that exhibits H-bonding with an amino acid backbone and a highest docking score of (-8.8 kcal / mole) which is higher than that of Fexaramine (-8 kcal / mole) where Akuammigine and Isoteropodine shows the lowest binding affinity value of (-6 kcal / mole) and (-6 kcal / mole). Furthermore 3-Isoajmalicine (-7 kcal / mole), Dihydrocorynantheol (-7.8 kcal / mole), Isomitraphylline (-7 kcal / mole), Isopterodine (-6 kcal / mole), Isorhynchophylline (-6.3 kcal / mole), Mitraphylline (-6.4 kcal / mole), Rhynchophylline (-6.7 kcal / mole), Pteropodine (-6.6 kcal / mole), Rotundifoline (-6.7 kcal / mole), Speciophylline (-7.5 kcal / mole), Uncarine F (-6.8 kcal / mole) showed a slightly lower docking score than Fexaramine.

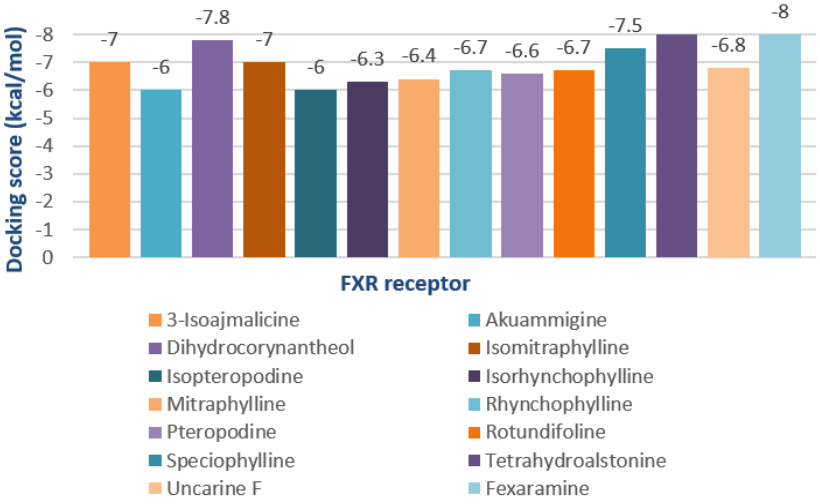
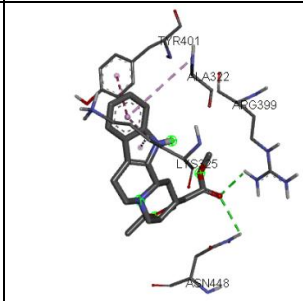
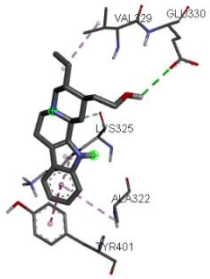
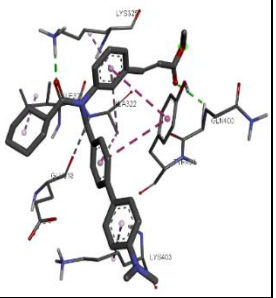
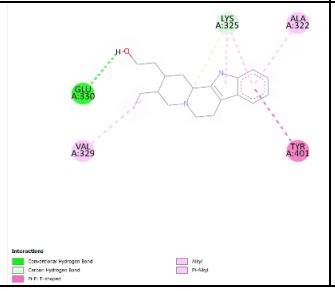
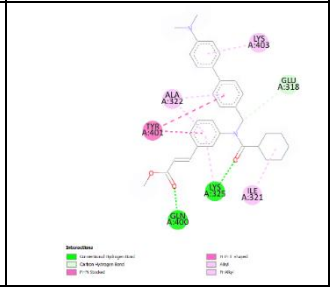


Figure 8. Binding affinity of FXR receptor with active compounds of *Mitragyna parvifolia* and synthetic ligand

Table 6. Represents docking interaction of FXR receptor with active compounds of *Mitragyna parvifolia* and synthetic ligand

Ligands	Binding affinity	Interaction with Amino acids
3-Isoajmalicine	-7	ILE-472, LYS-325
Akuammigine	-6	LEU-324, PHE-312
Dihydrocorynantheol	-7.8	GLU-330, VAL-329, TYR-401, ALA-322, LYS-325
Isomitraphylline	-7	ARG-459
Isopteropodine	-6	LYS-424, PRO-431
Isorhynchophylline	-6.3	GLY-326, TRP-473, VAL-329, ALA-452
Mitraphylline	-6.4	ASN-448, GLY-326, LYS-325, ASP-474
Rhynchophylline	-6.7	GLN-400, GLY-326, TYR-401, LYS-325, TRP-473, ILE-472, HIS-449, ALA-452
Pteropodine	-6.6	LYS-325, GLN-400
Rotundifoline	-6.7	GLY-326, LYS-325, TYR-401, ILE-472, ALA-452
Speciophylline	-7.5	TRP-473
Tetrahydroalstonine	-8.8	ARG-399, ASN-448, ALA-322, LYS-325, TYR-401
Uncarine F	-6.8	ASP-474, ALA-322, LYS-325
Fexaramine	-8	LYS-403, ALA-322, GLU-318, TYR-401, LYS-325, GLN-400, ILE-321

Table 7. 3D and 2D structure of the two higher binding affinities of active contituents and binding ligand on FXR receptor

Structures	Chemical constituents		Standard (Fexaramine)
	Tetrahydroalstonine	Dihydrocorynantheol	
3D			
2D			

3.3.4. Molecular Docking of MPs ligands with PPARα receptor

These docking studies have revealed that Pteropodine (-7.7 kcal / mole), Mitraphylline (-7.6 kcal / mole), and Uncarine F (-7.7 kcal / mole) has more negative binding energy compared to the reference ligand Fenofibrate (-7 kcal / mole). Further Tetrahydroelstonine and Akuammigine showed similar binding affinity as standard with a docking score of (-7 kcal / mole). The other ligands i.e. 3-Isoajmalicine (-6.8 kcal / mole), Dihydrocorynantheol (-6.7 kcal / mole), Isomitraphylline (-6.6 kcal / mole), Isopteropodine (-6.3 kcal / mole), Isorhynchophylline (-6 kcal / mole), Rhynchophylline (-6.6 kcal / mole), Rotundifoline (-6.4 kcal / mole), Speciophylline (-6.6 kcal / mole) exhibited a bit lower docking score compare to Fenofibrate.

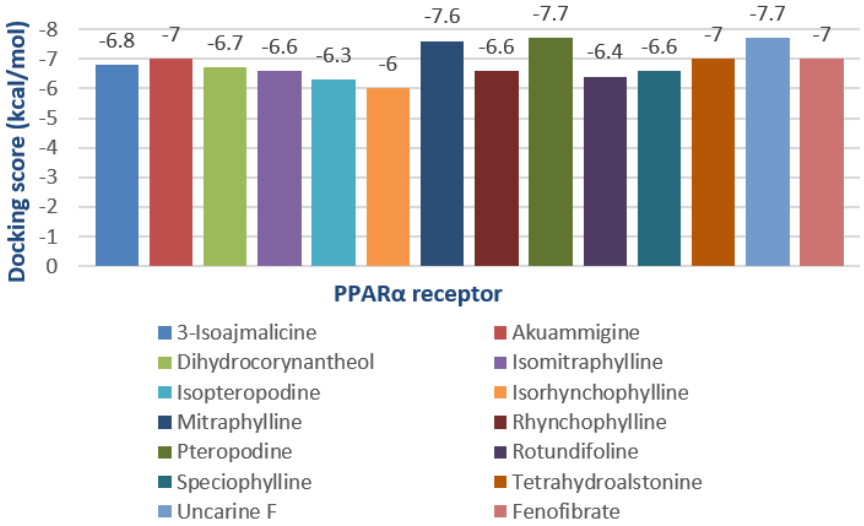
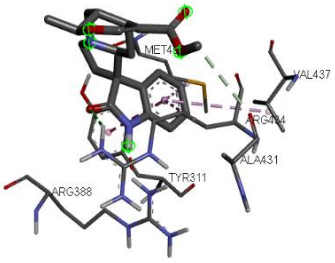
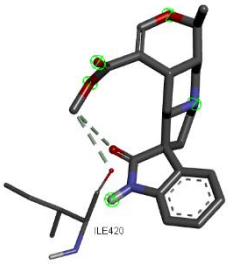
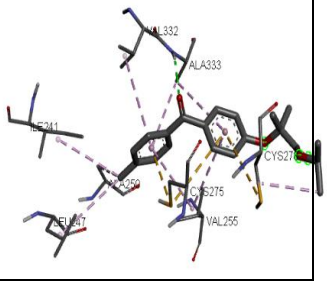


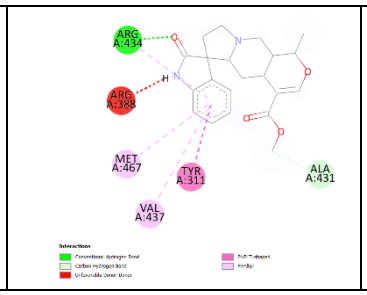
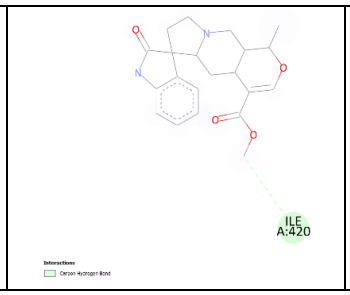
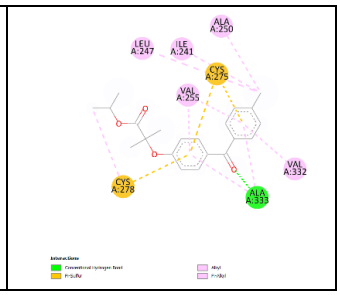
Figure 9. Binding affinity of PPARα with active compounds of *Mitragyna parvifolia* and synthetic ligand

Table 8. Represents docking interaction of PPARα receptor with active compounds of *Mitragyna parvifolia* and synthetic ligand

Ligands	Binding affinity	Interaction with Amino acids
3-Isoajmalicine	-6.8	LYS-204, LEU-410
Akuammigine	-7	ASP-328, VAL-240, PHE-338, LEU-229
Dihydrocorynantheol	-6.7	ASP-328, VAL-240, PHE-338
Isomitraphylline	-6.6	ARG-434, TYR-311
Isopteropodine	-6.3	GLY-231, ASN-326, VAL-240, PHE-338
Isorhynchophylline	-6	ASN-336, ILF-228
Mitraphylline	-7.6	ARG-434, ARG-388, VAL-437, MET-467, TYR-311, ALA-431
Rhynchophylline	-6.6	ARG-434, ARG-388, VAL-437, MET-467, TYR-311
Pteropodine	-7.7	ARG-434, ARG-388, MET-467, VAL-437, TYR-311, ALA-431
Rotundifoline	-6.4	PRO-357, GLU-439, LEU-436
Speciophylline	-6.6	ASN-336, ASN-236, ILE-228, LYS-224
Tetrahydroalstonine	-7	SER-373, ASN-415, HIS-411, SER-414, LEU-410, ALA-207, VAL-407
Uncarine F	-7.7	ILE-420
Fenofibrate	-7	ALA-250, ILE-241, LEU-247, CYS-275, VAL-255, VAL-332, ALA-333, CYS-278

Figure 10. 3D and 2D structure of the two higher binding affinities of active contituents and binding ligand on PPARα receptor

Structures	Chemical constituents		Standard (Fenofibrate)
	Pteropodine	Uncarine F	
3D			

2D			

3.4 Lipophilicity:

Lipophilicity is measured by using a combined set of Log P values, that is average of all Log P values and also known consensus Log P. For iLOGP, Rhynchophylline (3.68) revealed as most lipophilic whereas Dihydrocorynantheol (2.71) as least lipophilic. For XLOGP3, Dihydrocorynantheol (3.2) observed highest value followed by Isomitraphylline (1.62), Isopteropodine (1.62), Mitraphylline (1.62), Pteropodine (1.62), Speciophylline (1.62), and Uncarine F (1.62) showed lowest value. In case of WLOGP, Dihydrocorynantheol (2.79) showed higher value while Isomitraphylline (1.11), Isopteropodine (1.11), Mitraphylline (1.11), Pteropodine (1.11), Speciophylline (1.11), and Uncarine F (1.11) showed lower value. For MLOGP, Dihydrocorynantheol (2.71) observed highest value followed by Isomitraphylline (1.8), Isopteropodine (1.8), Mitraphylline (1.8), Pteropodine (1.8), Speciophylline (1.8), and Uncarine F (1.8) showed lower value. For SILCOS-IT, Dihydrocorynantheol (3.59) showed higher value while Isomitraphylline (1.67), Isopteropodine (1.67), Mitraphylline (1.67), Pteropodine (1.67), Speciophylline (1.67), and Uncarine F (1.67) showed lower value.

Table 9. Lipophilicity characteristics of proposed chemical constituents in relation with the synthetic ligand.

Ligand	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log P
3-Isoajmalicine	3.2	2.75	2.47	2.13	2.78	2.67
Akuammigine	3.2	2.75	2.47	2.13	2.78	2.67
Dihydrocorynantheol	2.71	3.2	2.79	2.71	3.59	3
Isomitraphylline	3.29	1.62	1.11	1.8	1.67	1.9
Isopteropodine	3.29	1.62	1.11	1.8	1.67	1.9
Isorhynchophylline	3.68	2.31	1.75	2.02	2.54	2.46
Mitraphylline	3.29	1.62	1.11	1.8	1.67	1.9
Rhynchophylline	3.68	2.31	1.75	2.02	2.54	2.46
Pteropodine	3.29	1.62	1.11	1.8	1.67	1.9
Rotundifoline	3.65	1.95	1.45	1.49	2.07	2.12
Speciophylline	3.29	1.62	1.11	1.8	1.67	1.9
Tetrahydroalstonine	3.2	2.75	2.47	2.13	2.78	2.67
Uncarine F	3.29	1.62	1.11	1.8	1.67	1.9
Atorvastatin	3.81	4.96	6.54	3.48	6.15	4.99
Ezetimibe	3.47	3.96	4.7	4.76	4.72	4.32
Fenofibrate	3.67	5.17	4.68	3.54	4.95	4.4
Fexaramine	5	6.88	6.46	4.66	6.02	5.8

3.5 Solubility:

According to ESOL model and Ali et al. all the 13 phytochemicals predicted as soluble compounds viz. 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopteropodine, Isorhynchophylline, Mitraphylline, Rhynchophylline, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroalstonine and Uncarine F. According to SILICOS- IT 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isorhynchophylline, Rhynchophylline, Rotundifoline and Tetrahydroalstonine predicted as soluble and remaining showed moderate water solubility. Comparing the solubility results of chemical constituents with the synthetic drug, all compound exhibits superior performance, as indicated by Table-11.

Table 10. Water Solubility properties of the chemical constituents from *Mitragyna parvifolia* in comparison with the commercial one

Ligands	ESOL				Ali				SILICOS- IT			
	Log S	Solubility		Class	Log S	Solubility		Class	Log S	Solubility		Class
		mg/ml	mol/L			mg/ml	mol/L			mg/ml	mol/L	
3-Isoajmalicine	-3.88	4.63E-02	1.31E-04	S	-3.55	9.92E-02	2.81E-04	S	-4.38	1.47E-02	4.16E-05	MS
Akuammigine	-3.88	4.63E-02	1.31E-04	S	-3.55	9.92E-02	2.81E-04	S	-4.38	1.47E-02	4.16E-05	MS
Dihydrocorynantheol	-3.81	4.61E-02	1.55 E-04	S	-3.7	6.01 E-02	2.01 E-04	S	-4.88	3.91 E-02	1.31 E-05	MS
Isomitraphylline	-3.18	2.45E-01	6.65E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Isopteropodine	-3.18	2.45E-01	6.65 E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Isorhynchophylline	-3.51	1.19E-01	3.11 E-04	S	-3.37	1.63 E-01	4.23 E-04	S	-4.48	1.27 E-02	3.30 E-05	MS
Mitraphylline	-3.18	2.45E-01	6.65 E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Rhynchophylline	-3.51	1.19E-01	3.11 E-04	S	-3.37	1.63 E-01	4.23 E-04	S	-4.48	1.27 E-02	3.30 E-05	MS
Pteropodine	-3.18	2.45E-01	6.65E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Rotundifoline	-3.37	1.69E-01	4.22 E-04	S	-3.42	1.51 E-01	3.76 E-04	S	-3.89	5.11 E-02	1.28 E-04	MS
Speciophylline	-3.18	2.45E-01	6.65 E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Tetrahydroalstonine	-3.88	4.63E-02	1.31 E-04	S	-3.55	9.92 E-02	2.81 E-04	S	-4.38	1.47 E-02	4.16 E-05	MS
Uncarine F	-3.18	2.45E-01	6.65 E-04	S	-2.66	8.11 E-01	2.20 E-03	S	-3.96	4.01 E-02	1.09 E-04	S
Atorvastatin	-5.99	5.78E-04	1.03 E-06	MS	-7.05	5.03 E-05	9.00 E-08	PS	-9.13	4.17 E-07	7.46 E-10	PS
Ezetimibe	-4.92	4.91E-03	1.20 E-05	MS	-4.94	4.74 E-03	1.16 E-05	MS	-7.21	2.55 E-05	6.22 E-08	PS
Fenofibrate	-5.23	2.14E-03	5.92 E-06	MS	-6.02	3.44 E-04	9.53 E-07	PS	-6.51	1.12 E-04	3.10 E-07	PS
Fexaramine	-6.95	5.53E-05	1.11 E-07	PS	-7.74	9.09 E-06	1.83 E-08	PS	-8.66	1.09 E-06	2.19 E-09	PS

Note: PS- Poorly soluble, S- Soluble, MS- Moderately soluble

3.6 In silico Pharmacokinetic Studies:

The overall in silico pharmacokinetics predictions showed high level of GI absorption for all the ligands except the synthetic drug atorvastatin. The blood-brain permeability was observed for Akuammigine, Dihydrocorynantheol, Isorhynchophylline, Rhynchophylline, Tetrahydroalstonine and the standard drug Ezetimibe, Fenofibrate while Isomitraphylline, Isopteropodine, Mitraphylline, Pteropodine, Rotundifoline, Speciophylline, Uncarine F and the synthetic drug Atorvastatin, Fenofibrate were observed negative permeability. In this study, it has been found that active chemical constituents are not impermeable with skin because of their higher negative log Kp value. The phytoconstituents 3-Isoajmalicine, Akuammigine, Dihydrocorynantheol, Isomitraphylline, Isopteropodine, Mitraphylline, Pteropodine, Rotundifoline, Speciophylline, Tetrahydroalstonine, Uncarine F and the synthetic ligands Atorvastatin, Ezetimibe, Fexaramine showed p-glycoprotein substrate activity while Rhynchophylline, Isorhynchophylline and synthetic ligand Fenofibrate did not show p-glycoprotein substrate activity. To detect inhibitory activity for cytochrome p450 as CYP1A2, all 9phytoconstituents showed non-inhibitors except the synthetic ligand Fenofibrate; for CYP2C19 all phytoconstituents showed non-inhibitors except the synthetic ligand Atorvastatin, Ezetimibe, Fexaramine; in case of CYP2C9, all ligands showed non-inhibitors except except the marketed drug Fexaramine and Fenofibrate; for CYP2D6 all ligands were showed inhibitors and for CYP3A4 all ligands were observed non-inhibitors except Rhynchophylline, Isorhynchophylline, Atorvastatin, Ezetimibe and Fexaramine. The estimated bioavailability disclose that all the chemical constituents have same bioavailability (i.e. 0.55).

Table 11. ADME analysis of thirteen selected ligands along with four selected standard drug using Swiss ADME tool

nd	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Kp (cm/s) Log	Bioavailability
3-Isoajmalicine	↑	+	+	-	-	-	+	-	-6.5	0.55
Akuammigine	↑	+	+	-	-	-	+	-	-6.5	0.55
Dihydrocorynantheol	↑	+	+	-	-	-	+	-	-5.85	0.55
Isomitraphylline	↑	-	+	-	-	-	+	-	-7.4	0.55
Isopteropodine	↑	-	+	-	-	-	+	-	-7.4	0.55
Isorhynchophylline	↑	+	-	-	-	-	+	+	-7.01	0.55
Mitraphylline	↑	-	+	-	-	-	+	-	-7.4	0.55
Rhynchophylline	↑	+	-	-	-	-	+	+	-7.01	0.55
Pteropodine	↑	-	+	-	-	-	+	-	-7.4	0.55
Rotundifoline	↑	-	+	-	-	-	+	-	-7.36	0.55
Speciophylline	↑	-	+	-	-	-	+	-	-7.4	0.55
Tetrahydroalstonine	↑	+	+	-	-	-	+	-	-6.5	0.55
Uncarine F	↑	-	+	-	-	-	+	-	-7.4	0.55
Atorvastatin	↓	-	+	-	+	-	+	+	-6.19	0.56
Ezetimibe	↑	+	+	-	+	-	+	+	-5.99	0.55
Fe-fibrate	↑	+	-	+	+	+	+	-	-4.83	0.55
Fexaramine	↑	-	+	-	+	+	+	+	-4.44	0.55

Note: High-↑, low-↓, yes-(+), no-(-)

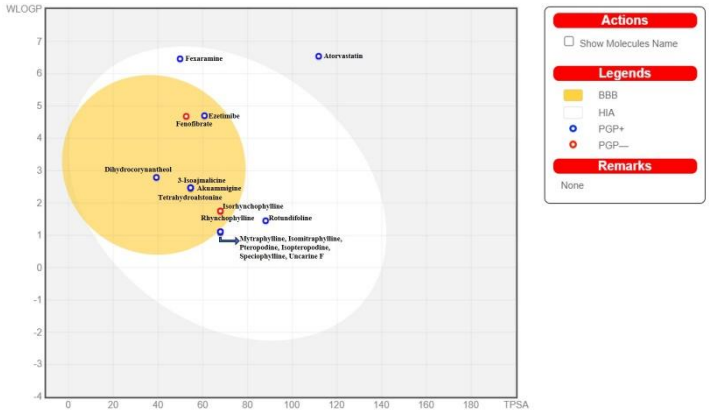


Figure 11. Evaluation of predicted BOILED-Egg model of the *Mitragyna parvifolia* compound

3.7 Drug-likeness of the ligands:

According to the Lipinski's rule of 5, an orally active drug cannot have more than one infraction of the following specifications.: MW ≤ 500 DA, H-bond acceptors ≤ 10, H-bond donors ≤ 5, and log P ≤ 5. Additional significant variables were also estimated, including polar surface area (TPSA), molar refractivity and rotatable bonds. A compound should have a TPSA of less than 140Å and less than 10 rotatable bonds. All the ligands have molecular weight less than 500 and less than 10 rotatable bonds except Atorvastatin. Drug molecules carrying a molecular weight of <500 are easier to transport and exhibit better absorption than those with a higher molecular weight.

Table 12. The drug-likeness of the phytoconstituents of *Mitragyna parvifolia* along with four synthetic ligands

Ligand	Molecular weight	TPSA	Molar refractivity	Mlog	Rotatable bonds	Num. of H-bond donors	Num. of H-bond acceptors
3-Isoajmalicine	352.43	54.56	103.47	2.13	2	1	4
Akuammigine	352.43	54.56	103.47	2.13	2	1	4
Dihydrocorynantheol	298.42	39.26	95.24	2.71	3	2	2
Isomitraphylline	368.43	67.87	106.47	1.8	2	1	5
Isopteropodine	368.43	67.87	106.47	1.8	2	1	5
Isorhynchophylline	384.47	67.87	113.39	2.02	5	1	5
Mitraphylline	368.43	67.87	106.47	1.8	2	1	5
Rhynchophylline	384.47	67.87	113.39	2.02	5	1	5
Pteropodine	368.43	67.87	106.47	1.8	2	1	5
Rotundifoline	400.47	88.1	115.42	1.49	5	2	6
Speciophylline	368.43	67.87	106.47	1.8	2	1	5
Tetrahydroalstonine	352.43	54.56	103.47	2.13	2	1	4
Uncarine F	368.43	67.87	106.47	1.8	2	1	5
Atorvastatin	558.64	111.79	158.26	3.48	13	4	6
Ezetimibe	409.43	60.77	112.97	4.76	6	2	5
Fenofibrate	360.83	52.6	97.98	3.54	7	0	4
Fexaramine	496.64	49.85	152.7	4.66	10	0	3

3.8 Prediction of Toxicity:

The toxicity was estimated on the basis of several targets that are connected to unfavourable drug-reactions. 3-Isoajmalicine, Akuammigine, Isomitraphylline, Isopteropodine, Isorhynchophylline, Mitraphylline, Rhynchophylline, Pteropodine, Speciophylline, Tetrahydroalstonine, and Uncarine F has claimed to be carcinogenic. 3-Isoajmalicine, Akuammigine and Tetrahydroalstonine, are found to have Immunotoxicity with a toxicity class of 3. All the phytoconstituents has shown negative Mutagenicity, Cytotoxicity and hepatotoxicity where Atorvastatin and Ezetimibe showed hepatotoxicity with LD50 of 5000 mg/kg and 1350 mg/kg, has class 5 and class 4 toxicity respectively. Table 14 displays the evaluated toxicity for all the ligands.

Table 13. Toxicity analysis of thirteen selected ligands along with four synthetic drug using Pro Tox II

Ligand	HT	CG	IT	MG	CT	AhR[1]	AR[2]	ARLBD[3]	Aromatase	ER[4]	ER-LBD[5]	PPA R-γ[6]	nrf2/ ARE[7]	HSE[8]	MMP[9]	p5315[10]	ATAD 5[11]	LD50[12] mg/kg	TC[13]
3-Isoajmalicine	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	3
Akuammigine	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	3
Dihydrocoryn antheol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	760	4
Isomitraphylli ne	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Isopteropodine	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Isorhynchophy lline	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	325	4
Mitraphylline	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Rhynchophylli ne	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	325	4
Pteropodine	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Rotundifoline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	325	4
Speciophylline	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Tetrahydroalst onine	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	3
Uncarine F	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	406	4
Atorvastatin	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	5000	5
Ezetimibe	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1350	4
Fenofibrate	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	4
Fexaramine	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	500	4

DISCUSSION

Modern drug discovery requires evaluating the efficacy of dynamic molecules and their ability to effectively reach the desired target site in a reactive state. This process involves conducting cellular, animal, and human clinical trials, which are both expensive and perhaps potentially hazardous. The estimation of a drug's pharmacokinetic properties i.e. absorption, distribution, metabolism, and excretion (ADME) is currently supported by computer-aided drug discovery; they provide fast, reliable, and predictive data and support experimental methods.

In this work, we assessed the ADME and toxicity characteristics of the bioactive phytoconstituents that found in *Mitragyna parvifolia,* using the Swiss ADME and Protox-II online tool. This programme is easily accessible and allows for simplified result analysis, even for those who are not experts in CADD. Using the Swiss ADME and Protox-II online tool, a total of 13 active phytoconstituents of *Mitragyna parvifolia* were examined to investigate various charecteristics like lipophilicity (Table 10), water solubility (Table 11), pharmacokinetic parameters (Table 12), Evaluation of predicted BOILED-Egg model (Figure 10), drug likeness (Table 13) and toxicity study (Table 14) respectively.

The possible interactions between the small active phytocompounds and proteins could lead to the creation of novel treatments for a wide range of pathologies. These concepts, supported by long experience of traditional wisdom and the use of indigenous resources to address health issues, are widely used in Ayurveda, Traditional Chinese Medicines, Yunani medicines as well as other indigenous centres which focus on local medicinal products. Therefore, based on its peculiar characteristics of exhibiting strong functional groups, *Mitragyna parvifolia* is a compound used in several medical treatments. The present study revealed a similar type of observation about the interaction with the four different target protein i.e. HMG-CoA reductase, NPC1L1, FXR and PPARα by displaying a notable binding energy difference in comparison with the synthetic drug Atorvastatin, Ezetimive, Fexaramine and Fenofibrate.

HMG-CoA reductase inhibitors commonly known as Statins, are the most commonly used anti-hyperlipidemic drugs that reduce cardiovascular mortality by inhibiting cholesterol biosynthesis. It has

been discovered that the alkaloids 3-Isoajmalicine and Tetrahydroalstonine shared the same amino acid (ASN-518 and TYR-514) as Atorvastatin (Table 2), indicating inhibition of enzyme activity. Even though 3-Isoajmalicine and Atorvastatin shared some hydrophobic amino acid residues, their greater negative binding energy may have been caused by the alkaloid's increased disruption of amino acid residues surrounding the C-terminus. According to this in silico investigation, the active phytochemicals derived from the formulation of *Mitragyna parvifolia* may have the ability to inhibit HMG-CoA reductase. However, to learn more about these phytochemicals' ability to reduce cholesterol levels, further laboratory studies are needed (i.e. in vitro as well as in vivo).

With NPC1L1 protein, as we found in the results (Table 4), the alkaloids Mitraphylline and Rotundifoline shared the common amino acid residue with the standard Ezetimibe (ILE-218, ASN-204). The stronger extent of interaction between the alkaloid and NPC1L1 protein may be related to the fewer hydrophobic amino acid residues. Interactions between ezetimibe and the protein have been shown to move the N-terminal domain, allowing for cholesterol movements as demonstrated by Huang *et al.* The amino acid hydrophobicity has already been associated with the parameter flexibility. The in silico docking study revealed that because of their higher binding affinity with NPC1L1 receptor, the phytoconstituents of *Mitragyna parvifolia* may be used to regulate and mitigate hypercholesterolemia.

In the docking study with FXR it has been revealed that Tetrahydroalstonine showed the highest binding affinity (Table 6) and also shared three common amino acid residues with the synthetic drug Fexaramine (ALA-322, LYS-325, TYR-401). The virtual screening's findings are consistent with the hypothesis that the active chemical compounds may bind to the same enzyme target as Fexaramine.

In case of PPARα, the results of docking studies showed that Pteropodine and Uncarine F has shown more negative binding affinity compared to the standard ligand Fenofibrate. The process by which a PPARα ligand fits into the binding site appears to be determined by its three-dimensional structure. Overall, these results suggest that the phytoconstituents in *Mitragyna parvifolia* may be able to reduce dyslipidemia via PPARα inhibition.

4. CONCLUSION

The prediction of binding affinity, lipophilicity, solubility, pharmacokinetics, drug-likeness and toxicity study revealed that the active compounds of *Mitragyna parvifolia* can be a promising lead compound for a new drug candidate for anti-hyperlipidemic phytomedicine. This study is limited by the fact that many factors are involved in lipid metabolism and hyperlipidemia control, and that HMG CoA, NPC1L1, FXR and PPAR are not the only targets. Therefore, it is reasonable to assume that *Mitragyna parvifolia* might potentially affect other proteins involved in lipid metabolism by blocking or activating them. Further, additional research, i.e. in vitro and in vivo trials on the same constituents, is required to confirm our results and the clear picture of the mechanism of action by which the medicinal plant demonstrated their antihyperlipidemic effects. In short, our findings strongly support the potential use of phytoconstituents from *Mitragyna parvifolia* as a medicinal herb that might be taken in daily life to prevent hyperlipidemia, as compared to currently prescribed and marketed drugs like atorvastatin, since these phytoconstituents have no adverse effects.

5. REFERENCES

[1]. Who M. Myocardial infarction and coronary deaths in the health organisation. Circulation. 1994;90:583-12.

[2]. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. The Journal of clinical investigation. 1985 Mar 1;75(3):809-17.

[3]. Paolisso G, Ferrannini E, Sgambato S, Varricchio MI, D'Onofrio FE. Hyperinsulinemia in patients with hypercholesterolemia. The Journal of Clinical Endocrinology & Metabolism. 1992 Dec 1;75(6):1409-12.

[4]. Bennani-Kabchi N, Fdhil H, Cherrah Y, Kehel L, El Bouayadi F, Amarti A, Saïdi M, Marquié G. Effects of Olea europea var. oleaster leaves in hypercholesterolemic insulin-resistant sand rats. Therapie. 1999 Nov 1;54(6):717-23.

[5]. Esakkimuthu S, Nagulkumar S, Darvin SS, Buvanesvaragurunathan K, Sathya TN, Navaneethakrishnan KR, Kumaravel TS, Murugan SS, Shirota O, Balakrishna K, Pandikumar P. Antihyperlipidemic effect of iridoid glycoside deacetylasperulosidic acid isolated from the seeds of Spermacoce hispida L.-A traditional antiobesity herb. Journal of ethnopharmacology. 2019 Dec 5;245:112170.

[6]. Expert Panel on Detection E. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). Jama. 2001 May 16;285(19):2486-97.

[7]. Dalwadi PP, Patani PV. Anti hyperlipidemic activity of Tephrosia purpurea plant extracts in poloxomer 407 induced hyperlipidemic rats. International Journal of Pharmacological Research. 2014;4(4):186-93.

[8]. Tacherfiout M, Petrov PD, Mattonai M, Ribechini E, Ribot J, Bonet ML, Khettal B. Antihyperlipidemic effect of a Rhamnus alaternus leaf extract in Triton-induced hyperlipidemic rats and human HepG2 cells. Biomedicine & Pharmacotherapy. 2018 May 1;101:501-9.

[9]. Shellard EJ, Phillipson JD, Gupta D. The Mitragyna species of Asia. 13. The alkaloids of the leaves of *Mitragyna parvifolia* (Roxb.) Korth. obtained from India. Planta Med. 1968 Dec;16(4):436-45.

[10]. Houghton PJ, Shellard EJ. THE MITRAGYNA SPECIES OF ASIA. Planta medica. 1974 Sep;26(06):104-12.
[11]. Pandey R, Singh SC, Gupta MM. Heteroyohimbinoid type oxindole alkaloids from *Mitragyna parvifolia*. Phytochemistry. 2006 Oct 1;67(19):2164-9.
[12]. Choudhary GP, Jain AP. A Review on *Mitragyna parvifolia* (Roxb.) Korth. -An Indian Medicinal Plant. International Journal of Pharmacy and Pharmaceutical Research. 2016 Aug;7(1):175-84.
[13]. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015 Jul 22;20(7):13384-421